# PHYSIOLOGICAL ROLE OF RENAL $I_1$ IMIDAZOLINE RECEPTORS IN THE RENAL EXCRETION OF WATER AND ELECTROLYTES IN THE RAT

BY

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

MASTER OF SCIENCE

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## DEDICATION

This thesis is dedicated to Ms. Mary Annan who has cheerfully tolerated the time I have not spent with her.

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## ABSTRACT

Recent studies have described the existence of a novel class of receptors which recognize compounds with the imidazoline moiety. These receptors have been termed imidazoline receptors and may mediate some of the effects previously attributed to $\alpha_2$-adrenoceptor stimulation. There are two subtypes of the imidazoline receptor. The $I_1$ imidazoline receptor has been defined by moxonidine and clonidine binding whereas the $I_2$ imidazoline receptor has been defined by idazoxan binding. Moxonidine demonstrates at least a 700-fold higher affinity for renal $I_1$ imidazoline receptors over $\alpha_2$-adrenoceptors. The effect of $I_1$ imidazoline receptor stimulation in the kidney, and the role of a G protein and prostaglandins in this response, were determined. Briefly, uninephrectomized rats were anaesthetized 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 renal artery for the direct intrarenal infusion of saline (vehicle) or moxonidine. Moxonidine (0, 0.3, 1 and 3 nmol/kg/min) produced a dose related increase in urine flow rate and sodium excretion without altering blood pressure and creatinine clearance. This diuretic and natriuretic action was abolished by pertussis toxin pretreatment. The response

to another diuretic, furosemide, was not altered by pertussis toxin pretreatment. This finding suggested that this effect of the pertussis toxin was not a non-specific effect. In another series of experiments, intraperitoneal administration of indomethacin (5 mg/kg) abolished the subsequent natriuretic response to an intrarenal infusion of moxonidine (1 nmol/kg/min). Concurrent administration of prostaglandin $E_2$ to the indomethacin pretreated rats restored the natriuretic effect of moxonidine. Since pertussis toxin inactivates a G protein, the results suggest that the $I_1$ imidazoline receptor was coupled to a G protein, most conceivably the Gi protein. As well, the results with indomethacin are consistent with the fact that prostaglandins play a permissive role in the renal actions of $I_1$ imidazoline receptor agonists such as moxonidine.

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# GENERAL INTRODUCTION

## Preamble

Blood pressure regulation is under the influence of the brain, heart and kidney. The latter is responsible for the long term regulation of blood pressure through its handling of water and sodium (Guyton, 1972). The kidney regulates blood pressure through a number of mechanisms. One mechanism involves the control of the extracellular fluid volume, which in turn has important effects on blood volume, cardiac output, and arterial pressure (Guyton, 1972, 1980). A second involves the activation of the renin-angiotensin system, which increases the pressure by constricting the peripheral arterioles (Coleman and Guyton, 1975). Angiotensin also stimulates the adrenal cortex to augment aldosterone production, which in turn increases sodium reabsorption and leads to fluid retention. The fluid volume control mechanism has been postulated to be more important in long term control by the kidneys which enables the kidney to override other arterial pressure regulators such as nervous and endocrine factors (Guyton, 1972).

The fluid/volume system for arterial pressure control is a very simple one, when the body contains too much extracellular fluid, the arterial pressure rises. The increased pressure in turn has a direct effect increase sodium and water excretion by the kidneys, thus returning the volume back to normal resulting in a normalization of

pressure. In the phylogenetic history of animal development, this renal-body fluid mechanism for pressure control is a very primitive one. It is fully operative in one of the lowest of vertebrates, the hagfish. This animal has a very low arterial blood pressure, only 8 to 14 mm Hg, and its pressure increases almost directly in proportion to its blood volume. The hagfish continually drinks sea water, which is absorbed into its blood, increasing the blood volume and also the pressure. However, when pressure rises too high, the kidney simply excretes excess volume into the urine and relieves the pressure. At low pressure, the kidney excretes far less fluid than is ingested. Therefore, the volume and pressure build up again to the normal levels.

Throughout the ages, this primitive mechanism of pressure control has survived almost exactly as it functions in the hagfish; in the human, kidney output of water and salt is just as sensitive to pressure changes as in the hagfish, if not more so. Indeed an increase in arterial pressure in the human of only a few millimeters of mercury can double the output of water, which is called a pressure diuresis, and also double the output of salt, which is called a pressure natriuresis. In the human and rat, the renal-body fluid system for arterial pressure control is hypothesized as a potentially the fundamental basis for long term arterial pressure control. Newer antihypertensive agents that directly influence the renal-body fluid control

of arterial pressure have obvious advantages over other antihypertensives.

Salt balance also plays a very important role in the renal body fluid schema for arterial pressure regulation. Experimental studies have shown that an increase in salt intake is far more likely to elevate the arterial pressure than is an increase in water intake (Coleman and Guyton 1969, Coleman 1970). This happens because water is normally excreted by the kidneys almost as rapidly as it is ingested, but salt often is not excreted so easily. As it accumulates in the body, salt can indirectly increase the extracellular fluid volume for several reasons two of which are listed below.

1. When there is excess salt in the body, the osmolality of the body fluids increases, and this in turn stimulates the thirst centre, making the person drink extra amounts of water to dilute the extracellular salt to a normal concentration. This obviously increases the extracellular fluid volume.
2. The increase in osmolality of the extracellular fluid also stimulates the hypothalamic-posterior pituitary gland secretory mechanism to secrete increased quantities of antidiuretic hormone (vasopressin) (Langston et al., 1963, Blessing 1986). The antidiuretic hormone in turn causes the kidneys to reabsorb greatly increased quantities of water from the renal tubular fluid before it is excreted as urine,

thereby diminishing the volume of urine while increasing the extracellular fluid volume.

Thus, the amount of salt that accumulates in the body is the main determinant of the extracellular fluid volume. Because only small increases in extracellular fluid can often increase the arterial pressure greatly, the accumulation of even a small amount of extra salt in the body can lead to considerable elevation of the arterial pressure (Anderson, 1984).

For these reasons, antihypertensive agents that target the kidney and thereby prevent the accumulation of salt and water in the body will be most desirable.

**Historical Perspectives**

Ever since Alquist (1948) proposed that adrenoceptors be subclassified into $\alpha$ and ß subtypes on the basis of their pharmacology, different subclasses of the $\alpha$ and ß adrenoceptors have been discovered using receptor binding studies, subtype selective antagonists and autoradiography (Berthelson and Pettinger, 1977; McPherson and Summers 1981; Bylund, 1985; Boyajian et al., 1987). The ß-adrenoceptors were further subdivided into $ß_1$ and $ß_2$ subtypes by Lands et al., (1967) and the $\alpha$-adrenoceptors subdivided into $\alpha_1$ and $\alpha_2$ classes (Starke and Langer, 1979).

Radioligand binding techniques have helped in detecting and quantitating $\alpha$-adrenoceptors in the kidney. Whereas, $\alpha_1$-adrenoceptors have been studied with the specific $\alpha_1$-

adrenoceptor antagonist prazosin, $\alpha_2$-adrenoceptors have been characterized with radiolabelled agonists such as $^3$H-clonidine (McPherson and Summers, 1981) and antagonists such as rauwolscine (Neylon and Summers 1985), yohimbine (Insel et al., 1985) and idazoxan (Boyajian et al., 1987; Coupry et al., 1987; Parini et al., 1989). The functional and biochemical characteristics of the $\alpha$-adrenoceptors in the brain and kidney were well elucidated in the 1970's and early 1980's. In these studies a trend began to emerge. It was observed that closely associated with these $\alpha_2$-adrenoceptors were non-adrenergic sites which were insensitive to catecholamines but recognized compounds with the imidazoline structure. The heterogeneous nature of these receptors became evident from pharmacological and physiological studies done on them (Boyajian et al., 1987, Coupry et al., 1987, Ernsberger et al., 1987, Parini et al., 1989). These studies were facilitated by the advent of more specific $\alpha_2$-adrenoceptor antagonists such as yohimbine, rauwolscine, idazoxan and p-aminoclonidine, the latter two having an imidazoline structure.

Utilizing radiolabelled $\alpha_2$-adrenoceptor antagonists such as yohimbine and rauwolscine, Cheung et al., (1982) showed that differences exist in the affinity of $\alpha_2$-adrenoceptors found in the rat brain and those in human platelet membranes for these ligands. The possibility that yohimbine and rauwolscine bound in part to $\alpha_1$-adrenoceptors in the rat brain but not in platelets was excluded by

showing that prazosin, an $\alpha_1$-adrenoceptor antagonist, generated displacement curves with slopes close to unity (Cheung et al., 1982). A slope of unity suggests that there is no cooperativity at the binding sites. The demonstration of the existence of heterogeneity of $\alpha_2$-adrenoceptors was confirmed by binding studies with $\alpha_2$-agonists such as clonidine, norepinephrine and epinephrine. These compounds also demonstrated a lower affinity for $\alpha_2$-adrenoceptors from rat brain compared to those from human platelets.

One criticism against the use of $^3$H-agonists in characterizing $\alpha_2$-adrenoceptors was that they preferentially labelled a high affinity state, and so the use of these ligands may have provided a quantitatively and qualitatively misleading profile of total $\alpha_2$-adrenoceptors (Hoffman et al., 1980). However, the antagonists were not associated with this problem.

Following the above studies, Petrash and Bylund, (1986) used prazosin and oxymetazoline inhibition of yohimbine binding to further define $\alpha_2$-adrenoceptor subtypes in human cerebral cortex, cerebellum, caudate nucleus and platelet membrane. They demonstrated that only one subtype was found in cortex, cerebellum and platelets but the binding characteristics seen in platelets differed from those observed in the cortex and cerebellum. The data from the caudate nucleus, however, were consistent with the presence of two receptor subtypes, in that they demonstrated binding characteristics observed in both platelets and cortex.

The $\alpha_2$-adrenoceptor in the platelets were labelled as $\alpha_{2a}$ since they had a higher affinity for oxymetazoline. Those in the cortex were labelled as $\alpha_{2b}$ due to a greater affinity for prazosin than for oxymetazoline. Based on this criteria it was concluded that both $\alpha_{2a}$ and $\alpha_{2b}$ adrenoceptors were found in the caudate nucleus. The observation that both prazosin and oxymetazoline inhibited yohimbine binding in the caudate nucleus in a manner consistent with two classes of binding sites could have been challenged since negative cooperativity and different affinity states of a single receptor could have been offered as an alternative explanation. In negative cooperativity, the binding of a ligand to a receptor reduces the affinity of the latter for similar ligands.

**Studies with Idazoxan**

Idazoxan (an imidazoline compound) has been described as a potent and selective $\alpha_2$-adrenoceptor antagonist with an $\alpha_2/\alpha_1$ selectivity ratio superseding that of most other $\alpha_2$-adrenoceptor blocking agents. Therefore, tritiated idazoxan was prepared and used as a radioligand for $\alpha_2$-adrenoceptors (Boyajian et al., 1987; Yablonsky et al., 1988; Wikberg, 1989, Langin and Lafontan, 1989). These studies consistently demonstrated that idazoxan labelled more sites than rauwolscine or yohimbine in male rabbit urethra (Yablonsky et al., 1988), male guinea pig cerebral cortex (Wikberg and Uhlen, 1990) and rabbit adipocytes (Langin and

Lafontan, 1989). Moreover, these extra sites labelled by idazoxan were non-adrenergic since idazoxan was not displaced by catecholamines. The binding of idazoxan to these sites was not inhibited by serotonin, histamine, dopamine or carbachol indicating that the site was not related to either serotoninergic, histaminergic, dopaminergic, cholinergic or adrenergic receptors.

In an attempt to further characterize the $\alpha_2$-adrenoceptors in renal proximal tubules, where they seem to modulate the sodium reabsorption (Pettinger et al., 1985), Coupry et al., (1987) performed binding studies in purified basolateral membranes from rabbit kidney. They reported that $^3$H-idazoxan labelled both $\alpha_2$-adrenoceptor and non-adrenergic binding sites in basolateral membranes from rabbit kidney.

Competition studies, which better characterized the heterogeneity of $^3$H-idazoxan binding, demonstrated that compounds with an imidazoline structure (tolazoline, tramazoline) completely inhibited the idazoxan binding while only 25% of binding was affected by epinephrine and non-imidazoline $\alpha_2$-adrenergic compounds (Coupry et al., 1987).

The non-homogeneity of the $\alpha_2$ receptors was further investigated in rat and human kidney (Michel and Insel, 1989b). They compared the pharmacological properties of the binding site for $^3$H-idazoxan with those of $^3$H-rauwolscine in rat and $^3$H-yohimbine in human renal cortical membranes. They confirmed previous findings that idazoxan binds to two

sites, the $\alpha_2$-adrenoceptor and an additional non-adrenergic binding site. The affinity of idazoxan for both sites was similar. However, their studies showed that these additional sites did not interact with imidazoline drugs such as p-aminoclonidine (PAC), moxonidine and clonidine. Although, their work demonstrated the presence of additional idazoxan binding sites in other tissues such as human platelets, myometrium, and HEL cells, these extra sites were not observed in three cell lines (MDCK, BC $3H_1$, Jurkat cells) lacking $\alpha_2$-adrenoceptors. However, these studies were inconclusive in determining whether the additional idazoxan binding site was a subtype of $\alpha_2$ adrenoceptor or distinct from it. The non-adrenergic idazoxan binding site in rabbit kidney recognized not only imidazoline compounds but also guanidinium analogs such as guanabenz, amiloride, 5-(M-ethyl-N-isopropyl) amiloride and phenamylamiloride (Coupry et al., 1989a, b). This binding site was labelled as imidazoline-guanidinium receptive site (IGRS). To determine whether this IGRS and the $\alpha_2$-adrenoceptor represented distinct proteins, Parini et al., (1989) solubilized and partially characterized the two binding sites in rabbit kidney. Utilizing heparin-agarose or lectin affinity chromatography, the two receptors were physically separated indicating that the two binding sites were distinct entities. To determine ligand recognition properties of the IGRS, competition binding studies were

performed with $^{3}$H-idazoxan after saturating $\alpha_2$-adrenoceptor binding sites with 10 uM rauwolscine.

