The Importance of the Renal Sympathetic Nerves in the Natriuretic Response to Imidazoline Receptor Agonists

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FACULTY OF GRADUATE STUDIES

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The Importance of the Renal Sympathetic Nerves in the Natriuretic Response to Imidazoline Receptor Agonists

BY

Deni Pirnat

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

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Abstract

In our preliminary dose – response study two I_1-imidazoline receptor agonists (rilmenidine and moxonidine) and two α_2-adrenoceptor agonists (clonidine and guanfacine) were investigated. We wanted to establish the dose for each drug that would produce a constant and significant increase in urine flow rate and sodium excretion with minimal changes in blood pressure and heart rate. Based on those studies, we selected rilmenidine (10 nmol/kg/min) and guanfacine (10 nmol/kg/min) for our further studies. The renal sympathetic nerves have been proposed to be important in the renal actions of imidazoline receptor and α_2-adrenoceptor agonists. We therefore determined the effects of acute renal denervation on the diuretic and natriuretic actions of rilmenidine, guanfacine and furosemide. The dose of furosemide (0.1 mg/kg) was selected based on the previous experiments in our laboratory. Male Sprague-Dawley rats underwent unilateral nephrectomy 7 to 10 days prior to the experimental day. Animals were anesthetized with pentobarbital. A tracheotomy was performed and the animal allowed breathing spontaneously. The carotid artery was cannulated for blood pressure and heart rate monitoring and the left jugular vein was cannulated for infusion of study drugs. The left kidney was exposed by a flank incision and the ureter was cannulated for the collection of urine. The kidney was denervated surgically and by painting the renal artery with phenol (10%) in 95% ethyl alcohol. Intravenous administration of guanfacine (10 nmol/kg/min) increased urine flow rate, sodium excretion and osmolar clearance.

Similarly, rilmenidine (10 nmol/kg/min) and furosemide (0.1 mg/kg) also increased urine flow rate, sodium excretion and osmolar clearance. Following denervation, rilmenidine was associated with a decrease in urine flow rate, sodium excretion, and a slight increase in free water clearance. Guanfacine was still associated with a slight increase in urine flow rate but this was secondary to an increase in free water clearance and not an increase in osmolar clearance. However, furosemide remained at similar levels as it was before denervation. These results indicate the importance of intact renal sympathetic nerves for the renal actions of imidazoline receptor and α_2-adrenoceptor agonists.

GENERAL INTRODUCTION

The most important function of the kidney is to regulate the composition of intracellular fluid and stabilize the extracellular fluid volume to maintain homeostasis in the body. The kidney controls water and electrolyte balance, excretes metabolic waste products, and releases erythropoietin which increases erythropoiesis through the formation of the hormone erythropoietin and produces a number of hormones (prostaglandins, kinins, angiotensin II) important in the regulation of blood pressure. Both hormonal and neural mechanisms are an integral part of the kidney function. It is the level of activation of the non-neural pathways and the intensity of the renal sympathetic nerve activity that regulate the degree of activity between these two mechanisms (Kopp and DiBona, 1993). The neural mechanisms normally maintain a certain degree of vasoconstriction, which allows the central nervous system to react promptly to rapid changes in arterial blood pressure by either vasodilatation or vasoconstriction. The regulation of the vessel tone in the kidney is assured through the interaction of the renal sympathetic nerves and the baroreceptor function and the macula densa mechanism in the control of renin secretion (Kopp and DiBona, 1993). Sympathetic noradrenergic neurons have been found to be distributed to the afferent and efferent arterioles, the juxtaglomerular apparatus and tubules. The existence of significant parasympathetic innervation has not been reported. Although biochemical and morphological studies indicate that

there are some dopamine-containing neurons within the kidney, the hypothesis that alterations in certain renal functions are dopamine related remains uncertain (DiBona, 1990a). Therefore, in this section we will mainly focus on the importance of the renal sympathetic nerves (intrinsic innervation, neurotransmitters and receptors) for maintenance of homeostasis.

Innervation of the Kidney

Anatomy

Intrinsic innervation:

The efferent intrinsic innervation of the kidney represents an extension of the central control system (central nervous system) that responds to peripheral and central afferent inputs. Although a number of techniques have been used, electron and fluorescent microscopy have helped the most in the final clarification of intrinsic renal innervation. The efferent (unmyelinated) sympathetic nerves enter the hilus of the kidney accompanied by the renal artery and vein and reach all segments of the renal vasculature. This adrenergic innervation is distributed throughout the renal cortex and medulla, with the highest density in the juxtamedullary region and the lowest density in the nephron tubules (Barajas et al., 1992). Direct renal nerve stimulation results in an increased net renal venous outflow of norepinephrine derived from renal nerve terminals. The importance of norepinephrine as a neurotransmitter was highlighted by the finding of a significant decrease in the renal norepinephrine concentration (95%) following chronic renal denervation (DiBona and Sawin, 1983, Fernandez-Repollet et al.,

1985) and the increased norepinephrine concentration in the venous blood after renal sympathetic nerve stimulation (Kopp et al., 1983). The low density of renal tubular innervation has been proposed to be insufficient to fully explain the tubular effects (Luff et al., 1992). It has also been proposed that a substantial release of norepinephrine into the renal interstitium may be the explanation as to how a neurotransmitter would reach the tubular epithelial cells (Barajas et al., 1984). Recently, DiBona (2000) proposed that the sympathetic renal nerves consist of functionally specific fiber groups that separately innervate tubules, juxtaglomerular granular cells and vessels. This allows for a different intensity of renal nerve stimulation of some fiber groups compared to others. This may explain how the sympathetic renal nerves produce different effects on a variety of renal functional responses including tubular function.

The sensory afferent (myelinated) renal nerves are localized in the corticomedullary connective tissue of the pelvic region and the major vessels (Barajas et al., 1992). They project into ipsilateral dorsal root ganglia and the dorsal horn in the spinal cord, as well as, to medullar and hypothalamic sites that also receive afferent nerve fibers from the carotid sinus (DiBona, 1985). The sensory innervation serves as one source of peripheral afferent input to assure the role of the kidney in the homeostatic regulation of body fluid volume.

Extrinsic innervation:

Different labeling methods have helped in the understanding of the extrinsic innervation of the kidney. Particularly, in the rat, acetylcholinesterase

was used in the tracing of efferent and afferent nerve fibers between the kidney and the celiac plexus, splanchnic nerves, the lumbar splanchnic nerves and the intermesenteric nerve plexus (Drukker et al., 1987). The use of different labeling techniques allowed for the tracing into the central nervous system (Menetrey and Basbaum, 1987; Vollanueva et al., 1991; Barajas et al., 1992). Of the sympathetic premotor nuclei, the rostral ventrolateral medulla (RVLM), A5 area, caudal raphe nuclei and paraventricular nucleus (PVN) in the hypothalamus were found to be regulatory centers of the renal sympathetic nerve activity (Ding et al., 1993). The RVLM is the most important for cardiovascular reflexes and in generating tonic sympathetic tone. For the generation of sympathetic tone in the RVLM, three mechanisms have been proposed: 1) sensitivity of the RVLM to minimal changes in local pH, PCO_2 and PO_2 to cause change in sympathetic nerve activity and consequently arterial blood pressure (Dampney, 1994); 2) pacemaker-like, rhythmic oscillations in the RVLM, that do not exist in the other brain structures (Sun et al., 1988); and 3) the network of a global oscillating system in different regions of the brain controlled by the RVLM to produce sympathetic tone (Gattone et al., 1986, Zhong et al., 1993). However, the physiological significance of these mechanisms is still questionable.

Function

Short-term arterial pressure regulation:

Unlike the RVLM that is directly projected through sympathetic motor neurons to produce sympathetic tone, other cell groups alter sympathetic tone

via interneurons that connect with sympathetic premotor neurons. Baroreceptors and chemoreceptors represent the first mechanism that is sensitive to changes in blood pressure. The afferent fibers from these receptors are an integral part of the glossopharyngeal capital nerve (arterial baroreceptors and chemoreceptors) and vagus nerve (cardiac baroreceptors and chemoreceptors). These terminate in the nucleus tractus solitarius (NTS). NTS neurons can only respond to one source of stimuli at the time (Donoghue et al., 1985). Lesion of the NTS completely abolishes the response to baroreceptors and the increase in arterial blood pressure (Colombary et al., 1996). These findings suggest an importance of the NTS in the baroreceptor response. The NTS has projections to the intermediolateral column (IML) and to sympathetic premotor neurons in the RVLM and others cell groups such as the rostral ventromedial medulla (RVMM), PVN, caudal ralphe nuclei and the A5 noradrenergic group (van-Zwieten and Chalmers, 1994). Baroreceptors in the carotid sinuses and the aortic arch respond to stretch of the vessel as a result of an increase in arterial pressure. The stimulation of the baroreceptors triggers an impulse transmission via the glossopharyngeal nerve (from carotid sinus) or vagus nerve (from aortic arch) to the medulla and to higher centers. Increased firing rate from the baroreceptors inhibits vasoconstrictor regions, causing peripheral vasodilation and resulting in blood pressure decrease. Stimulation of the vagus nerve results in a decrease in heart rate and blood pressure. Stimulation of cardiopulmonary baroreceptors located in the atria, ventricles and pulmonary vessel results in an inhibition of vasoconstrictor tone of resistance vessels. Chemoreceptors located in the

carotid and aortic bodies respond to changes in pH, PO_2 and PCO_2. However, the effect produced by chemoreceptors is of less importance compared to the direct response of the vasomotor region of the medulla. In addition, peripheral chemoreceptors become effective when blood pressure drops more than 80 mm Hg (Guyton et al., 1972).

Long-term arterial pressure regulation:

In the long-term arterial pressure regulation, the kidney plays a major role. The major importance of the kidney in the long-term regulation of blood pressure comes from the fact that a renal mechanism can reach infinite gain, if there is enough time for its control functions to reach the equilibrium point. Guyton and co-authors described this infinite gain pressure control mechanism for the first time in 1972. It is based on the effects of arterial pressure on the renal output of water and salt.

Renal function curve:

Figure 1 shows a renal function curve that explains the arterial pressure regulating mechanism in which changes in arterial blood pressure directly effect output of water and salt from the kidneys (solid curve of Figure 1). The standard "net" level of salt and water intake is represented by dashed line. In the normal person, at the mean arterial pressure of 100 mmHg, the net intake equals renal output, meaning that equilibrium has been reached. The point on Figure 1 where the two curves cross is called the equilibrium point. Any decrease in the blood

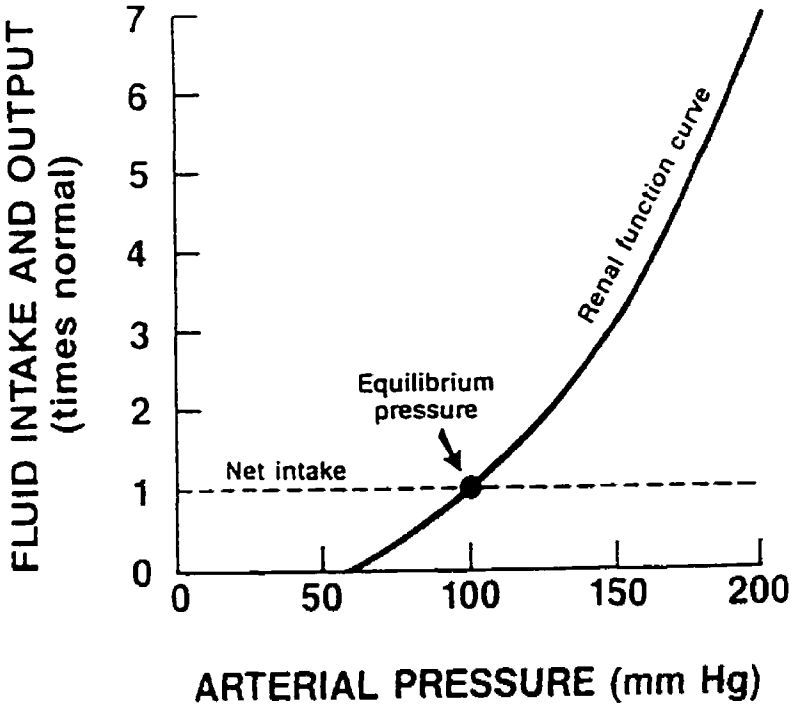

Figure 1. A typical renal function curve, showing the effect of arterial pressure on renal output of fluid; this figure also shows that the arterial pressure will be regulated to the "equilibrium point" where the function curve equilibrates with the fluid intake level. (Reprinted from Guyton, 1990.)

pressure will produce a decrease in urine volume (ie. fluid retention) in order to bring the arterial blood pressure back to 100 mmHg. At the arterial pressure of approximately 50 mm Hg, urine water and electrolyte output reaches zero. Alternatively, if arterial blood pressure rises above this point, urine output will increase rapidly and return the blood pressure to the equilibrium point. Thus, there is only one point where the input equals the output and this point is referred to as the "equilibrium point". In the example shown (Figure 1), this point is found at a blood pressure of 100 mm Hg. Whenever non-equilibrium temporarily occurs, renal output of water and salt will always come back to complete equilibrium with the intake of water and salt. This amount of infinite correction of the pressure abnormality that always reproaches the equilibrium point is known as the "infinite gain principle" (Guyton, 1990). It is rare that changes in water intake effect changes in arterial pressure. However, salt gain or loss alters arterial blood pressure since extracellular fluid volume depends directly on accumulated salt. For example, increased salt in the body causes osmolality of the body fluid to increase. This will stimulate both, the thirst center to increase water intake and vasopressin release to increase water reabsorption. Both mechanisms will lead to an increase in extracellular fluid volume.

Renin-angiotensin system:

Although the renal function curve is very important for understanding the long-term regulation of arterial pressure, this phenomenon was based on studies performed in the isolated kidney. In vivo however, there are other pressure

controlling mechanisms that may affect the renal function curve. One of these is the renin-angiotensin system. The renin-angiotensin system is a unique control system that is sensitive to blood pressure and blood volume changes. Renin is formed in granules and stored in modified smooth muscle cells close to the afferent arteriole in juxtaglomerular cells. These juxtaglomerular cells are in contact with specialized epithelial cells of the distal tubule, the macula densa, that are sensitive to low plasma sodium concentrations. Also, a decrease in pressure in the afferent arterioles reduces the stretch of the juxtaglomerular cells, thereby causing renin release. Therefore, when a drop in arterial blood pressure decreases glomerular filtration rate, renin is released into the blood stream. Angiotensinogen, in the presence of renin, is cleaved to form the decapeptide, angiotensin I. A further step in this pathway is the formation of the octapeptide angiotensin II in the presence of angiotensin converting enzyme. Angiotensin II, as a potent vasoconstrictor, is important for vascular tone of the renal arterioles and is important in tubular sodium reabsorption. Angiotensin II also indirectly affects sodium excretion by stimulating the secretion of aldosterone. This combination of direct and indirect actions of angiotensin II on sodium excretion makes the renin-angiotensin system one of the most powerful long-term systems in the regulation of body fluids and blood pressure (Hall et al., 1990).

Hypertension:

The kidney and sympathetic innervation play an important role in the regulation of body fluid homeostasis and cardiovascular regulation. Thus, many

factors such as neural regulation of the kidney function, hormonal regulation or disease of the kidney itself may affect this regulatory mechanism resulting in hypertension. In the early stage of essential hypertension, prior to a detectable blood pressure increase, the function of the kidney appears "normal". However, the functional curve of the kidney in the person with essential hypertension is not "normal". In order to have a normal salt and water output, a person with essential hypertension must have a blood pressure set at a higher level than a normal person. If we assume that the person with essential hypertension has a mean arterial pressure of 150 mm Hg, the renal functional curve will have a similar shape as a normal one, but shifted to the right. Compared to the normal function curve, the functional curve will reach a zero urine output at a pressure level of 110 mm Hg instead of 50 mm Hg. If the water and salt intake is increased in this person, it will cause a high output with a moderate increase in arterial pressure. Having this in mind we can see that the kidney does not necessarily need to be diseased but the function of the kidney in essential hypertension is not normal and the reason may be found in many extrarenal factors (Guyton, 1990).

The renal sympathetic nerves are found to participate greatly in the regulation of renal function. Both the efferent and the afferent renal nerves are involved in the regulation of volume homeostasis. Efferent renal sympathetic nerve activity is known to control urine sodium excretion by altering renal vasoconstriction, glomerular filtration rate, tubular sodium and water reabsorption and renin release (Kopp and DiBona, 1986; Kopp et al., 1987; DiBona, 1987;

1989). In experimental animals, changes in dietary sodium intake affect efferent renal sympathetic nerve activity (DiBona and Sawin, 1985). During expansion of blood volume in conscious rats, increase in sodium excretion was associated with a decrease in renal sympathetic nerve activity. These changes were greater in rats treated with a low sodium diet compared to those treated with normal or high sodium diet. The abnormality between urine sodium excretion and the development of hypertension in experimental animals is associated with a higher level of arterial pressure for any given level of urinary sodium excretion in hypertensive animals compared to control (Katholi, 1983). Studies with some experimental animals found that alterations in renal nerve activity caused changes in tubular sodium reabsorption and urine sodium excretion. The expansion in blood volume was associated with increased urine flow and sodium excretion. After bilateral renal denervation, the diuretic and natriuretic responses were lowered (DiBona and Sawin, 1985; Morita and Vatner, 1985; Peterson et al., 1988). Denervation also attenuated the severity of the hypertension in animal models of hypertension (Winternitz and Oparil, 1982). Studies mentioned above and many others confirmed the importance of the efferent sympathetic nerves in the development of hypertension by regulating sodium excretion. There has also been evidence of afferent sympathetic nerve importance in the reflex modulation of sympathetic nerve activity. In hypertensive animals, increased levels of norepinephrine were reduced after renal denervation. It was proposed that afferent renal nerves may change the levels of norepinephrine in medullar and hypothalamic centers important for autonomic cardiovascular regulation and

indirectly affect peripheral sympathetic tone (Winternitz and Oparil, 1982; Katholi, 1983). However, there is insufficient evidence for afferent renal nerve involvement in the pathology of human hypertension (Katholi, 1983).

Receptors

As stated above, the efferent intrinsic innervation of the kidney represents an extension of the central control system. The peripheral afferent input serves to assure the role of the kidney in the homeostatic regulation of body fluid volume. As a result, it would be beneficial to review the neurotransmitters associated with renal sympathetic nerve terminals and the specific receptors involved in the alteration of renal function. Although there is evidence that the renal nerves contain dopamine, it is not clear if dopamine is involved in the regulation of the renal function (DiBona, 1990a). Consequently, dopamine will not be discussed further at this time.

The neurotransmitter norepinephrine is stored in the form of a molecular complex with ATP in the granular vesicles formed in the cell bodies and transported down the neuron to the terminal of the sympathetic renal nerve. Presynaptic receptors are located on the nerve terminal and they modulate release of neurotransmitter. Postsynaptic receptors are located near the synaptic cleft. Extrasynaptic receptors are located peripherally to postsynaptic receptors and they are affected by the amount of released norepinephrine from the nerve terminal and by circulating norepinephrine and/or epinephrine. Renal nerve stimulation triggers the opening of calcium channels and the subsequent

entry of calcium into the nerve terminal. This results in an emptying of the norepinephrine from the granulated vesicle into the synaptic cleft. Norepinephrine binds to a specific receptor on a structural renal element causing alteration in renal function (DiBona and Kopp, 1997).

Adrenoceptor types:

Ahlquist (1948) classified adrenoceptors into α and β adrenoceptors. This classification originated from experiments in which Ahlquist studied five catecholamines: epinephrine (E), methylepinephrine (MeE), norepinephrine (NE), methylnorepinephrine (MeNE) and isoproterenol (I), in eight physiological assays and observed different potency in their pharmacological response. The experiment was designed to determine relative activity of the studied agents based on excitation or inhibition and any variations in the potency order between them. Ahlquist (1948) ranked the compounds in order, from the most to the least potent and noticed two different orders of potency: E, NE, MeNE, MeE, I, and I, E, MeE, MeNE, NE. This indicated that two distinct receptor systems may exist and they were identified as α- and β-adrenoceptors.

Subclasification of adrenoceptors:

Following nerve stimulation and the release of neurotransmitter in the synaptic cleft, norepinephrine has been found bound to postsynaptic α-adrenoceptors. However, norepinephrine binds to presynaptic adrenoceptors (autoreceptors) as well to inhibit further release of neurotransmitter (Langer,

1974; 1977). This study and studies that followed resulted in a further subclasification of α-adrenoceptors in to α_1- (postsynaptic, excitatory) and α_2- (presynaptic, inhibitory) adrenoceptors (Berthelsen and Pettinger, 1977). Under normal circumstances, α_2-adrenoceptors appear to be located extrasynaptically and do not mediate the effects of sympathetic neuronally released norepinephrine (Pettinger et al., 1985). Adrenoceptors were subsequently classified into three major types: α_1, α_2 and β (Wikberg et al., 1992). This pharmacological classification was based on three criteria: 1) the selective agonists for each of the three receptor subtypes showed a high affinity for one subtype compared to the other two types; 2) each adrenoceptor subtype coupled to a second messenger system through G-proteins (α_1 through G_q, α_2 through G_i and β through G_s); and 3) molecular structural differences and similarities under the three receptor subtypes. Advanced pharmacology and molecular biology resulted in the further subclassification of the β- as well as the α_1- and α_2- adrenoceptors into several subtypes (Bylund, 1995).

β-adrenoceptor subtypes:

The β-adrenoceptors are subdivided into three subtypes; β_1, β_2, and β_3 (Emorine et al., 1989). The first subdivision of the β-adrenoceptors into β_1 and β_2 was published in 1967. This division was based only on pharmacological criteria. Lands and coworkers (1967) compared effects of several sympathomimetic amines on bronchodilation and vasodilatation as well as cardiac force and fatty acid mobilization. They found two different orders of potency and suggested

subdivision of the β-adrenoceptors into β_1- and β_2-subtypes. The β_1-subtype, primarily localized in the adipose tissue, produced fatty acid mobilization and increased force of contraction in the heart. The β_2-subtype, localized in smooth muscles, produced bronchodilation and vasodilatation. This pharmacologically based classification of the β-adrenoceptors was later confirmed by cloning (Dixon et al., 1986; Frielle et al., 1987). In 1989 the third β-adrenoceptor (β_3) was discovered in adipose tissue and like the β_1 was involved in lipolysis. Expression of ligand binding properties for an atypical β-adrenoceptor subtype was compared to the human β_3-adrenoceptor expressed in Chinese hamster ovary cells. The human β_3-adrenoceptor responded to six β adrenoceptor agonists and stimulated intracellular adenylate cyclase activity. The rank order of potency of agonists in functional and binding studies was different from those of β_1- and β_2-subtypes (Emorine et al., 1989). Recently, the existence of a new, β_4-adrenoceptor was proposed. It was located in the heart and was pharmacologically different from the three already known β-adrenoceptors (Molenaar et al., 1997). This receptor has not yet been cloned.

α_1-adrenoceptor subtypes:

The α_1-adrenoceptors were previously subdivided into three subtypes based on pharmacological criteria and three subtypes based on cloning (Schwinn et al., 1990; Lomasney et al., 1991; Minneman and Esbenshade, 1994). Studies with different species and different tissues recognized a new relationship between cloned α_1-adrenoceptors and those defined pharmacologically (Ford et

al., 1994). To avoid confusion, α_1-adrenoceptors were later subdivided by the International Union of Pharmacology Subcommittee on Nomenclature for Adrenoceptors into three pharmacologically defined α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}) that represented the three cloned subtypes, α_{1a} (formerly called α_{1c}), α_{1b} (formerly called α_{1b}) and α_{1d} (formerly called $\alpha_{1a/d}$) (Hieble et al., 1995). Recently, an additional α_1-adrenoceptor (α_{1L}) has been proposed (Ford et al., 1996). Different tissues contain different subtypes of the α_1-adrenoceptors (Weinberg et al., 1994). For example, α_{1B}-adrenoceptor messenger RNA (mRNA) was found by in situ hybridization in the S_3 segment of proximal tubules and the thick-ascending limb of the loop of Henle in the rat kidney. The same method detected α_{1D} adrenoceptor mRNA in the internal blood vessels in the rat (Meister et al. 1994). The presence of α_1-adrenoceptors in the human kidney remains unclear. While some authors report presence of the α_1-adrenoceptor subtypes in the human kidney (Weinberg et al., 1994), others have not be able to identify it in quantifiable amounts (Stephanson and Summers, 1986). It is possible that on a protein level, the human kidney expresses very few if any α_1-adrenoceptors (Michel and Rump, 1996).

α_2-adrenoceptor subtypes:

The α_2-adrenoceptor subtypes were identified on the basis of functional studies as well as radioligand binding studies. More recently, the evidence for subclasification of the α_2-adrenoceptors comes from the studies that involve molecular cloning techniques. As the result of these studies, four

pharmacological and three molecular α_2-adrenoceptor subtypes exist according to the Fourth International Union of Pharmacology Nomenclature of Adrenoceptors (Bylund et al., 1994).

Four pharmacologically identified α_2-adrenoceptor subtypes include the α_{2a}-, α_{2b}-, α_{2c}- and α_{2d}-subtypes (Bylund, 1992). In the human platelet and the HT29 cell the α_{2a}-adrenoceptor subtype was found for which prazosin, spiroxatrine and ARC-239 had low affinity, while oxymetazoline had a high affinity. The α_{2b}-adrenoceptor subtype, from NG108 cell lines and neonatal rat lung, was found to have opposite rank order of affinity compared to the α_{2a}-adrenoceptor subtype for the same compounds. This subtype displayed a high affinity for prazosin, spiroxatrine and ARC-239 and a low affinity for oxymetazoline (Bylund et al., 1988). A third α_{2c}-adrenoceptor subtype was found in the opossum kidney cell line and natively in HepG2 and SKN-MC cell lines. This receptor had high affinity for prazosin and a low affinity for oxymetazoline but was pharmacologically different from the α_{2b}-adrenoceptor subtype. The α_{2c}-adrenoceptor subtype was also found to have a high affinity for rauwolscine, BAM1303 and WB4101 (Murphy and Bylund, 1988; Schaak et al., 1997). A fourth, α_{2d}-adrenoceptor subtype was found in rat salivary gland and bovine pineal gland. This subtype had a low affinity for BAM1303 and mianserin and a moderate affinity for rauwolscine and yohimbine (Michel et al., 1989; Simonneux et al., 1991).

