

RENAL PHARMACOLOGY OF CALCITONIN GENE-RELATED PEPTIDE

by

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Abstract

The existence of calcitonin gene-related peptide (CGRP) nerve fibres and CGRP receptors in the kidney and the coupling of the receptors to adenylyl cyclase suggest a possible role for CGRP in the regulation of renal microcirculation, electrolyte transport and water homeostasis. This study investigates the dose-effect relationship of CGRP on renal haemodynamics and tubular excretion in Inactin-anaesthetized, Sprague-Dawley rats. Renal arterial infusion of CGRP (0.3-300 pmol/kg/min) did not affect mean arterial pressure (MAP) or heart rate (HR). Low doses of CGRP increased renal blood flow (RBF), arterial conductance and glomerular filtration rate (GFR) but the highest dose reduced RBF and conductance without affecting GFR. High doses of CGRP also increased urine flow, and excretion of Na^+ and K^+ .

To investigate the contributions of endothelium-derived nitric oxide in the renal actions of CGRP, the nitric oxide synthase inhibitor, N^{G} -nitro-L-arginine methyl ester (L-NAME, 2 or 20 $\mu\text{mol/kg}$) was injected into the renal artery prior to the infusion of CGRP. The renal vasodilator but not the constrictor effect of CGRP was inhibited by both doses of L-NAME. The increase in GFR by CGRP was attenuated by the low dose and abolished by the high dose of L-NAME. L-NAME did not inhibit the diuretic, natriuretic or kaliuretic effects elicited by high doses of CGRP. The involvement of the renin-angiotensin system, renal sympathetic nerves and kidney prostaglandins in CGRP induced renal effects were also investigated via renal intra-arterial injection of the angiotensin II receptor antagonist losartan (0.3 and 3 $\mu\text{mol/kg}$), the α -adrenoceptor antagonist phenoxybenzamine (3 $\mu\text{mol/kg}$), the ganglion blocker mecamylamine (1

$\mu\text{mol/kg}$), and the cyclo-oxygenase inhibitor indomethacin ($3 \mu\text{mol/kg}$), respectively. The vasodilator effect of low doses of CGRP and the increase in GFR were not affected by losartan, phenoxybenzamine, mecamylamine or indomethacin. The vasoconstrictor effect induced by high doses of CGRP was blocked only by phenoxybenzamine, suggesting that it is due to noradrenaline release from sympathetic nerve terminals. CGRP's diuretic, natriuretic and kaliuretic effects were similarly inhibited by losartan, phenoxybenzamine and mecamylamine. This inhibition was primarily due to low perfusion caused by the reductions in MAP and RBF. Pre-treatment with indomethacin prevented CGRP's kaliuretic effect.

The effects of the specific CGRP1 receptor antagonists CGRP (8-37) (1 and 10 nmol/kg) and the putative CGRP receptor antagonist, $[\text{tyr}^0]\text{CGRP}(28-37)$ (3 and 30 nmol/kg) on the renal vascular and tubular effects of CGRP were also examined. Following renal arterial injection of CGRP (8-37) or $[\text{tyr}^0]\text{CGRP}(28-37)$, a high dose of CGRP (300 pmol/kg/min) markedly reduced MAP and increased HR. CGRP(8-37) completely but $[\text{Tyr}^0]\text{CGRP}(28-37)$ incompletely inhibited the vasodilatation and increments in GFR elicited by low doses of CGRP. Both blockers abolished the renal vasoconstriction but did not inhibit the diuresis, natriuresis or kaliuresis elicited by high but non-hypotensive doses of CGRP.

The study also compared the renal effects of adrenomedullin (AM), a novel vasodilator peptide which shows homology with CGRP, with those of CGRP. When injected into the renal artery, only the highest dose of AM reduced MAP whereas the highest two doses of CGRP reduced MAP. Both AM and CGRP induced similar increases in arterial conductance at all doses except for the highest dose of AM (1 nmol/kg), which

caused greater vasodilatation than did CGRP. Both peptides produced similar durations of vasodilatation. CGRP's diuretic and natriuretic effects were significantly greater than those of AM. CGRP, but not AM increased K^+ excretion and decreased urine osmolality. Our results show that AM is a more efficacious renal vasodilator but it produces less diuresis and natriuresis than does CGRP. The possible involvement of CGRP receptors in AM renal actions was also examined. Renal arterial infusion (0.001 to 1 nmol/kg) of AM did not alter MAP or HR but dose-dependently increased RBF and arterial conductance, GFR, urine flow and excretion of Na^+ as well as K^+ . AM's renal vascular and tubular effects were not inhibited by either the low or the high dose of CGRP(8-37) or [Tyr⁰]CGRP(28-37). Therefore, the renal vascular and tubular effects of AM are not mediated via the activation of CGRP1 receptors.

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Abbreviations

AM	adrenomedullin
cAMP	cyclic adenosine 3',5'-monophosphate
CGRP	calcitonin gene related peptide
EDRF/NO	endothelium-derived relaxing factor/nitric oxide
EDTA	ethylenediaminetetraacetate
GFR	glomerular filtration rate
HC%	blood haematocrit value
HR	heart rate
i.a.	intra-arterial
i.p.	intraperitoneal
i.v.	intravenous
L-NAME	n ^G -nitro-L-arginine methyl ester
MAP	mean arterial pressure.
mRNA	messenger ribonucleic acid
POB	phenoxybenzamine
RBF	renal blood flow.
SE	standard error of the mean
SP	sampling period
V	urine flow rate
U _K V	urinary potassium excretion rate
U _{Na} V	urinary sodium excretion rate
U _{osm}	urine osmolality