Although both the $\alpha_2$-adrenoceptor and the IGRS recognized imidazoline/guanidinium compounds, the IGRS recognized them with a rank order of affinity different from that exhibited by $\alpha_2$-adrenoceptors. Idazoxan binding was not inhibited by epinephrine, serotonin or dopamine at competing ligand concentrations of 10 uM.

**Studies With p-Aminoclonidine, Clonidine and Moxonidine**

As shown in the above discussion, several studies with idazoxan demonstrated the existence of non-adrenergic, catecholamine insensitive sites in brain and kidney of man, rat, pig and rabbit. However, the physiological role of these sites was not determined.

Parallel studies done in the mid 1980's spearheaded by Bousquet et al., (1984), Ernsberger et al., (1987; 1988a; 1989) and Ruffolo et al., (1977; 1982; 1983) using clonidine and its related compounds also showed the existence of non-adrenergic imidazoline preferring sites in the brain stem of several species of animals, and that these sites may participate in vasomotor regulation within the rostral ventrolateral medulla.

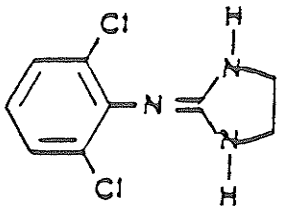
In 1984, Bousquet et al., made a very intriguing observation concerning the relationship between central $\alpha_2$-adrenoceptor stimulation and blood pressure regulation. They reported that after microinjections of certain $\alpha$-

adrenergic drugs (fig. 1) into the nucleus reticularis lateralis of anesthetized cats, compounds such as α-methyl norepinephrine, a selective $\alpha_2$-adrenoceptor agonist (0.1 - 10 ug/kg) failed to lower blood pressure. Alternatively, potent $\alpha_1$-adrenoceptor agonists such as cirazoline and ST 587 produced dose-dependent hypotensive effects similar to that observed with clonidine. They concluded that $\alpha_2$-adrenoceptor selective catecholamines were not active in the nucleus reticularis lateralis region, whereas, imidazoline based compounds induced hypotensive effects regardless of their affinity for the different α-adrenoceptor subtypes. This finding suggested the possible existence of sites in this region of the brain that preferentially bound compounds with an imidazoline structure. This site, and not $\alpha_2$-adrenoceptors, was responsible for blood pressure lowering.

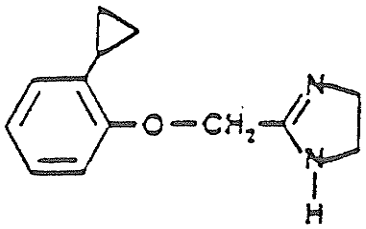
Prior to this study, Ruffolo and co-workers (1977) had studied the structure activity relationship between the imidazoline α-agonists and phenylethylamines in producing contractions in the rat vas deferens. They noted that repeated administration of imidazolines to rat vas deferens caused desensitization of the contractile response after the seventh dose. However, phenylethylamines produced contractions in the vas deferens which were refractory to the imidazolines. This observation provided evidence for the different mode of interaction of imidazolines and phenylethylamines at α-adrenoceptors. However, these studies failed to determine whether the imidazoline

Figure 1.

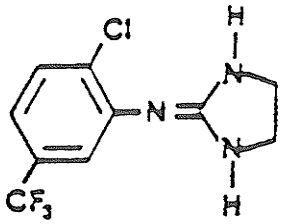
Chemical structures of imidazoline compounds and a phenylethylamine, α-MNE (α-methylnorepinephrine).



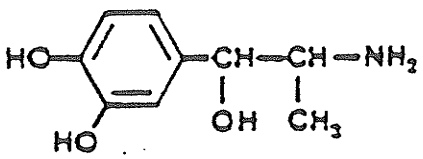
CLONIDINE



CIRAZOLINE



ST 587



α-MNE

adrenergic agonists act at allosteric sites of the α-adrenoceptor or somewhere else.

The first confirmation of the existence of specific imidazoline sensitive binding sites insensitive to catecholamines was made by the study of $^{3}$H-p-aminoclonidine binding to membranes prepared from the ventrolateral area, including the nucleus reticularis lateralis, of the bovine medulla (Ernsberger et al., 1987). In these preparations, it was demonstrated that only 70% of the sites labelled by $^{3}$H-p-aminoclonidine could be displaced by the phenylethylamines. The remaining phenylethylamine insensitive sites were sensitive to imidazoline based α-adrenoceptor agonists as well as certain molecules bearing an imidazole moiety which was structurally similar to the imidazoline ring, such as histamine and cimetidine. These non-adrenergic imidazoline binding sites appeared to be concentrated in the ventrolateral medulla, as compared to the frontal cortex, a finding consistent with an action of clonidine on medullary imidazoline receptors to lower blood pressure.

Utilizing $^{3}$H-clonidine, Bricca et al., (1989) confirmed that about 25% of the binding sites in bovine ventral medulla were insensitive to displacement by norepinephrine. In whole rat brainstem, no norepinephrine insensitive binding could be detected while in the area in human brain corresponding to the nucleus reticularis lateralis, norepinephrine, epinephrine or α-methylnorepinephrine had

virtually no effect on $^3$H-clonidine binding. However, imidazoline agonists such as cirazoline, and imidazoline antagonists such as idazoxan, produced potent displacement.

The ability of a series of $\alpha_2$-adrenoceptor agonists, including both imidazolines and phenylethylamines, to lower blood pressure or heart rate upon direct injection to the nucleus reticularis lateralis in anesthetized rats correlated well with their ability to inhibit the non-adrenergic component of $^3$H-p-aminoclonidine binding in bovine lateral medulla. The ability to lower heart rate did not correlate with their ability to inhibit central $\alpha_2$-adrenoceptors in bovine brain (Ernsberger et al., 1990). This suggested a functional role for this binding site which was labeled by $^3$H-p-aminoclonidine.

Recent experiments have shown that another imidazoline agonist radioligand, $^3$H-moxonidine, could label the sites identified by $^3$H-clonidine or $^3$H-p-aminoclonidine. In addition to their location in the central nervous system, these sites could also be detected in the renal medulla and adrenal chromaffin tissue (Ernsberger et al., 1991).

**Summary**

Radioligand binding studies have helped in our understanding of $\alpha_2$-adrenoceptor heterogeneity and the subsequent discovery of imidazoline receptors. Differences in the sites labelled by purported $\alpha_2$-adrenoceptor antagonists and agonists were noticed in the 1980's. $^3$H-

idazoxan, an imidazoline $\alpha_2$-adrenoceptor antagonist consistently labelled non-adrenergic sites in addition to the $\alpha_2$-adrenoceptors. On the other hand, non-imidazoline $\alpha_2$-adrenoceptor antagonists such as $^3$H-rauwolscine and $^3$H-yohimbine bound only $\alpha_2$-adrenoceptors. Because idazoxan has the imidazoline nucleus, it was suggested that the extra non-adrenergic sites labelled by this compound were the imidazoline receptors. Rauwolscine and yohimbine lack the imidazoline nucleus, hence their inability to label the imidazoline receptor. $^3$H-p-aminoclonidine and moxonidine, both imidazoline compounds, also bound non-adrenergic imidazoline sites. However, the imidazoline sites recognized by idazoxan are different from the imidazoline sites recognized by p-aminoclonidine and moxonidine. This observation led to the suggestion that heterogeneity exists in the imidazoline receptors.

**In Vivo Renal Studies**

*In vivo* experiments in which adrenergic imidazoline compounds have been directly infused into the renal artery of anesthetized uninephrectomized rats have consistently shown that these compounds act at more than one site and/or receptor. For example, low intrarenal infusion rates of clonidine increased free water clearance, while higher infusion rates also increased osmolar clearance (Blandford and Smyth, 1988).

Additional studies have indicated further that clonidine may be acting at more than one site and/or receptor. Although intrarenal infusion of clonidine produced an increase in urine flow rate secondary to an increase in free water clearance, intravenous infusions were found to increase osmolar clearance (Blandford and Smyth 1989). Furthermore, Smyth et al., (1992) showed that purported $\alpha_2$-agonists, namely, clonidine, 2,6-dimethylclonidine (2,6-DMC) and UK 14,304 showed different potencies in their ability to increase solute excretion and free water clearance. 2,6-DMC was the most potent at increasing osmolar clearance, whereas, clonidine was the most potent at increasing free water clearance. Conversely, clonidine had little effect on osmolar clearance and 2,6-DMC had little effect on free water clearance. This apparent reverse rank order of potency for clonidine and 2,6-DMC indicated the possibility that two receptors were involved in these responses. In a water loaded rat model, clonidine failed to change the proximal segment reabsorption of solute, however, 2,6-DMC produced a dose related decrease in the amount of solute reabsorbed in the proximal tubule for any given level of fluid delivery (Smyth et al., 1992). The effects of 2,6-DMC were not abolished by an antagonist of vasopressin $V_2$ receptors, whereas, the effects of clonidine were abolished by the $V_2$ antagonist. These studies suggested that as with central administration of various $\alpha_2$-adrenoceptor agonists, the effects in the periphery also

indicated more than one site and/or receptor may be involved.

To determine the physiological role of the imidazoline receptor in the kidney, Allan et al., (1993) infused moxonidine directly into the renal artery of anaesthetized rats. Moxonidine was chosen, since it displayed a 700-fold greater affinity for $I_1$ imidazoline receptors as compared to $\alpha_2$-adrenoceptors in receptor binding studies (Ernsberger et al., 1991). They reported that moxonidine increased urine flow rate through increases in osmolar clearance and not through an increase in free water clearance as documented for $\alpha_2$-adrenoceptor agonists. This effect was attenuated by idazoxan the imidazoline receptor selective antagonist but not by rauwolscine the $\alpha_2$-adrenoceptor selective antagonist.

The above findings were consistent with the work of Bidet et al., (1990) with isolated renal tubular cells from rabbits. They found that $^{22}Na^+$ entry into proximal tubule cells could be blocked by imidazoline derivatives such as idazoxan, and cirazoline. Cirazoline is an imidazoline receptor agonist whereas idazoxan is considered to be an antagonist at these same sites. It is therefore surprising that these two compounds had the same effect on sodium transport across the proximal cells. These results can probably be explained if we begin to think of idazoxan as a partial agonist at the imidazoline receptor.

In summary, limited studies have been performed in vivo on renal function of $I_1$ imidazoline receptors. Initial

studies with clonidine and 2,6-dimethylclonidine suggested that two receptors were involved in their renal responses. Renal $\alpha_2$-adrenoceptor stimulation caused an increase in free water clearance whereas stimulation of another site possibly the I1 imidazoline receptor caused an increase in osmolar clearance (solute excretion). Further studies with the highly selective $I_1$ imidazoline compound moxonidine confirmed the fact that $I_1$ imidazoline receptor stimulation produced an increase in solute excretion.

**Imidazoline Receptor Subtypes**

Heterogeneity in the imidazoline recognition sites has been reported. Michel et al., (1989a, b) reported that the $^3$H-idazoxan binding at non-adrenergic sites in rat kidney was not displaced by other imidazolines such as clonidine and moxonidine. However, UK14,304, tolazoline and guanabenz bound competitively at these sites with affinities similar to those at $\alpha_2$-adrenoceptors. Coupry et al., (1989) also demonstrated that idazoxan binding was inhibited by amiloride and potassium. On the other hand, Ernsberger et al., (1991) reported that p-aminoclonidine binding sites were not recognized by guanabenz, amiloride and other guanidino compounds in rat kidney and bovine ventrolateral medulla. These studies identified two subclasses of imidazoline receptors on the basis of differences in the pharmacological characteristics of the ligands used. At the First International Symposium on Imidazoline Preferring

Receptors, a uniform nomenclature for these two major subtypes of imidazoline receptors was adopted (Michel and Ernsberger, 1992). Sites labelled by $^3$H-clonidine and its analogs were termed $I_1$ sites. Non-adrenergic $^3$H-idazoxan labelled sites were called $I_2$. The "I" designation was meant to encompass not only imidazolines but also related structures including guanidines and oxazolines, all of which are potential ligands at these sites. The $I_1$ sites have been reported to mediate the central blood pressure lowering effects of clonidine and related compounds (Ernsberger et al., 1990). Also, $I_1$ sites in the kidney may mediate increases in fractional sodium excretion in distinction to the increases in free water clearance mediated by $\alpha_2$-adrenoceptor stimulation (Smyth et al., 1988, 1992; Allan et al., 1993). The description of the physiological role of $I_2$ receptors has been nebulous. They have been detected in mitochondrial outer membrane in rat liver (Tesson et al., 1991). These studies indicated that the $I_2$ receptor was located intracellularly.