Three cloned α_2-adrenoceptor subtypes include α_2-C10 (Kobilka et al., 1987), α_2-C2 (Weinshank et al., 1990) and α_2-C4 (Regan et al., 1988). The gene

from the human platelet α_2-adrenoceptor was cloned. Analysis of this gene was verified by the binding of a variety of the α_2-adrenergic ligands and suggested that the gene for the human platelet α_{2a}-adrenoceptor subtype was located on chromosome 10 (α_2-C10). Similarly, the gene for the α_{2b}-adrenoceptor subtype was identified on chromosome 4 (α_2-C4) and for the α_{2c}-adrenoceptor subtype on chromosome 2 (α_2-C2). These human α_2-adrenoceptor genes were identical to the pharmacologically identified α_2 adrenoceptor subtypes α_{2a}, α_{2b}, and α_{2c}. The RG20 clone, isolated from rat brain, was structurally similar to the α_2-C10 (subtype α_{2a}) showing high similarity based on the predicted amino acid sequence. However, several compounds, such as rauwolscine, yohimbine, BAM 1303, and SKF 104078, showed that the RG20 clone clearly differed from the α_{2a}-adrenoceptor subtype but was pharmacologically similar to the α_{2d}-adrenoceptor subtype (Lanier et al., 1991).

α_2-adrenoceptors in the kidney:

The various radioligand binding studies in the rat kidney were used to identify α_2-adrenoceptors in the cortex and in the outer medulla (McPherson and Summers, 1981; Muntz et al., 1986). The α_2-adrenoceptors appeared to be present in the cortical arterioles, glomeruli and proximal tubules. Also, functional studies demonstrated an inhibitory function of the α_2-adrenoceptors on cAMP levels in proximal convoluted tubules, cortical collecting tubules and medullary collecting tubules in isolated rat nephrons (Umemura et al., 1985). Radioligand binding studies also showed that of the α_2-adrenoceptor subtypes, 86% were of

the α_{2b}-subtype and 14% were of the α_{2a}-subtype in the rat. The presence of the α_{2c}-adrenoceptor subtype was not confirmed despite detection of the α_{2c}-subtype mRNA (Uhlen and Wikberg, 1991).

In the human, mRNA from all three subtypes has been reported (Perala et al., 1992). However, at the protein level, only a minor presence of the α_{2b}- or α_{2c}-subtype has been suggested (Motomura et al., 1989). The majority of the α_2-adrenoceptor subtype in the human kidney belongs to the α_{2a}-subtype (Neylon and Summers, 1985).

Function of the α_2-adrenoceptors:

Studies in various tissues showed that stimulation of both, α_1- and α_2-adrenoceptors could affect several physiological functions such as vasoconstriction, reabsorption of water and electrolytes in proximal tubules and collecting ducts and modulation of the activities of renin, vasopressin, prostaglandins, erythropoietin and parathyroid hormone (Schmitz et al., 1981; Garg, 1992). Both α_1- and α_2-adrenoceptor subtypes were found in the rat renal plasma membranes in a ratio of approximately 3 : 1. Schmitz and coworkers (1981) found that only the α_1-subtype mediated vasoconstriction of renal arterioles. Studies that followed however showed that both α_1- and α_2-adrenoceptors modulate vascular tone. For example, Gellai and Ruffolo (1987) demonstrated that an intravenous infusion of α_1 or α_2-adrenoceptor agonists decreased renal plasma flow equally. In another study in conscious normotensive Wistar rats, renal blood flow was first reduced by an intrarenal

injection of norepinephrine. Several α_1- and α_2-adrenoceptor antagonists were then used to define the receptors responsible for the vascular effects of the norepinephrine. In a second group of rats the α_2-adrenoceptor-antagonist rauwolscine was administered at a dose that failed to block the renal vasoconstrictor response to the α_1-adrenoceptor agonist phenylephrine. This established the α_2-adrenoceptor selectivity of the antagonist. Rauwolscine partially blocked the norepinephrine response. The decrease in renal blood flow produced by an α_2-adrenoceptor agonist guanabenz was abolished with rauwolscine, suggesting again that the vasoconstriction was a result of both α_1- and α_2-adrenoceptors (Wolff et al., 1989). These studies unmasked the renal vasoconstrictor action of α_2-adrenoceptors in the rat.

The α_2-adrenoceptors are also involved in water and sodium excretion. This may involve antagonism of vasopressin. Krothapalli and Suki (1984) showed in isolated rabbit cortical tubules that clonidine and norepinephrine inhibited vasopressin mediated water reabsorption. This effect of clonidine was blocked by yohimbine (α_2-adrenoceptor antagonist) but not by prazosin, indicating α_2-adrenoceptor involvement. Studies in the isolated perfused kidney showed that the antidiuretic and antinatriuretic effects of vasopressin could be reversed by an infusion of epinephrine. This effect of epinephrine was blocked with an α_2-adrenoceptor antagonist yohimbine (Smyth et al., 1985). The study indicated that stimulation of α_2-adrenoceptors reversed the effects of vasopressin. It was interesting that the inhibition of vasopressin-stimulated cAMP by α_2-adrenoceptor agonists observed in the rat kidney have not been

displayed in other species and humans (Edwards et al., 1992). A number of studies have demonstrated that stimulation of α_2-adrenoceptors by systemic administration of α_2-adrenoceptor agonists such as BHT-933 or UK-14,304 was followed by an increase in water and sodium excretion (Stanton et al., 1987; Gellai and Ruffolo, 1987; Smyth et al., 1992). Another study in vivo from Blandford and Smyth (1988a) examined the effects of an intrarenal infusion of the α_2-adrenoceptor agonist clonidine in the rat. The study demonstrated an increase in both water and sodium excretion that may have been due to a direct antagonism of the renal effects of vasopressin. The decrease in central release of vasopressin may be another mechanism of action since the α_2-adrenoceptors in the central nervous system are also involved in the regulation of water and sodium excretion. One study showed that norepinephrine could inhibit vasopressin release from a rat hypothalamo-neurohypophyseal explant in vitro (Armstrong et al., 1982). In the same year, Strandhoy and coworkers (1982) examined the effects of the α_2-adrenoceptor agonist guanabenz on water and sodium excretion in a dog. They found that guanabenz increased both water and sodium excretion while only water excretion was reduced after infusion of an α_2-adrenoceptor antagonist yohimbine. They proposed that two different sites and/or mechanisms of action may be involved. Blandford and Smyth (1988a) came to a similar conclusion in the study where they investigated the dose-response relationship between intrarenal clonidine infusion and water and sodium excretion in the rat. The increase in water excretion at the low dose of clonidine may be due to antagonism of the renal effects of vasopressin. Sodium

excretion however, observed only at higher doses of clonidine, may involve the decrease in central release of vasopressin, antagonism of renal effects of vasopressin or a mechanism unrelated to vasopressin (increase in blood pressure). A similar dose-response study showed that intravenously administered clonidine was more potent in the increase of sodium excretion than an intrarenal infusion of clonidine. These studies also indicated involvement of an extrarenal site of action (Blandford and Smyth, 1989). This finding and the fact that adrenergic stimulation was associated with an increase in prostaglandin synthesis (Matsumura et al., 1986) inspired the study in which the effects of intrarenal clonidine were observed in rats pretreated with a cyclooxygenase inhibitor indomethacin (Blandford and Smyth, 1991). Both water and sodium excretion were further increased after clonidine infusion in the indomethacin-pretreated animals at all infusion rates. An increase in urine volume was associated with an increase in osmolar clearance but not free water clearance. The infusion of prostaglandin E_2 reversed those effects, suggesting that the infusion of the α_2-adrenoceptor agonist clonidine may increase the synthesis of prostaglandin E_2, which in turn produced a decrease in water and sodium excretion. This study also supported the postulate of two separate sites and/or mechanisms of action for α_2-adrenoceptor agonists, one that mediated free water clearance and the another that mediated solute excretion. Blandford and Smyth (1992) therefore compared the rank order of potency for three α_2-adrenoceptor agonists (clonidine, UK 14,304 and 2,6-DMC) on free water clearance and osmolar clearance. Clonidine, as expected, appeared to be most potent in

increasing urine volume secondary to an increase in free water clearance. In contrast, 2,6-DMC was most potent in increasing urine volume by an increase in osmolar clearance. This effect of 2,6-DMC occurred in the proximal tubule, independent of vasopressin. In addition, a specific V_2 vasopressin receptor antagonist attenuated the effects of clonidine but not the effect of 2,6-DMC. This study proposed the possible existence of two separate α_2-adrenoceptors in the kidney.

As mentioned before, functional and radioligand binding studies (Uhlen and Wikberg, 1991) and later molecular cloning techniques (Bylund et al., 1994) helped in the identification of α_2-adrenoceptor subtypes. Intengan and Smyth (1996) demonstrated in Sprague-Dawley rats that intrarenal infusion of the α_2-adrenoceptor-agonist clonidine increased water and sodium excretion by increasing free water and osmolar clearance. Following pretreatment with the selective α_1-adrenoceptor antagonist prazosin, that possesses high affinity for the α_{2b} subtype, the increase in free water clearance was abolished. The increase in osmolar clearance was unaltered by prazosin. On the contrary, the increase in osmolar clearance was altered when rats were pretreated with the opioid receptor antagonist naltrexone. In these animals the increase in free water clearance remained intact. This study suggested the possibility of α_{2a}- and α_{2b}-adrenoceptor-subtype involvement in osmolar and free water clearance respectively following clonidine administration. Several studies found that alteration of the α_{2a}-adrenoceptor in rat and man was related to development of hypertension (Pettinger et al., 1982; Lockette et al., 1995). Intengan and Smyth

(1997b) found that the selective α_{2a}-adrenoceptor agonist, guanfacine produced a dose-related increase in urine volume and sodium excretion in normotensive Wistar rats that was attenuated in spontaneously hypertensive rats. This effect may be due to alteration of the α_{2a}-adrenoceptor or secondary to changes in blood pressure. In one kidney, one clip hypertensive rats however, guanfacine increased urine volume due to an increase in osmolar clearance, similar to normotensive rats without alteration in blood pressure or creatinine clearance. This study indicated that a defective α_{2a}-adrenoceptor subtype may be the reason for the absence of an increase in sodium excretion following guanfacine administration in spontaneously hypertensive rats. Therefore, the α_{2a}-adrenoceptor subtype may be an important element in the development of hypertension in spontaneously hypertensive rats.

Imidazoline receptors:

A novel class of receptor, the imidazoline receptor has been proposed based on radioligand binding studies and in vivo experiments. Bousquet and coworkers (1984) published the first study that proposed the involvement of the imidazoline receptor in lowering sympathetic nerve activity from the central nervous system.

Prior to this study, it was believed that all clonidine-like selective α_2-adrenoceptor agonists, when administered directly to specific sites within the brain, produce a hypotensive effect by acting on the α_2-adrenoceptor. In contrast, selective α_1-adrenoceptor agonists were not expected to decrease

blood pressure. In this study, Bousquet et al. (1984) compared the effects of clonidine and the highly selective α_2-adrenoceptor agonist α-methylnorepinephrine (α-MNE) with cirazoline and ST 587, proposed as the most potent α_1-adrenoceptor agonists. These drugs were administered directly into the nucleus reticularis lateralis (NRL) of the normotensive cat. Surprisingly, while clonidine, cirazoline and ST 587 produced a hypotensive effect, the α-MNE had no effect on the arterial blood pressure. It appeared that the hypotensive activity of these compounds did not depend on their selectivity for the α_1- or α_2-adrenoceptor. When the chemical structures of these α-adrenergic drugs were compared, only the α-MNE did not possess an imidazoline ring in its structure. It was concluded that most likely, the agents with an imidazoline structure had stimulated a unique receptor in the NRL in order to decrease arterial blood pressure (Bousquet et al., 1984). These putative receptors were initially called imidazoline-preferring sites.

The binding studies that followed brought new evidence that the hypotensive action of clonidine and clonidine-like substances was at least in part mediated through stimulation of the purported imidazoline binding sites in the ventrolateral medulla (Ernsberger et al., 1987). These studies used a radio-labeled analog of clonidine, [^{3}H]para-aminoclonidine ([^{3}H]PAC), to label binding sites in membranes prepared from the bovine ventrolateral medulla. This location appeared to be the major site of the hypotensive action of clonidine (Bousquet and Schwartz, 1983). [^{3}H]PAC bound not only to α_2-adrenoceptors but also to non-adrenergic sites. Norepinephrine (α_2-agonist) displaced [^{3}H]PAC

from approximately 70% of the binding sites in the ventrolateral medulla. The remaining 30% of the sites were termed "norepinephrine-insensitive" binding sites and were found to display a high affinity for clonidine and clonidine-like imidazoline compounds. These norepinephrine-insensitive sites were termed imidazoline binding sites. When compared with the frontal cortex, it was found that the ventrolateral medulla contained a higher number of imidazoline binding sites. It was proposed that the hypotensive effect of clonidine in the ventrolateral medulla was mediated at these sites (Ernsberger et al., 1987).

The radioligand binding studies performed by Boyajian and coworkers (1987) showed that two antagonist reported to be selective for the α_2-adrenoceptor, idazoxan and rauwolscine, had two different binding distribution patterns in the rat brain. They compared distributions of binding sites labeled by these two α_2-adrenoceptor antagonists ([^{3}H]idazoxan and [^{3}H]rauwolscine). This study brought anatomical evidence indicating that [^{3}H]idazoxan labelled a heterogenous population of α_2-adrenoceptor sites or sites similar to the α_2-adrenoceptor. However, only a small population of these sites was selectively labelled by [^{3}H]rauwolscine. This was confirmed by the pharmacological characterization of the different binding properties of [^{3}H]idazoxan and [^{3}H]rauwolscine (Boyajian and Leslie, 1987). It was shown that the regions of the brain which were receiving noradrenergic innervation were labelled by [^{3}H]idazoxan, while several areas receiving dopaminergic innervation were labeled most densely by [^{3}H]rauwolscine. In 1988 Hamilton, Reid and Yakubu also compared the binding of two α_2-adrenoceptor ligands, this time [^{3}H]idazoxan

and [^{3}H]yohimbine, to rabbit kidney and brain. They also found that [^{3}H]idazoxan and [^{3}H]yohimbine had two different binding patterns indicating the possibility of two unique binding sites.

Rilmenidine and moxonidine represent second generation antihypertensive drugs that bind these putative imidazoline-binding sites. Bousquet and coworkers (1992) studied the effects of rilmenidine in the NRL of the anesthetized rabbit. Rilmenidine like clonidine exhibited hypotensive effects, except the effect produced by rilmenidine was two times more potent in the NRL as compared to the effect of clonidine. Additional binding studies showed that rilmenidine compared to clonidine was two to three times more selective for the imidazoline preferring receptors than for α_2-adrenoceptors. Another interesting study investigated the importance of the imidazoline receptor for the antihypertensive effects of moxonidine, rilmenidine and clonidine (Chan et al, 1996). Originally moxonidine and rilmenidine (second-generation centrally acting antihypertensive agents) were developed to be more selective α_2-adrenoceptor agonists than clonidine. However, it appeared that they possessed a greater affinity for the "imidazoline preferring" non-adrenergic sites (Bricca et al., 1989).

A problem with many of these studies has been the utilization of antagonists and agonists that may potentially interact with both the α_2-adrenoceptor and imidazoline receptor. Experiments were required that established the selectivity of the agonist/antagonists in the study preparations. In this regard Chan and Head (1996) designed a study in which they first determined the central dose of antagonists (imidazoline/α_2-adrenoceptor

antagonist, efaroxan and α_2-adrenoceptor antagonist, 2-methoxyidazoxan (2-MI)) which produced an equal reversal of the hypotension produced by α-methyldopa (α_2-adrenoceptor agonist). Then they determined the ability of these selected antagonists to reverse the hypotension produced by moxonidine, rilmenidine or clonidine. Similar to α-methyldopa, the hypotensive effect produced by clonidine was reversed by both selected antagonists. In contrast, efaroxan appeared to be more effective at reversing the effects of rilmenidine and moxonidine than 2-MI. It was concluded that the hypotensive effect produced by rilmenidine and moxonidine was mediated mainly by an action on central imidazoline receptors compared to clonidine that acted mostly on central α_2-adrenoceptors. It was also concluded from this study that imidazoline receptors and α_2-adrenoceptors may be located on the same autonomic pathway involved with blood pressure regulation. When alternative routes of administration were used, similar results were obtained, suggesting that the route of administration was not an important consideration in the role played by these different receptors (Chan at al., 1996).

The selectivity of compounds for the imidazoline sites over the α_2-adrenoceptors may have important clinical consequences. Rilmenidine and moxonidine are currently approved agents for the treatment of hypertension in France and Germany (Michel and Ernsberger, 1992). At present, sedation and dry mouth limit the use of clonidine in the treatment of hypertension. Sedation, one of the side effects of clonidine, has been proposed to be mediated by binding to α_2-adrenoceptors in the locus coeruleus (Szabo et al., 1996; Hunter et al., 1997). Binding to α_2-adrenoceptors in the salivary glands produces dry mouth

(van-Zwieten, 1997). In contrast, rilmenidine and moxonidine have demonstrated a selectivity for I_1-imidazoline receptors over α_2-adrenoceptors and have been associated with much less sedation and dry mouth (Kirch, et al., 1990; Ernsberger et al., 1992; Wenzel et al., 1998).

A growing number of studies in this field has brought forward a variety of nomenclatures for these non-adrenergic receptors. Heterogeneity of these receptors has been recognized by Michel and Insel (1989), who proposed a uniform nomenclature (Michel and Ernsberger, 1992) based on pharmacological studies. The imidazoline receptors have been divided into an I_1-subtype, based on selective binding of [^{3}H]PAC, and an I_2-subtype, based on the selective binding of [^{3}H]idazoxan. Based on the affinities for cimetidine and amiloride, further subclassification of I_1-receptors (I_{1A} and I_{1B}) and I_2-receptors (I_{2A} and I_{2B}) was proposed (Hamilton, 1995). The I_1-imidazoline receptors were found in different species, including human, and in different tissues and cell types. Within the kidney, I_1-receptors were expressed along the nephron (proximal, distal and collecting tubule) whereas α_2-adrenoceptors were found mainly in the proximal tubule (Ernsberger et al., 1990). Both, α_2-adrenoceptors and I_1-receptors were found on secretory and epithelial cells in the pancreatic islets and on the platelet (Pilatz et al., 1991; Pilatz and Sletten, 1993). The I_2-subtype has been found to be part of the enzyme monoamine oxidase-B to which it was exclusively bound and most likely involved in the release of insulin (Parini et al., 1996). The identification and isolation of the I_1-subtype has remained elusive, because many agents used for identification of these receptors share affinity for both receptor

classes, producing similar or identical physiological responses (Hieble and Ruffolo, 1995). Thus a similar binding orientation among different classes of the receptors created a problem in the drawing of a sharp line between the I_1-imidazoline receptor and the other receptor classes. Existence of the imidazoline receptors has yet to be confirmed by cloning.

Functional studies:

Functional studies from our laboratory have provided additional evidence that α_2-adrenoceptors are unique from imidazoline receptors. We mentioned previously that clonidine was acting through the imidazoline-preferring sites in the NRL (Bousquet et al., 1984). However, functional studies from our laboratory showed that in contrast to the CNS, in the rat kidney clonidine does not appear to be an I_1-imidazoline receptor agonist. Initial studies by Blandford and Smyth (1988a) utilized an intrarenal infusion of clonidine to attempt to selectively stimulate renal α_2-adrenoceptors. At low doses, clonidine increased urine flow rate by increasing free water clearance. When a higher dose of clonidine was infused both sodium and water excretion was increased. This indicated that the low and higher infusion rates were altering urine flow rate by two distinct mechanisms. In these studies, the increase in solute excretion was associated with an increase in blood pressure. A number of studies had demonstrated that the renal effects of α_2-adrenoceptor stimulation were mediated through antagonism of the renal actions of arginine vasopressin (Smyth et al., 1985; Strandhoy et al., 1983; Krothapalli and Suki, 1984). To eliminate the possibility

that the increased blood pressure contributed to the increase in solute excretion, a study was performed with a V_2 vasopressin receptor antagonist for two main reasons. First, if the α_2-adrenoceptor agonists were acting by blocking the renal action of vasopressin, the specific V_2 vasopressin receptor antagonist should mimic the effects of the α_2-adrenoceptor agonists. The V_2 vasopressin receptor antagonist should produce a similar increase in free water and osmolar clearance as the α_2-adrenoceptor agonists but without an increase in blood pressure. Second, pretreatment with the V_2 vasopressin receptor antagonist should block the effects of the α_2-adrenoceptor agonists. It was shown that the V_2 vasopressin receptor antagonist was indeed able to mimic the effects of α_2-adrenoceptor agonists, suggesting that the α_2-adrenoceptor agonists may act through the vasopressin antagonism. Also, an increase in osmolar clearance followed by the V_2 vasopressin antagonist supported the hypothesis that the α_2-adrenoceptor agonists may produce an increase in osmolar clearance independent of the increase in blood pressure. Moreover, the response to the α_2-adrenoceptor agonist clonidine was blocked by the V_2 vasopressin receptor antagonist suggesting the importance of the renal effects of vasopressin for the action of clonidine (Blandford and Smyth, 1990). Although these studies suggested that clonidine was acting through vasopressin antagonism, it was still not clear if clonidine was acting at sites separate from the imidazoline receptors in the kidney. In this regard, pretreatment with a V_2 vasopressin receptor antagonist failed to alter the response to moxonidine, suggesting that clonidine was not acting by the same mechanisms as moxonidine (Allan et al., 1993). This

suggested that the effects of clonidine were dependent on endogenous vasopressin, whereas the effects of moxonidine were independent. This indicated that these two compounds were possibly acting at two separate sites.

Ernsberger et al., 1992 showed that moxonidine displayed a 600 times greater affinity for the imidazoline receptor over the α_{2b}-adrenoceptor in the rat kidney. Therefore, the increase in solute excretion following an intrarenal infusion of moxonidine may be mediated through receptors distinct from the α_2-adrenoceptor. In a study by Allan et al., 1993, moxonidine increased urine flow rate, sodium excretion and osmolar clearance but not free water clearance. In contrast, the diuresis produced by the low dose of the α_2-adrenoceptor agonist clonidine was accompanied by an increase in free water clearance. This supported the hypothesis that moxonidine and clonidine were acting on two separate sites. Similar results were obtained with the I_1-imidazoline receptor agonist rilmenidine (Smyth and Penner, 1995).

The difference between clonidine and the I_1-imidazoline receptor agonists was further supported in the one kidney one clip model of hypertension. The response to clonidine remained intact while the response to moxonidine and rilmenidine was attenuated in this study, showing that the I_1-imidazoline receptor agonists and α_2-adrenoceptor agonists were not the same (Li and Smyth, 1993; Li et al., 1994).

Another study from Blandford and Smyth (1991) used rats previously treated with indomethacin (cyclooxygenase inhibitor) to observe the role of prostaglandins in the response to an intrarenal infusion of clonidine. The

natriuretic effect of clonidine was potentiated by the indomethacin pretreatment. Darkwa and Smyth (1995) followed a similar experimental procedure for moxonidine. In that study, the natriuretic effect of moxonidine was antagonized by indomethacin. Collectively, these studies again demonstrated that in the kidney the I_1-imidazoline receptor agonist (moxonidine) was acting at a different site than the α_2-adrenoceptor agonist (clonidine). An increase in free water clearance following low dose of clonidine appeared to be due to stimulation of the α_{2b}-adrenoceptors and endogenous vasopressin antagonism (Bylund, 1985; Intengan and Smyth, 1996). An increase in osmolar clearance following a higher dose of clonidine was mediated by α_{2a}-adrenoceptor stimulation (Intengan and Smyth, 1997a). This increase in osmolar clearance was not the same as that produced by moxonidine. For the osmolar response, Allan et al. (1993), found that idazoxan selectively blocked the effects of moxonidine, whereas rauwolscine was more selective in the blockade of clonidine.

Central vs. peripheral sites of action:

Imidazoline receptors have been identified in the central nervous system as well as in the periphery, including the kidney (Ernsberger et al., 1992). The kidney plays an important role in the regulation of blood pressure. This role of the kidney can be influenced by antihypertensive agents acting directly on the kidney or by acting centrally to decrease peripheral sympathetic nerve activity (Head, 1995; Schafer et al., 1995). It is not fully understood whether a direct and/or central action is involved. A series of studies from our laboratory have

demonstrated that the effect of central administration of the I_1-imidazoline receptor agonist moxonidine was not the same as the peripheral administration of moxonidine. In these studies, the natriuretic response of stimulation of central sites was compared to the natriuretic response due to stimulation of receptors at peripheral sites.

Intracerebroventricular (ICV) administration of a low dose of the I_1-imidazoline receptor agonist moxonidine produced a natriuresis without a decrease in blood pressure and heart rate (Penner and Smyth, 1994a; 1995). An increase in free water clearance was obtained at a high dose of moxonidine. Pretreatment with ICV idazoxan (selective I_1-imidazoline receptor antagonist) completely inhibited the natriuresis produced with ICV moxonidine (Penner and Smyth, 1994b). Previously, our laboratory had shown that intrarenal administration of moxonidine also produced a natriuresis (Allan et al., 1993). However, pretreatment with ICV idazoxan only in part blocked this effect of moxonidine (Smyth and Penner, 1998). In contrast, intravenous administration of idazoxan completely blocked the action of intrarenal moxonidine but had no effect on ICV moxonidine (Smyth and Penner, 1998). Thus, ICV administrated idazoxan had a greater blocking effect on ICV moxonidine. Conversely, intravenous idazoxan was more effective at blockade of intravenous moxonidine. These studies suggested two separate sites of action for moxonidine, central and peripheral. In these studies, the kidney was recognized as an important peripheral site of action. However, it is hard to determine to what extent central or/and peripheral sites are involved since centrally acting agents may alter the

function of the kidney either directly or by decreasing the renal sympathetic nerve activity. A decrease in the renal sympathetic nerve activity will increase sodium excretion due to inactivation of renal α_1-adrenoceptors (Kopp and DiBona, 1992). Thus a study was designed in which the α_1-adrenoceptor antagonist prazosin was administered intravenously prior to moxonidine. This would determine if the change in stimulation of the α_1-adrenoceptors, secondary to changes in renal sympathetic nerve activity, was responsible for the change in sodium excretion. It appeared that prazosin abolished the response to ICV moxonidine (Penner and Smyth, 1995) but the response to intrarenal moxonidine remained unchanged (Penner and Smyth, 1994b) indicating once again two unique sites of action. Finally, Penner and Smyth (1995) performed renal denervation to determine if the renal effects followed by central administration of moxonidine were mediated through a decrease in sympathetic nerve activity. In the sham rats, ICV moxonidine produced an increase in urine volume, sodium excretion, osmolar clearance and free water clearance. Following denervation, sodium excretion and osmolar clearance were completely abolished, indicating the importance of intact renal nerves. However, urine volume was still increased due to an increase in free water clearance that remained at a level similar to that recorded in the sham animals.

