INTERRELATIONSHIP OF RENAL α2-ADRENOCEPTORS AND

VASOPRESSIN IN THE REGULATION OF SODIUM AND WATER

EXCRETION IN THE RAT

BY

DOROTHEA E. BLANDFORD

A thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology and Therapeutics
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This thesis is dedicated to my husband, Ted - with all of my love.

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ABSTRACT

In the rat kidney, α2-adrenoceptors predominate numerically over α1-adrenoceptors in a ratio of approximately 2 to 1. While α1-adrenoceptors mediate renal nerve stimulation-induced sodium retention, the physiological role of the α2-adrenoceptor, in the regulation of sodium and water excretion, is less clear. Therefore, the effects of α2-adrenoceptor stimulation and antagonism were evaluated in an anesthetized rat preparation. Briefly, uninephrectomized rats were anesthetized, and the carotid artery and jugular vein cannulated for the measurement of blood pressure and the infusion of saline (97 ul/min) respectively. The left kidney was exposed and the ureter cannulated for the collection of urine. A 31 gauge needle was inserted into the aorta, advanced into the renal artery and secured with glue. This allowed the direct intrarenal arterial infusion of the α2-adrenoceptor agonist, clonidine or the α2-adrenoceptor antagonist, yohimbine. Blockade of the α2-adrenoceptor by yohimbine resulted in a decrease in sodium and water excretion, and an increase in urine osmolality. Further experiments characterized the dose-response relationship between an intrarenal infusion of clonidine, and the effects on sodium and water excretion. At low infusion rates, clonidine only increased free water clearance. Both these effects are consistent with the antagonism of the renal effects of vasopressin. Higher infusion rates of clonidine, however, also increased sodium excretion. This dose-related dissociation of water and then solute excretion suggests that another mechanism may be involved in the renal response to α2-adrenoceptor stimulation. The enhanced natriuretic response to clonidine administered intravenously as compared to intrarenally, is consistent with this hypothesis. The natriuresis does not appear to be secondary to an increase in the release of atrial natriuretic peptide, however, the potentiation of the natriuretic effects of clonidine following indomethacin

pretreatment suggests that renal prostaglandins may be involved. Administration of a specific V2 antagonist also produced a dose-related increase in free water clearance at low doses, while higher doses also increased sodium excretion. In the presence of the V2 antagonist, the effects of high intrarenal infusion rates of clonidine on sodium excretion were attenuated. These results suggest that the renal effects of α2-adrenoceptor stimulation with clonidine may involve two different sites or mechanisms of action.

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GENERAL INTRODUCTION

Historical Perspectives

Our knowledge of adrenergic pharmacology has increased substantially since the initial subclassification of adrenoceptors into α and β subtypes was proposed by Ahlquist (1948). Ahlquist differentiated adrenoceptors on the basis of their pharmacology rather than on the basis of their function, as had been proposed earlier (Dale, 1906). By comparing the rank order of potency of five catecholamines in eight different physiological assay systems, Ahlquist discovered a similar rank order of potency of the catecholamines in five of the test systems, while the other three test systems exhibited a markedly different rank order of potency of the catecholamines. He postulated that the two different rank orders of potency represented two different receptor subtypes which he named α and β. This pharmacological classification scheme was subsequently validated by the use of antagonist drugs which selectively blocked either the α or the β-adrenoceptor-stimulated response. The β-adrenoceptors were further subdivided into $\beta1$ and $\beta2$ receptor subtypes by Lands et $al.$ (1967). This subdivision also had a pharmacological basis, since it resulted from a comparison of the rank order of potency of 12 agonists in several isolated organ systems. This subclassification has also since been substantiated by the development of subtype selective antagonists and receptor binding studies (Minneman et $al.$, 1979).

Similarly, α-adrenoceptors have been subdivided into $\alpha1$ and $\alpha2$ subtypes, however this identification was much more difficult. The initial subclassification was based on the presumed anatomical distribution of the α-adrenoceptors within the synapse. Presynaptic α-adrenoceptors were inhibitory in function while

postsynaptic α-adrenoceptors were excitatory. Subsequent work then demonstrated that presynaptic and postsynaptic α-adrenoceptors were not identical (Dubocovich and Langer, 1974, Starke *et al.*, 1974). Consequently, Langer (1974) proposed that the postsynaptic α-adrenoceptor which mediated the response in an effector organ be referred to as α1, while the presynaptic α-adrenoceptor that regulated transmitter release be referred to as α2. Generally, this anatomical subclassification holds true, however, sufficient evidence exists to indicate that not all α1-adrenoceptors are located postsynaptically (Kobinger and Pichler, 1980), and not all α2-adrenoceptors are located presynaptically (Timmermans and VanZwieten, 1981). Particularly in blood vessels, postsynaptic α2-adrenoceptors mediating vasoconstriction have been demonstrated to coexist along with postsynaptic α1-adrenoceptors (DeMay and Vanhoutte, 1981; Docherty and McGrath, 1980; Drew and Whiting, 1979; Timmermans *et al.*, 1979). Thus, the anatomic subdivision of α-adrenoceptors was not useful as a definition of subtypes. Recognizing this, Berthelson and Pettinger (1977) suggested both a functional and pharmacological basis for classifying α-adrenoceptor subtypes. Accordingly, α1- and α2-adrenoceptors mediate excitatory and inhibitory responses respectively. The functional classification, however, also lacked general applicability since it became apparent that some excitatory receptors should be included in the α2-adrenoceptor classification. A third attempt to define α-adrenoceptor subtypes was based on a biochemical approach. According to this definition, α1-adrenoceptors mediate effects secondary to an elevation of intracellular calcium, while α2-adrenoceptors mediate effects secondary to the inhibition of adenylate cyclase (Fain and Garcia-Sainz, 1980; Wikberg, 1979). However, shortly after this biochemical approach was proposed several investigators demonstrated that pressor responses to α2-adrenoceptor agonists could be blocked by calcium channel antagonists such as verapamil, diltiazem and nifedipine (Cavero *et al.*, 1983; Timmermans *et al.*, 1984; van Meel *et al.*, 1981a; 1981b). Furthermore,

contractions of isolated smooth muscles elicited by α2-adrenoceptor activation were reduced by lowering the calcium concentration and by calcium channel antagonists (Hicks *et al.*, 1985; Jim and Matthews, 1985; Medgett and Rajanayagam, 1984). Thus it appears that inhibition of adenylate cyclase is not the only mechanism by which stimulation of the α2-adrenoceptor can produce a physiological effect. At present, the definition of α1- versus α2-adrenoceptor subtypes is based on a pharmacological subclassification, namely the potency of the specific α-adrenoceptor antagonists prazosin and yohimbine, as originally suggested by Berthelson and Pettinger (1977). At the α1-adrenoceptor, prazosin is more potent than yohimbine, whereas the reverse is true at α2-adrenoceptors (Bylund and U'Prichard, 1983).

α-Adrenoceptor activation influences a number of diverse physiological functions in many mammalian species. A detailed review of the known and proposed function of α1- and α2-adrenoceptors in various organs and tissues is beyond the scope of this introduction Instead, only the effects of α1- and α2-adrenoceptors in the kidney will be addressed here, with a greater emphasis placed on α2-adrenoceptors.

Localization and Mechanisms of Action of α-Adrenoceptors

The existence of α-adrenoceptors in the kidney had been suspected for many years, since α-adrenergic drugs produce a variety of renal effects. The functions and locations of α-adrenoceptors in the kidney are now beginning to be understood. Radioligand binding techniques have been particularly useful for characterizing and quantitating α-adrenoceptors. In the kidney, α1-adrenoceptors have been studied with [^{3}H]prazosin (McPherson and Summers, 1981; 1982; Schmitz *et al.*, 1981; Snavely and Insel, 1982). α2-Adrenoceptors, on the other hand, have been identified and characterized with [^{3}H]clonidine (Jarrott et al, 1979; McPherson and

Summers, 1981), [^{3}H]yohimbine (Schmitz *et al.*, 1981; Snavely and Insel, 1982), [^{3}H]rauwolscine (McPherson and Summers, 1983) and [^{3}H]idazoxan (Boyajian *et al.*, 1987). These studies indicate that α1- and α2-adrenoceptors coexist in the kidneys of a variety of mammalian species, but the number, proportion and distribution of each α-adrenoceptor subtype may vary from species to species.

While there is clear evidence for the presence of postjunctional α2-adrenoceptors as well as α1-adrenoceptors on the smooth muscle of many vascular preparations (McGrath *et al*, 1982), neither ligand binding studies nor functional studies have thus far given very much detailed information on the distribution of α-adrenoceptor subtypes along the renal vasculature. Autoradiographic studies of rat kidney slices suggest a predominance of α1-adrenoceptors associated with the renal vasculature (Stephenson and Summers, 1985; 1986). In addition, these same studies indicated a high concentration of α2-adrenoceptors associated with the intima in the renal vasculature of the rat kidney. It is believed that α1- and α2-adrenoceptors coexist in the renal vasculature. These receptors appear to mediate a vasoconstrictor response and thereby would contribute to the modulation of renal blood flow.

Initial investigations of the α-adrenoceptor subtype mediating the renal vascular responses to exogenously administered agonists suggested an almost exclusive role of α1-adrenoceptors in the renal vasculature of the rat (Schmitz *et al.*, 1981), cat (Drew and Whiting, 1979) and the dog (Horn *et al.*, 1982). More recent experiments in the anesthetized rat (Wolff *et al.*, 1987) and in the extra-corporally blood perfused rat kidney (DiBona and Sawin, 1987) were consistent with this notion. However, in both of these studies, and in the studies of Horn *et al.* (1982) and Wolff *et al.* (1984) in both the dog and the rat, clonidine produced a small constriction of the renal vasculature. Addition of specific α2-adrenoceptor antagonists significantly attenuated the renal vasoconstriction induced by either

clonidine or guanabenz, suggesting that, at least in the dog and the rat, both α1- and α2-adrenoceptors are involved in mediating the vasoconstrictor response. Both α-adrenoceptor subtypes may therefore modulate renal blood flow. Significant renal vasoconstriction has also been documented by α2-adrenoceptor activation in the rabbit (Hesse and Johns, 1984). Recently, these studies have been expanded to investigate the role of the α2-adrenoceptor in mediating renal vasoconstriction in the conscious rat. Gellai and Ruffolo (1987) demonstrated that equipressor doses of cirazoline and B-HT 933, specific α1- and α2-adrenoceptor agonists respectively, were equally effective in decreasing effective renal plasma flow. Moreover, in the studies of Wolff *et al.* (1989), intrarenal arterial bolus doses of guanabenz, above 10 *ng*, decreased renal blood flow. This dose response curve was shifted to the right by the specific α2-adrenoceptor antagonists idazoxan and rauwolscine, clearly demonstrating a role for α2-adrenoceptors in mediating renal vasoconstriction.

In the rat, localization of [^{3}H]prazosin binding by autoradiography demonstrates that the majority of α1-adrenoceptors in the kidney are associated with proximal tubules where they are believed to mediate renal nerve stimulation-induced sodium reabsorption (Bello-Reuss *et al.*, 1976). A small population of α2-adrenoceptors has been identified in membrane preparations from glomeruli, however the contribution of glomerular α2-adrenoceptor binding to that in whole cortex is small. Most of the α2-adrenoceptors appear to be associated with renal tubules. Autoradiographic studies indicate that [^{3}H]rauwolscine binding in rat kidney cortical sections is also largely confined to proximal tubules (McPherson and Summers, 1983). Further studies using intact proximal tubular cells have shown that the α2-adrenoceptor is the predominant α-adrenoceptor subtype present in this nephron segment (Insel *et al.*, 1985). Moreover, in a renal membrane preparation, α2-adrenoceptors have been shown to predominate numerically over α1-adrenoceptors (Pettinger *et al.*, 1982; Schmitz *et al.*, 1981; Smyth *et al.*, 1984).

Denervation did not alter the number of α2-adrenoceptors, which suggested the possibility that these receptors were postsynaptic in location. Alternatively, denervation could result in a decrease in presynaptic α2-adrenoceptors. If the postsynaptic α2-adrenoceptors are innervated, receptor upregulation would occur with denervation. Thus, while postsynaptic α2-adrenoceptor number could increase, and presynaptic α2-adrenoceptor number could decrease, the net effect observed in a renal membrane preparation would be unaltered. Nevertheless, these α2-adrenoceptors could participate in the regulation of the tubular reabsorption of sodium and water or in vascular smooth muscle contraction (Horn *et al.*, 1982; Pettinger *et al.*, 1982; Sakakibara *et al.*, 1982; Young and Kuhar, 1980). Interestingly, this numerical predominance of α2- over α1-adrenoceptors has been shown to be enhanced in genetically hypertensive rats (Pettinger *et al.*, 1982; Sanchez and Pettinger, 1981). The increase in α2-adrenoceptor density in the spontaneously hypertensive rat (SHR) has recently been found to be primarily associated with the proximal tubules (Stanko and Smyth, in press). The significance of this elevation in renal α2-adrenoceptors in genetically hypertensive rats has not been described.

The effects of α1-adrenoceptors in the kidney appear to be mediated by the mobilization of phosphatidylinositol turnover, which leads to the formation of diacylglycerol and inositol 1,4,5-triphosphate which subsequently promotes calcium release from intracellular stores (Berridge and Irvine, 1984; Garcia-Sainz *et al.*, 1980; Putney, 1987). Whether this is the only mechanism whereby α1-adrenoceptor stimulation increases cytosolic calcium levels has recently been questioned (Han *et al.*, 1987). Nevertheless, the increase in cytosolic calcium is believed to activate protein kinase C which then triggers gluconeogenesis, a process which is known to be calcium-dependent, and electrolyte transport.

α2-Adrenoceptors on the other hand, have been demonstrated to decrease cellular adenosine 3',5'-cyclic monophosphate (cAMP) levels by inhibiting the membrane bound adenylate cyclase (Exton, 1982; Jakobs et al., 1981) However, it is not known whether this mechanism is the most important mechanism whereby stimulation of the α2-adrenoceptor produces a physiological effect. As mentioned previously, calcium influx appears to be involved in the vasoconstrictor action of α2-adrenoceptor agonists (Hicks *et al.*, 1985; Jim and Matthews, 1985; van Meel *et al.*, 1981a; 1981b). Nevertheless, inhibition of adenylate cyclase and the subsequent cAMP accumulation appears to be the predominant mechanism by which the effects of α2-adrenoceptor stimulation are mediated in the renal tubules (Pettinger *et al.*, 1987).

Renal Nerve Stimulation and Renal Denervation Studies

Activation of renal α-adrenoceptors produces a variety of effects including vasoconstriction, sodium and water retention, stimulation and inhibition of renin release and gluconeogenesis (DiBona, 1982; Gottschalk, 1979; Insel and Snavely, 1981; Kim *et al.*, 1980). Renal denervation has been known for some time to be associated with a natriuresis and diuresis in the anesthetized animal (Kaplan and Rapoport, 1951). The mechanism by which this occurs has only been elucidated in the last decade. Renal denervation in anesthetized rats produced a five to six-fold increase in the excretion of sodium. While this occurred in the absence of changes in glomerular filtration rate and renal blood flow, the increase was accompanied by a decrease in fractional and absolute water reabsorption in the proximal tubule (Bello-Reuss *et al.*, 1975). These results suggested that the diuresis and natriuresis associated with renal denervation are mediated by a direct effect of adrenergic neural tone to enhance proximal tubular sodium and water reabsorption.

Stimulation of the sympathetic nerves or administration of α-adrenoceptor agonists produces vasoconstriction that is not uniform, and blood flow is redistributed from cortical to medullary areas. Direct electrical nerve stimulation has been demonstrated to decrease the renal excretion of sodium. This occurred in the absence of changes in glomerular filtration rate and renal blood flow, and in the distribution of intrarenal blood flow, as measured by the radioactive microsphere technique (DiBona, 1977). Following cessation of stimulation, sodium excretion returned to control levels. Very clearly then, the change in sodium excretion resulted from a change in tubular reabsorption without a change in intrarenal hemodynamics. This effect could be blocked by either phenoxybenzamine, an α-adrenoceptor antagonist (Zambraski *et al.*, 1976) or guanethidine, an adrenergic neuron-blocking agent (Slick *et al.*, 1975). This suggested that α-adrenoceptor stimulation directly increased sodium reabsorption.

Renal α-adrenoceptors also play an important role in the release of renin. Although renin is primarily secreted from the macula densa cells of the juxtaglomerular apparatus, there is some evidence that renin is also produced in the glomerulus (Beierwaltes *et al.*, 1980). Low frequency renal nerve stimulation in dogs has been shown to increase the release of renin via β1-adrenoceptors (Kopp *et al.*, 1980; Osborn *et al.*, 1981). At higher frequencies of stimulation, renin release may be stimulated secondary to increased intrarenal production of prostaglandins. This effect is believed to be mediated predominantly by α1-adrenoceptors (Hisa *et al.*, 1985; Hisa and Satoh, 1981; Kopp *et al.*, 1981). Furthermore, inhibition of renin release by α2-adrenoceptors has also been suggested (Keeton and Campbell, 1980; Pettinger *et al.*, 1976). It is likely that this effect is mediated through a decrease in cAMP production. Pretreatment of rats with pertussis toxin, which ADP-ribosylates the inhibitory guanine nucleotide-binding protein (Katada and Ui, 1981), abolished

the α2-adrenoceptor-mediated decrease in renin secretion (Pedraza-Chaverri *et al.*, 1984).

As mentioned above, renal nerve stimulation is known to cause release of renin and prostaglandins from the kidney. Since these substances might influence tubular sodium reabsorption, DiBona *et al.* (1977) and Zambraski and DiBona (1976) investigated the possibility that these humoral agents might mediate the nerve stimulation-induced natriuresis. There was no inhibition of the antinatriuretic response to subpressor nerve stimulation following the administration of a competitive angiotensin II antagonist or indomethacin. Therefore, the response to nerve stimulation does not appear to be mediated by the intrarenal action of circulating angiotensin or prostaglandins.

In clearance and micropuncture studies, renal nerve stimulation resulted in an increase in sodium reabsorption in the proximal convoluted tubule in the absence of vascular or glomerular changes (Bello-Reuss *et al.*, 1976). Thus, renal sympathetic nerve activity appears to increase sodium reabsorption, primarily in the proximal tubule. Moreover, Bencsath *et al.* (1972; 1976) have demonstrated that the effects of renal nerve stimulation on tubular reabsorption of sodium can be dissociated from those on renin release. The antinatriuretic effects of renal nerve stimulation are therefore most likely mediated by a direct effect on the renal tubular epithelial cells, rather than by an indirect effect on renal hemodynamics, a change in the activity of the renin-angiotensin system, or a change in the synthesis of renal prostaglandins.

These studies, however, did not identify the α-adrenoceptor subtype mediating these effects. Osborn *et al.* (1983) analyzed the antinatriuretic effect of low level sympathetic nerve stimulation of renal nerves in the dog. Renal arterial infusions of prazosin, but not yohimbine or rauwolscine attenuated the antinatriuretic response. Thus, they concluded that the tubular effects of subpressor

sympathetic nerve stimulation were mediated by α1-adrenoceptors. Similarly, Hesse and Johns (1985) demonstrated that intrarenal infusions of norepinephrine increased renal tubular sodium reabsorption in the rabbit. On the basis of the use of selective antagonists, they concluded that α1-adrenoceptors mediated this response. Finally, Smyth *et al.* (1985b) demonstrated that in the isolated perfused rat kidney, the antinatriuretic effect of subpressor renal nerve stimulation could be antagonized by the α1-adrenoceptor antagonist, prazosin. Moreover, this study provided evidence that renal tubular α2-adrenoceptors cannot be activated by sympathetic nerve stimulation, but rather by circulating catecholamines only. These three independent observations provided strong support for a tubular α1-adrenoceptor mechanism which mediated the antinatriuretic effects of low level renal nerve stimulation. The α2-adrenoceptor, on the other hand, was not activated by stimulation of the sympathetic nerves and appeared to be coupled to the adenylate cyclase-cAMP system.

In Vitro Studies

As mentioned previously, renal α2-adrenoceptors were activated by circulating catecholamines (Smyth *et al.*, 1985b) and appeared to exert their effects through the inhibition of adenylate cyclase. Several *in vitro* studies indicate that the effects of α2-adrenoceptors are mediated through the inhibition of cAMP generation, particularly that increased by various hormones. Early studies demonstrated that α2-adrenoceptor agonists prevented vasopressin-induced cAMP accumulation in renal membranes (Beck *et al.*, 1972; Kurokawa and Massry, 1973a) and inhibited parathyroid hormone-induced increase in cAMP levels in rat kidney cortex suspensions (Guder and Rupprecht, 1975; Kurokawa and Massry, 1973b). More recently, Umemura *et al.* (1985; 1986a; 1986b) were able to identify the specific effects of α2-adrenoceptor stimulation, in relation to cAMP accumulation,

in specific nephron segments using isolated intact nephron segments instead of fractured cell preparations.

In the glomerulus, parathyroid hormone and serotonin have been shown to increase the production of cAMP. Stimulation of α2-adrenoceptors attenuated this parathyroid hormone and serotonin-induced increase in cAMP (Umemura *et al.*, 1986a). Prostaglandin E2, histamine and adenosine also increase cAMP levels in the glomerulus, but this increase was not attenuated by α2-adrenoceptor stimulation. Thus, the ability of α2-adrenoceptor stimulation to inhibit cAMP formation is dependent upon the particular hormone which activates the adenylate cyclase enzyme complex.

In the proximal tubule, the site where the majority of α2-adrenoceptors are located, α2-adrenoceptor stimulation with epinephrine inhibits the parathyroid hormone-stimulated increase in cAMP (Umemura *et al.*, 1985). This effect was antagonized by the specific α2-adrenoceptor antagonist yohimbine, but not by the specific α1-adrenoceptor antagonist prazosin. This effect, however, was confined to the proximal convoluted tubule. In the proximal straight tubule, α2-adrenoceptor stimulation was unable to inhibit the parathyroid hormone-induced increase in cAMP. This suggested that α2-adrenoceptors in this segment of the nephron are not coupled to parathyroid hormone.

There is a general agreement that at the level of the renal proximal tubule, catecholamines enhance sodium and water reabsorption (Bello-Reuss, 1980). *In vivo* micropuncture studies in the rat indicate that sodium reabsorption is affected by α-adrenoceptor agonists, although the subtype of the α-adrenoceptor mediating this effect was not defined. Recently, Nord *et al.* (1987) demonstrated that in a suspension of isolated rabbit renal tubular cells, α2-adrenoceptor agonists enhanced ^{22}Na influx . Yohimbine, but not prazosin completely blocked the norepinephrine-induced increase in sodium influx. Although catecholamines enhance proximal

tubular sodium and water reabsorption, the stimulated transport pathway had previously not been described. The observation that an amiloride analogue abolished the norepinephrine-induced sodium influx indicated that α2-adrenoceptors enhance sodium reabsorption by stimulation of the brush border sodium-hydrogen antiporter. Although this observation is very thought-provoking, it was not an integral consideration before the investigations presented in this thesis were completed. Since completion of the experiments, however, it has gained new consideration, as it may help to explain some of the observed results. It is therefore discussed in further detail in Section 8 (General Discussion).

As in the proximal tubule, the effects of α2-adrenoceptor stimulation in the loop of Henle are also diverse, depending upon where the α2-adrenoceptors are located. In the thin descending limb, prostaglandin E2 has been shown to increase cAMP production, presumably through the stimulation of adenylate cyclase. α2-Adrenoceptor stimulation attenuates this increase in cAMP (Umemura *et al.*, 1986b, Torikai and Kurokawa, 1981; 1984). While prostaglandin E2 alters fluid and electrolyte transport in the thick ascending limb and collecting tubule (Stokes, 1981), the functions of prostaglandin E2 in the descending limb have not been identified. The thin descending limb of the loop of Henle is an integral part of the countercurrent multiplication process which is responsible for the generation and maintenance of medullary hypertonicity. If prostaglandin E2 should alter the permeability characteristics of the thin descending limb to water and/or solutes, possibly via cAMP, then medullary hypertonicity could be impaired, and consequently sodium and water excretion could be altered (Kokko and Rector, 1972). In the thick ascending limb, adenylate cyclase is activated by vasopressin in the medullary portion, and by both vasopressin and parathyroid hormone in the cortical portion (Morel *et al.*, 1981; Umemura *et al.*, 1985). However, α2-

adrenoceptor agonists are ineffective in reversing the increase in cAMP accumulation in the thick ascending limb of the loop of Henle.

Although α2-adrenoceptors are predominantly located within the proximal convoluted tubules, the major function described to date for these receptors appears to result from an effect within the cortical and medullary collecting tubules. It has recently been shown that epinephrine and norepinephrine can inhibit vasopressin-stimulated cAMP accumulation in the intact cortical collecting tubule and in the medullary collecting tubule via an α2-adrenoceptor mechanism (Chabardes et al., 1984; Umemura et al., 1985). In intact cell preparations, glucagon, calcitonin, isoproterenol and vasopressin all activated adenylate cyclase and increased cAMP formation. However, only when cortical or medullary collecting tubule adenylate cyclase was activated by vasopressin, did α2-adrenoceptor stimulation decrease cAMP accumulation (Chabardes et al., 1984; Umemura et al., 1985). These results demonstrate hormone specificity of α2-adrenoceptor inhibition of cAMP accumulation in the collecting tubule. However, the functional role of these receptors in the modulation of vasopressin-induced cAMP accumulation had not been identified.

In isolated rabbit cortical collecting tubules, α-adrenoceptor agonists have been shown to directly inhibit vasopressin-mediated water reabsorption at the level of the renal tubule (Krothapalli et al, 1983; Krothapalli and Suki, 1984). This effect could be blocked by a specific α2-, but not by a specific α1-adrenoceptor antagonist. Using a similar preparation in the rat, Chabardes et al. (1984) demonstrated an inhibition of vasopressin-induced cAMP accumulation by nonspecific α-adrenoceptor agonists. This effect was blocked by yohimbine, but not by prazosin. Thus, these biochemical and physiological studies suggested that vasopressin increases water reabsorption in the cortical collecting tubule by increasing the formation of cAMP. α2-Adrenoceptor stimulation inhibits the vasopressin-induced

increase in cAMP and thereby inhibits the vasopressin-stimulated water reabsorption. In the isolated perfused rat kidney, stimulation of α2-adrenoceptors with epinephrine antagonized the effects of vasopressin on both water and sodium excretion. The blockade of the effects of epinephrine by yohimbine was confirmation that these renal effects were mediated through α2-adrenoceptors (Smyth *et al.*, 1985a). These studies found that vasopressin decreases urinary sodium excretion. This has, however, become an area of contention, since *in vivo* studies seem to suggest that vasopressin is natriuretic (Johnson *et al.*, 1979; Martinez-Maldonado *et al.*, 1971; Pierce *et al.*, 1984). Nevertheless, vasopressin increases sodium chloride reabsorption in the medullary ascending loop of Henle in the mouse (Hebert *et al.*, 1980; 1981) and in the rat (Jacobsen, 1981). In isolated cortical collecting tubules, vasopressin stimulates sodium reabsorption (Reif *et al.*, 1986; Schlatter and Schafer, 1987). Presumably, the increase in sodium excretion observed with α2-adrenoceptor stimulation in the isolated perfused rat kidney (Smyth *et al.*, 1985a) is due to an inhibition of vasopressin-mediated sodium reabsorption, since epinephrine alone was without effect.

In Vivo Renal Function Studies

Studies with both isolated cortical collecting tubules and the isolated perfused rat kidney have indicated that the effects of α2-adrenoceptor stimulation were mediated through the inhibition of cAMP formation (Chabardes *et al.*, 1984; Krothapalli and Suki, 1984; Smyth *et al.*, 1984; 1985a). This has been confirmed by the measurement of cAMP levels in single nephron segments and in glomeruli of the rat (Umemura *et al.*, 1985; 1986a, 1986b). The effects of α2-adrenoceptor stimulation are thus dependent on whether the hormonally activated cAMP was mediating an increase or decrease in urine volume and sodium excretion. The physiological effect of inhibition of cAMP formation by renal α2-adrenoceptor

stimulation would be dependent on the predominating effect of the endogenously produced cAMP. However, caution must be exercised when extrapolating the results of *in vitro* experiments to an *in vivo* situation. Use of isolated collecting tubules demands that the nephron be cut apart, and thus the collecting tubule is functionally destroyed. Even the isolated perfused rat kidney preparation, in which glomerular-tubular linkages are still intact, is not without problems. While the preparation eliminates extraneous factors such as alterations in the central release, degradation and/or excretion of vasopressin and other circulating hormones, it is the subtle interplay of all these factors that must be considered when one tries to identify the physiological function of the α2-adrenoceptor. A second disadvantage of the isolated perfused kidney is that the normally hypertonic medullary interstitium may be profoundly altered (Lieberthal *et al.*, 1987). It is therefore not surprising that somewhat different results have been documented in *in vivo* experiments. Moreover, the results of *in vivo* studies have been conflicting, likely due to technical differences between studies.

Intravenous administration of clonidine, an α2-adrenoceptor agonist, has resulted in an increase in renal water excretion in previous studies in anesthetized dogs (Humphreys and Reid, 1975; Olsen, 1976) and in the rat (Miller, 1980). This effect could be due to central inhibition of vasopressin release or a direct inhibition of vasopressin at the level of the renal tubule. Studies, in which the mechanism of this diuresis was investigated in the dog, have suggested it may be due to an inhibition of vasopressin release. This may be possible as a consequence of a direct inhibitory action on the hypothalamic neurons responsible for vasopressin synthesis and release (Reid *et al.*, 1979). Similarly, Humphreys and Reid (1975) could not detect any water diuresis when clonidine was infused intravenously into acutely hypophysectomized dogs. Their results indicated that clonidine causes water diuresis through inhibition of vasopressin release, possibly via an indirect pathway

mediated by the α-adrenergic effects of clonidine on the circulation. Additional support for this view is found in two other studies. Barr and Kauker (1979) found that clonidine did not prevent the antidiuretic action of vasopressin given to rats with diabetes insipidus, and concluded that their results were consistent with an inhibition of vasopressin release. More direct evidence came from Roman *et al.* (1979) who demonstrated a transient decrease in plasma vasopressin of clonidine-treated rats, along with the observation that infusion of vasopressin was capable of blocking the water diuresis invoked by clonidine. However, several studies in which vasopressin has been measured in blood or urine of dogs or rats after clonidine-induced diuresis have failed to find any detectable changes in plasma vasopressin concentrations (Gullner, 1979; Olsen, 1976; Solez *et al.*, 1980), and thus have not been able to substantiate this hypothesis. Solez *et al.* (1980) have shown that intravenous administration of clonidine causes an increase in free water clearance even when it was administered into rabbits that were pretreated with vasopressin, suggesting that clonidine inhibits the antidiuretic action of vasopressin at the tubular level.

While the above studies are in agreement concerning the effects of clonidine in inducing a water diuresis, the same is not true for the effects of clonidine on solute excretion. Clonidine is also able to affect electrolyte excretion, although the early evidence of this was contradictory and ill-defined. Electrolyte excretion was shown to be either increased, decreased or unaltered under the influence of clonidine. Olsen (1976) reported a four-fold increase in sodium excretion in conscious dogs, while others observed a decrease in urinary sodium excretion in anesthetized dogs (Chrysanthakopoulous and Lavender, 1975; Humphreys and Reid, 1975). Still others found no change in electrolyte excretion (Reid *et al.*, 1975). In the rat, sodium excretion has been reported to be enhanced by clonidine (Barr and Kauker, 1979; Miller, 1980).

Recent studies have looked at the effects of other α2-adrenoceptor agonists. Strandhoy *et al.*, (1982) demonstrated that intrarenal infusions of guanabenz in anesthetized dogs, significantly increased urine flow and sodium excretion. Urine osmolality was decreased to 530 mOsm/kg (from 1503 mOsm/kg), and plasma vasopressin levels were similarly decreased to approximately 5.25 *pg*/ml (from 7.92 *pg*/ml). They concluded that although this decrease in plasma vasopressin concentration could contribute to a diuresis, the reduced plasma vasopressin levels should still be adequate for a urine concentration greater than that observed. Thus, they concluded that the guanabenz-induced diuresis and natriuresis was due to both a decrease in the central release of vasopressin, as well as an inhibition of the effects of vasopressin at the renal tubular level. An interesting observation from this study was that an intravenous infusion of guanabenz was more potent in inducing the observed diuresis and natriuresis than a direct infusion into the renal artery.

Finally, Gellai and Ruffolo (1987) examined the renal effects of B-HT 933, a specific α2-adrenoceptor agonist in conscious normotensive rats. B-HT 933 significantly increased urine flow and sodium excretion, but did not alter potassium and urea excretion. Urine osmolality was decreased to hypotonic levels (172 ± 8 mOsm/kg) and free water clearance was increased. Since the B-HT 933-induced diuresis and natriuresis was not affected by the ganglionic blocker hexamethonium, the authors concluded that α2-adrenoceptor stimulation in conscious rats modulates the reabsorption of water and sodium at the site of the renal nephron, possibly through an interaction with vasopressin. In a follow-up micropuncture study, Stanton *et al.* (1987) demonstrated that the B-HT 933-induced diuresis was due to the inhibition of water and electrolyte reabsorption beyond the late distal tubule, strongly suggesting a role for α2-adrenoceptors in the modulation of vasopressin action in the collecting tubule.

What appears to be clear from the above mentioned studies, is that stimulation of α2-adrenoceptors *in vivo* is associated with an increase in both sodium and water excretion (Roman *et al.*, 1979; Miller, 1980; Strandhoy *et al.*, 1982; Gellai and Ruffolo, 1987; Stanton *et al.*, 1987). The exact mechanism whereby which α2-adrenoceptor stimulation mediates these effects remains unclear. A criticism of the use of specific agonists is that the observed effect may not be present under physiological conditions, but only in the presence of high levels of activation of the receptors. Thus, while the above studies have demonstrated a pharmacological role for α2-adrenoceptors, a physiological role for α2-adrenoceptors remains unclear. Pharmacological stimulation of the α2-adrenoceptors by intravenous, intrarenal or subcutaneous administration, and in only one or two doses which are either pressor or produce a hypotonic urine, has failed to clearly define a physiological function of these receptors. The question remains as to whether these receptors are tonically active *in vivo*. A direct method to determine whether renal α2-adrenoceptors play a physiological role in the regulation of sodium and water excretion would be by the infusion of a specific α2-adrenoceptor antagonist, such as yohimbine, into the renal artery. In order to observe an effect in the presence of an antagonist, the system must be active. Unfortunately, such experiments have produced conflicting results. Osborn *et al.* (1983) demonstrated that renal arterial infusions of either yohimbine or rauwolscine did not affect renin release or sodium and water excretion. Moreover, the antinatriuretic response to low frequency renal nerve stimulation was also unaffected by yohimbine or rauwolscine. In contrast, Fildes *et al.* (1985) demonstrated that yohimbine, administered as an intrarenal infusion, produced a significant increase in sodium and water excretion. The earlier observation suggests that α2-adrenoceptors do not function physiologically in the neuronal regulation of sodium reabsorption, while the latter suggests they function to retain sodium in the dog. A major difference between these studies is the infusion rate of yohimbine

used to block α2-adrenoceptors. Osborn *et al.* (1983) used an infusion rate of 0.46 ug/kg/min, a rate which may not have been great enough to effectively block α2-adrenoceptors (van Meel *et al.*, 1981c), while Fildes *et al.* (1985) used three infusion rates, each 20-200 times greater than that used by Osborn *et al.* (1983). At this rate of yohimbine infusion, it is possible that α1-adrenoceptors, as well as α2-adrenoceptors may have been blocked.

Purpose of the Present Studies

The initial purpose of the following experiments (specifically sections 2 & 3), was to determine a physiological role for the numerically predominant α2-adrenoceptor, and to characterize these receptors in more detail than had previously been done, using an *in vivo* preparation. Specifically, the effects of α2-adrenoceptor antagonism and stimulation on the renal excretion of sodium and water was to be determined. It was originally hypothesized that if α2-adrenoceptor stimulation antagonized the renal effects of vasopressin, and thereby increased the excretion of sodium and water, then blockade of these receptors would results in an antidiuresis and antinatriuresis. This would then, be consistent with the unmasking of the endogenous function of the α2-adrenoceptors.

Upon completion of these two sets of experiments, it became apparent that the renal effects of α2-adrenoceptor stimulation, in relation to sodium and water excretion, may be mediated by two independent mechanisms of action or at two independent sites. Thus, the focus of the experiments shifted slightly, and the purpose then was to determine what these two independent sites or mechanisms of action might be. In this process, the role of atrial natriuretic peptide, renal prostaglandins and vasopressin was determined. Consequently, the purpose of the experiments presented in this thesis is twofold.

2

Renal α2-Adrenoceptor Blockade Decreases Sodium and Water Excretion in the Anesthetized Rat

Synopsis:

The reported effects of renal α2-adrenoceptor blockade on sodium and water excretion have been inconsistent. The effect of an intrarenal infusion of an α2-adrenoceptor antagonist in rats undergoing two distinct levels of diuresis and natriuresis were therefore studied. The two levels of diuresis and natriuresis was achieved by the intravenous infusion of saline at two different rates, namely 97 ul/min and 24 ul/min. Renal excretion of sodium and water was studied in anesthetized rats that had been unilaterally nephrectomized (right kidney) 10 days prior to the experimental day. In the presence of the lower rate of saline infusion (24 ul/min), an intrarenal infusion of the α2-adrenoceptor antagonist yohimbine (25.6 $n$mol/kg/min) resulted in no change in urine flow rate or sodium and potassium excretion. In the presence of a modest diuresis, due to the higher level of saline infusion, intrarenal yohimbine resulted in a decrease in urine flow rate, sodium excretion and free water clearance. These effects of yohimbine were not found in adrenalectomized rats. The ability to demonstrate an effect of renal α2-adrenoceptor blockade was dependent on the baseline level of sodium and water excretion. Moreover, these results suggested that renal α2-adrenoceptors may mediate the inhibition of the renal action of vasopressin through adrenal catecholamines.

INTRODUCTION

In renal plasma membranes, $\alpha 2$-adrenoceptors have been shown to be numerically predominant over $\alpha 1$-adrenoceptors in a ratio of approximately 2:1 (Schmitz *et al.*, 1981). Renal $\alpha 1$-adrenoceptors mediate renal nerve stimulation-induced sodium retention and vasoconstriction (DiBona, 1982; Pettinger *et al.*, 1985; Smyth *et al.*, 1985b). A physiological function for the numerically predominant $\alpha 2$-adrenoceptors remains unclear.

Studies in the isolated perfused rat kidney have indicated that the effects of $\alpha 2$-adrenoceptor stimulation were mediated through inhibition of cAMP formation (Smyth *et al.*, 1984; 1985a). Thus, the effects of $\alpha 2$-adrenoceptor stimulation were dependent on whether the hormonally activated cAMP was mediating an increase (via arachidonic acid) or decrease (via vasopressin) in urine volume (Pettinger *et al.*, 1987; Smyth *et al.*, 1985a). In the *in vivo* situation, a number of hormones which activate the adenylate cyclase system would be present. The physiological effect of inhibition of cAMP formation by renal $\alpha 2$-adrenoceptor stimulation would be dependent on the predominating effect of the endogenously produced cAMP. At present, the endogenous agonist for $\alpha 2$-adrenoceptors has been proposed to be a circulating catecholamine, most conceivably epinephrine (Sawyer *et al.*, 1985; Smyth *et al.*, 1985b; Yamaguchi and Kopin, 1980).

A direct method to determine whether renal $\alpha 2$-adrenoceptors play a physiological role in the regulation of sodium and water excretion would be to infuse a specific $\alpha 2$-adrenoceptor antagonist into the renal artery. To date, such experiments have produced conflicting results. In the anesthetized dog, an intrarenal infusion of yohimbine has been reported to increase (Fildes *et al.*, 1985) or have no effect (Osborn *et al.*, 1983) on the excretion of sodium and water in the urine.

We therefore studied the effect of an intrarenal infusion of an α2-adrenoceptor antagonist yohimbine, on the excretion of sodium and water in anesthetized rats undergoing two distinct levels of sodium and water excretion. Intrarenal yohimbine decreased sodium and water excretion in the presence of a modest natriuresis and diuresis. These results demonstrate that the ability of α2-adrenoceptor blockade to decrease sodium and water excretion in the kidney is dependent on the basal rate of sodium and water excretion. The physiological function of renal α2-adrenoceptors may be related to the inhibition of the renal tubular actions of vasopressin. Intrarenal yohimbine in adrenalectomized rats failed to alter sodium and water excretion, suggesting that epinephrine was conceivably the endogenous α2-adrenoceptor agonist.

METHODS

1. Renal Function Experiments

Male Sprague Dawley rats (Charles River Canada Inc., St. Constant, P.Q. or the University of Manitoba breeding colony, whose breeders are replaced every three months with new breeders from Charles River Canada Inc.) weighing 200-225 g were unilaterally nephrectomized (right kidney) under ether anesthesia 7 to 10 days prior to the day of the experiment. Animals were allowed free access to water (tap water) and chow (Purina Rat Chow) and housed at $23^{o}C$ with a 12/12h light/dark cycle. On the day of the experiment, rats were anesthetized with pentobarbital (Nembutal, BDH Chemicals Ltd., Poole, England; 50-60 mg/kg i.p.). Additional anesthetic was administered in a 0.05 ml bolus intravenous dose of 3 mg/kg as needed. The animals were placed on a thermostatically controlled heating blanket. A rectal thermometer connected to a Harvard Animal Blanket Control Unit was used to maintain body temperature at $37.5^{o}C$. A tracheotomy was performed and the animal was placed on a ventilator if required. The left carotid artery was cannulated with a polyethylene catheter (PE60) for the measurement of blood pressure with a Statham pressure transducer (Model P23Dc) connected to a Grass polygraph Model V. The left jugular vein was cannulated with either one or two lines. In all experiments, one polyethylene catheter (PE160) was inserted for the continuous infusion of saline. In some experiments, an additional line (PE20) was also inserted for the intravenous administration of additional agents. The left kidney was exposed by a flank incision, and the ureter cannulated (PE50). A 31 gauge needle was advanced through the aorta into the renal artery and secured with glue (Superglue; Lepage Ltd., Bramalea, Ontario). Vehicle (saline) or yohimbine (Sigma Chemical Co., St. Louis, MO) was infused through this line into the renal artery at a rate of 13.6 ul/min. Saline was infused intravenously (jugular vein

catheter) at either 24 ul/min or 97 ul/min to produce two distinct levels of sodium and water excretion. Following a 45 min stabilization period, 5 consecutive 15 min urine samples were collected into pre-weighed tubes. Thus, the urine collection periods were 45-60, 60-75, 75-90, 90-105 and 105-120 min after the start of the stabilization period. Urine volume was determined gravimetrically. A plasma sample was obtained at the end of the experiment.

Two separate sets of experiments were conducted. In the first set of experiments, saline was infused into two groups of rats at either 24 or 97 ul/min immediately following the start of the stabilization. This established the natriuretic and diuretic effect of the higher rate of saline infusion. In another two groups of rats, saline was again infused at either 24 or 97 ul/min. After the first urine collection yohimbine, an α2-adrenoceptor antagonist, was infused directly into the renal artery at 25.6 $n$mol/kg/min (0.01 mg/kg/min) for the final four collection periods. In rats receiving the higher infusion rate of saline, α2-adrenoceptor blockade with yohimbine was found to have an effect on water and sodium excretion. Therefore a second set of experiments was completed in which rats were acutely adrenalectomized to determine if circulating epinephrine was the endogenous α2-adrenoceptor agonist. Plasma aldosterone and corticosteroid concentrations were maintained at fixed levels throughout the experiment by the continuous intravenous infusion of aldosterone (20 ng/min; Sigma Chemical Co., St. Louis, MO) and hydrocortisone (20 ug/min; Sigma Chemical Co., St. Louis, MO) to establish physiological levels as previously described (Roman and Cowley, 1985). These drugs were dissolved in saline containing 1% bovine serum albumin which was delivered with the saline at a rate of 97 ul/min (i.e., total drug and saline delivery rate was 97 ul/min). The control group received an equal volume of vehicle infused directly into the renal artery, while the other group received

intrarenal yohimbine immediately after the first urine collection as in the above experiments.

Sodium and potassium concentrations in plasma and urine were determined with a Beckman Klina Flame Photometer. Creatinine concentrations were determined with a Beckman Creatinine Analyzer Model 2. Urine osmolality was determined with a micro Osmette (Precision Systems).

Data were analyzed by a two way repeated measures analysis of variance (saline x dose), and where the main effect x time interactions were significant, the locus of the differences was determined by the least significant difference test, with Type I error rate controlled experiment-wise by Dunn's procedure (Winer, 1971). Data are presented as the means $\pm$ the standard error of the mean (S.E.M.). Each group represents 5 to 6 animals.

2. Radioligand Binding Study

One of the underlying assumptions of the *in vivo* renal function experiments was that the removal of one kidney did not alter the α2-adrenoceptor density in the remaining kidney. In order to test this assumption, the α2-adrenoceptor density in both the kidney removed during the nephrectomy, and in the remaining kidney (after a period of 7 to 10 days) was measured with a radioligand binding technique. Renal plasma membranes from whole kidneys were prepared by the method of Schmitz *et al.* (1981). [^{3}H]Rauwolscine (0.50-20.0 nM) was used to construct Scatchard plots in duplicate for each assay. The left kidneys from five male Sprague Dawley rats were removed, immediately frozen in liquid nitrogen and stored at -80°C. Ten days later, the remaining kidneys were also removed, immediately frozen in liquid nitrogen and stored at -80°C. On the day of the assay, the kidney was thawed, minced in cold sucrose buffer (0.25 M sucrose, 5 mM Tris-HCl, 1 mM MgCl$_2$, pH 7.40), and homogenized 4 times with a polytron for 10 s. After filtering

through gauze, the homogenate was centrifuged for 10 min at 482 x g (2000 rpm) at 4^OC. The pellet was discarded and the supernatant was centrifuged for 10 min at 29,000 x g (15,500 rpm). The pellet was washed twice by resuspension in buffer K (25 mM NaH_2PO_4, 25 mM K_2HPO_4, 1 mM $MgCl_2$), homogenized with a Teflon pestle and recentrifuged at 29,000 x g. The final pellet was resuspended and homogenized in the appropriate amount of buffer K (6.8 ml buffer K/g kidney weight). Membrane protein was determined according to the procedure of Lowry *et al.* (1951) using bovine serum albumin as the standard.

[^{3}H]Rauwolscine (72.2 Ci/*m*mol, NEN Research Products, DuPont, Boston MA) was used to label α2-adrenoceptors. Binding assays were performed by incubating 200 *u*l of renal plasma membrane (protein concentration 3.2-4.2 mg/ml), 50 *u*l of [^{3}H]rauwolscine (0.50-20.0 *n*M) and 50 *u*l of either buffer K or phentolamine (10 *u*M) to yield a final volume of 300 *u*l. All experiments were performed in duplicate. The mixture was incubated for 30 min at 25^OC. The reaction was terminated by the addition of 5 ml of ice-cold buffer and immediate filtration through Whatman GF/C glass fibre filters. The filters were washed with 2 additional 5 ml aliquots of cold buffer, placed in scintillation vials, and counted in 4 ml of triton-toluene aqueous scintillation cocktail, with a counting efficiency of 40% in a gamma scintillation counter. The radioligand and phentolamine were diluted to the appropriate concentrations in the assay buffer. Specific binding was determined as the binding which was in excess of nonspecific binding in the presence of 10 *u*M phentolamine. Analysis of the saturation experiments was performed with LIGAND, a computer assisted iterative curve fitting programme. The receptor density and dissociation constants were compared using paired *t*-tests.

RESULTS

1. Effect of unilateral nephrectomy on α2-adrenoceptor density

The renal α2-adrenoceptor density, as determined by Scatchard analysis of equilibrium binding data, in pre-and post-nephrectomy kidneys is shown in fig. 2.1. Ten days after initial nephrectomy there was no change in α2-adrenoceptor density (236 ± 49 vs. 221 ± 44 fmol/mg protein; $p>0.05$). In addition, there was no change in the K_d in the remaining kidney after nephrectomy (2.2 ± 0.4 nM) as compared to the control kidney (2.0 ± 0.4 nM).

2. Effect of intravenous saline infusion on electrolyte excretion

At the beginning of the stabilization period (i.e., immediately following the surgical procedure) saline was infused into the jugular vein at either 24 or 97 ul/min and maintained for the duration of the experiment.

The blood pressures and creatinine clearances in the rats receiving the two rates of saline infusion were similar (fig 2.2). However, in the group receiving the higher rate of saline infusion (97 ul/min), urine flow rate (40 ± 10 vs. 9 ± 1 ul/min), sodium excretion (7.14 ± 1.60 vs. 1.03 ± 0.18 uEq/min) and the fractional excretion of sodium (3.93 ± 0.79 vs. 0.46 ± 0.11) were increased ($p<0.05$) as compared to the rats receiving the lower rate of saline infusion (fig. 2.3). As well, osmolar clearance was increased in the rats receiving the high rate of infusion (88.2 ± 11.0 vs. 40.7 ± 11.9 ul/min) (fig 2.4). Potassium excretion (fig. 2.3), urine osmolality (fig. 2.4) and the change in free water clearance (fig. 2.4) were unaltered. Thus, this established two distinct levels of electrolyte excretion on which the effects of intrarenal yohimbine could be studied.

3. Effect of intrarenal yohimbine infusion on electrolyte excretion in rats undergoing a low level of excretion

In the rats given the low rate of saline infusion, blood pressure (125 ± 6.7 vs. 107 ± 3.3 mm Hg) and creatinine clearance (1.94 ± 0.2 vs. 1.57 ± 0.2 ml/min) were slightly, but not significantly, decreased in the group of animals receiving the intrarenal infusion of yohimbine as compared to the controls (fig. 2.5). In addition, urine flow rate (9 ± 1 vs. 9 ± 1 ul/min), sodium excretion (0.75 ± 0.12 vs. 1.03 ± 0.18 uEq/min), potassium excretion (3.13 ± 0.52 vs. 2.66 ± 0.38 uEq/min) (fig 2.6), urine osmolality (1400 ± 151 vs. 1251 ± 285 mOsm), osmolar clearance (40.7 ± 11.9 vs. 44.2 ± 5.2 ul/min) and the change in free water clearance (4.49 ± 4.3 vs. 0.97 ± 6.7 ul/min) (fig. 2.7) were similar to the control group. These data were obtained during the fourth collection period (90-105 min) and are representative of the lack of difference observed between groups during the other collection periods.

4. Effect of intrarenal yohimbine infusion in rats undergoing a mild diuresis

In these experiments, saline was administered at 97 ul/min to induce a modest diuresis. As above, the fourth collection period is presented. These data are representative of the observed differences between groups. The blood pressure and creatinine clearance were similar in the control group and in the rats receiving the intrarenal infusion of yohimbine (fig. 2.8). In the presence of the mild diuresis, the intrarenal infusion of yohimbine (25.6 nmol/kg/min) produced a decrease ($p<0.05$) in urine flow rate (40 ± 10 vs. 18 ± 2 ul/min) (fig. 2.9), sodium excretion (7.14 ± 1.60 vs. 3.22 ± 0.39 uEq/min) (fig. 2.9) and the fractional excretion of sodium (3.93 ± 0.79 vs. 1.41 ± 0.24) (data not shown) as compared to the respective control group. Potassium excretion was unaltered (fig. 2.9). Urine osmolality was increased (800 ± 104 vs. 1262 ± 165 mOsm), and osmolar clearance was unaltered (88.2 ± 11.0 vs. 67.9 ± 2.7 ul/min) in the rats receiving the yohimbine (fig. 2.10).

Free water clearance (fig. 2.10) decreased ($p<0.05$) in the group receiving the yohimbine infusion as compared to the control rats (-20.5 ± 7.2 vs. 2.84 ± 3.0 ul/min).