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To Mohamed and Hossam-Eldin

1. INTRODUCTION

1.1. Historical: discovery of CGRP

In 1981, Rosenfeld and co-workers (Rosenfeld et al., 1981) showed that serially transplanted rat cells from medullary thyroid carcinoma have a spontaneous ability to switch from a high to low producing state by increasing the size of mRNA. These investigators cloned the altered mRNA and located its sequence on the map of the calcitonin gene. They predicted and isolated a 37 amino acid peptide named calcitonin gene-related peptide (CGRP)(Amara et al., 1982). In 1984, on the basis of the predicted sequence of rat CGRP, a new peptide with a similar structure was isolated from the human medullary thyroid carcinoma (Morris et al., 1984). The diversity of the calcitonin gene regulatory process was further revealed by gene duplication. Human CGRP obtained from medullary thyroid carcinoma was first isolated and sequenced as the α -form (Morris et al., 1984). The second gene, also located on chromosome 11, called β -calcitonin/CGRP gene, encode the β -CGRP sequence which is expressed both in human and rats (Steenberg et al., 1985, Brain et al., 1986). β -CGRP varies from the α -form by one and three amino acids in rats and humans, respectively (Alevizaki, 1986). Both α -and β -CGRP are similar in biological activity (Steenbergh et al., 1986). During the last decade, the production, receptors, distribution and biological actions of CGRP have been the subject of innumerable studies (for reviews see Goodman and Iverson, 1986; Yamamoto and Tohyama, 1989; Ziadi et al., 1990a; Poyner, 1992a, Preibisz, 1993).

1.2. Chemical structure of CGRP

Human CGRP has 37 amino acids and structural homology with the 32 amino acid peptide human calcitonin. Both peptides have an overall positive charge and share a common C-terminal amide and a disulphide bridge near the N-terminal. Whereas the calcitonins exhibit divergence in structure among species, the known CGRP sequences are highly conserved. Interestingly, salmon calcitonin exhibits greater structure similarity to human CGRP than to human calcitonin (for review, see Zaidi et al., 1987; Breimer et al., 1988). Human α -CGRP differs from rat α -CGRP by four amino acids (positions 1, 3, 25 and 35). Human β -CGRP differs from human α -CGRP by three amino acids (positions 3, 22 and 25). Only two residues in rat β -CGRP (positions 1 and 25) differ from those of human α -CGRP (Zaidi et al., 1987). The structures of four mammalian CGRP molecules are as follows (* indicates difference from human α -CGRP):

A. Human α -CGRP:

NH₂-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asp-Val-Gly-Ser-Lys-Ala-Phe-CONH₂

B. Human β -CGRP:

NH₂-Ala-Cys-Asn* -Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Met* -Val-Lys-Ser* -Asn-Phe-Val-Pro-Thr-Asp-Val-Gly-Ser-Lys-Ala-Phe-CONH₂

C. Rat α -CGRP:

NH₂-Ser^{*}-Cys-Asn^{*}-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asp^{*}-Asn-Phe-Val-Pro-Thr-Asp-Val-Gly-Ser-Glu^{*}-Ala-Phe-CONH₂

D. Rat β -CGRP:

NH₂-Ser^{*}-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asp^{*}-Asn-Phe-Val-Pro-Thr-Asp-Val-Gly-Ser-Lys-Ala-Phe-CONH₂

1.3. Localization of CGRP

1.3.1. Nervous system

Rosenfeld and colleagues (1983) demonstrated that the facial, hypoglossal, peripenduncular and parabrachial nuclei contained large amounts of mRNA for CGRP. Immunocytochemical studies revealed an even more extensive distribution of the peptides (Kawai et al., 1985). Specific CGRP-rich pathways have been traced using retrograde tracing procedures in combination with immunocytochemistry, and the distribution infers possible functional roles for the peptide. Three major CGRP-containing tracts have been shown to connect the thalamic and hypothalamic nuclei to the limbic system (Kawai et al., 1985): 1) from the insular cortex to the ventromedial thalamic nucleus (Shimada et al., 1985a), 2) from the lateral septal area to the hypothalamus (Sakanaka et al., 1985), and 3) from the ventral hypothalamic surface to the caudate nucleus to join a band of fibres that pass beneath the globus pallidus

(Kawai et al., 1985). CGRP-rich fibres of the forebrain and diencephalon are derived from the parabrachial area (Rosenfeld et al., 1983; Shimada et al., 1985b) and the ventral surface of the hypothalamus (Sakanaka et al., 1985). Two other projections have also been identified, one between the dorsolateral hypothalamus and the ventromedial nucleus of the thalamus, and the other originating from the ventral hypothalamus projecting to the red nucleus of the striae terminalis, central amygdaloid nucleus, and the far lateral hypothalamus (Shimada et al., 1985a&b). In the lateral tract, CGRP is thought to be co-localized with cholecystokinin in single cells (Inagaki et al., 1984; Kawai et al., 1985). In the somatomotor and branchiomotor nuclei, especially those of the hypoglossal and facial nerves, CGRP is co-localized with acetylcholine in single cells (Takami et al., 1985a). CGRP is also co-localized with substance P in single cells whose fibres project from trigeminal nerve and tractus solitarius (Lee et al., 1985a; Wanaka et al., 1987). Similarly, CGRP and gamma aminobutyric acid appear to co-exist in Purkinje cells of the cerebellum (Kawai et al., 1985).

In the spinal cord, CGRP-rich structures are mainly confined to laminae I, II, V and X of the dorsal horn, tract of Lissauer, and small fibres throughout the gray matter (Gibson et al., 1984). This pattern of distribution is somewhat similar to that of substance P, another predominantly sensory neurotransmitter (Gibson et al., 1981). In addition, CGRP-rich structures are found in the dorsolateral nucleus of the white matter, fasciculus proprius, around intermediolateral cells of the thoracic region, and dense clusters in the sacral segment of the spinal cord around the parasympathetic

nuclei and the spinal canal (Gibson et al., 1984). Apart from a few exceptions, the inter-species distribution pattern of CGRP in the spinal cord is fairly constant, suggesting that functional constraints may have led to the conservation of structure (Gibson et al., 1984).