Collectively, the above cited work strongly supports two postulates. First these studies suggest the α_2-adrenoceptors to be unique from the I_1-imidazoline receptors. Second, the results from these studies are consistent with the postulate that I_1-imidazoline agonists may act at two separate sites to increase

urine flow rate and sodium excretion. The central sites appear to be preferentially stimulated by ICV moxonidine, but selectively blocked by renal denervation, ICV idazoxan or intravenous prazosin. On the other hand, the peripheral sites appear to be blocked by intravenous idazoxan.

Study Proposal

The importance of the sympathetic nervous system in the regulation of blood pressure is well documented. The same is true for the imidazoline receptors that have been identified in the central nervous system as well as in other tissues, including the kidney. The imidazoline receptors located centrally, as well as peripherally, mediate an increase in sodium excretion. In the previous studies from our laboratory, an I_1-imidazoline receptor agonist, moxonidine, was intensively studied in a series of experiments. It was shown that centrally acting moxonidine may affect the kidney either by a decrease in the sympathetic nerve activity or/and by acting directly on the kidney. To determine which mechanism is predominant and a possible interaction with adrenoceptors would be difficult. A renal denervation study from our laboratory has demonstrated the importance of the renal sympathetic nerves for the natriuretic effect of the centrally administered moxonidine. However, the effect of renal denervation on the effect of peripheral moxonidine has not been performed by our laboratory. Such a study would be a definitive approach to determine if a peripheral site of action for moxonidine independent of the sympathetic nervous system does exist.

It has been clearly demonstrated that the natriuretic effects following peripheral administration of the I_1-imidazoline receptor agonist, rilmenidine were abolished by prior renal denervation. This would strongly indicate that a direct tubular effect does not exist (Kline and Cechetto, 1993). This was at odds with the proposal by Penner and Smyth (1997) of a central and peripheral site of

action. Since the peripheral response to denervation has not been tested in our laboratory, in the present thesis I will clarify the importance of the sympathetic nerves in the natriuretic response to peripherally administered I_1-imidazoline receptor agonists. These studies will utilize the same in vivo preparation as that documented previously from our laboratory.

We therefore investigated the hypothesis that renal denervation would decrease the response to an intravenous infusion of an imidazoline receptor agonist.

Dose Response Study of Rilmenidine, Guanfacine, Moxonidine and Clonidine in Anesthetized Rats

INTRODUCTION

The natriuretic and diuretic actions of α_2-adrenoceptor agonists and I_1-imidazoline receptor agonists have been previously documented in our laboratory (Blandford and Smyth, 1988b; Allan et al., 1993; Li et al., 1994; Intengan and Smyth, 1996; 1997a; 1997b). Clonidine, a general α_2-adrenoceptor agonist, increased urine flow at low doses secondary to an increase in free water clearance. At higher doses, osmolar clearance was also increased (Blandford and Smyth, 1988a). This study, as well as others (Smyth et al., 1992) suggested that these actions of clonidine were mediated at two possible sites and/or receptors. Interestingly, radioligand binding studies had identified two different subtypes of α_2-adrenoceptors ($\alpha_{2a/d}$ and α_{2b}) in the rat kidney (Uhlén and Wikberg 1991). Intengan and Smyth (1996), further demonstrated that the increase in free water following clonidine could be selectively antagonized by prazosin, an α_1-adrenoceptor antagonist with a high affinity for the α_{2b}-subtype (Bylund, 1985). Naltrexone, an opioid antagonist, attenuated the ability of clonidine to increase osmolar clearance but not free water clearance. Based on these studies it was assumed that clonidine increased free water clearance secondary to interaction with the α_{2b}-subtype. It was further postulated that the osmolar effect of clonidine was mediated by another α_2-adrenoceptor, most conceivably the α_{2a}-subtype. This was confirmed by the demonstration that guanfacine, a

selective α_{2a}-adrenoceptor agonist, increased osmolar but not free water clearance and this effect was blocked by the selective α_{2a}-adrenoceptor antagonist RX-821002 (Intengan and Smyth, 1997b).

A novel class of receptor, the imidazoline receptor, has been proposed based on radioligand binding studies and in vivo experiments. A number of compounds previously classified as α_2-adrenoceptor agonists have been found to have an even greater affinity for the imidazoline receptor (Bousquet et al., 1984; 1992; Boyajan et al., 1987). Allan et al. (1993) showed that moxonidine, an I_1-imidazoline receptor agonist, increased urine flow rate, sodium excretion and osmolar clearance but not free water clearance, an effect similar to that reported for α_{2a}-adrenoceptor agonists (Intengan and Smyth, 1996; 1997b). However, in the 1 kidney 1 clip model of hypertension, the response to I_1-imidazoline receptor agonists (rilmenidine and moxonidine) was significantly attenuated whereas the response to clonidine remained intact (Li et al., 1994). As well, naltrexone, at a dose that significantly attenuated the natriuretic response to guanfacine and clonidine (Intengan and Smyth, 1996; 1997b) failed to alter the response to moxonidine (Intengan and Smyth, 1997a). Similarly, pretreatmant with a V_2 vasopressin receptor antagonist that completely attenuated the response to clonidine (Blandford and Smyth, 1990), failed to alter the response to moxonidine (Allan et al., 1993). Collectively, these and other studies suggested that clonidine and moxonidine were acting at two different receptors to increase osmolar clearance, the α_{2a}-adrenoceptor and I_1-imidazoline receptor respectively.

In the present study, we propose to determine the natriuretic dose-response relationship for two imidazoline receptor agonists (rilmenidine and moxonidine) and two α_2-adrenoceptor agonists (clonidine and guanfacine). The purpose of these studies was to re-establish the relationship between these agonists and changes in renal function. However, we also wished to determine the optimal dose that produced a significant increase in urine flow rate with a minimal change in blood pressure and heart rate for an I_1-imidazoline receptor agonist and an α_2-adrenoceptor agonist. These doses would then be utilized for subsequent renal denervation studies.

Methods

General experimental preparation

The experimental protocol has been previously described by Blandford and Smyth (1988b). Male Sprague-Dawley rats (175-225 g) were obtained from the University of Manitoba (Charles River Breeding Stock) and care provided according to The Canadian Council on Animal Care. The animals were kept in cages (two or three rats per cage) at 22°C with a 12 h light/dark cycle and fed a standard Purina rat chow diet with free access to tap water.

Seven to ten days prior to the experimental day, under ether anesthesia (Mallinckrodt), a right flank incision was performed. The right renal artery and vein, and ureter were occluded together by a simple knot with 4-0 surgical silk (Stevens and Sons) and the right kidney was removed. The muscle layer was closed with 4-0 silk and Michel suture clips were used to secure the skin. After the incision was sutured, a subcutaneous injection of 3.0 μg buprenorphine was administered for analgesia. Following nephrectomy, animals were placed overnight in individual cages to recover.

On the day of the acute experiment (7 to 10 days following nephrectomy) the rats (280-340 g) were anesthetized with an intraperitoneal injection of 50 mg/kg of pentobarbital (Nembutal, BDH Chemical Ltd., Poole, England). During the experiment, an additional bolus dose of pentobarbital (5.0 mg/kg, i.v.) was provided as needed (response to tail pinch or return of blink reflex). The rats

were placed on a thermostatically controlled heating blanket. The Harvard Animal Blanket Control Unit with a rectal thermometer probe was used to maintain body temperature at 37°C. The animals were tracheotomized and intubated with a Clay Adams polyethylene catheter (PE-240) to allow spontaneous breathing or to connect to a ventilator (Harvard rodent ventilator, model 683) if necessary. The left carotid artery was cannulated with a polyethylene catheter (PE-50) and connected to a Cobe pressure transducer, which was connected to a Grass polygraph Model 5D for blood pressure and heart rate monitoring. The left jugular vein was cannulated with a polyethylene catheter (PE-160) for the continuous infusion of saline (0.9% NaCl) at 97 μl/min (Sage syringe infusion pump). This produced a moderate diuresis and allowed for the administration of additional anesthetic as required. The remaining kidney was exposed by a left flank incision and the ureter was cannulated with a polyethylene catheter (PE-10). This allowed for timed urine collections into pre-weighted vials. A 31-gauge stainless steel needle was inserted into the renal artery and secured with super glue (Mastercraft, Toronto, Ontario). The needle was connected with a polyethylene catheter to the Harvard sage infusion pump for the intrarenal infusion (3.4 μl/min) of the study drugs.

Protocol

After completion of surgery, the continuous intravenous infusion of saline (97 μl/min) was initiated and continued to the end of the experiment. The rats were allowed to stabilize for 45 minutes (time = 0 – 45 min) before the first 30-minute collection of urine (time = 45 – 75 min). Pre-weighted 1.5 ml Eppendorf

centrifuge vials were used to collect urine and no intervention was performed during this period of time. Therefore, this first urine collection served as a control for comparing the baseline renal function of all animals. Also, based on previous studies from our laboratory, only animals that had initial urine flow rates between 3 and 30 µl/min were included for final analyses. The second 30-minute urine collection (time = 75 – 105 min) immediately followed the first collection. At this time an infusion of study drug or saline vehicle (3.4 µl/min) was started and continued for the duration of the experiment. The third 30 minute urine collection (time = 105 – 145 min) was obtained as the previous two collections. During each collection, blood pressure and heart rate were recorded continuously and documented at the midway point of each collection period.

Analysis

After the last urine collection, a blood sample was collected from the carotid artery into a borosilicate tube. One drop of heparin (Leo Laboratories) had been previously added into the tube to prevent blood clotting. A centrifuge was used to separate the plasma. Blue dye was injected through the renal artery line to confirm proper positioning of the needle. Creatinine levels in the plasma and urine were analyzed with a Beckman Creatinine Analyzer, osmolality with a Precision Systems Micro Osmometer and the sodium concentrations with a Nova Electrolyte Analyzer (13+).

All data were presented as the mean ± standard error and analyzed by repeated measures analysis of variance (ANOVA) followed by The GLM Procedure, Least Squares Means, to identify significant differences. Significance

is denoted in figures by *, which represents a $p<0.05$ and **, which represents $p<0.01$ compared to the control group.

Drugs

The drugs used in this study and their sources are: moxonidine (gift from Beiersdorf, AG, Hamburg, Germany); guanfacine (gift from Wyeth Ayerst); clonidine (Sigma Chemical Company, St. Louis, MO, USA); and rilmenidine (gift from Servier, France).

Results

Four agonists were investigated in this preliminary dose–response study (rilmenidine, guanfacine, moxonidine and clonidine). These studies established the dose for each drug that produced a consistent and significant increase in urine flow rate and sodium excretion with minimal changes in blood pressure and heart rate. The results have been presented as the mean ± S.E. of three to eight experiments for each infusion rate. The specific numbers for each group have been included in the figures. Also, the results have been presented as the absolute values as well as the difference between the baseline and final collection period to emphasizes the treatment differences between the studied groups.

Hemodynamic and Renal Effects of Rilmenidine

Following intrarenal administration of rilmenidine at 10 and 30 nmol/kg/min, blood pressure, heart rate and creatinine clearance remained at similar levels as the first control collections through the whole experiment for all treatments (Figure 1.1 and 1.1a). The group receiving 10 nmol/kg/min rilmenidine started at a higher blood pressure and this pressure remained elevated through the experiment (Figure 1.1). However, the rilmenidine infusion did not alter the blood pressure from the control collection levels (Figure 1.1a). At the same time, rilmenidine was associated with a significant increase in urine flow rate and sodium excretion in a dose-dependent manner (Figure 1.2 and

1.2a). Osmolar clearance was increased significantly at the higher infusion rate (Figure 1.3 and 1.3a).

Hemodynamic and Renal Effects of Guanfacine

In the experiments with guanfacine, a dose of 3 nmol/kg/min showed significant differences from the control group. Therefore, we did not proceed with higher doses of this drug.

As shown in Figure 1.4 and 1.4a, neither blood pressure nor creatinine clearance was altered by guanfacine treatment as compared to the control group. However, heart rate was decreased following guanfacine treatment (Figure 1.4 and 1.4a). Administration of guanfacine increased urine flow rate, sodium excretion (Figure 1.5 and 1.5a) and osmolar clearance (Figure 1.6 and 1.6a), but not free water clearance (Figure 1.6 and 1.6a).

Hemodynamic and Renal Effects of Moxonidine

When we investigated moxonidine (3 and 10 nmol/kg/min), blood pressure ($p=0.23$) and heart rate ($p=0.33$) failed to change significantly from the control group. Moxonidine failed to altered renal function at these doses investigated (Figures 1.7, 1.8, 1.9 and 1.7a, 1.8a, 1.9a).

Hemodynamic and Renal Effects of Clonidine

The starting dose of clonidine for this study was chosen, based on previous experiments from our laboratory, to alter renal function with minimal effects on blood pressure and heart rate.

Intravenous administration of clonidine (3 nmol/kg/min) slightly but non significantly increased blood pressure and decreased heart rate (Figure 1.10 and

1.10a) as compared to the control group. Creatinine clearance remained at a value similar to the control group (Figure 1.10 and 1.10a). Compared to the control group, 3 nmol/kg/min of clonidine increased urine flow rate (Figure 1.11 and 1.11a) secondary to an increase in free water clearance (Figure 1.12 and 1.12a). Clonidine did not alter osmolar clearance (Figure 1.12 and 1.12a).

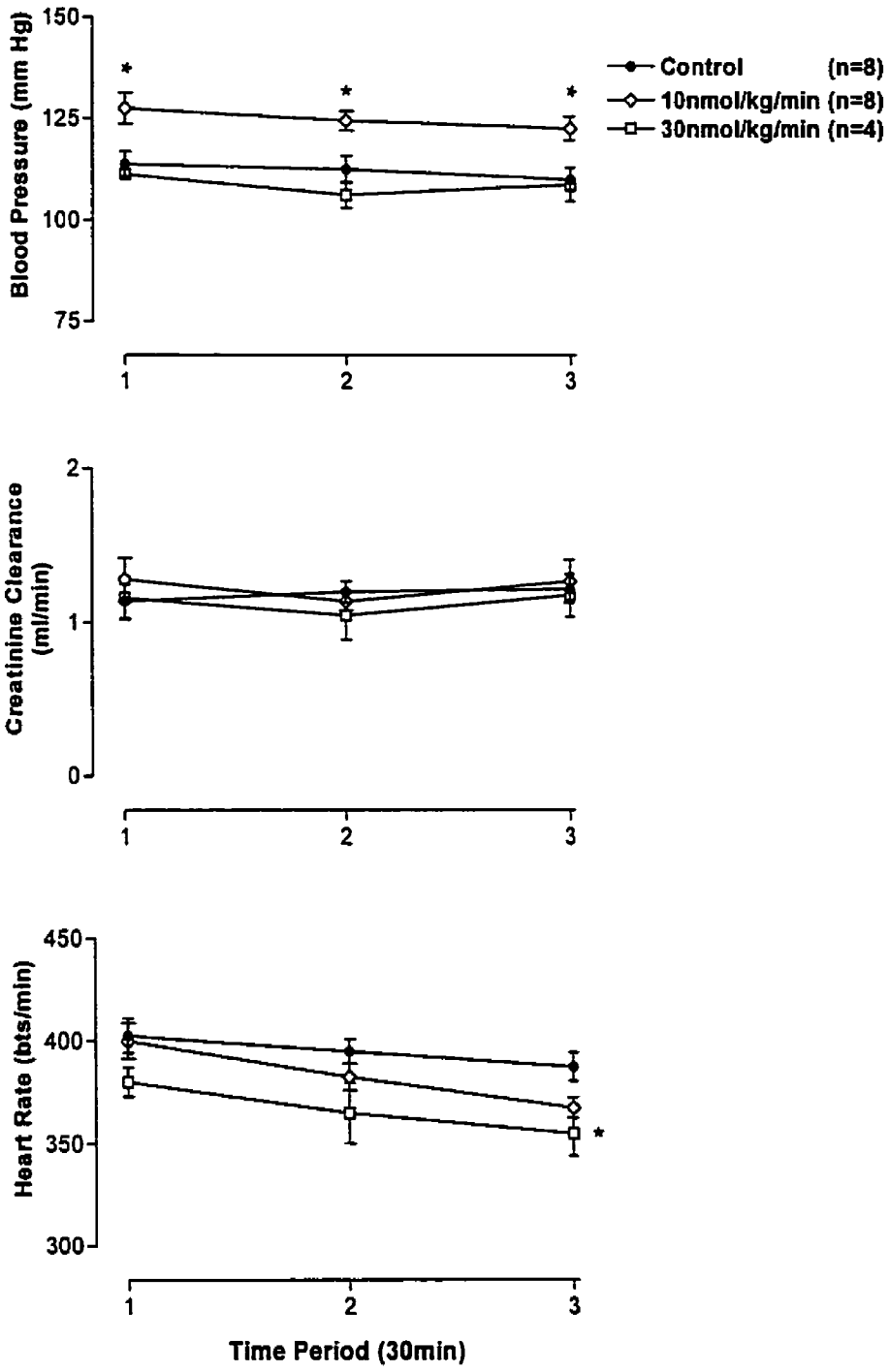

Figure 1.1. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or rilmenidine (10, 30 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

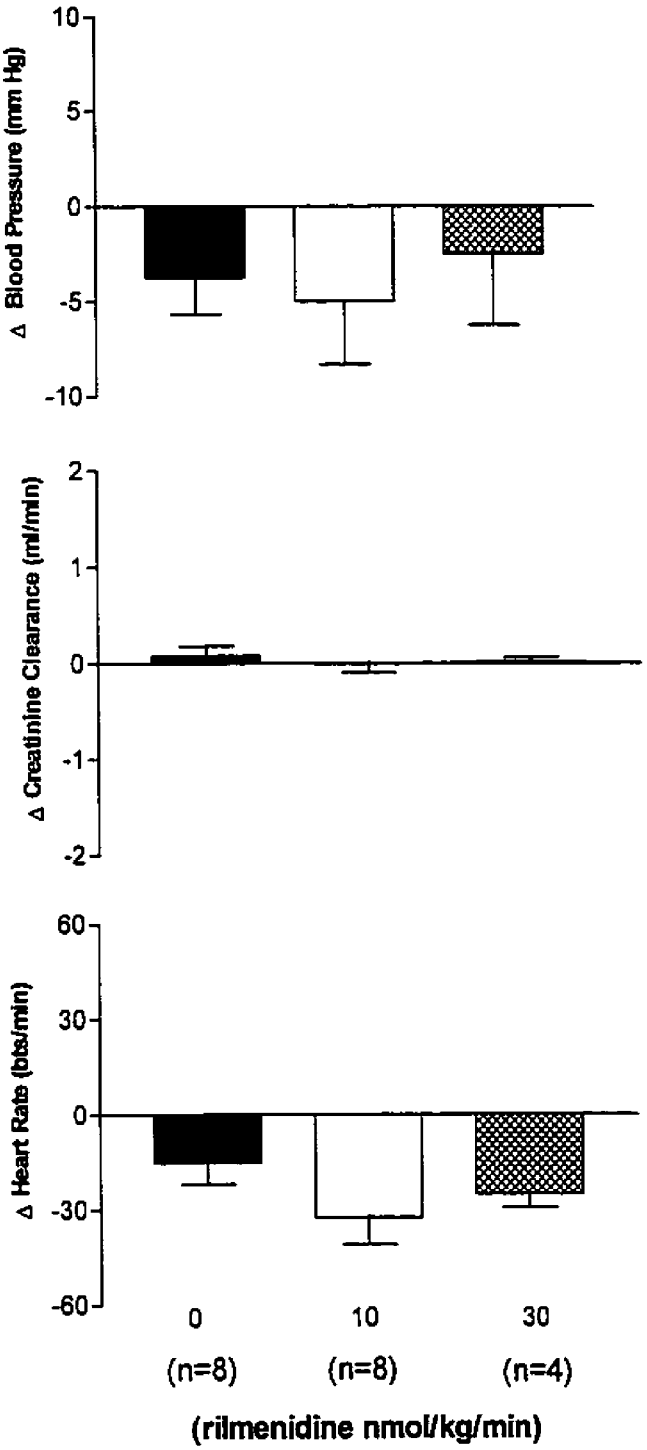

Figure 1.1a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or rilmenidine (10, 30 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

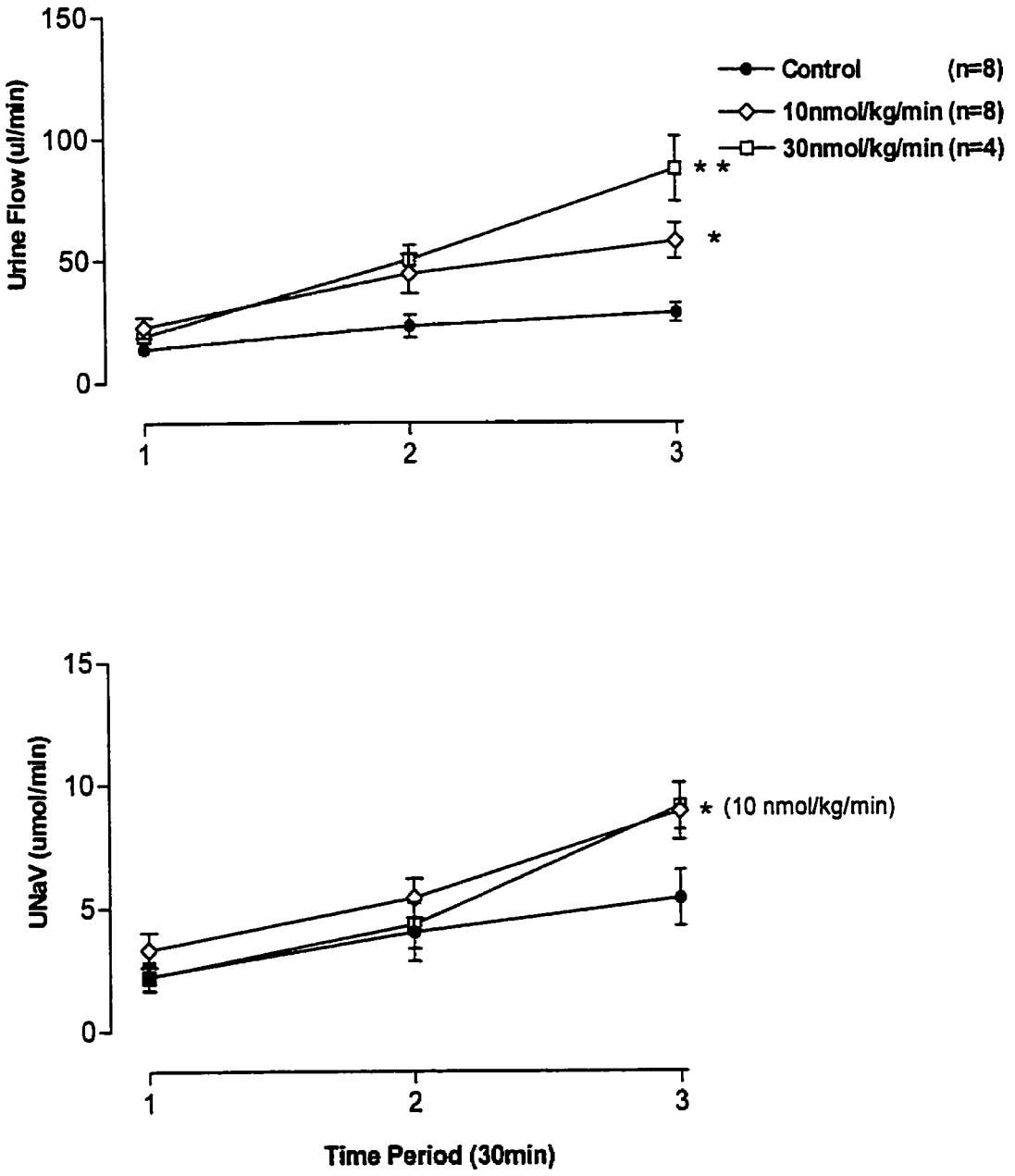

Figure 1.2. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or rilmenidine (10, 30 nmol/kg/min) on urine flow, and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

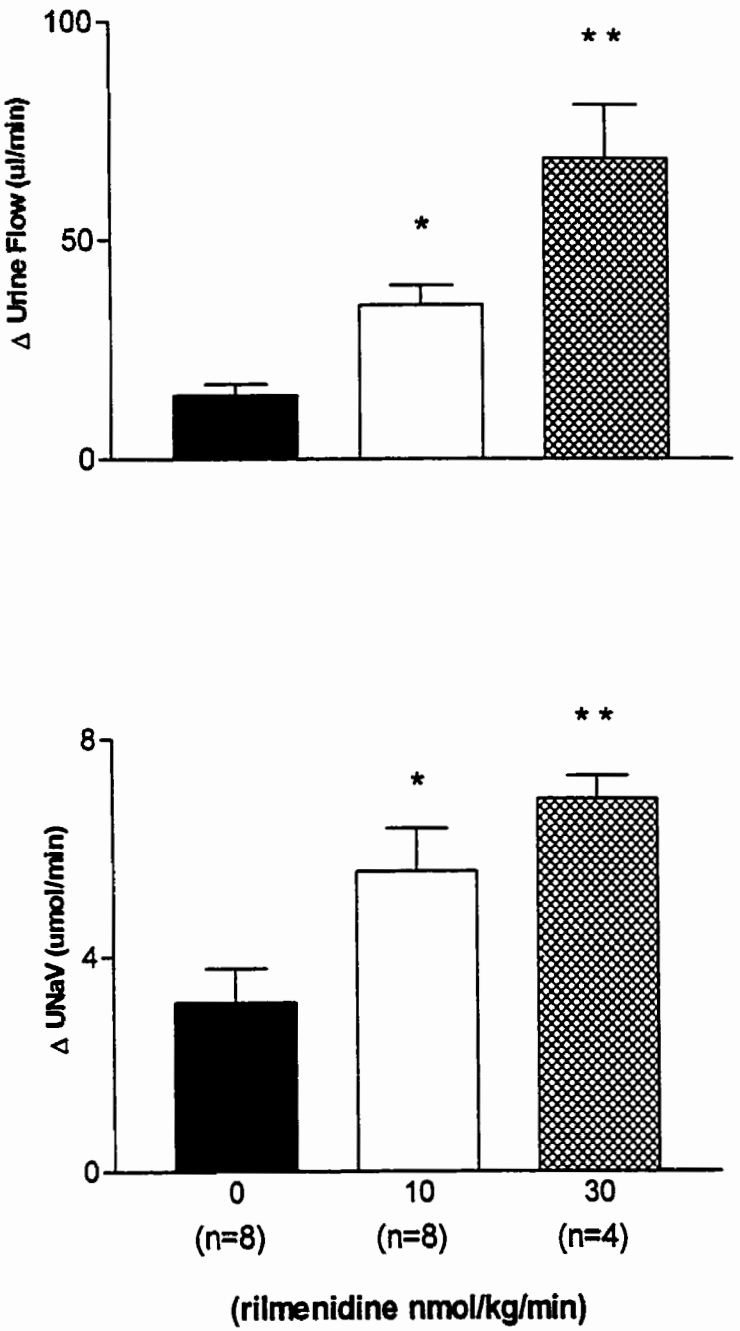

Figure 1.2a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or rilmenidine (10, 30 nmol/kg/min) on urine flow, and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