**Clonidine Displacing Substance** (CDS)

A problem with the imidazoline receptor concept concerns its insensitivity to cathecholamines. The question remains as to what neurotransmitter would activate the imidazoline receptor. Since these sites were not found to be adrenergic, histaminergic, serotoninergic or cholinergic,

none of the known neurotransmitters could be identified with these novel receptors.

Atlas and Burstein, (1984), using ion-exchange chromatography, zone electrophoresis and high-performance liquid chromatography first isolated and partially purified an endogenous compound from calf brain which specifically displaced bound $^{3}$H-clonidine in rat brain membranes and specifically bound $^{3}$H-yohimbine in human platelet membranes. Since both clonidine and yohimbine are commonly used to probe $\alpha_2$-adrenoceptors, it was suggested that the new compound might have a structural resemblance to $\alpha_2$-adrenoceptor ligands. This compound was termed clonidine displacing substance (CDS) and further characterization revealed that it was a non-catechol, non-protein and low molecular weight compound. In a follow up study, Bousquet et al (1986) investigated the role of CDS in the central regulation of blood pressure. They reported that topical application of CDS directly in the nucleus reticularis lateralis region of anaesthetised cats regularly elevated mean arterial blood pressure by about 40%. Pretreatment of anaesthetised rabbits with intracisternal CDS shifted the dose-response curve for injected clonidine to the right. They suggested that CDS was an endogenous antagonist for the hypotensive effect of clonidine, at least in the nucleus reticularis lateralis region. However, if CDS was an antagonist, this still left the question as to what was the endogenous agonist.

Meely et al., (1986) also isolated a substance from bovine brain which displaced $^{3}$H-clonidine binding to rat brain membranes and inhibited $^{3}$H-para-aminoclonidine binding to receptors in bovine ventrolateral medulla membranes. However, their form of CDS produced cardiovascular effects opposite to that reported by Bousquet et al., (1986). The preparation of Meely et al., (1986) produced a dose dependent fall in arterial blood pressure when microinjected into the C1 area of the rostral ventrolateral medulla in the rat. This effect was consistent with the central action of clonidine. Therefore, CDS was postulated to be the endogenous ligand for the imidazoline receptor. Possibly, one of the preparations of the CDS contained impurities which also affected blood pressure regulation.

It has been reported that CDS appears to be elevated in the serum of patients with pregnancy-induced hypertension (Kreisberg et al., 1987). This suggested that CDS may play a role in the pathogenesis of hypertension. However, it is not clear whether the increased levels of CDS caused the hypertension or were a reflex mechanism to lower blood pressure. A number of physiological functions have been attributed to CDS. These include contraction of smooth muscle in rat gastric fundus (Felsen et al., 1987), inhibition of the twitch response in rat vas deferens (Diamant and Atlas, 1986) and inhibition of epinephrine-induced aggregation of human platelets (Diamant et al., 1987).

Dontenwill et al., (1987) raised polyclonal antibodies against clonidine. The interaction between the antibodies and clonidine was strongly inhibited by CDS suggesting that CDS structurally resembled clonidine and its analogs. The importance of their work lies in the fact that in future these antibodies may help in the characterization of imidazoline receptors and in the search for the endogenous ligand for them.

Since the initial submission of the draft of this thesis, an endogenous clonidine displacing substance has been isolated (Li et al., 1994). The isolation of this substance, termed "agmatine", will allow studies which may describe the physiological function of these receptors.

**Signal Transduction of Imidazoline Receptors**

The signal transduction mechanism mediating the effects of the $\alpha_2$-adrenoceptor has been well elucidated. In particular, the $\alpha_2$-adrenoceptor has been found coupled to the Gi protein. The signal transduction mechanism that mediates the effects of the imidazoline receptors has not been extensively studied. The effects of GTP on the binding of various imidazolines to non-adrenergic sites have been studied (Paris et al., 1989, Michel et al., 1989a). Michel et al., (1989a) reported that binding of the imidazoline UK14,304 to non-adrenergic $^3$H-idazoxan binding sites was not affected by GTP and sodium. This observation indicated that the non-adrenergic $^3$H-idazoxan binding site was not coupled

to a G protein. Paris et al., (1989) reported that addition of Gpp(NH)p strongly inhibited the binding of $^{3}$H-clonidine and $^{3}$H-UK14,304 to HT29 cell $\alpha_2$-adrenoceptors but that it required 25 fold more Gpp(NH)p to inhibit 50% of $^{3}$H-UK14,304 binding compared to $^{3}$H-clonidine binding. Ernsberger et al., (1993) reported in an abstract that $^{3}$H-moxonidine binding to bovine ventrolateral medulla membranes was inhibited by Gpp(NH)p. This implies that the $I_1$ sites are G protein coupled. However, the specific G protein subunit is yet to be determined.

**Clinical Significance of Imidazoline Receptors**

There is increasing recognition of the role of the sympathetic nervous system in the pathogenesis of hypertension. Various classes of antihypertensive agents have as their target a reduction in the sympathetic activity or blockade of adrenergic receptors. Agents that act within the central nervous system to inhibit sympathetic nervous system activity have been in use for some time as clinically effective anti-hypertensive agents (Weber et al., 1990). Clonidine and α-methyldopa represent the first generation of such agents but are not considered as first line agents owing to a high incidence of side effects such as sedation and dry mouth. The vasodepressor action as well as these unpleasant side effects have been ascribed to agonist action at $\alpha_2$-adrenoceptors (Timmermans and van Zwieten, 1982; Timmermans et al., 1981). Previous efforts to separate the

vasodepressor actions from the sedative effects proved unsuccessful. Moxonidine and rilmenidine represent the second generation centrally acting anti-hypertensive agents (Ollivier et al., 1992). The advantage they have over the first generation agents is that they are less associated with sedation and dry mouth. Since these are both $I_1$ agonists, the implication is that vasodepression can be dissociated from sedation and other adverse effects elicited by central $\alpha_2$-adrenoceptor activation. The kidney plays an important role in the long term regulation of blood pressure through its regulation of water and solute excretion. Renal $I_1$ imidazoline receptor stimulation produces diuresis and natriuresis. These are physiological processes that culminate in a reduction in arterial pressure. Obviously then, $I_1$ agonists such as moxonidine and rilmenidine will have another advantage over other anti-hypertensive agents in that, apart from a central vasodepressor action, they have a peripheral action on the kidney which enhances excretion of water and sodium (Allan et al., 1993).

Moxonidine has already been released to the West German market for the treatment of hypertension. Clinical trials indicate that it has several other advantages over existing anti-hypertensives. For example, unlike the ß-blockers, it does not change blood glucose and lipid profiles (Weimann and Rudolph, 1992). Neither does it precipitate asthma in asthmatics. When used over a long

term it promotes regression of hypertrophied left ventricles (Ollivier et al., 1992).

**Statement of the problem**

Traditionally, the vasodepressor action of centrally acting anti-hypertensive agents has been attributed to an agonist action at $\alpha_2$-adrenoceptors. Recent evidence however, suggests that the $I_1$ imidazoline receptor may in part mediate the central antihypertensive effects of imidazoline compounds such as moxonidine and rilmenidine. The kidney plays an important role in the long term regulation of arterial blood pressure as outlined above. Anti-hypertensive agents that target the kidney may have added advantage over others. The identification of $I_1$ imidazoline receptors in the kidney prompted us to determine if the renal $I_1$ imidazoline receptor plays any role in the renal excretion of water and solutes.

Previous studies showed that moxonidine caused an increase in urine flow rate and sodium excretion most likely by a direct action on the renal $I_1$ imidazoline receptor. In another series of experiments the signal transduction mechanism involved in these physiological effects was studied. Specifically, we determined whether Gi proteins are involved.

Previous studies in the laboratory showed that indomethacin, an inhibitor of prostaglandin synthesis, potentiated the diuresis and natriuresis caused by renal $\alpha_2$-

adrenoceptor activation. Although $\alpha_2$-adrenoceptors are distinct from imidazoline receptors they both produce diuresis when activated. Therefore, we looked at the role of prostaglandins on the physiological effect of stimulation of renal $I_1$ imidazoline receptors.

**Purpose of the Present Studies:**

The imidazoline receptor has been classified into $I_1$ and $I_2$ subtypes. The $I_1$ imidazoline receptor shows high affinity for clonidine and its analogs and moxonidine whereas the $I_2$ imidazoline receptor shows high affinity for idazoxan (Michel and Ernsberger, 1992). Recent studies in our laboratory have indicated that moxonidine, an $I_1$ imidazoline receptor agonist causes diuresis and natriuresis in rats.

The aims of the present proposal were as follows.

1. To confirm the natriuretic effect of $I_1$ imidazoline receptor stimulation in the kidney by the direct intrarenal arterial infusion of moxonidine.
2. To determine whether this pharmacological and/or physiological effect was mediated through a G protein, by pretreatment with pertussis toxin.
3. To determine whether prostaglandins were involved by pretreatment with indomethacin and attempting to reverse its effects by an infusion of prostaglandin $E_2$.

# II MATERIALS AND METHODS

## Experimental Animals

Male Sprague-Dawley rats (225-250g) were obtained from University of Manitoba Central Animal Care, Winnipeg, Manitoba, Canada (Charles River Breeding Stock). They were housed at 22 $^{o}$C (relative humidity 50%) with a light-dark cycle changing at 7 a.m. and 7 p.m.. They were fed normal Purina rat chow and given tap water for drinking (ad libitum).

## Experimental Preparation

Prior to the day of the experimemt (7 to 12 days), the right kidney was removed under ether anaesthesia through a right flank incision. Xylocaine gel (Lidocaine hydrochloride 2%) was applied to the incision wound to alleviate post-operative pain. On the day of the experiment, rats were anaesthetised with pentobarbitone (Nembutal 50mg/kg; intraperitoneal). Additional anaesthetic was given as a bolus through the catheter in the jugular vein as needed. Animals were placed on a Harvard Animal Blanket Control Unit and the rectal thermometer connected to this blanket was inserted to maintain body temperature at 37.5 $^{o}$C. A tracheostomy was performed leaving the animal to breathe spontaneously through a PE-240 tube. The left carotid artery was cannulated with PE-60 tubing and connected to a Statham pressure transducer Model P23Dc and

Grass Model 5 Polygraph for the continuous monitoring of blood pressure and heart rate. Also the left jugular vein was cannulated with PE-160 tubing for the infusion of normal saline at 97 *ul*/min. The remaining left kidney was exposed through a flank incision and the ureter cannulated (PE 10) for the collection of urine into pre-weighed tubes. A 31 gauge stainless steel needle was advanced through the aorta into the renal artery for the intra-renal infusion of either saline (vehicle) or moxonidine in saline with a Harvard sage pump.

**Experimental Protocol**

Following surgery the rat was allowed to stabilize for a period of 45 minutes prior to the first urine collection. The first 15 minute urine collection served as the control period to ensure that the surgical procedure had not altered renal function. An intra-renal infusion of moxonidine solution or saline (vehicle) was started after the control collection and maintained for the rest of the experiment. During this infusion, four additional 15 minute urine collections were obtained into pre-weighed tubes.

At the end of the experiment, about 5 ml of blood was obtained through the carotid artery catheter and the plasma was separated and frozen. Proper placement of the needle in the renal artery was confirmed by the injection of methylene blue dye into the renal artery. Urine and plasma osmolality were measured with a Precision Systems Micro

Osmometer; creatinine concentration with a Beckman Creatinine 2 Analyzer; sodium and potassium concentration with a Nova Biomedical Electrolyte Analyzer Model 13.

The above general procedure was adopted in all the experiments with the following modifications:

A) Dose-Response Studies.

In these experiments, increasing doses of moxonidine (0.3, 1, 3 nmol/kg/min) or saline (vehicle) were infused intrarenally (3.4 ul/min) at the beginning of the second collection period and maintained for the duration of the experiment during which four consecutive 15 minute urine collections were obtained.

B) Pertussis Toxin Pretreated Animals.

In this series of experiments, pertussis toxin (3 ug/kg) was given to each rat via the tail vein 5 days prior to the experimental day from a stock solution of 3 ug/ml. Previous studies had indicated that a higher dose of pertussis toxin was required to effectively block the G protein (Pedraza-Chaverri et al., 1984). However, studies in our laboratory have documented significant effects of pertussis toxin treatment at a much lower dose (3 ug/kg) (Smyth et al., 1988). Six urine samples were collected at

15 minute intervals. Moxonidine in the saline solution (1 nmol/kg/min) was infused directly into the renal artery at the beginning of the second collection and maintained throughout the duration of the experiment. Preliminary results suggested that the diuretic effect of intra-renal moxonidine was attenuated by pertussis toxin pretreatment. Therefore, we determined whether the pertussis toxin treated rats would respond normally to other diuretics such as furosemide. This would rule out the fact that the decreased natriuretic effect of moxonidine was not general effect due to the animals being ill from the pertussis toxin treatment. Furosemide (0.1 mg/kg) was given as a bolus injection intravenously at the end of the fifth collection and a sixth 15 minute urine sample was collected.