In the sensory ganglia and primary afferents, it appears that nerve fibres rich in substance P and CGRP form a primary afferent neurone system comprising capsaicin-sensitive A and C fibre afferent nerves and medium-sized (type B) cells (Lee et al., 1985b). In the dorsal root, nodose, and trigeminal ganglia, 40% of all cells contain CGRP but only about half of these co-store substance P; moreover, all the cells that stain positive for substance P also do so for CGRP. The large cells only contain CGRP but no substance P, and these cells possibly participate in thermoreceptor and mechanoreceptor functions. Thus, the CGRP-containing cells in the spinal cord are possibly sensory neurons which are responsive to noxious and innocuous stimuli and those in the ganglia give rise to A as well as C afferent fibres (Gibson et al., 1984). The co-localization of CGRP with the tachykinins, and its depletion following capsaicin treatment (Diez guerra et al., 1987) suggest that CGRP may modulate or transmit sensory impulses.

A role for CGRP as an autonomic neurotransmitter has been suggested on the basis of its existence in the autonomic ganglia and the diffuse CGRP-rich innervations of the cardiovascular, gastrointestinal, urogenital and special sense systems. In autonomic sympathetic ganglia (celiac and superior cervical), CGRP is only localized in fibres and not the cell body. In the celiac ganglia, these are either "passing fibres"

that form thick bundles and co-store substance P or “terminating fibres” that form varicosities that do not co-store substance P. The latter possibly modulates ganglionic function. Clearly, CGRP-containing fibres outnumber those containing substance P. A similar distribution pattern of CGRP fibres exists in the parasympathetic ganglia as in the sympathetic ganglia (Yamamoto and Tohyama, 1989).

1.3.2. Cardiovascular system

In the heart, CGRP immunoreactivity is found in the pericardium and around, or parallel to, the coronary arteries. The fibres also exist in the heart muscle, particularly the papillary muscle (Mulder et al., 1985a). Although no positive nerve cell bodies are found in the sinoatrial node or atrioventricular nodes, CGRP immunoreactive fibres are seen within the nodes and conducting system. The nodal innervation may participate in regulating heart rate. Thus, CGRP released from the nonadrenergic, noncholinergic nerves innervating the sinus node may have a neurotransmitter role in cardio-acceleration (Mulder et al., 1985a). The distribution pattern of CGRP in the heart resembles that of substance P (Wharton et al., 1985) but differs from that of neuropeptide Y (Gu et al., 1983). Interestingly, the absolute amount of CGRP in the heart is lower than that in the vascular system (Mulder et al., 1985a).

In blood vessels, CGRP fibres are seen at the junction of the adventitia and media, and they penetrate into the muscle layer. Dense perivascular networks of CGRP are seen around the inferior vena cava, renal arteries, superior mesenteric arteries, femoral arteries and cerebral arteries (McCulloch et al., 1986; Mulder et al., 1985a&b; Wanaka et al., 1987). The innervation is often most marked at the origins of

the arteries; the carotid and aortic bifurcation are particularly richly innervated with CGRP-containing fibres (Mulderry et al., 1985a). The association of CGRP-rich structure with those containing vasoactive intestinal peptide, substance P and enkephalin at these sites are suggestive of the involvement of these peptides in cardiovascular control. In the venous system, dense CGRP-rich innervation exists in the inferior vena cava, the renal vein and femoral vein. It is of interest that whereas the distribution pattern of CGRP in the mesenteric vasculature is similar to that of substance P, only CGRP-fibres are present in the renal vessels (Della et al., 1983; Uddman et al., 1986); this may indicate a more specific role of CGRP in the control of renal blood flow. In the cerebral arteries, there is presence of either dense periadventitial fibre bands or fibres forming a meshwork in the adventitia (McCulloch et al., 1986; Wanaka et al., 1987). CGRP-containing fibres of the cerebral vascular system follow the same pattern as substance P-containing fibres, and in some of these fibres, the two peptides are co-localized (Edvinsson et al., 1983). In the carotid arterial system, CGRP-fibres arise from the trigeminal ganglia, whereas in the vertebrobasilar system, CGRP-fibres originate from similar cells of the cervical dorsal root ganglia (McCulloch et al., 1986; Wanaka et al., 1987).

1.3.3. Plasma

The first assessment of circulating CGRP in healthy human subjects was published by Girgis et al. (1985). In unextracted plasma, the mean level of CGRP immunoreactivity measured with specific rabbit antibody was around 90 pg/ml, and the level did not differ between men and women. The levels of CGRP in unextracted

plasma of normal subjects range from an average value of 81 to 140 pg/ml (Stevenson et al., 1986; Bendtsen et al., 1991; Valdemarsson et al., 1990). Unextracted serum tends to show higher levels of 137 to 367 pg/ml (Shifter 1991). The average CGRP levels in extracted plasma and serum in groups of healthy subjects range from 2 to 44 pg/ml (Trasforni et al., 1991). Undoubtedly, the establishment of a range of circulating CGRP levels in normal subjects is important in determining possible physiological and pathophysiological roles of CGRP. However, one must also realize that CGRP may function primarily as a neuropeptide rather than a circulating hormone, as circulating CGRP is mainly a spill-over of released transmitter from perivascular nerve terminals (Zaidi et al., 1985; Deiz Guerra et al., 1988).