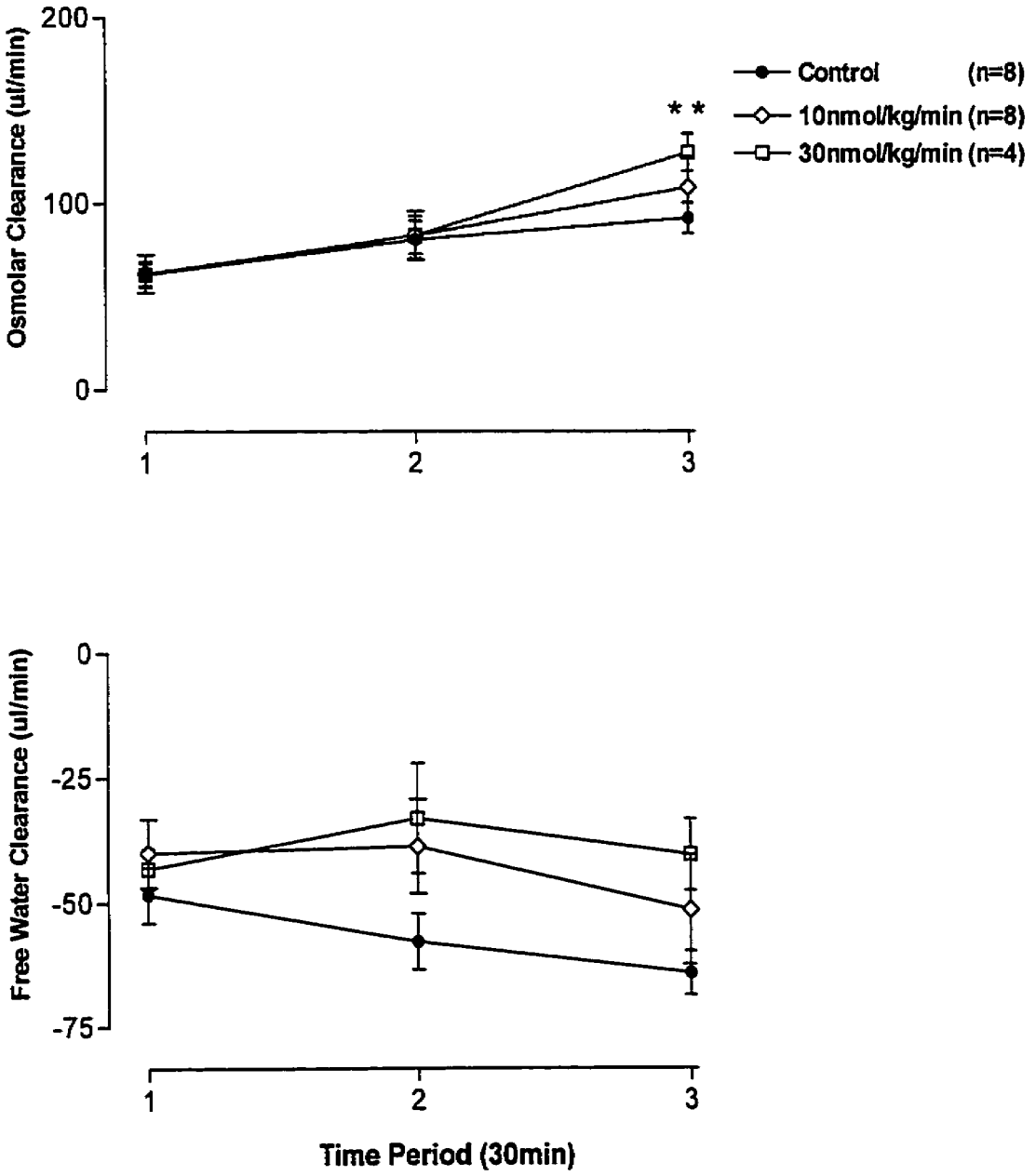

Figure 1.3. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or rilmenidine (10, 30 nmol/kg/min) on osmolar clearance and free water in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

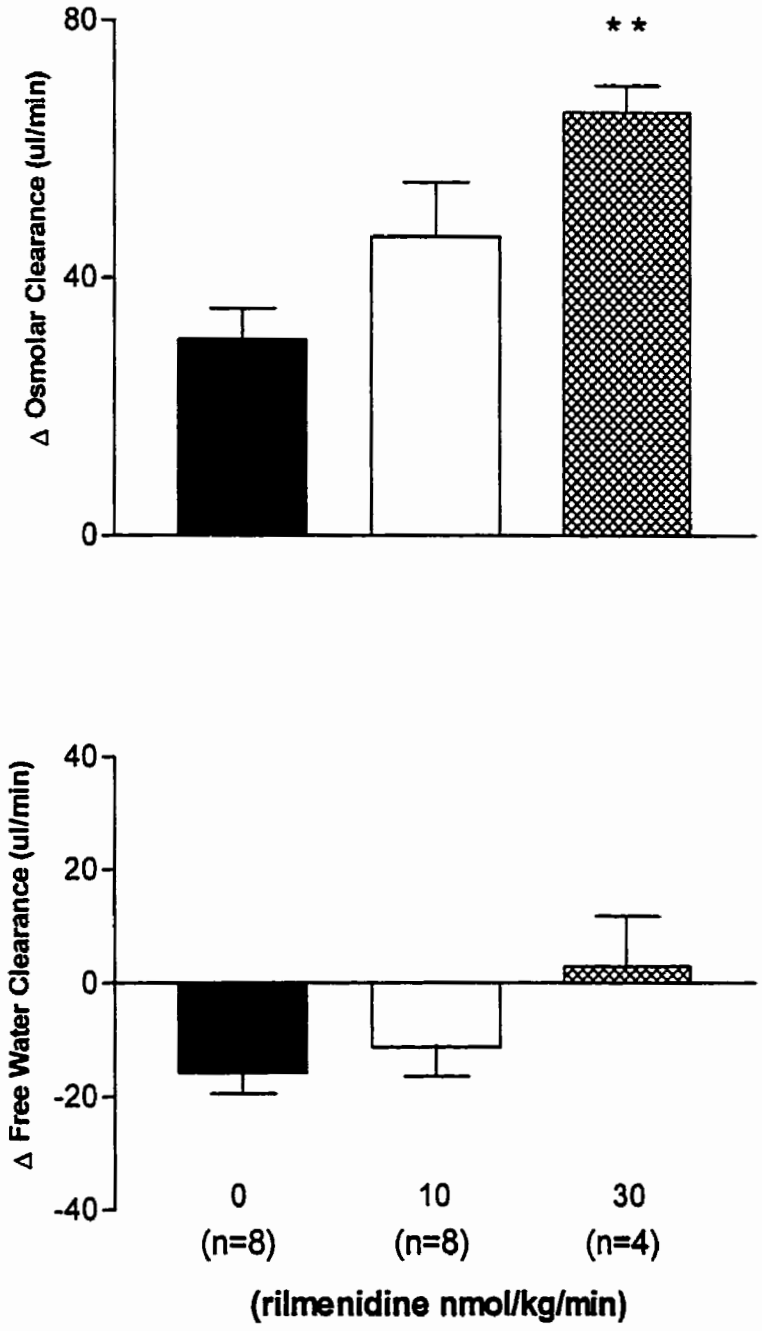

Figure 1.3a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or rilmenidine (10, 30 nmol/kg/min) on osmolar clearance and free water in male Spragu - Dawley rats. Data are presented as the mean ± S.E of the mean of the difference between the final collection and baseline values.

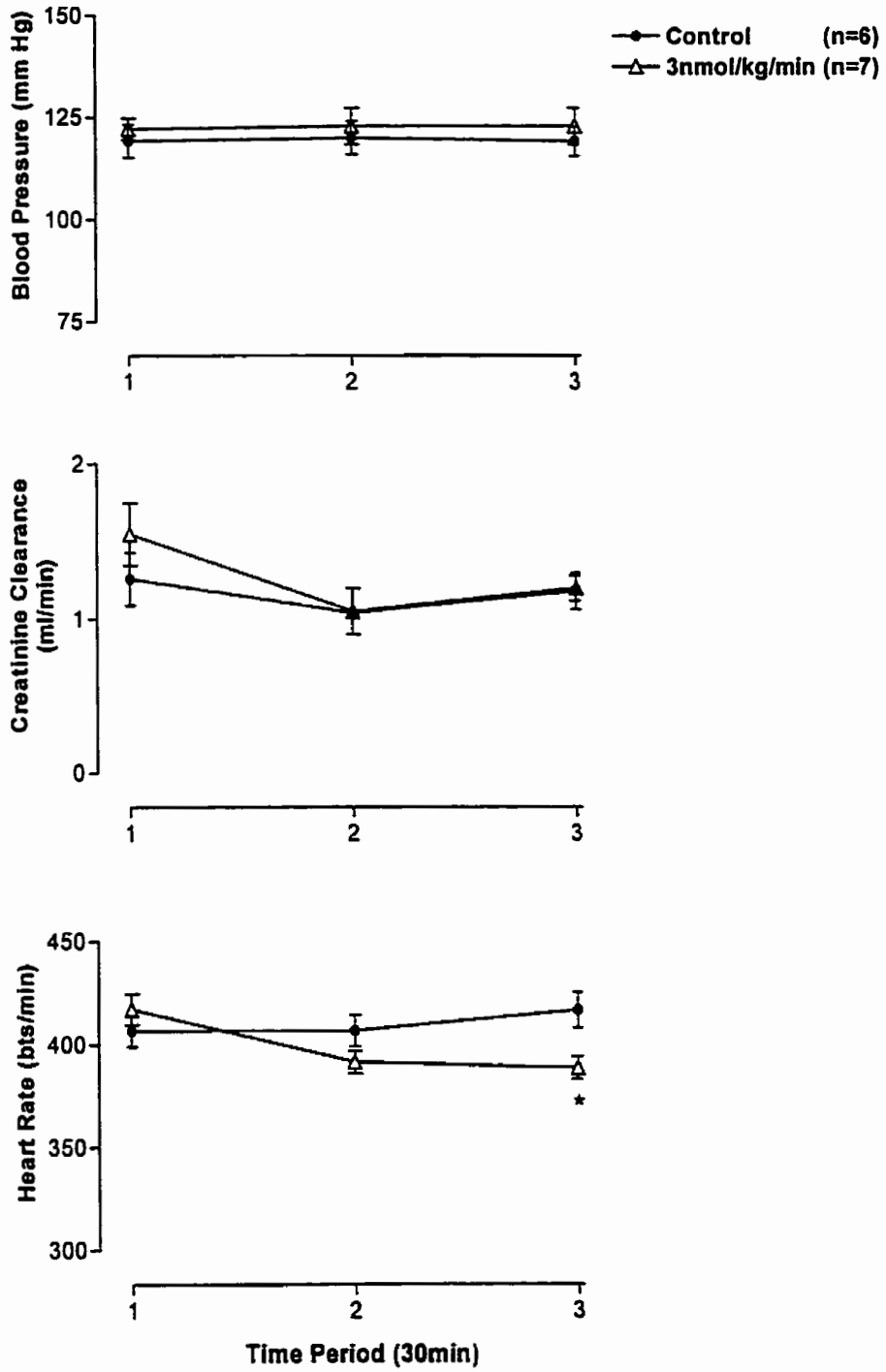

Figure 1.4. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or guanfacine (3 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

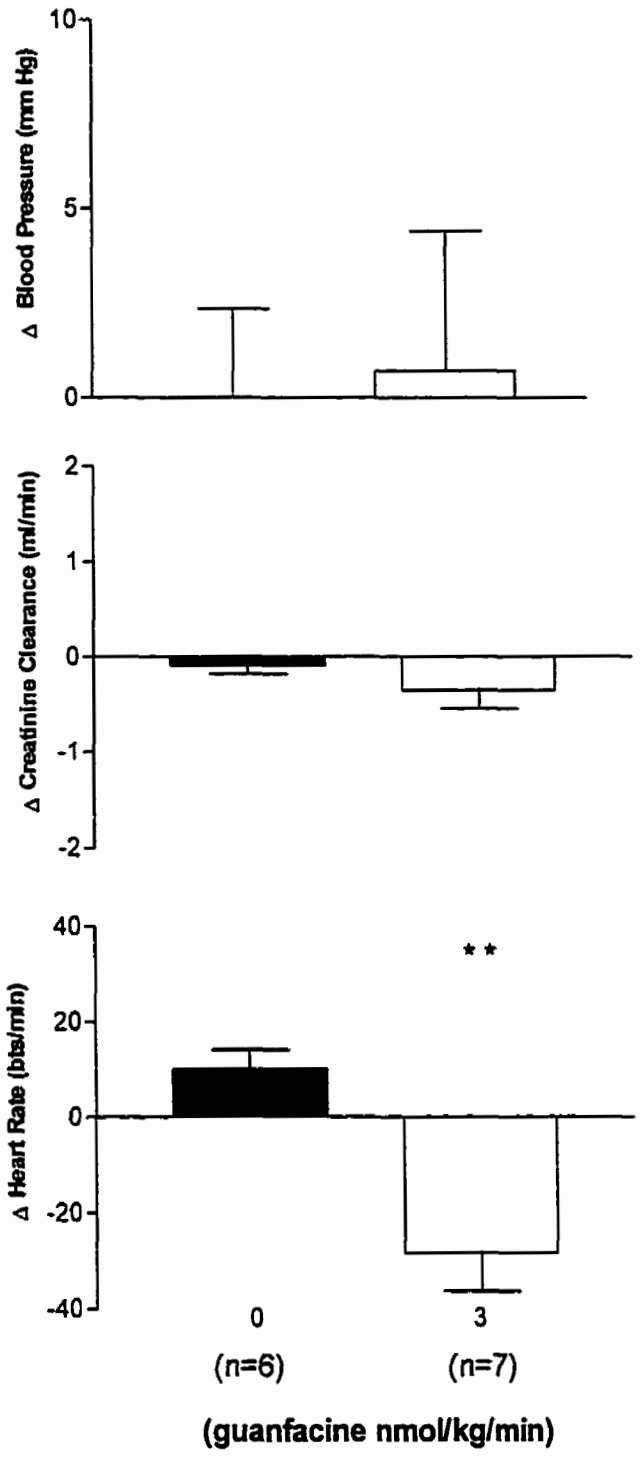

Figure 1.4a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or guanfacine (3 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

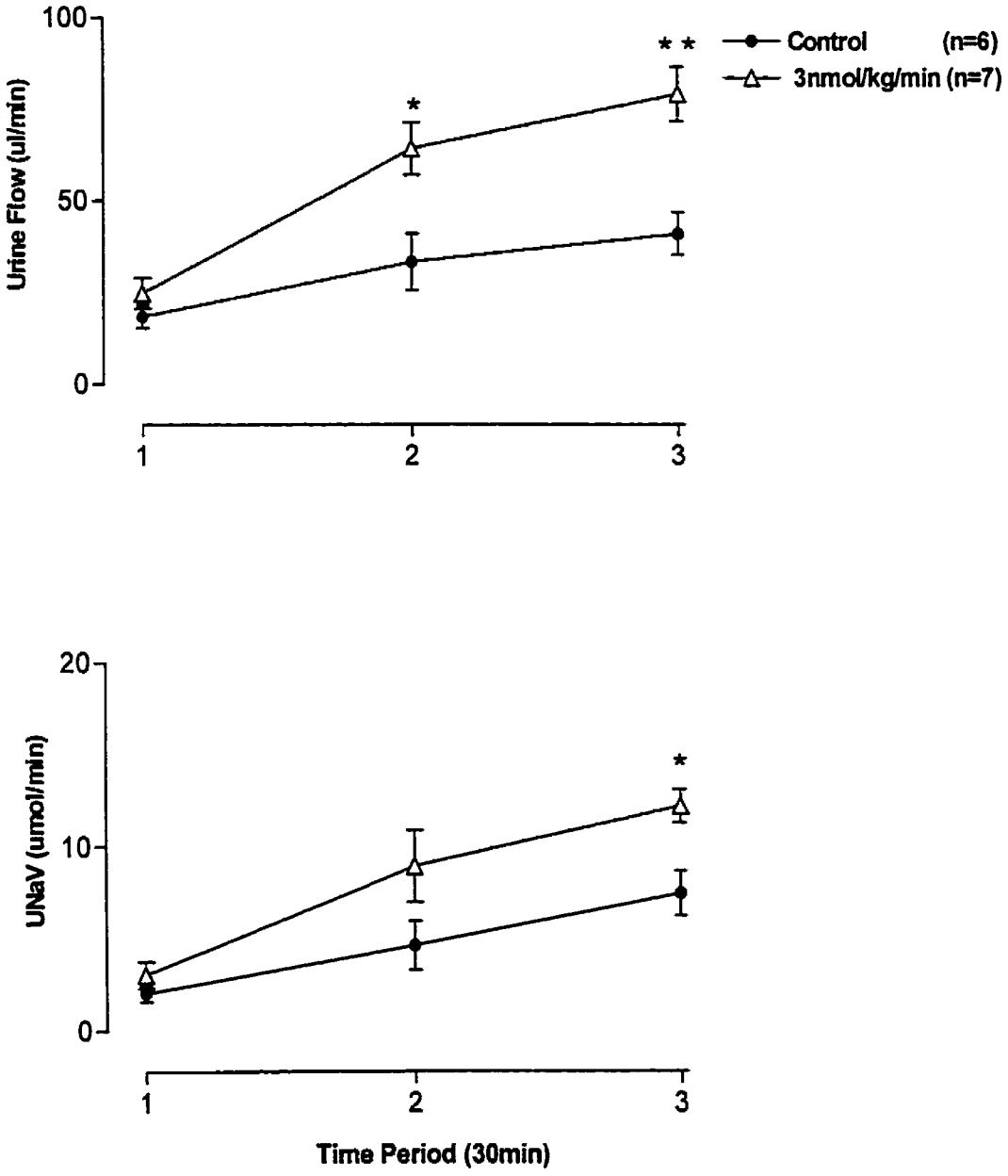

Figure 1.5. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or guanfacine (3 nmol/kg/min) on urine flow and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

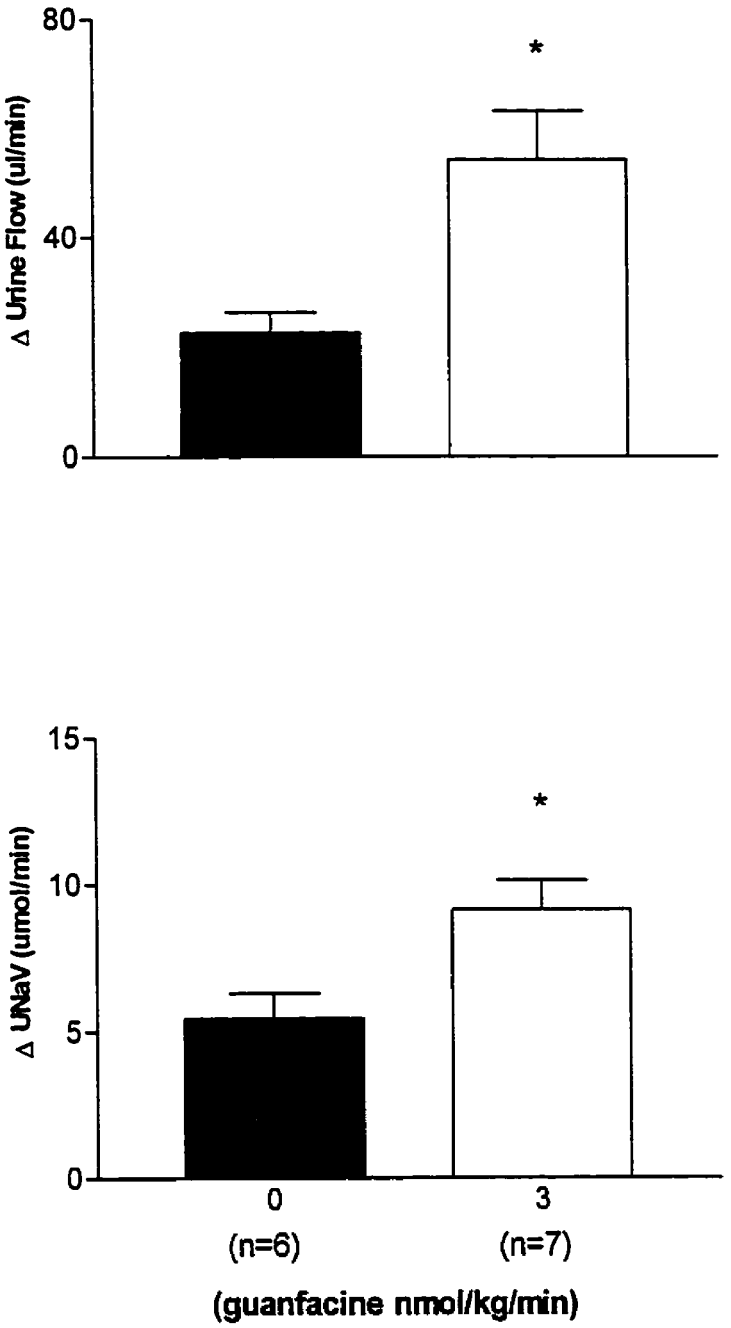

Figure 1.5a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or guanfacine (3 nmol/kg/min) on urine flow and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

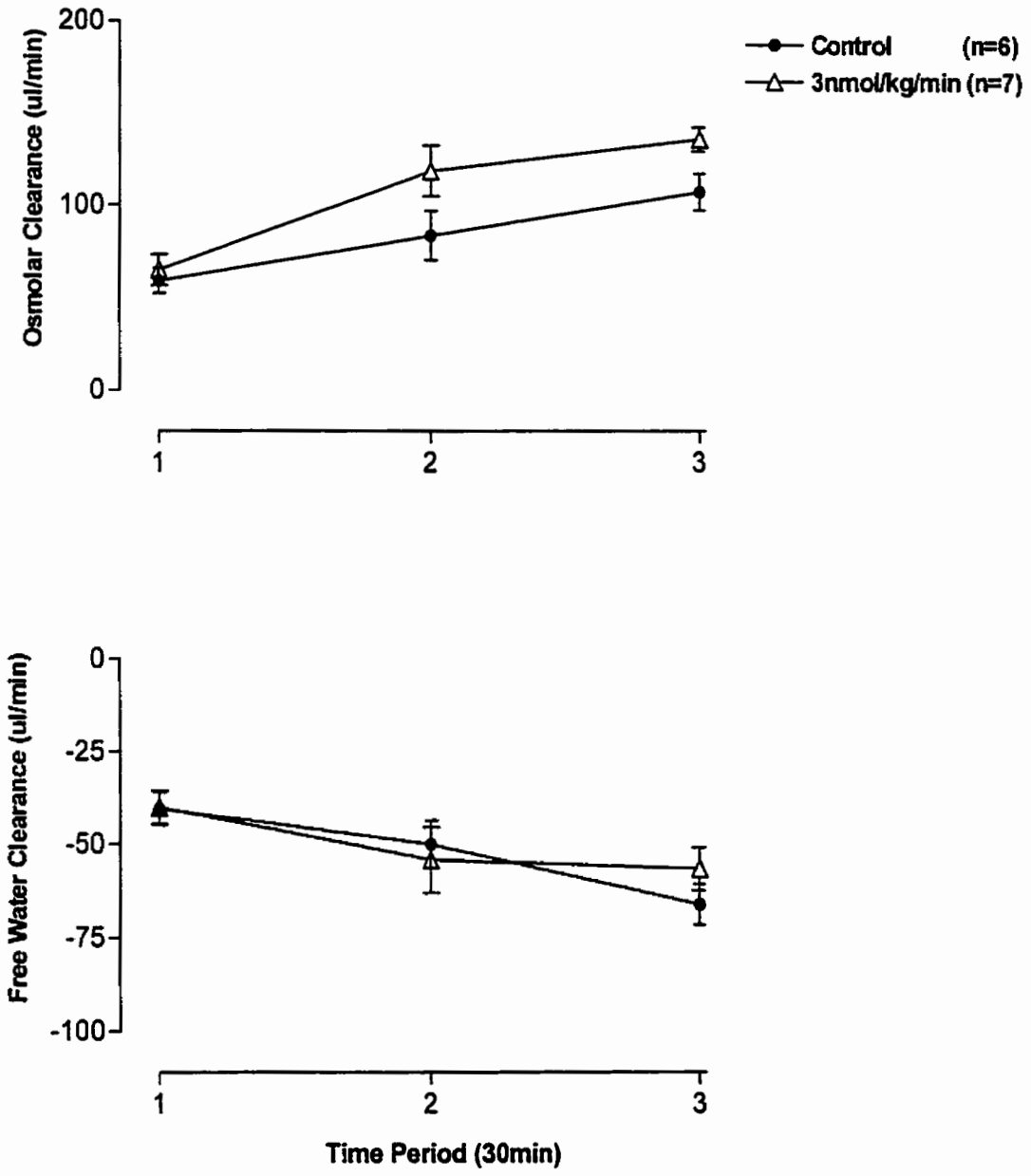

Figure 1.6. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or guanfacine (3 nmol/kg/min) on osmolar clearance and free water clearance in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