C) Indomethacin Pretreated Rats

The rats in this group were given either indomethacin (5 mg/kg), dissolved in physiological buffer (25 mM $NaH2PO_4$, 25 mM $K_2HPO_4$, 1 mM $MgCl_2$) at pH 7.4, or vehicle (0.3 ml) by intraperitoneal injection immediately following induction of anaesthesia. Indomethacin was dissolved to a concentration of 5 mg/ml in the buffer. We chose a dose of 5 mg/kg of indomethacin because previous studies indicated that this dose reduced the synthesis of renal prostaglandin $E_2$ by more than 80% (Hui and Falardeau, 1990). In another group of rats, in addition to the indomethacin pretreatment,

prostaglandin $E_2$ (1 ug/kg/min) was infused via the jugular vein at the rate of 3.4 ul/min by a Harvard sage pump at the beginning of the second urine collection and maintained throughout the period of the experiment. Blandford and Smyth, (1991) had previously shown that this dose of prostaglandin did not alter sodium excretion when infused via the jugular vein. Two urine samples were collected at 30 minute intervals after the first 15 minute control collection.

**Statistical Analysis**

Data are presented as the mean ± standard error (s.e) of the mean. Statistical analysis was performed with a repeated-measures ANOVA using SAS System Version 6.07. Significant interactions were further analyzed with a Least Squares Means Difference Test. A P value of less than 0.05 was deemed significant. The number of animals per group has been included in the table and figure legends.

**Drugs**

Moxonidine (supplied by Beiersdorf, AG, Hamburg, Germany), was dissolved in saline and stored in the fridge at a temperature of 4 $^{o}$C. Each preparation was used within a week.

Pertussis toxin was obtained from Sigma Chemical Company and dissolved in saline (3 ug/ml) and kept at a temperature below 0 $^{o}$C.

Indomethacin (Sigma Chemical Company) was dissolved in a few drops of a sodium hydroxide solution (1 N). Phosphate buffer (25 mM $NaH_2PO_4$, 25 mM $K_2HPO_4$, 1 mM $MgCl_2$ ) was then added and the final pH adjusted to 7.4 (hydrochloric acid, HCl 1N).

Furosemide (Sigma Chemical Company) was stored in the refrigerator at a temperature of 4 $^{o}$C.

Nembutal (British Drug House) was prepared in a final concentration of 50 mg/ml.

Xylocaine jelly (2%) (Astra Pharma Inc., Mississauga Ont).

## RESULTS

Dose-Response Relationship For Moxonidine:

In these series of experiments we examined the renal effects of increasing doses of moxonidine (0, 0.3, 1, 3 nmol/kg/min). The first urine collection for each experiment served as a preparation control. This allowed evaluation of baseline renal function for each experiment and the determination of altered data secondary to the surgical procedure. In all groups studied, the measured parameters did not differ among groups during this first collection period prior to any intervention (Table 1). The fourth collection period is representative of the differences observed among groups and consequently these data are presented in detail (fig 2 to 4).

No change in heart rate, blood pressure and creatinine clearance was observed at any of the infusion rates studied (fig 2). A dose related increase in urine flow rate and sodium excretion was observed (fig 3). A significant decrease in potassium excretion at the doses of 0.3 and 1 nmol/kg/min was seen. The increase in electrolyte excretion was reflected by an increase in osmolar clearance (fig 4). As well, there was an increase in free water clearance at the infusion rates of 1 and 3 nmol/kg/min (fig 4).

Table 1.

Baseline measurements during the first urine collection immediately prior to the infusion of moxonidine or saline.

**Table 1**

Baseline Values - Dose Response Experiments

| | Moxonidine nmol/kg/min | | | |
|---|---|---|---|---|
| | Control n=7 | 0.3 n=5 | 1 n=8 | 3 n=5 |
| Blood Pressure (mm Hg) | 112 ± 5 | 121 ± 2 | 115 ± 4 | 126 ± 3 |
| Creatinine Clearance (ml/min) | 1.95 ± 0.10 | 1.65 ± 0.38 | 1.81 ± 0.30 | 1.53 + 0.15 |
| Urine Volume (ul/min) | 15 ± 2 | 22 ± 3 | 21 ± 2 | 26 ± 2 |
| Sodium Excretion (uEq/min) | 1.3 ± 0.1 | 3.1 ± 0.5 | 3.3 ± 0.6 | 3.1 ± 0.6 |
| Potassium Excretion (uEq/min) | 4.7 ± 0.4 | 3.7 ± 0.4 | 4.2 ± 0.3 | 3.6 ± 0.4 |
| Free Water Clearance (ul/min) | -52 ± 5 | -41 ± 5 | -40 ± 3 | -41 ± 6 |
| Osmolar Clearance (ul/min) | 67 ± 5 | 63 ± 7 | 61 ± 4 | 64 ± 7 |

Figure 2.

Dose-response effects of intrarenal infusion of moxonidine (0 nmol/kg/min, n=7; 0.3 nmol/kg/min, n=5; 1 nmol/kg/min, n=8; 3 nmol/kg/min, n=5) on blood pressure, heart rate and creatinine clearance in the rat. Each group represents the mean $\pm$ standard error of the mean (vertical bars). Moxonidine at 0 nmol/kg/min represents vehicle (saline) infusion.

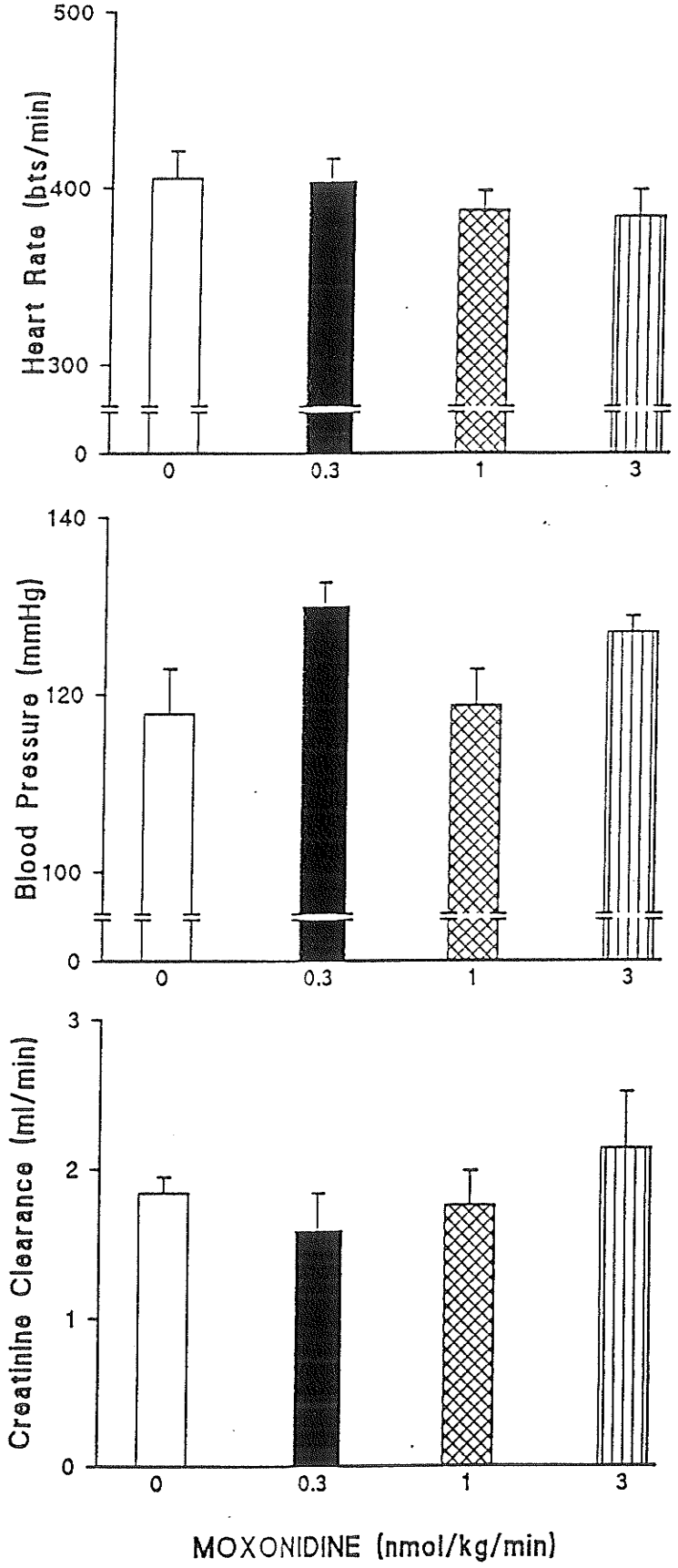

500
400
300
0
Heart Rate (bts/min)
0
0.3
1
3
140
120
100
0
Blood Pressure (mmHg)
0
0.3
1
3
3
2
1
0
Creatinine Clearance (ml/min)
0
0.3
1
3
MOXONIDINE (nmol/kg/min)

Figure 3.

Dose-response effects of intrarenal infusion of moxonidine (0 nmol/kg/min, n=7; 0.3 nmol/kg/min, n=5; 1 nmol/kg/min, n=8; 3 nmol/kg/min, n=5) on urine flow rate, potassium and sodium excretion in the rat. Each group represents the mean $\pm$ standard error of the mean (vertical bars). * denotes $P<0.05$ versus control vehicle.

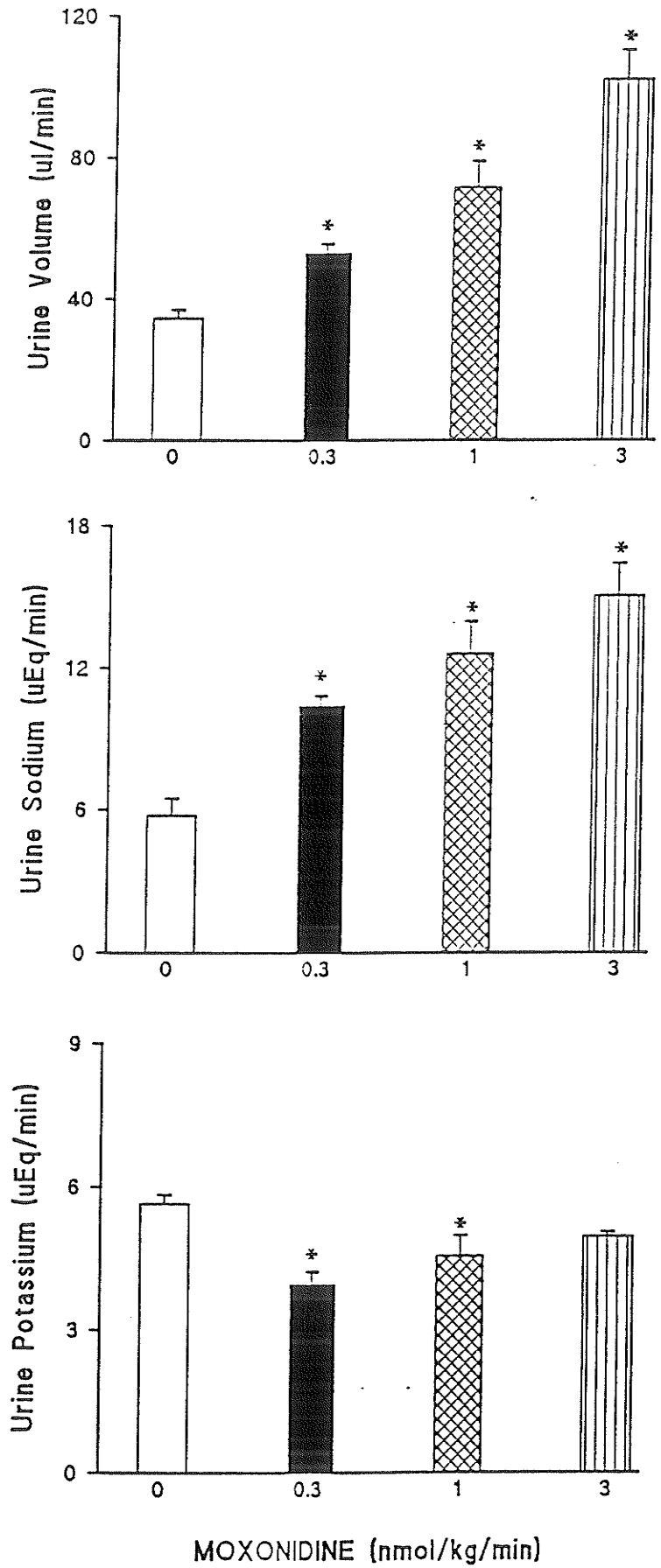
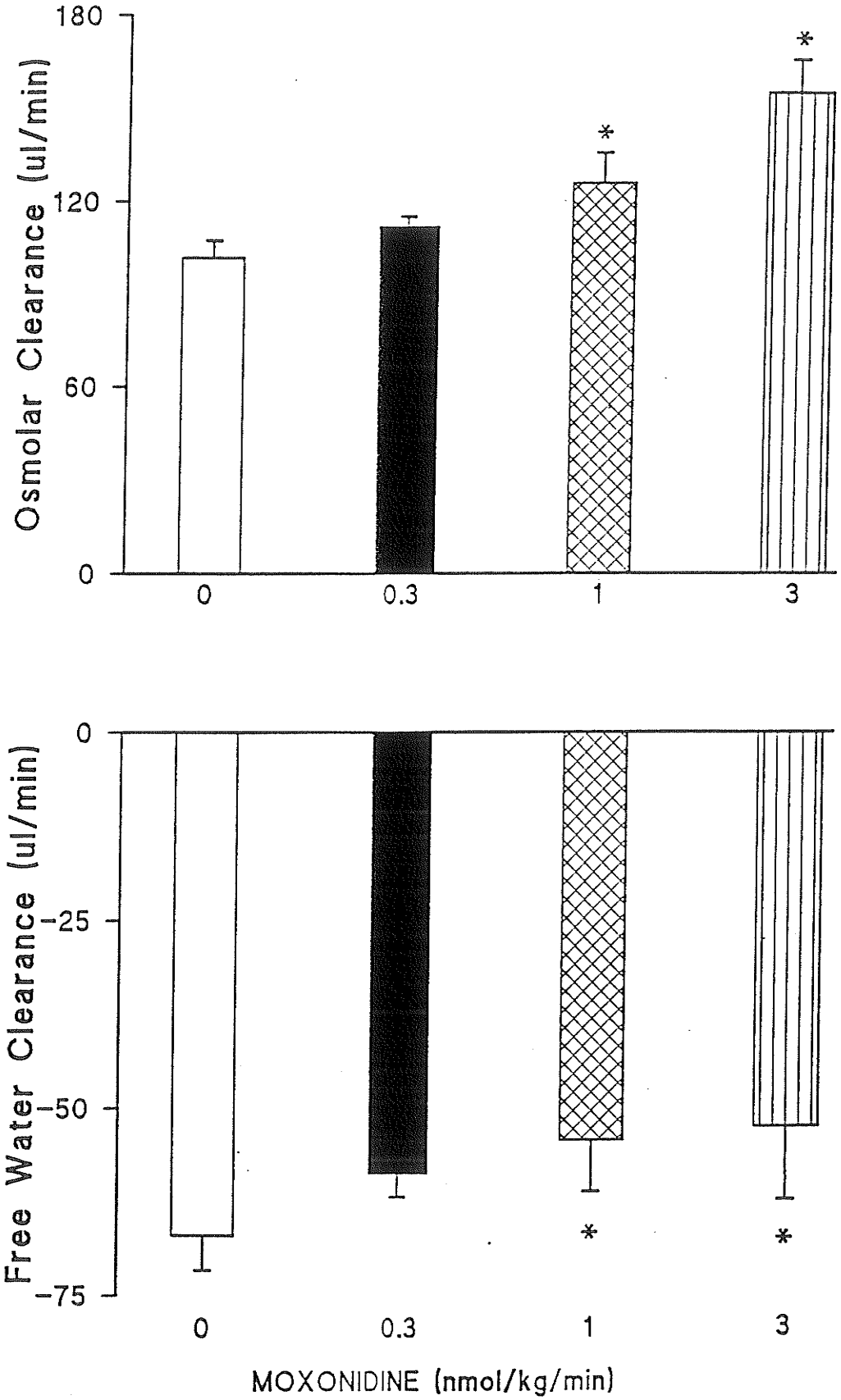