5. Effect of intrarenal yohimbine infusion in acutely adrenalectomized rats

In these experiments, rats were adrenalectomized. Again, as in the above experiments in which yohimbine decreased urine flow rate, saline was infused at 97 ul/min. The effects of yohimbine on urine flow or sodium excretion were attenuated (fig. 2.11). As well, blood pressure (130 ± 6 vs. 128 ± 4 mm Hg (fig. 2.12), creatinine clearance (1.4 ± 0.1 vs. 1.5 ± 0.1 ml/min (fig. 2.12), potassium excretion (4.5 ± 0.4 vs. 4.7 ± 0.6 uEq/min (fig. 2.11) and urine osmolality (574 ± 56 vs. 623 ± 84 mOsm (fig. 2.13) were unaltered. Again, these data represent the fourth collection period (90-105 min), and are representative of the observed differences between the two groups.
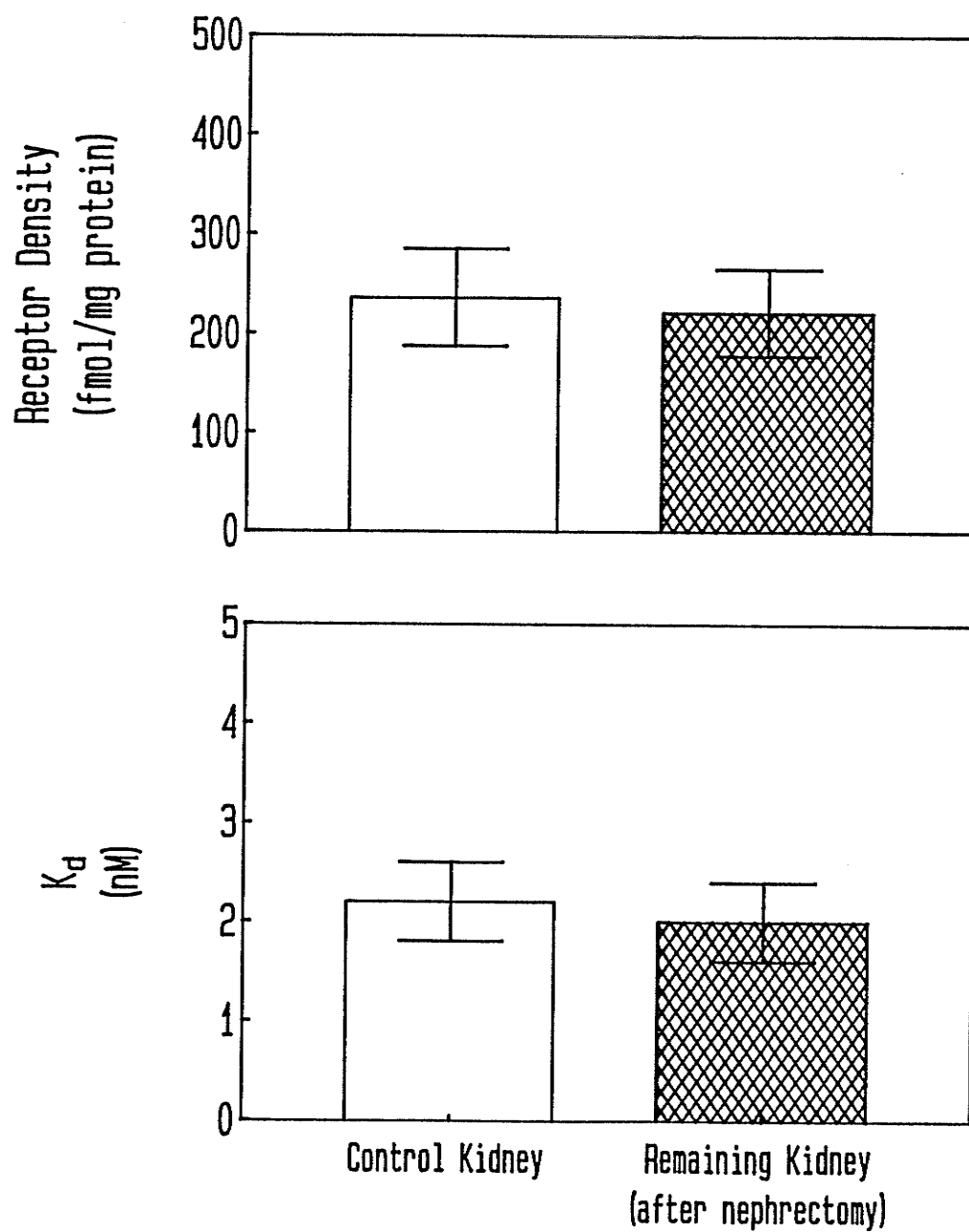
Fig. 2.1. Effect of nephrectomy on renal α2-adrenoceptor density (upper graph) and K_d (lower graph). Values represent the means $\pm$ S.E.M.
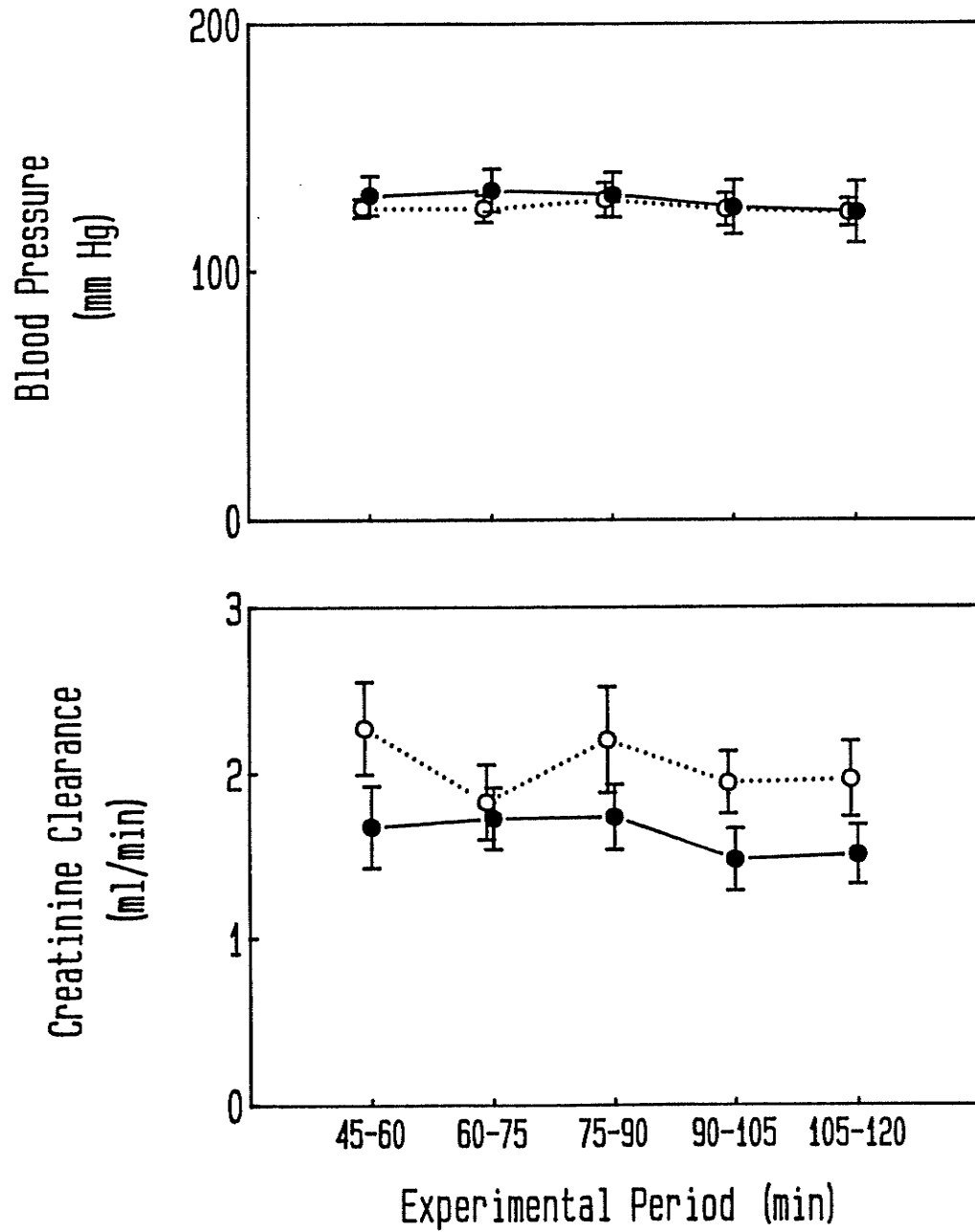
Fig. 2.2. Effect of two intravenous infusion rates of saline on blood pressure and creatinine clearance. (O) 24 ul/min, n=5; (●) 97 ul/min, n=7.
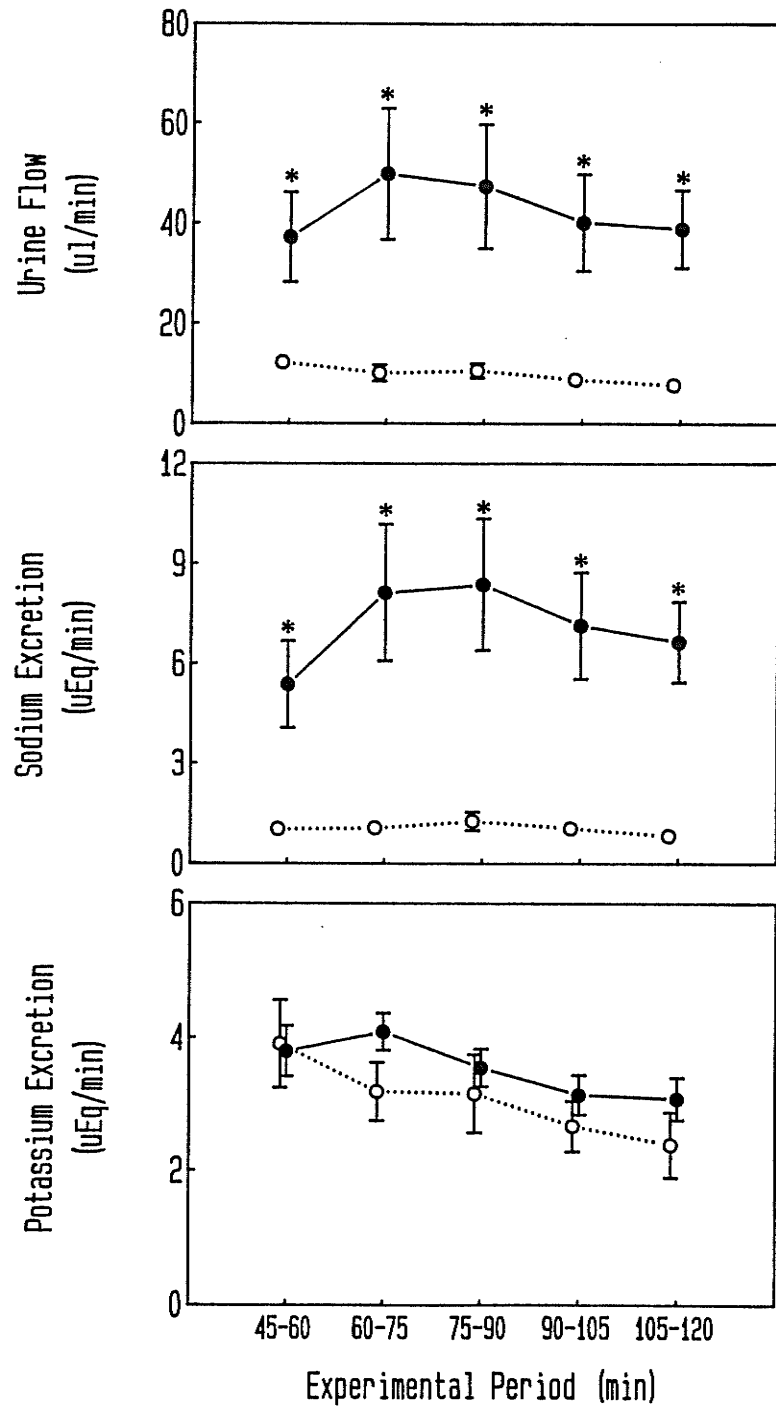
Fig. 2.3. Effect of two intravenous infusion rates of saline on urine flow rate, sodium excretion and potassium excretion. (O) 24 *ul*/min, n=5; (●) 97 *ul*/min, n=7.
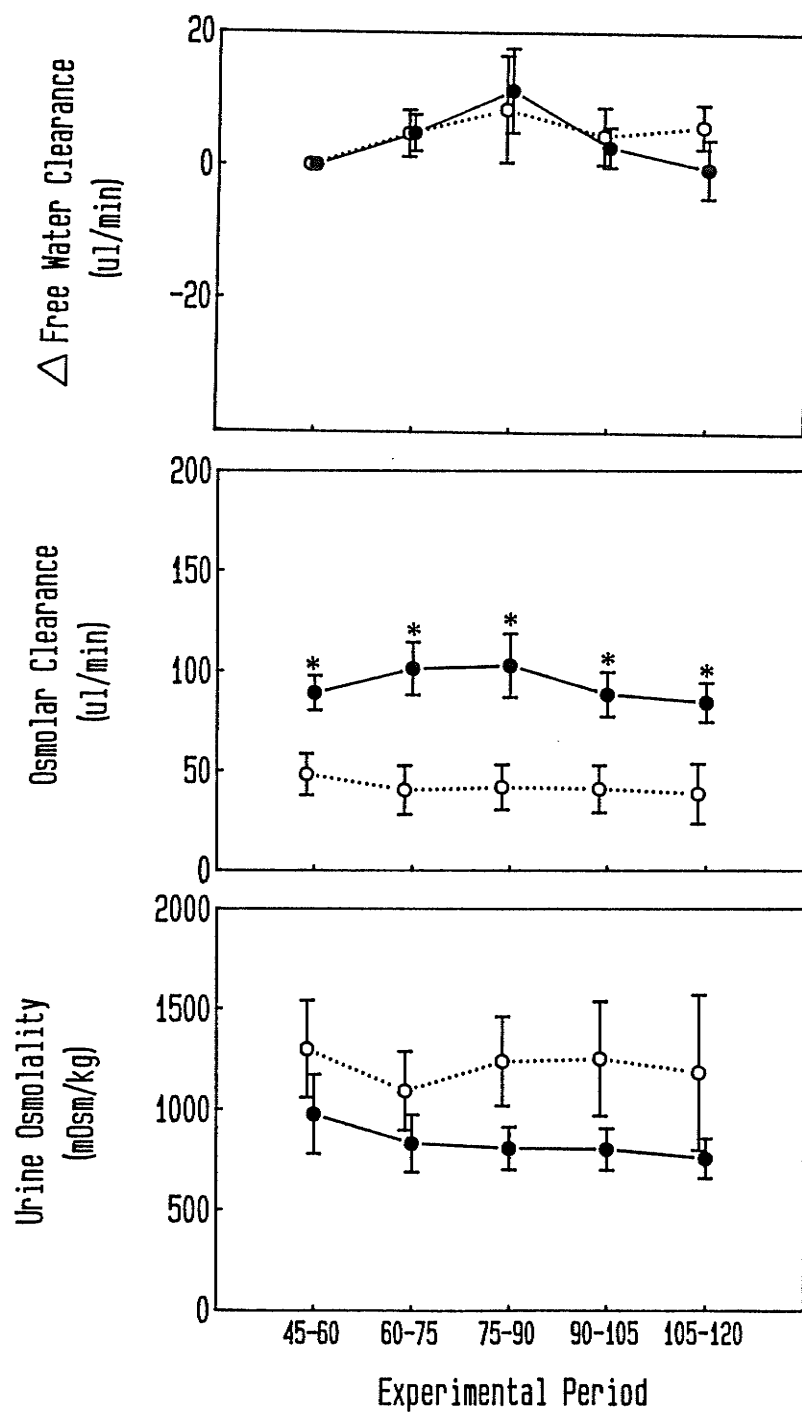
Fig. 2.4. Effect of two intravenous infusion rates of saline on the change in free water clearance, osmolar clearance and urine osmolality. (O) 24 ul/min, n=5; (●) 97 ul/min, n=7.
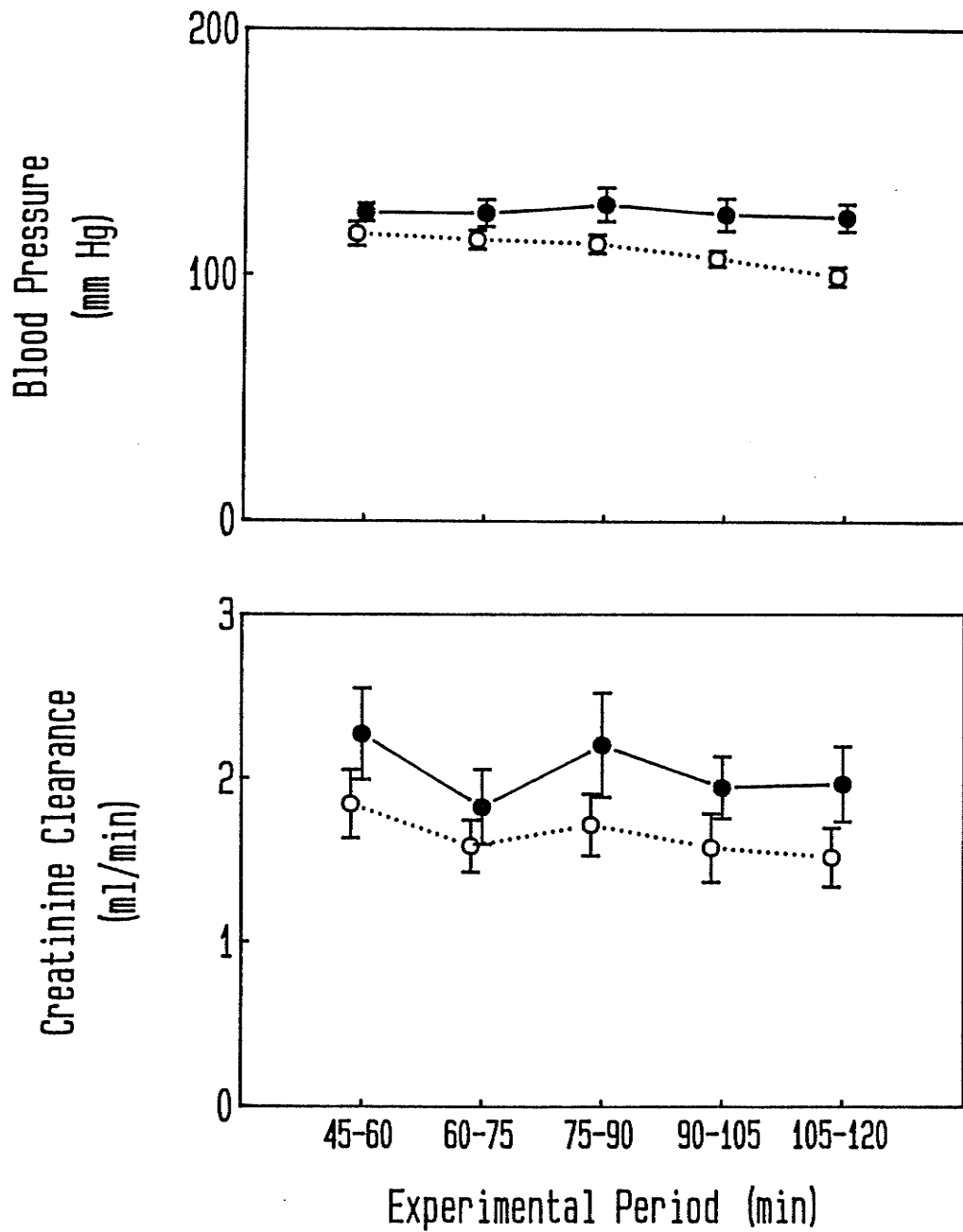
Fig. 2.5. Effect of an intrarenal infusion of yohimbine (25.6 $n$mol/kg/min) on blood pressure and creatinine clearance in rats receiving an i.v. infusion of saline (24 ul/min). The yohimbine infusion was started immediately following the first urine collection. (●) 24 ul/min i.v. saline control, n=5; (○) 24 ul/min i.v. saline plus intrarenal yohimbine, n=6.
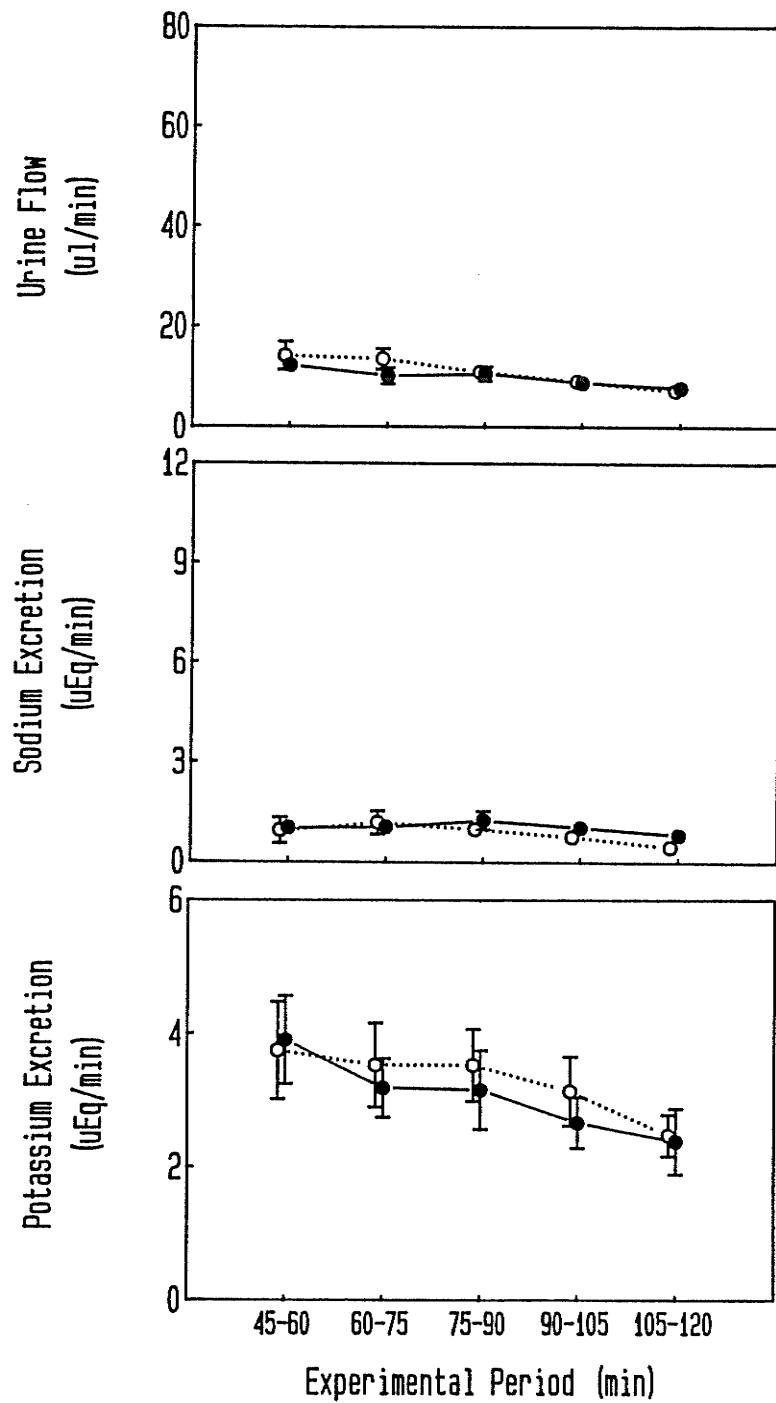
Fig. 2.6. Effect of an intrarenal infusion of yohimbine (25.6 *n*mol/kg/min) on urine flow rate, sodium excretion and potassium excretion in rats receiving an i.v. infusion of saline (24 *u*l/min). (●) Vehicle control, n=5; (○) intrarenal yohimbine, n=6.
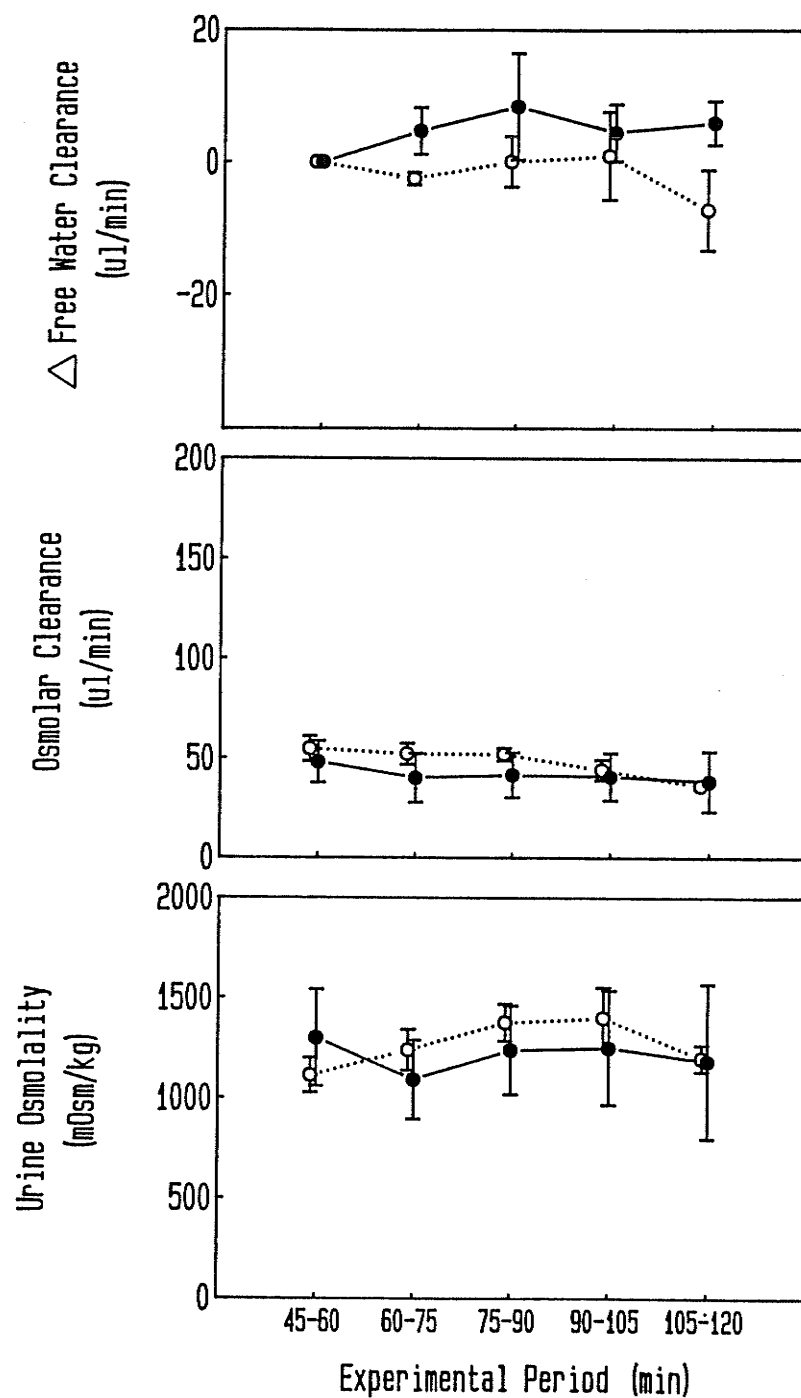
Fig. 2.7. Effect of an intrarenal infusion of yohimbine (25.6 $n$mol/kg/min) on the change in free water clearance, osmolar clearance and urine osmolality in rats receiving an i.v. infusion of saline (24 ul/min). (●) Vehicle control, n=5; (○) intrarenal yohimbine, n=6.
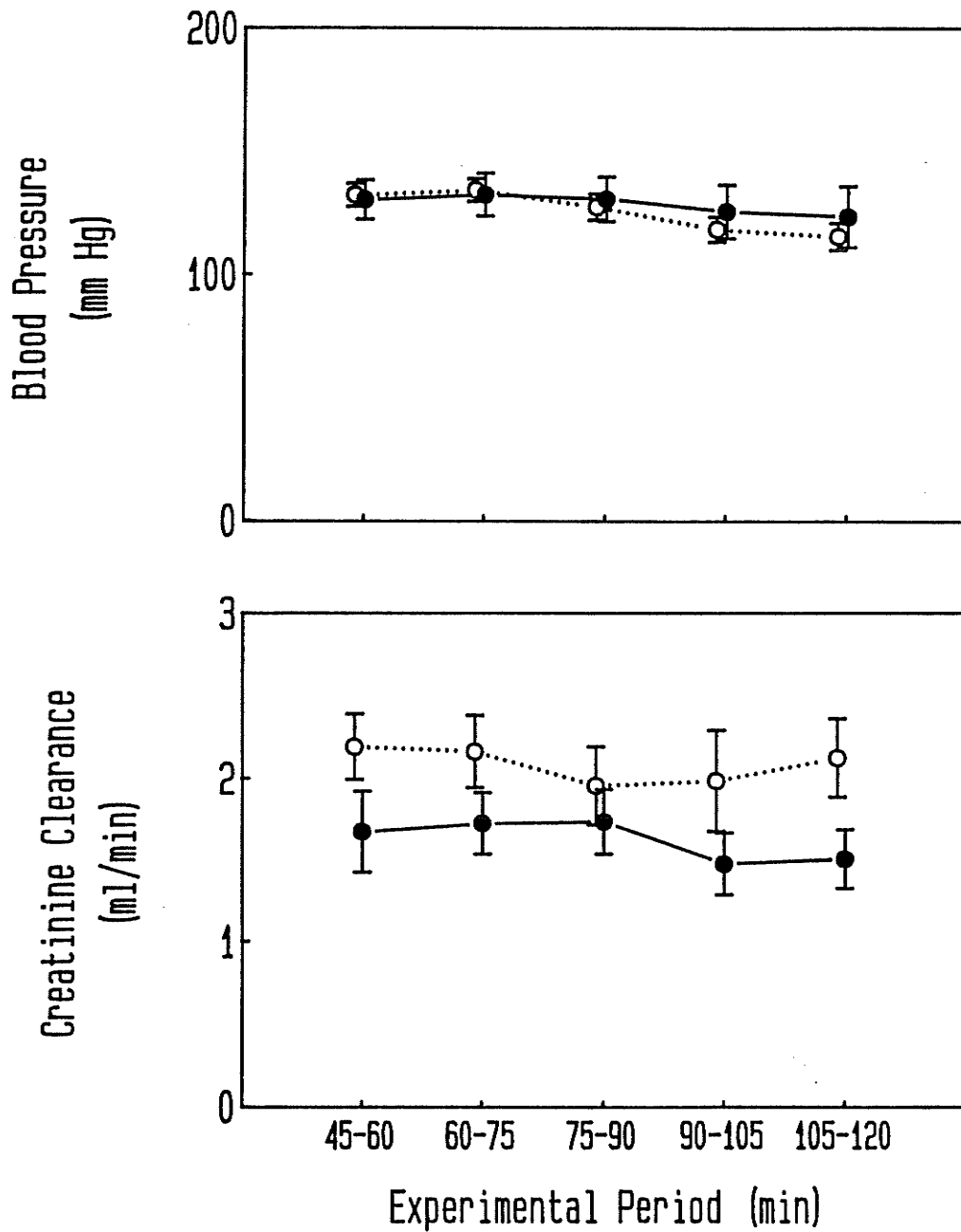
Fig. 2.8. Effect of an intrarenal infusion of yohimbine (25.6 $n$mol/kg/min) on blood pressure and creatinine clearance in rats receiving an i.v. infusion of saline (97 ul/min) to induce a modest diuresis. (●) Vehicle control, n=7; (○) intrarenal yohimbine, n=5.
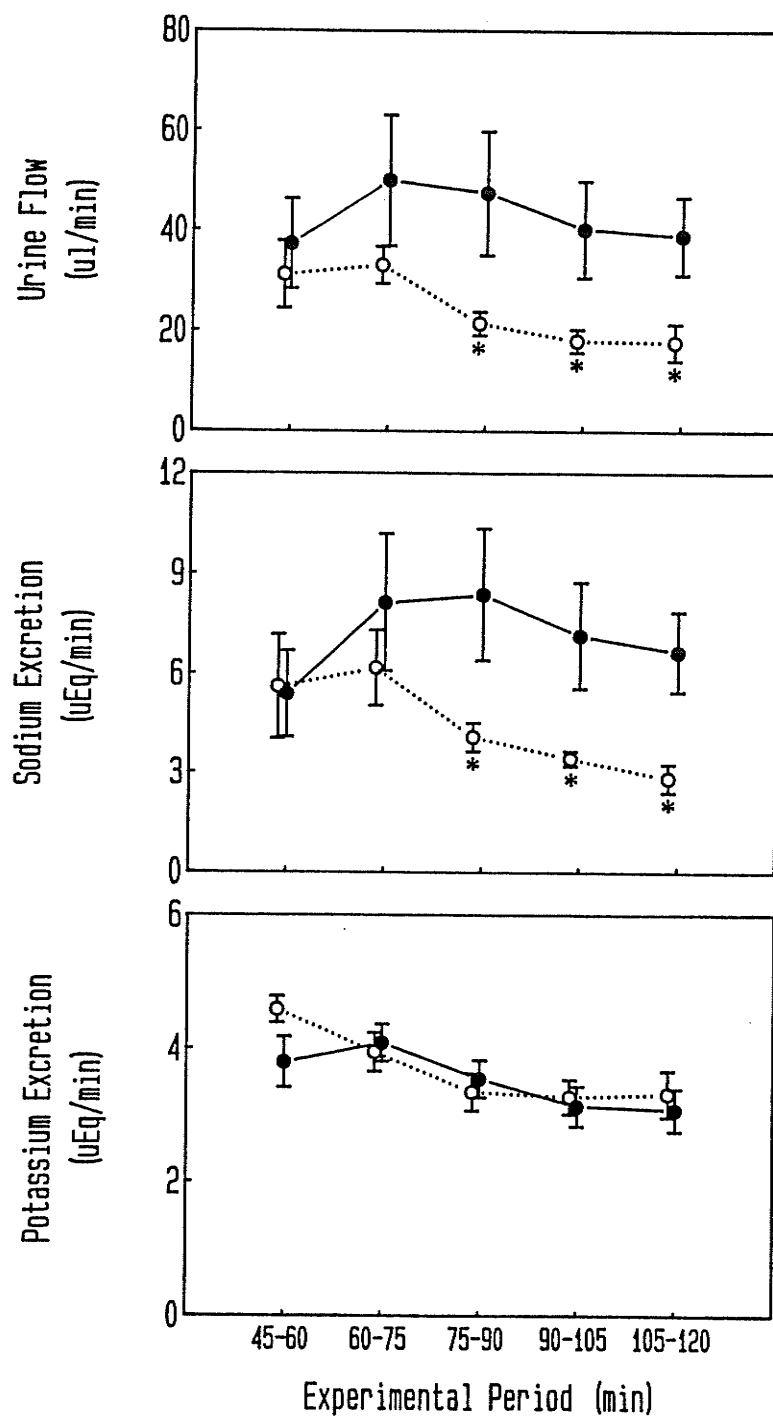
Fig. 2.9. Effect of an intrarenal infusion of yohimbine (25.6 nmol/kg/min) on urine flow rate, sodium excretion and potassium excretion in rats receiving an i.v. infusion of saline (97 ul/min) to induce a modest diuresis. (●) Vehicle control, n=7; (○) intrarenal yohimbine, n=5.
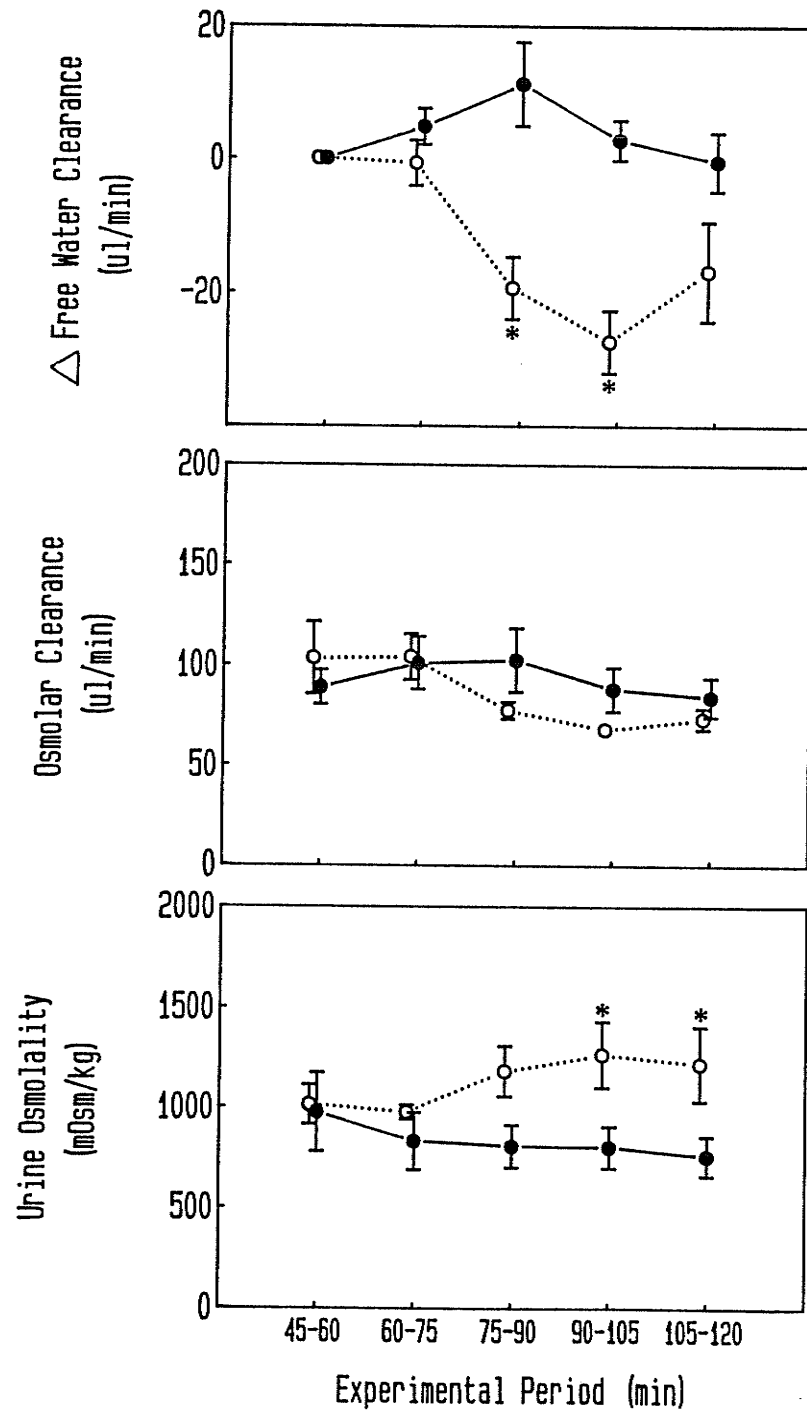
Fig. 2.10. Effect of an intrarenal infusion of yohimbine (25.6 nmol/kg/min) on the change in free water clearance, osmolar clearance and urine osmolality in rats receiving an i.v. infusion of saline (97 ul/min) to induce a modest diuresis. (●) Vehicle control, n=7; (○) intrarenal yohimbine, n=5.
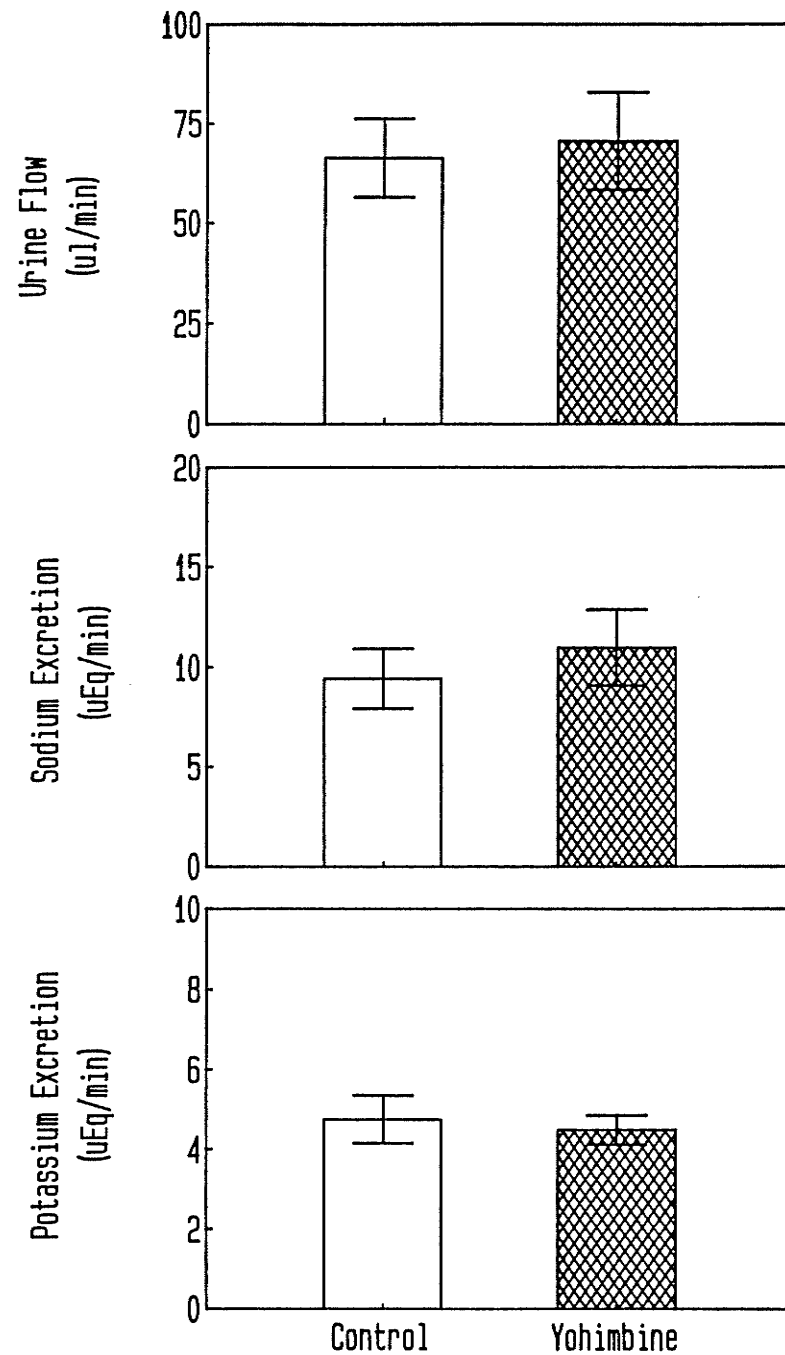
Fig. 2.11. Effect of an intrarenal yohimbine (25.6 $n$mol/kg/min) infusion on urine flow rate, sodium excretion and potassium excretion in adrenalectomized rats. Open bars represent control, n=5; cross-hatched bars represent yohimbine infusion, n=5.
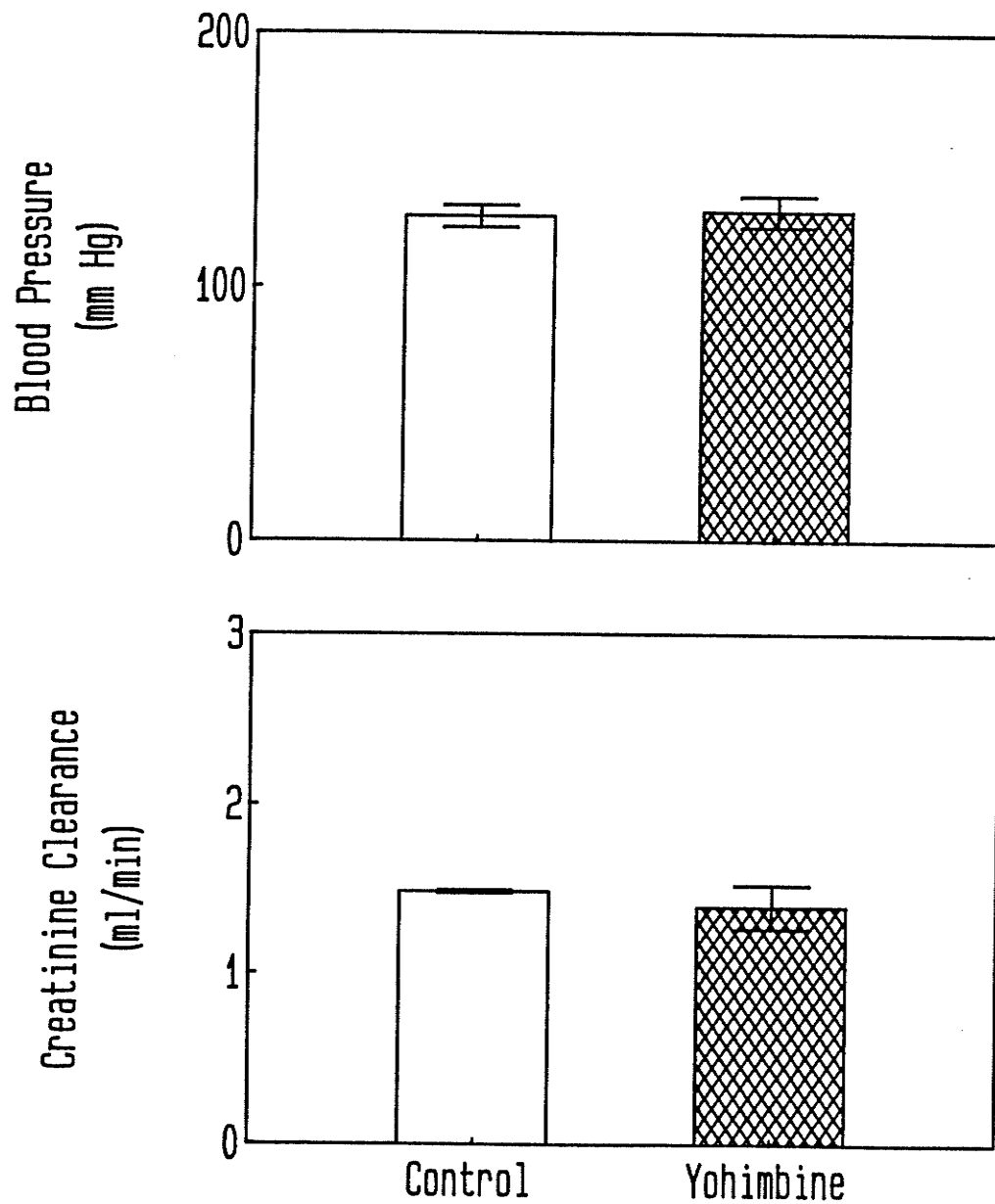
Fig. 2.12. Effect of an intrarenal yohimbine (25.6 *n*mol/kg/min) infusion on blood pressure and creatinine clearance in adrenalectomized rats. Open bars represent control, n=5; cross-hatched bars represent yohimbine infusion, n=5.
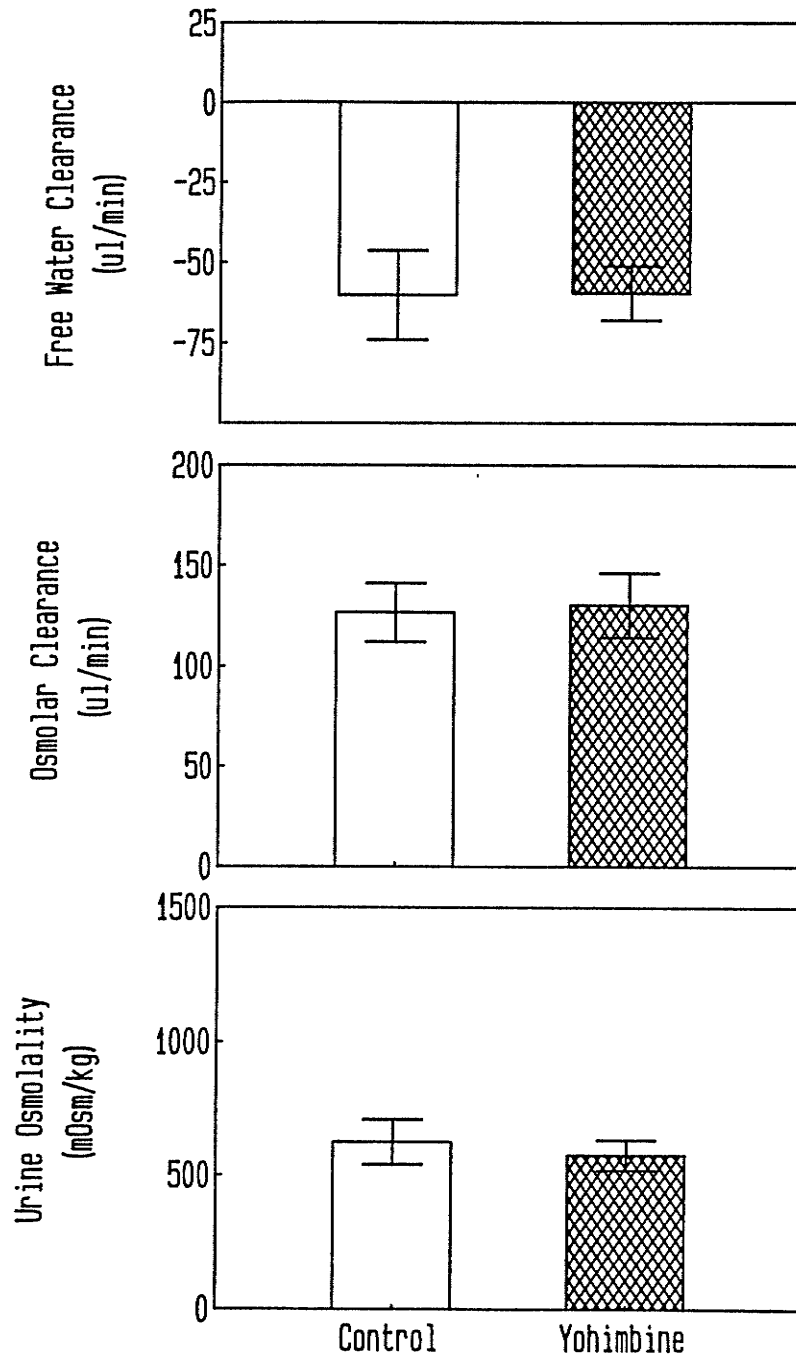
Fig. 2.13. Effect of an intrarenal yohimbine (25.6 $n$mol/kg/min) infusion on free water clearance, osmolar clearance and urine osmolality in adrenalectomized rats. Open bars represent control, n=5; cross-hatched bars represent yohimbine infusion, n=5.

DISCUSSION

In the present study, the predominant function of α2-adrenoceptors, as related to sodium and water excretion, was investigated by the infusion of a specific α2-adrenoceptor antagonist, yohimbine, Yohimbine was infused directly into the renal artery in an attempt to avoid any systemic changes in blood pressure which may also directly alter urine electrolyte excretion. While it is possible that the long half life of yohimbine may eventually result in similar plasma levels with intrarenal and intravenous infusions, the higher concentration at the site of infusion may be important. A specific antagonist rather than an agonist was used in an attempt to unmask the endogenous activity of the renal α2-adrenoceptor. A criticism of the use of specific agonists may be that the effect observed may not be present under physiological conditions, but only in the presence of high levels of activation of the receptors. Conversely, to observe an effect in the presence of an antagonist, the system must be active. In the present study, the rats were unilaterally nephrectomized 7 to 10 days prior to the experiment day. This was done to reduce surgical stress at the time of the experiment (i.e., small flank incision; one ureter cannulated vs. laparotomy; two ureters cannulated), and to allow for a more accurate measurement of urine flow rate than a bladder catheter would permit due to dead space.

Since the nature of the present study was to determine a physiological role for α2-adrenoceptors, it was necessary to establish that the removal of one kidney did not alter the α2-adrenoceptor density in the remaining kidney. Using radioligand binding studies, this underlying assumption was tested. No significant change in renal α2-adrenoceptor density occurred as a result of the nephrectomy. The effect of yohimbine was investigated in the presence of two distinct levels of sodium and water excretion, as well as in acutely adrenalectomized rats. The

animals were adrenalectomized to eliminate circulating epinephrine which has been implicated as the endogenous α2-adrenoceptor agonist (Sawyer *et al.*, 1985; Smyth *et al.*, 1985a; Yamaguchi and Kopin, 1980).

Intrarenal yohimbine failed to decrease sodium and water excretion in rats receiving the low rate of saline infusion. This may be related to the already low level of sodium and water excretion present in these animals. In these rats, the low baseline level of excretion would have been difficult to decrease further. Rats receiving the higher rate of saline infusion had an increased level of sodium and water excretion. In these rats, yohimbine decreased sodium and water excretion. The mechanism by which the yohimbine exerted this effect is not clear. It appears that yohimbine may have reversed the α2-adrenoceptor-mediated antagonism of the renal effects of vasopressin. In the rats receiving the high rate of saline infusion, yohimbine decreased free water clearance without an accompanying change in osmolar clearance. This would be consistent with an increase in activity of the renal effects of vasopressin. α2-Adrenoceptor blockade may be potentiating the renal effects of vasopressin (vide infra). The mechanism by which yohimbine decreased the excretion of sodium is not known. In the adrenalectomized animals, yohimbine failed to demonstrate this antidiuretic and antinatriuretic effect. In the absence of endogenous α2-adrenoceptor activation, yohimbine would not be expected to decrease urine and sodium excretion. Thus, these results are consistent with the adrenal medulla as the source of the endogenous α2-adrenoceptor agonist.

The relationship between α2-adrenoceptor activation and vasopressin activity has been well documented. In *in vivo* preparations, vasopressin has been shown to decrease water and increase sodium excretion (Johnson *et al.*, 1979; Martinez-Maldonado *et al.*, 1971; Pierce *et al.*, 1984). α2-Adrenoceptor agonists when infused intravenously, inhibit the renal effects (Krothapalli *et al.*, 1983; Krothapalli and Suki, 1984; Smyth *et al.*, 1985a; Strandhoy *et al.*, 1982) and the central release (Barr

and Kauker, 1979; Strandhoy *et al.*, 1982) of vasopressin. α2-Adrenoceptor agonists such as clonidine and guanabenz only reverse the effects of vasopressin on water excretion. These agonists produce a further increase in sodium excretion (Barr and Kauker, 1979; Strandhoy *et al.*, 1982; 1983). The reason α2-adrenoceptor agonists reverse the effect on water excretion but potentiate the sodium excretion is not known.

Previous studies in which yohimbine was infused into the renal artery of the dog (Fildes *et al.*, 1985) have been criticized in that the rate of infusion may have also blocked α1-adrenoceptors (DiBona *et al.*, 1986). In the rat, stimulation of renal α1-adrenoceptors increases sodium and water retention (Smyth *et al.*, 1985b). Acute blockade of these receptors would be expected to produce an increase in sodium and water excretion (Osborn *et al.*, 1983). This is the opposite of that observed in the present study, suggesting renal α1-adrenoceptors were not antagonized. Studies in the rabbit have demonstrated that an intrarenal dose of yohimbine, approximately 30 times that used in the present study, did not alter the renal vasoconstrictor response to an α1-adrenoceptor agonist (Hesse and Johns, 1984). The response to an α2-adrenoceptor agonist was significantly attenuated. These results, however, may be difficult to extrapolate into the rat model since no α2-adrenoceptor-mediated renal vasoconstriction has been found in the anesthetized rat (Wolff *et al.*, 1987).

The interpretation of the data must consider effects on presynaptic α2-adrenoceptors. Stimulation of presynaptic α2-adrenoceptors decreased the release of norepinephrine from the nerve endings in the periphery (Langer, 1977) and in the rat kidney (Jeffries *et al.*, 1987). In this study, the intrarenal yohimbine may have blocked presynaptic α2-adrenoceptors and enhanced the release of norepinephrine from the nerve ending. This norepinephrine may have activated postjunctional α1-adrenoceptors and decreased sodium and water excretion (Smyth *et al.*, 1985b),

however, the complete attenuation of the effects of yohimbine following adrenalectomy indicates this is unlikely. It is also uncertain whether the enhancement of the antinatriuretic effect of renal nerve activity would have decreased free water clearance. As well, previous studies have failed to demonstrate a potentiation of the antinatriuretic effect of renal nerve stimulation following $\alpha 2$-adrenoceptor blockade (DiBona and Sawin, 1987).