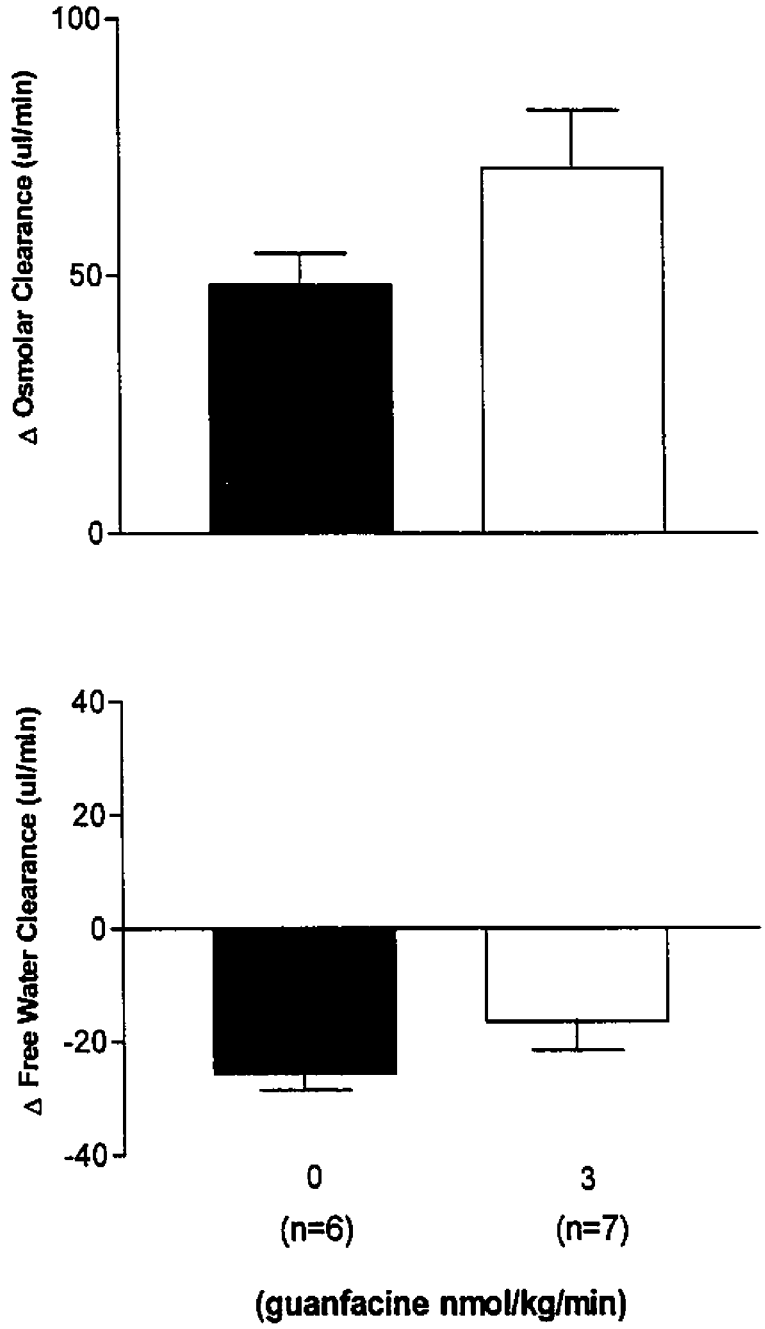

Figure 1.6a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or guanfacine (3 nmol/kg/min) on osmolar clearance and free water clearance in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

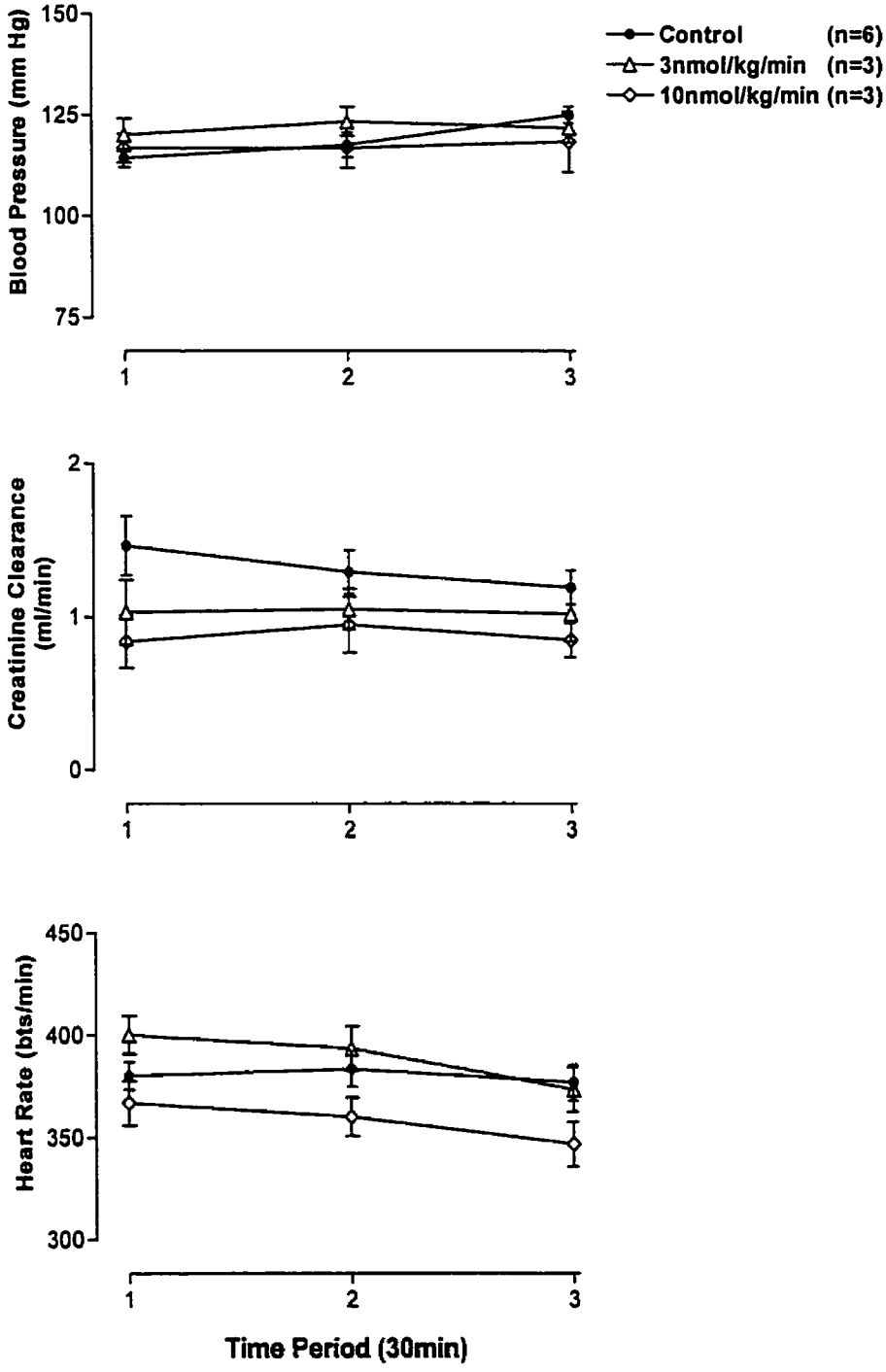

Figure 1.7. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or moxonidine (3 and 10 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

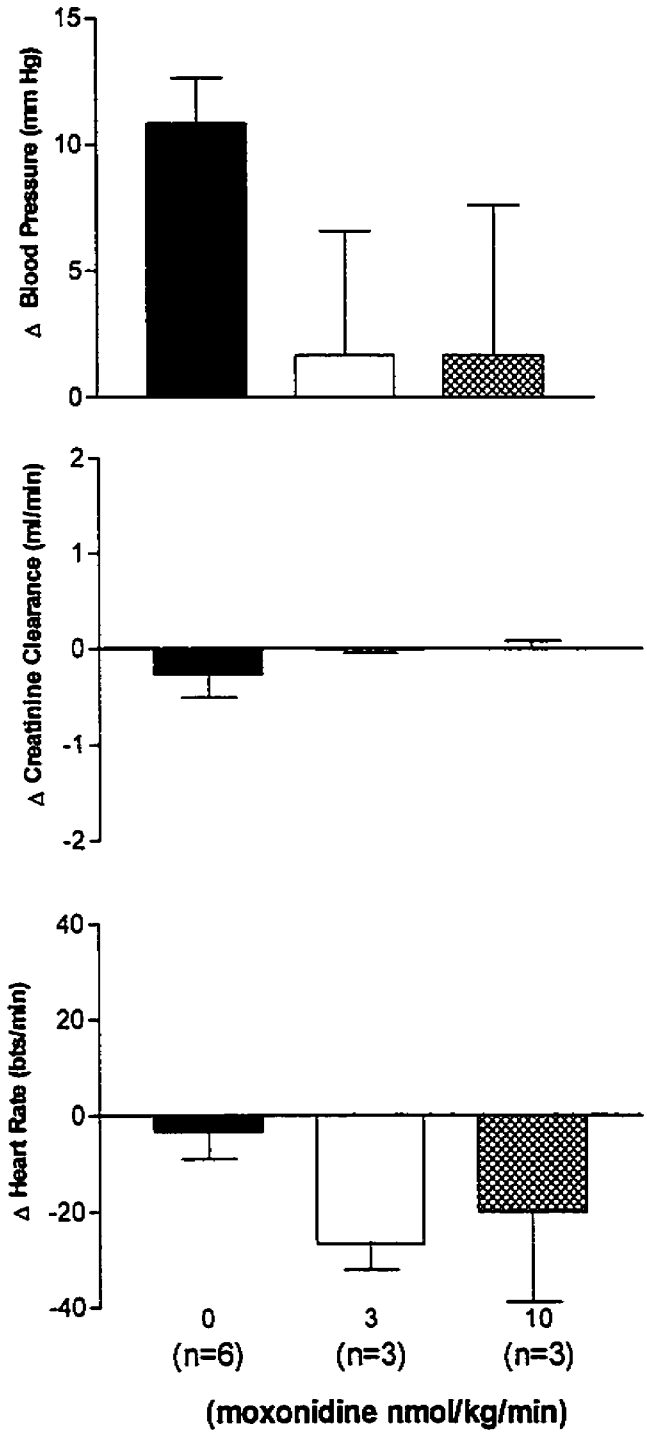

Figure 1.7a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or moxonidine (3, 10 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

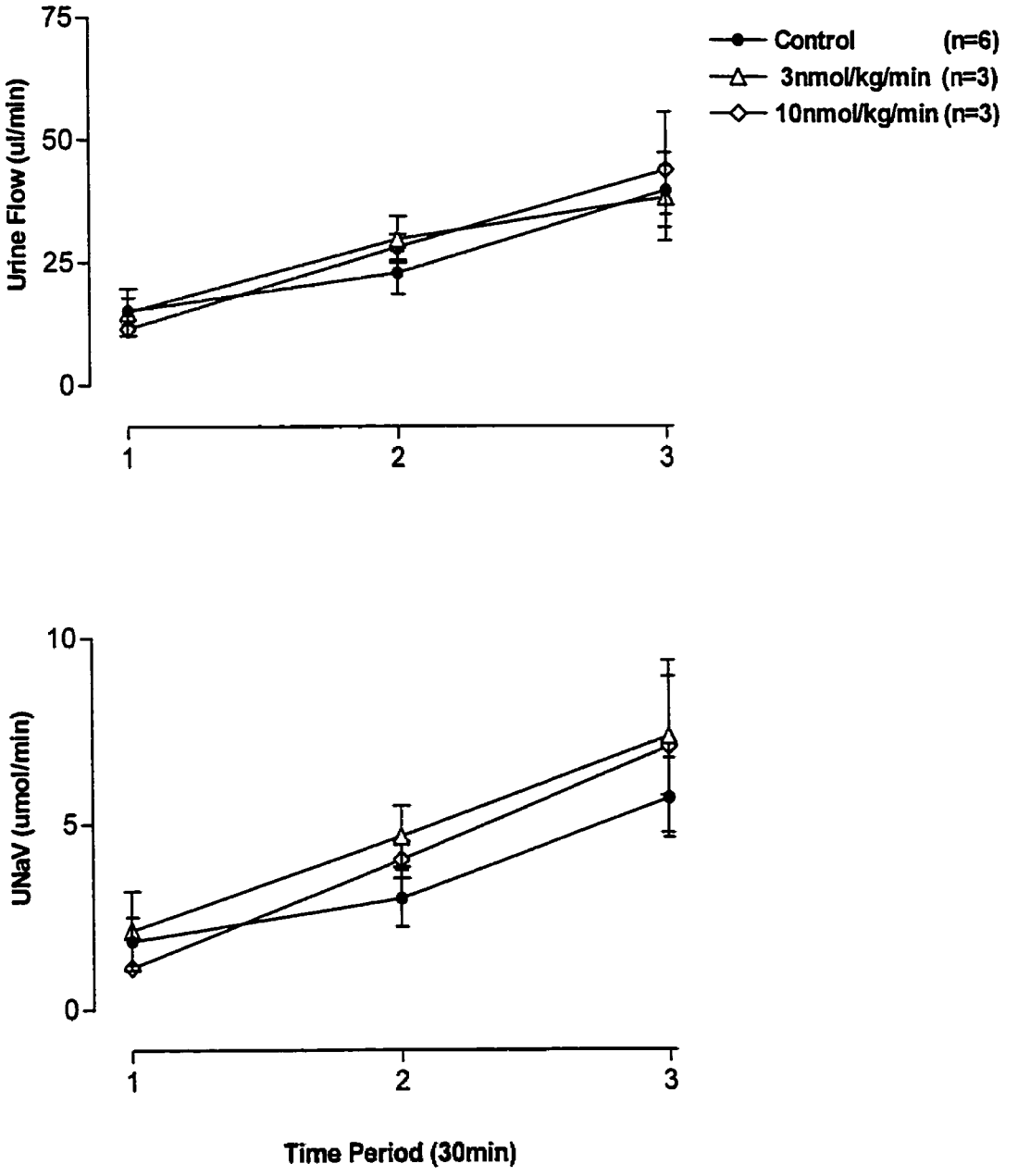

Figure 1.8. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or moxonidine (3 and 10 nmol/kg/min) on urine flow and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

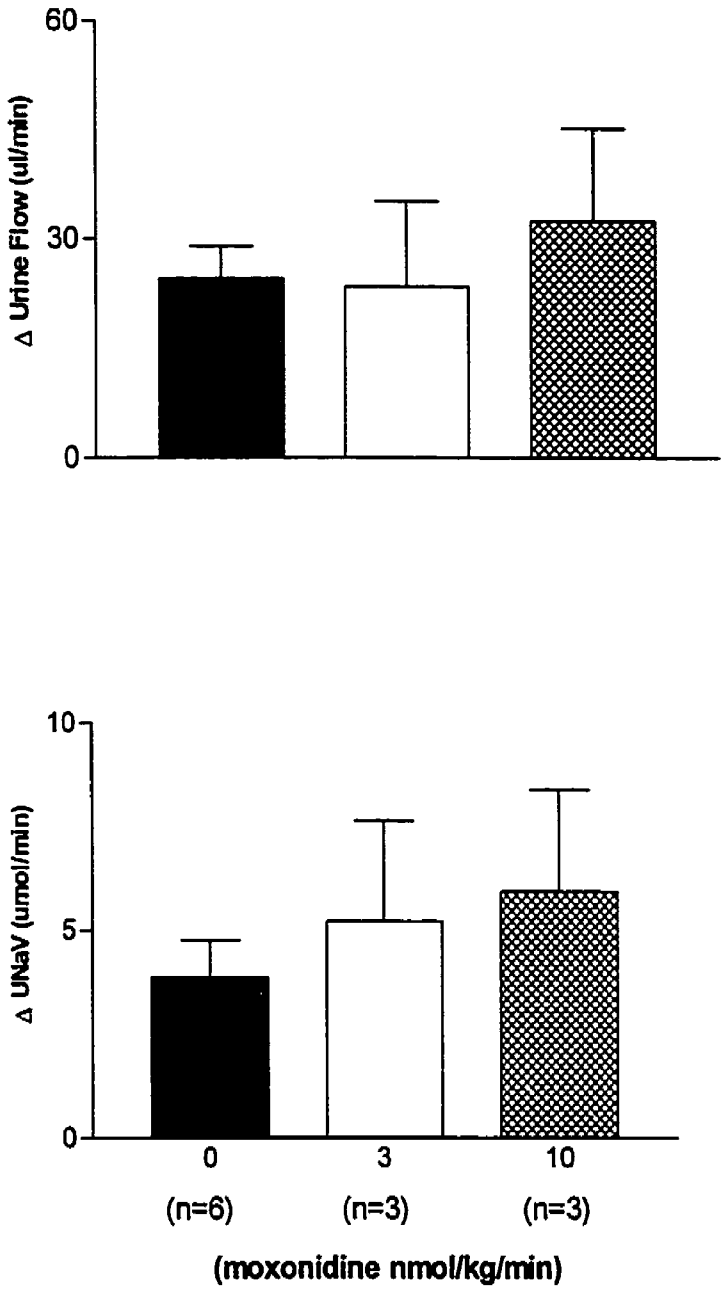

Figure 1.8a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or moxonidine (3, 10 nmol/kg/min) on urine flow and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

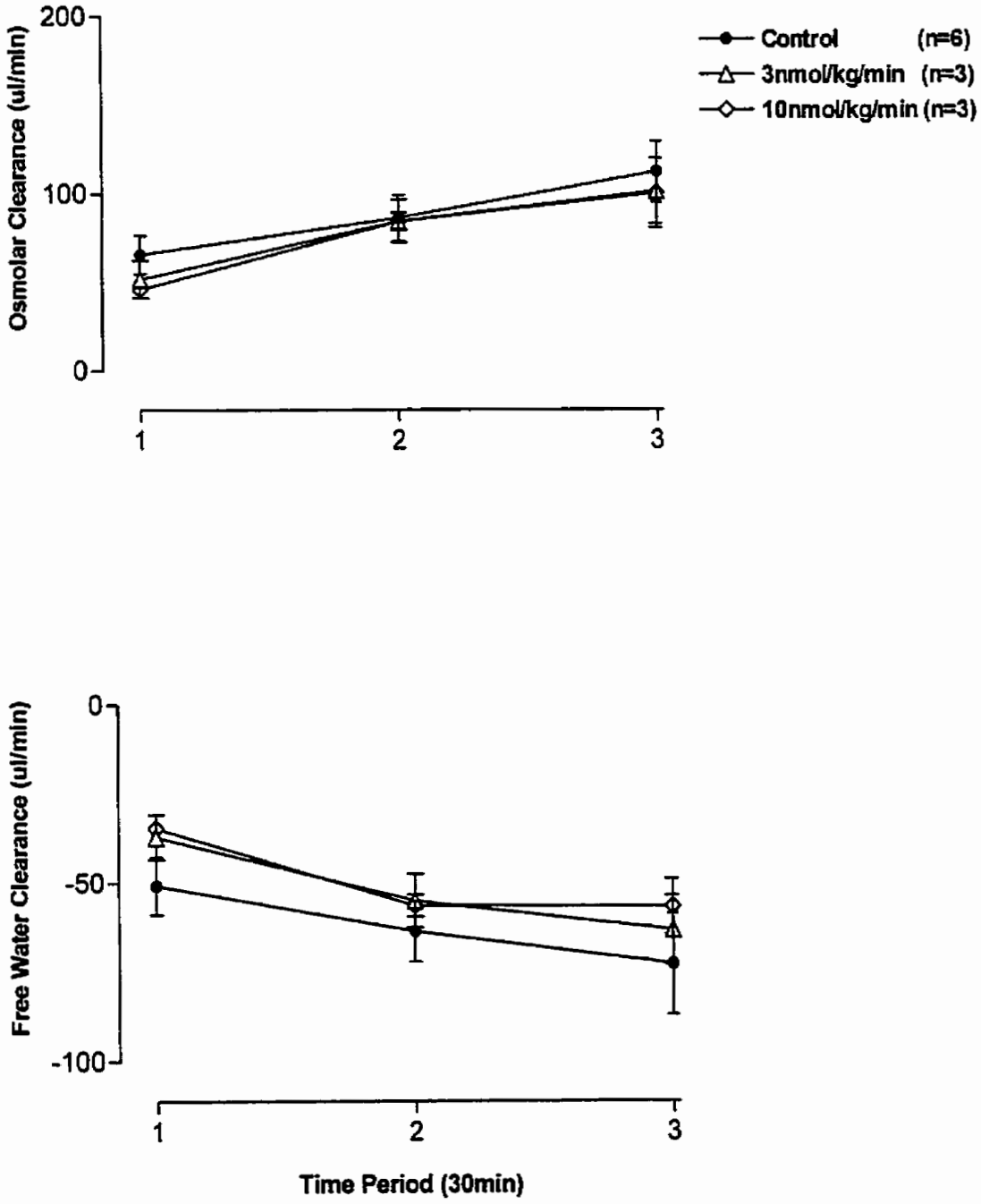

Figure 1.9. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or moxonidine (3 and 10 nmol/kg/min) on osmolar clearance and free water clearance in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

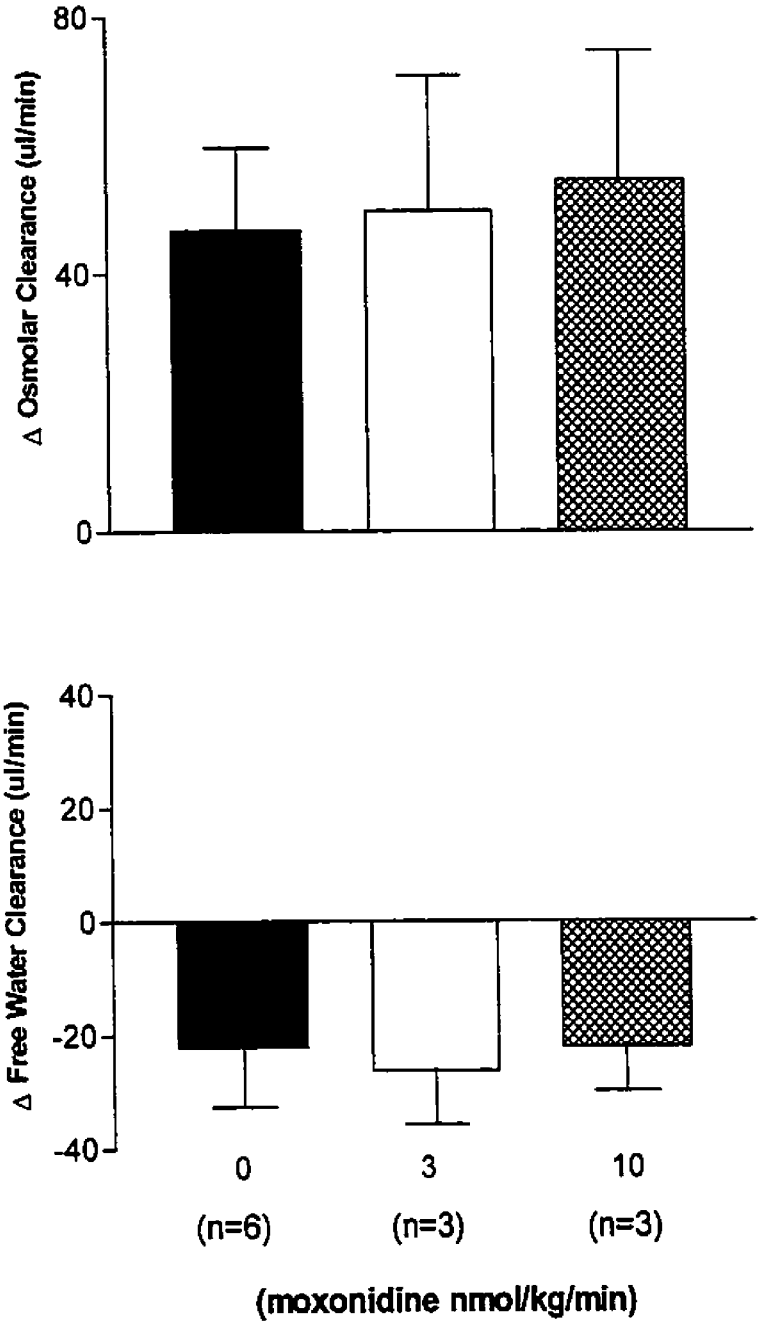

Figure 1.9a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or moxonidine (3, 10 nmol/kg/min) on osmolar clearance and free water clearance in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

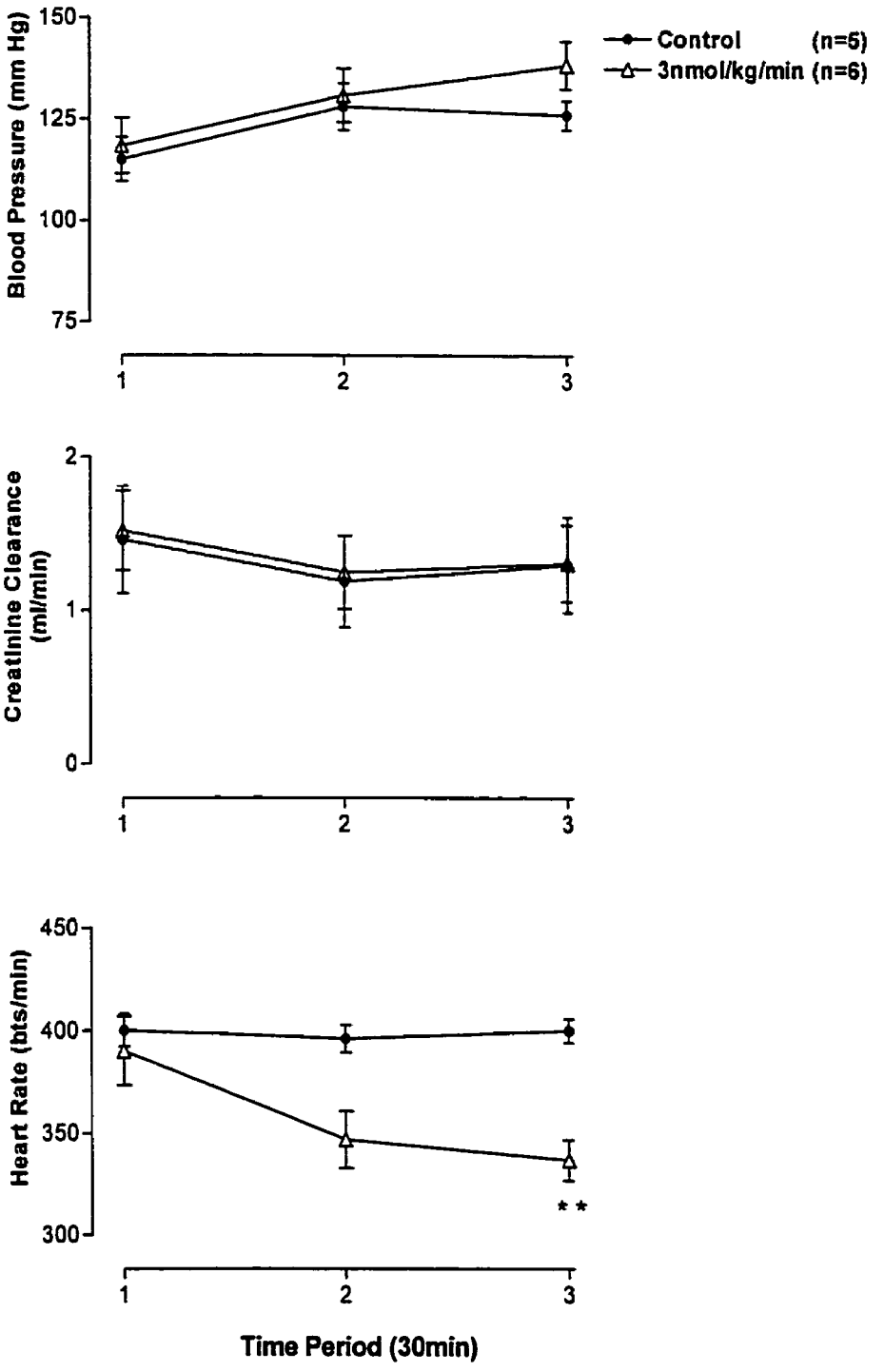

Figure 1.10. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or clonidine (3 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E.