1.3.4. Thyroid gland

Various groups have reported the presence of large quantities of immunoreactive CGRP in the normal rat and human thyroid (Tschopp et al., 1985; Zaidi et al., 1985; MacIntyre et al., 1987a). CGRP is present in most normal and malignant thyroid C cells (Sikri et al., 1985). It is predominantly localized with calcitonin in the same granules of heavily granulated C cells. However, there is also a significant population of cells that stain positively for CGRP but have no affinity for calcitonin (Sikri et al., 1985). The pituitary in human has a higher content of CGRP than does the thyroid but the reverse occurs in rats (MacIntyre, 1984, MacIntyre et al., 1987a&b). Besides calcitonin and CGRP, C-cells of the thyroid contain other peptides and proteins such as somatostatin (Sikri et al., 1985), gastrin releasing peptide (Yamaguchi et al., 1984), corticotropin (Charpin et al., 1982), pre-opiomelanocortin

(Steenbergh et al., 1984) and the enzymes dopa-decarboxylase and histaminase (Lippman et al., 1982).

1.3.5. Gastrointestinal tract

CGRP is distributed in nerves supplying the gastrointestinal tract and its vasculature (Mulder et al., 1985b; Rodrigo et al., 1985). In the oesophagus, both sensory (capsaicin-sensitive) and motor CGRP-fibres have been detected (Rodrigo et al., 1985). CGRP-rich sensory nerve fibres form extensive sub- and intra-epithelial plexuses in the gastrointestinal tract that are stretch-sensitive (Robles et al., 1983). CGRP-positive nerve fibres are also found in the smooth and striated (motor end plate) muscles of the oesophagus. CGRP-rich fibres originate from the nodose ganglia (sensory) and nucleus ambiguus (Motor). In addition, high levels of immunoreactive-CGRP exist in the pylorus, descending colon and rectum, with lesser amounts in the pancreas. CGRP-rich nerve fibres of the gut are also found in the circular muscle, submucosa of blood vessels, muscularis mucosa as well as mucosa; they often lie in association with non-immunoreactive ganglion cells (Mulder et al., 1985b).

1.4. CGRP receptors

Specific high affinity binding sites for CGRP have been identified throughout the nervous system, including the cerebellum, brainstem and the spinal cord. Similarly, high affinity binding sites for CGRP have been characterized in peripheral organs such as the heart, spleen, liver, kidney, skeletal muscles and macrophages (Sigrist et al., 1986; Dennis et al., 1990; Goltzman and Mitchell, 1985; Stangel et al., 1993). CGRP binding in the liver, spleen, heart, skeletal muscle, astrocytes and macrophages is

linked to adenylyl cyclase activation (Sigrist et al., 1986; Goltzman and mitchell, 1985; Vignery et al., 1991; Laufer and Chnageux, 1989; Yamaguchi et al., 1988a&b). In macrophages, CGRP also stimulates a Na^+/H^+ -exchange through a protein kinase C-dependent mechanism (Vignery et al., 1991). CGRP enhances phosphoinositide turnover in skeletal muscle cells, but this effect is also mediated in part by cAMP (Laufer and Changeux, 1989).

1.4.1. CGRP antagonists

Biochemical and functional studies show that some fragments of CGRP have antagonistic actions against CGRP. The C-terminal fragment, CGRP (8-37), was first described by Chiba et al. (1989) to cause a parallel rightward-shift in the dose vs cAMP production curve in response to human α -CGRP in rat liver plasma membranes. CGRP (8-37) has also been shown to be a competitive antagonist at vascular CGRP receptors in various *in vitro* preparations. CGRP (8-37) reversibly antagonized relaxation responses to CGRP in the isolated perfused rat kidney (Castellucci et al., 1993; chin et al., 1994), perfused rat mesenteric arterial bed (Han et al., 1990a; Claing et al., 1992), porcine coronary artery (Foulkes et al., 1991; Franco-Cereceda, 1992), and in rat basilar artery (Kitazono et al., 1993). CGRP (8-37) also inhibited vasodilatation *in vivo* elicited by CGRP in the rabbit (Hughes and Brain, 1991) and rat (Escott and Brain, 1994) skin beds and in cat cerebral arterioles (Wei et al., 1992). In conscious rats, CGRP(8-37) inhibited vasodilatation responses to CGRP in the renal and hindquarter beds and vasoconstrictor response to CGRP in the mesenteric bed (Gardiner et al., 1990a). Additionally, CGRP(8-37) antagonized the positive inotropic

effect of CGRP in guinea pig isolated left atrium and relaxant response to CGRP on rat vas deferens (Maggi et al, 1991).

[Tyr⁰]CGRP(8-37) was first described by Chakder and Rattan (1990) as a CGRP receptor antagonist in the opossum internal anal sphincter. [Tyr⁰]CGRP(8-37) antagonized all forms of CGRP tested in this preparation but had markedly different potencies for human α - and β -CGRP, rat CGRP and rat [tyr⁰]CGRP. The results suggest that [tyr⁰]CGRP(8-37) has an affinity as high as that of CGRP(8-37) against the human CGRP-preferring receptors. This compound also antagonized the CGRP-induced increases in cAMP levels in neuroblastoma cells (van Valen et al., 1990). [Tyr⁰]CGRP(8-37) inhibited the CGRP-induced amylase secretion in the isolated acini from the guinea pig pancreas (Manton et al., 1990). [Tyr⁰]CGRP(8-37) was over an order of magnitude less potent than CGRP (8-37) in displacing CGRP binding in rat liver plasma membranes (Yamaguchi et al., 1988a&b).

Rovero et al., (1992) reported that the short C-terminal fragments of human α -CGRP, CGRP (19-37) and CGRP (23-37), and the N-terminally-acetylated derivative, AcCGRP (19-37), produced rightward displacements of the positive inotropic dose-response curves of human α -CGRP on guinea pig isolated left atrium without depressing the maximal response.