Previous studies have proposed that effects of renal $\alpha 2$-adrenoceptor stimulation may be mediated through inhibition of cAMP formation (Smyth et al., 1984, 1985a). In the isolated perfused kidney, $\alpha 2$-adrenoceptor stimulation has been shown to increase (Smyth et al., 1985a) or decrease (Pettinger et al., 1985) the excretion of sodium and water. These results were dependent on the infusion of vasopressin and arachidonic acid respectively. The predominant effect in the whole animal, however, was not determined. The results of the present study suggest that the predominant role of renal $\alpha 2$-adrenoceptors in the regulation of water and/or sodium excretion was mediated by the antagonism of the effects of vasopressin. A number of hormones mediate their effects through the activation of the adenylate cyclase system in the kidney (Morel et al., 1981, Pettinger et al., 1987). These include parathyroid hormone, calcitonin, vasopressin and prostaglandins. In isolated nephron segments, $\alpha 2$-adrenoceptor stimulation may attenuate the formation of cAMP by these hormones (Pettinger et al., 1987). Thus, renal $\alpha 2$-adrenoceptors may serve other functions in the kidney unrelated to sodium and/or water excretion.

Finally the effect of yohimbine in the present study may have been secondary to elevations in vasopressin and circulating catecholamines as a result of the stress induced by anesthesia and surgery (Bonjour and Malvin, 1970; Bridle et al., 1983). This cannot be determined since plasma vasopressin and catecholamine levels were not measured in the present study. Moreover, the relationship between vasopressin,

anesthesia and surgical stress has not been systematically evaluated (Cowley and Liard, 1987). Thus, in the least, the present study indicates that under situations of stress, $\alpha 2$-adrenoceptors may play a significant role in the regulation of the renal effects of vasopressin. Whether this occurs in the unstressed animal remains to be determined.

In summary, renal $\alpha 2$-adrenoceptor antagonism produced a decrease in sodium and water excretion. This effect is consistent with yohimbine blocking the antagonistic activity of $\alpha 2$-adrenoceptors on the renal effects of vasopressin. These receptors may play a tonal role in the regulation of the renal effects of vasopressin in the anesthetized rat. The attenuation of the effects of yohimbine following adrenalectomy suggest that circulating epinephrine may be the endogenous $\alpha 2$-adrenoceptor agonist.

3

Dose Selective Dissociation of Water and Solute Excretion after Renal α2-Adrenoceptor Stimulation

The majority of the data in this section have been presented at the Canadian Society of Clinical Investigation meetings, Winnipeg, 1987 (Clin. Invest. Med. 10:C288, 1987). These data have also been previously published. Blandford, D.E. and Smyth, D.D., J. Pharmacol. Exp. Ther. 247:1181-1186, 1988.

Synopsis:

In vitro studies have demonstrated an antagonism of the renal effects of vasopressin with α2-adrenoceptor stimulation. Whether the effect of α2-adrenoceptor stimulation, in relation to sodium and water excretion, *in vivo* is mediated through independent mechanisms is unclear. The dose-response relationship between renal α2-adrenoceptor stimulation (clonidine) on water and electrolyte excretion was evaluated in anesthetized rats. Rats were nephrectomized unilaterally 7 to 10 days before the experiment day to allow isolation of renal function. A baseline level of sodium and water excretion was established by the infusion of saline (97 ul/min i.v.). In separate groups of rats, clonidine was infused directly into the renal artery at 0 (vehicle), 0.1, 0.3, 1 or 3 ug/kg/min at a rate of 3.4 ul/min. The lower doses (0.1, 0.3 and 1 ug/kg/min) produced a dose-related increase in urine flow and free water clearance and a decrease in urine osmolality. Electrolyte or solute excretion was not altered at these infusion rates even though urine flow rate increased 4-fold. The highest dose investigated (3 ug/kg/min) increased urine flow rate (9-fold) and sodium excretion (4-fold). Free water clearance and osmolar clearance were also increased. The effects of clonidine were attenuated by yohimbine but not prazosin, indicating these effects were mediated by α2-adrenoceptor stimulation. These results demonstrate a dose-related selectivity of α2-adrenoceptor stimulation for water and sodium excretion. The increase in water excretion at the lower infusion rates would be consistent with the antagonism of the renal effects of vasopressin. The potent natriuresis observed only at higher doses indicates another mechanism may be involved.

INTRODUCTION

In the previous section, blockade of the α2-adrenoceptor with yohimbine decreased urine volume, free water clearance and sodium excretion, and increased urine osmolality. This suggested that the endogenous activation of α2-adrenoceptors may inhibit the renal actions of vasopressin. To further examine this possibility, the effect of exogenous α2-adrenoceptor stimulation with clonidine was examined.

A number of previous studies have described the effects of α2-adrenoceptor stimulation on the renal excretion of sodium and water. *In vivo*, stimulation of α2-adrenoceptors has been associated with an increase in both sodium and water excretion (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). *In vitro* studies in the isolated perfused rat kidney (Smyth *et al.*, 1985a) and in isolated nephron segments (Krothapalli *et al.*, 1983; Krothapalli and Suki, 1984) have demonstrated similar effects and proposed that these effects of renal α2-adrenoceptor stimulation were dependent on the presence of vasopressin.

The renal effects of vasopressin have been shown to be mediated through the formation of cAMP (Krothapalli and Suki, 1984; Umemura *et al.*, 1985). In the isolated perfused kidney (Smyth *et al.*, 1985a) and in isolated cortical collecting tubules (Krothapalli *et al.*, 1983) vasopressin decreased sodium and water excretion. In these studies α2-adrenoceptor stimulation inhibited the vasopressin-induced activation of adenylate cyclase and the effects of vasopressin on sodium and water excretion. These studies suggested that the effects of α2-adrenoceptor activation in the kidney were due to the inhibition of vasopressin-mediated increases in cAMP production.

Earlier studies by Strandhoy *et al.* (1982) indicated that the effects of renal α2-adrenoceptor stimulation on sodium and water excretion appeared to involve independent mechanisms. This contention has been supported by studies which have suggested that the effects of α2-adrenoceptor stimulation may be unrelated to vasopressin (Baranowska *et al.*, 1987; Leander *et al.*, 1985).

If the changes in sodium and water excretion after α2-adrenoceptor stimulation were mediated by different independent mechanisms (Strandhoy *et al.*, 1982), then a dissociation in sensitivity of these mechanisms to α2-adrenoceptor stimulation may be anticipated. Previous studies have utilized only one or two doses to describe the effect of α2-adrenoceptor agonists. As well, studies which have investigated the dose-response relationship have infused the α2-adrenoceptor agonist intravenously. The enhanced efficacy of α2-adrenoceptor agonists administered intravenously as compared to intrarenally (Strandhoy *et al.*, 1982) indicates that systemic effects may be a problem in these studies. Consequently, these previous studies may have failed to observe an existing differential effect of α2-adrenoceptor stimulation on water and sodium excretion.

The dose-response relationship between the intrarenal infusion of an α2-adrenoceptor agonist (clonidine) and the excretion of sodium and water was therefore investigated. The dose-related dissociation in the excretion of water and sodium suggests that α2-adrenoceptor stimulation may be mediating these effects at different sites in the kidney and/or by two independent mechanisms.

METHODS

As previously described (Section 2), animals in these experiments were surgically prepared to determine either (a) the pressor response to intravenous and intrarenal infusions of the α2-adrenoceptor agonist, clonidine (0, 0.1, 0.3, 1, 3, 10, 30 and 100 ug/kg/min; Sigma Chemical Co., St. Louis, MO) or (b) the *in vivo* response to a continuous intrarenal infusion of vehicle (saline) or clonidine (0.1, 0.3, 1 or 3 ug/kg/min) at a rate of 3.4 ul/min.

1. <u>Pressor responsiveness</u>. The pressor response to intravenous and intrarenal infusions of the α2-adrenoceptor agonist clonidine (0, 0.1, 0.3, 1, 3, 10, 30 and 100 ug/kg/min) was determined. After the surgical procedure, the preparation was allowed to stabilize for 30 min. Intravenous infusions were administered by the jugular vein. For the intrarenal infusion, a flank incision was made and a 31 gauge needle advanced through the aorta into the renal artery and secured with glue. Each infusion rate was maintained until a steady state in blood pressure was achieved.

2. <u>Renal function</u>. The renal effects of an intrarenal infusion of vehicle (saline) or clonidine (0.1, 0.3, 1 or 3 ug/kg/min) were determined. In addition to the surgery previously described, an electromagnetic flow probe (2.0-2.5 mm, North Carolina 100 series) was placed around the renal artery for the measurement of renal blood flow with a Carolina Analog Blood Flow Meter (Model FM501). After a 45 min stabilization period, a 15 min control urine collection was obtained into a preweighed tube. This was followed by the continuous infusion of vehicle or clonidine at 3.4 ul/min. During these infusions four consecutive 15 min urine

collections were obtained. Throughout the entire experiment, saline was infused at a rate of 97 ul/min.

In the above experiments, the increase in sodium and water excretion observed following the administration of clonidine (3 ug/kg/min) may have been secondary to the increase in blood pressure (approximately 25 mm Hg). Therefore, these experiments were repeated using a vascular occluder (a micrometer screw clamp) to clamp the descending aorta proximal to the renal artery. The carotid artery as well as the femoral artery were cannulated to record blood pressure proximal and distal to the clamp. The blood pressure recorded distal to the clamp in the femoral artery represented the blood pressure at the level of the renal artery. Thus, in the face of increased systemic pressure, the clamp was screwed down to maintain a constant renal perfusion pressure. Sham experiments were also conducted where the occluder was positioned around the descending aorta, but was not screwed down with increases in systemic blood pressure. The femoral artery was cannulated (PE60) for the measurement of post-occlusion blood pressure. Following the infusion of clonidine, the aorta was partially occluded so that the blood pressure measured in the femoral catheter remained constant with control values.

To determine whether the diuretic and natriuretic effects of clonidine were mediated by α2-adrenoceptors, the response to clonidine (1 and 3 ug/kg/min) was repeated in the presence of prazosin (0.01 mg/kg; Pfizer Canada Inc., Kirkland, Ontario), an α1-adrenoceptor antagonist, or yohimbine (1 mg/kg), an α2-adrenoceptor antagonist (van Meel et al., 1981c). The protocol was as described earlier except immediately after the surgical procedure prazosin or yohimbine was administered as a bolus intravenous dose. In this series of experiments prazosin failed to alter the response to clonidine, suggesting either α1-adrenoceptors were not involved or alternatively these receptors were not effectively blocked at the dose

used. The effectiveness of α1-adrenoceptor blockade by prazosin (0.01 mg/kg) was evaluated in pithed rats. Rats were pithed under pentobarbital anesthesia by inserting a blunt stainless steel rod (approximately 1.5-2.0 mm in diameter) through the orbit of the left eye and foramen magnum down into the vertebral canal as described by Gillespie *et al.* (1970). Immediately after pithing, the trachea cannula was attached to a Harvard Apparatus Animal Ventilator. After a 30 min stabilization period, the pressor response to clonidine and phenylephrine (0.1, 0.3, 1, 3, 10, 30 and 100 ug/kg/min) was determined in the absence and presence of prazosin. Only one dose response curve was performed on an individual rat.

Statistical analyses were performed with analysis of variance followed by Duncan's multiple comparison test to determine the location of significance. For the second (aortic clamp) and third (pretreatment with yohimbine or prazosin) sets of experiments, data were again analyzed by analysis of variance (drug x clamp; drug x condition respectively). Significant interactions were analyzed further with simple main effects analyses and Tukey HSD tests (Winer, 1971). Data are expressed as the mean $\pm$ the S.E.M.

RESULTS

1. Effect of clonidine on blood pressure

The disparate effects of intravenous and intrarenal infusions of clonidine are shown in figure 3.1. Intrarenal infusion rates of clonidine at 3 $ug/kg/min$ or greater produced a significant increase in blood pressure. Intravenous infusion of clonidine resulted in a slight depressor response (not statistically significant) at lower infusion rates (0.3, 1 and 3 $ug/kg/min$) which reversed to pressor at the two highest doses examined (30 and 100 $ug/kg/min$) (fig. 3.1).

2. Effect of intrarenal clonidine infusion on electrolyte excretion.

The intrarenal infusion of clonidine resulted in a dose-related increase in urine flow rate (fig. 3.2) and free water clearance (fig. 3.3). Clonidine at 0.3 and 1 $ug/kg/min$ specifically increased urine flow rate 2.5- and 4-fold, respectively. Free water clearance was also increased. These lower doses of clonidine failed to alter solute excretion as indicated by the lack of changes in sodium and potassium excretion (fig. 3.2) and osmolar clearance (fig. 3.3). Blood pressure and renal blood flow were unaltered (fig. 3.4). At an infusion rate of 1 $ug/kg/min$, clonidine significantly decreased creatinine clearance. The highest dose of clonidine studied (3 $ug/kg/min$) produced a further increase in urine flow rate (9.5-fold) and free water clearance. However, this high dose also increased sodium excretion (4-fold), potassium excretion and osmolar clearance. This high dose was slightly pressor, reaching a level of significance during the fourth collection period. Creatinine clearance was decreased but this did not reach a level of statistical significance. Renal blood flow was unaltered (fig. 3.4).

The dose-dependent dissociation in the clonidine-induced alterations in water and then sodium excretion is shown in figure 3.5. Clonidine infusions of 0.3

and 1 $ug/kg/min$ increased urine flow rate with no change in sodium excretion. The maximal infusion rate tested increased both water and sodium excretion. A similar trend was found when free water clearance and osmolar clearance were compared (fig. 3.5) indicating an increase in solute excretion only at the highest dose investigated.

3. Effect of clonidine with the use of the aortic clamp.

Since blood pressure was significantly elevated with the high infusion rate of clonidine, these studies were repeated using an aortic clamp to maintain normal renal perfusion pressures. At 3 $ug/kg/min$, clonidine increased blood pressure. When the aortic clamp was used, blood pressure, as measured in the femoral artery, was unaltered (fig. 3.6). Heart rate was decreased with clonidine, irrespective of whether or not the aortic clamp was used, while creatinine clearance was unaltered (fig. 3.6). The effects on urine flow, sodium excretion and potassium excretion are shown in figure 3.7. Urine flow and sodium excretion were increased, both with and without the use of the aortic clamp. The absolute increase in urine flow and sodium excretion following the use of the aortic clamp is attenuated. These results indicate that some, but not all of the effects of clonidine on sodium and water excretion could be attributed to an increase in systemic blood pressure and renal perfusion pressure. Potassium excretion was unaltered. Free water clearance was elevated with clonidine, and this effect appears to be mediated independently of the change in blood pressure (fig. 3.8). Conversely, osmolar clearance was increased only when the systemic blood pressure was elevated (fig. 3.8). Finally, urine osmolality was decreased with clonidine, and this decrease occured regardless of whether the aortic clamp was used or not (fig. 3.8).

4. Effect of pretreatment with yohimbine or prazosin.

The intrarenal infusion of clonidine in the presence of $\alpha 1$- or $\alpha 2$-adrenoceptor blockade by pretreatment with prazosin or yohimbine, respectively, had no significant effect on creatinine clearance (fig. 3.9) or potassium excretion (fig. 3.10). Clonidine increased blood pressure at 3 $ug/kg/min$ in both control and prazosin pretreated animals. Pretreatment with yohimbine attenuated this increase in blood pressure (fig. 3.9). Heart rate was decreased by both infusion rates of clonidine. Pretreatment with prazosin did not alter this effect, while pretreatment with yohimbine attenuated the decrease in heart rate at the 1 $ug/kg/min$ infusion rate of clonidine (fig. 3.9).

Pretreatment with yohimbine resulted in a significant attenuation of the renal effects of clonidine on urine flow and sodium excretion (fig. 3.10). Furthermore, the effect of clonidine on free water clearance and urine osmolality was also attenuated (fig. 3.11). Pretreatment with prazosin failed to attenuate the dose-related increase in urine flow and sodium excretion (fig. 3.10). As well, while urine osmolality and osmolar clearance were unaltered in the presence of prazosin, the response to free water clearance was partially decreased (fig. 3.10). The failure of prazosin to alter the renal effects of clonidine was not related to an inadequate blockade of $\alpha 1$-adrenoceptors since the pressor response to phenylephrine was significantly attenuated by this dose of prazosin (fig. 3.12). Prazosin (0.01 mg/kg) attenuated the pressor response to phenylephrine (ED_{50} mm Hg = 7.5 $\pm$ 1.4 vs. 32.2 $\pm$ 2.2 $ug/kg/min$, p<0.05), but not to clonidine ED $_{50}$m Hg = 1.9 $\pm$ 0.2 vs. 3.9 $\pm$ 1.8 $ug/kg/min$). The data suggest that the observed effects of clonidine were mediated by $\alpha 2$-adrenoceptors.
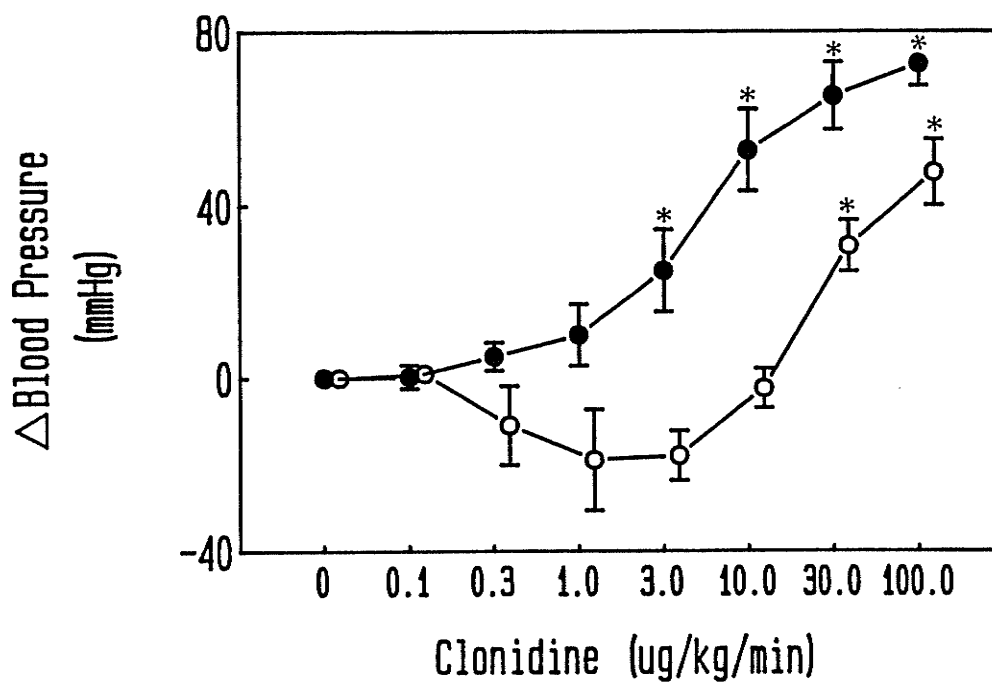
Fig. 3.1. Effect of (○) intravenous, n=5; and (●) intrarenal n=7 infusions of clonidine on blood pressure.
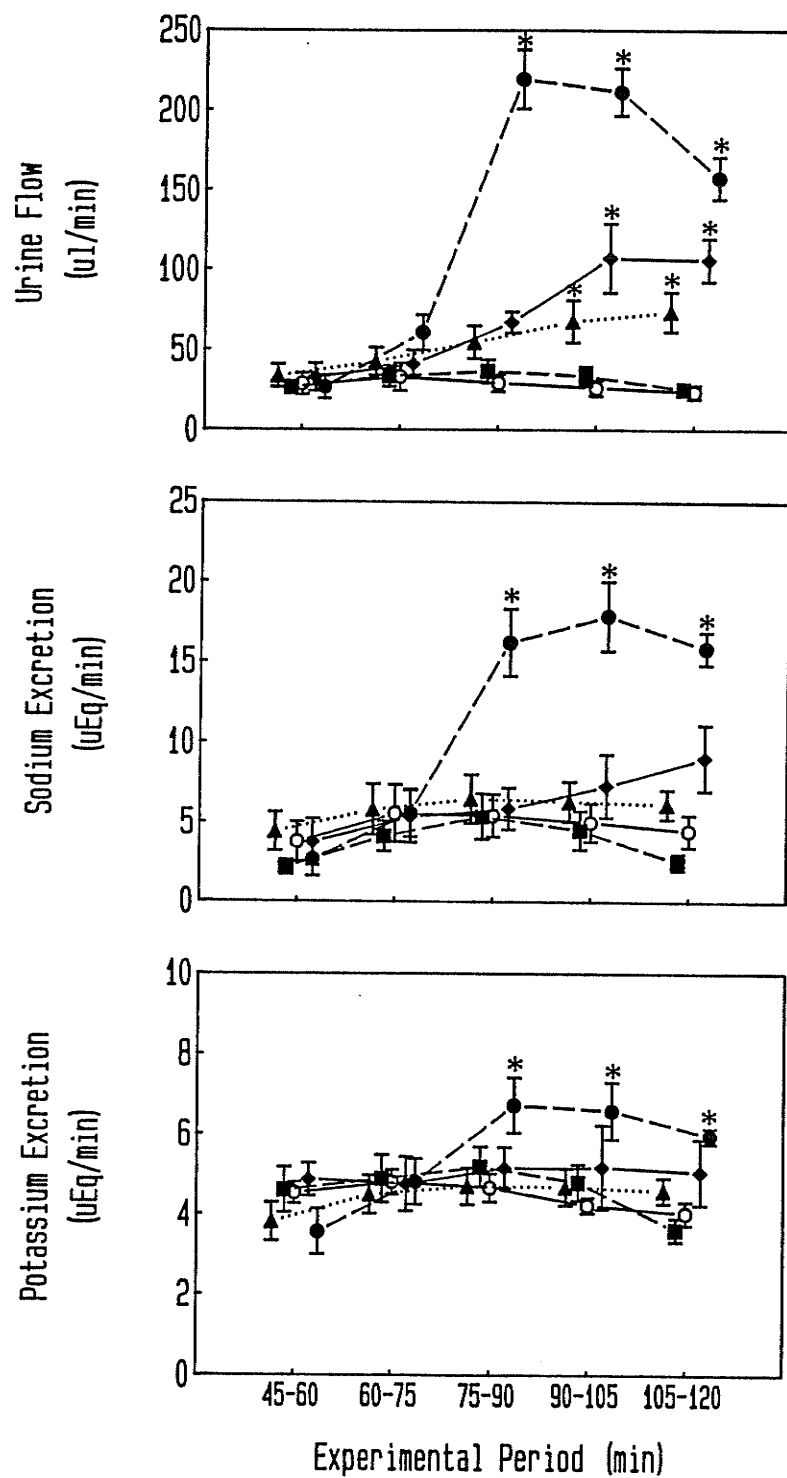
Fig. 3.2. Effect of an intrarenal infusion (3.4 *ul*/min) of clonidine on urine flow rate, sodium excretion and potassium excretion. (o) Saline vehicle, n=5; (■) 0.1 *ug*/kg/min clonidine, n=6; (▲) 0.3 *ug*/kg/min clonidine, n=8; (◆) 1 *ug*/kg/min clonidine, n=5; (●) 3 *ug*/kg/min clonidine, n=7.
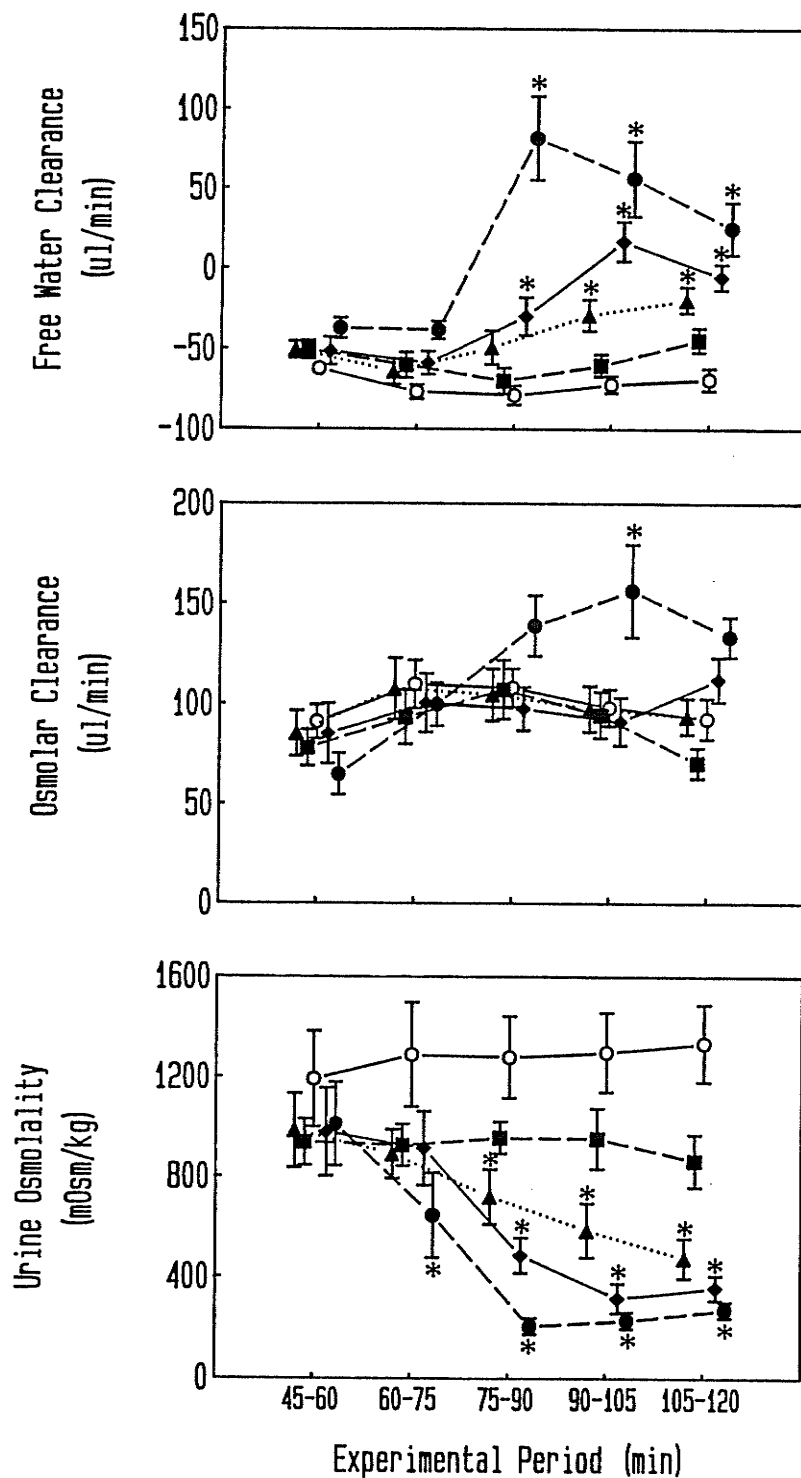
Fig. 3.3. Effect of an intrarenal infusion (3.4 *u*l/min) of vehicle or clonidine on free water clearance, osmolar clearance and urine osmolality. (○) Saline vehicle, n=5; (■) 0.1 *u*g/kg/min clonidine, n=6; (▲) 0.3 *u*g/kg/min clonidine, n=8; (◆) 1 *u*g/kg/min clonidine, n=5 (●) 3 *u*g/kg/min clonidine, n=7.
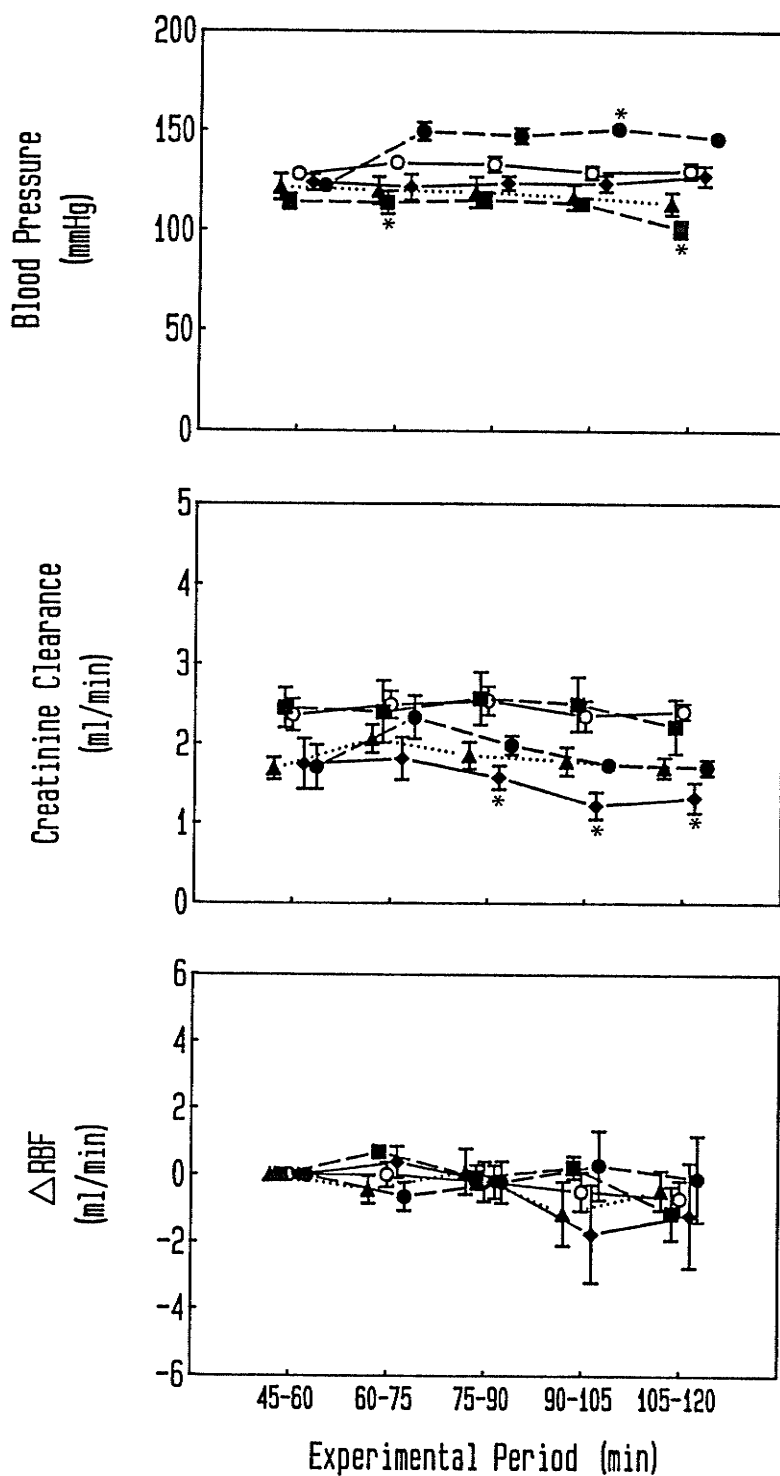
Fig. 3.4. Effect of an intrarenal infusion (3.4 *u*l/min) of vehicle or clonidine on blood pressure, creatinine clearance and change in renal blood flow (△RBF). (○) Saline vehicle, n=5; (■) 0.1 *u*g/kg/min clonidine, n=6; (▲) 0.3 *u*g/kg/min clonidine, n=8; (◆) 1 *u*g/kg/min clonidine, n=5; (●) 3 *u*g/kg/min clonidine, n=7.
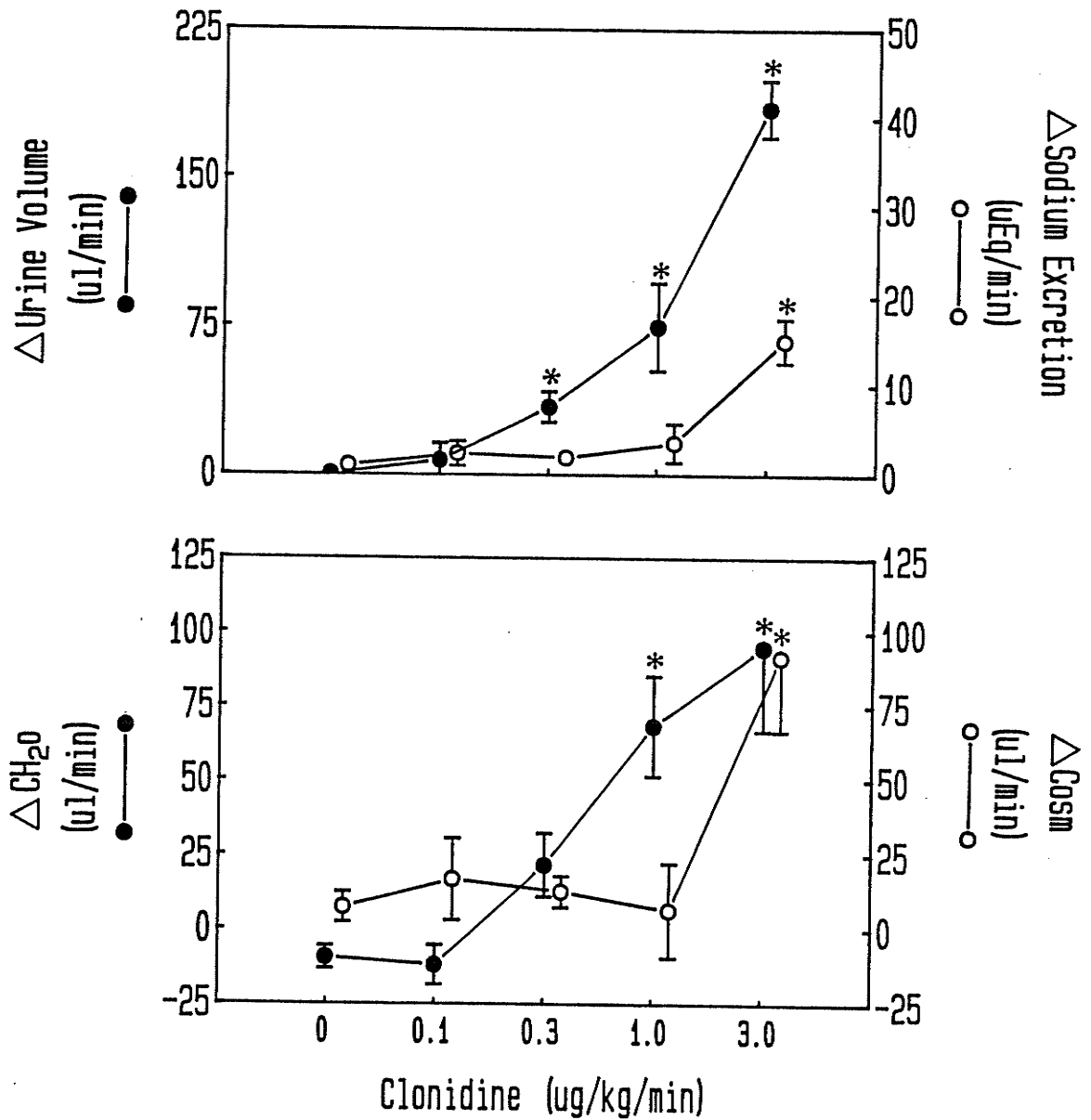
Fig. 3.5. Effect of an intrarenal infusion (3.4 ul/min) of increasing doses of clonidine on changes in (●) urine flow rate, and (○) sodium excretion, and on changes in (●) free water clearance (CH_2O), and (○) osmolar clearance (Cosm). These data represent the changes observed from the first collection period (control) to the fourth, and are representative of the observed differences between groups. Each infusion rate represents the mean ± S.E.M. of 5 to 8 experiments.
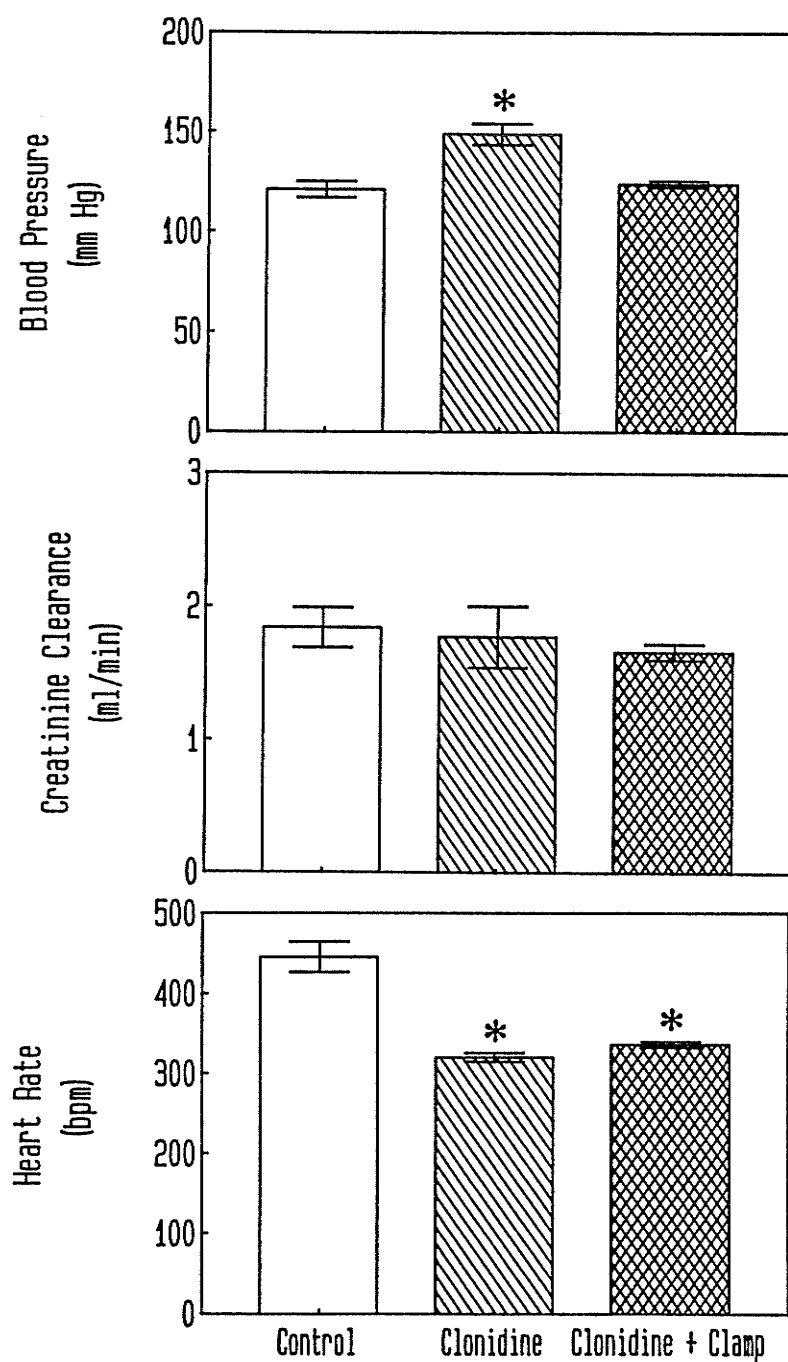
Fig. 3.6. Effect of a 3 $ug/kg/min$ infusion of clonidine with and without the use of a vascular occluder (clamp), on blood pressure, creatinine clearance and heart rate. Open bars represent vehicle control, n=4; hatched bars represent the clonidine infusions without the clamp, n=5; cross-hatched bars represent the clonidine infusions with the clamp, n=5.
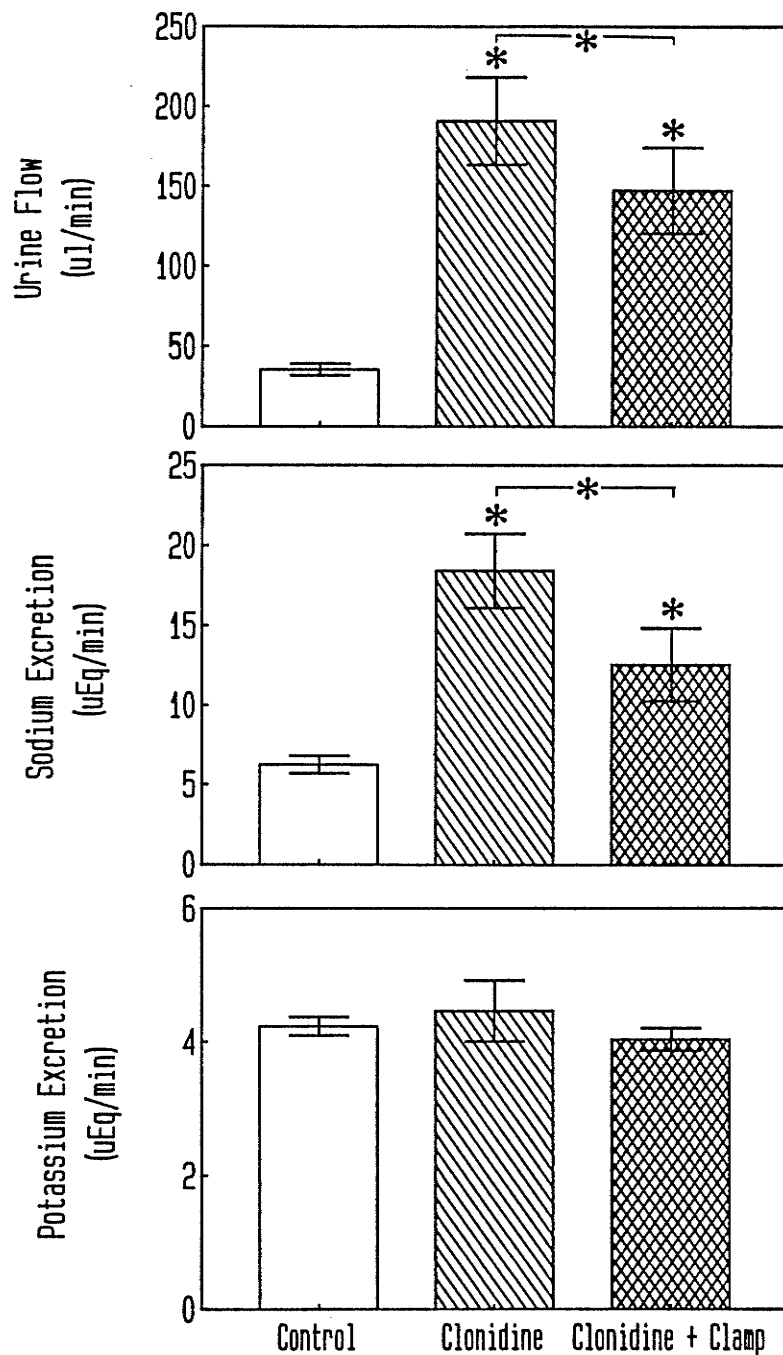
Fig. 3.7. Effect of a 3 ug/kg/min infusion of clonidine with and without the use of a vascular occluder (clamp) on urine flow rate, sodium excretion and potassium excretion. Open bars represent vehicle control, n=4; hatched bars represent clonidine infusions without the clamp, n=5; cross-hatched bars represent clonidine infusions with the clamp, n=5.
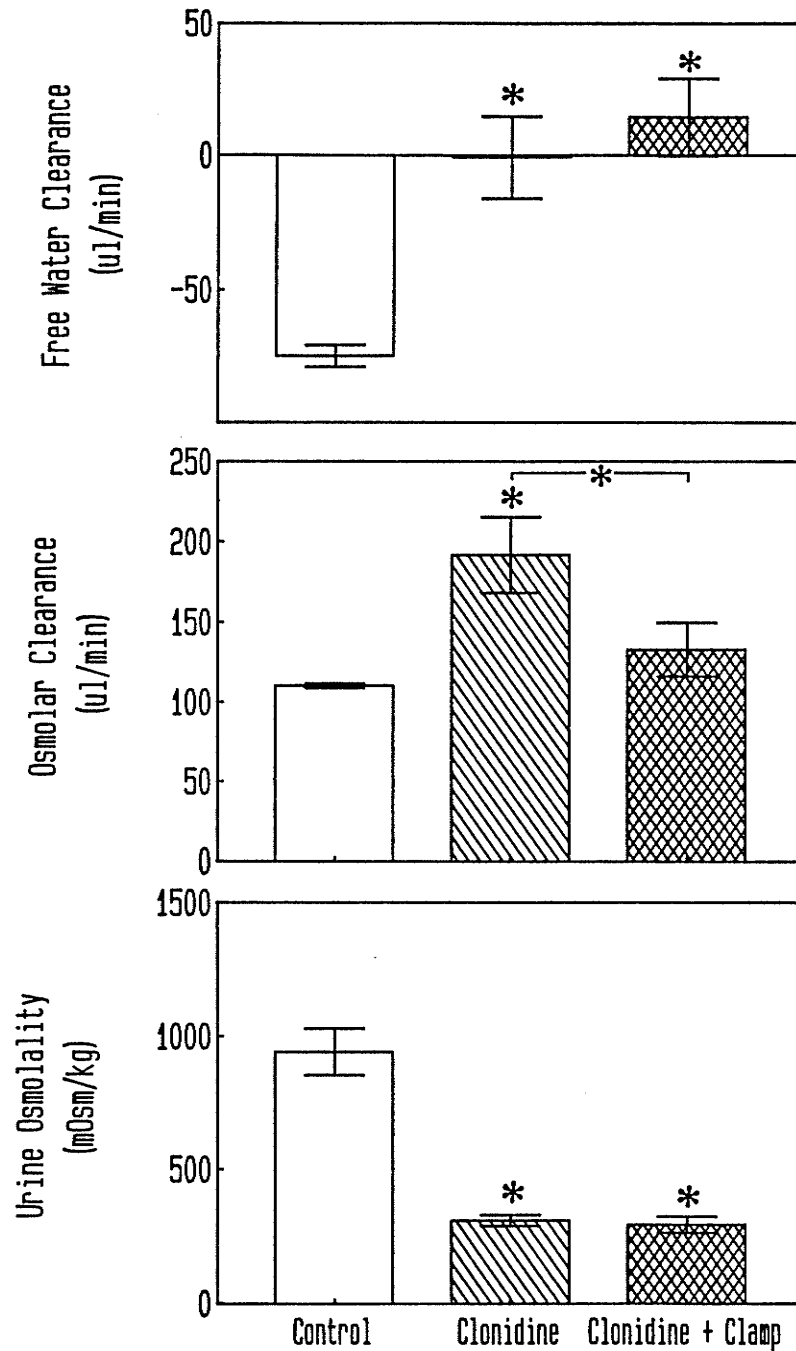
Fig. 3.8. Effect of a 3 ug/kg/min infusion of clonidine with and without the use of a vascular occluder (clamp) on free water clearance, osmolar clearance and urine osmolality. Open bars represent vehicle control, n=4; hatched bars represent clonidine infusions without the clamp, n=5; cross-hatched bars represent clonidine infusions with the clamp, n=5.
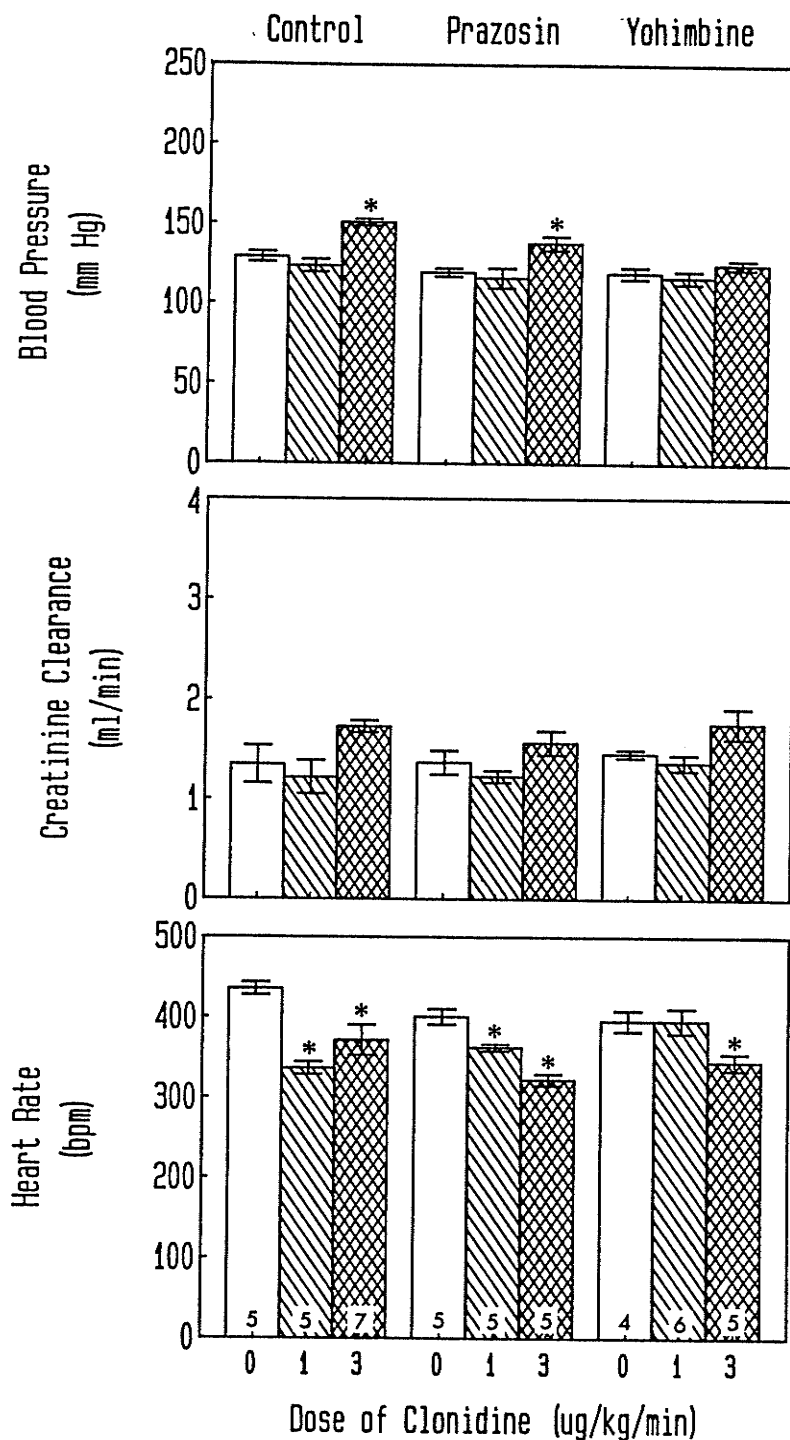
Fig. 3.9. Effect of an intrarenal infusion (3.4 *ul*/min) of clonidine with either yohimbine or prazosin pretreatment on blood pressure, creatinine clearance and heart rate. Open bars represent control (vehicle); hatched bars represent clonidine, 1 *ug*/kg/min; cross hatched bars represent clonidine, 3 *ug*/kg/min. These data were obtained from the 4th collection period (90-105 min), and are representative of the observed differences between groups.
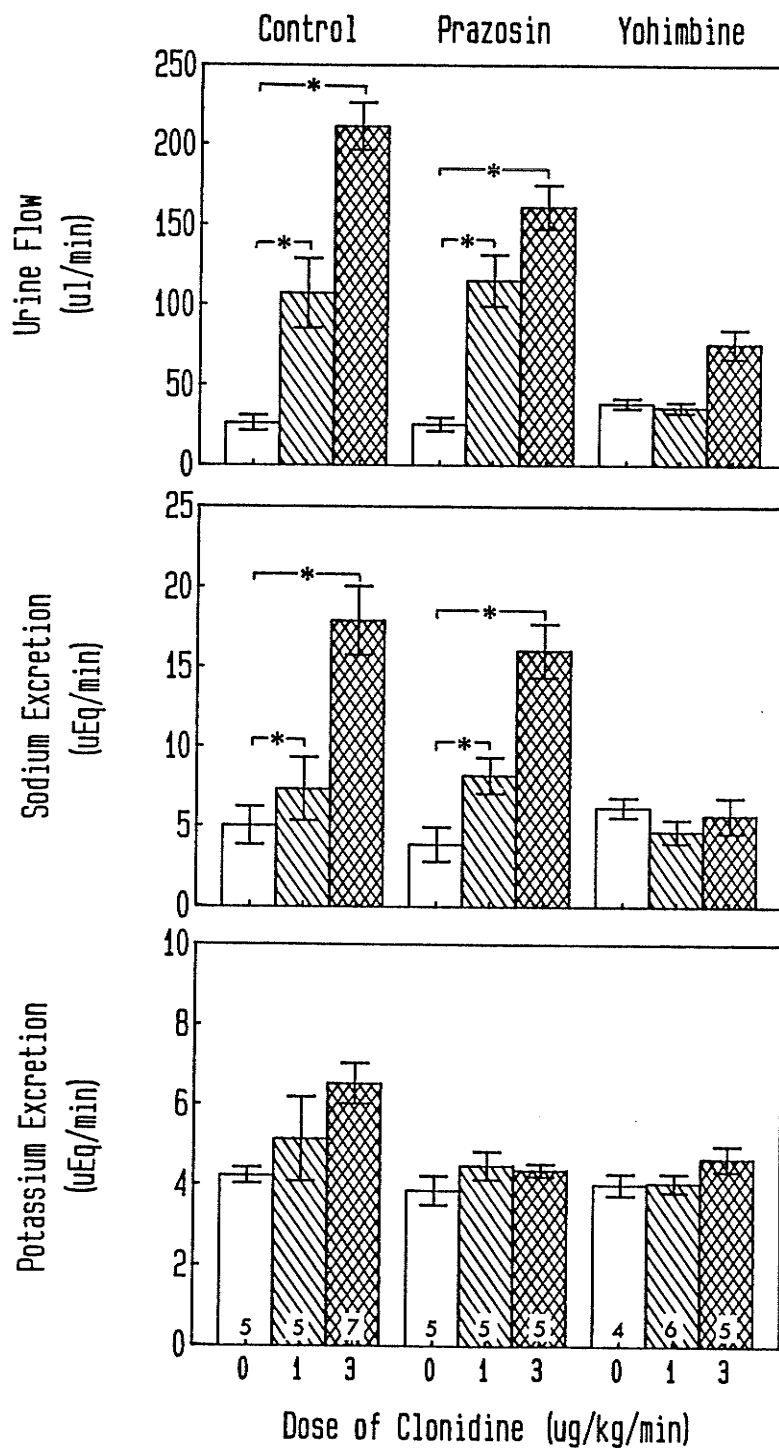
Fig. 3.10. Effect of an intrarenal infusion (3.4 ul/min) of clonidine with either yohimbine or prazosin pretreatment, on urine flow rate, sodium excretion and potassium excretion. Open bars represent control, (vehicle); hatched bars represent clonidine, 1 ug/kg/min; cross-hatched bars represent clonidine, 3 ug/kg/min.
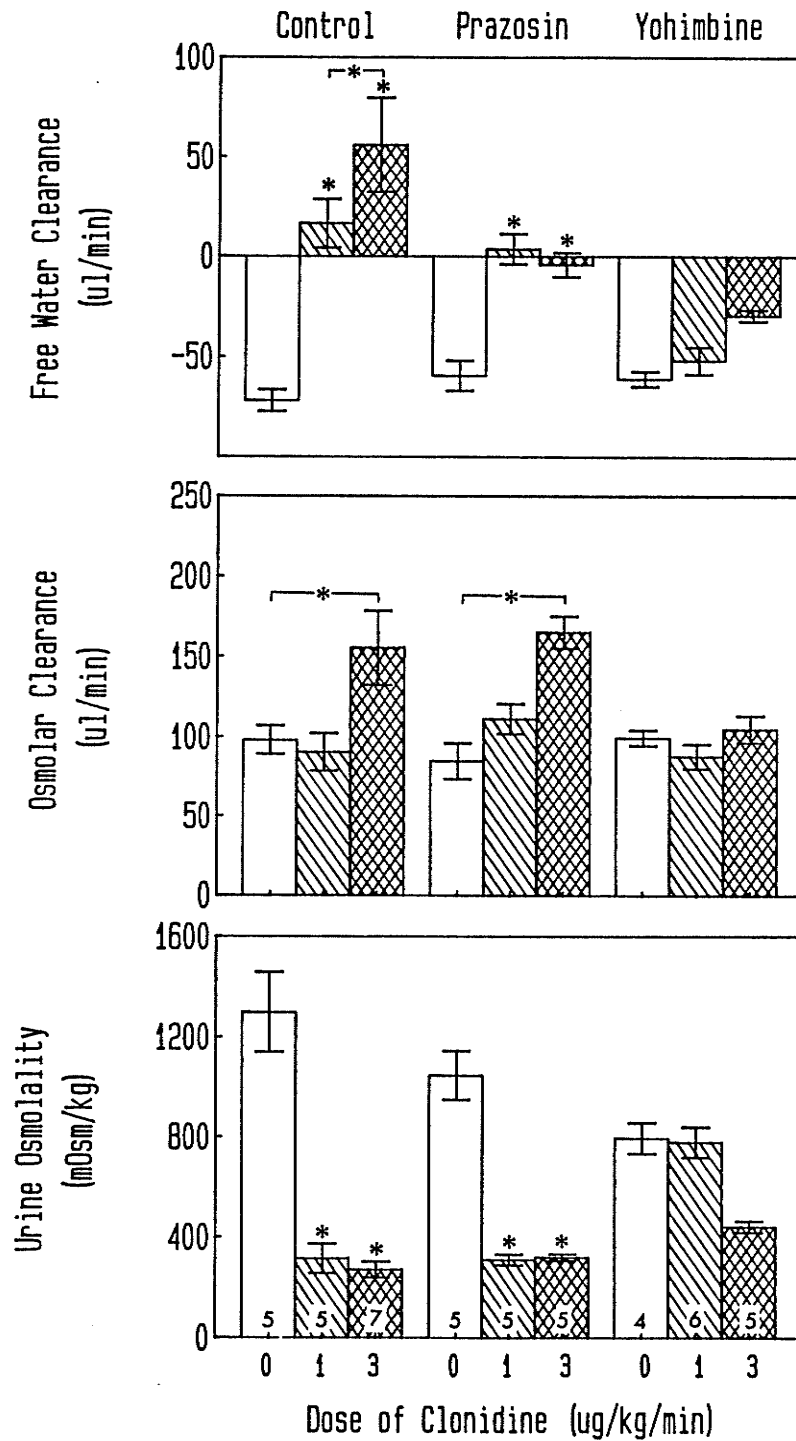
Fig. 3.11. Effect of an intrarenal infusion (3.4 ul/min) of clonidine with either yohimbine or prazosin pretreatment, on free water clearance, osmolar clearance and urine osmolality. Open bars represent control (vehicle); hatched bars represent clonidine, 1 ug/kg/min; cross-hatched bars represent clonidine, 3 ug/kg/min.
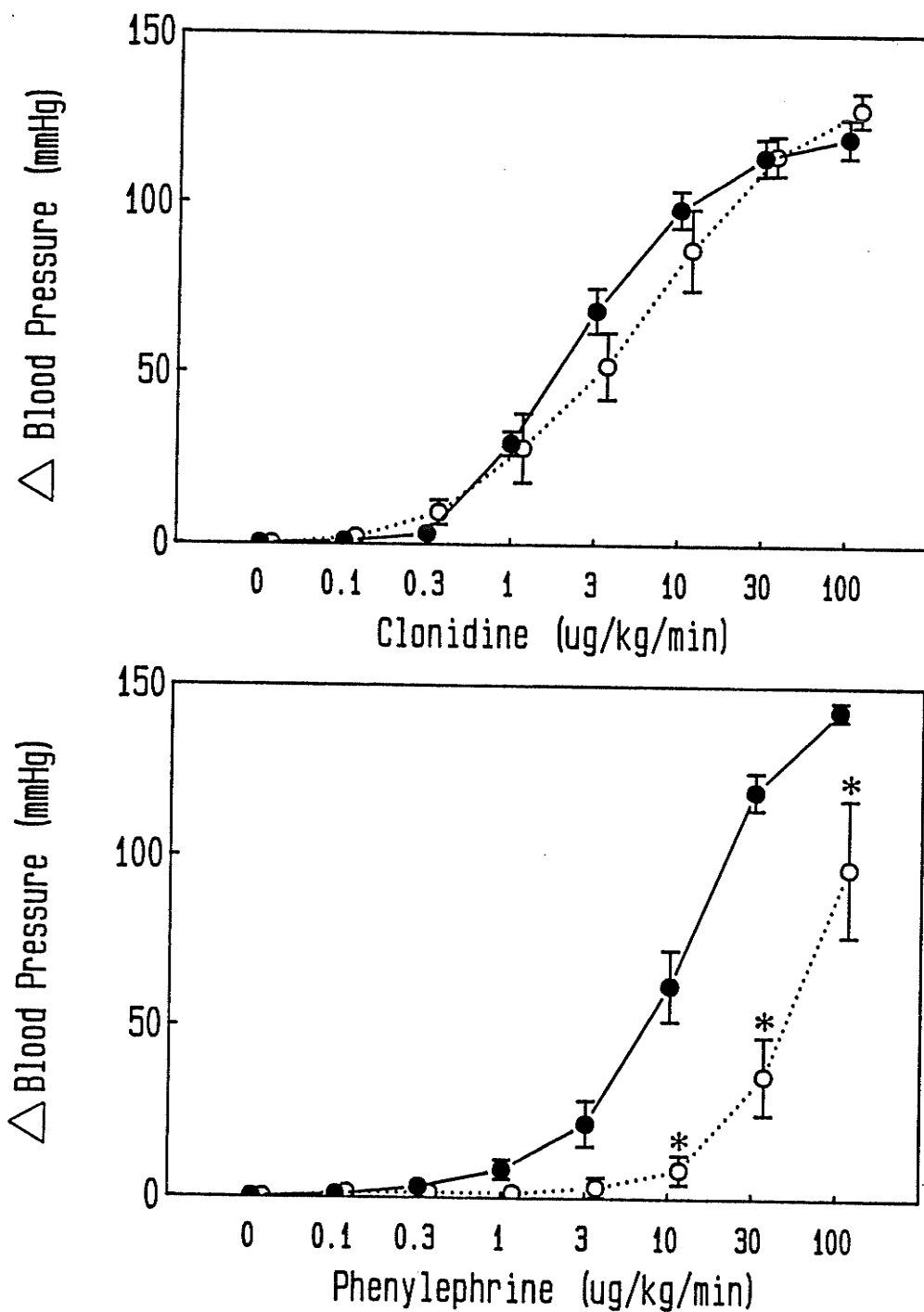
Fig. 3.12. Effect of 0.01 mg/kg bolus i.v. dose of prazosin on the pressor response to clonidine and phenylephrine in the pithed rat. (●) Control, n=6; (○) prazosin, n=4.