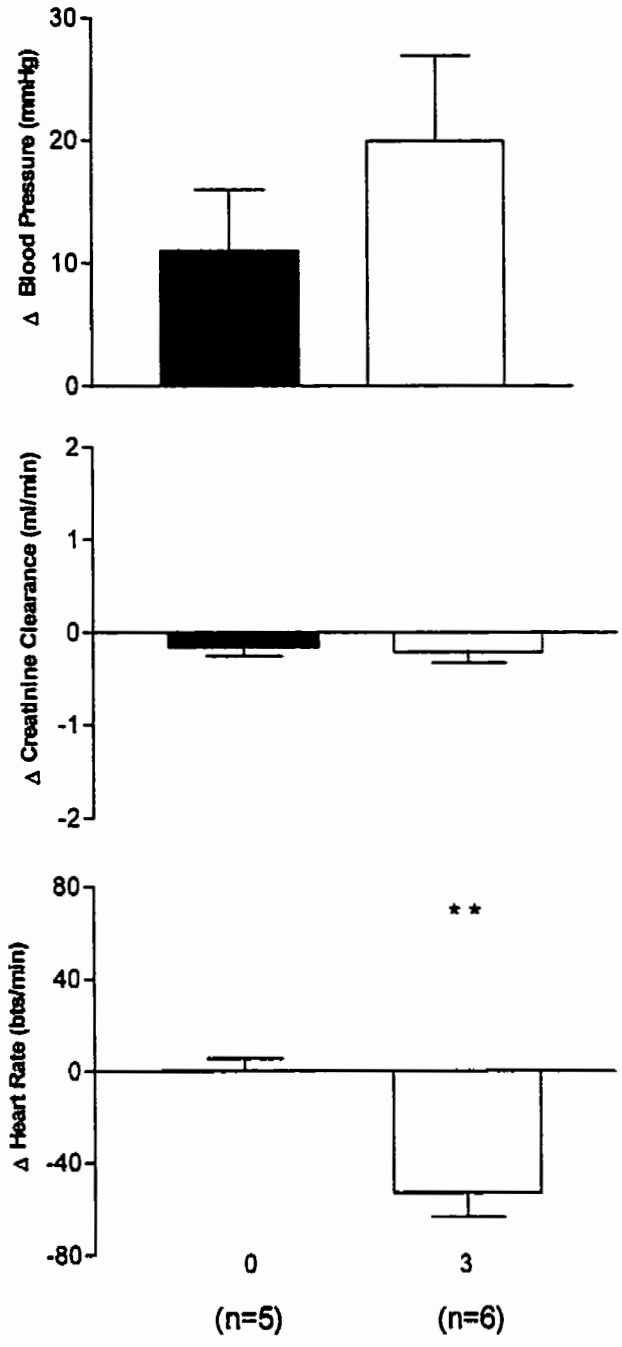

Figure 1.10a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or clonidine (3 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline value.

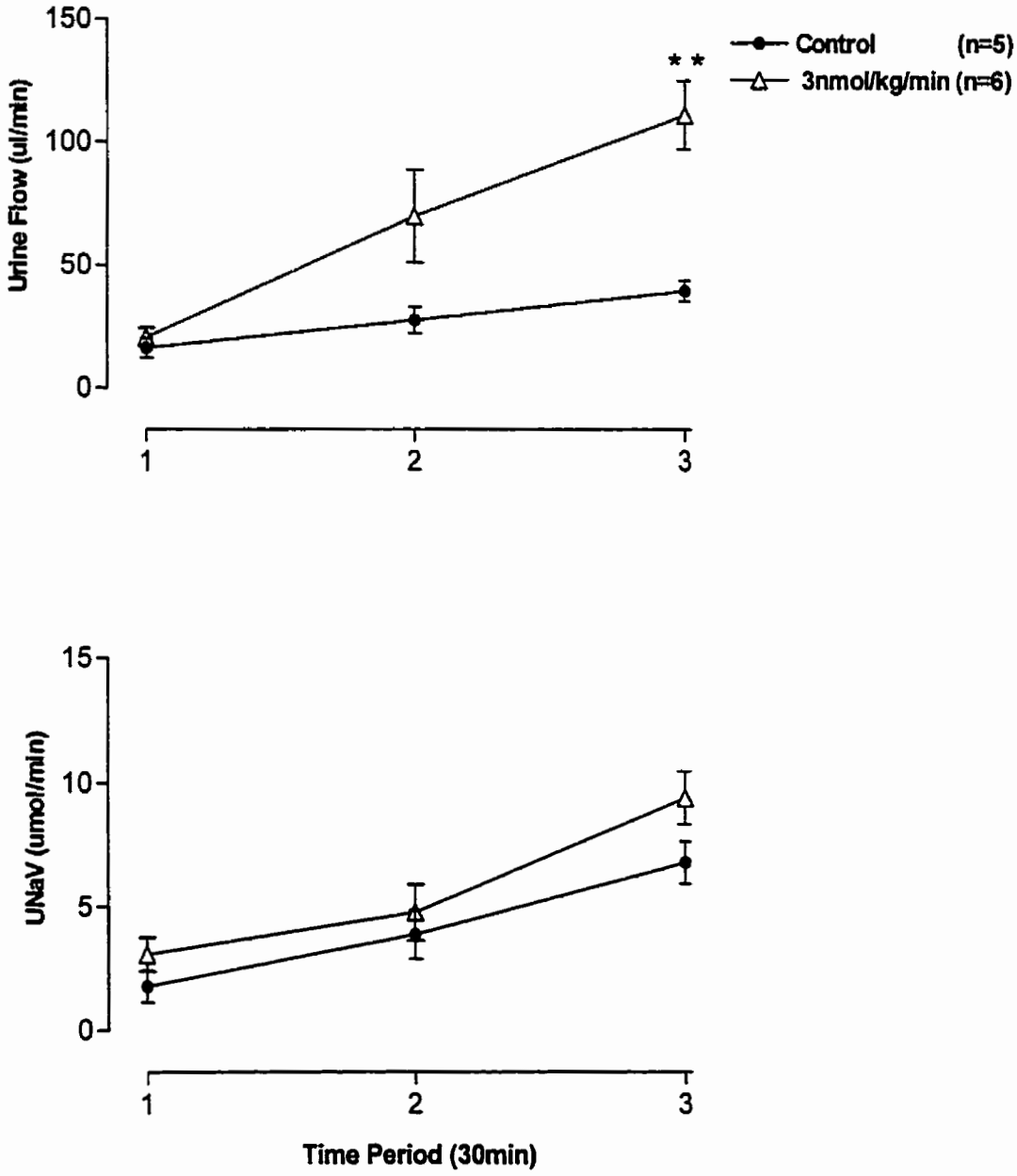

Figure 1.11. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or clonidine (3 nmol/kg/min) on urine flow and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E .

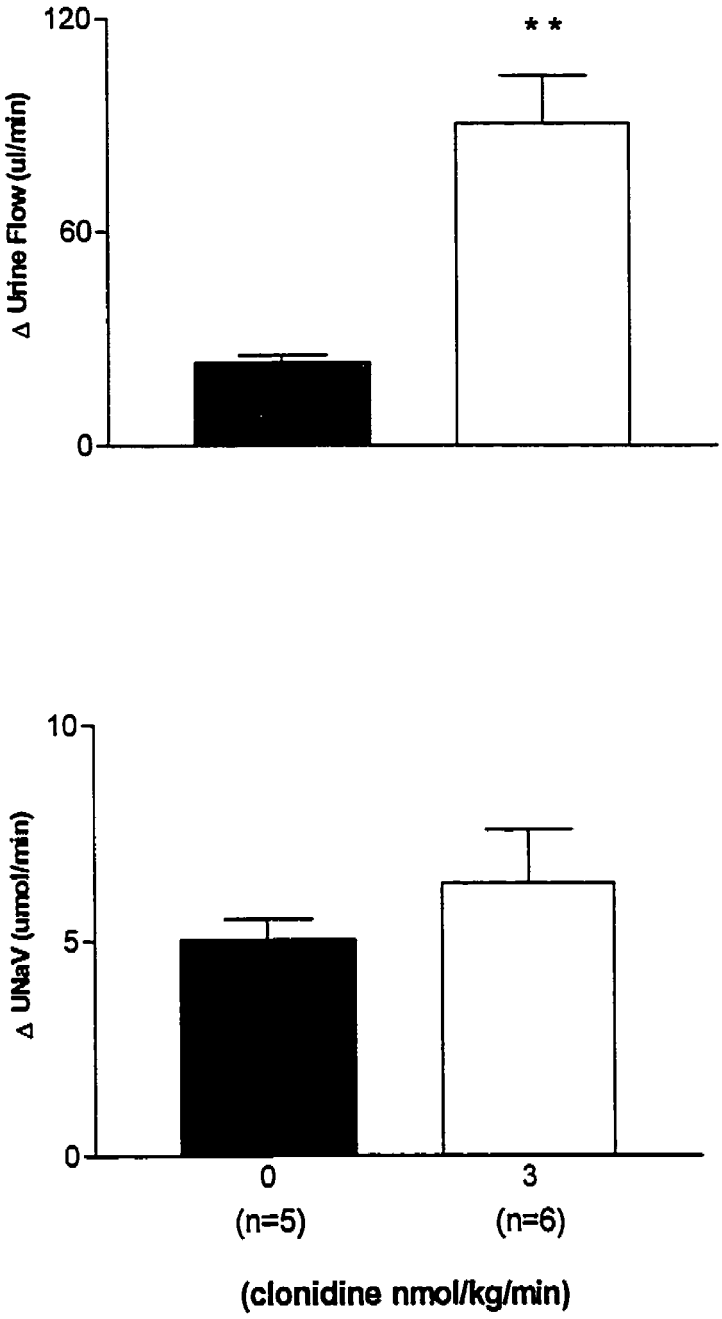

Figure 1.11a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or clonidine (3 nmol/kg/min) on urine flow and sodium excretion in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline value.

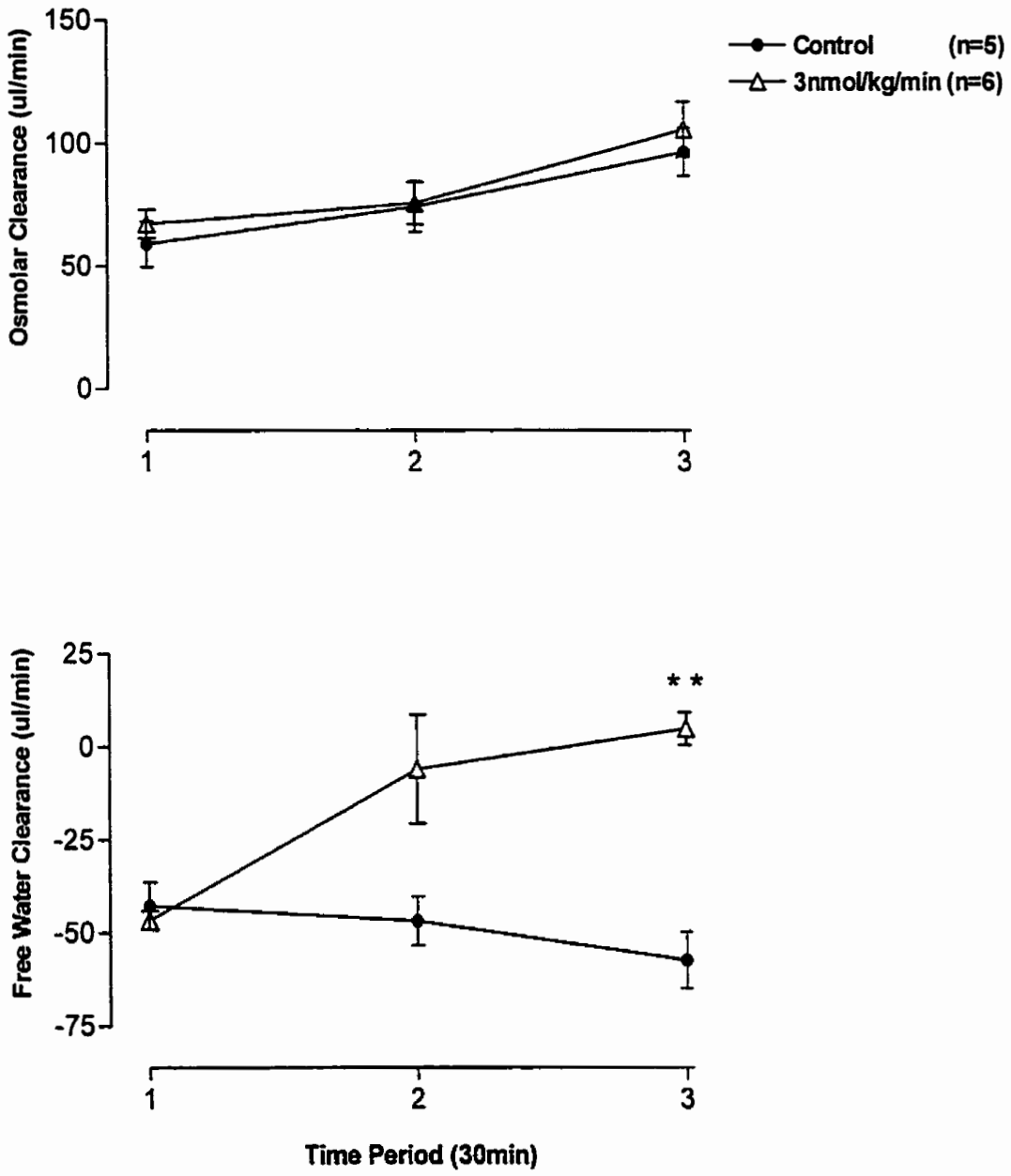

Figure 1.12. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or clonidine (3 nmol/kg/min) on osmolar clearance and free water clearance in male Sprague-Dawley rats. Data are presented as the mean ± S.E .

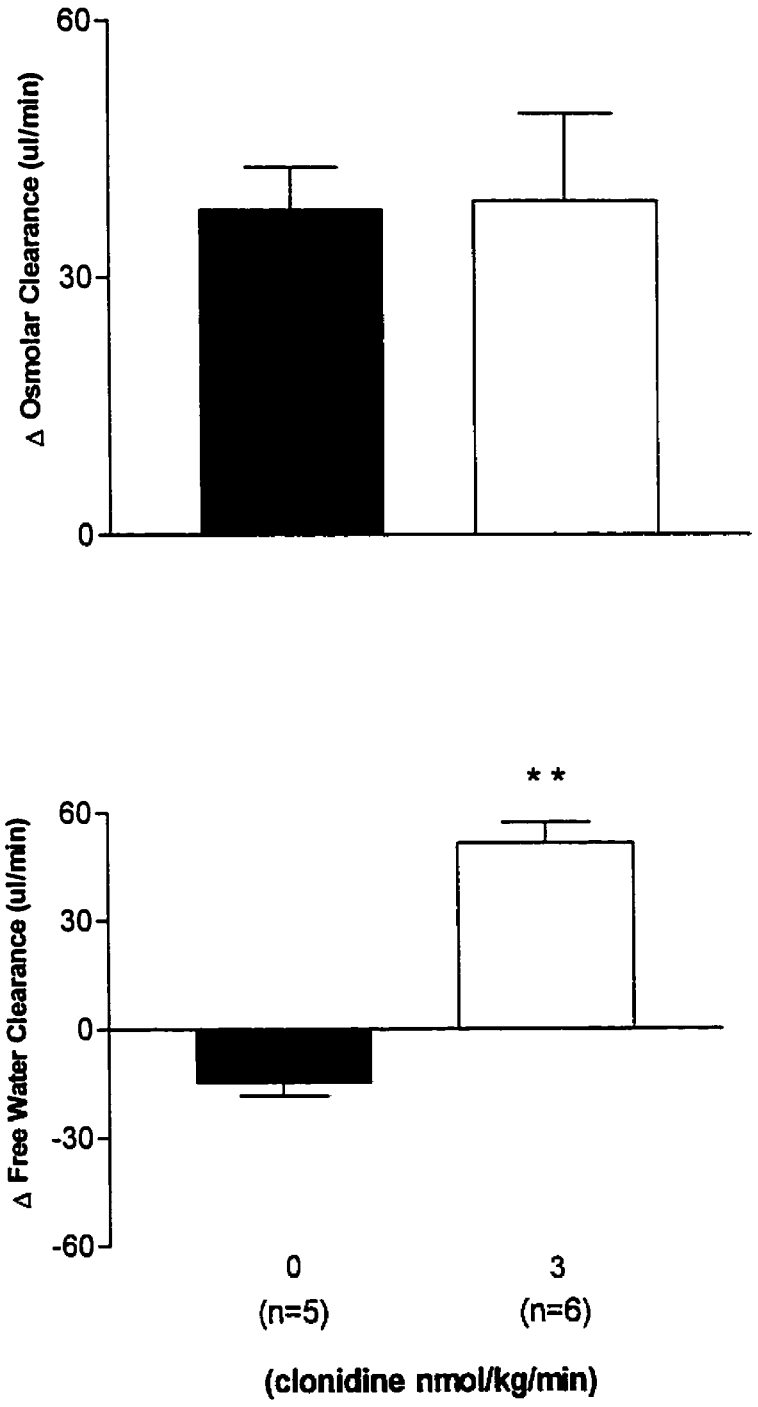

Figure 1.12a. Dose-response effects of intrarenal infusions of vehicle (0.9% saline) or clonidine (3 nmol/kg/min) on osmolar clearance and free water clearance in male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values.

Discussion

The effects of rilmenidine, as an antihypertensive drug, were previously attributed to activation of α_2–adrenoceptors (van-Zwieten, 1988). However, radioligand binding studies and in vivo experiments suggested that the effects of second generation antihypertensive drugs, including rilmenidine, may be due to activation of I_1–imidazoline receptors (Bousquet et al., 1984, 1992). Rilmenidine has at least a 3 fold greater affinity for the I_1–imidazoline receptor than for the α_2–adrenoceptor (Gomez et al., 1991; Ernsberger et al., 1992). It was concluded by direct measurement of sympathetic nerve activity and plasma catecholamines that rilmenidine lowered sympathetic tone in conscious rabbits (Szabo and Urban, 1995). In the present study, however, we searched for a dose of rilmenidine that would alter blood pressure and heart rate minimally. The doses of 10 and 30 nmol/kg/min were successful. It was also previously reported that rilmenidine increased urine flow rate and sodium excretion in a dose-dependent manner and that the increase in urine flow is associated with an osmolar clearance increase rather than an increase in free water clearance (Li, et al., 1994). An increase in free water clearance in our study may be due to α_{2b}–adrenoceptor activity since all I_1–imidazoline receptor agonists also have some affinity for the α_2–adrenoceptor (Hieble and Ruffolo, 1995).

As previously observed in our laboratory, guanfacine (3 nmol/kg/min), showed an increase in urine flow rate, sodium excretion and osmolar clearance but not an increase in free water clearance (Intengan and Smyth, 1997b).

As previously reported (Allan et al., 1993; Li, et al, 1994), in normotensive rats, moxonidine and rilmenidine produced a dose-dependent increase in urine flow, sodium excretion and osmolar clearance. However, in our study, moxonidine, at doses similar to those used previously (3 and 10 nmol/kg/min), failed to alter renal function. Since the drug was provided from an old stock we speculated that age of drug may have been a problem. Therefore we have not proceed further with moxonidine.

Clonidine, in the rat, increased urine flow rate by increasing free water clearance and osmolar clearance (Blandford and Smyth, 1988a; 1991). At the dose used in our study, clonidine failed to alter osmolar clearance. This may be due to the low dose of clonidine studied. It has been previously demonstrated that low doses of clonidine only increase free water clearance (Blandford and Smyth, 1988a). A higher dose of clonidine may be required to alter osmolar clearance. It might suggest the existence of two distinct receptors and/or sites involved in this response (Blandford and Smyth, 1989). Since in our study a low dose of 3 nmol/kg/min of clonidine produced an increase in blood pressure and decrease in heart rate, we did not proceed with higher doses of this drug.

Based on these studies, we selected rilmenidine (10 nmol/kg/min) and guanfacine (10 nmol/kg/min) to further investigate the effects of renal denervation on the renal actions of I_1–imidazoline receptor and α_2–adrenoceptor agonists respectively.

Renal Effects of Rilmenidine, Guanfacine and Furosemide in Sham and Acute Renal Denervated Rats

INTRODUCTION

As discussed in our preliminary study, there are a number of reports from our laboratory as well as others that investigated the natriuretic response to stimulation of I_1-imidazoline receptors and α_2–adrenoceptors (Blandford and Smyth, 1988a; 1988b; Allan et al., 1993; Li et al., 1994; Intengan and Smyth, 1996, 1997a; 1997b; Hohage et al., 1997a; 1997b; Gellai and Ruffolo, 1987). It has been also documented that a decrease in the peripheral sympathetic nerve activity plays a crucial role in regulation of blood pressure (Head, 1995; Schafer et al., 1995) and in producing a natriuretic response (Smyth and Penner, 1999).

Functional studies and radioligand binding experiments have identified imidazoline receptors different from α_2–adrenoceptors (Bousquet et al., 1984, 1992; Boyajan et al., 1987; Allan et al., 1993; Intengan and Smyth, 1996, 1997a; 1997b). Imidazoline receptors have been located in the kidney as well as in the central nervous system (Ernsberger et al., 1992). It has been shown that imidazoline receptors in the central nervous system were involved in the lowering of the sympathetic nerve activity (Bousquet et al., 1984; Ernsberger et al., 1987). Thus the kidney may be involved in regulation of the blood pressure secondary to a decrease in sympathetic nerve activity (DiBona and Kopp, 1997; Smyth and Penner, 1999). In the General Introduction, we presented a series of studies from our laboratory that had compared the effects of the central and peripheral

administration of the I_1–imidazoline receptor agonist, moxonidine (Penner and Smyth, 1994a; 1994b; 1995; 1997; Allan et al., 1993; Smyth and Penner, 1998). It appears that the imidazoline receptors located centrally as well as peripherally increased sodium excretion when stimulated. Intact renal nerves appeared to be important for the natriuretic effect of centrally administered moxonidine. Thus it was proposed that the effect of peripheral administration of moxonidine may be due to a direct tubular effect. Following denervation (Penner and Smyth, 1995) sodium excretion and osmolar clearance were completely attenuated compared to the sham operated animals, indicating the importance of an intact renal nerve. However, the increase in free water clearance was at a level similar to that observed before denervation. Although the mechanism by which these centraly acting compounds increased free water clearance has not been determined, this effect appeared to be independent from the renal nerves. Penner and Smyth (1997) examined the effects of another I_1–imidazoline receptor agonist, rilmenidine, in denervated rats and obtained similar results.

Kline and Cechetto, (1993) reported that renal denervation completely attenuated the natriuretic response to an intravenous infusion of rilmenidine. These findings were not consistent with the studies from our laboratory. Experimental and surgical procedures that Kline and Cechetto utilized in this study were different from the one that utilized in our present study. In the present study, we investigated the effects of an intravenous infusion of rilmenidine in renal denervated rats using the experimental and surgical procedure previously documented from our laboratory (Penner and Smyth, 1997).

Methods

The general procedure has been described in detail previously in the "General experimental preparation" section. Male Sprague-Dawley rats had undergone unilateral nephrectomy under ether anesthesia and were allowed to recover for seven to ten days. On the experimental day, animals were anesthetized with pentobarbital, then tracheotomized and allowed to breath spontaneously. The left carotid artery was cannulated for heart rate and blood pressure monitoring. The left jugular vein was cannulated with two separate catheters. The first catheter (PE-160) was advanced directly into the jugular vein for the continuous infusion of saline (0.9% NaCl) at 97 μl/min (Sage syringe infusion pump) to produce a moderate diuresis. This also allowed for the administration of additional anesthetic as required. The second catheter (PE-50) was inserted into the first line with a 21-gauge needle for the infusion of saline vehicle or the study drug at 3.4 μl/min using the Harvard sage infusion pump (Harvard Apparatus CO. Dover, Mass. Model NO 600-000). The left kidney was exposed by a flank incision and the ureter was cannulated for the collection of urine. The kidney was denervated surgically by cutting and stripping all visible nerves from the renal artery and by painting the renal artery and vein with 10% phenol (Sigma Chemical Company St. Louis, MO, USA) in 95% ethyl alcohol (Commercial Alcohols Inc., Brampton, Ontario) .Control sham animals were treated similar with the exception that the nerves were not cut and the renal

artery and vein were not painted with 10% phenol. Following a 45-minute stabilization period, the intravenous infusion of saline (97 μl/min) was initiated and continued to the end of experiment. After the first 30 minute control urine collection, rilmenidine (10 nmol/kg/min), guanfacine (10 nmol/kg/min), furosemide (1.67 μg/kg/min for total dose of 0.1 mg/kg over one hour, Sabex) or saline (0.9% NaCl) was infused constantly during the second and third collection.

The results have been presented as the differences between the first (control) and third (treatment) urine collection. The data were presented as the mean ± standard error and analyzed by repeated measures analysis of variance (ANOVA) followed by The GLM Procedure, Least Squares Means, to identify significant differences. Significance is denoted in figures by *, which represents a $p<0.05$ and **, which represents $p<0.01$ compared to control group.