Urine Volume (ul/min)
120
80
40
0
0
0.3
1
3
Urine Sodium (uEq/min)
18
12
6
0
Urine Potassium (uEq/min)
9
6
3
MOXONIDINE (nmol/kg/min)

Figure 4.

Dose-response effects of intrarenal infusion of moxonidine (0 nmol/kg/min, n=7; 0.3 nmol/kg/min, n=5; 1 nmol/kg/min, n=8; 3 nmol/kg/min, n=5) on osmolar clearance and free water clearance in the rat. Each group represents the mean $\pm$ standard error of the mean (vertical bars). * denotes $P<0.05$ versus control.


180
120
60
0
Osmolar Clearance (ul/min)
*
*
0
0.3
1
3
0
-25
-50
-75
Free Water Clearance (ul/min)
*
*
0
0.3
1
3
MOXONIDINE (nmol/kg/min)

Pertussis Toxin Pretreatment Experiments:

In these series of experiments we examined one method to potentially imply the signal transduction mechanism by which moxonidine mediated the above renal effects. Specifically, we determined whether the $I_1$ imidazoline receptors were coupled to a G protein. Pertussis toxin ADP-ribosylates the Gi protein and thereby inactivates it. To determine whether the renal effects of moxonidine were mediated through the Gi protein, we pretreated rats with low doses of pertussis toxin (3 ug/kg/min) five days prior to the administration of moxonidine. Previous studies in our laboratory (Smyth et al., 1988) found that this dose was effective in inducing an effect yet did not alter the stability of the preparation. The first urine collection served as the control collection and there was no difference between groups during this first collection period (Table 2). Data from all collection periods have been presented (fig 5, 6 and 7). Pretreatment of rats with pertussis toxin abolished the diuretic and natriuretic effects of moxonidine (fig 5). However, there was no significant difference in potassium excretion (fig 5), blood pressure, heart rate or creatinine clearance (fig 6). Moxonidine also caused a small increase in free water clearance but this effect was abolished by pertussis toxin pretreatment (fig 7). It is possible that the pertussis toxin might have produced a non specific effect on the rats rendering them unresponsive to all diuretics. To challenge this notion we infused

Table 2.

Baseline measurements during the first urine collection in the presence and absence of pertussis toxin pretreatment and immediately prior to the infusion of moxonidine or saline.

**Table 2**

Baseline Values - Pertussis Toxin Experiment

| | Control n=7 | P.T. Saline n=5 | Moxonidine n=6 | P.T. Moxonidine n=5 |
|---|---|---|---|---|
| | | | Moxonidine nmol/kg/min | |
| Blood Pressure (mm Hg) | 112 ± 5 | 115 ± 6 | 115 ± 5 | 120 ± 4 |
| Creatinine Clearance (ml/min) | 1.95 ± 0.10 | 2.71 ± 0.38 | 1.81 ± 0.33 | 2.14 ± 0.47 |
| Urine Volume (ul/min) | 15 ± 2 | 15 ± 3 | 21 ± 2 | 14 ± 3 |
| Sodium Excretion (uEq/min) | 1.3 ± 0.1 | 0.9 ± 0.1 | 3.3 ± 0.7 | 3.1 ± 0.7 |
| Potassium Excretion (uEq/min) | 4.7 ± 0.4 | 3.5 ± 0.5 | 4.2 ± 0.3 | 3.1 ± 0.7 |
| Free Water Clearance (ul/min) | -52 ± 5 | -43 ± 4 | -40 ± 3 | -50 ± 10 |
| Osmolar Clearance (ul/min) | 67 ± 5 | 58 ± 4 | 61 ± 4 | 64 ± 13 |

Figure 5.

Effects of intrarenal infusion of moxonidine 1 nmol/kg/min in the presence (n=5) and absence (n=6) of pertussis toxin 3ug/kg, a G protein inhibitor, on urine volume, sodium and potassium excretion in the rat as compared to the vehicle control group (n=7). Each group represents the mean $\pm$ standard error of the mean. * denotes $P<0.05$ versus control. Mox = Moxonidine; P.T. = Pertussis Toxin.

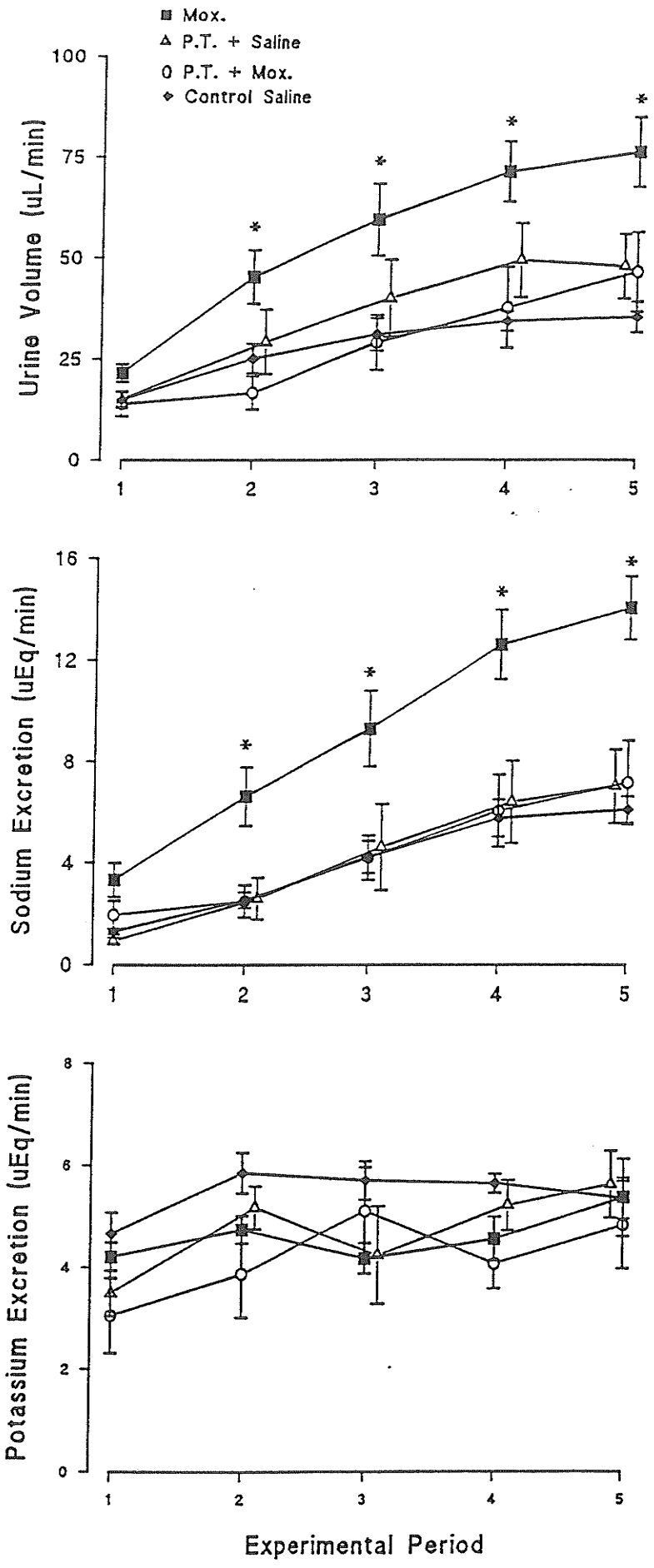

Mox.
P.T. + Saline
P.T. + Mox.
Control Saline
Urine Volume (uL/min)
100
75
50
25
0
Sodium Excretion (uEq/min)
16
12
8
4
0
Potassium Excretion (uEq/min)
8
6
4
2
0
1
2
3
4
5
*
Experimental Period

Figure 6.

Effects of intrarenal infusion of moxonidine 1 nmol/kg/min in the presence (n=5) and absence (n=6) of pertussis toxin 3ug/kg, a G protein inhibitor, on blood pressure, heart rate and creatinine clearance in the rat as compared to the vehicle control group (n=7). Each group represents the mean ± standard error of the mean. * denotes $P<0.05$ versus control. Mox = Moxonidine; P.T. = Pertussis Toxin.

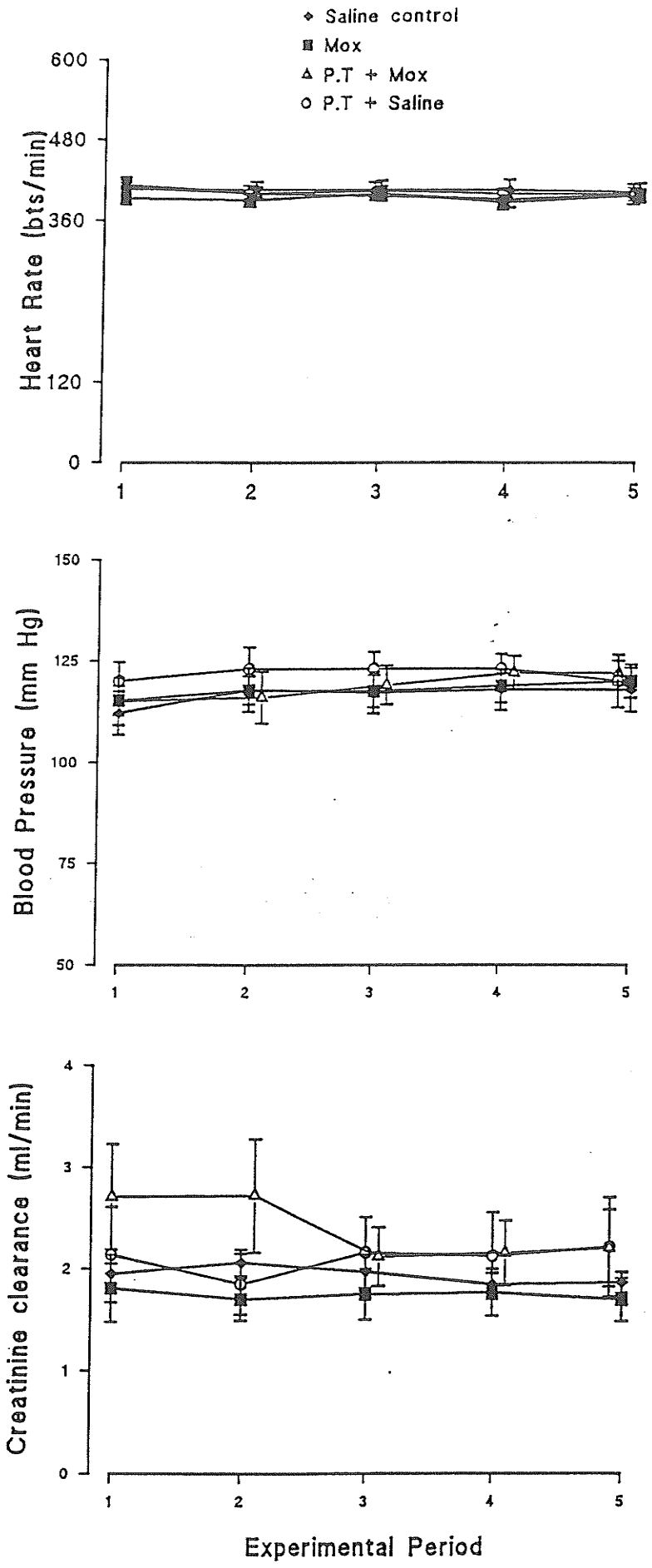

Saline control
Mox
P.T + Mox
P.T + Saline
Heart Rate (bts/min)
600
480
360
120
0
Blood Pressure (mm Hg)
150
125
100
75
50
Creatinine clearance (ml/min)
4
3
2
1
0
1
2
3
4
5
Experimental Period

Figure 7.