DISCUSSION

Previous studies have reported an enhanced renal response to clonidine when administered systemically as compared to an intrarenal infusion (Strandhoy *et al.*, 1982). Similarly, we have found a disparity between the pressor response to clonidine when infused into the jugular vein and infused directly into the renal artery. The intrarenal infusion produced only a pressor response, whereas the intravenous infusion produced an initial depressor response, which was not statistically significant, followed by a pressor response at higher infusion rates. An intrarenal infusion was used in an attempt to avoid systemic effects of the α2-adrenoceptor agonist which may have indirectly altered electrolyte excretion. The intrarenal infusion of clonidine at 0.3, 1 and 3 *ug/kg/min* produced a dose-related increase in urine volume. However, an increase in sodium and potassium excretion and an increase in osmolar clearance was only observed at the highest infusion rate investigated (3 *ug/kg/min*). Furthermore, clonidine produced a dose-related decrease in urine osmolality and an increase in free water clearance.

The mechanism by which clonidine mediated this dose-dependent effect on water and then solute excretion is not known. The clear dissociation of the effects of clonidine on sodium and water excretion, suggests two independent mechanisms may be involved as previously postulated (Strandhoy *et al.*, 1982). Yohimbine pretreatment (1 mg/kg), shown previously to block α2-adrenoceptors (van Meel *et al.*, 1981c), attenuated the renal response to clonidine. Pretreatment with an α1-adrenoceptor selective dose of prazosin (0.01 mg/kg) failed to attenuate these renal effects of clonidine. This suggests that the renal effects of clonidine, in this preparation, are mediated by α2-adrenoceptors.

The ability of α2-adrenoceptor stimulation to increase both the excretion of sodium and water has been well documented both *in vivo* (Gellai and Ruffolo, 1987;

Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982) and *in vitro* preparations (Krothapalli *et al.*, 1983; Krothapalli and Suki, 1984; Smyth *et al.*, 1984; 1985a). In contrast to the present study, these previous studies failed to report a dose-dependent dissociation of the effects of an α2-adrenoceptor agonist on water and sodium excretion. The apparent discrepancy may have occurred for two reasons; the high doses investigated in previous studies and/or the route of drug administration.

Studies utilizing only one or two doses of an α2-adrenoceptor agonist have observed a concomitant increase in sodium and water excretion (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). These studies administered doses which were pressor and/or produced a hypotonic urine. This same effect was observed in the present study only with the maximal infusion rate investigated. This was approximately 30 to 100 times greater than the dose which selectively increased urine volume but not sodium excretion. Moreover, the studies of Strandhoy *et al.* (1982) demonstrated that the intravenous infusion of an α2-adrenoceptor agonist produced a greater effect on sodium and water excretion than when the same dose was administered directly into the renal artery. These results suggested that the intravenous route of administration may have additional systemic and/or central effects. Thus, previous studies which investigated high intravenous doses of an α2-adrenoceptor agonist would have failed to observe the dose-related dissociation in water and sodium excretion.

The mechanism(s) by which clonidine selectively increases water and then sodium excretion is (are) unknown. Previous studies have proposed that the effects of α2-adrenoceptor agonists were mediated by the inhibition of the renal effects of vasopressin (Smyth *et al.*, 1985a; Strandhoy *et al.*, 1982). The effects of vasopressin on sodium and water excretion are variable depending on the preparation utilized. *In vitro* studies with the isolated perfused rat kidney (Lieberthal *et al.*, 1987; Smyth

et al., 1985a) and in isolated nephron segments (Krothapalli *et al.*, 1983; Reif *et al.*, 1986) have demonstrated a decrease in water and sodium excretion after the administration of vasopressin. In *in vivo* preparations, vasopressin has been shown to decrease water excretion but increase urinary sodium excretion (Balment *et al.*, 1986b; Fejes-Toth and Szenasi, 1981; Martinez-Maldonado *et al.*, 1971). The reason for the discrepancy between *in vivo* and *in vitro* preparations in relation to sodium excretion is unclear. It may be related to the secondary release of a factor in the *in vivo* situation, such as oxytocin (Balment *et al.*, 1986a) and/or prostaglandins (Lieberthal *et al.*, 1987). The dose-dependent effects of α2-adrenoceptor agonists may be due to a dose-dependent difference in antagonism of these other factors.

Clonidine was infused directly into the renal artery to avoid systemic effects. The decrease in heart rate observed at all infusion rates studied and the increase in blood pressure at the high infusion rate, indicate extrarenal effects may have been involved. At the high infusion rate, blood pressure increased approximately 25 mm Hg with an increase in urine flow rate to 211 $\pm$ 16 ul/min as compared to 26 $\pm$ 5 ul/min in the control group. Roman and Cowley (1985) have reported that a similar increase in blood pressure only increased urine flow from approximately 20 ul/min in the control group to 40 ul/min. Moreover, when renal perfusion pressure was maintained constant during infusions of clonidine (3 ug/kg/min) by the use of an aortic occluder above the renal artery, clonidine still increased urine flow and sodium excretion. The absolute increase in urine flow and sodium excretion in the blood pressure controlled group was slightly, but significantly less than that observed in the group whose renal perfusion pressure was not held constant. Thus, the contribution of the increased blood pressure to the results is minimal. Some, but certainly not all of the effects of clonidine seen in this study can be attributed to a pressure-diuresis and a pressure-natriuresis phenomenon. Similarly, the decreased heart rate observed in the present study indicated that clonidine may also have had

a central effect which would have decreased the level of activity of the sympathetic nervous system (Koepke and DiBona, 1986; Pettinger, 1975). This effect would decrease the level of activity of renal α1-adrenoceptors which mediate sodium and water retention (Smyth *et al.*, 1985b). This appears unlikely, inasmuch as a decrease in renal α1-adrenoceptor activity would not have also produced a decrease in urine osmolality and an increase in free water clearance as seen in the present study.

Prazosin pretreatment failed to alter the response to clonidine suggesting that α1-adrenoceptors were not involved. Moreover, the remaining hypertrophied kidney in chronically uninephrectomized rats functions as if "physiologically denervated" (Szenasi *et al.*, 1988), suggesting any central actions of clonidine would have had negligible effects on renal nerve activity. Alternatively, clonidine may inhibit the central release of vasopressin as suggested by Barr and Kauker (1979). A more recent study demonstrated that clonidine does not reduce plasma vasopressin in the rat (Leander *et al.*, 1985).

In addition to the central effects, it is possible that clonidine may have stimulated presynaptic α2-adrenoceptors. This would result in a decreased release of norepinephrine in the kidney (Jeffries *et al.*, 1987), causing a decrease in the level of activity of α1-adrenoceptors which mediate sodium and water retention in the rat (Smyth *et al.*, 1985b). As discussed above, this site of action would not be consistent with the observed decrease in urine osmolality and increase in free water clearance.

In summary, stimulation of renal α2-adrenoceptors with low infusion rates of clonidine produced an increase in water excretion. At higher infusion rates of clonidine, sodium and potassium excretion were also increased. The observed effect on free water clearance at lower infusion rates is consistent with the postulate that the renal effects of α2-adrenoceptor stimulation are mediated by the inhibition of

the renal effects of vasopressin. However, the increase in both sodium and water excretion at higher doses of clonidine may involve another mechanism.

4

Enhanced Natriuretic Potency of Intravenous Clonidine: Extrarenal Site of Action?

<u>Synopsis</u>:

Other laboratories have demonstrated that intravenous administration of clonidine resulted in a concomitant increase in water and sodium excretion. In contrast, in the previous section, low intrarenal infusion rates of clonidine were shown to selectively increase water excretion, whereas higher infusion rates were required to increase solute excretion. This suggested that the renal effects of $\alpha 2$-adrenoceptor stimulation may be mediated at two independent sites or by two independent mechanisms of action. To determine if one of these sites or mechanisms of action was an extrarenal one, the dose response curve for an intravenous infusion of clonidine on water and solute excretion was compared to that of an intrarenal infusion of clonidine. Uninephrectomized rats were anesthetized and the remaining kidney isolated for the collection of urine. Clonidine (0.1, 0.3, 1 or 3 ug/kg/min) or vehicle (saline) was administered either intravenously or intrarenally. Both intravenous and intrarenal administration of clonidine produced a dose selective dissociation of water and solute excretion, that is, at low infusion rates only urine volume was increased. Higher infusion rates were required to increase sodium excretion. In addition, intravenous administration of clonidine was more potent in producing a natriuresis, suggesting that the renal effects may be, in part, secondary to additional peripheral and/or central effects of this agonist following this route of administration.

INTRODUCTION

Previous studies have shown that stimulation of α2-adrenoceptors *in vivo* is associated with an increase in both sodium and water excretion (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). These effects have been attributed to either a direct antagonism of the renal effects of vasopressin, a decrease in the central release of vasopressin and/or other independent mechanisms unrelated to vasopressin (Baranowska *et al.*, 1987; Leander *et al.*, 1985).

Strandhoy *et al.* (1982) proposed that the effects of renal α2-adrenoceptor stimulation on sodium and water excretion appeared to be mediated by independent mechanisms. If the changes in sodium and water excretion following α2-adrenoceptor stimulation are indeed mediated by different mechanisms, then a dissociation in sensitivity of these mechanisms to α2-adrenoceptor agonists would be expected to occur. In the previous section, a dose selective dissociation of water and then sodium excretion following α2-adrenoceptor stimulation with clonidine infused directly into the renal artery was reported (Blandford and Smyth, 1988b - Section 3). A diuretic effect was observed at low infusion rates of clonidine (0.3 and 1 ug/kg/min), while a natriuretic effect was observed only at the maximal infusion rate tested (3 ug/kg/min). These results were consistent with the postulate that α2-adrenoceptor stimulation may be mediating the increase in water and sodium excretion at different sites in the kidney and/or by two independent mechanisms.

If the natriuretic effect of α2-adrenoceptors is mediated predominantly at renal sites, then one would anticipate that a direct intrarenal infusion would have greater potency. Conversely, if an extrarenal site is involved, then an intravenous infusion would be expected to be similar or have a more potent effect on sodium excretion than an intrarenal infusion. Therefore, in the present study, the renal

effects of intravenously infused clonidine were compared to the effects of clonidine infused directly into the renal artery.

METHODS

As previously described, (Section 2) male Sprague Dawley rats (200-225 g) were unilaterally nephrectomized (7 to 10 days prior to the experiment day) and surgically prepared to compare the difference between the renal response to intravenous and intrarenal infusions of the α2-adrenoceptor agonist, clonidine. The left jugular vein of the rats receiving intravenous infusions of clonidine was cannulated with two lines; (PE160) for the infusion of saline (97 ul/min) and (PE20) for the continuous infusion of vehicle or clonidine. For the rats receiving infusions directly into the renal artery, only one line was placed into the jugular vein (PE160) for the infusion of saline. For the direct intrarenal infusion of the clonidine, the left kidney was exposed by a flank incision and the ureter cannulated (PE50). A 31 gauge needle was advanced through the aorta into the renal artery and secured with glue. Finally, an electromagnetic flow probe was placed on the renal artery for the measurement of renal blood flow.

Following a 45 min stabilization period, urine was collected for 15 min in a pre-weighed tube. This was followed by the continuous infusion of vehicle (saline) or clonidine (0.1, 0.3, 1 or 3 ug/kg/min) at a rate of 3.4 ul/min into either the jugular vein or the renal artery. Only one infusion rate was investigated in each rat. Four consecutive 15 min urine samples were collected during this infusion. Urine volume was determined gravimetrically. A plasma sample was obtained at the end of the experiment.

Statistical analyses of the intravenous infusions versus the intrarenal infusions were performed with a two-way, repeated measures analysis of variance (route of administration x dose of clonidine). Significant interactions (i.e., where the dose of clonidine had different effects depending on the route of administration) were analyzed further with simple main effects analyses and Tukey HSD tests

(Winer, 1971). Data are presented as the mean $\pm$ S.E.M., and are taken from the last three collection periods (i.e., 15 to 60 min after the infusion of clonidine was begun). Each group represents 5-8 animals.

RESULTS

Both intrarenal and intravenous administration of clonidine produced a dose-related dissociation of water and solute excretion (fig. 4.1). Intrarenal infusion rates of 0.3 and 1 ug/kg/min significantly increased urine flow rate without an accompanying increase in sodium excretion. At the maximal infusion rate tested (3 ug/kg/min), both urine flow rate and sodium excretion were increased. Similarly, an intravenous infusion of 0.3 ug/kg/min clonidine significantly increased urine flow without a concomitant increase in sodium excretion. At higher intravenous infusion rates (1 and 3 ug/kg/min), clonidine increased both urine flow rate and sodium excretion. The effects on free water clearance and osmolar clearance are parallel to that of urine volume and sodium excretion respectively (fig. 4.2).

For the purpose of comparison, intravenous and intrarenal infusions of clonidine were analyzed by a two-way, repeated measures analysis of variance (route of administration x dose of clonidine). A significant interaction effect was noted for blood pressure. The intrarenal infusion of clonidine only increased blood pressure at the maximal infusion rate tested (fig. 4.3). Intravenous infusions significantly decreased blood pressure at 0.1 ug/kg/min and increased blood pressure at 1 and 3 ug/kg/min clonidine (fig. 4.3). Significant main effects (route of administration) were observed for both heart rate and creatinine clearance. Heart rate was decreased to a greater extent by clonidine administered intravenously as compared to an intrarenal administration (fig. 4.4). Creatinine clearance, also, was decreased when clonidine was administered intravenously as compared to an intrarenal administration (fig. 4.3). Renal blood flow was unaltered (data not shown).

A significant dose-related increase in urine flow rate was observed, with urine flow increased at 0.3, 1 and 3 ug/kg/min clonidine irrespective of the route of administration (fig. 4.4). At the 1 ug/kg/min infusion rate, the intravenous

administration of clonidine had a greater effect on urine flow rate as compared to the intrarenal route. Sodium excretion demonstrated a significant main effect (route of administration) and interaction effect. The intrarenal infusions of clonidine increased sodium excretion only at the maximal dose tested (3 ug/kg/min), while the intravenous infusion increased sodium excretion at both 1 and 3 ug/kg/min (fig. 4.4). Moreover, intravenous infusions of clonidine at 0.3, 1 and 3 ug/kg/min had a greater effect on sodium excretion than clonidine administered directly into the renal artery. The same trend was noted for osmolar clearance. Intrarenal clonidine only increased osmolar clearance at the maximal dose tested, while the intravenous infusion increased osmolar clearance at 1 and 3 ug/kg/min (fig. 4.5). Again, clonidine administered intravenously had a greater effect on osmolar clearance than intrarenally administered clonidine at 0.3, 1 and 3 ug/kg/min.

Free water clearance was increased at all doses tested, and this increase was observed irrespective of the route of administration (fig. 4.5). Urine osmolality was decreased at all doses, again, irrespective of the route of administration (fig. 4.5), and, potassium excretion was unaltered (fig. 4.4).
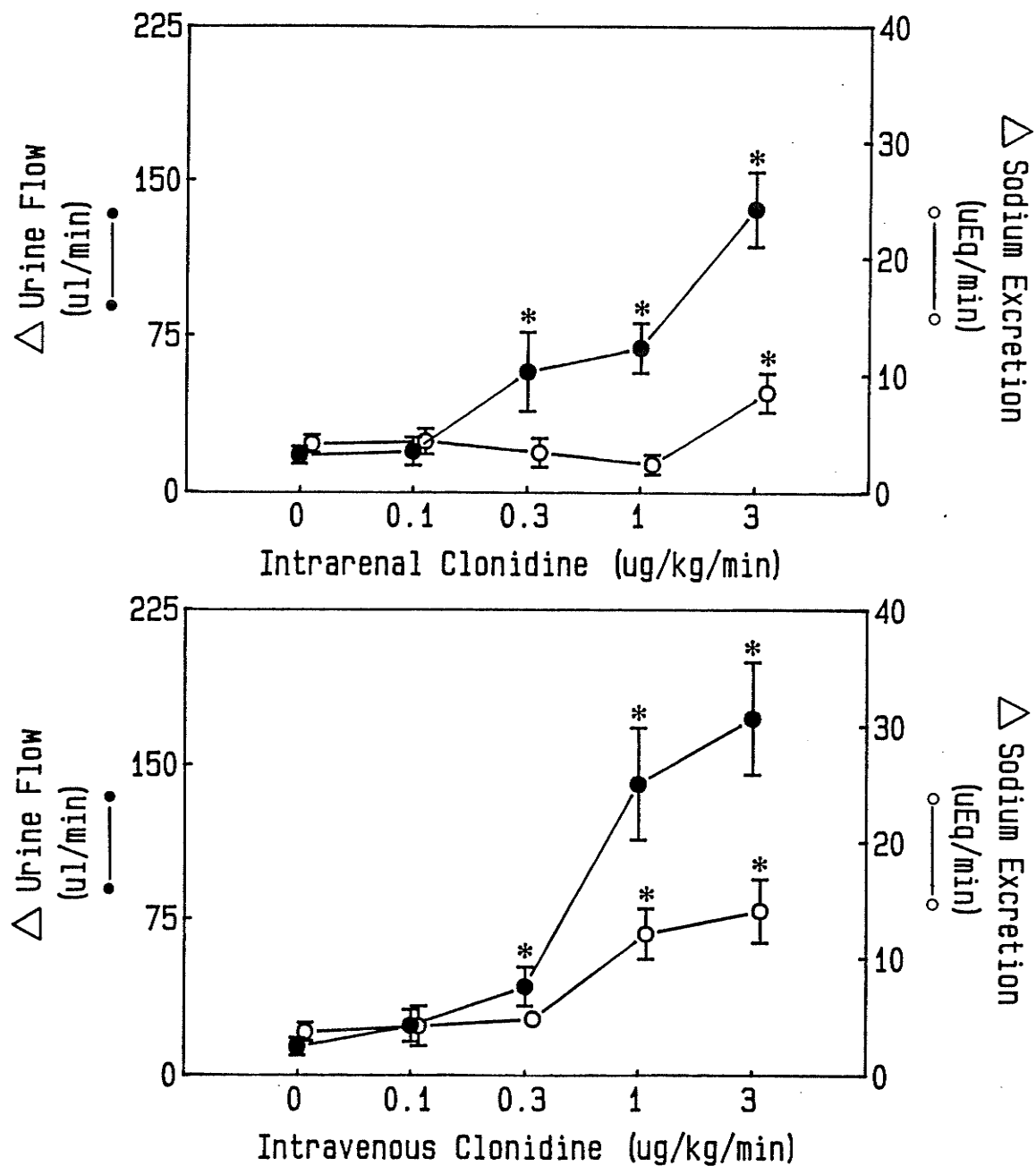
Fig. 4.1. Effect of an intrarenal (upper panel) and intravenous (lower panel) infusion (3.4 ul/min) of vehicle (saline) or increasing doses of clonidine on (●) urine flow rate and (○) sodium excretion. These data represent the changes observed from the first collection period (control) to the mean of the last three collection periods. ✱ p<0.05 for individual points vs. the control group. Each point represents 5 to 8 experiments.
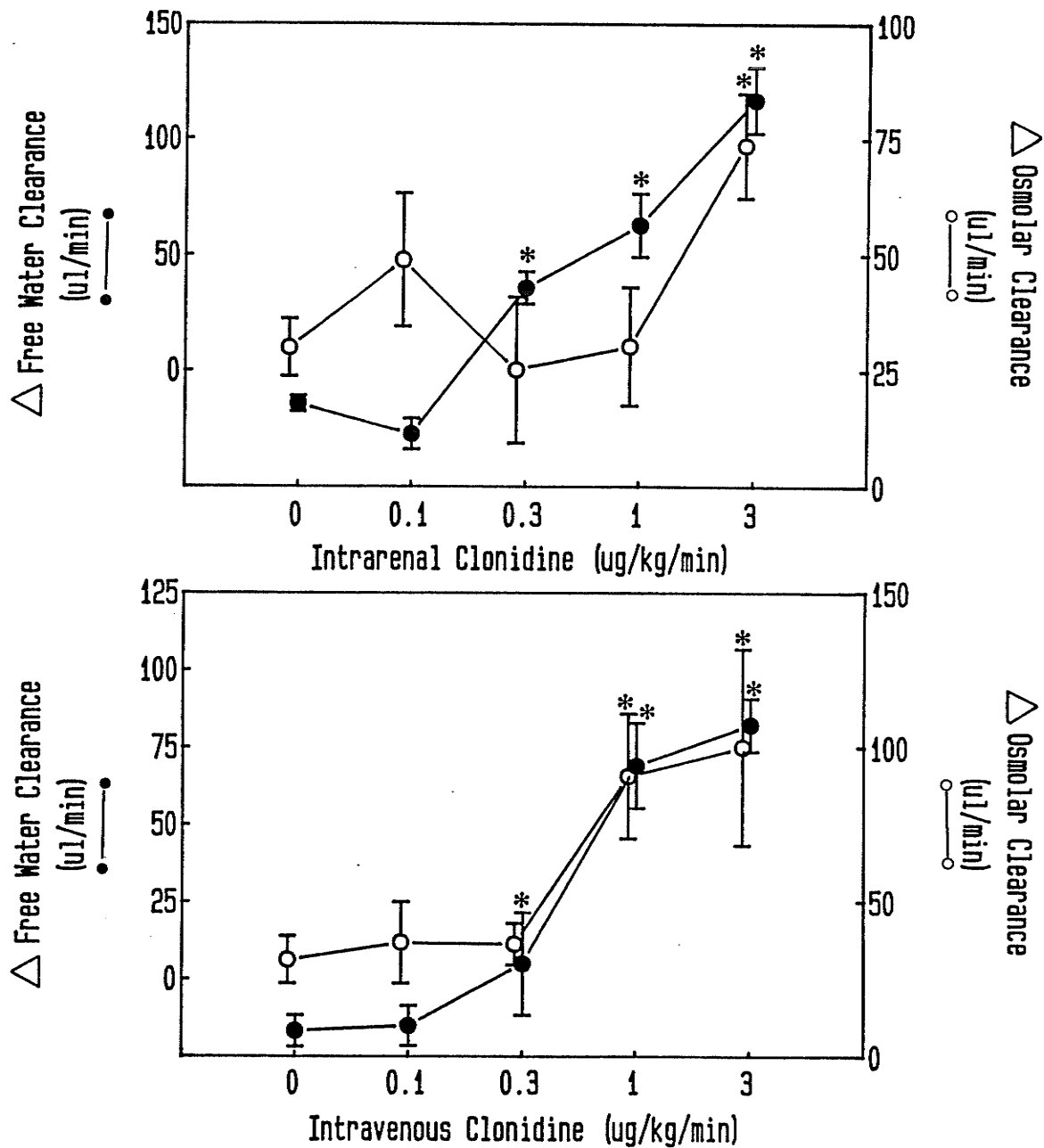
Fig. 4.2. Effect of an intrarenal (upper panel) and intravenous (lower panel) infusion (3.4 *ul*/min) of vehicle (saline) or increasing doses of clonidine on (●) free water clearance and (○) osmolar clearance. These date represent the changes observed from the first collection period (control) to the mean of the last three collection periods. ∗ p<0.05 for individual points vs. the control group. Each point represents 5 to 8 experiments.
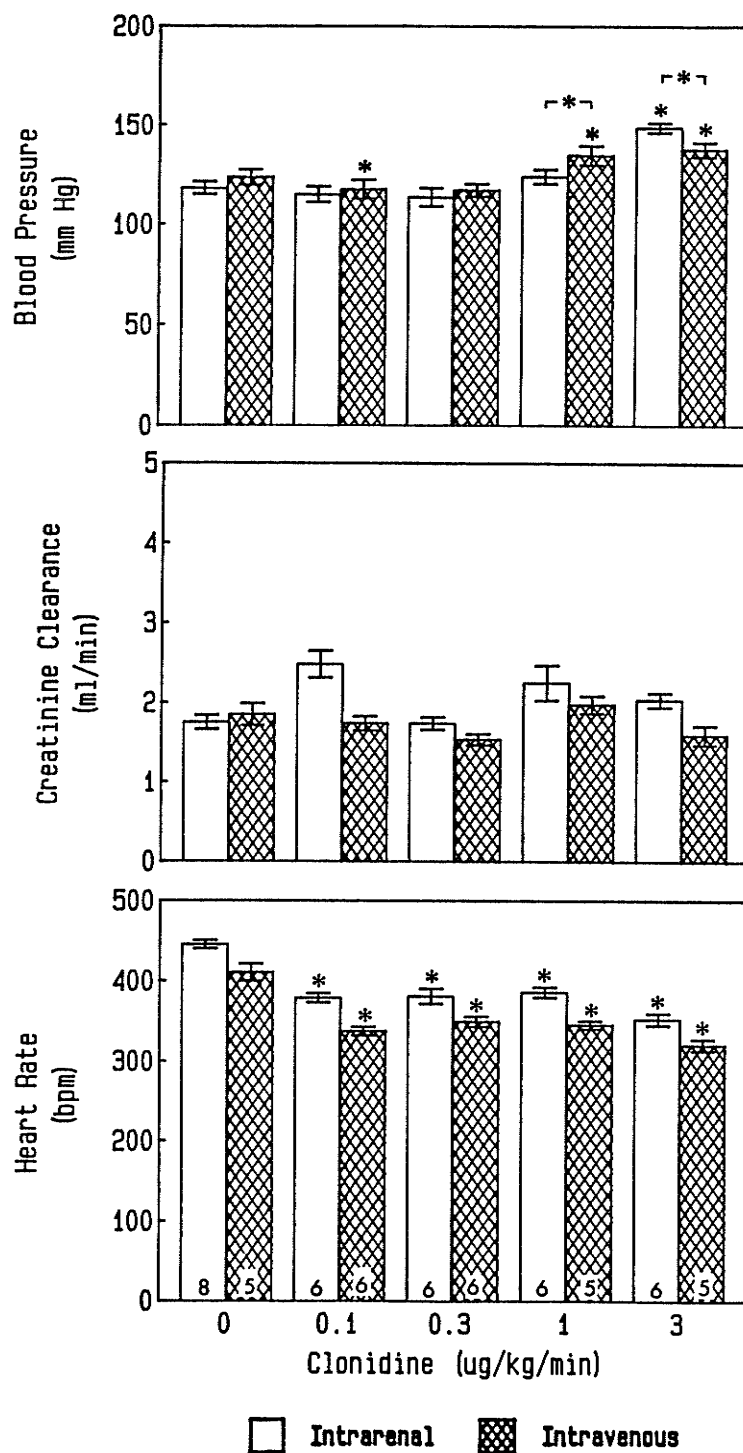
Fig. 4.3. Effect of clonidine administered by intrarenal (open bars) and intravenous (cross-hatched bars) infusions (3.4 ul/min) on blood pressure, creatinine clearance and heart rate. The number of animals studied at each infusion rate are presented in the bars on the bottom graph. * $p<0.05$ for individual points vs. the control group; ⌐*¬ $p<0.05$ for the intravenous infusion of clonidine vs. the intrarenal infusion of clonidine.
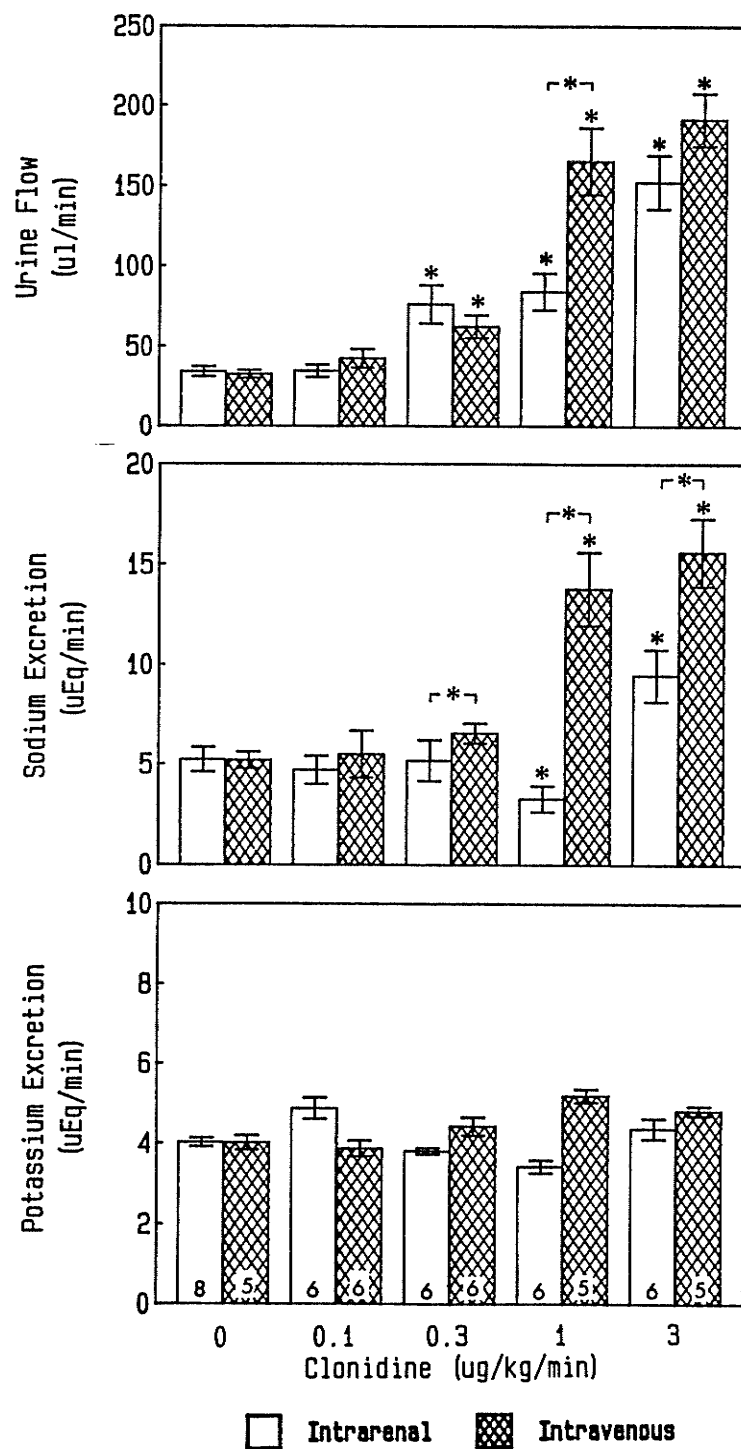
Fig. 4.4. Effect of clonidine administered by intrarenal (open bars) and intravenous (cross-hatched bars) infusions (3.4 ul/min) on urine flow, sodium excretion and potassium excretion. * p<0.05 for individual points vs. the control group; ⌐*¬ for the intravenous infusion of clonidine vs. the intrarenal infusion of clonidine.
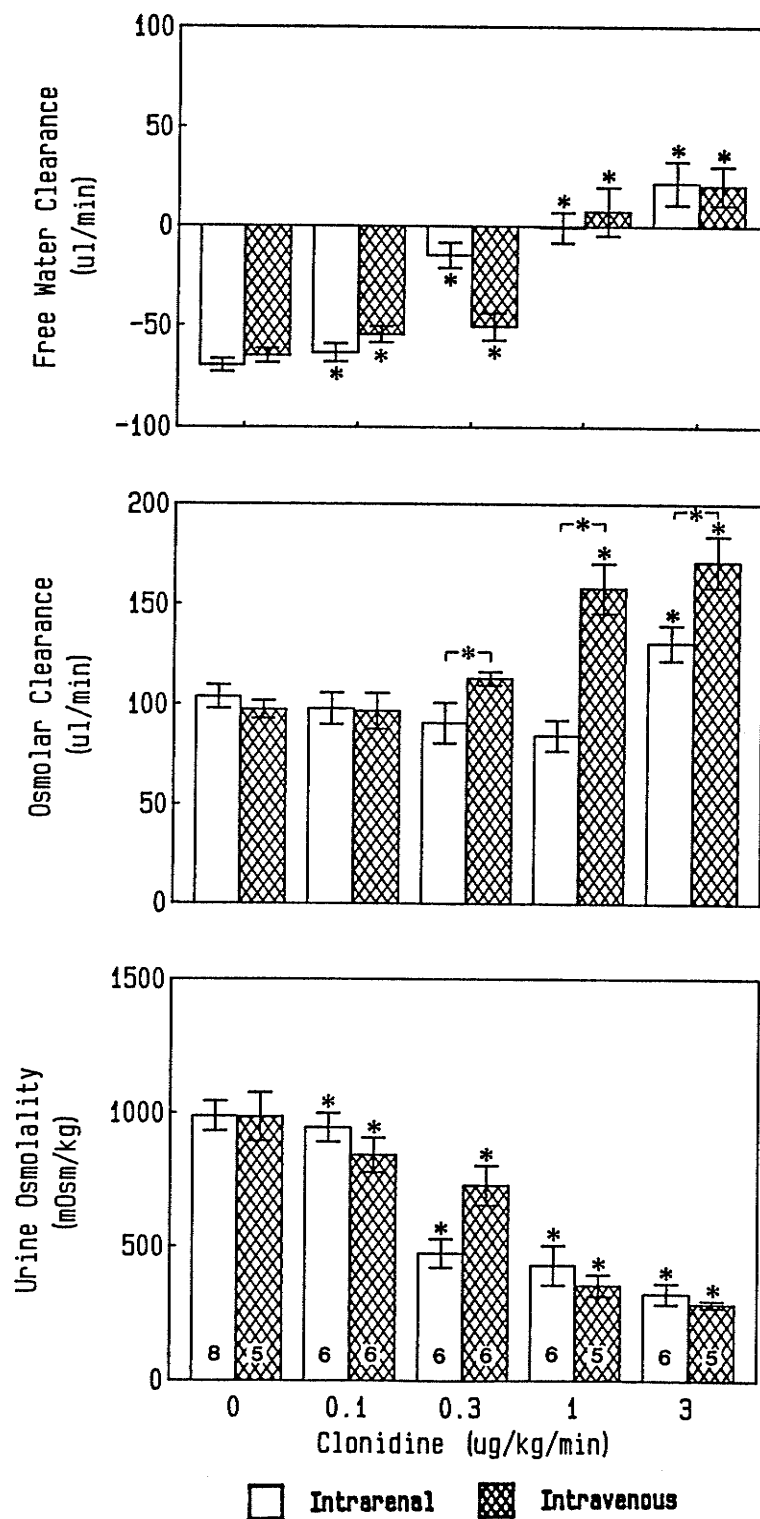
Fig. 4.5. Effect of clonidine administered by intrarenal (open bars) and intravenous (cross-hatched bars) infusions (3.4 ul/min) on free water clearance, osmolar clearance and urine osmolality. * p<0.05 for the individual points vs. the control group; ⌐*¬ p<0.05 for the intravenous infusion of clonidine vs. the intrarenal infusion of clonidine.

DISCUSSION

Previous studies have reported on the renal effects of α2-adrenoceptor agonists administered systemically (Barr and Kauker, 1979; Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). In most of these studies, only one or two doses of the α2-adrenoceptor agonists were utilized, and this dose was generally pressor and/or produced a hypotonic urine. All these studies, however, demonstrated a concomitant increase in water and sodium excretion following α2-adrenoceptor stimulation. This is in contrast to the results of the previous section which reported a clear dose-related dissociation of water and then solute excretion following α2-adrenoceptor agonist administration directly into the renal artery (Blandford and Smyth, 1988b - Section 3). This is consistent with the findings of Strandhoy *et al.*, (1982) who reported an enhanced diuretic and natriuretic response to a single dose of guanabenz infused intravenously as compared to a direct intrarenal arterial infusion. This suggested that sodium and water excretion following α2-adrenoceptor stimulation may involve an extrarenal site of action. The present study characterized the dose response to α2-adrenoceptor stimulation following an intravenous infusion of clonidine. This response was compared to that of an intrarenal infusion of clonidine. If an extrarenal site of action mediated the response to an intrarenal infusion of clonidine, then one would anticipate a similar or an enhanced response following intravenous infusion. Conversely, if the effects were mediated only at renal sites, then an intravenous infusion would have a similar or a lower potency than an intrarenal infusion.

In the present study, an intrarenal infusion of clonidine was compared to an intravenous infusion of clonidine. An intrarenal infusion of clonidine produced a dose-related dissociation of water and solute excretion which was consistent with

that reported in the previous section (Blandford and Smyth, 1988b - Section 3). Moreover, an intravenous infusion of clonidine also produced a similar, but weaker dose-related dissociation. The same infusion rates of clonidine, whether administered by an intravenous or intrarenal route, increased urine volume and water excretion. However, one intravenous infusion rate (1 $ug/kg/min$) significantly increased urine flow as compared to the same rate of an intrarenal arterial infusion of clonidine. Sodium excretion and osmolar clearance differed between the two routes of administration in that lower infusion rates increased sodium excretion when given by the intravenous route but not when given by the intrarenal route. While the intrarenal infusions increased sodium excretion only at the maximal infusion rate tested, the intravenous infusions had a greater potency, increasing sodium excretion at both 1 and 3 $ug/kg/min$. This suggests that independent mechanisms may be mediating sodium and water excretion following the infusion of an α2-adrenoceptor agonist.

These results are in contrast to those previously observed, where infusion of α2-adrenoceptor agonists produced a concomitant increase in both urine volume and sodium excretion (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). The reason for this discrepancy is unclear. It may be related to the fact that α2-adrenoceptor agonists in many of these studies were administered in only one or two doses which were pressor or produced a hypotonic urine. As such, the threshold dose for the selective increase in urine volume may have been missed.

The mechanisms by which clonidine selectively increased water and then sodium excretion, and has an apparent greater potency when given intravenously, remains unclear. Vasopressin increases cAMP production in the medullary and cortical segments of the thick ascending limb of Henle and in the collecting tubules (Umemura *et al.*, 1985). In the collecting tubules, vasopressin increases the

permeability to water (Krothapalli *et al.*, 1983). In the thick ascending limb vasopressin increases sodium reabsorption (Hebert *et al.*, 1984). However, α2-adrenoceptor stimulation has only been associated with an inhibition of vasopressin-induced cAMP formation in the cortical collecting tubule (Umemura *et al.*, 1985a). Moreover, α2-adrenoceptor stimulation has also been associated with a decrease in water permeability in this segment (Krothapalli and Suki, 1984). Consequently, a low intrarenal infusion rate of clonidine would conceivably antagonize the effects of vasopressin in this segment and decrease water permeability (Krothapalli and Suki, 1984) thereby selectively increasing water excretion. Vasopressin has been reported to increase sodium reabsorption in a number of nephron segments (Elalouf *et al.*, 1984; Hebert *et al.*, 1984; Reif *et al.*, 1986). Higher intrarenal infusion rates of clonidine may have decreased the central release of vasopressin. This has been previously reported for the α2-adrenoceptor agonist, clonidine (Barr and Kauker, 1979; Roman *et al.*, 1979). This in turn would have decreased the antinatriuretic effect of vasopressin in the kidney and consequently increased sodium excretion. On the other hand, the intravenous infusion of clonidine may have functioned predominantly to decrease the central release of vasopressin. This would have decreased both the antidiuretic (cortical collecting tubule) and antinatriuretic (thick ascending limb) effects of vasopressin.

One can also consider the postulate that the systemic levels of clonidine were lower during intrarenal infusions as compared to intravenous infusions, since it is likely that a proportion of the intrarenally infused clonidine was ultrafiltered and eliminated. As such, it is possible that the differences may be of a pharmacokinetic, rather than a pharmacodynamic nature. However, since plasma vasopressin and circulating clonidine levels were not measured in the present study, this contention remains speculative. It is interesting to note, however, that the dose of clonidine which has been previously shown to inhibit vasopressin release (Roman *et al.*, 1979)

is similar to the maximal infusion rate used in the present study. On the other hand, in a more recent study, another α2-adrenoceptor agonist, BHT-933 was found to have had no effect on plasma vasopressin levels (Gellai and Edwards, 1988).

The natriuretic effect of clonidine may have been mediated by a number of other extrarenal factors independent of vasopressin. Firstly, Baranowska *et al.*, (1987) demonstrated that clonidine, in a bolus dose of 50 *ug/kg*, significantly increased the plasma levels of atrial natriuretic peptide. Thus, low doses of clonidine administered directly into the renal artery may result in an antagonism of the renal effects of vasopressin, producing a diuresis. High doses may stimulate the release of atrial natriuretic peptide resulting in both an increase in urine volume and sodium excretion. This would also be consistent with the increased natriuretic potency of clonidine following an intravenous infusion as compared to an intrarenal infusion. Secondly, Balment *et al.*, (1986) demonstrated that the natriuretic response to vasopressin *in vivo* is potentiated by concurrent administration of oxytocin. While the role of oxytocin in this preparation is unknown, it is conceivable that clonidine may alter the release of the neurohypophysial hormones such that the oxytocin to vasopressin ratio is altered. If the net effect is a relative increase in oxytocin levels, this may result in a synergistic action between oxytocin and vasopressin to enhance sodium excretion. This synergistic effect has been demonstrated in the rat (Balment *et al.*, 1986a; Conrad *et al.*, 1986; Edwards and LaRochelle, 1984).

Intravenous clonidine decreased heart rate at all infusion rates studied and further decreased heart rate at the maximal infusion rate tested (3 *ug/kg/min*). Blood pressure was decreased at the low infusion rates and increased at the high infusion rates. These results suggest both a central and systemic effect of clonidine. A central effect of clonidine could result in a decrease in the level of activity of the sympathetic nervous system (Koepke and DiBona, 1986), whereas a systemic effect

could result in stimulation of presynaptic α2-adrenoceptors in the kidney (Jeffries et al., 1987). Both of these effects would decrease the release of norepinephrine in the kidney. This would subsequently decrease the level of activation of renal α1-adrenoceptors resulting in an increase in sodium and water excretion (Smyth *et al.*, 1985). While plausible, this appears unlikely since a decrease in renal α1-adrenoceptor activity is not consistent with a decrease in urine osmolality and an increase in free water clearance as observed in the present study. In addition, the remaining hypertrophied kidney in chronically uninephrectomized rats functions as if physiologically denervated (Szenasi *et al.*, 1988) and consequently any central actions of clonidine would have had negligible effects on renal nerve activity. The greater decrease in heart rate observed at the maximal infusion rate tested may be secondary to the increased blood pressure. However, the contribution of the increased blood pressure (15 mm Hg in this particular study) to the observed renal effects would also be expected to be negligible (Roman and Cowley, 1985; Section 3).

In summary, the comparison of α2-adrenoceptor stimulation following either intravenous or intrarenal infusions of clonidine produced a dose-related dissociation of water and solute excretion. This is in contrast to the concomitant increase in water and solute excretion following α2-adrenoceptor stimulation by clonidine and the other α2-adrenoceptor agonists administered intravenously as previously described (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). Further, the intravenous infusions appear to be more potent for the excretion of sodium. These results are consistent with the postulate that water excretion may be mediated by direct renal effects (antagonism of vasopressin), whereas solute excretion may be mediated by other extrarenal factors. Moreover, these studies indicate that the investigation of the physiological role of renal α2-adrenoceptors by an intravenous infusion of agonists may be inappropriate.

5

Clonidine-Induced Diuresis and Natriuresis Does Not Involve Atrial Natriuretic Peptide Release

The data in this section have been presented at the Federation of American Societies for Experimental Biology meetings, Las Vegas, 1988 (FASEB J. 2(4):A793, 1988).

Synopsis:

In previous sections (Sections 3 & 4) low doses of intrarenal clonidine increased only the excretion of water. Higher doses also increased sodium and potassium excretion. It was postulated that low doses of clonidine antagonized the renal effects of vasopressin, while higher doses may release atrial natriuretic peptide (ANP). In this series of experiments, the effects of an intrarenal infusion of clonidine on renal function and on plasma ANP levels were determined. Unilaterally nephrectomized rats were anesthetized, and the carotid artery and jugular vein cannulated for the recording of blood pressure and the infusion of saline (97 ul/min) respectively. The left kidney was exposed, and the ureter cannulated for the collection of urine. A 31-gauge needle was advanced into the renal artery for the infusion of vehicle (saline) or clonidine (1, 3 or 10 ug/kg/min) at 3.4 ul/min. Plasma samples were taken both before and during the infusion of clonidine, for the measurement of plasma ANP levels (radioreceptor assay, Amersham). Low doses of clonidine (1 ug/kg/min) increased only urine volume and free water clearance. Higher doses of clonidine (3 and 10 ug/kg/min) increased both sodium and water excretion and osmolar clearance. Although ANP levels appeared to be slightly elevated at the higher infusion rates, no relationship was found between sodium excretion and ANP levels. These results indicate that, at the doses tested, ANP is not involved in the clonidine-induced diuresis and natriuresis.

INTRODUCTION

Renal α2-adrenoceptor stimulation has been shown to increase the excretion of both sodium and water (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.*, 1987; Strandhoy *et al.*, 1982). This has been attributed to a direct antagonism of the renal effects of vasopressin (Krothapalli *et al.*, 1984; Smyth *et al.*, 1985a). Alternatively, α2-adrenoceptor agonists may function to decrease the endogenous release of vasopressin (Barr and Kauker, 1979) or to both decrease the central release as well as antagonize the renal effects of vasopressin (Strandhoy *et al.*, 1982). In the Brattleboro rat which lacks endogenous vasopressin, infusion of α2-adrenoceptor antagonists resulted in an antinatriuresis and an antidiuresis (Farjam and Greven, 1989), suggesting that the effects of α2-adrenoceptor stimulation may not be directly linked to vasopressin. In this regard clonidine, an α2-adrenoceptor agonist has been shown to increase sodium and water excretion (Miller, 1980), as well as to increase the plasma levels of atrial natriuretic peptide (ANP) (Baranowska, 1987). This increased level of ANP may account for the diuresis and natriuresis. Thus, a number of alternate explanations exist for the natriuretic action of α2-adrenoceptor agonists.