Results

The results have been presented as the difference between the baseline (first) and final (third) collection period. By presenting the delta values, the quantity of differences between study groups was emphasized. Baseline values (first collection period) before any study drug or saline was infused have been presented (Table 2.1, 2.2 and 2.3). No significant differences were found in baseline levels compared to data from the control groups. However, urine flow rate and sodium excretion and osmolar clearance were increased in the denervated control group as compared to the sham group for the first urine collection (Table 2.2).

Renal Effects of Rilmenidine in Sham vs. Acute Denervated Rats

The dose of rilmenidine (10 nmol/kg/min) that altered renal function with minimal changes in blood pressure and heart rate was selected based on the previous section. Blood pressure decreased approximately 10 mmHg in sham rats and heart rate decreased approximately 30 beats/min in both sham and denervated rats. Creatinine clearance remained at similar levels before and after denervation (Figure 2.1).

In sham treated rats, rilmenidine (10 nmol/kg/min) increased urine flow rate and sodium excretion although the increase in sodium excretion failed to reach significance ($p=0.068$). Following denervation, rilmenidine was associated with a slight decrease in urine flow rate and a significant reduction sodium

excretion (Figures 2.2). Also, in rilmenidine treated sham rats, osmolar clearance, but not free water clearance was increased. In denervated animals, rilmenidine was associated with a decrease in osmolar clearance and an increase in the free water clearance by the kidney (Figure 2.3).

Renal Effects of Guanfacine in Sham vs. Acute Denervated Rats

Similar to rilmenidine, the dose of 10 nmol/kg/min of guanfacine was selected based on the previous section. Following intravenous infusion of guanfacine, blood pressure and creatinine clearance remained at similar levels in both, sham and denervated animals as that of the control groups. Nevertheless, a decrease in heart rate of approximately 40 beats/min was found in both sham and denervated rats (Figure 2.4). In sham animals guanfacine increased urine flow, sodium excretion and osmolar clearance (Figures 2.5 and 2.6). Following denervation, guanfacine was still associated with an increase in urine flow rate. This may have been secondary to an increase in free water clearance which approached a level of significance. An increase in osmolar clearance was not observed following denervation (Figures 2.5 and 2.6).

Renal Effects of Furosemide in Sham vs. Acute Denervated Rats

The dose of furosemide (0.1 mg/kg) was selected based on the previous experiments in our laboratory that altered renal function with minimum changes in blood pressure and heart rate. Furosemide decreased creatinine clearance in sham and denervated rats (Figure 2.7). Furosemide increased urine flow, sodium excretion and osmolar clearance (Figures 2.8 and 2.9). Following denervation, furosemide significantly increased urine flow, sodium excretion and

osmolar clearance. The increase in the denervated animals was similar to the increase observed in sham animals.

	sham control (n = 6)	sham rilmenidine (n = 6)	denervated control (n = 6)	denervated rilmenidine (n = 6)
Blood pressure (mm Hg)	123 ± 3	120 ± 4	117 ± 5	114 ± 4
Creatinine clearance (ml/min)	1.4 ± 0.1	1.5 ± 0.3	1.1 ± 0.1	1.3 ± 0.1
Heart rate (bts/min)	380 ± 5	373 ± 10	387 ± 4	387± 6
Urine volume (ul/min)	11 ± 0	10 ± 2	12 ± 2	11 ± 3
Sodium excretion (umol/min)	0.7 ± 0.1	0.9 ± 0.3	1.2 ± 0.3	1.5 ± 0.8
Osmolar clearance (ul/min)	49 ± 3	47 ± 7	44 ± 5	50 ± 10
Free water clearance (ul/min)	-38 ± 3	-37 ± 5	-32 ± 4	-39 ± 7

Table 2.1. Baseline values obtained from the first urine collection before intravenous infusion of vehicle (0.9% saline) or rilmenidine (10 nmol/kg/min) in sham and denervated male Sprague-Dawley rats. Results are presented as the mean ± S.E.

	sham control (n = 6)	sham guanfacine (n = 6)	denervated control (n = 6)	denervated guanfacine (n = 6)
Blood pressure (mm Hg)	117 ± 2	118 ± 3	115 ± 2	114 ± 3
Creatinine clearance (ml/min)	1.7 ± 0.1	1.3 ± 0.1	1.5 ± 0.2	1.7 ± 0.2
Heart rate (bts/min)	377 ± 7	387 ± 4	380 ± 0	377 ± 7
Urine volume (ul/min)	9 ± 1	12 ± 3	23 ± 2*	23 ± 3
Sodium excretion (umol/min)	1.2 ± 0.3	1.6 ± 0.5	3.7 ± 0.9*	3.3 ± 0.7
Osmolar clearance (ul/min)	54 ± 4	53 ± 7	81 ± 11	65 ± 12
Free water clearance (ul/min)	-44 ± 3	-41 ± 5	-58 ± 9	-42 ± 10

Table 2.2. Baseline values obtained from the first urine collection before intravenous infusion of vehicle (0.9% saline) or guanfacine (10 nmol/kg/min) in sham and denervated male Sprague-Dawley rats. Results are presented as the mean ± S.E. (* denotes $p<0.05$ for the denervated control group versus the sham control)

	sham control (n=6)	sham furosemide (n=6)	denervated control (n=6)	denervated furosemide (n=6)
Blood pressure (mm Hg)	117 ± 2	125 ± 3	115 ± 2	118 ± 3
Creatinine clearance (ml/min)	1.7 ± 0.1	2.1 ± 0.2	1.5 ± 0.2	2.9 ± 0.5
Heart rate (bts/min)	377 ± 7	410 ± 8	380 ± 0	390 ± 9
Urine volume (ul/min)	9 ± 1	10 ± 1	23 ± 2	20 ± 3
Sodium excretion (umol/min)	1.2 ± 0.3	1.3 ± 0.3	3.7 ± 0.9	2.4 ± 0.5
Osmolar clearance (ul/min)	54± 4	58 ± 4	81 ± 11	80 ± 8
Free water clearance (ul/min)	-44 ± 3	-48 ± 3	-58 ± 9	-60 ± 5

Table 2.3 Baseline values obtained from the first urine collection before intravenous infusion of vehicle (0.9% saline) or furosemide (0.1 mg/kg) in sham and denervated male Sprague-Dawley rats. Results are presented as the mean ± S.E.

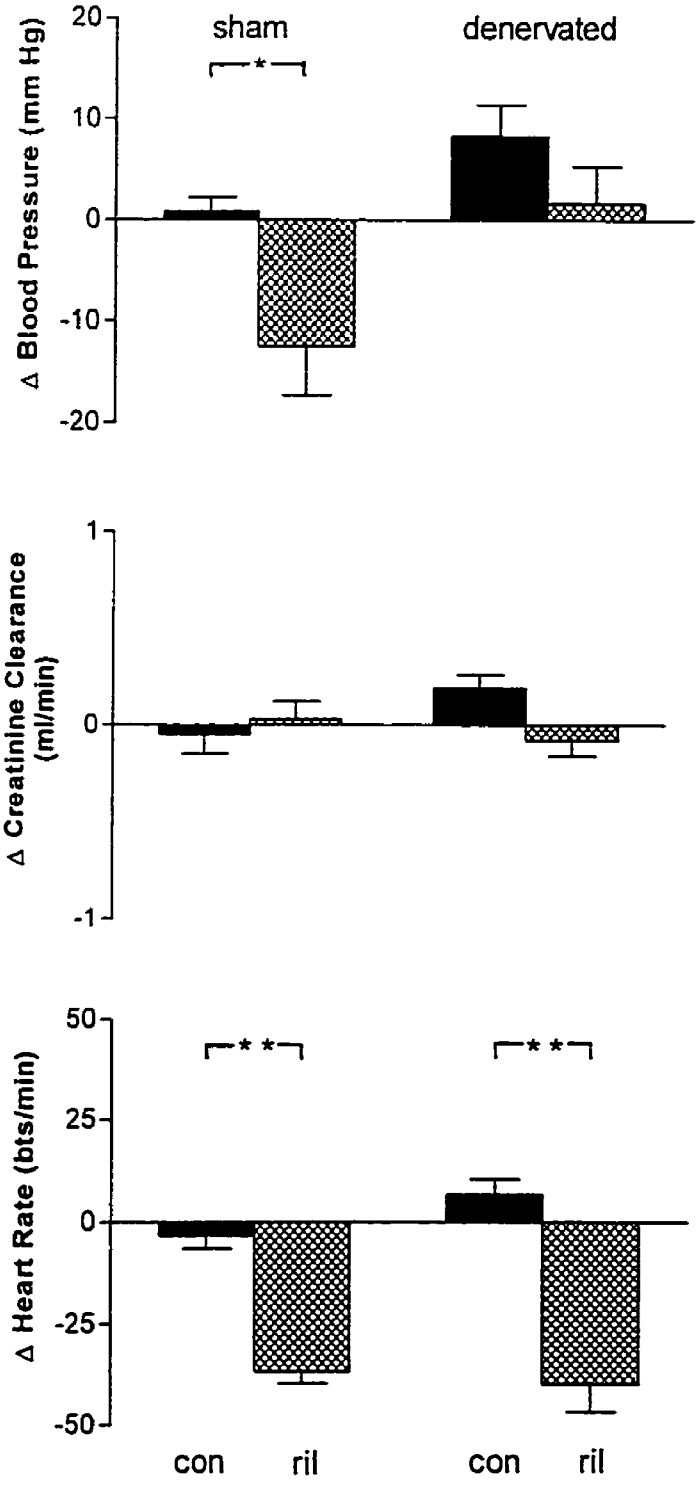

Figure 2.1. Effects of intravenous infusions of vehicle (0.9% saline) or rilmenidine (10 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in sham and denervated male Sprague - Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values of six experiments.

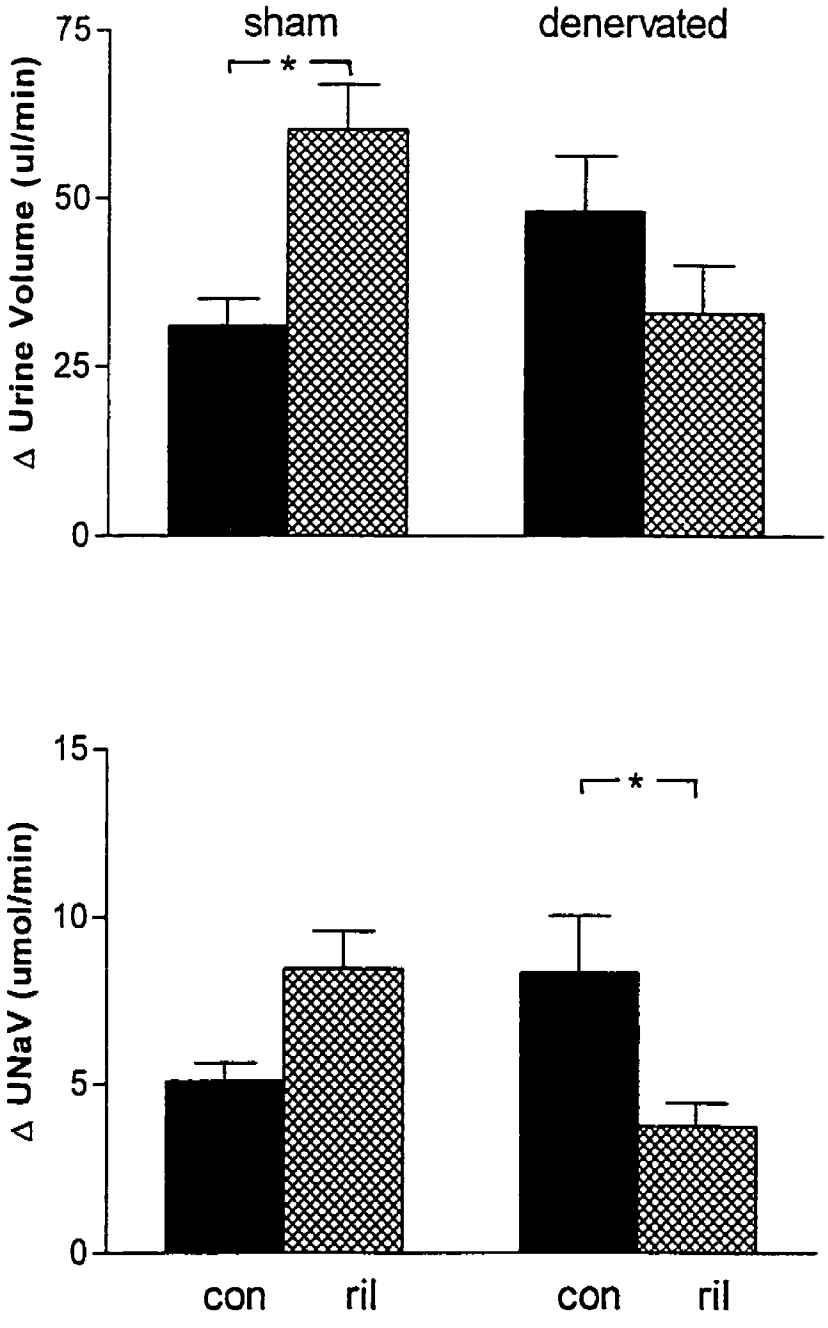

Figure 2.2. Effects of intravenous infusions of vehicle (0.9% saline) or rilmenidine (10 nmol/kg/min) on urine flow and sodium excretion in sham and denervated male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values of six experiments.

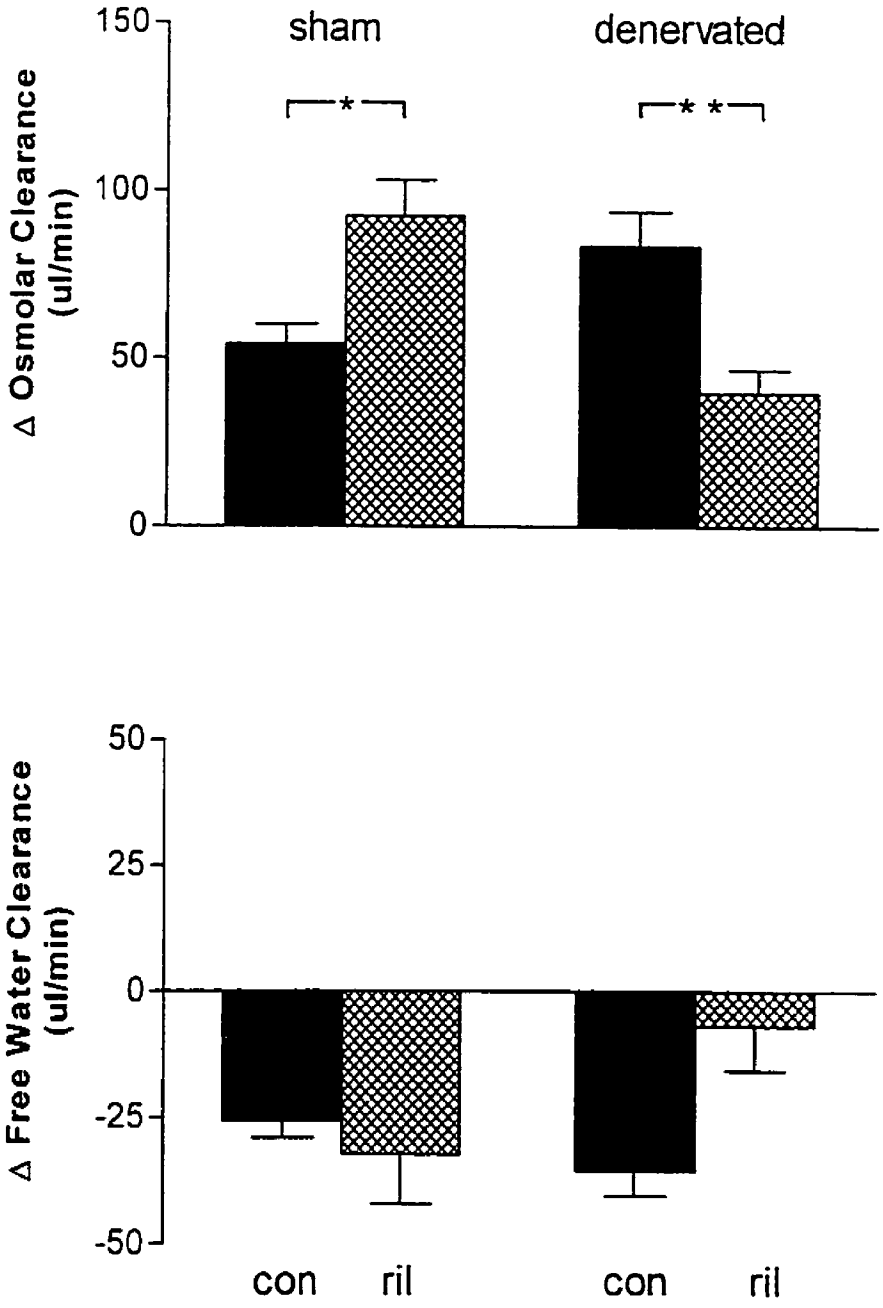

Figure 2.3. Effects of intravenous infusions of vehicle (0.9% saline) or rilmenidine (10 nmol/kg/min) on osmolar clearance and free water in sham and denervated male Sprague - Dawley rats. Data are presented as the mean ± S.E of the mean of the difference between the final collection and baseline values of six experiments.

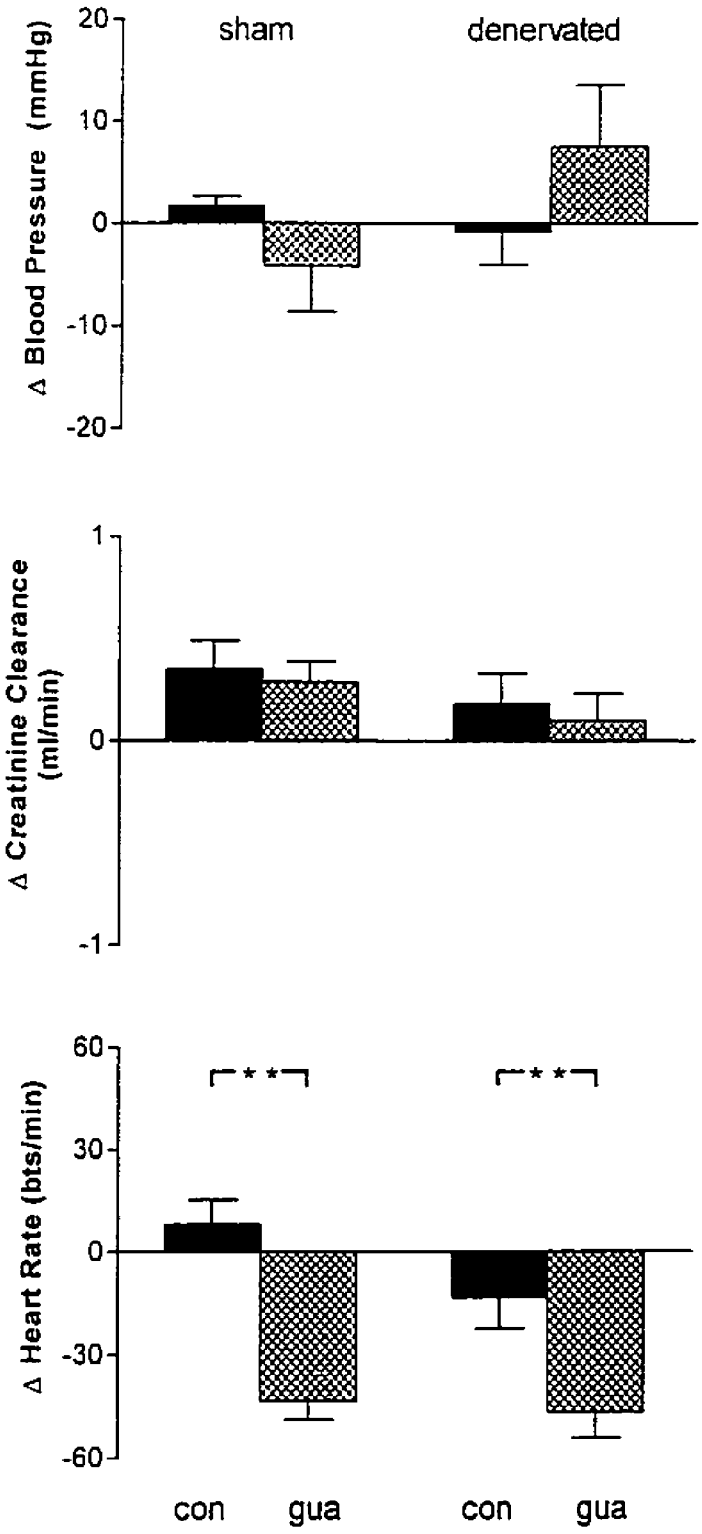

Figure 2.4. Effects of intravenous infusions of vehicle (0.9% saline) or guanfacine (10 nmol/kg/min) on blood pressure, creatinine clearance and heart rate in sham and denervated male Sprague - Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values of six experiments.

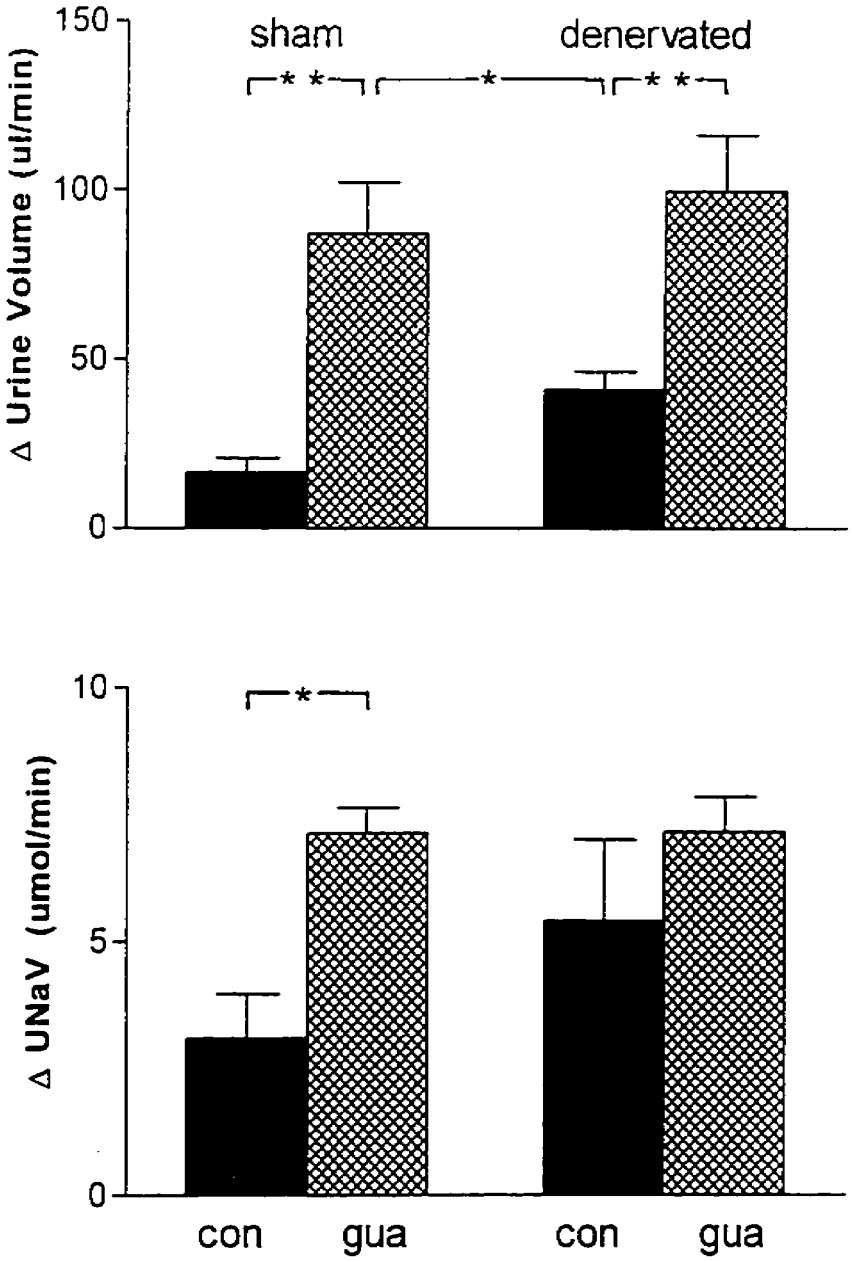

Figure 2.5. Effects of intravenous infusions of vehicle (0.9% saline) or guanfacine (10 nmol/kg/min) on urine flow and sodium excretion in sham and denervated male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values of six experiments.