1.4.2. CGRP agonists

Various forms of CGRP have varying potencies among different tissues. Human β -CGRP appears to be more potent than either human α -CGRP or rat α -CGRP in eliciting a fall in the resting tension of opossum internal anal sphincter (Chakder and

Rattan, 1990) and in stimulating cAMP production in sarcoma cells (van Valen et al., 1989). In contrast, human and rat α - and β -CGRP appears to be equipotent in stimulating cAMP production in neuroblastoma cells and L6 myocytes (van Valen et al, 1990; Poyner et al., 1992b). It has been suggested that CGRP receptors can be divided into subtypes preferring β -CGRP or α -CGRP. In an autoradiographic study, [125 I]human β -CGRP was found to label a slightly different set of neuronal structures than did [125 I]human α -CGRP (Henke et al., 1987). In the stomach, human α -CGRP and human β -CGRP have opposite effects on acid secretion (Beglinger et al., 1988; Bauerfeind et al., 1989). Differences have been also noted between human and rat forms of CGRP. In the guinea pig isolated right atrium, rat CGRP is 10 times more potent than human CGRP in causing positive chronotropy, but the two forms are equipotent in producing positive inotropy (Marshall et al., 1986).

[Tyr⁰]-CGRP is reported to be 2-5 times less potent than the parent CGRP molecule in relaxing opossum internal anal sphincter and in stimulating cAMP production in L6 myocytes (Chakder and Rattan, 1990; Poyner et al., 1992b). [Tyr⁰]-CGRP is 10 times less potent than human α -CGRP in eliciting chronotropic effect on the isolated guinea pig left atrium (Dennis et al., 1989) and in stimulating adenylyl cyclase in neuroblastoma cells (van Valen et al, 1990). In contrast, [Tyr⁰]-CGRP has 10-fold higher binding affinity than human α -CGRP in the rat brain and cerebellum (Dennis et al., 1989).

Dennis et al. (1989) reported that acetoamido-methylcysteine_{2,7}-CGRP ([Cys(ACM)_{2,7}]-CGRP) is a selective CGRP₂ (CGRP(8-37)-insensitive) receptor

agonist. [Cys(ACM)_{2,7}]-CGRP was virtually inactive in cardiac tissues with EC₅₀>710 nM but had EC₅₀ of 76 nM in eliciting relaxations in the rat vas deferens. These investigators proposed that [Cys(ACM)_{2,7}]-CGRP was selective for the CGRP (8-37) low affinity sites. In support of this hypothesis, Poyner et al. (1992b) reported that CGRP induced cAMP production in L6 myocytes are sensitive to inhibition by CGRP(8-37) but was not stimulated by [Cys(ACM)_{2,7}]-CGRP (Poyner et al., 1992b). [Cys(ACM)_{2,7}]-CGRP can also be used to discriminate between the subtypes of binding sites in radioligand binding assays. It binds with similar affinities to rat brain and spleen membranes, but has less affinity in the nucleus accumbens relative to the medial frontal cortex (Dennis et al., 1989).

1.4.3. CGRP receptor subtypes

Accumulating evidence suggests the existence of heterogeneous CGRP receptors. The evidence is largely based on the existence of different potencies between molecular forms of CGRP and the different blocking efficacy of the CGRP antagonists CGRP (8-37). It is believed that there are at least two subtypes of CGRP receptors (Dennis et al., 1989; 1990; Mimealut et al., 1991; Giuliani et al., 1992; Stangl et al., 1993; for review see Poyner, 1992a). CGRP1 and CGRP2 binding sites were designated on the basis of affinity of the sites to the C-terminal fragment, CGRP(8-37) (Chiba et al., 1989, Dennis et al., 1989; 1990). CGRP(8-37) was proposed as a selective antagonist at the CGRP1 receptor (Chiba et al., 1989; Maggi et al., 1991) while possessing weak antagonistic activity at CGRP2 receptors. On the other hand, [Cys(ACM)_{2,7}]-CGRP is believed to selectively activate CGRP2 receptors with an EC₅₀

of about 1/100th that of human α -CGRP (Dennis et al; 1990). CGRP1 and CGRP2 receptors are typified by those present in the guinea pig heart and rat vas deferens, respectively (Dennis et al., 1990). In conclusion, there appears to be multiplicity of CGRP receptors but the definite classification of these receptors has to await the development of selective subtypes of CGRP antagonists.

1.4.4. Cross-interaction between CGRP, calcitonin and amylin receptors

Calcitonin, CGRP and amylin are all members of the calcitonin gene peptides. Although each one of these peptides has its own distinct receptors, they cross-react with receptors of a different class due to the similar chemical structures. Salmon calcitonin acts on calcitonin receptors at nanomolar concentrations but CGRP receptors at micromolar range (Poyner et al., 1992a). Calcitonin and CGRP have similar potencies in certain tissues but this may be due to the simultaneous expression of receptors for both peptides (Vignery et al., 1991). Calcitonin and CGRP bind with high affinity to the same sites in the nucleus accumbens of the rat (Dennis et al., 1989).

The potency of amylin varies among tissues. It is less potent than CGRP in the cardiovascular system (Brain et al., 1990) and in activating adenylyl cyclase in rat skeletal myocytes and liver plasma membrane (Poyner et al., 1992b; Morishita et al., 1990). These actions are most likely due to cross-interaction of amylin with CGRP receptors as they were blocked by low concentrations of CGRP(8-37) (Poyner et al., 1992b). In contrast, amylin has potent metabolic actions that are not due to cross-interaction with CGRP receptors (Wang et al., 1991; MacIntyre et al., 1991).