A previous section documented a dose-related dissociation in the actions of clonidine (Blandford and Smyth, 1988b - Section 3). It was postulated that two independent mechanisms or sites of action may be involved. The selective increase in water excretion at low infusion rates would be consistent with either an antagonism of the renal effects of vasopressin or a decrease in the central release of vasopressin. The increase in both sodium and water excretion at the high infusion rates, however, may involve another mediator. In the present study we postulated that the natriuretic effect of a high infusion rate of clonidine may be secondary to an increase in the release of ANP. Therefore plasma ANP levels were measured in the

presence of three levels of clonidine infusion; a lower infusion rate which increased only water excretion and two high infusion rates which would be expected to be associated with increased solute excretion.

METHODS

1. *In Vivo* Renal Function

Male Sprague Dawley rats, weighing 200-225 g, were unilaterally nephrectomized (right kidney; ether anesthesia) 7 to 10 days prior to the experiment. On the day of the experiment, the rats were anesthetized with pentobarbital (Nembutal; 50 mg/kg i.p.) and surgically prepared as described previously (Section 2). Following surgery and a 45 min stabilization period, a 15 min control urine sample was collected, and a 0.5 ml blood sample was taken. This was followed by the continuous infusion of clonidine (1, 3 or 10 ug/kg/min) at a rate of 3.4 ul/min. During these infusions, three consecutive 15 min urine samples were collected into pre-weighed tubes. Thus the experimental urine collection periods were 60-75, 75-90 and 90-105 min after the start of stabilization. Two additional 0.5 ml plasma samples were taken; one at the end of the second urine collection period (after the start of the clonidine infusion), and one at the end of the experiment.

Plasma samples used in the measurement of plasma ANP levels were placed into tubes containing 1 mg EDTA/ml of blood and 100 ul (500 KIU/ml whole blood) protease inhibitor (Aprotinin)/ml of blood. Blood samples were spun at 4°C at 2000 x g for 30 min, and immediately thereafter separated and stored at -15°C. ANP levels were then determined with a radioreceptor assay (Amersham).

2. Atrial Natriuretic Peptide Radioreceptor Assay

The plasma sample was purified by direct Sep-pak chromatography using the following procedure. The Sep-pak C18 cartridge was pre-washed with 10 ml ethanol:water:acetic acid (90:6:4) and 10 ml 4% acetic acid at a flow rate of less than 5 ml/min. The plasma sample was applied to the cartridge, washed with 5 ml

4% acetic acid and eluted with 3 ml of the ethanol:water:acetic acid solution. The sample was then dried.

Radioreceptor assays were performed by incubating 200 ul of tracer (30 kBq, 0.8 uCi[3-[^{125}I]iodotyrosyl^{28}]αANP), 100 ul of plasma sample and 200 ul of receptor (prepared from bovine adrenal glands) to yield a final volume of 500 ul. All experiments were performed in duplicate. The mixture was incubated for 90 min at 25°C. The receptor bound fraction was then separated by 30 min of centrifugation at >2000 x g at 4 ^{O}C. The supernatant was discarded, the tubes drained for 5 min and counted for 60 sec in a gamma scintillation counter.

Statistical analyses for both the renal function studies and the radioreceptor assay were performed with an analysis of variance. Significant effects were further analyzed with Tukey HSD tests. Data are expressed as the mean $\pm$ S.E.M.

RESULTS

1. Renal function

Blood pressure and creatinine clearance was monitored at 15 min intervals throughout the experiment. There was no difference in either blood pressure or creatinine clearance between the groups during the control urine collection (fig. 5.1). However at both 3 and 10 ug/kg/min infusions of clonidine, blood pressure and creatinine clearance were significantly increased from the respective controls during the second and third collection period (fig. 5.1). Heart rate was decreased (fig. 5.1) in all groups immediately after the start of clonidine infusion irrespective of the infusions rate utilized. Moreover, there was no difference between the three infusion rates of clonidine at any given time period.

Electrolyte and water excretion during the control collection period (45-60 min) was not different between groups (fig. 5.2). Following this control urine collection, the intrarenal infusions of clonidine (1, 3 or 10 ug/kg/min) were begun. Intrarenal infusions of clonidine produced a dose-related increase in urine volume and sodium excretion that was most evident during the first and second collection period after the infusion of clonidine was begun (i.e., 60-75 and 75-90 min) (fig. 5.2). Moreover, clonidine infusions of 3 and 10 ug/kg/min increased urine flow rate and sodium excretion as compared to the 1 ug/kg/min infusion of clonidine. By the third collection period, however, this difference between the doses of clonidine was not significant. Potassium excretion was unaltered by 1 ug/kg/min infusion of clonidine, increased by 3 ug/kg/min infusion of clonidine during the second and third collection periods and increased by 10 ug/kg/min infusion of clonidine during the third collection period, each compared to its respective control collection. However, there was no significant difference between the groups at any given time period (fig. 5.2).

Free water clearance was increased in a dose-related manner (fig. 5.3). While there was no difference between groups during the control collection, free water clearance was increased by 3 ug/kg/min infusion of clonidine as compared to 1 ug/kg/min. Similarly, 10 ug/kg/min clonidine increased free water clearance as compared to 3 ug/kg/min infusions of clonidine. Osmolar clearance was unaltered by the 1 ug/kg/min infusion of clonidine, increased by the 3 ug/kg/min infusion of clonidine during the third and fourth collection periods, and further increased by the 10 ug/kg/min infusion rate of clonidine during all experimental collection periods, each compared to its respective control collection (fig. 5.3). Again, there was no difference between the groups at any given time period. Urine osmolality was significantly decreased with clonidine irrespective of the infusion rate. Moreover, there was no difference between the groups at any time period.

2. Plasma ANP levels

Plasma ANP levels were measured after the control period, prior to the infusion of clonidine, as well as 30 min after the infusion of clonidine was begun. Overall, clonidine increased plasma levels of ANP as compared to pre-clonidine ANP levels, irrespective of the infusion rate of clonidine used (fig. 5.4). There was however, no difference between the groups, and thus this increase may simply reflect a time effect. Moreover the ANP values are well within the normal range reported for rat plasma (20-550 pmol/l). To determine whether there was any association between the natriuretic effect of clonidine and plasma ANP levels, the data was plotted on a scattergram (fig. 5.5), and a regression analysis was performed. The correlation coefficient was determined to be 0.49 suggesting that there is no correlation between the natriuretic effect of clonidine and plasma ANP levels.
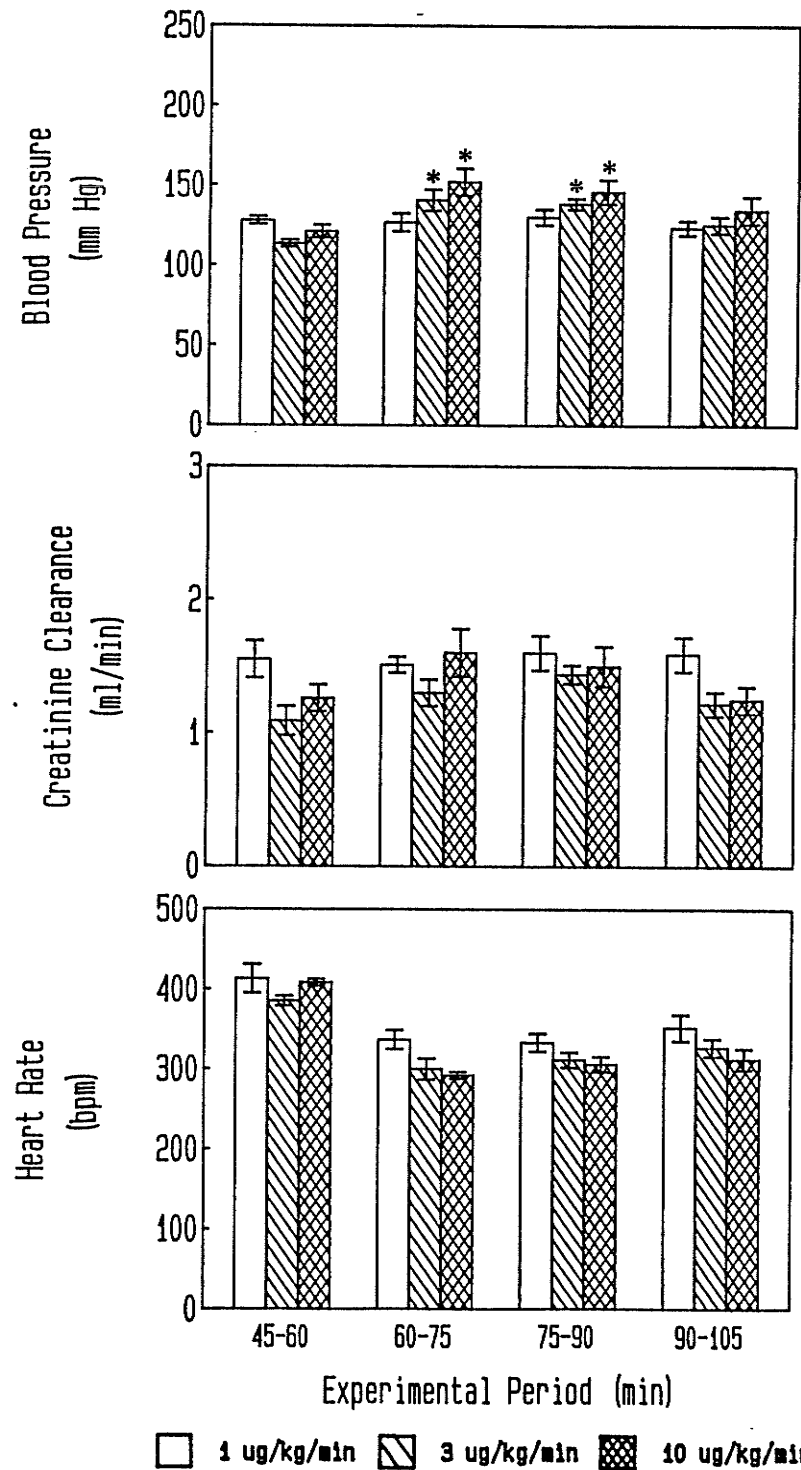
Fig. 5.1. Effect of intrarenal infusions of clonidine on blood pressure, creatinine clearance and heart rate. The open bars represent 1 ug/kg/min clonidine, n=6; the hatched bars represent 3 ug/kg/min clonidine, n=6, and the cross-hatched bars represent 10 ug/kg/min clonidine, n=5. These data represent the mean ± S.E.M. of the 2nd collection period.
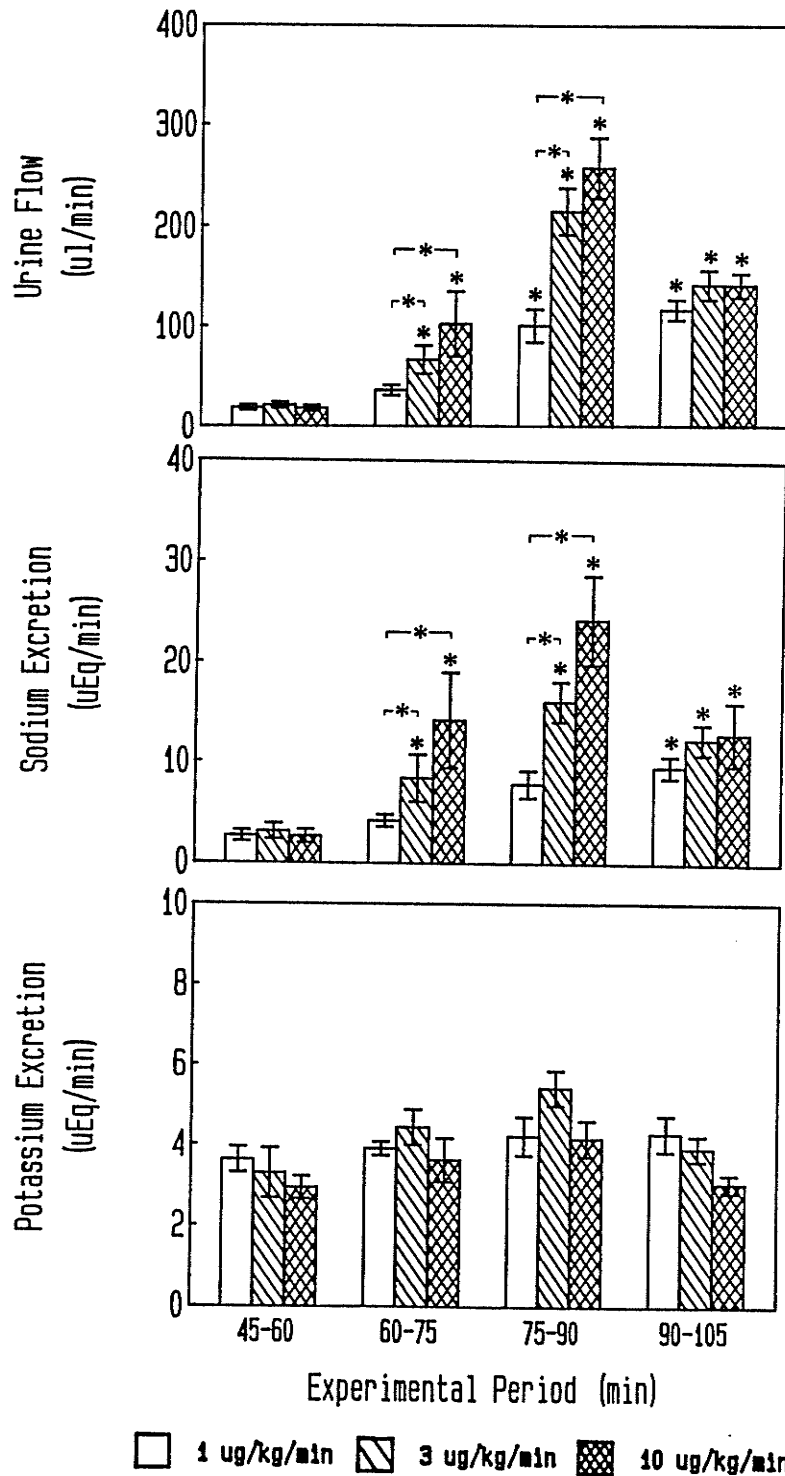
Fig. 5.2. Effect of intrarenal infusions of clonidine on urine flow rate, sodium excretion and potassium excretion. Open bars represent 1 ug/kg/min clonidine, n=6; hatched bars represent 3 ug/kg/min clonidine, n=6; and cross-hatched bars represent 10 ug/kg/min clonidine, n=5. * p<0.05 for individual point vs. its respective control (45-60 min).
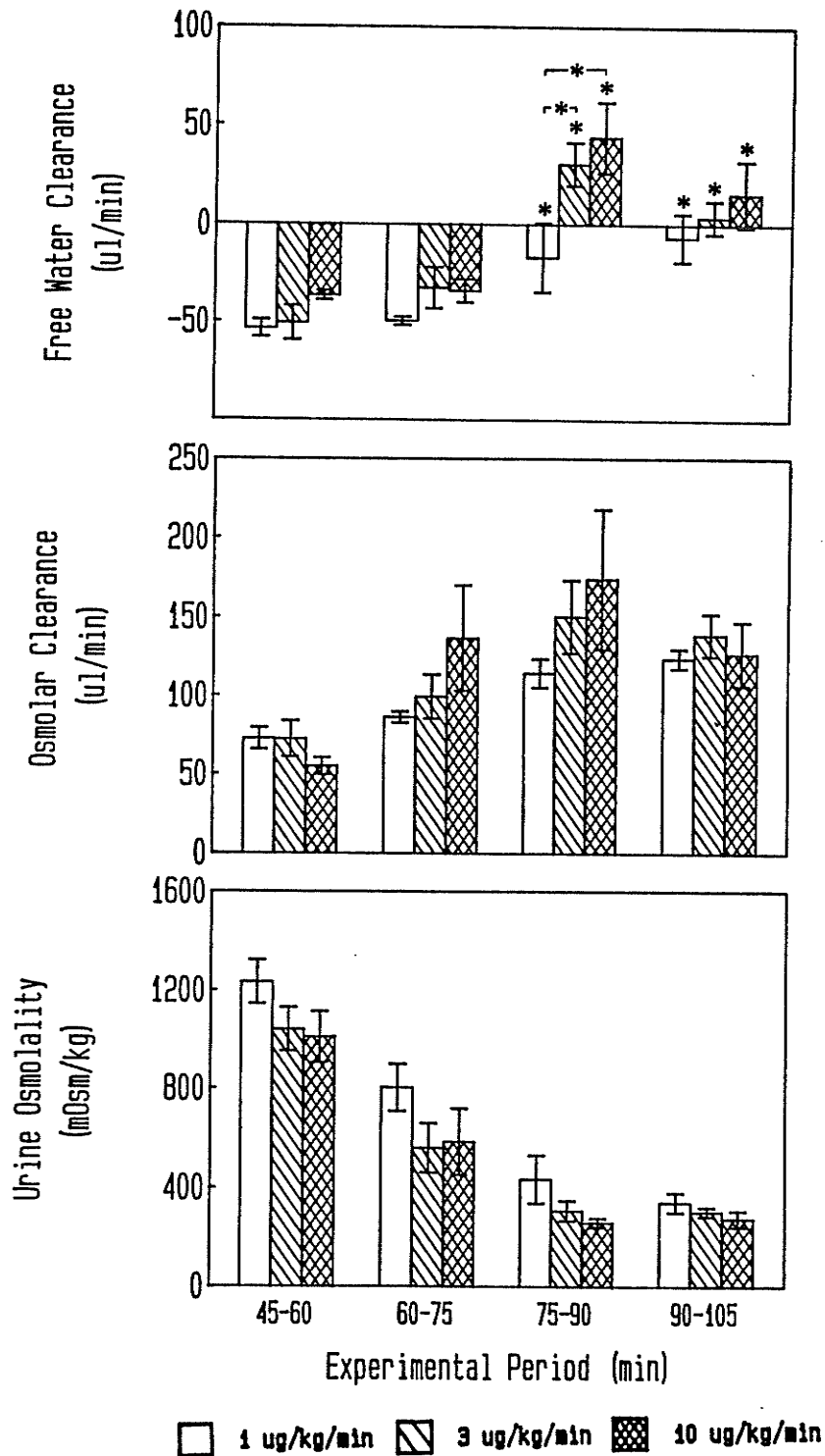
Fig. 5.3. Effect of intrarenal infusions of clonidine on free water clearance, osmolar clearance and urine osmolality. Open bars represent 1 ug/kg clonidine, n=6; hatched bars represent 3 ug/kg/min clonidine, n=6; and cross-hatched bars represent 10 ug/kg/min clonidine, n=5. * p<0.05 for individual point vs. its respective control (45-60 min).
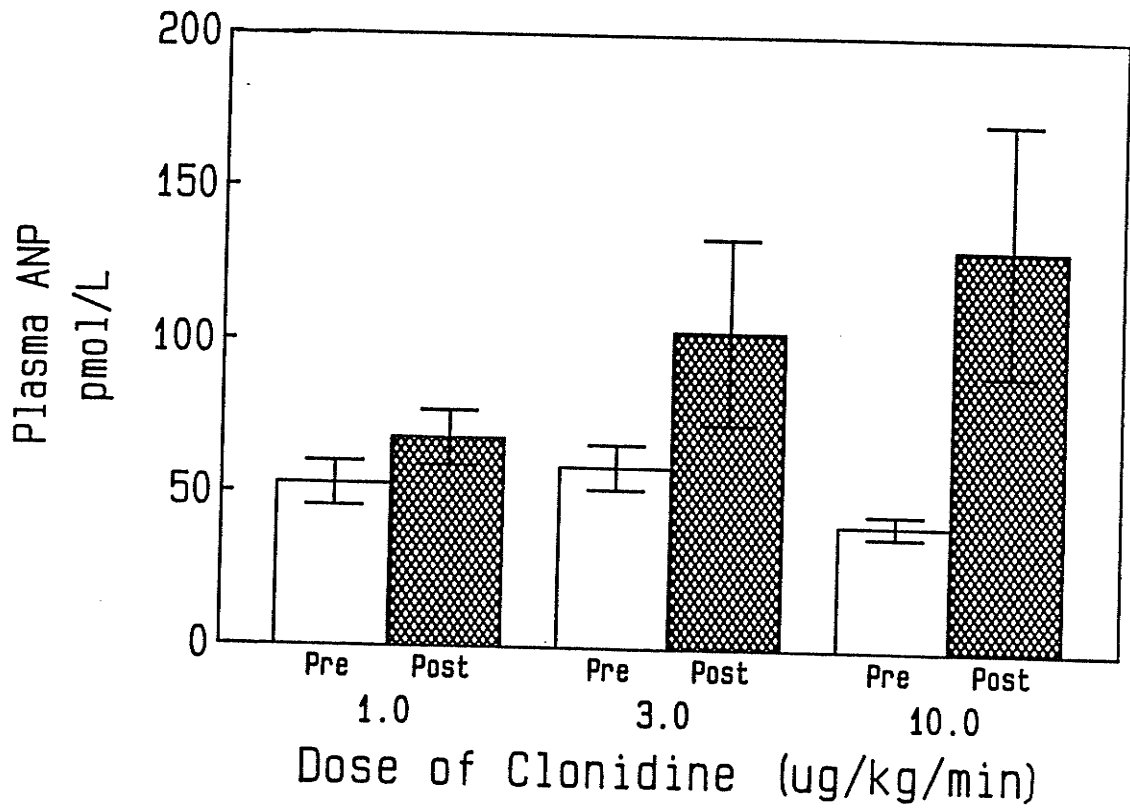
Fig. 5.4. Effect of clonidine (1, 3, or 10 ug/kg/min) on plasma levels of ANP. Open bars represent ANP levels before the infusion of clonidine was begun, while the cross-hatched bars represent ANP levels 30 min after the infusion of clonidine was begun.
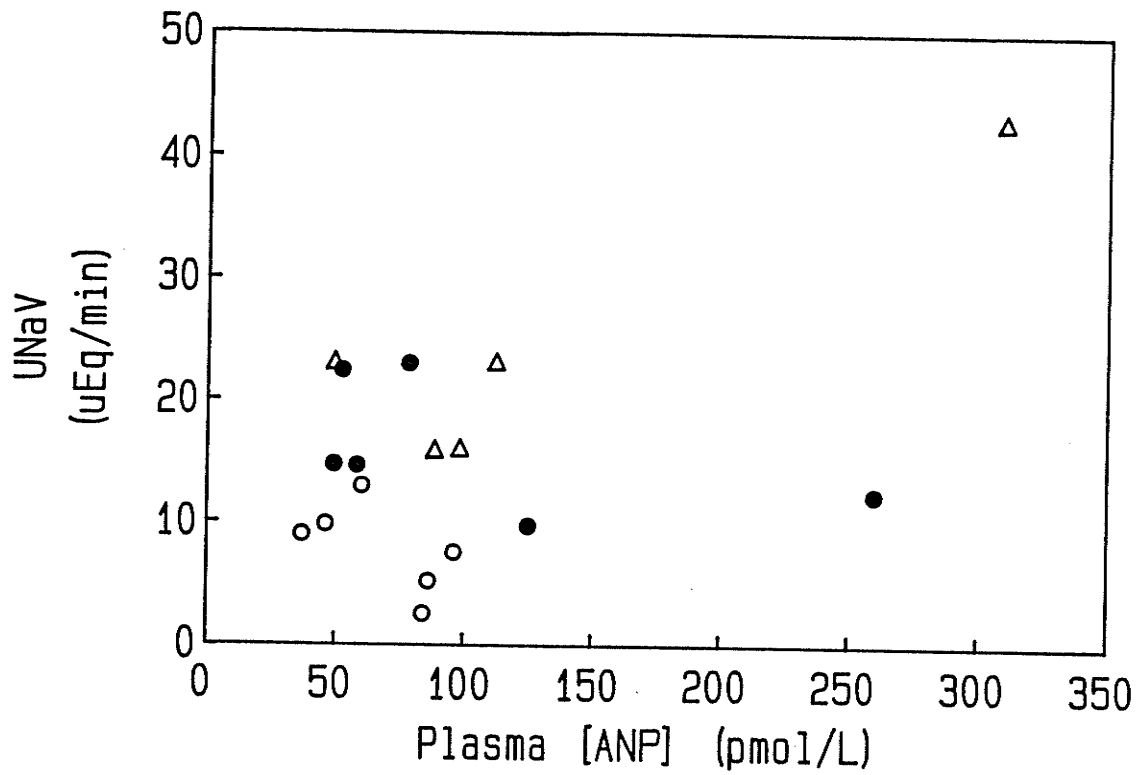
Fig. 5.5. Scatterplot of plasma ANP levels (*p*mol/l) plotted against sodium excretion at (○) 1 *u*g/kg/min infusions of clonidine, (●) 3 *u*g/kg/min infusions of clonidine and at (△) 10 *u*g/kg/min infusions of clonidine. r=0.49.

DISCUSSION

In 1981, DeBold demonstrated that mammalian atrial extracts contained a potent diuretic and natriuretic factor located in specific secretory-like granules of atrial cardiocytes. This factor was subsequently identified as a number of structurally related atrial natriuretic peptides (Flynn *et al.*, 1983; Kangawa and Matsuo, 1984). Recently, Baranowska *et al.* (1987) demonstrated that clonidine, an α2-adrenoceptor agonist, significantly increased plasma levels of ANP in normally hydrated, conscious rats. This increment was postulated to correlate with the diuretic and natriuretic actions of clonidine. As reported in the previous section, clonidine produced a dose-related dissociation of water and solute excretion (Blandford and Smyth, 1988b - Section 3). High infusion rates of clonidine (> 3 ug/kg/min) were necessary to increase urine flow rate and free water clearance as well as sodium excretion and osmolar clearance. These results and others (Strandhoy *et al.*, 1982) suggest that the effects of renal α2-adrenoceptor stimulation on sodium and water excretion may be mediated by two different mechanisms of action. Therefore, low infusion rates of clonidine may inhibit the renal effects of vasopressin while high infusion rates may result in the release of ANP, which then increases both water and sodium excretion.

In the present study, intrarenal infusions of clonidine (1, 3 and 10 ug/kg/min) produced a dose-related increase in urine flow rate and sodium excretion. Both 3 and 10 ug/kg/min infusions of clonidine increased urine flow rate as compared to the 1 ug/kg/min infusion of clonidine. Similarly, free water clearance was also increased in a dose-related manner. Clonidine at 3 ug/kg/min increased osmolar clearance during the third and fourth collection period, while 10 ug/kg/min increased osmolar clearance during all experimental collection periods as compared to the respective control collection periods. These results are

consistent with the previously reported results for intrarenal infusions of clonidine. Moreover, these effects have previously been shown, in this thesis, to be mediated by α2-adrenoceptors (Blandford and Smyth, 1988b - Section 3). In that study, the effects of clonidine on urine volume and sodium excretion were attenuated by yohimbine, an α2-adrenoceptor antagonist, but not by prazosin, an α1-adrenoceptor antagonist.

While the present results of the effects of clonidine on the excretion of water and sodium are similar to those previously reported, some minor discrepancies exist. What is most obvious is the fact that urine flow rate, sodium excretion and free water clearance dropped drastically during the fourth collection period in the presence of high clonidine infusion rates. This is most likely due to two technical difficulties. First, two 0.5 ml blood samples were taken, one immediately prior to this last collection period. Secondly, the rate of urine flow was so great with both the 3 and 10 $ug/kg/min$ infusion rates of clonidine, that the animals were unable to maintain that rate for an extended period of time without adequate fluid replacement. This, coupled with the removal of approximately 5% of the total blood volume of the rat may have compromised the stability of the preparation. This reasoning is supported by the fact that the clonidine-induced increase in blood pressure and creatinine clearance at 3 and 10 $ug/kg/min$ infusion rates is decreased in the fourth collection period to values that are consistent with control values.

The clonidine-induced increase in blood pressure and creatinine clearance may seem to suggest that the changes in water and electrolyte excretion are secondary to vascular or glomerular changes. While this possibility cannot be ruled out in the present study, Roman and Cowley (1985) have reported that a similar increase in blood pressure (25 mm Hg) would only increase urine flow rate by approximately 20 ul/min. Thus, the contribution of the increased blood pressure to the present results is negligible. This is supported by the results of the experiments

which maintained a normal renal perfusion pressure by the use of a vascular occluder (Section 3).

In the present study, plasma ANP levels were not significantly increased with high infusion rates of clonidine. This is in contrast to the results of Baranowska *et al.* (1987). In that study, a bolus intravenous injection of 50 ug resulted in a 20-fold elevation in plasma ANP levels. This maximal elevation of ANP release was observed 10 min after the injection of clonidine. Clearly, the results of the present study are inconsistent. This may, however, simply reflect different methodologies. In the present study clonidine was administered intrarenally over a period of 45 min. Consequently, it is conceivable that plasma levels of clonidine may not have been sufficient to induce ANP release even at the maximal infusion rate tested. As demonstrated by Baranowska *et al.* (1987), doses of clonidine less than 50 ug were unable to increase ANP levels above normal levels. Alternatively, we may have missed the peak plasma concentration of ANP with our sampling. At 30 min after the administration of clonidine, plasma ANP levels were not much different from control (Baranowska *et al.*, 1987). Peak plasma levels of ANP occurred between 5 and 11 minutes. Such a pattern of ANP release with clonidine would be consistent with the time course of the natriuretic and diuretic effects of clonidine in the present study.

In summary, the results of the present study suggest that the diuretic and natriuretic effects of α2-adrenoceptor stimulation with clonidine do not involve the release of ANP. The results, however, do not exclude the potential importance of ANP at higher infusion rates. The physiological relevance of ANP release at these high levels of α2-adrenoceptor stimulation remain to be determined.

6

Potentiation of the Natriuretic Effect of Clonidine Following Indomethacin in the Rat

The data in this section have been presented at the Canadian Society of Clinical Investigation meetings, Edmonton, 1989 (Clin. Invest. Med. 12:B69, 1989). These data have also been submitted for publication.

<u>Synopsis</u>:

Previous sections have demonstrated a diuretic effect of clonidine at low intrarenal infusion rates with a natriuretic effect being observed at high infusion rates ($\geq$ 3 ug/kg/min). The natriuresis at high infusion rates may have been secondary to increased renal prostaglandin production. Therefore, the effects of indomethacin (a cyclooxygenase inhibitor) on the response to clonidine were evaluated in the anesthetized rat. Intrarenal infusions of vehicle (saline) or clonidine (0.1, 0.3, 1 or 3 ug/kg/min) were examined both in the presence and absence of pretreatment with indomethacin (5 mg/kg; i.p.). As found in previous sections, clonidine produced a dose-related increase in urine volume and free water clearance at 0.3, 1 and 3 ug/kg/min as compared to the vehicle group. Sodium excretion and osmolar clearance however, was increased only at the highest infusion rate investigated. Following indomethacin pretreatment, clonidine produced a greater increase in urine volume at each infusion rate investigated. The indomethacin pretreatment also resulted in a potentiation of the natriuretic effect of clonidine at all infusion rates investigated. Interestingly, this was associated with an increase in osmolar clearance but not free water clearance. These effects of indomethacin were reversed by the infusion of prostaglandin E2. An infusion of prostaglandin E2 attenuated the indomethacin induced increase in both urine flow and sodium excretion, indicating that the effects of indomethacin were mediated by prostaglandin inhibition. These results suggest that endogenous prostaglandin production attenuated the renal effects of clonidine. As well, these studies suggest that in the presence of α2-adrenoceptor stimulation, prostaglandin E2 mediates an antidiuretic and antinatriuretic effect.

INTRODUCTION

Previous studies reported earlier in this thesis, have demonstrated a dose related dissociation in water and sodium excretion following an intrarenal infusion of clonidine, an α2-adrenoceptor agonist (Blandford and Smyth, 1988b - Section 3; 1989a - Section 4). These results were in contrast to a number of other studies which demonstrated concomitant increases in water and sodium excretion following the systemic administration of α2-adrenoceptor agonists. Results already presented in this thesis are consistent with the postulate (Strandhoy *et al.*, 1982) that the effects of renal α2-adrenoceptor stimulation on sodium and water excretion may be mediated at two different sites or by two different mechanisms of action. The effect of clonidine on water excretion at low infusion rates was consistent with an antagonism of the renal effects of vasopressin. The mechanism by which high infusion rates of clonidine increased sodium excretion remained unclear. The fact that intravenous administration of clonidine was more potent in producing a natriuresis (Blandford and Smyth, 1989a - Section 4) suggests that the effects of clonidine in increasing sodium excretion may be secondary to additional actions of this agonist.

One explanation for the natriuresis seen with a high infusion rate of clonidine involves the possible role of the acid-stable, fat soluble prostaglandins. Early studies have demonstrated that prostaglandins have diuretic and natriuretic actions (Fine and Trizna, 1977; Iino and Imas, 1978; Johnston *et al.*, 1967; Stokes and Kokko, 1977). In addition, it has been shown that prostaglandin synthesis is increased during adrenergic stimulation (Diz *et al.*, 1981; Dunham and Zimmerman, 1970; Matsumura *et al.*, 1986). Therefore the increased sodium excretion observed at a high infusion rate of clonidine may be secondary to an increase in the synthesis of prostaglandins. Thus, the effects of an intrarenal infusion of clonidine were

investigated in the presence and absence of indomethacin, a cyclooxygenase inhibitor.

METHODS

Male Sprague Dawley rats (200-225 g) were unilaterally nephrectomized (right kidney, ether anesthesia) 7-10 days prior to the day of the experiment. On the experiment day, they were surgically prepared as described previously (Section 2). Vehicle (0.3 ml) or indomethacin (5 mg/kg; Sigma Chemical Co., St. Louis, MO) dissolved in a physiological buffer (25 mM NaH_2PO_4, 25 mM K_2HPO_4, 1 mM $MgCl_2$) at a pH of 7.40 was administered intraperitoneally immediately following induction of anesthesia. Following surgery, the rats underwent a 45 min stabilization period, followed by a 15 min period where urine was collected into a pre-weighed tube. This was to serve as a preparation control to ensure that each rat had the same level of renal function, and to determine whether indomethacin pretreatment produced any additional effects in the absence of clonidine. This was followed by the continuous infusion of vehicle (saline) or clonidine (0.1, 0.3, 1 or 3 ug/kg/min) at a rate of 3.4 ul/min directly into the renal artery. Only 1 infusion rate was investigated in each rat. Two consecutive 30 min urine collections were obtained during the infusion. Again, throughout the experiment, saline was infused at a rate of 97 ul/min.

Preliminary experiments indicated that indomethacin pretreatment altered the renal response to clonidine. This was consistent with endogenous prostaglandins altering the response. If this was correct, then it would be expected that an infusion of a prostaglandin should reverse the effects of indomethacin. In another group of rats prostaglandin E2 was infused intravenously (through a second jugular catheter, PE20) at 1 ug/kg/min at a rate of 3.4 ul/min (dissolved in ethyl alcohol at 100 mg/ml and diluted in physiological buffer at a pH of 7.4) in addition to the indomethacin (5 mg/kg i.p.) and clonidine (1 ug/kg/min). Prostaglandin E2 was also given as a continuous intravenous infusion which was begun at the same time as

the clonidine and maintained for the duration of the experiment. The rest of the protocol is identical to the one previously described.

Statistical analyses of the first collection period (preparation controls) was performed to confirm that surgery or indomethacin had not altered renal function. This data was analyzed by a two way analysis of variance. Statistical analyses of the experimental data were performed with a two-way repeated measures analysis of variance (presence or absence of indomethacin x dose of clonidine). Significant interaction effects (i.e., where the dose of clonidine had different effects depending on whether indomethacin was present or not) were further analyzed with simple main effects analyses and Tukey HSD tests (Winer, 1971). Data are presented as the mean $\pm$ S.E.M., and are taken from the second 30 min collection period, which is representative of the observed differences. The data from the experiments where the animals were given the intravenous infusion of prostaglandin E2 in the presence and absence of indomethacin were analyzed by a one-way repeated measures analysis of variance. Significant effects were further analyzed with Tukey's HSD tests. Again, the data are presented as the mean $\pm$ the S.E.M., and are taken from the second collection period.

RESULTS

1. Preparation Controls

This data was analyzed to confirm that all rats had the same level of renal function. All groups were similar with respect to the measured baseline parameters. Specifically, there was no difference between any groups with respect to blood pressure, creatinine clearance, heart rate, renal blood flow, urine volume, sodium and potassium excretion, free water clearance, osmolar clearance and urine osmolality (data not shown). These results indicate that all rats did have the same level of renal function. Moreover, the results also suggest that pretreatment with indomethacin, in the absence of clonidine, had no effects of the excretion of sodium and water.

2. Response to Intrarenal Clonidine Following Indomethacin Treatment

A significant main effect (dose of clonidine) was found for blood pressure (fig. 6.1). Blood pressure was significantly elevated in the two groups of animals receiving a 3 $ug/kg/min$ infusion of clonidine, both in the presence and absence of indomethacin. Blood pressure was slightly, but not significantly elevated in the animals receiving a 1 $ug/kg/min$ infusion of clonidine, particularly in the group pretreated with indomethacin. Vascular and glomerular function were unaltered by pretreatment with indomethacin or treatment with clonidine as evidenced by no changes in renal blood flow (as measured by an electromagnetic flow meter) and glomerular filtration rate (as measured by creatinine clearance) (fig 6.1). Heart rate was also unaltered by the experimental conditions (data not shown).

Significant main effects (both presence of indomethacin and dose of clonidine) were noted for both urine flow rate and sodium excretion (fig. 6.2). Indomethacin enhanced both the diuretic and natriuretic effects of clonidine.

Clonidine produced a dose related increase in urine flow rate at 0.3, 1 and 3 ug/kg/min both with and without indomethacin pretreatment. However, at the 0.3 and 1 ug/kg/min infusion rates of clonidine, indomethacin potentiated the effect on urine flow rate. Sodium excretion was increased at both the 0.3 and 1 ug/kg/min infusion rate in the animals pretreated with indomethacin as compared to controls. Moreover, a significant interaction effect was also noted for sodium excretion. In the absence of indomethacin pretreatment, clonidine increased sodium excretion only at 3 ug/kg/min, whereas in the presence of indomethacin, clonidine significantly increased sodium excretion at 0.3, 1 and 3 ug/kg/min as compared to the saline control (0 clonidine). A similar trend was noted with osmolar clearance (fig. 6.3). Intrarenal infusions of clonidine increased osmolar clearance at a dose of 1 and 3 ug/kg/min in the animals that were treated with indomethacin. In the absence of indomethacin, clonidine increased osmolar clearance only at the maximal dose tested (fig. 6.3) Potassium excretion was unaltered by the intrarenal infusions of clonidine (fig. 6.2).

Free water clearance was increased at all doses studied, and this increase was observed regardless of whether the animals were pretreated with indomethacin (fig. 6.3). Although not statistically significant (p=0.057), the animals that were pretreated with indomethacin demonstrated a smaller increase in free water clearance as compared to the vehicle pretreated group. Finally urine osmolality was decreased at all doses tested, and this decrease was observed irrespective of whether the animals were pretreated with indomethacin or not (fig. 6.3).

3. Response to Indomethacin Following Prostaglandin E2 Infusion

The previous experiments indicated that the observed effects may be due to inhibition of prostaglandin synthesis, and as such, infusion of a prostaglandin would be expected to reverse these effects. Therefore, the effects of indomethacin on the

response to clonidine (1 ug/kg/min) in the presence of an intravenous infusion of prostaglandin E2 (1 ug/kg/min) were determined. This data was analyzed by a one way repeated measures analysis of variance. Rats receiving indomethacin, in the presence of clonidine, had a significantly increased urine flow rate as compared to the animals receiving clonidine alone (fig. 6.4). Addition of prostaglandin E2 attenuated the indomethacin-induced increase in urine flow rate. The same is true for sodium excretion and osmolar clearance. Pretreating the animals with indomethacin significantly increased sodium excretion (fig. 6.4) and osmolar clearance (fig. 6.5) from control, while addition of prostaglandin E2 attenuated the indomethacin-induced increase in both sodium excretion and osmolar clearance. These effects however, were seen in the presence of an elevated blood pressure (fig. 6.6). In the animals pretreated with indomethacin, clonidine increased blood pressure by approximately 18 mm Hg. This increase was attenuated by the addition of prostaglandin E2. Heart rate was unaltered (data not shown).

In the indomethacin pretreated group, free water clearance was increased only during the second collection period (i.e. interaction effect p=0.050). This increase was prevented by the concurrent administration of prostaglandin E2 (fig. 6.6). Urine osmolality was unaltered (fig. 6.5). Similarly, creatinine clearance and heart rate were also unaltered (fig. 6.6).
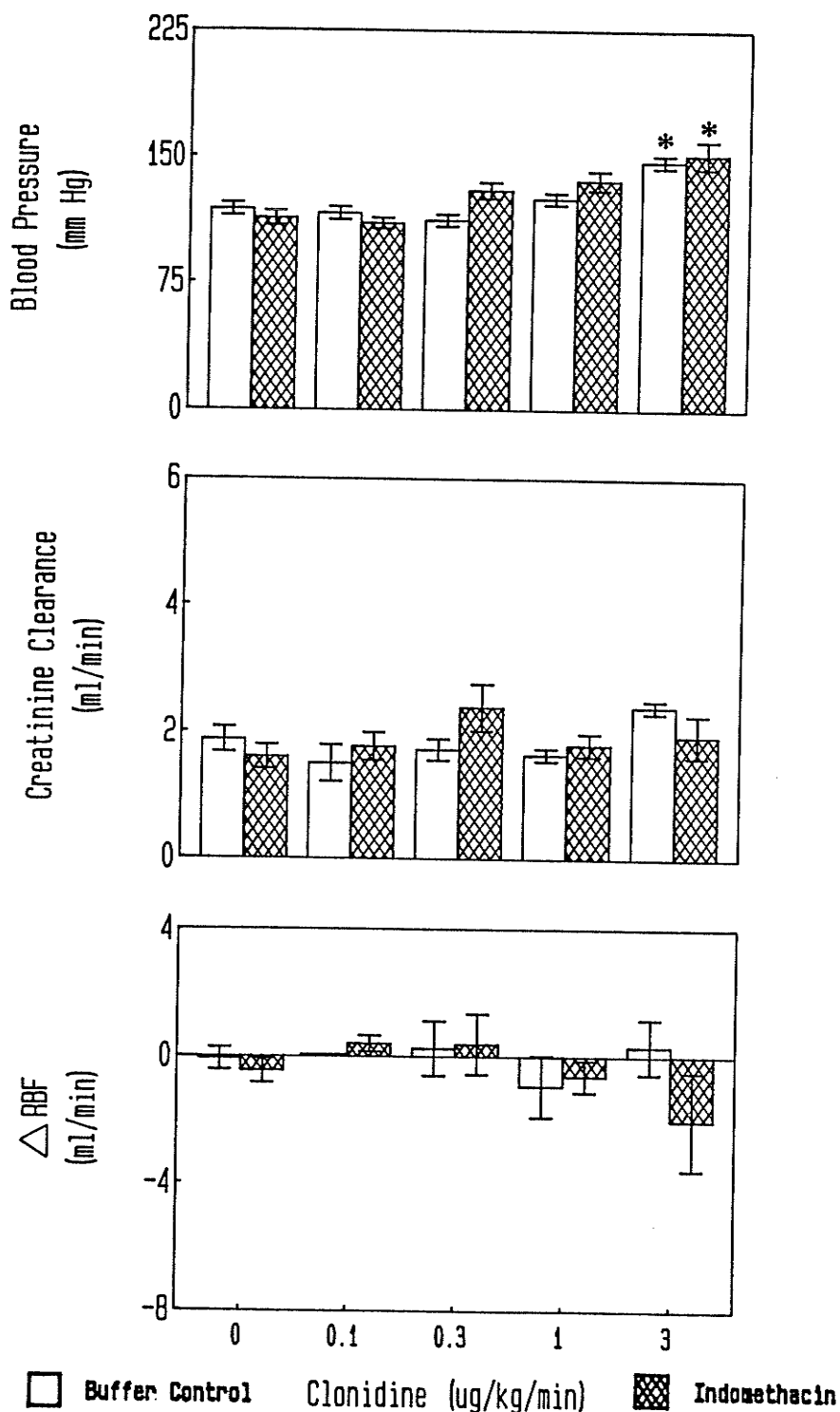
Fig. 6.1. Effect of clonidine infusions (0, 0.1, 0.3, 1 and 3 ug/kg/min) directly into the renal artery in the presence and absence of indomethacin on blood pressure, creatinine clearance and the change in renal blood flow (ΔRBF). The open bars represent control (vehicle), while the cross-hatched bars represent pretreatment with indomethacin. Each group contains 5 to 7 experiments. These data represent the mean ± S.E.M. of the second collection period.
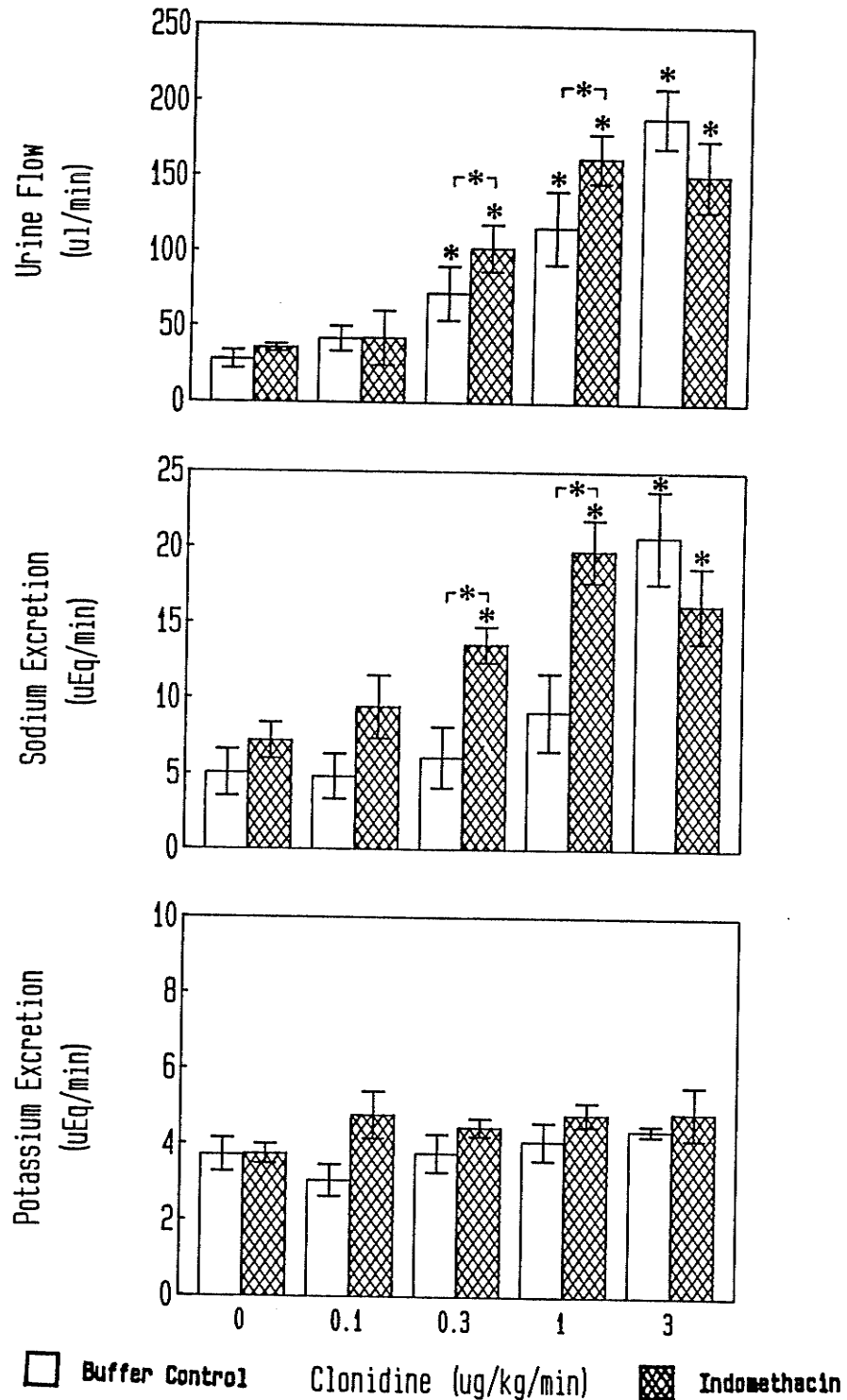
Fig. 6.2. Effect of clonidine infusions (0, 0.1, 0.3, 1 and 3 *ug*/kg/min) directly into the renal artery in the presence and absence of indomethacin on urine flow, sodium excretion and potassium excretion. The open bars represent control, while the cross-hatched bars represent pretreatment with indomethacin.
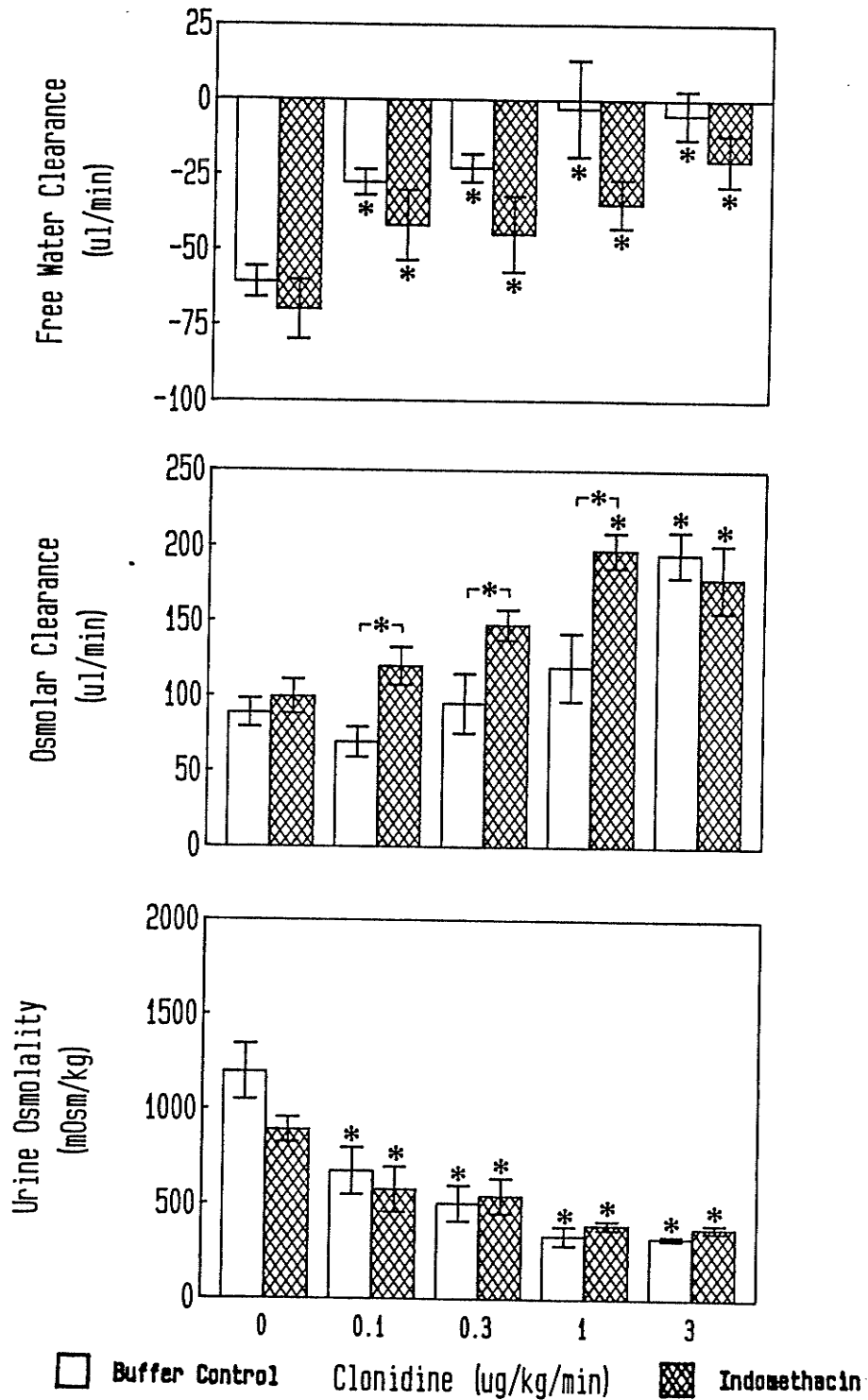
Fig. 6.3. Effect of clonidine infusions (0, 0.1, 0.3, 1 and 3 *ug*/kg/min) directly into the renal artery in the presence and absence of indomethacin on free water clearance, osmolar clearance and urine osmolality. The open bars represent control, while the cross-hatched bars represent pretreatment with indomethacin.
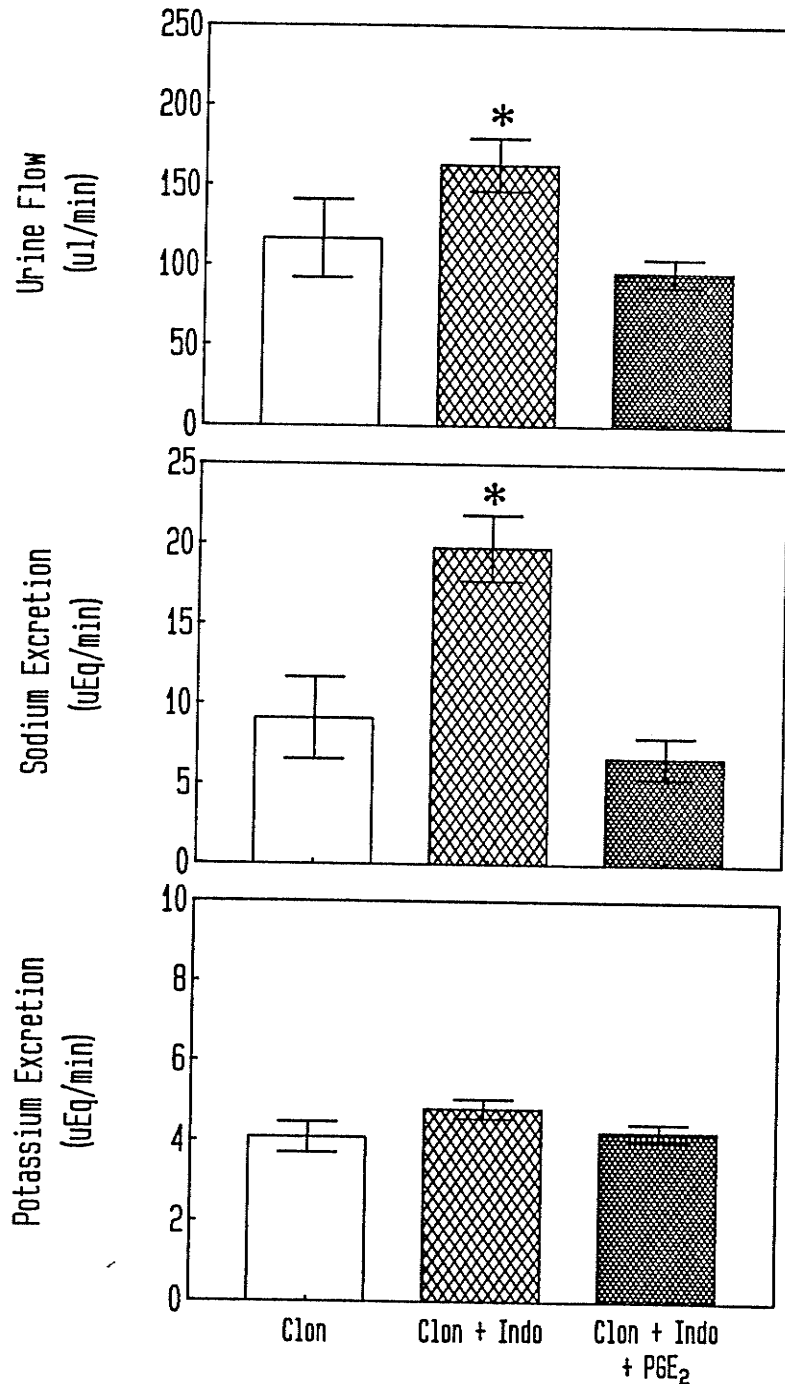
Fig. 6.4. Effect of a 1 ug/kg/min intrarenal infusion of clonidine in the presence and absence of both indomethacin and prostaglandin E2 (PGE2) on urine flow, sodium excretion and potassium excretion. Open bars represent control, n=5; cross-hatched bars represent pretreatment with indomethacin, n=6; and the dense cross-hatched bars represent pretreatment with indomethacin and concurrent administration of prostaglandin E2, n=6.
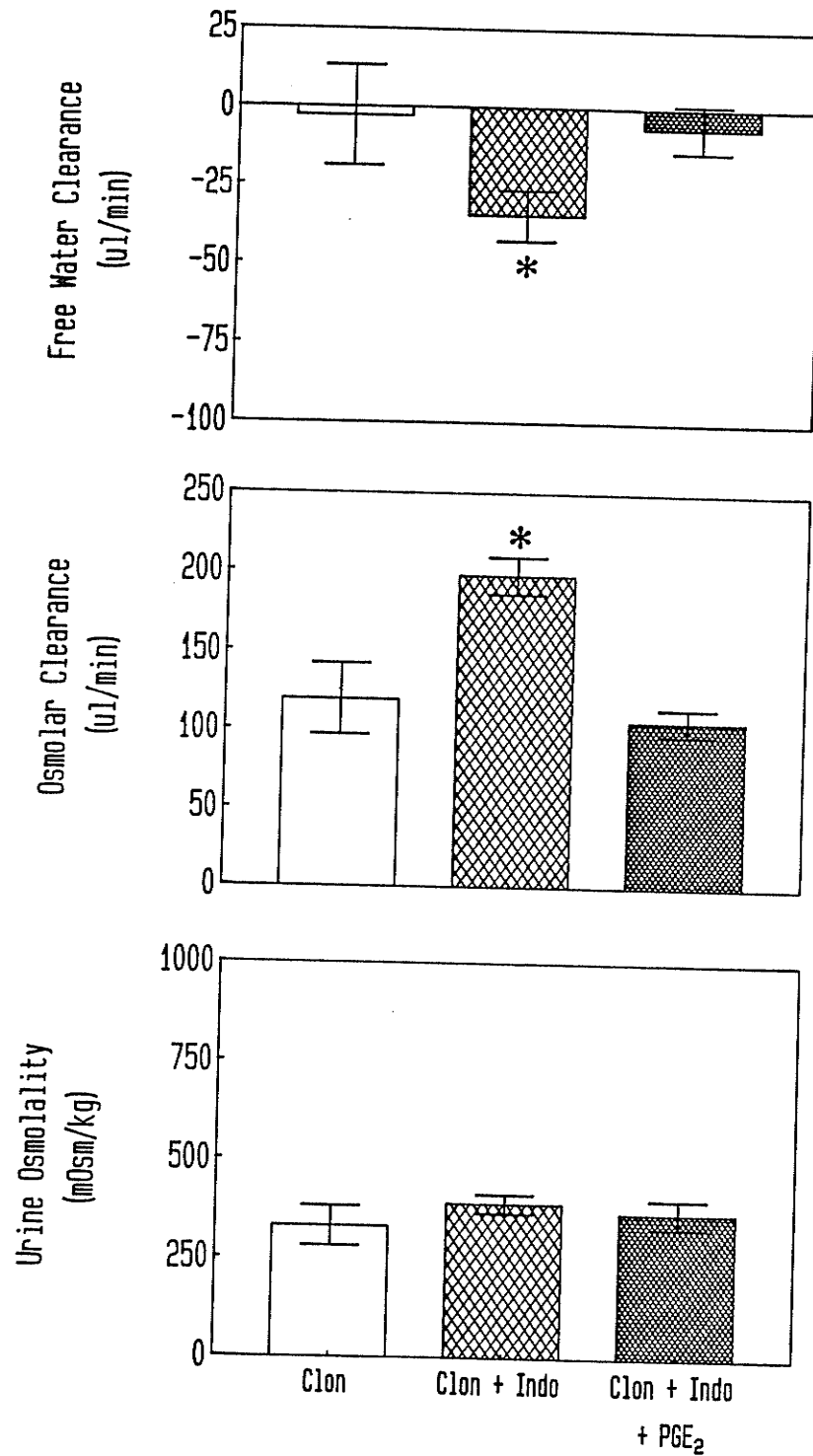
Fig. 6.5. Effect of a 1 ug/kg/min intrarenal infusion of clonidine in the presence and absence of both indomethacin and prostaglandin E2 (PGE2) on free water clearance, osmolar clearance and urine osmolality. Open bars represent control, n=5; cross-hatched bars represent pretreatment with indomethacin, n=6; and the dense cross-hatched bars represent pretreatment with indomethacin and concurrent administration of prostaglandin E2, n=6.
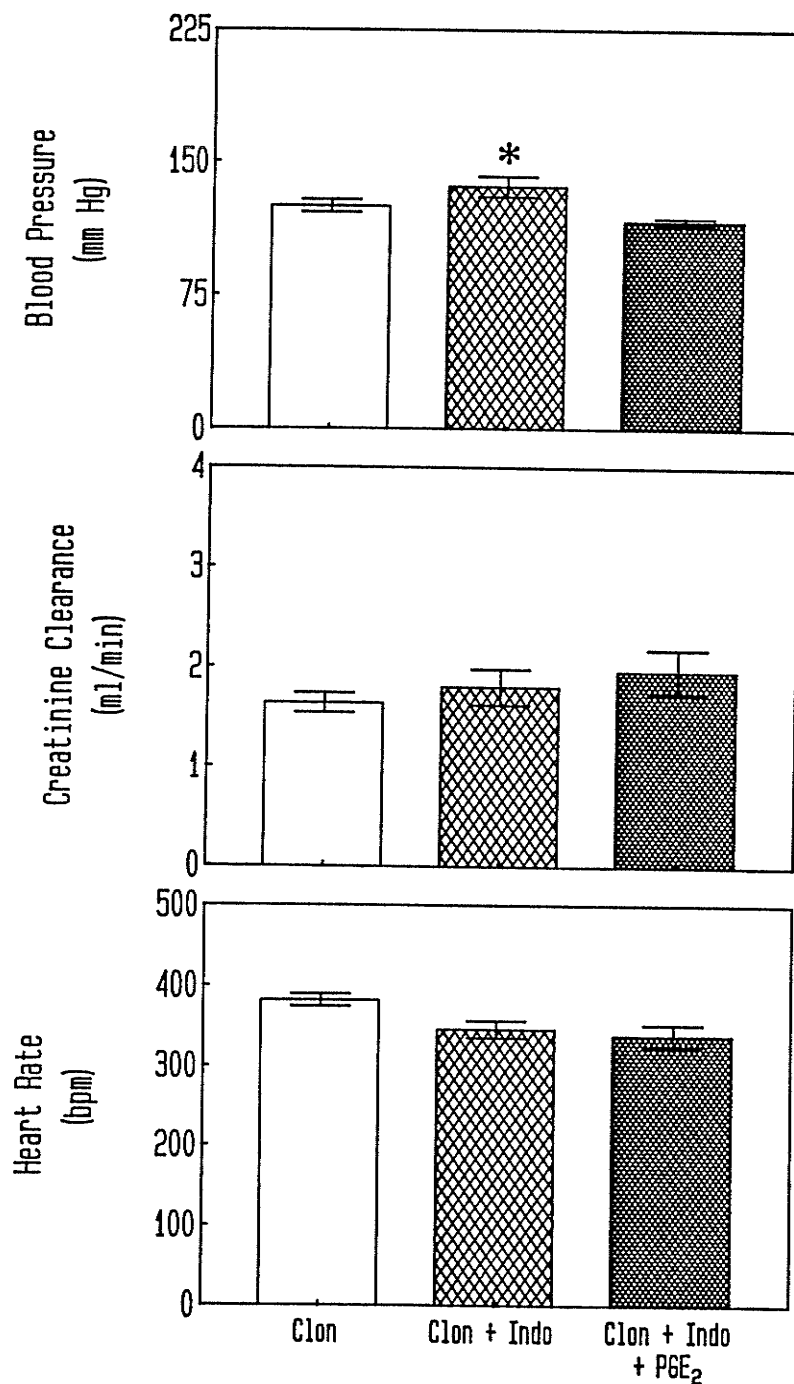
Fig. 6.6. Effect of a 1 ug/kg/min intrarenal infusion of clonidine in the presence and absence of both indomethacin and prostaglandin E2 (PGE2) on blood pressure, creatinine clearance and heart rate. Open bars represent control, n=5; cross-hatched bars represent pretreatment with indomethacin, n=6; and the dense cross-hatched bars represent pretreatment with indomethacin and concurrent administration of prostaglandin E2.