Effects of intrarenal infusion of moxonidine 1 nmol/kg/min in the presence (n=5) and absence (n=6) of pertussis toxin 3ug/kg, a G protein inhibitor, on osmolar clearance and free water clearance in the rat as compared to the vehicle control group (n=7). Each group represents the mean $\pm$ standard error of the mean. * denotes $P<0.05$ versus control. Mox = Moxonidine; P.T. = Pertussis Toxin.

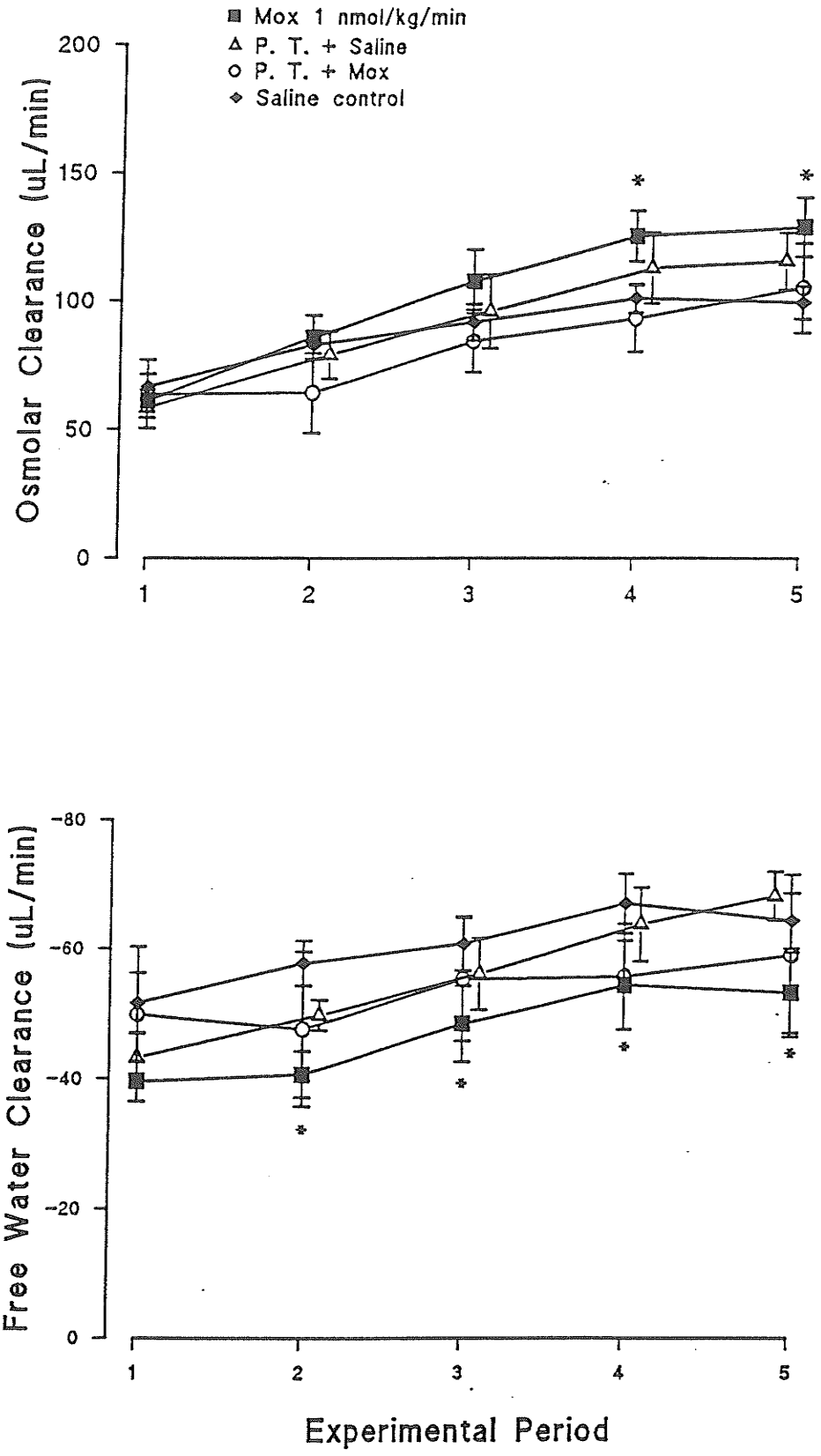

Mox 1 nmol/kg/min
P. T. + Saline
P. T. + Mox
Saline control
Osmolar Clearance (uL/min)
200
150
100
50
0
1
2
3
4
5
*
*
Free Water Clearance (uL/min)
-80
-60
-40
-20
0
1
2
3
4
5
*
*
*
*
Experimental Period

Table 3.

Response to furosemide (0.1 mg/kg) in rats which were pretreated saline (vehicle) or pertussis toxin (P.T.). Furosemide was also administered to a group of rats which had been pretreated with pertussis toxin and the response for moxonidine (Mox.) examined.

Table 3

Response to Furosemide

| | Saline n=5 | P.T. & Mox. n=5 | P.T. n=5 |
|---|---|---|---|
| Blood Pressure (mmHg) | 121 ± 5 | 123 ± 4 | 125 ± 3 |
| Heart Rate (bts/min) | 404 ± 7 | 393 ± 7 | 396 ± 7 |
| Urine Volume (ul/min) | 182 ± 17 | 190 ± 11 | 170 ± 16 |
| Urinary Sodium Excretion (uEq/min) | 26.5 ± 2.1 | 23.5 ± 4.2 | 20.7 ± 4.0 |
| Osmolar Clearance (ul/min) | 244 ± 15 | 261 ± 37 | 234 ± 26 |
| Free Water Clearance (ul/min) | -62 ± 10 | -71 ± 38 | -65 ± 17 |

furosemide (0.1 mg), a known diuretic which does not act through a G protein, into the jugular vein at the end of the fifth collection and collected urine for the next fifteen minutes. There was an increase in urine flow rate and sodium excretion in all the rats that were studied including those that failed to respond to moxonidine after the pertussis toxin pretreatment (Table 3).

Role of Prostaglandins in the Renal Response to Moxonidine:

Endogenously produced prostaglandin $E_2$ is known to modulate sodium excretion by the kidney but the exact mechanism is not fully understood. We used indomethacin to block the endogenous synthesis of prostaglandin $E_2$ and then determined the renal effects of $I_1$ imidazoline receptor stimulation with moxonidine (1 nmol/kg/min). Again, the first urine collection served as the preparation control. In all groups studied the measured parameters did not differ between groups during this first collection period prior to any intervention (Table 4). The third collection period represents differences observed among groups and consequently these data are presented in figures 8 to 10. As previously reported, moxonidine (1 nmol/kg/min) increased urine volume, sodium excretion and osmolar clearance (fig 9 and 10). Although blood pressure, creatinine clearance and heart rate (fig 8) did not change significantly during indomethacin pretreatment there was attenuation of the diuretic and natriuretic effects of moxonidine following

indomethacin pretreatment (fig 9). Free water clearance was also increased as compared to controls. In addition, indomethacin pretreatment abolished the increase in osmolar clearance produced by moxonidine (fig 10). Concurrent administration of prostaglandin $E_2$ to the indomethacin pretreated rats partially restored the natriuretic and diuretic effects of moxonidine (fig 9).

Table 4.

Baseline measurements during the first urine collection in the presence and absence of indomethacin and immediately prior to the infusion of moxonidine or saline. Mox. moxonidine; Buff. buffer; Indo. indomethacin; $PGE_2$ prostaglandin $E_2$.

**Table 4**

Baseline Values - Indomethacin Experiments

| | Control n=6 | Mox. & Buff. n=6 | Mox. & Indo. n=7 | Mox. & Indo. & $PGE_2$ n=5 | $PGE_2$ n=5 |
|---|---|---|---|---|---|
| Blood Pressure (mm Hg) | 112 ± 5 | 122 ± 2 | 122 ± 5 | 127 ± 2 | 114 ± 4 |
| Creatinine Clearance (ml/min) | 1.95 ± 0.10 | 1.60 ± 0.18 | 2.03 ± 0.27 | 1.65 ± 0.21 | 2.22 ± 0.22 |
| Urine Volume (ul/min) | 15 ± 2 | 14 ± 1 | 9 ± 2 | 23 ± 3 | 14 ± 1 |
| Sodium Excretion (uEq/min) | 1.3 ± 0.1 | 1.9 ± 0.4 | 1.7 ± 0.4 | 2.9 ± 0.4 | 0.8 ± 0.3 |
| Potassium Excretion (uEq/min) | 4.7 ± 0.4 | 2.7 ± 0.3 | 3.2 ± 0.6 | 3.6 ± 0.1 | 2.8 ± 0.3 |
| Free Water Clearance (ul/min) | -52 ± 5 | -40 ± 6 | -46 ± 7 | -50 ± 7 | -32 ± 9 |
| Osmolar Clearance (ul/min) | 67 ± 5 | 61 + 4 | 56 ± 8 | 73 ± 5 | 49 ± 9 |

Figure 8.

Effects of intrarenal infusion of moxonidine 1 nmol/kg/min in the presence (n=7) and absence (n=6) of indomethacin 5 mg/kg, and prostaglandin $E_2$ alone (n=5) on blood pressure, heart rate and creatinine clearance in the rat as compared to the vehicle control group (n=6). Each group represents the mean $\pm$ standard error of the mean. B = Phosphate buffer; M = Moxonidine ; I = Indomethacin; $PGE_2$ = Prostaglandin $E_2$ (1ug/kg/min); S = Saline.

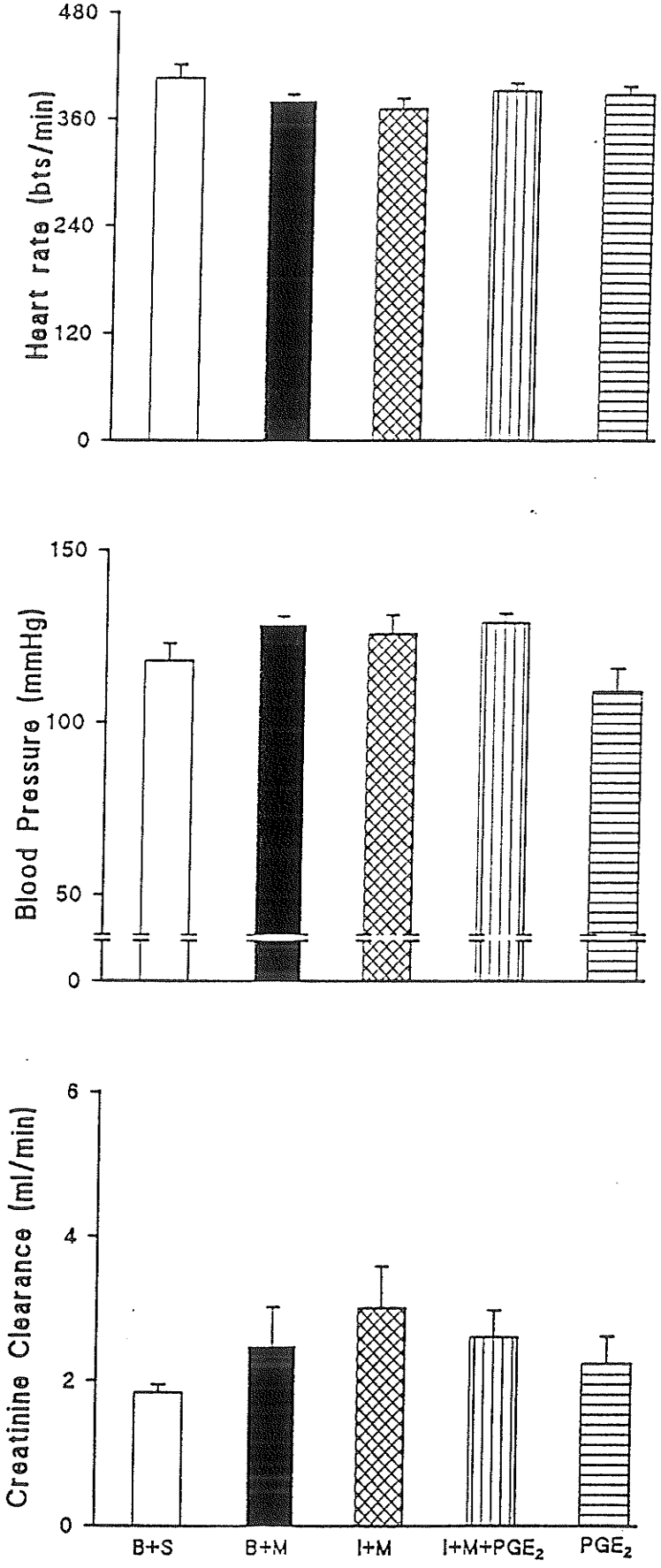

480
360
240
120
0
Heart rate (bts/min)
150
100
50
0
Blood Pressure (mmHg)
6
4
2
0
Creatinine Clearance (ml/min)
B+S
B+M
I+M
I+M+PGE2
PGE2

Figure 9.