1.5. CGRP biological activity

1.5.1. Neuronal effects

CGRP has been shown to activate neuronal pathways. When injected centrally, it increased noradrenergic sympathetic outflow (Fisher et al., 1983; Hasegawa et al., 1993; Messmer et al., 1993; Kuo et al., 1994) leading to increases in heart rate and blood pressure. It caused suppression of appetite (Tannenbaum and Goltzman, 1985), inhibited basal and stimulated gastric acid secretion (Lenz et al., 1985; Kraenzlin et al., 1985) and decreased intestinal motility (Fargeas et al., 1985). CGRP coexists with cholecystinin in the parabrachial-ventromedial hypothalamic pathway involved in the control of feeding, and receptors for these two peptides are present in the parabrachial region (Inagaki et al., 1986, Dennis et al., 1990). CGRP decreases growth hormone release via a central mechanism (Tannenbaum and Goltzman, 1985). Central administration of CGRP increased rectal temperature suggesting a role for CGRP in thermoregulation (Dennis et al., 1990). Centrally-injected CGRP decreased motor activity and caused catalepsy in rats, suggesting an interaction of CGRP with the dopaminergic neurones of the basal ganglia (Jolicoer et al., 1989). CGRP potentiates the release of substance P from the rat spinal dorsal horn neurones during mechanical nociception, suggesting the presence of presynaptic CGRP receptors on substance P neurones (Oku et al., 1987). Both substance P and CGRP increased the release of glutamate and aspartate in dorsal horn perfusate (Kangrga et al., 1990). Presynaptic CGRP receptors have also been suggested to be involved in the regulation of acetylcholine release (Schworer et al., 1991). CGRP also inhibited the

activity of substance P endopeptidase in human cerebrospinal fluid (Le Greves et al., 1985).

1.5.2. Cardiovascular effects

Tippins et al. (1984) were the first to demonstrate the positive myotropic effect of CGRP in the rat isolated auricle. CGRP increased both the force and rate of contraction of hearts from rat, man and guinea pig but not canine or rabbit (Marshall et al., 1986; Sigrist et al., 1986; Gennari and Fischer, 1986; Ishikawa et al., 1987; Lappe et al., 1987a). CGRP increased force of contraction of porcine ventricular muscles (Niyauchi et al., 1988). The myotropic action of CGRP is not mediated via adrenergic or histaminergic mechanisms or the release of prostaglandin, but is similar to that of capsaicin. The cardiac effects of capsaicin and CGRP are similar in appearance and duration and they both exhibit tachyphylaxis. Furthermore, the contractile response to capsaicin was abolished during tachyphylaxis to CGRP (Lundberge et al., 1985; Sigrist et al., 1986). It has been suggested that CGRP, following release from its cardiac nerves, acts directly on specific CGRP receptors to stimulate cAMP production (Sigrist et al., 1986). CGRP increased cAMP levels in atrial homogenates and myocytes grown in culture (Ishikawa et al., 1987, Wang and Fiscus, 1989). The rat and human α -forms of CGRP are 10 times more potent than the human β -form in increasing the rate of contraction in isolated rat hearts (Holman et al., 1986). CGRP has also been shown to increase coronary blood flow (Greenwald et al., 1986; Joyce et al., 1990).

CGRP is one of the most potent vasodilators known (Brain et al., 1985). The intradermal injection of femtomole-quantities of the peptide in human or rabbit skin

increased local blood flow for several hours (Brain et al., 1985). CGRP causes vascular relaxation in almost all vascular beds in different species either *in vitro* or *in vivo* (See review by Zaidi et al., 1990). CGRP dilates both resistance (DiPette et al., 1987; Lappe et al., 1987a,b; Han et al., 1990b; Gardiner et al., 1989; Abdelrahman et al., 1992) and capacitance vessels (Abdelrahman and Pang, 1992). CGRP induces vasorelaxation through a non-cholinergic, non-adrenergic mechanism (Han et al., 1990b). Endothelium-dependent as well as endothelium-independent mechanisms may be involved (see section 1.6.) depending on the vascular bed and the species of animals. CGRP stimulates adenylyl cyclase in vascular tissues suggesting the involvement of cAMP (Hirata et al., 1988; Kubota et al., 1985; Crossman et al., 1990). The targets for the cAMP protein kinase are unknown but ion channels may be involved. In rabbit mesentery, the dilatation caused by CGRP was inhibited by the potassium channel blocker glibenclamide (Nelson et al., 1990). However, in rat coronary artery, glibenclamide had no effect on CGRP-induced vasodilatation (Prieto et al., 1991). Glibenclamide also did not affect the hypotensive effect of CGRP in conscious rats (Abdelrahman et al., 1992). CGRP increased cGMP production in the rat abdominal aorta (Wang et al., 1991) and rat thoracic aorta (Fiscus et al., 1991). Release of cyclo-oxygenase products e.g. prostacyclins, can also be an alternative mediator for CGRP's relaxant action (Crossman et al., 1987). The presence of CGRP-containing nerve fibres around and penetrating inside blood vessels and the potency and efficacy of CGRP in eliciting vasodilatation suggest that CGRP may participate in the regulation of blood flow. The vasodilator action of CGRP may involve a variety of

mechanisms and second messengers.

1.5.3. Effects on the gastrointestinal tract

In addition to the central effect of CGRP in inhibiting gastric acid secretion and intestinal motility, a variety of peripheral CGRP effects on gastrointestinal motor function have been reported. CGRP inhibited gallbladder contractility *in vitro*, either in the basal condition or when stimulated by CCK-8 or substance P (Gibbins et al., 1985). This effect was tetrodotoxin-resistant, suggesting that it was mediated via direct inhibition by CGRP on gall bladder smooth muscle cells (Maggi et al., 1991). In the isolated guinea pig intestinal longitudinal muscle, CGRP caused relaxation of histamine-induced contractions as well as electrically-induced cholinergic contractions, suggesting a direct action on the smooth muscle (Bartho et al., 1987). In contrast, CGRP contracted circular smooth muscle of the guinea pig ileum which was sensitive to blockade by atropine, implying an indirect, neuronally-mediated action (Holezer, 1988). Therefore, within the intestine, CGRP may directly relax the longitudinal smooth muscle and/or stimulate contraction of circular smooth muscle via a neuronal mechanism. In addition, CGRP caused dose-dependent acetylcholine release from the guinea pig myenteric plexus suggesting that it acts as an excitatory neurotransmitter for the cholinergic myenteric plexus (Mulholland and Jaffer, 1990).