DISCUSSION

In the isolated perfused rat kidney (Smyth *et al.*, 1985) and in isolated cortical collecting tubules (Krothapalli *et al.*, 1983) α2-adrenoceptor stimulation inhibited vasopressin-induced activation of adenylate cyclase, as well as, the effects of vasopressin on sodium and water excretion. These *in vitro* studies indicated that the effects of α2-adrenoceptor activation in the kidney were due to the inhibition of the vasopressin mediated increase in cAMP production. However, other studies have proposed that the effects of renal α2-adrenoceptor stimulation on sodium and water excretion may be mediated at two different sites in the kidney or by two different mechanisms of action. Strandhoy *et al.* (1982), reported an enhanced diuretic and natriuretic response to guanabenz following an intravenous infusion as compared to an intrarenal infusion. Similar results were demonstrated with clonidine in the previous section (Blandford and Smyth, 1989a - Section 4). These studies suggested that water excretion following α2-adrenoceptor stimulation may be mediated by intrarenal factors; i.e., the inhibition of the renal effects of vasopressin, whereas sodium excretion may involve an extrarenal site or mechanism of action which is independent of vasopressin. This contention has been supported by studies which attribute the increase in sodium and water excretion after α2-adrenoceptor stimulation to a decrease in the central release of vasopressin (Barr and Kauker, 1979; Roman *et al.*, 1979), an increase in the synthesis of renal prostaglandins (Lieberthal *et al.*, 1987) and/or other independent mechanisms, possibly unrelated to vasopressin (Baranowska *et al.*, 1987; Leander *et al.*, 1985).

Since prostaglandin synthesis is increased during adrenergic stimulation (Diz *et al.*, 1981; Dunham and Zimmerman, 1970, Matsumura *et al.*, 1987), and since prostaglandins have been shown to have diuretic and natriuretic actions (Fine and Trizna, 1977; Iino and Imas, 1978; Johnston *et al.*, 1967; Stokes and Kokko, 1977),

the effects of an intrarenal infusion of clonidine, an α2-adrenoceptor agonist, were examined in the presence and absence of indomethacin, a cyclooxygenase inhibitor. It was anticipated that the natriuretic response seen with an intrarenal infusion of clonidine at a rate of 3 *ug/kg/min* (Blandford and Smyth, 1988), would be attenuated if this effect was due to a secondary increase in the synthesis of renal prostaglandins.

In the present study, indomethacin enhanced rather than attenuated both the diuretic and, to a greater extent, the natriuretic effects of clonidine. More specifically, while clonidine produced a dose-related increase in urine flow rate, both with and without indomethacin pretreatment, free water clearance was attenuated with the administration of indomethacin. Sodium excretion and osmolar clearance were enhanced. Thus, the potentiation of the effects of clonidine by indomethacin appear to be independent of water excretion. In the vehicle pretreated animals, sodium excretion was increased only at the maximal infusion rate tested. In the indomethacin pretreated animals however, sodium excretion was increased by clonidine at both the 0.3 and 1 *ug/kg/min* infusion rates as compared to both control animals (0 clonidine) and animals pretreated with the vehicle. Similar effects were seen for solute excretion (osmolar clearance). In addition, the indomethacin-induced potentiation of the effects of clonidine were attenuated by the concurrent administration of prostaglandin E2. These results suggest that the effects of clonidine may be attenuated by the synthesis of prostaglandins, which are antinatriuretic.

It has been suggested that prostaglandins may play an important role in modulating the renal actions of vasopressin. Vasopressin increases the production of cAMP within the epithelial cell. This increased accumulation of cAMP results in an increase in sodium reabsorption in the cortical and medullary segments of the thick ascending limb of the loop of Henle (Elalouf *et al.*, 1984; Hebert and Andreoli,

1984; Reif *et al.*, 1986), and an increase in the permeability of the collecting tubule to water. *In vitro* studies in the toad bladder (Orloff *et al.*, 1965) and in the isolated collecting duct (Grantham and Orloff, 1968) have demonstrated that prostaglandin E suppresses vasopressin-induced changes in water permeability, most likely by inhibiting vasopressin-activated adenylate cyclase. More recent studies have supported this contention by demonstrating that prostaglandins decrease vasopressin-induced cAMP accumulation in the rabbit cortical collecting tubule (Chabardes *et al.*, 1988). Prostaglandin synthesis inhibition with cyclooxygenase inhibitors, on the other hand, has been shown to enhance the ability of prostaglandins to antagonize the effects of vasopressin (Chabardes *et al*, 1988; Jackson *et al.*, 1980).

Similarly, in *in vivo* studies, inhibition of cyclooxygenase has been shown to result in an augmentation of the antidiuretic effects of vasopressin in the rat (Berl *et al.*, 1977; Lum *et al.*, 1977) and in the dog (Anderson *et al.*, 1975; Fejes-Toth *et al.*, 1977; Zambraski and Dunn, 1979). It would appear that the results reported here are in contrast with those previously reported, since pretreatment with indomethacin had no effect on water and sodium excretion in the absence of clonidine. However, the failure of indomethacin to alter our baseline levels may reflect a low endogenous synthesis of prostaglandins in the control experiments which was elevated following clonidine infusion. Alternatively, this may indicate that the dose of indomethacin these animals received was insufficient to block the synthesis of renal prostaglandins. While prostaglandin levels were not measured in the present study, others have reported that a similar dose of indomethacin inhibits prostaglandin synthesis under similar experimental conditions (Campbell *et al.*, 1979; Francisco *et al.*, 1982). Moreover, the fact that indomethacin potentiated the effects of $\alpha2$-adrenoceptor stimulation, and an infusion of prostaglandin E2 reversed the effects of indomethacin seems to indicate that the dose used was sufficient.

These results indicate that the effects of indomethacin were indeed mediated by an inhibition of prostaglandin synthesis.

It has been postulated that α2-adrenergic agonists also blunt the hydroosmotic effect of vasopressin in the isolated cortical collecting tubule of the rabbit (Krothapalli et al., 1983; Krothapalli and Suki, 1984) and the rat (Chabardes et al., 1983; Umemura et al., 1985) by inhibiting the generation of cAMP. This raises the possibility that α2-adrenoceptors decrease cAMP production by increasing the synthesis of prostaglandins which function as mediators of this inhibitory effect. As such, prostaglandin synthesis inhibition with indomethacin would be expected to result in an augmentation of the vasopressin-induced cAMP production, culminating in an antidiuresis. These effects however, were not observed in the toad bladder (Alvo et al., 1988), the rat cortical collecting tubule (Chabardes et al., 1988) or in the present study. Although free water clearance was statistically unaltered by indomethacin pretreatment in the present study, the data does suggest (p=0.057) that pretreatment with indomethacin may attenuate the ability of clonidine to increase free water clearance. Moreover, in the studies with concurrent prostaglandin E2 administration, this increase in free water clearance with indomethacin reached a level of statistical significance. Whether this is physiologically relevant is unclear. What is interesting is that urine flow rate following clonidine was increased rather than decreased following indomethacin pretreatment.

With respect to sodium excretion, prostaglandins have been shown to have natriuretic effects (Fulgraff and Meiforth, 1971; Iino and Imai, 1978; Johnston et al., 1967). The exact mechanism of action has not been delineated, and may be attributed to changes in renal hemodynamics, caused by their effects on vascular smooth muscle (Martinez-Maldonado et al., 1972), or by altering sodium transport by a direct action on the renal tubules, particularly the collecting tubules (Fulgraff

and Meiforth, 1971). More specifically, prostaglandin E2 suppresses sodium transport in the cortical and medullary collecting tubules (Fulgraff and Meiforth, 1971; Iino and Imai, 1978). Consequently, inhibition of prostaglandin synthesis would be expected to decrease the excretion of sodium. This was not seen in the present study. In the absence of clonidine, indomethacin had no effect on sodium excretion, suggesting that endogenous levels of prostaglandins are very low under these experimental conditions. In the presence of clonidine, indomethacin elevated, rather than decreased, the excretion of sodium, while the concurrent administration of prostaglandin E2 attenuated this indomethacin-induced effect. These results strongly suggest that clonidine may stimulate the synthesis of renal prostaglandins, which in turn clearly play an antinatriuretic role in the present study. These results are consistent with the work of Kirschenbaum and Stein (1975).

The mechanism by which renal prostaglandins decrease the excretion of sodium is unclear. Since blood pressure was elevated with high infusion rates of clonidine, particularly in the presence of indomethacin, it may be argued that the observed effects simply reflect changes in hemodynamics. Moreover, infusion of prostaglandin E2 decreased the indomethacin-induced increase in blood pressure. However, the observed effects on sodium excretion also occur at infusion rates of clonidine which produce no alterations in blood pressure. In addition, renal blood flow was not changed throughout all experiments. In the present study, osmolar clearance was increased with indomethacin while free water clearance was slightly decreased. This increase in solute excretion, in the absence of changes in water excretion and potassium excretion may indicate that the indomethacin-induced natriuresis may be due to diminished sodium reabsorption beyond the distal tubule.

Sodium transport has been shown to be either stimulated (Fassina *et al.*, 1976; Kirschenbaum and Stein, 1975; Lipson and Sharp, 1971) or inhibited (Al-Awqai and Greenough, 1972; Fulgraff and Meiforth, 1971; Iino and Imai, 1979) by

prostaglandins. These effects may be due to a direct action of the prostaglandin on the epithelia, but are diverse and appear to be dependent on the nature of the epithelia wherein sodium transport occurs. Accordingly, Leyssac *et al.* (1975) proposed that prostaglandins stimulate sodium transport in epithelia with high electrical resistance, whereas they inhibit sodium transport in epithelia with low electrical resistance. According to this hypothesis, it would be anticipated that prostaglandins would stimulate sodium transport in the collecting tubule since this segment belongs to the high resistance membranes (Helman *et al.*, 1971). Such effects of prostaglandins would be consistent with the present results.

In summary, pretreatment of the rats with indomethacin resulted in a potentiation, rather than an attenuation of the natriuretic response to clonidine. The ability of clonidine to increase urine volume was also potentiated by indomethacin, but the increase free water clearance was unaltered or even decreased. Moreover, infusion of prostaglandin E2 reversed the effects of indomethacin both on urine volume and sodium excretion. Taken together, these results suggest that clonidine may increase the synthesis of renal prostaglandins, and that these prostaglandins in turn, function not to increase water and sodium excretion, but rather to decrease water, and particularly sodium excretion in the anesthetized rat.

7

Role of Vasopressin in the Response to Intrarenal Infusions of α2-Adrenoceptor Agonists

The majority of the data in this section, have been presented at the Federation of American Societies for Experimental Biology meetings, New Orleans, 1989 (FASEB J. 3(3):A415, 1989). These data are also in press, J. Pharmacol. Exp. Ther. 1990.

Synopsis:

Previous studies have indicated that the effects of renal α2-adrenoceptor stimulation are mediated through the blockade of the renal effects of vasopressin. If this premise is correct, then 1) specific antagonists of the antidiuretic effect of vasopressin (V2 antagonists) should mimic α2-adrenoceptor stimulation, and 2) in the presence of V2 antagonists, the diuretic and natriuretic effect of clonidine should be attenuated. The renal effects of [d(CH$_2$)$_5$, D-Ile^2, Ile^4]-AVP, a specific V2 antagonist, were studied. On the experimental day, uninephrectomized rats were anesthetized (Nembutal) and the carotid artery and jugular vein cannulated for the recording of blood pressure and infusion of saline, respectively. The left kidney was exposed and the ureter cannulated. A 31-gauge needle was advanced into the renal artery to permit direct intrarenal infusion of study drugs. Intravenous bolus doses of the V2 antagonist (0, 1, 3, 10 or 30 nmol/kg) produced a dose-related increase in urine volume and free water clearance at all doses tested. Sodium excretion increased only at the higher doses (10 and 30 nmol/kg). This dose-related dissociation in water and then sodium excretion is similar to that observed following intrarenal clonidine infusions. In the presence of the V2 antagonist, clonidine (3 ug/kg/min) had no effect on urine volume or free water clearance, but significantly decreased the excretion of sodium from control. These results demonstrate that V2 antagonists mimic the effects of clonidine in the kidney. As well, in the absence of vasopressin (V2 antagonism), the effects of clonidine are attenuated. Moreover, they are also consistent with not only an antidiuretic role for endogenous vasopressin, but also an antinatriuretic one.

INTRODUCTION

Previous studies *in vitro* have demonstrated that stimulation of renal α2-adrenoceptors attenuates the increase in cAMP production as well as the antidiuretic effect of vasopressin (Krothapalli *et al.*, 1983; Smyth *et al.*, 1985). *In vivo*, the function of α2-adrenoceptors is less clear since a number of hormones increase cAMP production in specific nephron segments and this increase in cAMP may be attenuated by α2-adrenoceptor agonists. Consequently, the overall physiological effect of α2-adrenoceptor stimulation is dependent on the predominating effect of endogenously produced cAMP. A number of studies have reported on the renal effects of α2-adrenoceptor agonists administered systemically (Roman *et al.*, 1979; Miller, 1980; Strandhoy *et al.*, 1982; Gellai and Ruffolo, 1987; Stanton *et al.*, 1987). These studies demonstrated a concomitant increase in water and sodium excretion following α2-adrenoceptor stimulation. These effects were attributed to either a direct antagonism of the renal effects of vasopressin, a decrease in the central release of vasopressin and/or other mechanisms unrelated to vasopressin. In this thesis, a dose-related dissociation in water and sodium excretion has been demonstrated with increasing doses of clonidine (Blandford and Smyth, 1988b - Section 3).

These studies with clonidine were consistent with the previously proposed postulate (Strandhoy *et al.*, 1982) that the effects of renal α2-adrenoceptor stimulation on sodium and water excretion may be mediated by two different sites and/or mechanisms of action. Low doses may increase urine volume by the antagonism of the renal effects of vasopressin, while higher doses of clonidine may decrease the central release of vasopressin or may exert an effect by mechanisms independent of vasopressin (Leander *et al.*, 1985; Balment *et al.*, 1986; Baranowska *et al.*, 1986) to increase both urine volume and sodium excretion.

The renal effects of α2-adrenoceptor agonists appear to be mediated by the antagonism of the renal effects of vasopressin. Consequently, one would postulate that specific antagonists of the antidiuretic effect of vasopressin (V2 antagonists) should mimic the effects of α2-adrenoceptor agonists. However, clonidine, at a high infusion rate also increased sodium excretion. If the increase in sodium excretion is due to the antagonism of vasopressin, then this would imply that vasopressin is antinatriuretic *in vivo*, a notion that is strongly opposed at present. If, however, the effects of clonidine at high doses (3 ug/kg/min) are dependent on vasopressin, then in the presence of a V2 antagonist, the effects of clonidine should be attenuated. Therefore, we set out to first determine the renal effects of a specific V2 antagonist, $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]$-AVP, and second, to determine the effects of clonidine in the presence of vasopressin blockade with this V2 antagonist.

METHODS

As previously described (Section 2), male Sprague-Dawley rats (200-225 g) were unilaterally nephrectomized and surgically prepared. The left jugular vein was cannulated (PE160) for the infusion of saline (97 ul/min) and for the bolus administration (0.2 ml) of the V2 antagonist, $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]$-AVP (1, 3, 10 or 30 $n$mol/kg; Peninsula Laboratories Inc., Belmont, CA), or saline (vehicle). The left kidney was exposed by a flank incision and retracted ventrally. The ureter was cannulated. An electromagnetic flow probe was placed around the renal artery for the measurement of renal blood flow. At this point, the preparation was used to determine either the dose response to the V2 antagonist or the effects of clonidine in the presence of the V2 antagonist.

1. Dose Response to the V2 Antagonist

Following the surgical procedure, the animals were allowed to stabilize for 45 min, after which a 15 min control urine sample was collected. This was followed by the direct intravenous administration (over a period of approximately 1 min) of vehicle or increasing doses of the V2 antagonist (1, 3, 10 or 30 $n$mol/kg) in a total volume of 0.2 ml. Only one dose was investigated in each rat. Four consecutive 15 min urine collections were then obtained.

2. Effects of Clonidine in the Presence of a V2 Antagonist

In these experiments, a 31 gauge needle was advanced through the aorta into the renal artery for the direct continuous infusion of clonidine into the renal artery as previously described (Section 2). Following a 45 min stabilization period, a 15 min control collection was obtained. This was followed by the intravenous administration of vehicle (saline) or V2 antagonist (30 $n$mol/kg). This dose was

chosen since it produced a maximal decrease in urine osmolality to levels consistent with almost complete antagonism of vasopressin in the previous experiments. Following another 15 min stabilization period, the continuous intrarenal infusion of clonidine (1 or 3 ug/kg/min) was begun and maintained for the duration of the experiments. Again, four 15 min urine collections were obtained.

In the above experiments, the increase in sodium and water excretion observed following clonidine (3 ug/kg/min) may have been secondary to the increase in blood pressure (approximately 20 mm Hg). Moreover, the apparent antinatriuretic effect observed with clonidine in the above experiments with the V2 antagonist may have also been obscured by an increase in blood pressure. Consequently, these experiments were repeated using a vascular occluder (a micrometer screw clamp) to clamp the descending aorta proximal to the renal artery. The carotid and the femoral arteries were cannulated to record blood pressure proximal and distal to the clamp. The blood pressure recorded distal to the clamp in the femoral artery represented the blood pressure at the level of the renal artery. In the face of increased systemic pressure, the clamp was screwed down to maintain a constant renal perfusion pressure. Sham experiments were also conducted where the occluder was positioned around the descending aorta, but was not screwed down with increases in systemic blood pressure. The femoral artery was cannulated (PE60) for the measurement of post occlusion blood pressure. Following the infusion of clonidine, the aorta was partially occluded such that the blood pressure measured in the femoral catheter remained constant with control values.

Statistical analysis of the dose response to the V2 antagonist was performed with a one-way, repeated measures analysis of variance. Significant interactions were further analyzed with Tukey HSD tests. Data are presented as the mean ±

S.E.M. The experiments which were done to examine the effects of clonidine in the presence of the V2 antagonist as well as those using the vascular clamp were analyzed with a two-way, repeated measures analysis of variance (presence of V2 antagonist x dose of clonidine; presence of V2 antagonist x clonidine/clamp, respectively). Significant interactions were analyzed further with simple main effects analyses and Tukey HSD tests (Winer, 1971). Again, data are presented as the mean $\pm$ S.E.M., and are taken from the last three collection periods (i.e., 15 to 60 min after the infusion of clonidine was begun). Each group represents 6 to 8 animals unless otherwise specified.

RESULTS

1. Dose Response to the V2 Antagonist

The intravenous administration of the V2 antagonist, $[d(CH_2)_5, D\text{-}Ile^2, Ile^4]$-AVP, resulted in a dose-related increase in urine volume (fig. 7.1) and free water clearance (fig. 7.2). The V2 antagonist at 3, 10 and 30 $n$mol/kg increased urine volume. Free water clearance was increased, while urine osmolality was decreased at all doses tested. The lower doses of the V2 antagonist (1 and 3 $n$mol/kg) failed to alter solute excretion as indicated by no changes in sodium and potassium excretion (fig. 7.1) and osmolar clearance (fig. 7.2). Higher doses of the V2 antagonist (10 and 30 $n$mol/kg), however, significantly increased sodium excretion (fig. 7.1), but had no effect on potassium excretion. Osmolar clearance was decreased at all doses during the second collection period and was increased with 10 $n$mol/kg of the V2 antagonist during the final collection period. This represents a dose-related dissociation of water and sodium excretion following V2 antagonist administration. Blood pressure and creatinine clearance (fig. 7.3) were unaltered. Renal blood flow was decreased at 3, 10 and 30 $n$mol/kg in a dose-related manner (fig. 7.3). Filtration fraction was unaltered (data not shown).

2. Effects of Clonidine in the Presence of the V2 Antagonist

In this group of experiments, significant main effects (dose of clonidine) were found for blood pressure, the change in renal blood flow and heart rate (fig. 7.4). Blood pressure was significantly elevated in the groups of animals receiving intrarenal infusions of clonidine at 3 ug/kg/min, both in the presence and absence of the V2 antagonist. Renal blood flow, however, was decreased in these same groups (receiving 3 ug/kg/min intrarenal infusions of clonidine). Heart rate was decreased in a dose-related manner at both 1 and 3 ug/kg/min of clonidine, again

both in the presence and the absence of the V2 antagonist. Finally, creatinine clearance and filtration fraction (data not shown) was unaltered in all groups.

A significant interaction effect was noted for both urine flow rate and sodium excretion (fig. 7.5). For urine volume, in the absence of the V2 antagonist, clonidine produced a dose-related increased in urine flow rate. In the presence of the V2 antagonist, however, 1 ug/kg/min clonidine significantly increased urine volume from control, while 3 ug/kg/min clonidine did not alter urine volume. Moreover, the V2 antagonist alone significantly increased urine volume from control suggesting an antidiuretic effect of endogenous vasopressin. For sodium excretion, clonidine alone, in the absence of the V2 antagonist, increased sodium excretion only at the maximal dose tested. This, however, as previously stated was accompanied by a slight but significant elevation in blood pressure. In the presence of the V2 antagonist, 1 ug/kg/min clonidine did not change sodium excretion from control, whereas clonidine at 3 ug/kg/min decreased sodium excretion. This decrease in sodium excretion occurred in spite of an elevation in systemic blood pressure. In addition, by the third and fourth collection periods post infusion of clonidine, sodium excretion was significantly elevated with the V2 antagonist alone as compared to control, suggesting that vasopressin is also antinatriuretic. Potassium excretion was unaltered (fig. 7.5).

Free water clearance and urine osmolality also demonstrated a significant interaction effect (fig. 7.6), while osmolar clearance (fig. 7.6) was unaltered. Free water clearance showed a similar trend as did urine volume (i.e., dose-related increase). Clonidine produced a dose-related increase in free water clearance. In the presence of the V2 antagonist, 1 ug/kg/min clonidine significantly increased free water clearance from its respective control, while 3 ug/kg/min clonidine did not significantly alter free water clearance. Urine osmolality was significantly decreased by the intrarenal infusions of clonidine in the absence of the V2

antagonist. The V2 antagonist alone significantly decreased urine osmolality from control, and the addition of clonidine had no effect in further decreasing urine osmolality.

3. Aortic Clamp Experiments

From the preceding results, two questions arose: 1) can the increase in sodium excretion with 3 $ug/kg/min$ clonidine be attributed to an increase in blood pressure, and 2) if this is the case, in the absence of an increase in blood pressure would the decrease in sodium excretion seen with 3 $ug/kg/min$ clonidine in the presence of the V2 antagonist decrease further. To answer these questions, the experiments with a 3 $ug/kg/min$ intrarenal infusion of clonidine were repeated. A vascular occluder was used to clamp the descending aorta proximal to the kidney to maintain a constant renal perfusion pressure. This data was analyzed by a two-way, repeated measures analysis of variance.

A significant interaction effect was observed for blood pressure (fig. 7.7). In the absence of the V2 antagonist, the animals that received clonidine, and were also sham clamped (occluder positioned, but not screwed down with increasing systemic blood pressure) had a higher blood pressure (as measured in the femoral artery) than control animals (those not receiving clonidine) and those in which the aorta was partially occluded. The same trend was evident in the animals which received the intravenous administration of the V2 antagonist. In the presence of the V2 antagonist, in all three groups, (control, sham clamp and clamp), blood pressure was slightly elevated as compared to the control animals which did not receive the V2 antagonist.

Significant interaction effects were also noted for urine volume and sodium excretion (fig. 7.7). In the absence of the V2 antagonist, a 3 $ug/kg/min$ intrarenal infusion of clonidine increased urine volume in both the sham clamp and clamp

groups. However, the increase in urine volume following clonidine (3 $ug/kg/min$) was decreased by the prevention of the associated blood pressure increase. The same trend is apparent with sodium excretion, in that 3 $ug/kg/min$ clonidine significantly increased sodium excretion from control in both the sham clamp and clamp groups. Again, the absolute increase in sodium excretion was decreased in the group whose renal perfusion pressure was controlled. This suggests that part of the effect of clonidine on urine volume and sodium excretion may be attributed to an increase in systemic blood pressure. In the presence of the V2 antagonist, clonidine failed to increase urine volume further from control. Sodium excretion was decreased with 3 $ug/kg/min$ clonidine in both the sham clamp and clamp groups. Moreover, the absolute decrease in sodium excretion was not different between these two groups.
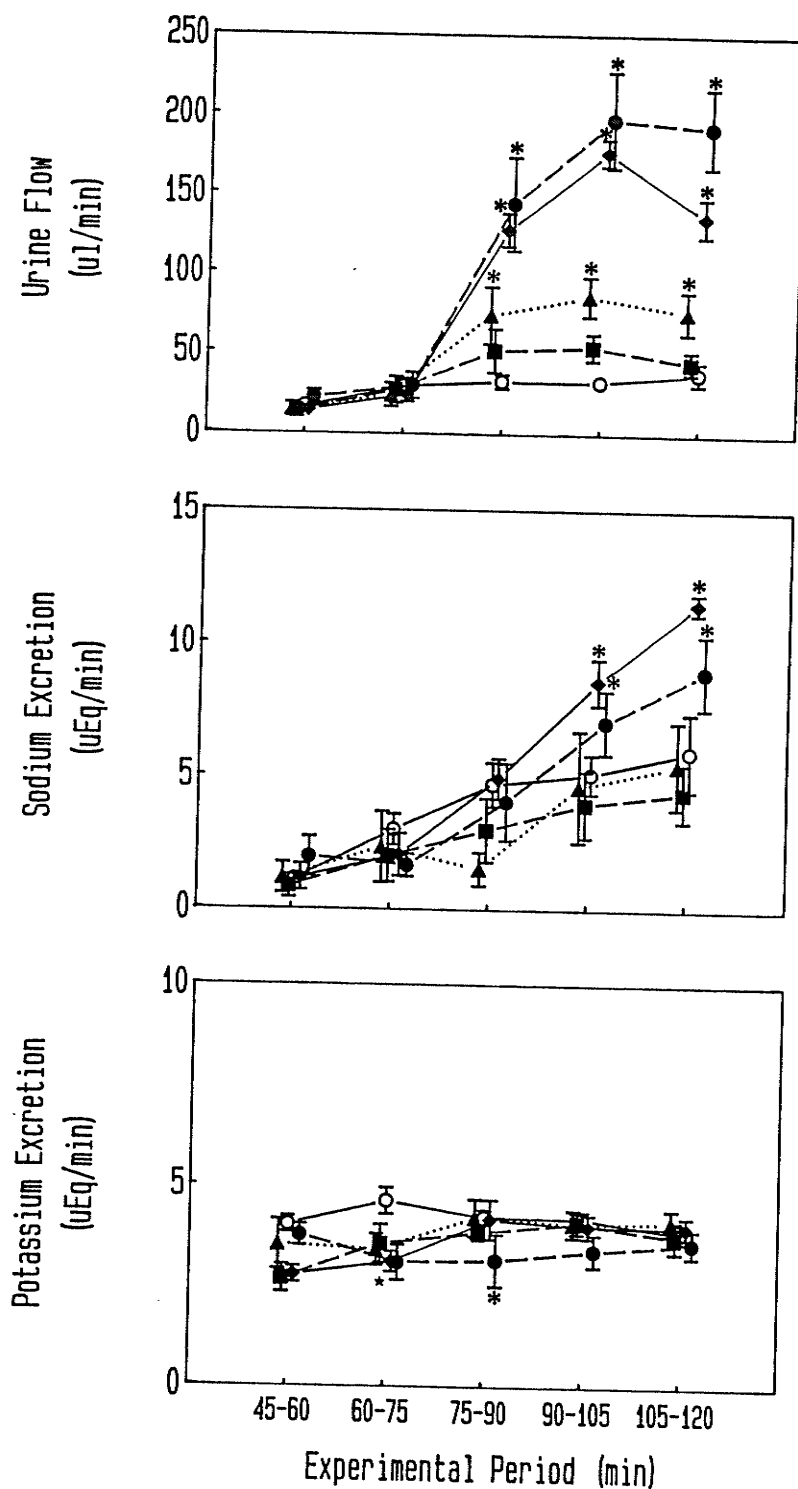
Fig. 7.1. Effect of intravenous administration of 0.2 ml of saline (vehicle) or [d(CH$_2$)$_5$,D-Ile^2,Ile^4]-AVP on urine flow, sodium excretion and potassium excretion. (O) saline vehicle, n=8; (■) 1 $n$mol/kg, n=6; (▲) 3 $n$mol/kg, n=6; (◆) 10 $n$mol/kg, n=6; (●) 30 $n$mol/kg, n=6.
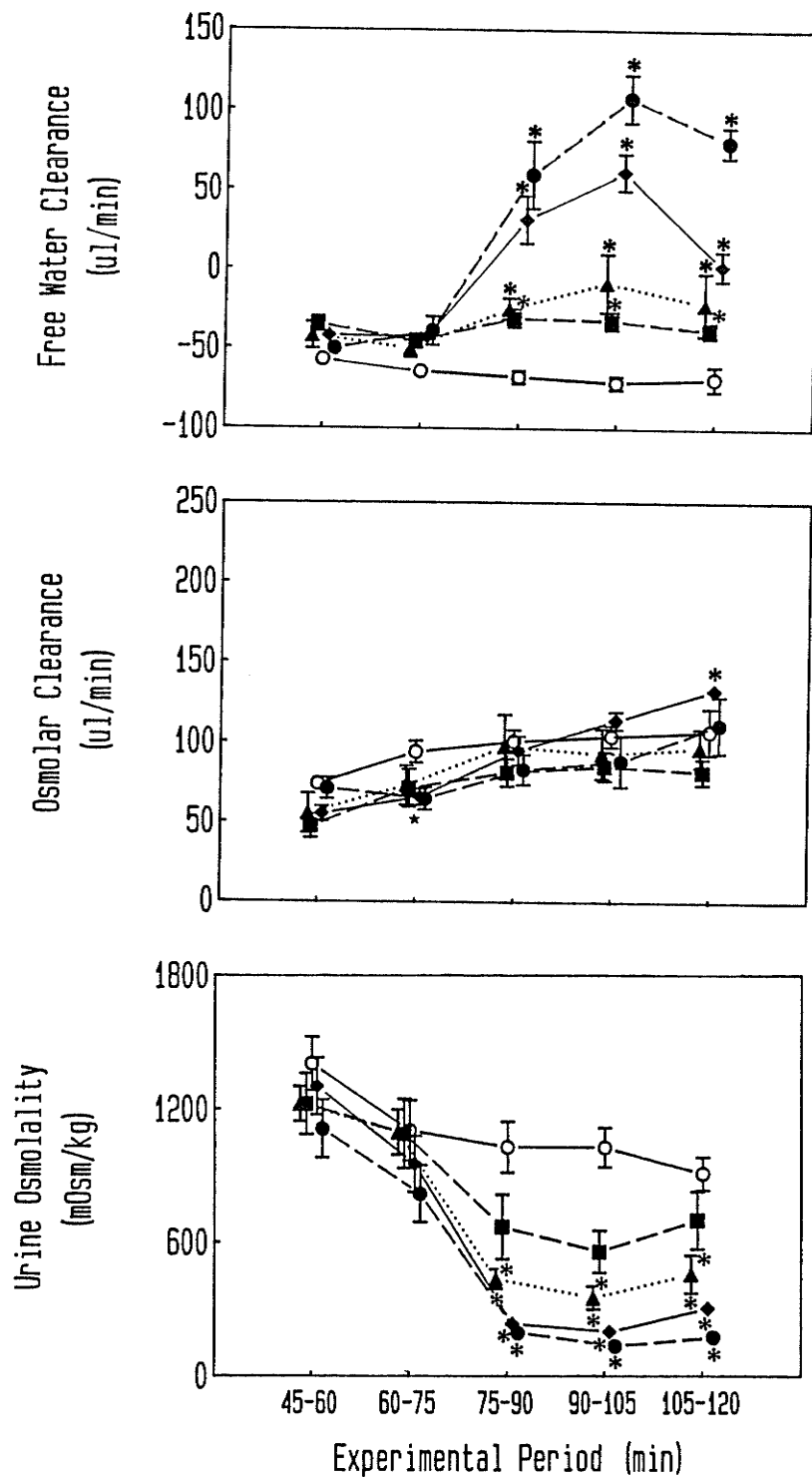
Fig. 7.2. Effect of intravenous administration of saline (0.2 ml) or [d(CH₂)₅,D-Ile²,Ile⁴]-AVP on free water clearance, osmolar clearance and urine osmolality. (○) saline vehicle, n=8; (■) 1 $n$mol/kg, n=6; (▲) 3 $n$mol/kg, n=6; (◆) 10 $n$mol/kg, n=6; (●) 30 $n$mol/kg, n=6.
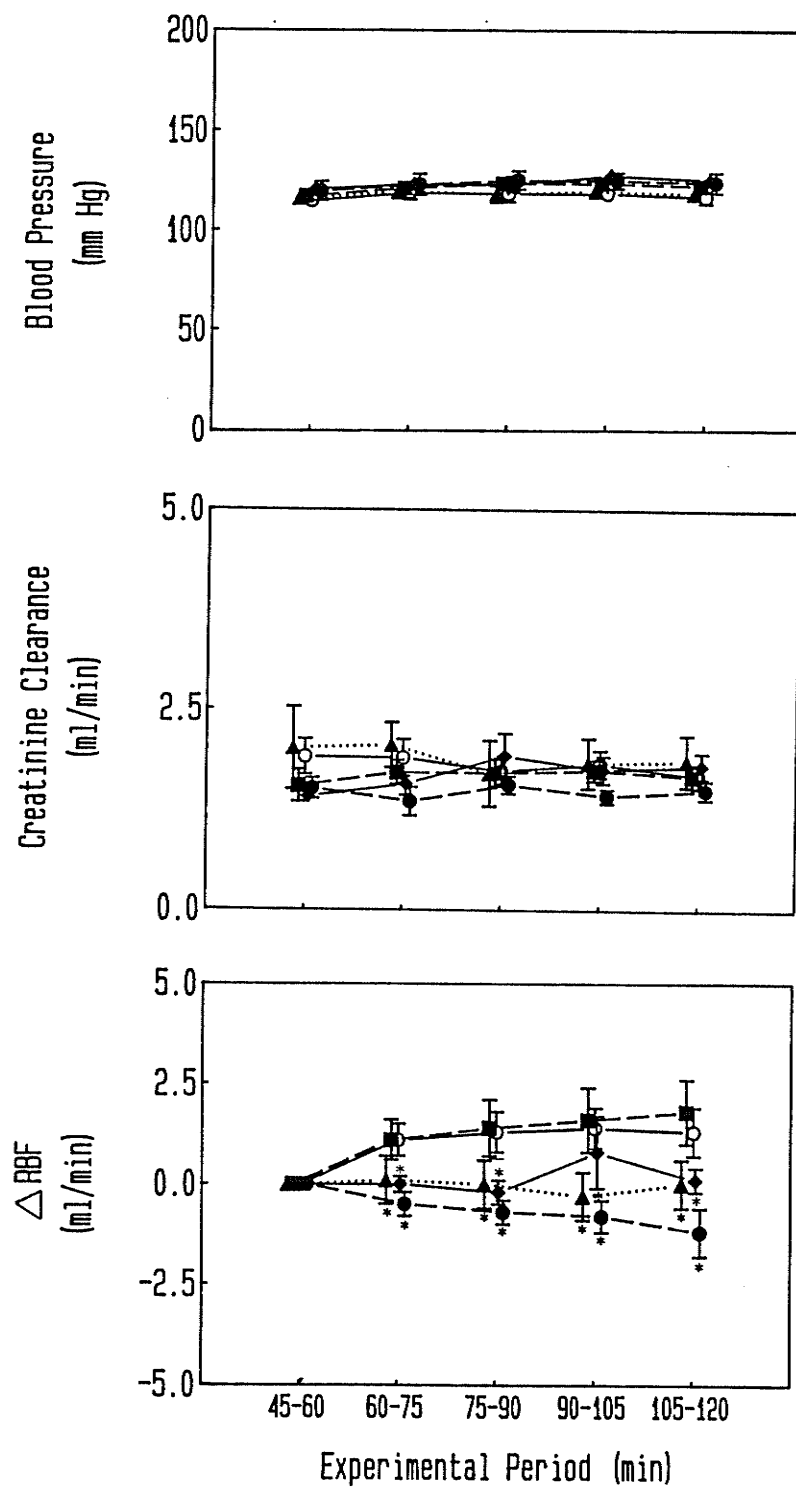
Fig. 7.3. Effect of intravenous administration of saline (0.2 ml) or [d(CH$_2$)$_5$,D-Ile^2,Ile^4]-AVP on blood pressure, creatinine clearance and the change in renal blood flow (ΔRBF). (O) saline vehicle, n=8; (■) 1 $n$mol/kg, n=6; (▲) 3 $n$mol/kg, n=6; (◆) 10 $n$mol/kg, n=6; (●) 30 $n$mol/kg, n=6.
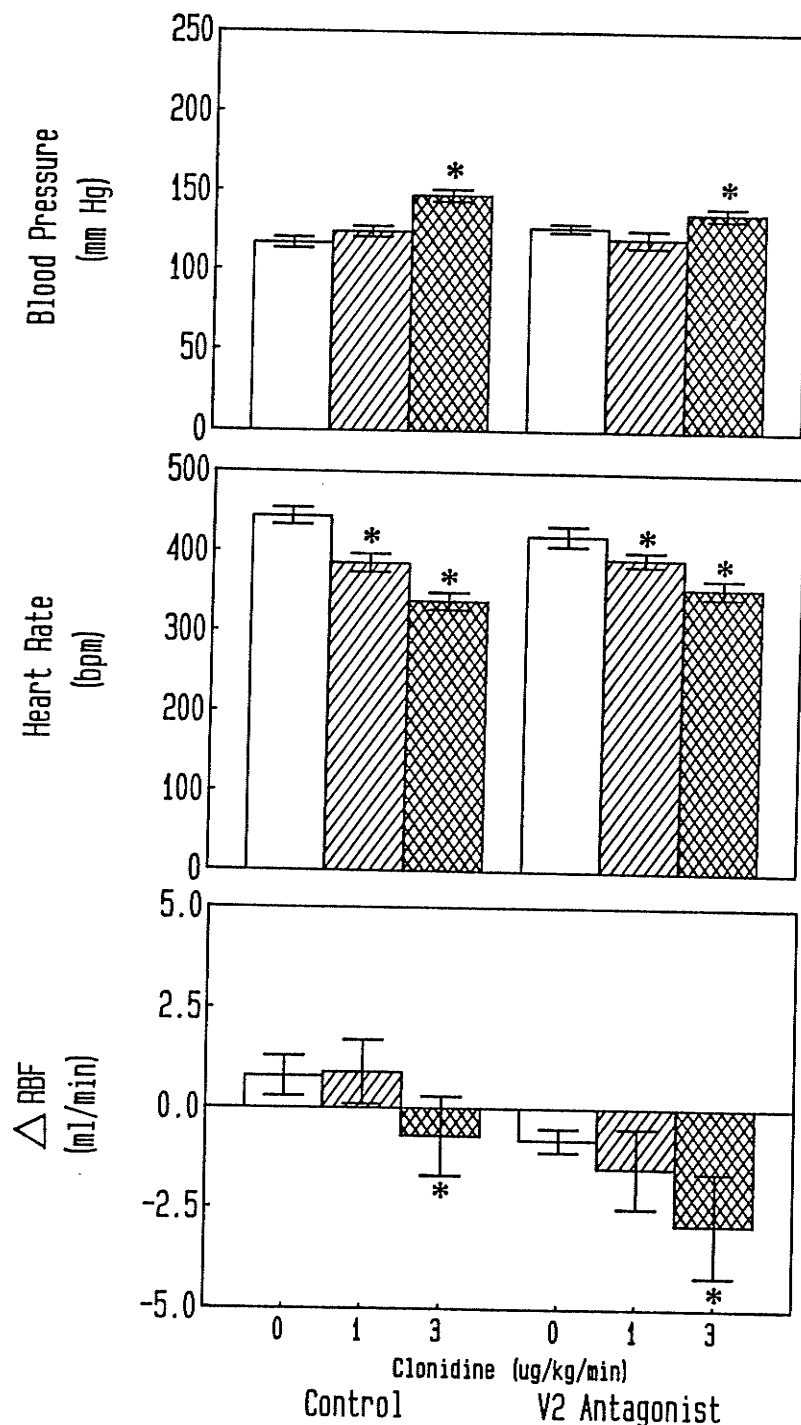
Fig. 7.4. Effects of an intrarenal infusion (3.4 ul/min) of clonidine in the absence and presence of [d(CH$_2$)$_5$,D-Ile^2,Ile^4]-AVP, on blood pressure, heart rate and the change in renal blood flow ($\triangle$RBF). Open bars represent control (vehicle), n=8 and 5 repectively; hatched bars represent clonidine 1 ug/kg/min, n=6 and 5 respectively; cross-hatched bars represent clonidine 3 ug/kg/min, n=6 and 6 respectively. These data represent the mean $\pm$ S.E.M. of the last three collection periods.
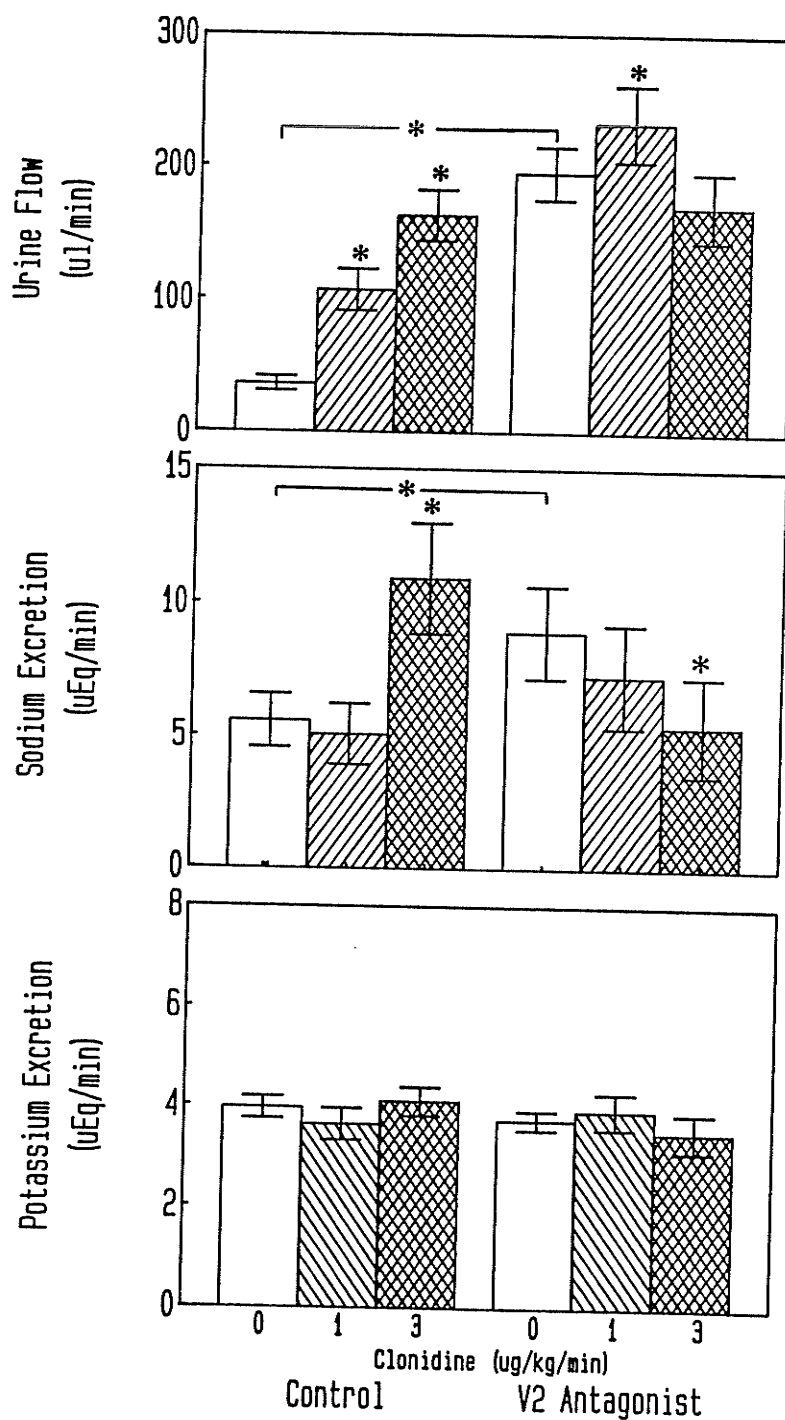
Fig. 7.5. Effect of an intrarenal infusion (3.4 ul/min) of clonidine in the absence and presence of [d(CH$_2$)$_5$,D-Ile^2,Ile^4]-AVP on urine flow, sodium excretion and potassium excretion. Open bars represent control, n=8 and 5 respectively; hatched bars represent clonidine 1 ug/kg/min, n=6 and 5 respectively; cross-hatched bars represent clonidine 3 ug/kg/min, n=6 and 6 respectively.
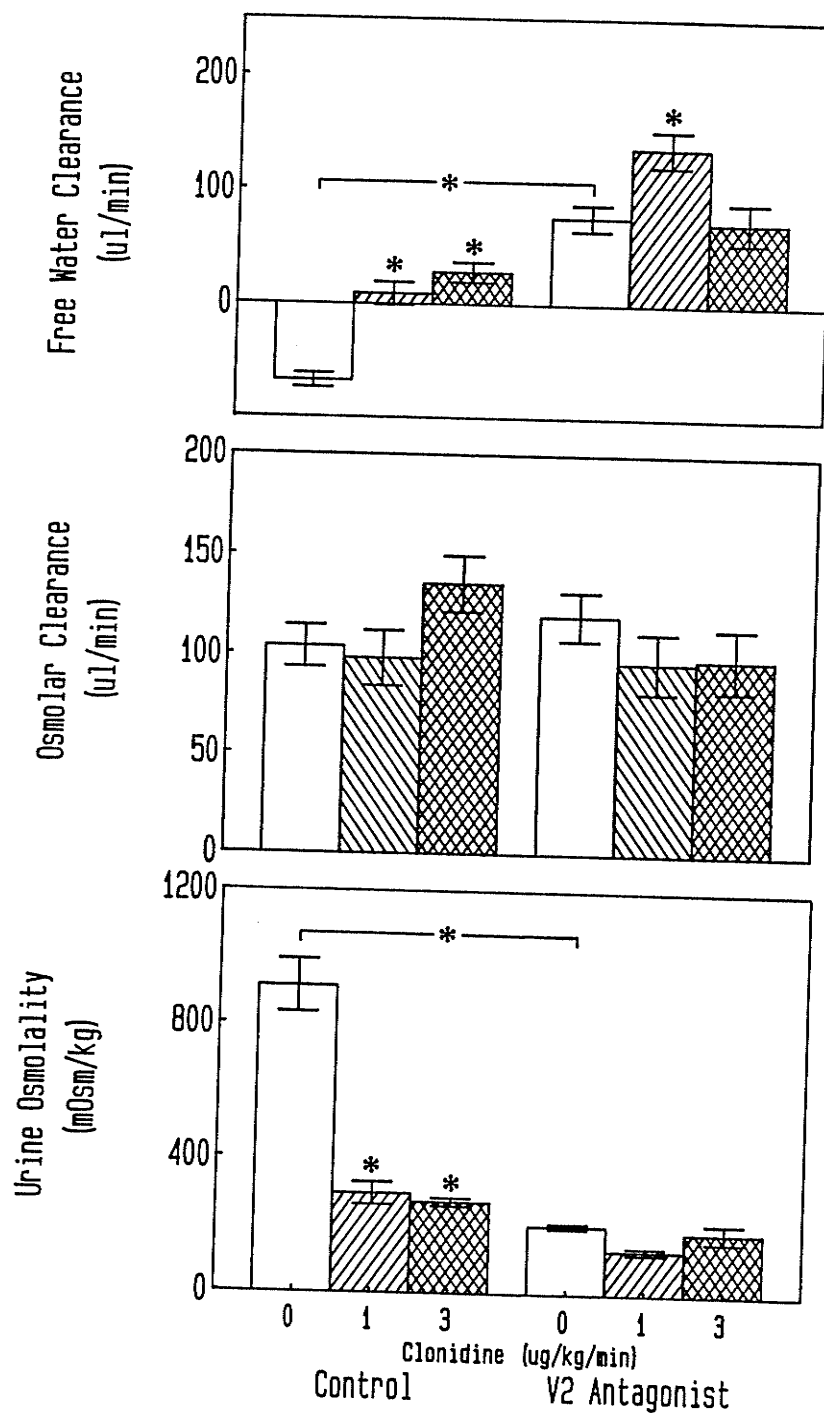
Fig. 7.6 Effect of an intrarenal infusion (3.4 ul/min) of clonidine in the absence and presence of $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]$-AVP on free water clearance, osmolar clearance and urine osmolality. Open bars represent control, n=8 and 5 respectively; hatched bars represent clonidine 1 ug/kg/min, n=6 and 5 respectively; cross-hatched bars represent clonidine 3 ug/kg/min, n=6 and 6 respectively.
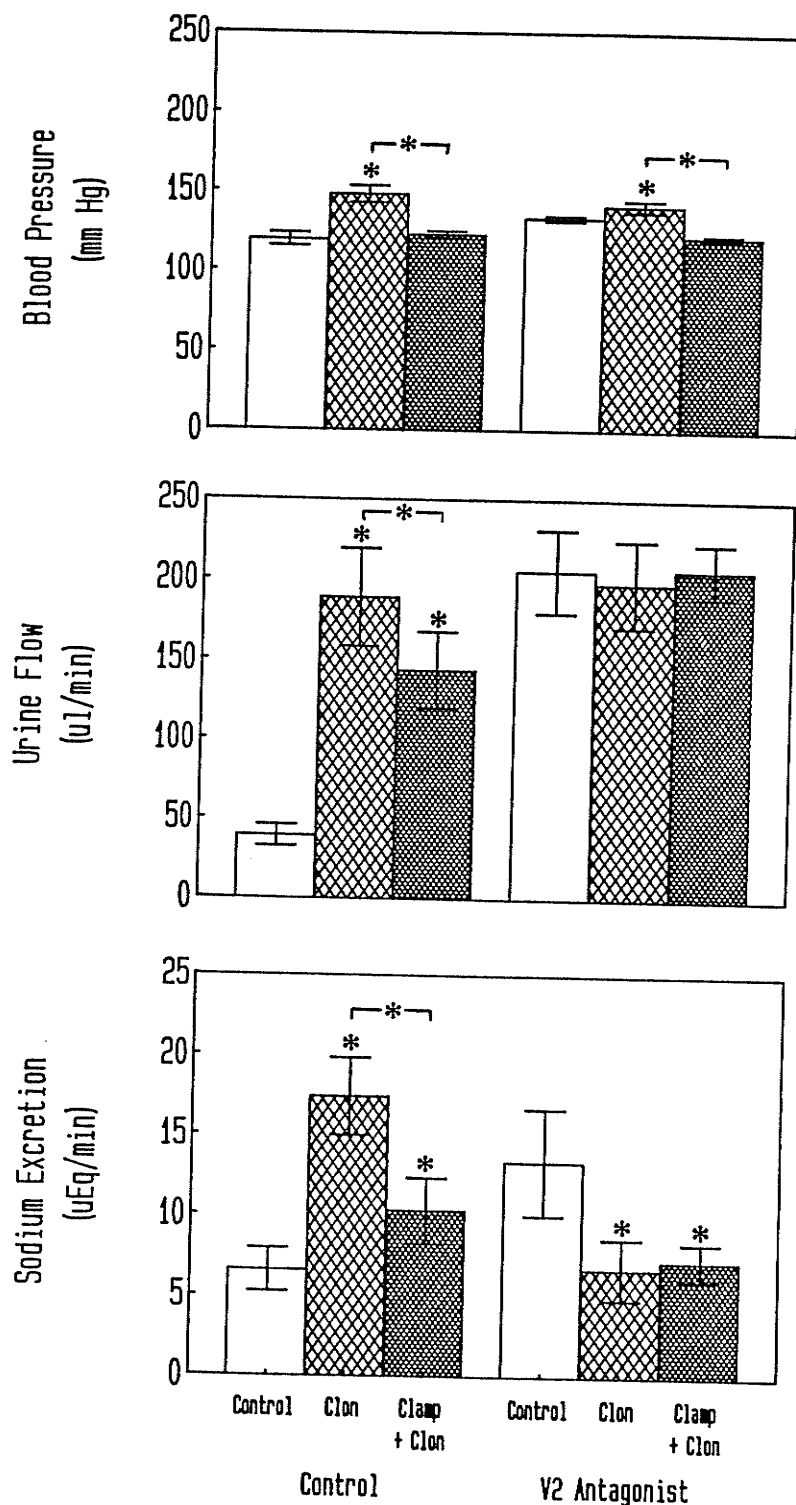
Fig. 7.7. Effect of a 3 $ug/kg/min$ infusion of clonidine with and without the use of a vascular occluder (clamp) in the absence and presence of $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]$-AVP, on blood pressure, urine flow and sodium excretion. Open bars represent control (vehicle); cross-hatched bars represent clonidine (3 $ug/kg/min$) without the clamp; dense cross-hatched bars represent clonidine (3 $ug/kg/min$) with the use of the clamp. These data represent the mean ± S.E.M. of the last three collection periods.