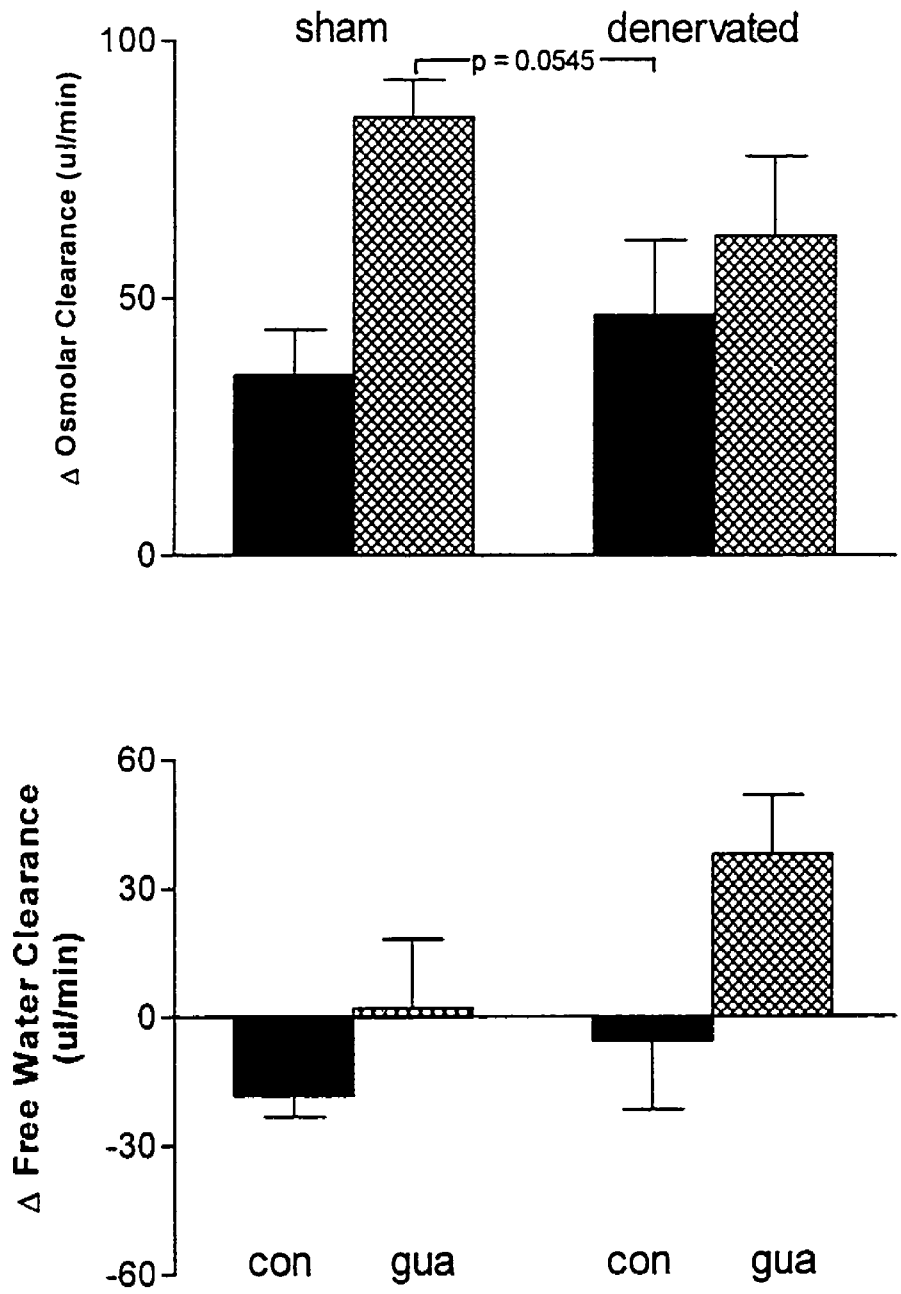

Figure 2.6. Effects of intravenous infusions of vehicle (0.9% saline) or guanfacine (10 nmol/kg/min) on osmolar clearance and free water in sham and denervated male Sprague - Dawley rats. Data are presented as the mean ± S.E of the mean of the difference between the final collection and baseline values of six experiments.

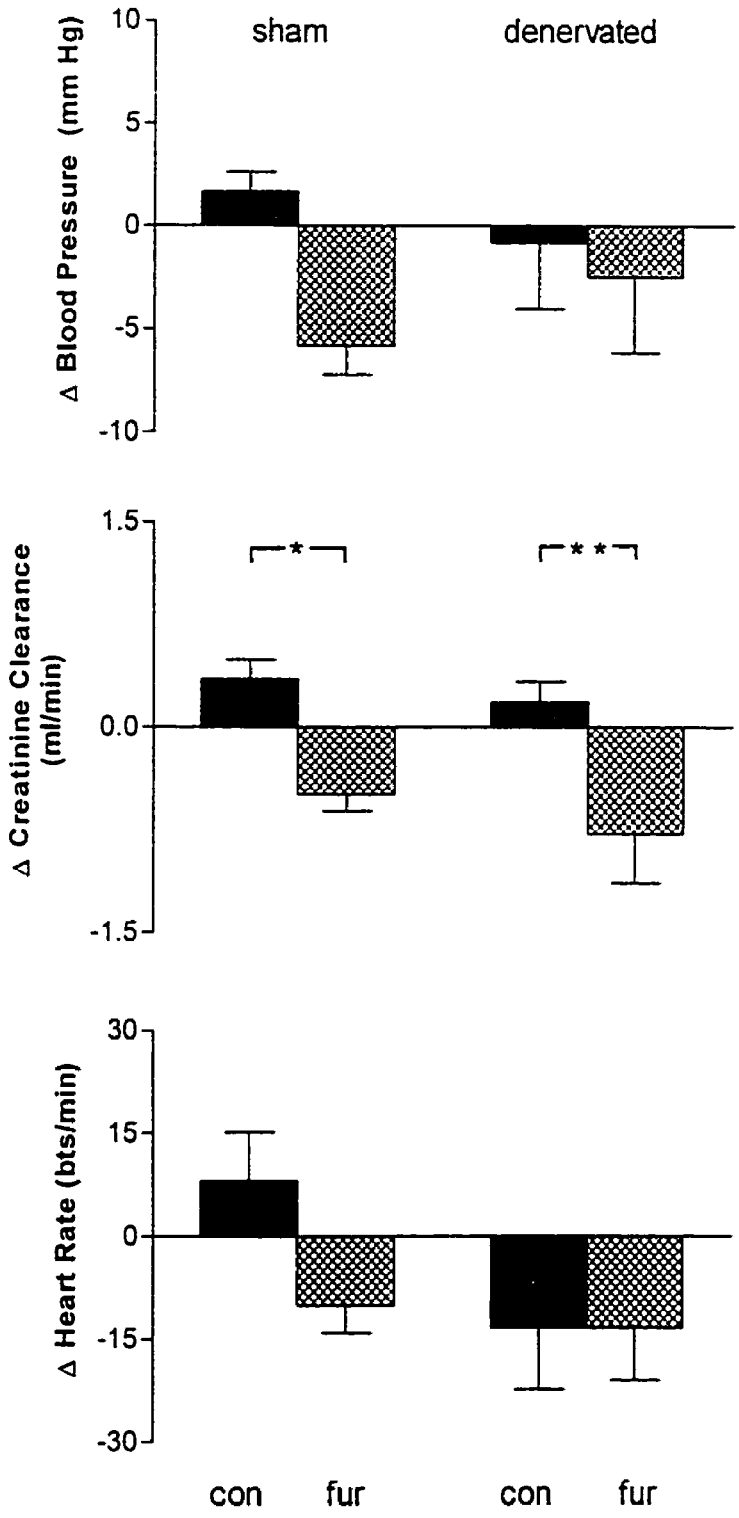

Figure 2.7. Effects of intravenous infusions of vehicle (0.9% saline) or furosemide (0.1 mg/kg) on blood pressure, creatinine clearance and heart rate in sham and denervated male Sprague - Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values of six experiments.

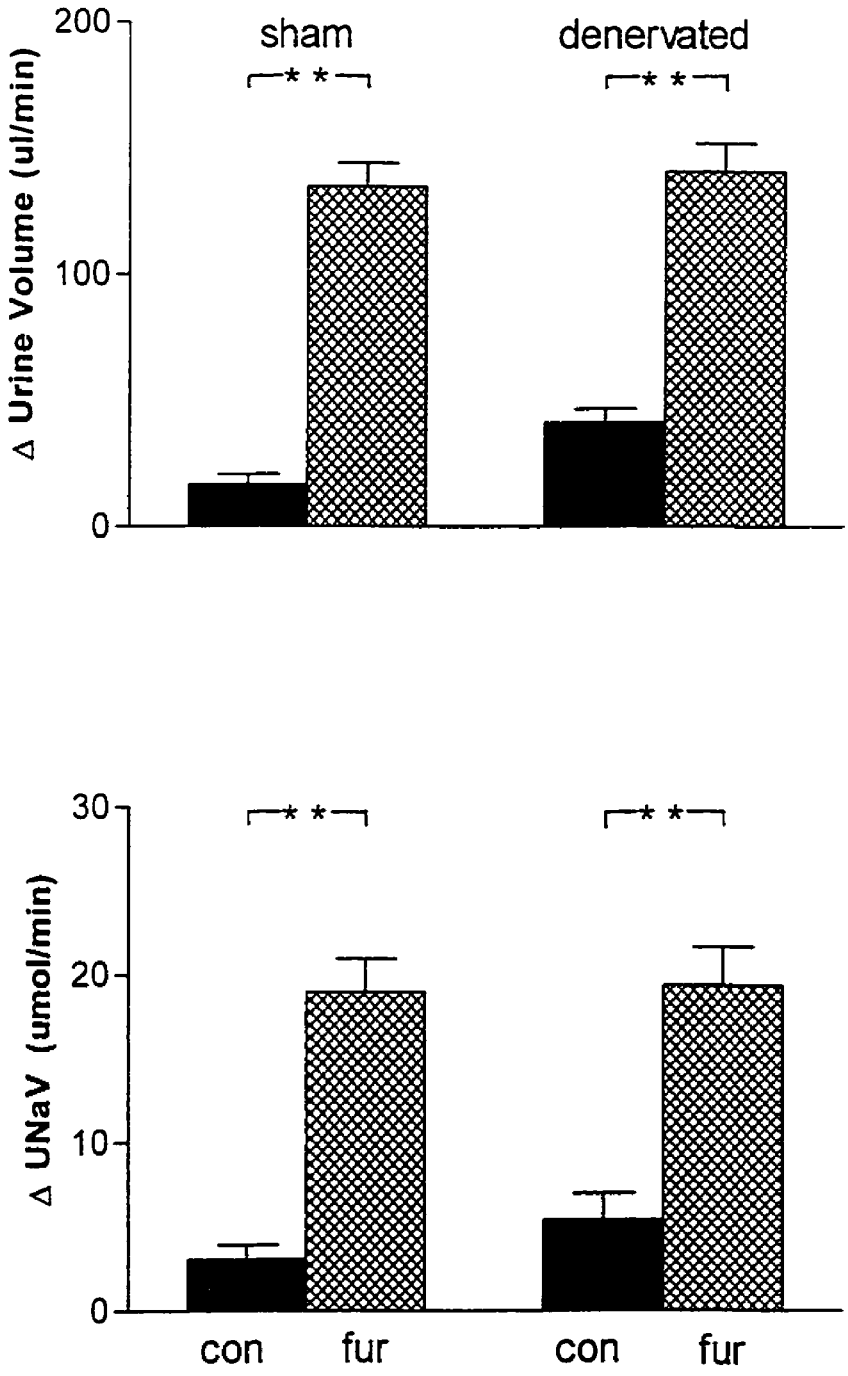

Figure 2.8. Effects of intravenous infusions of vehicle (0.9% saline) or furosemide (0.1 mg/kg) on urine flow and sodium excretion in sham and denervated male Sprague-Dawley rats. Data are presented as the mean ± S.E. of the mean of the difference between the final collection and baseline values of six experiments.

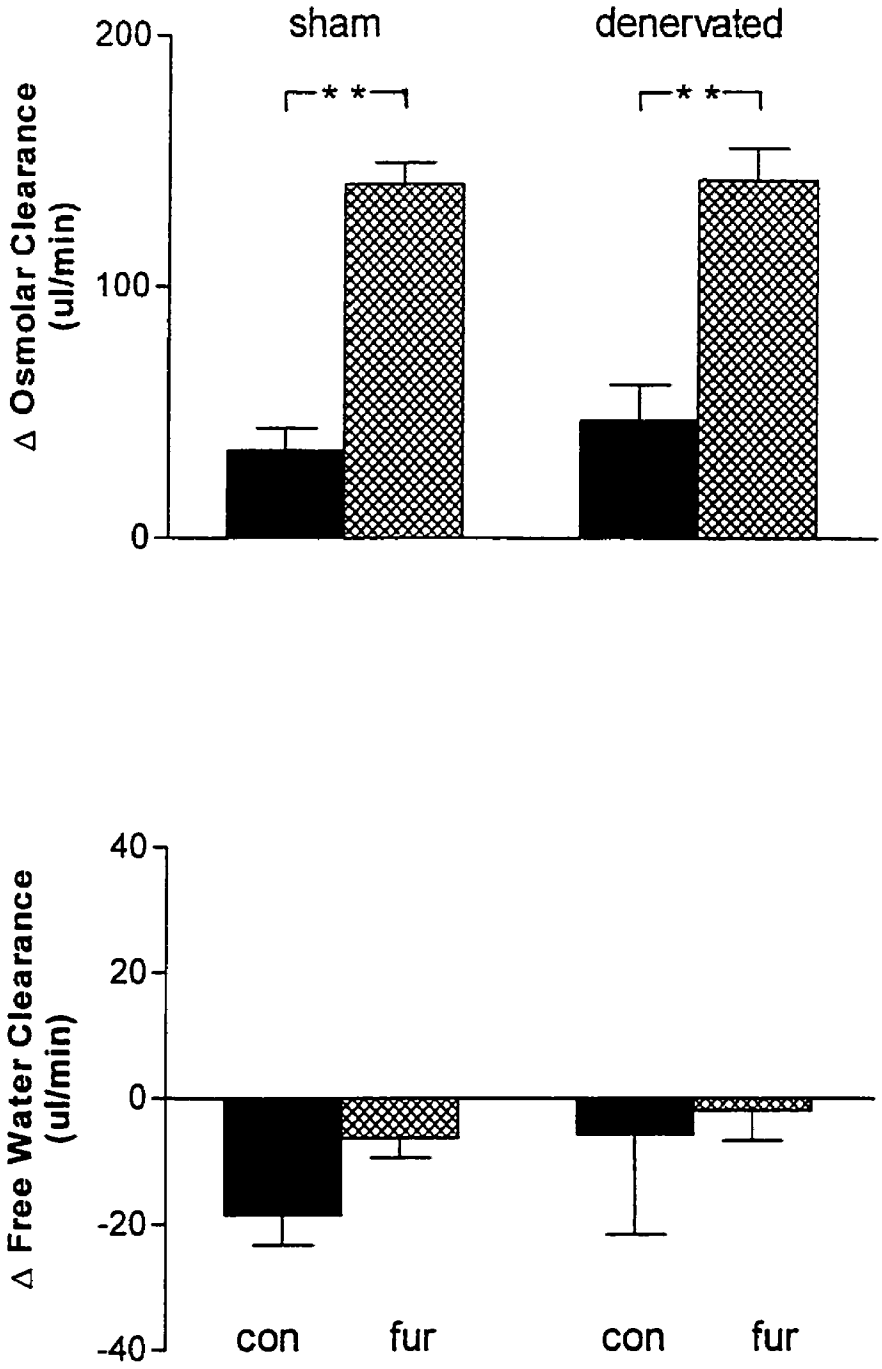

Figure 2.9. Effects of intravenous infusions of vehicle (0.9% saline) or furosemide (0.1 mg/kg) on osmolar clearance and free water in sham and denervated male Sprague -Dawley rats. Data are presented as the mean ± S.E of the mean of the difference between the final collection and baseline values of six experiments.

General Discussion

In previous sections we had identified a number of studies that supported the existence of the imidazoline receptor which was unique from the α_2–adrenoceptor (see General Introduction). In our initial studies, we determined the natriuretic dose–response relationship for both imidazoline receptor agonists (rilmenidine and moxonidine) and α_2–adrenoceptor agonists (clonidine and guanfacine). The purpose of these experiments was to select the optimal dose that produced a significant increase in urine volume and sodium excretion with a minimal effect on blood pressure and heart rate. Changes in blood pressure were avoided to minimize changes in renal perfusion pressure as contributing to the changes in urine volume. Based on those studies, rilmenidine (10 nmol/kg/min) was chosen to determine if the natriuretic response followed by intravenous infusion of an I_1-imidazoline receptor agonist was mediated through a central and/or a peripheral site. This was based on previous studies that supported the natriuretic response of the central acting imidazoline receptor agonists being secondary to a decrease in renal sympathetic nerve activity (Smyth and Penner, 1999). The natriuretic response may have been due to central as well as peripheral imidazoline receptors. Collectively, a number of studies from our laboratory had indicated that the effect of central administration was not the same as peripheral administration (Penner and Smyth, 1994a; 1994b, 1995, 1997; Allan et al., 1993; Smyth and Penner, 1998). Thus an additional mechanism, most likely within the kidney may be a logical explanation.

Kline and Cechetto (1993) demonstrated that renal denervation abolished the natriuretic response to an intravenous infusion of rilmenidine. In the rats with intact renal nerves, they obtained an increase in urine flow, sodium excretion, osmolar clearance and a decrease in free water clearance following intravenous rilmenidine. The denervation abolished all of these effects produced by rilmenidine. This indicated the importance of intact renal nerves for the natriuretic and diuretic response. They also suggested that α_2-adrenoceptors were not involved in the response to rilmenidine, since the decrease in free water clearance was observed in these experiments. Studies from our laboratory investigated the effects of ICV administration of moxonidine and rilmenidine in acute renal denervated rats (Penner and Smyth, 1997; Smyth and Penner, 1998). An increase in free water clearance was obtained which was most likely independent from the renal nerves. An increase in sodium excretion and osmolar clearance required intact renal nerves. The effects of denervation on the natriuretic response to a peripherally administrated I_1-imidazoline receptor agonist had not been determined in our laboratory. Therefore, in our present study we investigated the natriuretic response following the peripheral administration of an I_1-imidazoline receptor agonist, rilmenidine, in sham and renal sympathetic denervated rats. These studies were to determine if rilmenidine acts directly on the kidney or acts centrally through the renal sympathetic nerves. Results from our study found that following acute renal denervation, an intravenous infusion of rilmenidine decreased the urine flow rate, sodium excretion and osmolar clearance rather than producing an increase as

found in the sham-denervated animals. Free water clearance was increased in the denervated animals, most likely due to an α_2-adrenoceptor effect. This importance of intact renal nerves for the natriuretic response to a peripherally administered I_1 imidazoline receptor agonist, rilmenidine was in agreement with the work by Kline and Cechetto (1993).

Based on these studies, it was not clear if the effects of denervation were specific for I_1-imidazoline agonist. We therefore looked at the effects of denervation on the response to an α_2-adrenoceptor agonist, guanfacine. Unlike imidazoline receptor agonists, the renal effects of α_2-adrenoceptor agonist have been found to alter urine flow secondary to altering the renal effects of vasopressin. This has been documented in isolated renal tubules (Krothapalli and Suki, 1984; Umemura et al., 1985), isolated perfused kidney (Smyth, et al., 1985) and in whole animals (Strandhoy et al., 1982; Blandford and Smyth, 1988a). α_2-Adrenoceptor agonists have also been shown to act within the central nervous system to decrease sympathetic nerve activity and thereby increase urine flow rate (Garty et al., 1990). The studies in the isolated tubules and perfused kidney would suggest that the renal effects of α_2-adrenoceptor agonists should occur, at least in part, independant from the renal nerves. This contention, however, would be at odds with the studies of Kline and Mercer (1990) where renal denervation blocked the effect of an intravenous infusion of the α_2-adrenoceptor agonist BTH 933. We therefore determined the effects of denervation on the renal response to an intravenous infusion of guanfacine.

We followed the exact experimental procedure previously described for rilmenidine. Based on our preliminary studies, guanfacine was chosen at an infusion rate of 10 nmol/kg/min. In the intact animals, an intravenous infusion of guanfacine increased urine flow, sodium excretion and osmolar clearance. Following denervation guanfacine still increased urine flow, but this effect was now secondary to an increase in free water clearance. According to the results from this study, intact renal nerves appear to be important for the natriuretic response to an intravenous infusion of the α_2-adrenoceptor agonist, guanfacine.

These findings were not anticipated based on previous studies in isolated tubules and perfused kidney. We speculated that the renal denervation may have produced a non-specific decrease in natriuresis. We therefore repeated these studies with a natriuretic compound that should not be affected by denervation. Furosemide has been identified as a loop diuretic which does not act through a G-protein, but by inhibition of sodium, potassium and chloride ion cotransport of the luminal membrane of the ascending limb of the loop of Henle (Smith and Strack, 1995; Smith et al., 1996). The dose of furosemide (1.67 μg/kg/min for a total dose of 0.1 mg/kg over one hour) was selected, based on previous experiments in our laboratory, to alter renal function without affecting blood pressure and heart rate (Darkwa, 1994).

A selected dose of furosemide was administered intravenously as previously described for rilmenidine and guanfacine in intact and denervated rats. The response to the infusion of furosemide was similar in both groups of rats. The ability of furosemide to increase urine flow rate in denervated rats would

suggest that the denervation did not cause a general decrease in the ability of all natriuretic agents to increase sodium excretion. The effects of the denervation were non-specific.

Additional preliminary studies

Although the results have not been included with this thesis, I attempted a number of preliminary experiments to address why renal nerve stimulation attenuated the response to guanfacine and rilmenidine. A possible explanation for the ability of denervation to alter the response to guanfacine and rilmenidine may be that the renal nerves were playing a permissive role. We therefore completed a series of preliminary experiments where renal norepinephrine was replaced with a constant infusion in denervated rats. This approach was based on previous studies with norepinephrine and epinephrine in innervated and denervated kidney (Almgard and Ljungqvist, 1975; Kopp et al., 1983; Hisa et al., 1989; Janssen et al., 1989). We speculated that the infusion of norepinephrine would mimic the presence of the sympathetic renal nerves and return the response of the kidney to rilmenidine. This would be an indication that intact renal nerves indeed must be present for the natriuretic response of intravenous rilmenidine but that the effect was not primarily due to a decrease in renal sympathetic nerve activity.

In our first series of experiments with norepinephrine, we attempted to reverse the effects of denervation with an intrarenal infusion of norepinephrine. Our results showed an increase in blood pressure and heart rate with the norepinephrine infusion. The norepinephrine failed to return the natriuretic effect

of rilmenidine in these denervated rats. This study design did have some limitations. It is important to mention at this point that previous findings in vitro and in vivo showed that unlike norepinephrine released from a nerve terminals, an infusion of norepinephrine would be expected to stimulate both postjunctional and extrajunctional adrenoceptors. This may be a reason that the antinatriuretic effects were not obtained until the dose of norepinephrine was high enough to decrease renal blood flow and increase arterial blood pressure (Lang et al., 1993). We believe that if we are able to keep blood pressure and heart rate from increase during infusion of norepinephrine, we may be able to see different diuretic and natriuretic responses compared to the control group.

In another series of experiments with rilmenidine, guanfacine and furosemide an adjustable clamp was placed around abdominal aorta above the left renal artery to maintain renal perfusion pressure (Roman and Cowley, 1985). In these studies, when arterial blood pressure was increased due to the norepinephrine infusion, renal perfusion pressure was adjusted by tightening the clamp above the renal artery. This procedure maintained renal perfusion pressure at a constant level until the end of experiment.

The intravenous infusion of norepinephrine (3 nmol/kg/min) with and without a concomitant infusion of rilmenidine (10 nmol/kg/min), increased blood pressure significantly and to a similar extent. Femoral artery blood pressure (an indication of renal perfusion pressure), in the control group and treatment groups (norepinephrine or norepinephrine and rilmenidine) remained at similar levels due to the adjustment of the clamp placed around the abdominal aorta above the

left renal artery. Heart rate increased approximately 20 beats/min in animals infused with norepinephrine.

Following the intravenous infusion of norepinephrine, urine flow rate, sodium excretion and osmolar clearance remind at similar levels between the three groups. Intravenous administration of norepinephrine (3 nmol/kg/min) with rilmenidine (10 nmol/kg/min) and norepinephrine alone also failed to show a significant increase in free water clearance compared to control (data not shown). Thus these results, in contrast to our expectations, failed to further confirm the importance of renal sympathetic nerves for the renal action of I_1 imidazoline receptor agonists. However, the experimental procedure in this study involved much more surgical manipulation compared to previous studies. Thus we believe that it would be worth repeating this study with further development of the surgical preparation.

Further directions

In our initial dose-response study we determined the optimal dose of rilmenidine or guanfacine that produced a significant diuretic and natriuretic response with minimal change in blood pressure and heart rate. In future, we think that it would be worthy to look at a new dose-response study, this time to establish the maximal dose response of rilmenidine as well as guanfacine. We would then compare the maximal diuretic and natriuretic response following administration of these drugs to the diuretic and natriuretic effects of the renal denervated animals infused with saline only. If the diuretic and natriuretic effects were primarily due to a decrease in renal sympathetic nerve activity, then even

the highest response following the administration of rilmenidine or guanfacine should not be higher than the response obtained after total destruction of the renal nerves. If not, then we must assume that an additional mechanism exists. Although not conclusive, our results support this possibility. In the sham treated rats, the increase in urine flow rate (Figure 2.5) and osmolar clearance (Figure 2.6) following guanfacine was greater than that observed following denervation alone. If the guanfacine was acting solely by decreasing renal sympathetic nerve activity, it would not be expected to cause a greater effect than renal denervation alone. Data from this study indicated that a maximal dose of rilmenidine or guanfacine, with renal perfusion pressure control, may produce a significantly higher diuretic and natriuretic response than simple denervation alone.

Previous studies from our laboratory had compared effects of central and peripheral administration of the I_1-imidazoline receptor agonist, moxonidine (Penner and Smyth, 1994a; 1994b, 1995, 1997; Allan et al., 1993; Smyth and Penner, 1998). The studies suggested that the diuretic and natriuretic effect may have been produced by activation of I_1-imidazoline receptors in the CNS, and the decrease in the sympathetic nerve activity, as well as by acting directly on the I_1-imidazoline receptor into the kidney. Mukaddam-Daher and Gutkowska (2000) have provided evidence that the natriuretic effect of moxonidine was mediated by a release of atrial natriuretic peptide (ANP). ANP is a cardiac peptide that mediates a diuresis and natriuresis through its second messenger cGMP, thereby controlling fluid absorption in the proximal tubule of the kidney (Garvin, 1992). Interestingly, the renal nerves appeared to be important for the response

of ANP. Following denervation the diuresis and natriuresis in response to ANP was increased (Dowling et al., 1989; Pollock and Arendshorst, 1991; Christy et al., 1994). These studies suggested that an intravenous injection of moxonidine, by acting on the imidazoline receptors, produced a diuresis and natriuresis secondary to an increased level of plasma ANP and urinary cGMP. Thus the natriuretic and diuretic effects of the moxonidine in the kidney may be related to the increased level of the ANP (Mukaddam-Daher and Gutkowska, 2000). According to Christy et al. (1994), in innervated animals the renal nerve may antagonize renal response to ANP. Our results, however, do not support a role for ANP in the renal actions of rilmenidine. In denervated rats we would have anticipated an even greater diuretic and natriuretic response following the administration of moxonidine if these effects were in fact related to an increase in the ANP level. In our study, following intravenous infusion of the I_1-imidazoline receptor agonist rilmenidine, we found a decreased diuretic and natriuretic response in the denervated animals compared to those with intact renal nerves. Thus it would be interesting in the future, to investigate renal denervation and the renal action of ANP and see if denervation would also attenuate the response to ANP in our preparation.

Summary

In this thesis we have demonstrated the significance of intact renal sympathetic nerves for the natriuretic response to an intravenous infusion of an I_1-imidazoline receptor agonist, rilmenidine. Following a similar experimental

procedure we then examined an α_2-adrenoceptor agonist guanfacine, and showed that intact renal nerves were also important to produce the natriuretic response. This was a specific effect, as the response to furosemide was unaltered by denervation. Thus we have shown that denervation has a specific effect on the natriuretic activity of the rilmenidine and guanfacine.

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