Effects of intrarenal infusion of moxonidine 1 nmol/kg/min in the presence (n=7) and absence (n=6) of indomethacin 5 mg/kg, and prostaglandin $E_2$ alone (n=5) on urine volume, sodium excretion and potassium excretion in the rat. Each group represents the mean $\pm$ standard error of the mean. * denotes $P<0.05$ versus control. -*- denotes $P<0.05$ between groups. B = Phosphate Buffer; M = Moxonidine; I = Indomethacin; $PGE_2$ = Prostaglandin $E_2$ (1 ug/kg/min); S = Saline.

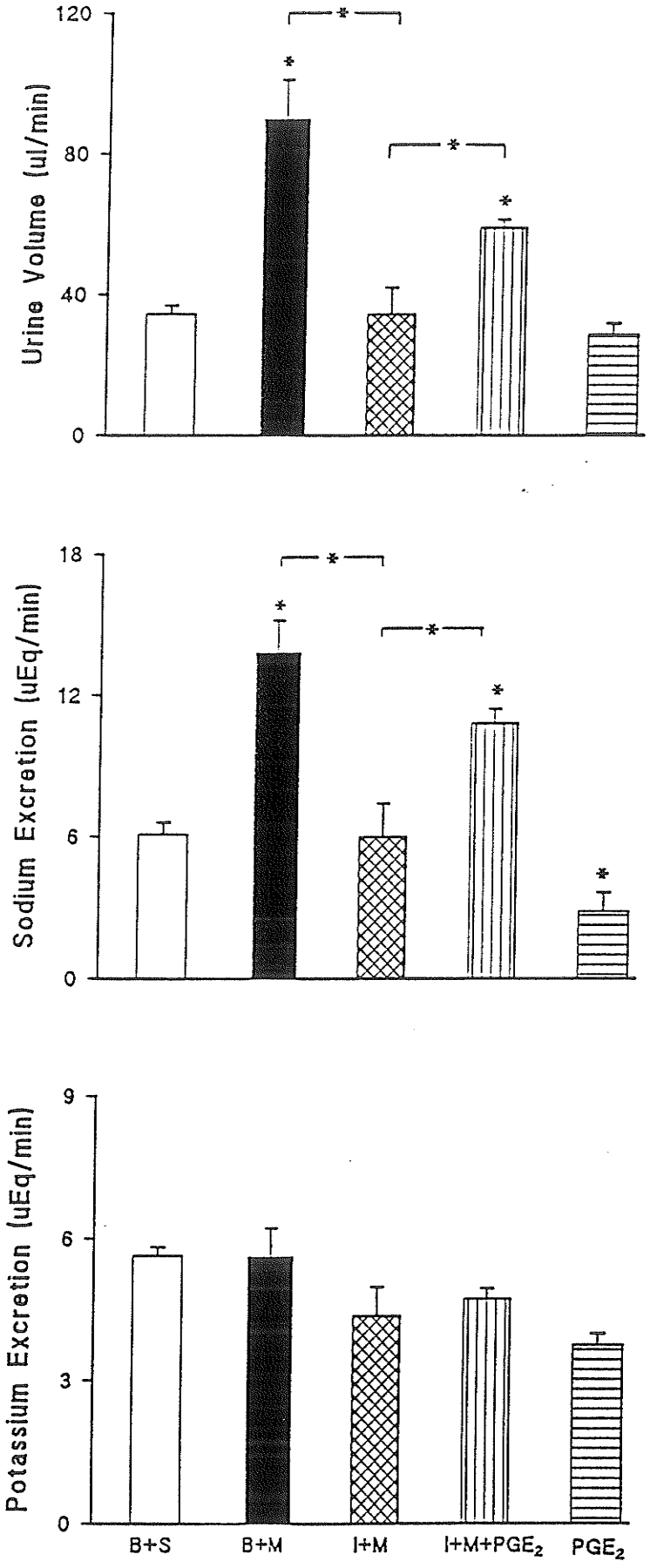
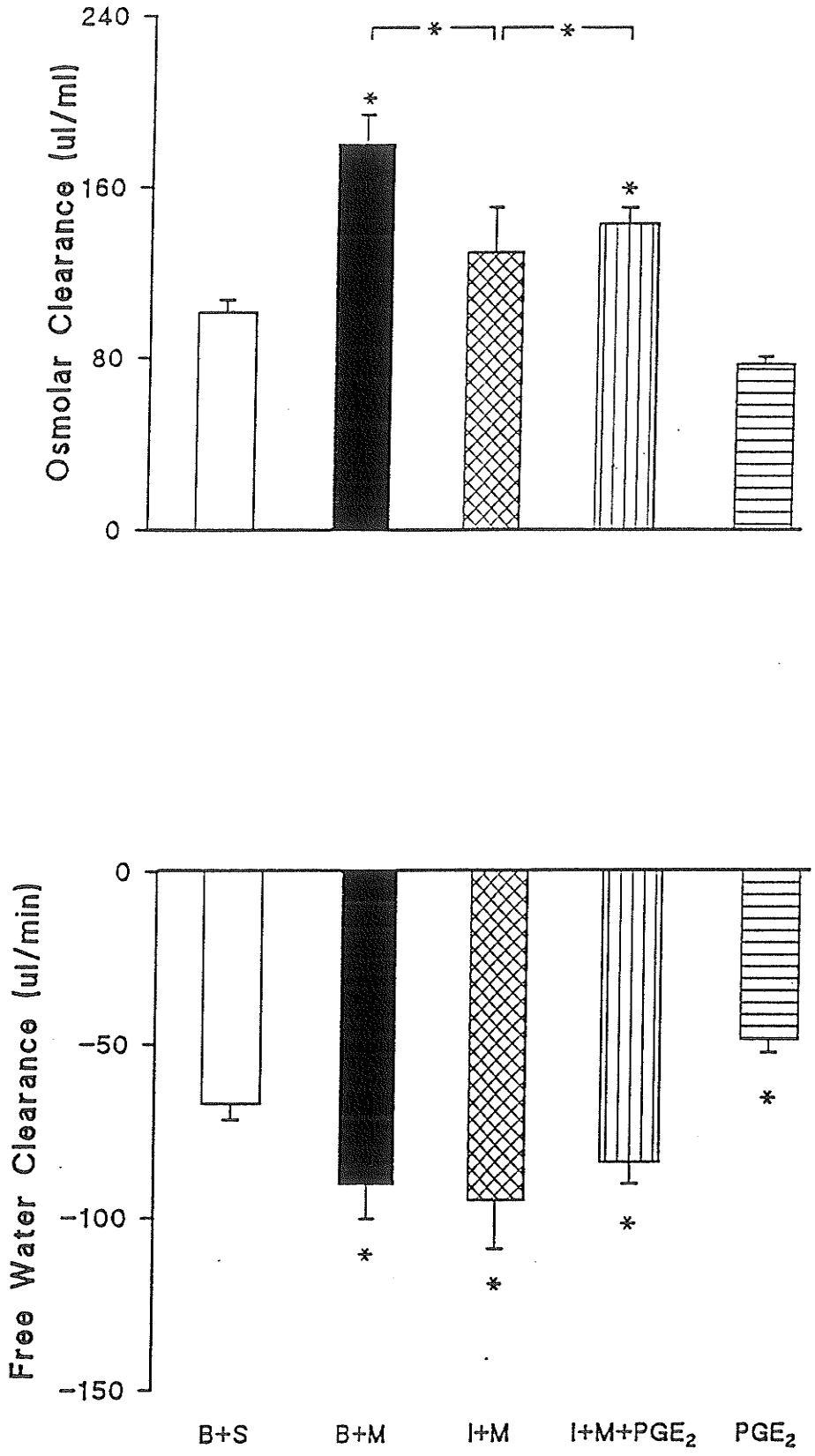

Urine Volume (ul/min)
120
80
40
0
Sodium Excretion (uEq/min)
18
12
6
0
Potassium Excretion (uEq/min)
9
6
3
0
B+S
B+M
I+M
I+M+PGE2
PGE2

Figure 10.

ffects of intrarenal infusion of moxonidine 1 nmol/kg/min in the presence (n=7) and absence (n=6) of indomethacin 5 mg/kg, and prostaglandin $E_2$ alone (n=5) on osmolar clearance and free water clearance in the rat. Each group represents the mean $\pm$ standard error of the mean. * denotes $P<0.05$ versus control. -*- denotes $P<0.05$ between groups. B = Phosphate Buffer; M = Moxonidine; I = Indomethacin; $PGE_2$ = Prostaglandin $E_2$ (1ug/kg/min); S = Saline.


Osmolar Clearance (ul/ml)
240
160
80
0
*
*
*
*
Free Water Clearance (ul/min)
0
-50
-100
-150
*
*
*
*
B+S
B+M
I+M
I+M+PGE2
PGE2

# DISCUSSION

It is now apparent that some of the effects previously attributed to $\alpha_2$-adrenoceptors were in fact due to stimulation of a novel class of receptors called imidazoline receptors or I receptors (Bousquet et al., 1984; Ernsberger et al., 1987; Allan et al., 1993). These receptors demonstrate a high preference for compounds with the imidazoline moiety such as clonidine, idazoxan and moxonidine. Based on pharmacological studies two subtypes of the imidazoline receptor have been proposed, the $I_1$ and $I_2$. The $I_1$ imidazoline receptor has been defined by clonidine and moxonidine binding, whereas, the $I_2$ imidazoline receptor has been defined by idazoxan binding (Michel and Ernsberger, 1992; Ernsberger, 1992). Previous studies had shown that intrarenal infusion of clonidine in anaesthetized rats produced an increase in urine flow rate secondary to an increase in free water clearance at low doses but at high infusion rates the increased urine excretion was secondary to an increase in osmolar clearance (Blandford and Smyth, 1988). Clonidine has a higher preference for the $\alpha_2$-adrenoceptor as compared to the $I_1$ imidazoline receptor (Lehmann et al., 1989). The effects of the low infusion rates of clonidine were consistent with $\alpha_2$-adrenoceptor stimulation. Clonidine has an imidazoline nucleus. For this reason, it was speculated that the higher

infusion rates stimulated the $I_1$ imidazoline receptors resulting in a natriuresis.

Moxonidine has over a 700 fold affinity and selectivity for renal $I_1$ receptors (Ernsberger et al., 1991, 1992) compared to $\alpha_2$-adrenoceptors. Therefore it was used as a tool for studying the $I_1$ imidazoline receptor function in the kidney. Our results have been consistent with Allan et al., (1993) who reported that intrarenal infusion of moxonidine caused a diuresis and natriuresis in a dose dependent fashion without any change in heart rate, blood pressure or creatinine clearance. Creatinine clearance was used as a measure of glomerular filtration rate. Blood pressure and creatinine clearance are two parameters which can alter urine volume and sodium excretion. The fact that these parameters remained constant at the infusion rates studied are consistent with the renal effects of moxonidine being due to direct stimulation of the $I_1$ imidazoline receptor. This observation was consistent with the work of Bidet et al., (1990) which showed that imidazoline compounds such as cirazoline and idazoxan inhibited the entry of $^{22}Na$ into proximal tubules of the isolated rabbit kidney. However, this work seemed to suggest that cirazoline, an imidazoline receptor agonist, and idazoxan, an imidazoline receptor antagonist, both have the same effects on renal membranes. This controversy may be resolved if one considers idazoxan to be a partial antagonist.

Recent studies in our laboratory compared the ability of three purported $\alpha_2$-adrenoceptor agonists to increase water (free water) and solute (osmolar) excretion (Smyth et al., 1992). Clonidine and 2,6-dimethylclonidine, two purported $\alpha_2$-adrenoceptor agonists (Fondacaro et al., 1989), displayed similar potencies in the ability to increase urine flow rate. However, the effects of clonidine were mediated primarily by an increase in free water clearance and the effects of 2,6-dimethylclonidine were mediated by an increase in osmolar clearance. Also, pretreatment with a specific $V_2$ vasopressin receptor antagonist completely attenuated the renal actions of clonidine but not of 2,6-dimethylclonidine. These findings were consistent with the existence of two subtypes of $\alpha_2$-adrenoceptors and/or two unique receptors. 2,6-Dimethylclonidine has since been found to possess a high affinity for $I_1$ imidazoline receptors (D.D. Smyth, personal communication).