1.5.4. Endocrine effects

Through a central effect, CGRP increases the secretion of adrenocorticotrophic hormone (ACTH) and vasopressin (AVP) (Brasilis et al., 1988), and inhibits the release of growth hormone (Netti et al., 1989). CGRP stimulates the release of atrial

natriuretic peptide (ANP) (Schiebinger and Sanra, 1989). Kurtz et al., (1989a) reported that CGRP stimulated renin release in normal human volunteers and *in vitro* in isolated rat renal juxtaglomerular cells. Murakami et al., (1989) showed that CGRP inhibited the release of aldosterone from *in vitro* isolated rabbit adrenal glomerulosa cells. In conscious dogs, CGRP elevated plasma renin activity but reduced aldosterone release (Murakami et al., 1991).

1.5.5. Actions on skeletal muscle

CGRP is co-localized with acetylcholine at motor nerve terminals (New and Mudge, 1986; Mora et al., 1989). CGRP increased the isometric twitch tension of the mouse diaphragm (Takami et al., 1985b&c). However, the main effect of CGRP on skeletal muscle is to increase phosphorylation of the nicotinic receptor and to increase the transcription of the α -subunit of the receptor, an effect mediated via cAMP (Moss et al., 1991). CGRP inhibited basal and insulin-stimulated glycogen synthesis and stimulated glycogenolysis (Leighton et al., 1989), suggesting that it may also regulate metabolism.

1.5.6. Role in inflammation

CGRP is one of the inflammatory mediators. Its pro-inflammatory action is via vasodilatation which potentiates the migration of the other autacoids that increase vascular permeability, since CGRP itself has little effect on vascular permeability. Thus, CGRP increases oedema formation and neutrophil accumulation in rabbit skin in response to the complement fragment, FMLP, leukotriene B₄, substance P, histamine and interleukin 1 (Buckley et al., 1991a,b; Hughes and Brain 1991).

1.5.7. Clinical relevance of CGRP

Several studies reported no difference in plasma levels of immunoreactive CGRP between men and women (see Preibisz, 1993). However, Valdemarsson et al., (1990) reported significantly lower plasma concentrations of CGRP in men than age-matched women during the menstrual cycle. In addition, CGRP concentrations was higher in women taking contraceptives than those not taking the pills or in men (Valdemarsson et al., 1990) and the difference could be due to hormone-induced volume expansion. Circulating CGRP levels in pregnant women at different gestational ages were higher than those in the control group or those at 5-7 days postpartum (Stevenson et al., 1986). In another study, CGRP immunoreactivity level was measured in healthy women before conception, on a monthly basis during pregnancy, and 24 h and 5 days after delivery (Saggese et al., 1990). A statistically significant increase in CGRP level was noted beginning the third month of gestation, and continued until 24 h postpartum, with the peak value occurring in the ninth month. CGRP levels were normalized on the fifth day after delivery (Saggese et al., 1990).

Increased CGRP levels are also seen in other chronic volume expansion states, such as hepatic cirrhosis (Bendtsen et al., 1991), congestive heart failure (Anand et al., 1991) and volume overload in patients with renal failure undergoing haemodialysis (Odar-Cederloef et al., 1991). Blood levels of CGRP were markedly elevated in patients with medullary thyroid carcinoma (MTC) to the extent that it can be used as a marker and predictor of metastasis and malignancy in this condition (Sikri et al., 1985).

Kawasaki et al., (1990a) reported an age-related decrease of CGRP-innervation

in mesenteric resistance vessels of the spontaneously hypertensive rat (SHR) and this decrease reached a peak at the established state of hypertension. These authors suggested that a defective CGRP-induced vasodilator mechanism might have contributed to the development and maintenance of hypertension in the SHR (Kawasali et al., 1990). Similarly, plasma CGRP concentrations were markedly lower in hypertensive patients compared to normal subjects, and CGRP levels increased following the normalization of blood pressure (Jian et al., 1989). In contrast to these findings, increased plasma CGRP concentrations were found in patients with essential hypertension, pheochromocytoma and primary aldosteronism; the increased levels might have been a compensatory reaction to elevated blood pressure (Masuda et al., 1992).

CGRP has been shown to have a beneficial effect in several cardiovascular disorders. The infusion of CGRP in patients with congestive heart failure increased cardiac output, reduced blood pressure, right arterial pressure as well as pulmonary artery wedge pressure but did not alter heart rate or cause the development of drug tolerance (Anand et al., 1991; Shekar et al., 1991). CGRP infusion also lowered peripheral vascular resistance in subarachnoid haemorrhage (Johnston et al., 1990) and Raynaud's phenomenon (Shawket et al., 1989; Bunker et al., 1990).

1.6. CGRP and EDRF/NO

Endothelium-dependent relaxing factor/nitric oxide (EDRF/NO) release has been suggested to be one of the mechanisms by which CGRP induces vascular relaxation. *In vitro* studies show that CGRP induced endothelium-independent

relaxation of human pulmonary artery (McCormack et al., 1989), bovine and human coronary arteries (Greenberg et al., 1987; Franco-Cerceda, 1991) and pig splenic arteries (Pernow, 1989). The relaxation effect of CGRP in isolated rat aortic rings was endothelium-dependent (Brain et al., 1985; Fiscus et al., 1991; Grace et al., 1987) and inhibited by the nitric oxide synthase inhibitors, N^G-monomethyl-L-arginine and N^G-nitro-L-arginine (Gray and Marshal, 1992). However, CGRP caused dose-dependent activation of adenylyl cyclase without elevation of cGMP levels in bovine aortic endothelial and smooth muscle cells, suggesting the lack of involvement of guanylyl cyclase (Crossman et al., 1990). There is regional variation in the dependence of EDRF/NO for the vasodilatation effect of CGRP. Prieto et al. (1991) showed that CGRP induced endothelium-dependent relaxation of isolated rat proximal epicardial coronary artery but endothelium-independent relaxation of the distal intramyocardial coronary artery (Prieto et al., 1991). CGRP-induced relaxation involved both endothelium-dependent and endothelium-independent mechanisms in the pig external iliac arteries (Samuelson and Jernbeck, 1991).