DISCUSSION

The effects of α2-adrenoceptor stimulation have been proposed to be mediated through the antagonism of the renal action of vasopressin (Gellai and Ruffolo, 1987; Miller, 1980; Roman *et al.*, 1979; Stanton *et al.* 1987; Strandhoy *et al.*, 1982). If this premiss was correct, then one would postulate that specific antagonists of the antidiuretic effect of vasopressin should produce the same effects as α2-adrenoceptor agonists. In this regard, in the present study, the dose response relationship of the V2 antagonist $[d(CH_2)_5,D\text{-}Ile^2,Ile^4]$-AVP, a highly potent and selective antidiuretic antagonist, (Manning *et al.*, 1984) was determined. Increasing i.v. bolus doses of the V2 antagonist (0, 1, 3, 10 or 30 *n*mol/kg) produced a dose-related increase in urine volume and free water clearance at lower doses (1 and 3 *n*mol/kg) with sodium excretion increased only at the higher doses (10 and 30 *n*mol/kg). This dose-related dissociation is similar to that reported for clonidine (Blandford and Smyth, 1988). This parallel is consistent with the postulate that the effects of clonidine were mediated through antagonism of the stimulatory effects of vasopressin on cAMP generation. Consequently, these results may seem to suggest that clonidine at high doses or infusion rates does not necessarily act independently of vasopressin as previously speculated (Blandford and Smyth, 1988b - Section 3; 1989a - Section 4). This would therefore imply that vasopressin plays an antinatriuretic role in *in vivo* preparations, a notion not generally accepted (Humphreys *et al.*, 1970; Johnson *et al.*, 1979; Martinez-Maldonado *et al.*, 1971; Pierce *et al.*, 1984; Schwartz and Reid, 1986; Smith *et al.*, 1979).

The increase in sodium excretion observed following the V2 antagonist is therefore somewhat surprising, given that vasopressin is believed to be natriuretic. It is, however, consistent with several other *in vivo* studies (Gellai *et al.*, 1984; Kinter *et al.*, 1986; Kinter *et al.*, 1987). However these studies focussed on the large

increases in urine volume and not the modest increase in sodium excretion. The absolute increase in sodium excretion in the present study could be due to an elevated rate of urine flow in the distal nephron segments (Wright *et al.*, 1977), inhibition of vasopressin-stimulated sodium reabsorption in the thick ascending limb of the loop of Henle, sodium and urea reabsorption in the cortical collecting tubule (Imbert-Teboul *et al.*, 1975; Imbert-Teboul *et al.*, 1978), or an inhibition of vasopressin-stimulated increase in the sodium conductance in the apical cell membrane, that is, an increase in sodium reabsorption in the rat cortical collecting tubule (Schlatter *et al.*, 1987). Thus, these results not only confirm the antidiuretic role for vasopressin, but also indicate an antinatriuretic one. However, given that urine osmolality decreases to levels lower than plasma osmolality, and that the vasopressin antagonist produced a dose-related increase in free water clearance (net positive free water clearance), it may be argued that V2 antagonists are true water diuretic or aquaretic agents as proposed by Kinter *et al.*, (1987). This does not necessarily rule out an antinatriuretic role for vasopressin, which would be consistent with the results of the *in vitro* experiments (Constantine *et al.*, 1982; Krothapalli *et al.*, 1983; Schlatter *et al.*, 1987; Smyth *et al.*, 1985).

This study is, as briefly mentioned previously, in contrast with other *in vivo* studies which suggest that vasopressin is natriuretic (Humphreys *et al.*, 1970; Johnson *et al.*, 1979; Martinez-Maldonado *et al.*, 1971; Pierce *et al.*, 1984; Schwartz and Reid, 1986; Smith *et al.*, 1979). These natriuretic effects may be related to elevations in glomerular filtration rate, or may be secondary to alterations in medullary solute concentrations or hydraulic gradients (Smith *et al.*, 1979). Infusions of vasopressin have also been shown to reduce renin secretion (Johnson *et al.*, 1973) and increase renal prostaglandin and kinin secretion (Nasjletti *et al.*, 1978), all of which could contribute to decreased renal tubular reabsorption of sodium. In addition, this natriuresis may be due to secondary increases in oxytocin,

which has been shown to function synergistically with vasopressin to increase sodium excretion (Balment *et al.*, 1986; Conrad *et al.*, 1986; Edwards and LaRochelle, 1984).

While there continues to be much controversy about whether vasopressin influences renal blood flow and glomerular filtration rate, in the present study the V2 antagonist decreased renal blood flow from control. These results suggest that endogenous vasopressin at renal tubular sites may increase renal blood flow. Alternatively, since V2-receptors are blocked, vasopressin would be unable to act at this site. It is possible that this displaced vasopressin is now available to bind with V1-receptors on blood vessels. Stimulation of these receptors would decrease renal blood flow. This hypothesis is however, inconsistent with the work of Gellai *et al.*, (1983, as cited by Valtin, 1987) where they demonstrated that inhibition of both the pressor and the antidiuretic effects of vasopressin had no effect on effective renal plasma flow in the Long Evans rat.

In the second series of experiments, the effects of clonidine were determined in the presence and absence of the renal effects of vasopressin using the maximal dose of the V2 antagonist used in the dose response study (30 *n*mol/kg). This dose was selected since it decreased urine osmolality to levels that are consistent with almost complete antagonism of vasopressin. A higher dose may have had some antivasopressor activity (Manning *et al.*, 1984). If the effects of clonidine at high doses are dependent on vasopressin, then in the presence of the V2 antagonist the effect of clonidine should be attenuated. Conversely, the large effect of clonidine on water and sodium excretion secondary to vasopressin inhibition may mask the effects of clonidine (sodium excretion or reabsorption) in other nephron segments.

As noted in previous experiments (Blandford and Smyth, 1988b - Section 3; 1989a - Section 4), clonidine alone resulted in a dose-related increase in urine volume and free water clearance. In the presence of the V2 antagonist alone, urine volume and free water clearance significantly increased from control demonstrating

again, as in the dose response relationship, the antidiuretic effect of vasopressin. Addition of 1 $ug/kg/min$ clonidine significantly increased both urine volume and free water clearance, while addition of 3 $ug/kg/min$ clonidine had no further effect, despite the fact that a 3 $ug/kg/min$ infusion of clonidine was accompanied by an increase in blood pressure (20 mm Hg). It is possible that the V2 antagonist may not have completely attenuated the antidiuretic effects of vasopressin, that is, the circulating levels of the drug may not have been sufficient to block all V2-receptors, and thus the addition of 1 $ug/kg/min$ clonidine may have further attenuated or antagonized the effects of vasopressin, resulting in a further increase in urine volume and free water clearance. However, given that, one would expect then, that a higher dose would have, at the very least, the same effect as the lower dose, if not a greater one. This did not occur.

With respect to sodium excretion, clonidine alone increased (doubled) sodium excretion only at the maximal dose (3 $ug/kg/min$). This was associated with an increase in blood pressure. The V2 antagonist alone increased sodium excretion as compared to control, suggesting again, that vasopressin, in addition to being antidiuretic is antinatriuretic. In the presence of the V2 antagonist, 3 $ug/kg/min$ clonidine significantly attenuated sodium excretion from control (i.e., V2 antagonist alone).

The mechanism by which clonidine produces these effects both in the presence and absence of vasopressin is not clear. In the presence of vasopressin, it is possible that the increase in systemic blood pressure may play a significant role in inducing a pressure-natriuresis. Similarly, the clonidine-induced decrease in sodium excretion seen in the presence of the V2 antagonist may be enhanced if the increase in blood pressure was prevented. Thus, we repeated the experiments with a 3 $ug/kg/min$ intrarenal infusion of clonidine and a vascular occluder to clamp the descending aorta to ensure that renal perfusion pressure remained constant. Both

in the presence and absence of the vascular occluder, clonidine alone increased urine volume and sodium excretion from control. However the absolute increase in urine volume and sodium excretion was attenuated in the absence of the V2 antagonist, suggesting that some of the effects of 3 ug/kg/min clonidine may be attributed to an increase in renal perfusion pressure. In the presence of the V2 antagonist, clonidine, with and without the use of the vascular occluder, had no effect on urine volume but decreased sodium excretion. Sodium excretion was not further attenuated by the use of the occluder. This could be due to the fact that clonidine decreased sodium excretion to baseline levels, which are already very low.

Baranowska *et al.*, (1987) demonstrated that clonidine in a bolus dose of 50 ug/kg significantly increased the plasma levels of atrial natriuretic peptide. Thus, high doses of clonidine may stimulate the release of atrial natriuretic peptide, resulting in both an increase in urine volume and sodium excretion. Alternatively, it has been suggested that the increase in atrial natriuretic factor may induce release of vasopressin from the rat posterior pituitary (Januszewicz *et al.*, 1985). This increase in vasopressin release may be of a sufficient magnitude to overcome the antagonism by the V2 antagonist, and thus exert its antidiuretic and antinatriuretic effects; decreasing urine volume (as compared to low doses of clonidine) and decreasing sodium excretion. However, other, more recent, *in vivo* studies demonstrate that atrial natriuretic factor, instead of inducing vasopressin release, may in fact inhibit its release (Lee *et al.*, 1987; Samson *et al.*, 1987). In addition, in our experiments where plasma ANP levels were measured (Smyth and Blandford, 1988 - Section 5), clonidine did not increase plasma ANP levels, suggesting that at least in the series of studies presented in this thesis, the natriuretic response to clonidine is not secondary to changes in circulating levels of ANP.

In summary, increasing i.v. doses of the V2 antagonist produced a dose-related increase in urine volume and free water clearance at all doses, with sodium

excretion increased only at the higher doses. This is similar to the dose-related dissociation in water and then solute excretion observed with increasing doses of clonidine. Intrarenal clonidine (1 ug/kg/min) in the presence of the V2 antagonist further increased urine volume and free water clearance, but not sodium excretion. In the presence of the V2 antagonist, intrarenal clonidine (3 ug/kg/min) had no effect on urine volume or free water clearance while sodium excretion was significantly decreased from control. This ability of V2 antagonists to mimic the effects of intrarenal clonidine suggests that clonidine may be mediating its effects through the antagonism of the renal effects of vasopressin. Moreover, they are consistent with not only an antidiuretic role for endogenous vasopressin, but also an antinatriuretic one. Finally, these results indicate that in the absence of vasopressin, stimulation of renal α2-adrenoceptors results in an antinatriuresis.

8

GENERAL DISCUSSION

In renal plasma membranes, α-adrenoceptors are known to be present with an approximate 2-fold greater density of $\alpha2$- over $\alpha1$-adrenoceptors (Sanchez and Pettinger, 1981; Schmitz et al., 1981). Renal $\alpha1$-adrenoceptors are generally believed to be important in mediating renal nerve stimulation-induced tubular sodium reabsorption (Osborn et al., 1982; 1983) and vasoconstriction (DiBona, 1982; Pettinger et al., 1985; Schmitz et al., 1981; Smyth et al., 1985b). The precise physiological role, however, of the numerically predominant $\alpha2$-adrenoceptors has not been delineated. Both $\alpha1$- and $\alpha2$-adrenoceptors have been proposed to alter electrolyte and fluid balance, yet their exact roles are not clearly understood. Smyth et al., (1985b) have proposed separate roles of α-adrenoceptor subtypes; renal nerve stimulation potentiates tubular water and sodium reabsorption via $\alpha1$-adrenoceptor stimulation, while $\alpha2$-adrenoceptors play an important role in the regulation of water and sodium excretion by modulating the actions of vasopressin and other hormones in the distal segments of the nephron.

Previous studies have described a close physiological association of renal $\alpha2$-adrenoceptors and vasopressin. Vasopressin, through stimulation of cAMP formation, has been shown to be antidiuretic and antinatriuretic in *in vitro* preparations, whereas in *in vivo* preparations vasopressin has been shown to be antidiuretic and natriuretic. The natriuretic effect of vasopressin, *in vivo*, has been postulated to be due to any of a number of alterations in vascular function, in glomerular function or in the circulating levels of general or local hormones (Cowley et al., 1988). Stimulation of $\alpha2$-adrenoceptors in the isolated perfused rat kidney (Smyth et al., 1985a) and in isolated cortical collecting tubules (Krothapalli et

al., 1983) inhibited the vasopressin-induced decrease in the excretion of sodium and water. These *in vitro* studies indicated that the effects of α2-adrenoceptors are mediated through the inhibition of cAMP production, particularly that increased by vasopressin. *In vivo*, there are a number of hormones which activate the adenylate cyclase-cAMP system in specific nephron segments. The function of these receptors is dependent on the pool of hormonally activated cAMP. However, the predominant function of these receptors *in vivo* was not known. In this regard, the series of experiments presented in this thesis have centered around the characterization of the renal α2-adrenoceptor. More specifically, the interrelationship of renal α2-adrenoceptors and vasopressin in the regulation of sodium and water excretion has been investigated using an intact, anesthetized rat preparation.

It was initially postulated that the infusion of an α2-adrenoceptor antagonist, yohimbine, directly into the renal artery would unmask the predominant endogenous activity of the α2-adrenoceptor. In the anesthetized rat, blockade of the α2-adrenoceptor with yohimbine resulted in a significant decrease in the excretion of sodium and water, and free water clearance, and an increase in urine osmolality (Blandford and Smyth, 1988a - Section 2). This study was one of the first descriptions of the blockade of the endogenous function of α2-adrenoceptors in the kidney. Since then, these results have been confirmed by Farjam and Greven (1989). In their study, the α2-adrenoceptor antagonists yohimbine and idazoxan decreased urine flow, and sodium and potassium excretion in both the intact and diabetes insipidus rat. Because the same effect was observed in the vasopressin-deficient rat, the authors concluded that vasopressin does not play a role in α2-adrenoceptor antagonist-induced antidiuresis. Thus, it would appear that α2-adrenoceptor stimulation exerts its effects on renal tubular sodium and water handling independently of vasopressin. This could represent additional evidence for

two sites or mechanisms of action of α2-adrenoceptor agonists. Alternatively, it may reflect an abnormality of the Brattleboro rat. Vasopressin deficiency in the Brattleboro rat is associated with a depletion of pituitary oxytocin content (Valtin *et al.*, 1965). In normal rats, elevated plasma osmolality is known to activate oxytocin releasing neurons (Brimble and Dyball, 1977), and raise plasma oxytocin concentrations (Balment *et al.*, 1980; Brimble *et al.*, 1978). In the Brattleboro rat, the raised plasma osmolality is associated with hypertrophy of the hypothalamic-hypophyseal neurons (Orkand and Palay, 1967), increased firing rate of the neurohypophyseal neurons (Dyball, 1974), and elevated plasma oxytocin levels (Brimble *et al.*, 1982; Boer *et al.*, 1988; Dogterom *et al.*, 1988; North *et al.*, 1982). While there have been numerous reports suggesting that oxytocin is capable of influencing renal function, the exact nature of this effect is unclear. Most investigators have reported that oxytocin causes an increase in urine flow (Jacobsen and Kellogg, 1956; Lees and Lockett, 1964; Rosas *et al.*, 1962), although an antidiuretic effect has also been observed in the normal rat (Sawyer and Valtin, 1967; Chan, 1965). When given in pharmacological doses, oxytocin has been found to significantly increase sodium excretion in the neurohypophysectomized rat (Balment *et al.*, 1986a; 1986b). When given in smaller, more physiological doses which produce plasma hormone levels consistent with those measured in an intact rat, oxytocin had no effect on the renal excretion of sodium. Therefore, given that plasma oxytocin levels are significantly elevated in the Brattleboro rat, and that oxytocin may have a diuretic and natriuretic effect when present in such large concentrations, the α2-adrenoceptor antagonists yohimbine and idazoxan may block the renal effects of oxytocin. However a link between α2-adrenoceptors and oxytocin has not yet been established. A direct way to determine if the α2-adrenoceptor antagonist-induced antidiuresis is independent of vasopressin would be by the selective ablation of the paraventricular and supraoptic nuclei of the

hypothalamus, the brain areas responsible for the production of vasopressin and oxytocin in normal rats. The renal effects of specific α2-adrenoceptor antagonists (and agonists) could then be determined both in the presence and absence of physiological concentrations of vasopressin. To determine what role, if any, oxytocin plays in the response to α2-adrenoceptor stimulation or antagonism, these studies could be repeated in the presence and absence of physiological concentrations of oxytocin.

Another possible explanation for the vasopressin independent effect observed by Farjam and Greven (1989) may be related to the ability of both yohimbine and idazoxan to bind to imidazoline-preferring receptors. This possibility may be more feasible, particularly since the dose of yohimbine used was 50 times greater than the dose used in the study presented in this thesis (Blandford and Smyth, 1988a - Section 2), and was administered as a bolus dose. This possibility is addressed later in more detail.

Further experiments presented in this thesis characterized these α2-adrenoceptors in detail by elucidating the dose-response relationship between an intrarenal infusion of clonidine, an α2-adrenoceptor agonist, and the effects on sodium and water excretion. By the study of the complete dose-response curve, two functions of these receptors, which had been missed in previous studies (due to the high doses utilized), were dissociated. At low infusion rates, clonidine only increased urine flow and free water clearance (Blandford and Smyth, 1988b - Section 3). These effects (both stimulation and antagonism of α2-adrenoceptors) were consistent with clonidine antagonizing the renal effects of vasopressin. Higher infusion rates of clonidine, however, also increased the excretion of sodium and osmolar clearance (Blandford and Smyth, 1988b - Section 3). This suggested that another mechanism or site of action may be involved in the response to α2-adrenoceptor agonists.

The natriuretic effect at high infusion rates suggested that an extrarenal effect may be involved. Alteratively, a vasopressin-independent effect or another site of action within the kidney may mediate this natriuretic effect. The enhanced natriuretic effect of clonidine when administered intravenously as compared to intrarenally (Blandford and Smyth, 1989a - Section 4) was strong evidence that an extrarenal site may mediate this natriuretic response. It was subsequently postulated that high infusion rates of clonidine may 1) increase the release of atrial natriuretic peptide (ANP), 2) increase the synthesis of renal prostaglandins, which have been shown to have natriuretic effects, or 3) decrease the central release of vasopressin. Alternatively, the natriuresis seen with high infusion rates of clonidine may be secondary to an increase in the release of oxytocin which has been shown to function synergistically with vasopressin to selectively increase sodium excretion in the neurohypophysectomized rat (Balment *et al.*, 1986b).

Measurement of plasma ANP levels indicated that the natriuresis seen with clonidine does not appear to be secondary to an increase in the release of ANP (Smyth and Blandford, 1988b - Section 5), at least with the infusion rates tested. On the other hand renal prostaglandins may be involved. The potentiation of the effects of clonidine following cyclooxygenase inhibition with indomethacin (Smyth and Blandford, 1989 - Section 6) is consistent with the endogenous production of prostaglandins playing an important regulatory role. The present results suggest that prostaglandin production, in response to clonidine infusion, has an apparent disparate effect. Free water clearance is increased whereas solute excretion (sodium) is decreased by these indomethacin-sensitive factors. The third possibility, i.e., a decrease in the central release of vasopressin, has not been addressed in this series of experiments. As discussed previously, α2-adrenoceptor stimulation has been associated with an inhibition of vasopressin-induced cAMP formation only in the cortical and medullary collecting tubules (Umemura *et al.*, 1985). Consequently,

α2-adrenoceptor stimulation with clonidine would antagonize the effects of vasopressin in this segment and decrease water permeability (Krothapalli and Suki, 1984). This would selectively increase water excretion. In a number of other nephron segments, including the thick ascending limb of the loop of Henle, vasopressin has been reported to increase sodium reabsorption (Hebert and Andreoli, 1984; Elalouf et al., 1984; Reif et al., 1986). As α2-adrenoceptors are not coupled to vasopressin receptors through the adenylate cyclase-cAMP system in these nephron segments, stimulation of α2-adrenoceptors would not be expected to reverse the effects of vasopressin on sodium excretion. If clonidine at high infusion rates decreases the endogenous release of vasopressin, as suggested by Barr and Kauker (1979) and Reid et al. (1979), the antinatriuretic effect of vasopressin would have been decreased. This would have increased both the antidiuretic (cortical and medullary collecting tubule) and antinatriuretic (thick ascending limb) effects of vasopressin. Since plasma vasopressin levels have not been measured in the present series of studies, this contention remains speculative. However recent studies have clearly documented that α2-adrenoceptor agonists have no effect on plasma vasopressin levels (Gellai and Edwards, 1988). More specifically, clonidine does not reduce plasma vasopressin levels in the rat (Leander et al., 1985).

The proposed effects of clonidine on oxytocin release have also not been investigated in this series of experiments, however, it represents another plausible avenue which needs further exploration. While there is no direct evidence as of yet, that α2-adrenoceptors increase the release of oxytocin, it is known that vasopressin and oxytocin can be released both simultaneously or independently depending upon the stimuli. Moreover, even when they are released simultaneously, they can be selectively regulated so that different quantities of hormone are released (Kasting, 1988). It is thus plausible that if α2-adrenoceptor stimulation decreases vasopressin release, the ratio of oxytocin to vasopressin would be increased. As mentioned

previously, the precise role of oxytocin in modulating renal function is unclear. It has however been established that oxytocin functions synergistically with vasopressin to increase sodium excretion in the rat (Balment *et al.*, 1986a; Conrad *et al.*, 1986; Edwards and LaRochelle, 1984).

As previously postulated, the results presented in this thesis indicate that two sites or mechanisms of action may exist for the renal effects of clonidine. The potentiation of the effects of clonidine following cyclooxygenase inhibition with indomethacin is consistent with the involvement of endogenous renal prostaglandins in antagonizing the natriuretic effect of this proposed second mechanism of action. Since prostaglandin synthesis is increased during adrenergic stimulation (Diz, *et al.*, 1981; Dunham and Zimmerman, 1970; Matsumura *et al.*, 1987), it stands to reason that clonidine would increase the synthesis of prostaglandins. Prostaglandins have long been believed to have effects at several sites involved in the regulation and maintenance of water balance, however the exact nature of their effects is unclear. They are believed to play an important role in modulating the renal actions of vasopressin, presumably by inhibiting the vasopressin-activated adenylate cyclase. The resultant decrease in cAMP formation, therefore, decreases the water permeability of the collecting duct (Grantham and Orloff, 1968). With respect to sodium handling, the results are at best controversial. Some studies support the concept that endogenous prostaglandins inhibit sodium transport (Leyssac *et al.*, 1975; Tannenbaum *et al.*, 1975), whereas others support the opposing standpoint, that prostaglandins enhance sodium transport (Kirschenbaum and Stein, 1976). Variations in hemodynamic changes, differences in experimental conditions and many other factors may be responsible for these discrepancies. Regardless of the exact nature of their function, it seems clear that renal prostaglandins alter water and sodium excretion, possibly by different mechanisms. Water excretion may be increased through prostaglandins by antagonism of the effects of vasopressin

(inhibition of vasopressin-induced adenylate cyclase), while sodium excretion may be modified by a direct tubular action of the prostaglandins. With respect to the present experiments with clonidine, preliminary studies in our laboratory suggest that the infusion rates used in this series of studies, increased the synthesis of renal prostaglandins. The effect of indomethacin, as well as that of prostaglandin E2 in the present study suggests that prostaglandins may be antinatriuretic (Smyth and Blandford, 1989; Section 6), which supports previous findings (Kirschenbaum and Stein, 1976). In addition, through the inhibition of the vasopressin-mediated increases in cAMP formation, prostaglandins would be expected to produce a water diuresis. This is consistent with the effects observed with low intrarenal infusion rates of clonidine. At high infusion rates of clonidine, sodium excretion was significantly elevated. This may reflect a predominance of the ability of prostaglandins to inhibit vasopressin-induced accumulation of cAMP over the ability of prostaglandins to alter tubular sodium reabsorption. This hypothesis would imply two sites of action. Moreover, it is conceivable that other $\alpha2$-adrenoceptor agonists may variably stimulate prostaglandin synthesis to a greater or lesser extent. Increased prostaglandin synthesis would result in a greater water or lesser solute excretion.

Ongoing experiments in our laboratory have recently uncovered a reverse rank order of potency for clonidine, UK 14,304 and 2,6-dimethyl-clonidine (Blandford *et al.*, 1990). A dose-related increase in sodium excretion and urine volume was observed with all three $\alpha2$-adrenoceptor agonists. Specifically, the rank order of potency for increasing urine flow was clonidine $\geq$ 2,6-dimethyl-clonidine > UK 14,304 and for increasing sodium excretion was 2,6-dimethyl-clonidine > clonidine $\geq$ UK 14,304. This apparent reverse rank order of potency was accentuated when solute and water excretion were analyzed. Free water clearance was most markedly increased with clonidine, followed by UK 14,304 with 2,6-

dimethyl-clonidine having little or no effect. Conversely, 2,6-dimethyl-clonidine increased osmolar clearance markedly, while clonidine and UK 14,305 produced a much smaller increase only at high infusion rates. These results strengthen our postulate that there may be two different sites of action for the renal effects of α2-adrenoceptors; one site linked to vasopressin, resulting in an increase in free water clearance, and another not coupled to vasopressin, which is associated with the increase in solute excretion. In this regard, it is interesting to speculate that clonidine, being more specific for water excretion would exert it effects at the site coupled to vasopressin while 2,6-dimethyl-clonidine, being more potent for solute (sodium) excretion may exert an effect at another site. Recall, however, that the administration of the V2 antagonist blocked the effects of all infusion rates of clonidine studied, suggesting that if two sites or mechanisms of action exist, both may be coupled to vasopressin.

This previously mentioned hypothesis is however, further strengthened by the fact the 2,6-dimethyl-clonidine, unlike clonidine increases urine volume without an increase in free water clearance (Blandford et al., 1990). Studies done in the presence of maximal doses of a V2-receptor antagonist, or in a water-loaded rat preparation, suggest that 2,6-dimethyl-clonidine increases solute excretion independent of vasopressin (Li et al., 1990). The results of the water-loaded rat preparation also demonstrate an increase in the delivery of filtrate from the proximal segments of the nephron. These results suggest that low infusion rates of 2,6-dimethyl-clonidine decrease sodium and water reabsorption in the proximal tubule of the rat, a site where vasopressin is without effect.

The exact nature of this proposed site in the proximal tubule is unknown. It is interesting to speculate that it may represent an α2-adrenoceptor subtype. α2-Adrenoceptor subtypes have recently been described (Bylund, 1985; Bylund, 1988; Bylund et al., 1988), and it is possible that as many as four subtypes exist; α2A, α2B,

α2c and a possible fourth subtype which has been proposed as a result of the cloning of α2-adrenoceptor subtypes by Lefkowitz and Regan (Kobilka *et al.*, 1987; Regan *et al.*, 1989). Recently, Stanko *et al.* (1990; Stanko and Smyth, in press) have characterized α2-adrenoceptors in a proximal tubular suspension. In their preparation, rauwolscine had a 2.7 times greater affinity than yohimbine, and prazosin had a relatively high affinity (K_i, 25 nM). These characteristics are consistent with that of an α2B subtype, which, in the neonatal rat lung was characterized by rauwolscine being 3 times more potent than yohimbine, and prazosin having a relatively high affinity. The effects of clonidine, in the studies presented in this thesis have been attributed to stimulation of the α2-adrenoceptor, since pretreatment with yohimbine attenuated the renal effects of clonidine (Blandford and Smyth, 1988b - Section 3). Prazosin had no effect on the clonidine-induced increase in urine flow rate and sodium excretion. The response of free water clearance, however was partially attenuated by prazosin pretreatment, primarily at the highest infusion rate investigated. As such, these results would also be consistent with an α2B-adrenoceptor subtype. It seems unlikely that the α2-adrenoceptor coupled to vasopressin may represent a different α2-adrenoceptor subtype. Nevertheless, it is possible that α2-adrenoceptors in different nephron segments may have different effects. In the collecting tubule, the α2-adrenoceptor is coupled to vasopressin. Stimulation of the α2-adrenoceptor in this segment may preferentially inhibit water reabsorption. In the proximal tubule, where vasopressin is without effect, stimulation of the α2-adrenoceptor may inhibit sodium reabsorption. On the other hand, the effect of clonidine on solute excretion may not occur at a proximal tubular site where α2-adrenoceptors have been characterized as being consistent with the α2B-adrenoceptor subtype. In the previously mentioned study (Blandford and Smyth, 1988b - Section 3), prazosin was unable to alter the

effects of clonidine on osmolar clearance. This however, is inconsistent with an α2B-adrenoceptor subtype, and thus may reflect a different α2-adrenoceptor subtype.

Alternatively, since both clonidine and 2,6-dimethyl-clonidine are imidazoline compounds, they may bind to and stimulate imidazoline receptors with different relative affinities. The existence of imidazoline-preferring adrenoceptors was first identified by Ruffolo *et al.* (1977). Moreover, the presence of both α2-adrenoceptors and imidazoline-binding sites in the basolateral membranes of rabbit renal proximal tubules has been demonstrated (Couprey *et al.*, 1989; Lechaud *et al.*, 1988). The functional role of these receptors, if any, in relation to electrolyte and water excretion, has not yet been identified. Moreover, since clonidine binds to both sites, it may be difficult to dissociate the effects at the α2-adrenoceptor from the effects of clonidine at an imidazoline receptor. In the present experiments with clonidine, the effects of clonidine have been attributed to the stimulation of α2-adrenoceptors since yohimbine, a specific α2-adrenoceptor antagonist, attenuated the clonidine-induced response. Ernsberger *et al.* (1988) have demonstrated that yohimbine also binds to imidazoline receptors with a relative high affinity. It is possible that the blockade of the actions of clonidine by yohimbine may be mediated at imidazoline sites. Interestingly, corynanthine, the yohimbine diastereoisomer, is much less potent (25-fold) than yohimbine at α2-adrenoceptors (IC_{50} (for $[^3H]$p-amino-clonidine) = 4700 ± 1500 vs. 179 ± 15 $n$mol/l respectively), but is equipotent at imidazoline sites in the ventrolateral medulla of the rat (Ernsberger *et al.*, 1988). On the other hand, rauwolscine appears to be selective for α2-adrenoceptors. It is less potent than both yohimbine and corynanthine at imidazoline sites, as it is unable to inhibit the binding of the imidazoline radioligand, idazoxan (IC_{50} > 100,000 $n$mol/l) (Lachaud *et al.*, 1988) It would be interesting to determine the rank order of potency for these α2-adrenoceptor antagonists on the inhibition of the renal effects of clonidine. This may allow us to determine which effects of clonidine

are mediated at imidazoline sites and which effects are mediated at the $\alpha 2$-adrenoceptor. Consequently, the effects of clonidine and 2,6-dimethyl-clonidine in producing a natriuresis may be mediated, in part, at an imidazoline-preferring receptor site. As such, 2,6-dimethyl-clonidine may have a greater relative affinity to this receptor than clonidine, and thus it is more potent in increasing solute excretion.

The effects of $\alpha 2$-adrenoceptor stimulation have been proposed to be mediated by the antagonism of the renal effects of vasopressin. If this premiss is correct, then one would postulate that specific antagonists of the antidiuretic effects of vasopressin (V2 antagonists) would produce the same effects as $\alpha 2$-adrenoceptor stimulation. Studies with the specific V2 antagonist, $[d(CH_2)_5, D\text{-}Ile^2, Ile^4]$-AVP, have demonstrated that blockade of the renal tubular vasopressin receptor mimics the effects of intrarenal infusions of clonidine. Increasing intravenous bolus doses of the V2 antagonist (0, 1, 3, 10 and 30 $n$mol/kg) produced a dose-related increase in urine volume and free water clearance at lower doses (1 and 3 $n$mol/kg) with sodium excretion increased only at the higher doses (Blandford and Smyth, 1989b - Section 7). This was similar to the dose-related dissociation in water and then sodium excretion observed with increasing infusion rates of clonidine (Blandford and Smyth, 1988b - Section 3), suggesting that the effects of clonidine, at high doses or infusion rates does not necessarily act independently of vasopressin as previously speculated (Blandford and Smyth, 1988b - Section 3; Blandford and Smyth, 1989 - Section 4). Moreover, these results further suggest that vasopressin plays an antinatriuretic role in *in vivo* preparations, a notion not generally accepted (Humphreys *et al.*, 1970; Johnson *et al.*, 1979; Martinez-Maldonado *et al.*, 1971; Pierce *et al.*, 1984; Schwartz and Reid, 1986, Smith *et al.*, 1979). As outlined previously, the natriuretic effects of vasopressin in the previous experiments may be related to elevations in glomerular filtration rate, or may be secondary to alterations

in medullary solute concentrations or hydraulic gradients (Smith *et al.*, 1979). Infusions of vasopressin have also been shown to reduce renin secretion (Johnson *et al.*, 1975) and kinin secretion (Nasjletti *et al.*, 1978), both of which could contribute to decreased renal tubular reabsorption of sodium. The natriuretic effects of vasopressin have not been consistent. Recently Cowley *et al.* (1988) demonstrated that chronic infusions of vasopressin resulted in a natriuresis only in the presence of volume expansion. This natriuresis is thus secondary to reflex hormonal effects and increased blood pressure. In addition, in experiments using the congenitally vasopressin deficient Brattleboro rat, infusions of vasopressin increased urine osmolality and decreased urine volume and sodium excretion, suggesting that vasopressin is antinatriuretic (Laycock and Williams, 1973). Studies utilizing specific antagonists of the renal tubular vasopressin receptors have shown large increases in urine volume, accompanied by only modest increases in sodium excretion (Gellai *et al.*, 1984; Kinter *et al.*, 1986; Kinter *et al.*, 1987). Given that urine osmolality was decreased to levels lower than plasma osmolality, and that a net positive free water clearance was observed, it may be argued that V2 antagonists are true water diuretic or aquaretic agents (Kinter *et al.*, 1987). Although sodium excretion was slightly elevated, the net effect of V2 antagonist administration may actually be sodium retention in relation to water. However, since total body sodium was not measured in the previous studies, this contention remains speculative. Alternatively, the absolute increase in sodium excretion, although somewhat masked by the phenomenal effect on water excretion, could be due to a number of factors. Sodium excretion could be due to an elevated rate of urine flow in the distal nephron segments (Wright, 1977), inhibition of vasopressin-stimulated sodium reabsorption in the thick ascending limb of the loop of Henle, an inhibition of sodium and urea reabsorption in the cortical collecting tubule (Imbert-Teboul *et al.*, 1975; 1978), or an inhibition of vasopressin-stimulated increase in sodium

conductance in the collecting tubule (Schlatter and Schafer, 1987). The results of the V2 antagonist experiments reported in this thesis are consistent with such possible actions of the V2 antagonists on sodium handling in the kidney.

The natriuretic effects of clonidine in the absence of endogenous vasopressin have also been investigated. Low infusions rates of clonidine in the presence of the V2 antagonist significantly increased urine volume and free water clearance with no effect on sodium excretion (Blandford and Smyth, 1989b - Section 7). In this study, high infusion rates of clonidine had no effect on urine volume and free water clearance, while sodium excretion was significantly decreased from control. This antinatriuretic effect of clonidine in the presence of a V2 antagonist may be the first *in vivo* observation of an α2-adrenoceptor which increases sodium and thus water reabsorption. The physiological significance of a sodium retaining α2-adrenoceptor (in the absence of vasopressin) is unclear. It may explain the increased density of α2-adrenoceptors in the spontaneously hypertensive rat (SHR) as compared to the Wistar-Kyoto rat (WKY). A recent study by Stanko and Smyth (in press) demonstrated that the increase in α2-adrenoceptor density in the SHR is located in the proximal tubule, where vasopressin is without effect. It may be argued that the effect of a sodium-retaining α2-adrenoceptor is normally masked by vasopressin in the intact rat. Only in pathological situations where α2-adrenoceptor density is significantly elevated, does this effect manifest itself. This argument, in and of itself is a weak one. Generally, a greater loss of water over sodium is observed with α2-adrenoceptor mediated antagonism of vasopressin. Michel *et al.* (1989) proposed that this differential excretion of sodium and water would result in a net increase in the concentration of sodium in the body. This increased total body sodium is the trigger for an increase in total peripheral resistance and thus, increased blood pressure. This hypothesis is consistent with the observation that the increase in α2-adrenoceptor density in SHR precedes the increase in blood pressure.

Consequently, a sodium retaining α2-adrenoceptor would be unnecessary for the development of increased blood pressure

Alternatively, recent investigations have supported a role of the Na^+ /H^+ antiporter in decreasing sodium excretion with α2-adrenoceptor stimulation. In a suspension of isolated rabbit renal proximal tubular cells, α2-adrenoceptors enhanced ^{22}Na-influx. The observation that an amiloride analogue, ethylisopropylamiloride, abolished the norepinephrine-induced sodium influx indicated that α2-adrenoceptors enhance sodium reabsorption by stimulation of the Na^+ /H^+ antiporter (Nord et al., 1987). This observation was recently extended to include rats, as well as α1- and other α2-adrenoceptor agonists (Gesek et al., 1989). Moreover, these observation were consistent with the measurement of oxygen consumption, a dynamic index of transcellular sodium transport in proximal and distal nephron segments (Gesek and Strandhoy, 1989). Since elevated cAMP levels have been shown to decrease antiporter activity (Pollock et al., 1986), Gesek and Schoolwerth (1990) and Gesek and Strandhoy (1990) have postulated that stimulation of α2-adrenoceptors, which increases cAMP, stimulates the antiporter. Functionally, this would be anticipated to increase sodium transport and result in a net decrease in sodium excretion. This might therefore account for the decrease in sodium excretion observed with clonidine in the absence of vasopressin. However, this postulate remains speculative since guanabenz has been shown to inhibit the Na^+ /H^+ exchanger. In this regard, Gesek and Schoolwerth (1990) have suggested that this may represent binding to an additional site other than the α2-adrenoceptor in the basolateral membrane. Stimulation of this receptor causes an inhibition of the antiporter. This would be consistent with recent reports of α2-adrenoceptor (Bylund, 1985) and imidazoline-preferring receptor (Michel and Insel, 1989) heterogeneity.

Summary:

The proposed mechanisms for the renal actions of clonidine, as suggested by the studies presented in this thesis, are depicted schematically in figure 8.1. Intrarenal arterial infusions of clonidine, at low infusion rates (< 3 ug/kg/min), resulted in an increase in urine volume and free water clearance. The increase in free water clearance is consistent with an antagonism of the renal effects of vasopressin (site (a) on diagram). Consequently, it seems likely that these effects of clonidine are mediated within the cortical and medullary collecting tubules where $\alpha2$-adrenoceptors have been shown to inhibit vasopressin-induced cAMP accumulation. This increase in free water clearance was blocked both by yohimbine, and to a lesser extent, by prazosin. These results are therefore consistent with the postulate that the renal effects of clonidine, in relation to free water clearance are mediated through an $\alpha2_B$-adrenoceptor subtype.

At high intrarenal arterial infusion rates of clonidine, a significant increase in sodium excretion and osmolar clearance was also observed. As suggested earlier, this natriuretic effect of clonidine may be due to an independent site or mechanism of action. We tentatively propose that this second site (site (b) on diagram) may represent another $\alpha2$-adrenoceptor subtype, an imidazoline-preferring receptor, or some other mechanism. Yohimbine blocked the effects on both sodium excretion and osmolar clearance. Since yohimbine binds to both $\alpha2$-adrenoceptors and imidazoline-preferring receptors, the exact nature of this proposed second site remains unclear. However it is possible that while clonidine acts preferentially at $\alpha2$-adrenoceptors, high doses may also stimulate the imidazoline-preferring adrenoceptor. The anatomical location for this second site remains questionable. A proximal tubular location has been suggested by the experiments of Li *et al.* (1990), which demonstrated that 2,6-dimethyl-clonidine, a clonidine analogue which is more potent than clonidine at increasing sodium excretion, exerts its effects within the
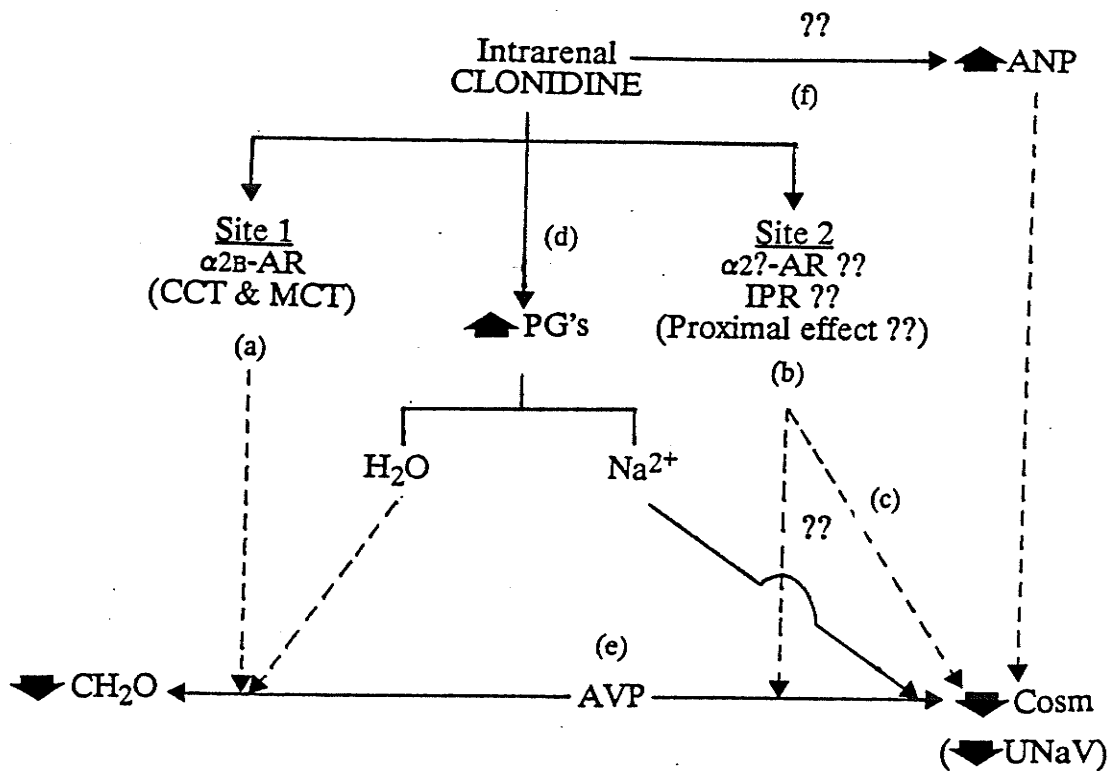
Fig. 8.1. Schematic for the proposed mechanisms of action for the renal effects of clonidine. For a discussion of the figure, please refer to the text. Atrial natriuretic peptide (ANP); vasopressin (AVP); renal prostaglandins (PG's); α_{2B}-adrenoceptor subtype (α_{2B}-AR); possible other α_2-adrenoceptor subtype (α_2?-AR); imidazoline-preferring receptor (IPR); free water clearance (CH20); osmolar clearance (Cosm); urinary sodium excretion (UNaV); cortical and medullary collecting tubules (CCT & MCT); —————→ stimulation, - - - - - - -→inhibition.

proximal tubule. A proximal tubular location however, may rule out another α2-adrenoceptor subtype since Stanko *et al.* (1990) have recently characterized α2-adrenoceptors in the proximal tubule, and have found them to be consistent with an α2B-adrenoceptor subtype. Whether the effects of clonidine in increasing sodium excretion and osmolar clearance at this second site are mediated by the antagonism of the antinatriuretic effects of vasopressin is a third item that remains questionable in this schematic (site (c) on diagram). The results of the study with the V2 antagonist suggest that the effects of clonidine at high infusion rates does not necessarily act independently of vasopressin since increasing doses of the V2 antagonist produced the same dose-related dissociation as the increasing infusion rates of clonidine (Blandford and Smyth, 1989b - Section 7). In contrast, the study of Farjam and Greven (1989) suggested that α2-adrenoceptor stimulation may exert its effects on renal tubular sodium and water handling independently of vasopressin, since both yohimbine and idazoxan decreased sodium and water excretion in the vasopressin-deficient Brattleboro rat. However, the results of their study may be attributable to the abnormality of the Brattleboro rat or to the blockade of both α2-adrenoceptors and imidazoline-preferring receptors as previously suggested. Both idazoxan (Lachaud *et al.*, 1988) and yohimbine (Ernsberger *et al.*, 1988) bind to the imidazoline-preferring receptor with a relative high affinity. Moreover, the dose of yohimbine was fifty times greater than the one used in the initial experiments presented in this thesis (Blandford and Smyth, 1988a - Section 2). The interactions of imidazoline-preferring receptors and vasopressin, if any, have not yet been established.