Another line of evidence, which suggests that the $\alpha_2$-adrenoceptor and the imidazoline receptor represent distinct sites with distinct functions, involved the use of idazoxan and rauwolscine at doses that were specific for the imidazoline site and the $\alpha_2$-adrenoceptor respectively. A number of studies had found that idazoxan bound with a high affinity to non-adrenergic sites and that these sites were different from $\alpha_2$-adrenoceptors (Michel et al., 1989b; 1990). Idazoxan but not rauwolscine blocked the enhancement of urine volume, sodium excretion and osmolar clearance

caused by moxonidine. However, rauwolscine at the same dose that failed to block the effects of moxonidine, attenuated the effects of clonidine. Similarly, idazoxan, at doses that blocked moxonidine, failed to alter the effects of clonidine. The selective blockade of these two agonists by rauwolscine and idazoxan provides support for the actions being mediated at two distinct sites.

These renal studies are consistent with previous studies in the brain where the imidazoline receptors were first discovered. In a classical experiment Bousquet et al (1984) demonstrated that only compounds with the imidazoline moiety reduced arterial blood pressure when they were microinjected into the nucleus reticularis lateralis of anaesthetized cats. A known $\alpha_2$-adrenoceptor agonist which lacked the imidazoline moiety such as alpha methyl noradrenaline failed to produce hypotension when applied to this same region. Therefore they suggested the existence of a novel class of receptors in the brain which were distinct from $\alpha_2$-adrenoceptors yet mediated lowering of arterial blood pressure. The present study confirms the existence of these receptors in the kidney which appear to mediate a natriuresis when stimulated.

Role of G Proteins:

Whereas, the signal transduction mechanisms of $\alpha_2$-adrenoceptors have been well elucidated, very little is known about the signal transduction mechanism of the

imidazoline receptors. Alpha$_2$-adrenoceptors have been demonstrated to be coupled to an inhibitory GTP binding protein, Gi (Nomura et al., 1985). Pertussis toxin is known to ADP-ribosylate certain G proteins such as GO and Gi, thereby inactivating them (Pedraza-Chaveri et al., 1984). Therefore it can be used to suggest that a given receptor is coupled to a G protein. It has been reported that clonidine inhibits catecholamine release from adrenal chromaffin cells and that this effect is attenuated by pretreatment with pertussis toxin (Ohara-Imaizumi et al., 1988). In contrast it was observed that the inhibitory action of hexamethonium, a nicotinic cholinergic antagonist, and of nifedipine, a voltage-sensitive Ca+ channel blocker, on acetylcholine-evoked release of catecholamines were not affected at all by the same treatment with pertussis toxin (Ohara-Imaizumi et al., 1988). Thus pertussis toxin seems to interfere selectively with the cellular mechanisms whereby clonidine inhibited acetylcholine-evoked release of catecholamines. This disparity of effects of pertussis toxin was consistent with the fact that inhibition by clonidine in adrenal chromaffin cells was a consequence of its binding to the pertussis toxin specific receptor. This receptor was probably different from the $\alpha_2$-adrenoceptor in pharmacological characteristics. Recent studies (Regunathan et al., 1993) have demonstrated that adrenal chromaffin cells do not express $\alpha_2$-adrenoceptors but rather express only $I_2$ imidazoline receptors. If we extrapolate this

finding to the data of Ohara-Imaizumi et al., (1988) then it seems probable that the $I_2$ imidazoline receptor was coupled to a G protein which was inactivated by pertussis toxin, most probably a Gi protein. Such a finding sharply contradicts the studies of Michel et al (1989b) who had previously reported that the imidazoline receptor was labelled by idazoxan and the $I_2$ imidazoline receptor was not coupled to a G protein.

In our present studies, the natriuretic and diuretic effects of moxonidine, the $I_1$ receptor agonist, were attenuated by pertussis toxin pretreatment, suggesting that the $I_1$ imidazoline receptor was coupled to a G protein, most conceivably a Gi protein. This observation was consistent with the results of Ernsberger et al., (1993). They demonstrated that $^3$H-moxonidine binding to bovine ventrolateral medulla membranes was altered by GTP analogs. These studies have suggested that the $I_1$ imidazoline receptor may be coupled to a G protein.

Role of Prostaglandins:

Previous studies in our laboratory have shown that indomethacin potentiated the natriuretic effect of $\alpha_2$-adrenoceptor stimulation (Blandford and Smyth, 1991). These results were in sharp contrast to our data with renal $I_1$ imidazoline receptor stimulation where we found attenuation of the natriuretic effects of $I_1$ receptor stimulation following indomethacin pretreatment. It follows then that

inhibition of prostaglandin synthesis will culminate in either an increase or a decrease in sodium excretion depending on whether the agonist has a higher affinity for the $\alpha_2$-adrenoceptor or the $I_1$ imidazoline receptor.

Prostaglandins have been associated with sodium transport in the kidney by various studies (Fulgraff and Meiforth, 1971; Iino and Imai, 1978; Stokes and Kokko, 1977) The exact mechanism of action has not been delineated. Prostaglandins may operate at three different levels to alter sodium excretion. First, they may produce changes in renal hemodynamics through their effects on vascular smooth muscle. Secondly, they may alter sodium transport by a direct action on the renal tubules, particularly the collecting tubules (Fulgraff and Meiforth, 1971). Thirdly, they may antagonize the renal effects 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. This is

consistent with more recent studies which have shown that prostaglandins decrease vasopressin-induced cAMP accumulation in the rabbit cortical collecting tubule (Chabardes et al., 1988). Smyth et al., (1985), using an isolated perfused rat kidney model, showed that $\alpha_2$-adrenoceptor stimulation inhibited vasopressin-induced activation of adenylate cyclase, as well as the effects of vasopressin on sodium and water excretion. Their results suggested that $\alpha_2$-adrenoceptor activation in the kidney led to the inhibition of the vasopressin mediated increase in cAMP production. It has been postulated that $\alpha_2$-adrenoceptor 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., 1984; Umemura et al., 1985) by inhibiting the generation of cAMP. Prostaglandin E reduced vasopressin induced cAMP levels. Consequently, it can be postulated that $\alpha_2$-adrenoceptor stimulation causes an increase in prostaglandin E synthesis which in turn reduced vasopressin induced cAMP. As a result sodium excretion was altered. It follows then that if prostaglandin synthesis was inhibited with indomethacin one would anticipate an increase in the levels of vasopressin induced cAMP levels and hence an increase in the renal effects of vasopressin. However, Blandford and Smyth (1991) reported an enhanced rather than an attenuated diuretic and natriuretic effects of clonidine following indomethacin pretreatment. The

mechanism by which this enhancement occurred has not been identified.

The signal transduction mechanism mediating $I_1$ imidazoline receptor stimulation has not been fully elucidated. However, it has been reported that the $I_1$ imidazoline receptor was coupled to a G protein (Ernsberger et al., 1993). Moreover, preliminary results from our present study indicate that it is coupled to a G protein, most conceivably the Gi protein. Since Gi receptor stimulation generally leads to a decrease in cAMP, we may speculate that $I_1$ receptor stimulation like $\alpha_2$-adrenoceptor stimulation may cause a decrease in vasopressin induced cAMP accumulation and hence reduce water and sodium reabsorption. From this point of view there should be no difference in the renal effects of $\alpha_2$-adrenoceptor stimulation and $I_1$ imidazoline receptor stimulation. However, Allan et al., (1993) reported that unlike $\alpha_2$-adrenoceptor stimulation, the renal effects of moxonidine were not blocked by vasopressin $V_2$ receptor antagonists whereas the effects of clonidine were blocked (Blandford and Smyth, 1990). This observation suggests that the $I_1$ imidazoline receptor functions independently of vasopressin. If prostaglandin $E_2$ reduces vasopressin induced cAMP levels then administration of prostaglandin $E_2$ should cause no differences in the renal effects of $\alpha_2$-adrenoceptor and $I_1$ imidazoline receptor stimulation since cAMP levels will be reduced in both situations. Thus, the observed differences cannot be

explained by a reduction in vasopressin induced cAMP levels. The interaction between renal prostaglandins and vasopressin on $\alpha_2$-adrenoceptor and $I_1$ imidazoline receptors does not appear to be enough to explain the observed differences.

Another level of interaction between prostaglandins and sodium excretion would be through regulation of renal blood flow. Prostaglandins cause renal vasodilatation which produces an increase in medullary blood flow and a subsequent increase in medullary interstitial hydrostatic pressure. Granger et al., (1984, 1988) reported that direct expansion of the renal interstitial volume by an injection of 2.5% albumin through a polyethylene matrix implanted in the kidney interstitium resulted in an increase in renal interstitial hydrostatic pressure and sodium excretion. Micropuncture studies revealed that the fractional delivery of sodium at the late proximal tubules was increased in response to this direct renal interstitial volume expansion and indicated that proximal tubules were involved in the natriuresis caused by elevated renal interstitial pressure (Haas et al., 1984). Furthermore, Yoshikazu et al., (1989) reported that renal interstitial volume expansion also increases urinary prostaglandin $E_2$ excretion and that the inhibition of prostaglandin synthesis blunted the natriuretic effect of elevated renal interstitial hydrostatic pressure. These data suggested that prostaglandins were involved in the natriuresis caused by elevated renal interstitial pressure. However, increased

renal blood flow per se does not lead to an increase in renal interstitial hydrostatic pressure (Haas et al., 1984). Intrarenal infusion of natural prostaglandin $E_2$ increased renal blood flow, renal interstitial hydrostatic pressure, and urinary sodium excretion but infusion of a prostaglandin analog which increased renal blood flow failed to increase renal interstitial hydrostatic pressure. This accounted for the lack of a natriuretic effect of this compound. Also, there are reports that significant increases in renal interstitial pressure do not necessarily reduce sodium reabsorption by the proximal tubules (Strandhoy et al., 1974).

$I_1$ Imidazoline receptors have not been found on renal blood vessels and it is not known whether moxonidine causes renal vasodilatation at the infusion rate studied since renal blood flow was not measured in our preparation. However, our studies indicated that prostaglandins were necessary for the renal effects of moxonidine. It has been suggested that increased renal interstitial hydrostatic pressure may lead to an increase in endogenous prostaglandin synthesis and that the increased synthesis of prostaglandins may play an important role in inhibiting sodium reabsorption by the renal tubules. Since changes in renal hemodynamics were not monitored in our studies, it is difficult to tell whether moxonidine caused increased renal blood flow and a consequent increase in renal prostaglandin synthesis. The dose of prostaglandin $E_2$ that was infused alone failed to

increase urine volume compared to controls. However, it caused a significant decrease in sodium excretion. This is consistent with the work of Kirschenbaum and Stein, (1976). However, this decrease in sodium excretion was not reflected in a decrease in osmolar clearance suggesting that another ion not measured in this experimental preparation may have been excreted in response to the prostaglandin. This interpretation would be consistent with the work of Stokes (1979) who showed that prostaglandin $E_2$ has a chloruretic effect by inhibiting chloride transport at the thick ascending limb.

Interestingly, Blandford and Smyth (1991) showed that prostaglandin synthesis inhibition potentiated the natriuretic effect of clonidine and concurrent administration of prostaglandin $E_2$ inhibited this response without any apparent change in renal blood flow. The above studies indicate that prostaglandin $E_2$ modulated sodium transport in the kidney following $\alpha_2$-adrenoceptor and $I_1$ imidazoline receptor stimulation but this was done in an opposing manner. Whereas our present data are consistent with the fact that prostaglandins play a permissive role in the renal effects of $I_1$ imidazoline receptors, previous studies in the same laboratory showed that they inhibited the renal effects of $\alpha_2$-adrenoceptor stimulation in the kidney. This apparent contradiction in function raises the question of how prostaglandins play these contrasting roles in the kidney. Differences in the anatomical distribution

of $\alpha_2$-adrenoceptors and $I_1$ imidazoline receptors have been reported. $\alpha_2$-Adrenoceptors numerically predominate over I1 receptors in the proximal tubules whereas the I1 receptors abound in the distal collecting tubules (Zakiel et al., 1993). Further, Coupry et al., (1989a) reported an asymmetrical distribution of the imidazoline receptors in the kidney. The basolateral border demonstrates a twelve fold higher density of imidazoline receptors compared to the brush border membrane. Whether these differences have anything to do with the contrasting role of prostaglandins in the regulation of sodium following stimulation of these receptors has yet to be determined.

It has been generally believed that in the absence of renal hemodynamic alterations, proximal tubular changes were not involved in the natriuretic tubular effect of the renal prostaglandins (Strandhoy et al., 1974, Leyssac et al 1975). The evidence for distal nephron actions of prostaglandins to influence sodium excretion comes partly from clearance experiments, and partly from studies of isolated tubular segments and other tissues such as frog skin which can be regarded as models for study of distal nephron segments. The diverse effects of prostaglandins on sodium transport across epithelia cells were noted by Leyssac et al., (1975). They proposed that prostaglandins would 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). It is yet to be determined whether the numerical relationship between $\alpha_2$-adrenoceptors and $I_1$ imidazoline receptors along the nephron contributes significantly to the differences in resistance to the tubular epithelia cells as proposed by Leyssac et al., (1975).

In summary, the results from the present study have demonstrated that moxonidine, an $I_1$ imidazoline receptor agonist produces a dose related increase in sodium excretion and urine flow rate. This response is coupled to a Gi protein and is modulated by prostaglandins. Further, the results of this thesis provide another piece of evidence that $\alpha_2$-adrenoceptors are distinct from imidazoline receptors in the rat kidney.

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