At site (d) in the schematic, clonidine has been shown to increase the synthesis of renal prostaglandins. Since the excretion of urinary prostaglandins has not been measured, this remains speculative. However, the experiments with indomethacin suggest this possibility (Smyth and Blandford, 1989 - Section 6). In

the present studies, infusions of prostaglandin E2 attenuated the indomethacin-induced increase in sodium excretion and osmolar clearance, and reversed the indomethacin-induced decrease in free water clearance. These results suggest that prostaglandins are diuretic, possibly by antagonizing the renal effects of vasopressin, as well as antinatriuretic, likely through a direct tubular action. Thus, prostaglandins are involved in the modulation of both sodium and water balance, possibly through independent mechanisms.

The enhanced natriuretic potency of intravenous clonidine (Blandford and Smyth, 1989 - Section 4) could also fit into this schematic. While we had originally postulated that this may represent an extrarenal site of action, it is nevertheless possible that it may reflect an intrarenal effect. An intrarenal infusion of clonidine may increase the synthesis of renal prostaglandins to an extent that they play an modulatory role in sodium and water handling. Intravenous infusions of clonidine may not, since clonidine concentrations within the kidney would presumably be less than that following an intrarenal arterial infusion. If prostaglandin synthesis is not sufficiently increased with an intravenous infusion of clonidine, the antinatriuretic effect of the prostaglandins would not be manifest, and this could account for the enhanced natriuretic response. In addition, if clonidine at high doses does act to decrease the central release of vasopressin, the increased systemic concentration of clonidine following an intravenous infusion would be more likely to produce decreased plasma vasopressin levels than an intrarenal infusion. This would consequently decrease the action of vasopressin in the cortical and medullary thick ascending limb of the loop of Henle, resulting in an increase in sodium excretion. As previously mentioned, plasma vasopressin and clonidine levels were not measured in the present series of studies. Moreover, the results obtained in other studies where plasma vasopressin levels were measured following α2-adrenoceptor stimulation are inconclusive.

The experiments outlined in Section 7 (Blandford and Smyth, 1989b) have demonstrated both an antinatriuretic and antidiuretic role for vasopressin. This is depicted at site (e) in the schematic. In the presence of the V2 antagonist, clonidine decreased the excretion of sodium. This effect is consistent with the schematic. Clonidine, by acting at both sites of action, antagonizes the renal actions of vasopressin. In addition, the antinatriuretic effect of the renal prostaglandins would also serve to further decrease sodium excretion. Finally, the proposed role of ANP is depicted at site (f). Whether the intrarenally administered clonidine increases plasma levels of ANP remains questionable. In the present experiments (Smyth and Blandford, 1988c - Section 5), clonidine was unable to significantly increase plasma levels of ANP. This does not however, exclude the potential importance of ANP release at higher infusion rates of clonidine.

The experiments presented in this thesis have uncovered a number of interesting results. These results may represent physiological or functional correlates to recent biochemical and molecular pharmacological advances in the field of adrenergic pharmacology. Clearly, these studies leave a number of unanswered questions. These could provide the basis for future investigations using an *in vivo* approach.

REFERENCES

Ahlquist, R.P.: A study of the adrenotropic receptors. Am. J. Physiol. 153:586-600, 1948.

Al-Awqati, W. and Greenough, W.B.: Prostaglandins inhibit intestinal sodium transport. Nature 238:26-27, 1972.

Alvo, M., Espinoza, A.M., Fernandes, V. and Marusic, E.T.: Alpha2-agonists block ADH action in toad bladder and this inhibition is not modified by indomethacin. Am. J. Physiol. 255:F74-F77, 1988.

Anderson, R.J., Berl, T., McDonald, K.M. and Schrier, R.W.: Evidence for an in vivo antagonism between vasopressin and prostaglandin in the mammalian kidney. J. Clin. Invest. 56:420-426, 1975.

Balment, R.J., Brimble, M.J. and Forsling, M.L.: Release of oxytocin induced by salt loading and its influence on renal excretion in the male rat. J. Physiol. (Lond.) 308:439-449, 1980.

Balment, R.J., Brimble, M.J. and Forsling, M.L.: Oxytocin release and renal actions in normal and brattleboro rats. Ann. N.Y. Acad. Sci. 394:241-253, 1982.

Balment, R.J., Brimble, M.J., Forsling, M.L., Kelly, L.P. and Musabayane, C.T.: A synergistic effect of oxytocin and vasopressin on sodium excretion in the neurohypophysectomized rat. J. Physiol. (Lond.) 381:453-464, 1986a.

Balment, R.J., Brimble, M.J., Forsling, M.L. and Musabayane, C.T.: The influence of neurohypophysial hormones on renal function in the acutely hypophysecomized rat. J. Physiol. (Lond.) 381:439-452, 1986b.

Baranowska, B., Gutkowska, J., Cantin, M. and Genest, J.: Plasma immunoreactive atrial natriuretic factor (IR-ANF) increases markedly after alpha2-adrenergic stimulation with clonidine in normally hydrated rats. Biochem. Biophys. Res. Commun. 143:159-163, 1987.

Barr, J.G. and Kauker, M.L.: Renal tubular site and mechanism of clonidine-induced diuresis in rats; clearance and micropuncture studies. J. Pharmacol. Exp. Ther. 209:389-395, 1979.

Beck, N.P., Reed, S.W., Murdough, M.V. and Davis, B.B.: Effects of catecholamines and their interaction with other hormones on cyclic 3',5'-adenosine monophosphate of the kidney. J. Clin. Invest. 51:939-944, 1972.

Beierwaltes, W.H., Schryver, S., Olsen, P.S. and Romen, J.C.: Interaction of the prostaglandin and renin-angiotensin systems in isolated rat glomeruli. Am. J. Physiol. 239:F602-F608, 1980.

Bello-Reuss, E.: Effect of catecholamines on fluid reabsorption by the isolated proximal convoluted tubule. Am. J. Physiol. 238:F347-F352, 1980.

Bello-Reuss, E., Colindres, R.E., Pastoriza-Munoz, E., Mueller, R.A. and Gottschalk, C.W.: Effects of acute unilateral renal denervation in the rat. J. Clin. Invest. 56:208-217, 1975.

Bello-Reuss, E., Trevino, D.L. and Gottschalk, C.W.: Effect of renal sympathetic nerve stimulation on proximal water and sodium reabsorption. J. Clin. Invest. 57:1104-1107, 1976.

Bencsath, P., Szalay, L., Debreczeni, L.A., Takacs, L.A., Vajda, L. and Fischer, A.: Denervation diuresis and renin secretion in the anaesthetized dog. Eur. J. Clin. Invest. 2:422-425, 1972.

Bencsath, P., Szalay, L. and Takacs, L.: Natriuresis and renin release by the denervated dog kidney during furosemide administration. Eur. J. Clin. Invest. 6:445-448, 1976.

Berl, T., Raz, R., Wald, H., Horowitz, J. and Czaczkes, W.: Prostaglandin synthesis inhibition and the action of vasopressin: Studies in man and rat. Am. J. Physiol. 232:F529-F537, 1977.

Berridge, M. J. and Irvine, R.F.: Inositol triphosphate, a novel second messenger in cellular transduction. Nature 312:315-321, 1984.

Berthelson, S. and Pettinger, W.A.: A functional basis for classification of alpha-adrenergic receptors. Life. Sci. 21:595-606, 1977.

Blandford, D.E. and Smyth, D.D: Renal $\alpha2$-adrenoceptor blockade decreases sodium and water excretion in the anesthetized rat. Eur. J. Pharmacol. 154:117-124, 1988a.

Blandford, D.E. and Smyth, D.D.: Dose selective dissociation of water and solute excretion following renal alpha2-adrenoceptor stimulation. J. Pharmacol. Exp. Ther. 247:1181-1186, 1988b.

Blandford, D.E. and Smyth, D.D.: Enhanced natriuretic potency of intravenous clonidine: Extrarenal site of action? Eur. J. Pharmacol. 174:181-188, 1989a.

Blandford, D.E. and Smyth, D.D.: The effects of intrarenal clonidine are mimicked by vasopressin (V2) antagonists. (Abstract) FASEB J. 3(3):A415, 1989b.

Blandford, D.E., Thom, S.L., Li, P. and Smyth, D.D.: Reverse rank order of potency for clonidine, 2,6-dimethyl-clonidine (DMC) and UK 14,304 on water and solute excretion in the rat. (Abstract) In press, Clin. Invest. Med., 1990.

Brimble, M.J. and Dyball, R.E.J.: Characterization of the responses of oxtyocin- and vasopresin-secreting neurones in the supraoptic nucleus to osmotic stimulation. J. Physiol. (Lond.) 271:253-271, 1977.

Brimble, M.J., Dyball, R.E.J. and Forsling, M.L.: Oxytocin release following osmotic activation of oxytocin neurones in the paraventricular and supraoptic nuclei. J. Physiol. (Lond.) 278:68-78, 1978.

Boer, G.J., van Heerikhuize, J. and van der Woude, T.P.: Elevated serum oxytocin of the vasopressin deficient Brattleboro rat is present throughout life and is not sensitive to treatment with vasopressin. Acta. Endocrinol. 117:442-450, 1988.

Bonjour, J.P. and Malvin, R.L.: Plasma concentrations of ADH in conscious and anesthetized dogs. Am. J. Physiol. 218:1128-1132, 1970.

Boyajian, C.L., Loughlin, S.E. and Leslie, F.M.: Anatomical evidence for alpha-2 adrenoceptor heterogeneity: Differential autoradiographic distribution of [³H]rauwolscine and [³H]idazoxan in rat brain. J. Pharmacol. Exp. Ther. 241:1079-1091, 1987.

Bridle, P.A., Brosniham, K.B., Speth, R.C., Goormastic, M. and Ferrario, C.M.: Basal levels of plasma epinephrine and norephinephrine in the dog. Hypertension 5 (Suppl. V), V128-V133, 1983.

Bylund, D.B.: Heterogeneity of alpha2-adrenergic receptors. Pharmacol. Biochem. Behav. 22:835-843, 1985.

Bylund, D.B.: Subtypes of alpha2-adrenoceptors: Pharmacological and molecular biological evidence converge. Trends Pharmacol. Sci. 9:356-361, 1988.

Bylund, D.B., Ray-Prenger, C. and Murphy, T.J.: Alpha-2A and alpha-2B adrenergic receptor subtypes: Antagonist binding in tissues and cell lines containing only one subtype. J. Pharmacol. Exp. Ther. 245:600-607, 1988.

Bylund, D.B. and U'Prichard, D.C.: Characterization of alpha-1 and alpha-2 adrenergic receptors. Int. Rev. Neurobiol. 24:343-422, 1983.

Campbell, W., Graham, R. and Jackson, E.: Role of renal prostaglandins in sympathetically mediated renin release in the rat. J. Clin. Invest. 64:448-456, 1979.

Cavero, I., Shepperson, F., Lefevre-Borg, F. and Langer, S.Z.: Differential inhibition of vascular smooth muscle responses to α1- and α2-adrenoceptor agonists by diltiazem and verapamil. Circ. Res. 52 (Suppl. 1):69-76, 1983.

Chabardes, D., Brick-Ghannam, C., Montegut, M. and Siaume-Perez, S.: Effect of PGE_2 and alpha-adrenergic agonists on AVP-dependent cAMP levels in rabbit and rat CCT. Am. J. Physiol. 255:F43-F48, 1988.

Chabardes, D., Montegut, M., Imbert-Teboul, M. and Morel, F.: Inhibition of alpha2-adrenergic agonists on AVP induced cAMP accumulation in isolated collecting tubule of the rat kidney. Mol. Cell. Endocrinol. 37:263-275, 1984.

Chan, W.Y.: Effects of neurohypophysial hormones and their deamino analogues on renal excretion of Na^+, K^+ and water in rats. Endocrinol. 77:1097-1104, 1965.

Chrysanthakopoulos, S.G. and Lavender, A.R.: Water diuresis from clonidine (Catapress). Proc. Soc. Exp. Biol. Med. 150:85-91, 1975.

Conrad, K.P., Gellai , M., North, W.G. and Valtin, H.: Influence of oxytocin on renal hemodynaics and electrolyte and water excretion. Am. J. Physiol. 251:F290-296, 1986.

Constantine, J.W., Lebel, W. and Archer, R.: Functional postsynaptic alpha2- but not alpha1-adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. Eur. J. Pharmacol. 85:325-329, 1982.

Coupry, I., Lachaud, V., Podevin, R-A., Koenig, E. and Parini, A.: Different affinities of α2-agonists for imidazoline and α2-adrenergic receptors. Am. J. Hypertens. 2:468-470, 1989.

Cowley, A.W. Jr., and Liard, J.F.: Cardiovascular actions of vasopressin, in : Vasopressin Principles and Properties, Eds. Gash, D.M. and Boer, G.J. (Plenum Press, New York) p. 392, 1987.

Cowley, A.W. Jr., Merrill, D.C., Smith, M.J. and Skelton, M.M.: Role of vasopressin in regulation of sodium excretion. Am. J. Med. Sci. 294:308-313, 1988.

Dale, H.H.: On some physiological actions of ergot. J. Physiol. (Lond.) 34:163-206. 1906.

DeBold, A.J., Borenstein, H.B., Veress, A.T. and Sonnenbery, H.: A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci. 28:89-94, 1981.

DeMay, J.G. and Vanhoutte, P.M.: Uneven distribution of postjunctional α1- and α2-like adrenoceptors in canine arterial and venous smooth muscle. Circ. Res. 48:875-884, 1981.

DiBona, G.F.: Neurogenic regulation of renal tubular sodium reabsorption. Am. J. Physiol. 233:F73-F81, 1977.

DiBona, G.F.: The functions of renal nerves. Rev. Physiol. Biochem Pharmacol. 94:75-181, 1982.

DiBona, G.F., Fildes, R.D., Eisner, G.M. and Jose, P.A.: Renal alpha-adrenoceptors and sodium excretion. Am. J. Physiol. (Letters to the Editor) 19:F949, 1986.

DiBona, G.F. and Sawin, L.L.: Role of renal alpha2-adrenergic receptors in spontaneously hypertensive rats. Hypertension 9:41-48, 1987.

DiBona, G.F., Zambraski, E.J., Aguilera, A.J. and Kaloyanides, G.J.: Neurogenic control of renal tubular sodium reabsorption in the dog. Circ. Res. 40:I-127 - I-130, 1977.

Diz, D.I., Baer, P.G. and Nasjletti, A.: Effect of norephinephrine and renal denervation on renal PGE_2 and kallikrein in rats. Am. J. Physiol. 241:F477-F481, 1981.

Docherty, J.R. and McGrath, J.C.: A comparison of pre- and postjunctional potencies of several α-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. Evidence for two types of postjunctional α-adrenoceptors. Arch. Pharmacol. 312:107-116, 1980.

Dogterom, J., van Wimersma Greidanus, T.B. and Swaab, D.F.: Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: Measurements in plasma and CSF of intact and hypophysectomised rats. Neuroendocrinol. 24:108-118, 1977.

Drew, G.M. and Whiting, S.B.: Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle in vivo. Br. J. Pharmacol. 67:207-215, 1979.

Dubocovich, M.L. and Langer, S.Z.: Negative feedback regulation of noradrenaline release by nerve stimulation in the perfused cat's spleen: Differences in potency of phenoxybenzamine in blocking the pre- and postsynaptic adrenergic receptors. J. Physiol. (Lond.) 237:505-519, 1974.

Dunham, E.W. and Zimmerman, B.G.: Release of prostaglandin like material from dog kidney during nerve stimulation. Am. J. Physiol. 219:1279-1285, 1970.

Dyball, R.E.J.: Single unit activity in the hypothalamo-neurohypophysial system of Brattleboro rats. J. Endocrinol. 60:135-143, 1974.

Edwards, B.R. and LaRochelle, F.T.: Antidiuretic effect of endogenous oxytocin in dehydrated Brattleboro homozygous rats. Am. J. Physiol. 247:F453-465, 1984.

Elalouf, J.M., Roinel, N. and DeRouffignac, C.: Effect of antidiuretic hormone on electrolyte reabsorption and secretion in distal tubules of rat kidney. Pfluegers Arch. 401:167-173, 1984.

Ernsberger, P., Giuliano, R., Willette, R.N., Granata, A.R. and Reis, P.J.: Hypotensive action of clonidine analogues correlates with binding affinity at imidazole and not alpha-2-adrenergic receptors in the rostral ventrolateral medulla. J. Hypertens. 6:S554-S557, 1988.

Exton, J.H.: Molecular mechanisms involved in α-adrenergic responses. Trends. Pharmacol. Sci. 3:111-115, 1982.

Fain, J.N. and Garcia-Sainz, J.A.: Role of phosphatidylinositol turnover in alpha-1 and adenylated cyclase inhibition in alpha-2 effects of catecholamines. Life. Sci. 26:1183-1194, 1980.

Farjam, A. and Greven, J.: Effects of the alpha2-adrenoceptor antagonists yohimbine and idazoxan on kidney function in intact and diabetes insipidis rats. Urol. Int. 44:255-259, 1989.

Fassina, G., Carpenedo, F. and Santi, R.: Effect of prostaglandin E_1 on isolated short-circuited frog skin. Life Sci. 8:181-187, 1987.

Fejes-Toth, G., Magyar, A. and Walter, J.: Renal response to vasopressin after inhibition of prostaglandin synthesis. Am. J. Physiol 232:F416-F423, 1977.

Fejes-Toth, G. and Szenasi, G.: The effect of vasopressin on renal tubular ^{22}Na efflux in the rat. J. Physiol. (Lond.) 318: 1-7, 1981.

Fildes, R.D., Eisner, G.M., Calcagno, P.L. and Jose, P.A.: Renal alpha-adrenoceptors and sodium excretion in the dog. Am. J. Physiol. 248:F128-F133, 1985.

Fine, L.G. and Trizna, W.: Influence of prostaglandins on sodium transport of isolated medullary nephron segments. Am. J. Physiol. 232:F383-F390, 1977.

Flynn, T.G., de Bold M.L. and de Bold, A.J.: The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochem. Biophys. Res. Commun. 117:859-865, 1983.

Francisco, L., Osborn, J. and DiBona, G.: Prostaglandins in renin release during sodium deprivation. Am. J. Physiol. 243:F537-F542, 1982.

Fulgraff, G. and Meiforth, A.: Effects of prostaglandin E_2 on excretion and reabsorption of sodium and fluid in rat kidneys (micropuncture studies). Pflugers Arch. 330:243-256, 1971.

Garcia-Sainz, J.A., Hoffman, B.B., Li, S., Lefkowitz, R.J. and Fain, J.N.: Role of alpha1-adrenoceptors in the turnover of phosphatidylinositol and alpha2-adrenoceptors in the regulation of cAMP accumulation in hampster adipocytes. Life Sci. 27:953-961, 1980.

Gellai, M. and Edwards, R.M.: Mechanism of α2-adrenoceptor agonist-induced diuresis. Am. J. Physiol. 255:F317-F323, 1988.

Gellai, M. and Ruffolo, R.R.: Renal effects of selective alpha-1 and alpha-2 adrenoceptor agonists in conscious, normotensive rats. J. Pharmacol. Exp. Ther. 240:723-728, 1987.

Gellai, M., Silverstein, J.H., Hwang, J.C., LaRochelle, F.T. Jr., and Valtin H.: Influence of vasopressin on renal hemodynamics in conscious Brattleboro rats. Am. J. Physiol. 246:F819-F827, 1984.

Gesek, F.A., Cragoe, D.J. Jr. and Strandhoy, J.W.: Synergistic alpha-1 and alpha-2 adrenergic stimulation of rat proximal nephron Na^+/H^+ exchange. J. Pharmacol. Exp. Ther. 249:694-700, 1989.

Gesek, F.A. and Schoolwerth, A.C.: Hormonal interactions with the proximal Na^+ - H^+ exchanger. Am. J. Physiol. 258:F514-F521, 1990.

Gesek, F.A. and Strandhoy, J.W.: Dual interactions between α_2-adrenoceptor agonists and the proximal Na+-H+ exchanger. Am. J. Physiol. 258:F636-F642, 1990.

Gesek, F.A. and Strandhoy, J.W.: Alpha adrenoceptor agonist stimulation of oxygen consumption in rat proximal and distal nephrons. J. Pharmacol. Exp. Ther. 249:529-534, 1989.

Gillespie, J.S., MacLaren, A. and Pollock, D.: A method of stimulating different segments of the automonic outflow from the spinal column to various organs in the pithed rat and cat. Br. J. Pharmacol. 40:257-267, 1970.

Gottschalk, C.W.: Renal nerves and sodium excretion. Ann. Rev. Physiol. 41:229-240, 1979.

Grantham, J.J. and Orloff, J. : Effect of prostaglandin E_1 on the permeability response to the isolated collecting tubule to vasopressin, adenosine 3',5'-monophosphate and theophylline. J. Clin. Invest. 47:1154-1161, 1968.

Guder, W.G. and Rupprecht, A.: Antagonism between parathyroid hormone and norepinephrine on cyclic adenosine-3',5'-monophosphate (cAMP) levels in isolated tubules from rat kidney cortex. Pfluegers Arch. 354:177-186, 1975.

Gullner, H.: Lack of suppression of vasopressin plasma levels by catapress. Pharmacol. Res. Commun. 11:39-44, 1979.

Han, C., Abel, P.W. and Minneman, K.P.: α1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329:333-335, 1987.

Hebert, S.C. and Andreoli, T.E.: Effects of antidiuretic hormone on cellular conductive pathways in mouse medullary thick ascending limb of Henle. II. Determinants of the ADH-mediated increases in transepithelial voltage and in net Cl^- absorption. J. Membr. Biol. 80:221-233, 1984.

Hebert, S.C., Culpepper, R.M. and Andreoli, T.E.: NaCl transport in mouse medullary thick ascending limbs I. Functional nephron heterogeneity and ADH-stimulated NaCl cotransport. Am. J. Physiol. 241:F412-F431, 1981.

Hebert, S.C., Friedman, P.A. and Andreoli, T.E.: The effects of antidiuretic hormone on cellular conductive pathways in mouse meduallary thick ascending limbs of Henle. I. ADH increases directly the K^+ conductance of apical membranes. J. Memb. Biol. 51:1-19, 1982.

Helman, S.I., Grantham, J.J. and Burg, M.B.: Effect of vasopressin on electrical resistance of renal cortical collecting tubules. Am. J. Physiol. 220:1825-1832, 1971.

Hesse, I.F.A. and Johns, E.J.: An in vivo study of the alpha-adrenergic subtypes on the renal vasculature of the anesthetized rabbit. J. Auton. Pharmacol. 4:145-152, 1984.

Hesse, I.F.A. and Johns, E.J.: The role of α-adrenoceptors in the regulation of renal tubular sodium reabsorption and renin secretion in the rabbit. Br. J. Pharmacol. 84:715-724, 1985.

Hicks, P.E., Tierney, C. and Langer, S.Z.: Preferential antagonism by diltiazem of α2-adrenoceptor mediated vasoconstrictor responses in perfused tail artery of spontaneously hypertensive rats. Naunyn Schmiedebergs Arch. Pharmacol. 328:388-395, 1985.

Hisa, H., Hayashi, Y. and Satoh, S.: Effects of blockades of alpha- and beta-adrenoceptors and dopamine receptors on renal nerve stimulation induced prostaglandin E_2 and renin release in anesthetized dogs. J. Pharmacol. Exp Ther. 235:481-486, 1985.

Hisa, H. and Satoh, S.: Role of renal prostaglandin in renin release during renal nerve stimulation in anesthetized dogs. Arch. Int. Pharmacodyn. Ther. 261:133-146, 1983.

Horn, P.T., Kohli, J.D., Listensky, J.J. and Goldbery, L.I.: Regional variation in the alpha adrenergic receptors in the canine resistance vessels. Naunyn-Schmiedebergs Arch. Pharmacol. 318:166-172, 1982.

Humphreys, M.J., Friedler, R.M. and Earley, L.E.: Natriuresis produced by vasopressin or hemorrhage during water diuresis in the dog. Am. J. Physiol. 219:658-665, 1970.

Humphreys, M.J. and Reid, I.A.: Suppression of antidiuretic hormone secretion by clonidine in the anesthetized dog. Kid. Int. 7:405-412, 1975.

Iino, Y. and Imai, M.: Effects of prostaglandins on sodium transport in isolated collecting tubules. Pflugers Arch. 373:125-132, 1978.

Imbert-Teboul, M., Chabardes, D., Montegut, M., Clique, A. and Morel, F.: Vasopressin-dependent adenylate cyclase in single segments of rabbit kidney tubule. Pflugers Arch. 357:173-186, 1975.

Imbert-Teboul, M., Chabardes, D., Montegut, M., Clique, A. and Morel, F.: Vasopressin-dependent adenylate cyclase activities in the rat kidney medulla: Evidence for two separate sites of action. Endocrinol. 102:1254-1261, 1978.

Insel, P.A. and Snavely, M.D.: Catecholamines and the kidney: Receptors and renal function. Ann. Rev. Physiol. 43:625-636, 1981.

Insel, P.A., Snavely, M.D., Healy, D.P., Munzel, P.A., Potenza, C.L. and Nord, D.P.: Radioligand binding and functional assays demonstrate postsynaptic alpha2 receptors on proximal tubules of rat and rabbit kidney. J. Cardiovasc. Pharmacol. 7 (Suppl 8):S9-S17, 1985.

Jackson, B.A., Edwards, R.M. and Dousa, R.P.: Vasopressin-prostaglandin interaction in isolated tubules from rat outer medulla. J. Lab. Clin. Med. 96:119-128, 1980.

Jacobson, H.N. and Kellogg, R.H.: Isotonic NaCl diuresis in rats. Antidiuresis and chloruresis produced by posterior pituitary extracts. Am. J. Physiol. 184:376-389, 1956.

Jakobs, K.H., Aktories, K. and Schultz, G.: Inhibition of adenylate cyclase by hormones and neurotransmittors. Adv. Cyclic Nucleotide Res. 14:173-187, 1981.

Januszewics, P., Gutkowska, J., deLean, A., Thibault, G., Garcia, R., Genest, J. and Cantin, M.: Synthetic atrial natriuretic factor induces release (possible receptor-mediated) of vasopressin from rat posterior pituitary. Proc. Soc. Exp. Biol. Med. 178:321-325, 1985.

Jarrott, B., Louis, W.J. and Summers, R.J.: The characteristics of ^{3}H-clonidine binding to an A-adrenoceptor in membranes from guinea pig kidney. Br. J. Pharmacol. 65:663-670, 1979.

Jeffries, W.B., Tam, L.T., Wang, Y., Smyth, D.D. and Pettinger, W.A.: Prazosin-induced alterations in renal alpha-adrenoceptor function. Hypertension 9, III-125 -III-129, 1987.

Jim, K.F. and Matthews, W.D.: Role of extracellular calcium in contractions produced by activation of postsynaptic alpha2-adrenoceptors in the canine saphenous vein. J. Pharmacol. Exp. Ther. 234:161-165, 1985.

Johnson, M.D., Kinter, L.B. and Beeuwkes, R.: Effects of AVP and DDAVP on plasma renin activity and electrolyte excretion in conscious dogs. Am. J. Physiol. 236:F66-F70, 1979.

Johnston, H.H., Herzog, J.P. and Lauler, D.P.: Effect of prostaglandin E_1 on renal hemodynamics, sodium and water excretion. Am. J. Physiol. 213:939-946, 1967.

Kangawa, K. and Matsuo, H.: Purification and complete amino acid sequence of α-human atrial natriuretic polypiptide (α-hANP). Biochem. Biophys. Res. Commun. 118:131-139, 1984.

Kaplan, S.A. and Rapoport, S.: Urinary excretion of sodium and chloride after splanchnicotomy: Effect on the proximal tubule. Am. J. Physiol. 164:175-181, 1951.

Kasting, N.W.: Simultaneous and independent release of vasopressin and oxytocin in the rat. Can. J. Physiol. Pharmacol. 66:22-26, 1988.

Katada, T. and Ui, M.: Islet-activating protein: A modifier of receptor-mediated regulation of rat islet adenylate cyclase. J. Biol. Chem. 256:8310-8317, 1981.

Keeton, T.K. and Campbell, W.B.: The pharmacologic alteration of renin release. Pharmacol. Rev. 32:81-227, 1980.

Kim, J.K., Linas, S.L. and Schrier, R.W.: Catecholamines and sodium transport in the kidney. Pharmacol. Rev. 31:169-178, 1980.

Kinter, L.B., Churchill, S., Stassen, F.L., Moore, M. and Huffman, W.: Vasopressin antagonism in the squirrel monkey (Saimiri sciuresu). J. Pharmacol. Exp. Ther. 241:797-803, 1987.

Kinter, L.B., Dytko, G., Ashton, D., McDonald, J., Huffman, W. and Stassen, F.: Discovery and therapeutic utility of vasopressin antagonists in rats. J.Cardiovas. Pharmacol. 8 (Suppl. 7): S36-S43, 1986.

Kirschenbaum, M.A. and Stein, J.H.: The effect of inhibition of prostaglandin synthesis on urinary sodium excretion in the conscious dog. J. Clin. Invest. 57:517-521, 1976.

Kobilka, B.K., Matsui, H., Kobilka, T.S., Yang-Feng, T.L., Francke, U., Caron, M.G., Lefkowitz, R.J. and Regan, J.W.: Cloning, sequencing, and expression of the gene coding for the human platelet α2-adrenergic receptor. Science 238:650-656, 1987.

Kobinger, W. and Pichler, L.: Investigation into different types of post- and presynaptic α-adrenoceptors at cardiovascular sites in rats. Eur. J. Pharmacol. 65:393-402, 1980.

Koepke, J.P. and DiBona, G.F.: Central adrenergic receptor control of renal function in conscious hypertensive rats. Hypertension 8:133-141, 1986.

Kokko, J.P. and Rector, F.C. Jr.: Countercurrent multiplication system without active transport in inner medulla. Kid. Int. 2:214-223, 1972.

Kopp, U., Aurell, M., Nilsson, I.M. and Ablad, B.: The role of beta-1 adrenoceptors in the renin release response to graded renal sympathetic nerve stimulation. Pfluegers Arch. 387:107-113, 1980.

Kopp, U., Aurell, M., Sjolander, M. and Ablad, B.: The role of prostaglandins in the alpha- and beta-adrenoceptor mediated renin release response to graded renal nerve stimulation. Pfluegers Arch. 391:1-8, 1981.

Krothapalli, R.K., Duffy, K.D., Senekjian, H. and Suki, W.: Modulation of the hydro-osmotic effect of vasopressin on the rabbit cortical collecting tubule by adrenergic agents. J. Clin. Invest. 72:287-294, 1983.

Krothapalli, R.K. and Suki, W.: Functional characterization of the alpha-adrenergic receptor modulating the hydro-osmotic effect of vasopressin on the rabbit cortical collecting tubule. J. Clin. Invest. 73:740-749, 1984.

Kurokawa, K., and Massry, S.G.: Interaction between catecholamines and vasopressin on renal medullary cAMP of rat. Am. J. Physiol. 225:825-829, 1973a.

Kurokawa, K., and Massry, S.G.: Evidence for two separate adenyl cyclase systems responding independently to parathyroid hormone and beta-adrenergic agonists in the renal cortex of rat. Proc. Soc. Expl Biol. Med. 143:123-126, 1973b.

Lachaud, V., Coupry, I., Podevin, R., Dausse, J., Koenig, E and Parini, A.: Interaction of clonidine and rilnenidine with imidazoline-preferring receptors. J. Hyperten. 6:S511-S513, 1988.

Laycock, J.F. and Williams, P.G.: The effect of vasopressin (Pitressin) administration on sodium, potassium and urea excretion in rats with and without diabetes insipidus (DI) with a note on the excretion of vasopressin in the DI rat. J. Endocrinol. 56:111-120, 1973.

Lands, A.M., Luduena, F.P. and Buzzo, H.J.: Differentiation of receptors responsiveness to isoproterenol. Life. Sci. 6:2241-2249, 1967.

Langer, S.Z.: Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23:1793-1800, 1974.

Langer, S.Z.: Presynaptic receptors and their role in the regulation of transmitter release. Br. J. Pharmacol. 60:481-497, 1977.

Leander, J.D., Zerbe, R.L. and Hart, J.C.: Diuresis and suppression of vasopressin by kappa opioids: Comparison with mu and delta opioids and clonidine. J. Pharmacol. Exp. Ther. 234:463-469, 1985.

Lee, J., Malvin, R.L., Claybaugh, J.R. and Huang, B.S.: Atrial natriuretic factor inhibits vasopressin secretion in conscious sheep. Proc. Soc. Exp. Biol. Med. 185:272-276, 1987.

Lees, P. and Lockett, M.F.: The influence of hypophysectomy and of adrenalectomy on the urinary changes induced by oxytocin in rats. J. Physiol. (Lond.) 171:403-410, 1964.

Leyssac, P.P., Christensen, P., Hill, R., Skinner, S.L.: Indomethacin blockade of renal PGE-synthesis: Effect on total renal and tubular function and plasma renin concentration in hydropenic rats and on their response to isotonic saline. Acta Physiol. Scand. 94:484-496, 1975.

Li, P., Penner, S.B., Blandford, D.E. and Smyth, D.D.: Renal alpha2-adrenoceptor mediated natriuresis and diuresis independent of vasopressin. (Abstract) In press, ISH, 1990.

Lieberthal, W., Vasilevsky, M.L., Valeri, C.R. and Levinsky, N.G.: Interactions between ADH and prostaglandins in isolated erythrocyte-perfused rat kidney. Am. J. Physiol. 252:F331-F337, 1987.

Lipson, L.C. and Sharp, G.W.G.: Effect of prostaglandin E_1 on sodium transport and osmotic water flow in the toad bladder. Am. J. Physiol. 220:1046-1052, 1971.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

Lum, G.M., Aisenbrey, G.A., Dunn, M.J., Berl, T., Schrier, R.W. and McDonald, K.M.: In vivo effect of indomethacin to potentiate the renal medullary cyclic AMP response to vasopressin. J. Clin. Invest. 59:8-13, 1977.

Martinez-Maldonado, M., Eknoyan, G. and Suki, W.N.: Natriuretic effects of vasopressin and cyclic AMP: Possible site of action in the nephron. Am. J. Physiol. 220:2013-2020. 1971.

Martinez-Maldonado, M., Tsaparas, N., Eknoyan, G. and Suki, W.N.: Renal actions of prostaglandins: Comparison with acetylcholine and volume expansion. Am. J. Physiol. 222:1147-1152, 1972.

Manning, M., Nawrocka, E., Misicka, A., Olma, A., Klis, W.A., Seto, J. and Sawyer, W.H.: Potent and selective antagonists of the antidiuretic response to arginine-vasopressin based on modifications of [1-(β-mercapto-β,β-pentamethylenepropionic acid),2-D-isoleucine, 4-valine] arginine-vasopressin at position 4. J. Med. Chem. 27:423-429, 1984.

Matsumura, Y., Ozawa, Y., Susuki, H. and Saruta, T.: Synergistic action of angiotensin II on norephinephrine-induced release from rat glomeruli. Am. J. Physiol. 250:F811-F816, 1986.

McGrath, J.C.: Evidence for more than one type of post-junctional α-adrenoceptor. Biochem. Pharmacol. 31:467-484, 1982.

McPherson, G.A. and Summers, R.J.: ^{3}H-Prazosin and ^{3}H-clonidine binding to α-adrenoceptors in membranes prepared from regions of rat kidney. J. Pharm. Pharmacol. 33:189-191, 1981.

McPherson, G.A. and Summers, R.J.: A study of α1-adrenoceptors in rat renal cortex: Comparison of [^{3}H]prazosin binding with α1-adrenoceptors modulating gluconeogenesis under physiological conditions. Br. J. Pharmacol. 77:177-184, 1982.

McPherson, G.A. and Summers, R.J.: Evidence from binding studies for α2-adrenoceptors directly associated with glomeruli from rat kidney. Eur. J. Pharmacol. 90:333-341, 1983.

Medgett, I.C. and Rajanayagam, M.A.S.: Effects of reduced calcium ion concentration and of diltiazem on vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in rat isolated tail artery. Br. J. Pharmacol. 83:889-898, 1984.

Michel, M.C. and Insel, P.I.: Are there multiple imidazoline binding sites? Trends Pharmacol. Sci. 10:342-344, 1989.

Michel, M.C., Insel, P.I. and Brodde, O.E.: Renal alpha2-adrenergic receptor alterations: a cause of essential hypertension? FASEB J. 3:139-144, 1989.

Miller, M.: Clonidine-induced diuresis in the rat: Evidence for a renal site of action. J. Pharmacol. Exp. Ther. . 214:608-613, 1980.

Minneman, K.P., Hedberg, A. and Molinoff, P.B.: Comparison of beta-adrenergic receptor subtypes in mammalian tissues. J. Pharmacol. Exp. Ther. 211:502-508, 1979.

Morel, F., Imbert-Teboul, M. and Chabardes, D.: Distribution of hormone dependent adenylate cyclase in the nephron and its physiological significance. Ann. Rev. Physiol. 43:569-581, 1981.

Nasjletti, A., McGraff, J.D. and Colina-Chourio, J.: Interrelation of the renal kallikrein-kinin systems and renal prostaglandins in the conscious rat. Circ. Res. 43:799-807, 1978.

Nord, E.P., Howard, M.J., Hafezi, A., Moradeshagi, P., Vaystub, S. and Insel, P.A.: Alpha2-adrenergic agonists stimulate Na$^+$-H$^+$ antiport activity in the rabbit renal proximal tubule. J. Clin. Invest. 80:1755-1762, 1987.

North, W.G., Gellai, M and Hardy, G.: Oxytocin and oxytocin-associated neurophysin evaluation by RIA in the brattleboro rat. Ann. N.Y. Acad. Sci. 394:167-172, 1982.

Olsen, U.B.: Clonidine-induced increase of renal prostaglandin activity and water diuresis in conscious dogs. Eur. J. Pharmacol. 36:95-101, 1976.

Orkand, P.M. and Palay, S.: Effect of treatment with exogenous vasopressin on the structural alterations in the hypothalamo-neurohypophysial system of rats with hereditary diabetes insipidus. Anat. Rec. 157:295-297, 1967.

Orloff, J., Handler, J.S. and Bergstrom, S.: Effect of prostaglandin (PGE$_1$) on the permeability response of toad bladder to vasopressin, theophylline, and adenosine 3',5'-monophosphate. Nature 205:397-401, 1965.

Osborn, L.J., DiBona, G.F. and Thames, M.D.: Beta-1 receptor mediation of renin secretion elicted by low frequency renal nerve stimulation. J. Pharmacol. Exp. Ther. 216:265-269, 1981.

Osborn, J.L., DiBona, G.F. and Thames, M.D.: Role of renal α2-adrenoceptor mediating renin secretion. Am. J. Physiol. 242:F620-F626, 1982.

Osborn, L.J., Holdaas, H., Thames, M.D and DiBona, G.F.: Renal adrenoceptor mediation of antinatriuretic and renin secretion responses to low frequency renal nerve stimulation in the dog. Circ. Res. 53:298-305, 1983.

Pedraza-Chaverri, J., Alatorre-Gonzales, M.C., Ibarra-Rubio, M. E., Pena, J.C. and Carcia-Sainz, J.A.: Effect of pertussis toxin on the adrenergic regulation of plasma renin activity. Life. Sci. 35:1683-1690, 1984.

Pettinger, W.A.: Clonidine, a new antihypertensive drug. N. Engl. J. Med. 293:1179-1180, 1975.

Pettinger, W.A., Keeton, T.K., Campbell, W.B. and Harper, D.C.: Evidence for a normal A-adrenergic receptor inhibiting renin release. Circ. Res. 38:338-346, 1976.

Pettinger, W.A., Sanchez, A., Saavedra, J., Haywood, J.R., Gandler, T. and Rodes, T.: Altered renal A2 adrenergic receptor regulation in genetically hypertensive rats. Hypertension. 4, Suppl II: II-188 - II-192, 1982.

Pettinger, W.A., Smyth, D.D. and Umemura, S.: Renal alpha2-adrenoceptors, their locations and effects on sodium excretion. J. Cardiovasc. Pharmacol. 7 (Suppl. 8):S24-S27, 1985.

Pettinger, W.A., Umemura, S., Smyth, D.D. and Jeffries, W.B.: Renal alpha2-adrenoceptors and the adenylate cyclase-cAMP system: Biochemical and physiological interactions. Am. J. Physiol. 252:F199-F208, 1987.

Pierce, E.T., Grekin, R.J. and Mouw, D.R.: Efferent role of ADH in CNS-induced natriuresis. Am. J. Physiol. 246:F32-F36, 1984.

Pollock, A.S., Warnock, D.G. and Strewler, G.F.: Parathyroid hormone inhibition of Na^+-H^+ antiporter activity in a cultured renal cell line. Am. J. PHysiol. 250:F217-F225, 1988.

Putney, J.W.: Formation and actions of calcium mobilizing messenger, inositol 1,4,5-triphosphate. Am. J. Physiol. 252:G149-G157, 1987.

Regan, J.W., Kobilka, T.S., Yang-Feng, T.L., Caron, M.C., Lefkowitz, R.J.: Cloning and expression of a human kidney cDNA for an alpha2-adrenergic receptor subtype. Proc. Natl. Acad. Sci. 85:6301-6305, 1988.

Reid, I.A., MacDonald, D.M., Pachnis, B. and Ganong, W.F.: Studies concerning the mechansim of suppression of renin secretion by clonidine. J. Pharmacol. Exp. Ther. 192:713-721, 1975.

Reid, I.A., Nolan, P.L., Wolf, J.A. and Kiel, L.C.: Suppression of vasopressin secretion by clonidine: Effect of α2-adrenoceptor antagonists. Endocrinol. 104:1403-1406, 1979.

Reif, M.C., Troutman, S.L. and Schafer, J.A.: Sodium transport by rat cortical collecting tubule. J. Clin. Invest. 77:1291-1298, 1986.

Roman, R.J. and Cowley, A.W. Jr.: Characterization of a new model for the study of pressure-natriuresis in the rat. Am. J. Physiol. 248:F190-F198, 1985.

Roman, R.J., Cowley, A.W. and Lechene, C.: Water diuretic and natriuretic effect of clonidine in the rat. J. Pharmacol. Exp. Ther. 211:385-393, 1979.

Rosas, R., Barnafi, L., Pereda, T. and Croxatto, H.: Effect of oxytocin structural changes on rat renal excretion of Na, K and water. Am. J. Physiol. 202:901-904, 1962.

Ruffolo, R.R., Turowski, B.S. and Patil, P. N.: Lack of cross-desensitization between structurally dissimilar α-adrenoceptor agonists. J. Pharm. Pharmacol. 29:378-380, 1977.

Sakakibara, Y., Fujiwara, M. and Muramatsu, I.: Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. Naunyn-Schmiedebergs Arch. Pharmacol. 319:1-7, 1982.

Samson, W.K., Aguila, M.C., Martinovic, J., Antunes-Rodrigues, J. and Norris, M.: Hypothalamic action of atrial natriuretic factor to inhibit vasopressin secretion. Peptides 8:449-454, 1987.

Sanchez, A. and Pettinger, W.A.: Dietary sodium regulation of blood pressure and renal α1- and α2-receptors in WKY and SHR. Life Sci. 29:2795-2802, 1981.

Sawyer, W.H. and Valtin, H.: Antidiuretic responses of rats with hereditary hypothalamic diabetes insipidus to vasopressin, oxytocin and nicotine. Endocrinol. 80:207-210, 1967.

Sawyer, R., Warnock, P. and Docherty, J.R.: Role of vascular alpha2-adrenoceptors as targets for circulating catecholamines in the maintenance of blood pressure in anesthetised spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 7:809-812, 1985.

Schlatter, E. and Schafer, J.A.: Electrophysiologic studies in principal cells of rat cortical collecting tubules: ADH increases the apical membrane Na^+ - conductance. Pflugers Arch. 409:81-92, 1987.

Schmitz, J., Graham, R., Sagalowsky, A. and Pettinger, W.A.: Renal alpha1-and alpha2-adrenergic receptors: Biochemical and pharmacological correlations. J. Pharmacol. Exp. Ther. 219:400-406, 1981.

Schwartz, J. and Reid, I.A.: Role of the vasoconstrictor and antidiuretic activities of vasopressin in inhibition of renin secretion in conscious dogs. Am. J. Physiol. 250:F92-F96, 1986.

Slick, G.L., Aguilera, A.J., Zambraski, E.J. DiBona, G.F. and Kaloyanides, G.J.: Renal noradrenergic transmission. Am. J. Physiol. 229:60-65, 1975.

Smith, M.J., Cowley, A.W. Jr., Guyton, A.C. and Manning, R.D.: Acute and chronic effects of vasopressin on blood pressure, electrolytes, and fluid volumes. Am. J. Physiol. 237:81-92, 1979.

Smyth, D.D. and Blandford, D.E.: Clonidine-induced diuresis and natriuresis does not involve atrial natriuretic protein release. (Abstract) FASEB J. 2(4):A793, 1988.

Smyth, D.D. and Blandford, D.E.: Potentiation of the natriuretic effect of clonidine following indomethacin in the rat. (Abstract) Clin. Invest. Med. 12:B69, 1989.

Smyth, D.D., Umemura, S. and Pettinger, W.A.: Alpha2-adrenoceptors and sodium reabsorption in the isolated perfused rat kidney. Am. J. Physiol. 247:F680-F685, 1984.

Smyth, D.D., Umemura, S. and Pettinger, W.A.: Alpha2-adrenoceptor antagonism of vasopressin-induced changes in sodium excretion. Am. J. Physiol. 248:F767-F772, 1985a.

Smyth, D.D., Umemura, S. and Pettinger, W.A.: Renal nerve stimulation causes alpha1-adrenoceptor-mediated sodium retention but not alpha2-adrenoceptor antagonism of vasopressin. Circ. Res. 57:304-311, 1985b.

Snavely, M.D. and Insel, P.A.: Characterization of alpha-adrenergic receptor subtypes in the rat renal cortex. Mol. Pharmacol. 22:532-546, 1982.

Solez, K., Ideura, T., Silvia, C.B., Hamilton, B. and Saito, H.: Clonidine after renal ischemia to lessen acute renal failure and microvascular damage. Kid. Int. 18:309-322, 1980.

Stanko, C.K. and Smyth, D.D.: Proximal tubular alpha-2 adrenoceptor density in the spontaneously hypertensive rat. In press, Am. J. Hypertens. 1990.

Stanko, C.K., Vandel, M.I., Bose, R. and Smyth, D.D.: Characterization of $\alpha 2$-adrenoceptors in the rat: proximal tubule, renal membrane and whole kidney studies. Eur. J. Pharmacol. 175:13-20, 1990.

Stanton, B., Puglisi, E. and Gellai, M.: Localization of alpha2-adrenoceptor-mediated increase in renal Na^+, K^+, and water excretion. Am. J. Physiol. 252:F1016-F1021, 1987.

Starke, K., Montel, H., Gayk, W. and Merker, R.: Comparison of the effects of clonidine on pre- and postsynaptic adrenoceptors in the rabbit pulmonary artery. Naunyn Schmildebergs Arch. Pharmacol. 285:133-150, 1974.

Stephenson, J.A. and Summers, R.J.: Light microscopic autoradiography of the distribution of [³H]rauwolscine binding to α2-adrenoceptors in rat kidney, Eur. J. Pharmacol. 116:271-278, 1985.

Stephenson, J.A. and Summers, R.J.: Autoradiographic evidence for a heterozygous distribution of [³H]prazosin in rat, dog and human kidney. J. Auton. Pharmacol. 6:109-116, 1986.

Stokes, J.G.: Integrated actions of the renal medullary prostaglandins in the control of water excretion. Am. J. Physiol. 240:F471-F480, 1981.

Stokes, J.B. and Kokko, J.P.: Inhibition of sodium transport by prostaglandin E_2 across the isolated perfused rabbit collecting tubule. J. Clin. Invest. 59:1099-1104, 1977.

Strandhoy, J.W., Morris, M. and Buckalew, Jr., W.M.: Renal effects of the antihypertensive, guanabenz, in the dog. J. Pharmacol. Exp. Ther. 221:347-352, 1982.

Strandhoy, J.W., Morris, M., Steg, B. and Buckalew, Jr., V.M.: Synergistic effect of modest volume expansion on the diuretic and natriuretic action of guanabenz. J. Pharmacol. Exp. Ther. 226:419-424, 1983.

Szenasi, G., Kottra, G., Bencsath, P. and Takacs, L.: Renal nerves in exaggerated water and sodium excretion by hypertropohied kidney of anesthetized rats. Am. J. Physiol. 254:F32-F37, 1988.

Tannenbaum, J., Splawinski, J.K., Oates, J.A. and Nies, A.S.: Enhanced renal prostaglandin production in the dog. 1. Effects on renal function. Circ. Res. 36:197-203, 1975.

Timmermans, P.B.M.W.M., Kwa, H.Y. and VanZwieten, P.A.: Possible subdivision of postsynaptic α-adrenoceptors mediating pressor responses in the pithed rat. Arch. pharmacol. 310:189-193, 1979.

Timmermans, P.B.M.W.M., Mathy, M.J., Thoolen, M.J.M.C., DeJonge, A., Willfert, B. and van Zwieten, P.A.: Invariable susceptibility to blockade by nifedipine of vasoconstriction to various α2-adrenoceptor agonists in pithed rats. J. Pharm. Pharmacol. 36:772-775, 1984.

Timmermans, P.B.M.W.M. and VanZwieten, P.A.: The postsynaptic α2-adrenoceptors. J. Auton. Pharmacol. 1:171-181, 1981.

Torikai, S. and Kurokawa, K.: Distribution of prostaglandin E_2-sensitive adenylate cyclase along the rat nephron. Prostaglandins 21:427-438, 1981.

Torikai, S. and Kurokawa, K.: Effect of PGE_2 on the cell cAMP content in the thin descending limb of Henle of the rat. Miner. Electrolyte Metab. 10:21-25, 1984.

Umemura, S., Marver, D., Smyth, D.D. and Pettinger, W.A.: Alpha2-adrenoceptors and cellular cAMP levels in single nephron segments from the rat. Am. J. Physiol. 249:F28-F33, 1985a.

Umemura, S., Smyth, D.D. and Pettinger, W.A.: $\alpha2$-Adrenoceptor stimulation and cellular cAMP levels in microdissected rat glomeruli. Am. J. Physiol. 250:F103-F108, 1986a.

Umemura, S., Smyth, D.D. and Pettinger, W.A.: Regulation of renal cellular cAMP levels by prostaglandins and $\alpha2$-adrenoceptor: Microdissection studies. Kid. Int. 29:703-707, 1986b.

Valtin, H.: Physiological effects of vasopressin on the kidney, in: Vasopressin, Principles and Properties, Eds. Gash, D.M. and Boer, G.J., (Plenum Press, N.Y.,) 1987.

Valtin, H., Sawyer, W.H. and Sokol, H.W.: Neurophpophysial principles in rats homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). Endocrinol. 77:701-706, 1965.

van Meel, J.C.A., DeJonge, A., Kalkman, H.O., Willfert, B., Timmermans, P.B.M.W.M. and van Zwieten, P.A.: Organic and inorganic calcium antagonists reduce vasoconstriction in vivo mediated by postsynaptic $\alpha2$-adrenoceptors. Naunyn Schmeideberg Arch. Pharmacol. 316:288-293, 1981a

van Meel, J.C.A., DeJonge, A., Kalkman, H.O., Willfert, B., Timmermans, P.B.M.W.M. and van Zwieten, P.A.: Vascular smooth muscle contration initiated by postsynaptic $\alpha2$-adrenoceptor activation is induced by an influx of extracellular calcium. Eur. J. Pharmacol. 69:205-208, 1981b

van Meel, J.C.A., DeJonge, A., Timmermans, P.B.M.W.M., and van Zwieten, P.A.: Selectivity of some alpha adrenoceptor agonists for peripheral alpha-1 and alpha-2 adrenoceptors in the normotensive rat. J. Pharmacol. Exp. Ther. 219:760-767, 1981c.

Wikberg, J.E.S.: The pharmacological classification of adrenergic alpha-1 and alpha-2 receptors and their mechanisms of action. Acta. Physiol Scand. Suppl. 468:1-110, 1979.

Winer, B.J.: Statistical Principles in Experimental Design, 2nd ed., (McGraw-Hill Book Co., New York), 1971.

Wolff, D.W., Buckalew, V.M. Jr. and Strandhoy, J.W.: Renal $\alpha1$- and $\alpha2$-adrenoceptor mediated vasoconstriction in dogs: Comparison of phenylephrine, clonidine, and guanabenz. J. Cardiovasc. Pharmacol. 6:S793-S798, 1984

Wolff, D.W., Colindres, R.E. and Strandhoy, J.W.: Unmasking sensitive $\alpha2$-adrenoceptor-mediated renal vasoconstriction in conscious rats. Am. J. Physiol. F1132-F1139, 1989.

Wolff, D.W., Gesek, F.A. and Strandhoy, J.W.: In vivo assessment of rat renal alpha adrenoceptors. J. Pharmacol. Exp. Ther. 241:472-476, 1987.

Wright, F.: Sites and mechanisms of potassium transport along the renal tubules. Kid. Int. 11:415-432, 1977.

Yamaguchi, I. and Kopin, I.J.: Differential inhibition of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses in pithed rats. J. Pharmacol. Exp. Ther. 214:275-281, 1980.

Young, W.S. and Kuhar, M.J.: Alpha2-adrenergic receptors are associated with renal proximal tubules. Eur. J. Pharmacol. 67:493-495, 1980.

Zambraski, E.J. and DiBona, G.F.: Angiotensin II in antinatriuresis of low level renal nerve stimulation. Am. J. Physiol. 231:1105-1110, 1976.

Zambraski, E.J., DiBona, G.F. and Kaloyanides, G.J.: Specificity of neural effect on renal tubular sodium reabsorption. Proc. Soc. Exp. Biol. Med. 151:543-546, 1976.

Zambraski, E.J. and Dunn, M.J.: Renal prostaglandin E_2 secretion and excretion in conscious dogs. Am. J. Physiol. 236:F552-F558, 1979.