In vivo studies suggest the vasodilatation effect of CGRP partially depends on EDRF/NO. The depressor effect of CGRP in conscious rats was also partially inhibited by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) (Abdelrahman et al., 1992). More recently, Amuchastegui et al. (1994) reported that L-NAME abolished the increases in renal plasma flow and glomerular filtration rate induced by intravenous infusion of CGRP in anaesthetized rats and the responses to CGRP in L-NAME treated rats were restored by L-arginine infusion.

1.7. CGRP and the renin-angiotensin system

CGRP increases plasma renin activity in conscious normal human volunteers (Kurtz et al., 1988), rats (Itabashi et al. 1988) as well as dogs (Murakami et al., 1989, 1991), the mechanism may involve indirect (hypotension-induced increase in renin release) as well as direct actions of CGRP. CGRP concurrently increased renin secretion and cAMP production from isolated rat renal juxtaglomerular cells (Kurtz et al., 1988) and isolated perfused rat kidney (Kurtz et al., 1989). CGRP also suppressed angiotensin-induced contraction in isolated rat renal mesangial cells. Contraction of renal mesangial cells is an important determinant of glomerular filtration rate as it reduces the filtering surface (Ichikawa et al., 1983). Thus, the relaxant effect of CGRP on renal mesangial cells may be a mechanism by which CGRP increases glomerular filtration.

1.8. CGRP and the autonomic nervous system

CGRP is known to elicit non-cholinergic non-adrenergic vasodilatation (Kawasaki et al., 1988; Han et al., 1990b; Nuki et al., 1994) which is abolished by tetrodotoxin and capsaicin, a pungent ingredient of red peppers and a peptidergic toxin (Buck and Burks, 1986). Despite the detection of CGRP-containing nerves within the autonomic ganglia, it has been demonstrated that pre-treatment with the ganglion blocker hexamethonium did not affect cardiovascular responses to intravenously administered CGRP, suggesting direct vasodilator action of CGRP (Andersson 1989). On the other hand, cerebro-ventricular injection of CGRP induced a prompt rise

in plasma noradrenaline levels, arterial pressure and heart rate, suggesting central stimulation of sympathetic outflow (Fisher et al., 1983; Hasegawa et al., 1993; Messmer et al., 1993; Kuo et al., 1994). In isolated perfused mesenteric beds, it has been shown that stimulation of perivascular nerves containing CGRP attenuates adrenergic vasoconstriction, suggesting direct action of CGRP on vascular smooth muscle (Kawasaki et al., 1990b&c). Therefore, it appears that the tone of mesenteric arteries is controlled not only by sympathetic adrenergic nerves but also by CGRP-containing nerves (Kawasaki et al., 1990b). It was further demonstrated that in the perfused mesenteric artery, neuronally released noradrenaline inhibits CGRP release via presynaptic α_2 -adrenoceptors located on CGRP nerves thereby attenuating CGRP-mediated vasodilatation (Kawasaki et al., 1990c). These results suggest reciprocal interaction between adrenergic and CGRP nerves and dual control in mesenteric arterial resistance.

1.9. CGRP and prostanoids

Brain et al. (1985) showed that indomethacin had no effect on CGRP-induced vasodilatation in rabbit skin *in vivo*. Indomethacin in microsphere studies also did not affect regional vasodilatation responses to intravenously administered CGRP in anaesthetized albino rabbits (Andersson 1989). These results suggest that CGRP-induced vasodilatation is independent of the release of prostanoids. In contrast, CGRP-induced relaxation of noradrenaline-precontracted rat aortic strips was partially inhibited by indomethacin (Brain et al., 1985). The initial flare produced by intradermal injection of CGRP in man is inhibited by aspirin (Barnes et al., 1987). The

discrepancies between different studies may be due to different profiles of prostaglandin production in endothelial cells of microvessels and large vessels (Charo et al., 1984). Crossman et al. (1987) reported that CGRP induced concentration-dependent release of prostacyclin and activation of adenylyl cyclase in cultured endothelial cells of umbilical vein.

1.10. CGRP and the kidney

1.10.1. Distribution of binding sites

CGRP-containing nerve fibres are found in high densities in the renal medulla, papilla and the cortex, with a higher concentration of immunoreactive CGRP in the medulla than the papilla and the cortex. Abundant immunoreactive CGRP was also found in muscular layer of the renal pelvis, in the proximity of arteries and arterioles including the juxtaglomerular apparatus and the periglomerular and peritubular space (Maggi et al., 1987; Geppetti et al., 1989a,b; see Review by Kurtz et al., 1989a) and in the ureter and bladder (Maggi et al., 1987). CGRP immunoreactivity is absent following neonatal treatment with capsaicin (Ghatei et al., 1984; 1985). Although the tissue concentration of CGRP in the urogenital tract was at least 10 times greater than that of substance P, the distribution of both is similar (Ghatei et al., 1984, 1985). CGRP is present in higher concentration in the kidney than any other neuropeptides except neuropeptide Y, whose concentration was similar to that of CGRP (Ghatei et al., 1985). Radioligand binding studies on membranes prepared from the medullary region of the porcine kidney displayed high affinity and high density (K_d , 0.12 nM, B_{max} , 127 fmol/mg protein) receptors for human and rat CGRP (Aiyar et al., 1991).

1.10.2. Second messenger system

It has been shown that CGRP stimulates renal cortical and medullary adenylyl cyclase activity in a concentration-dependent manner (Geppetti et al., 1989a&b; Aiyar et al., 1991) and elevates cAMP production (Kurtz et al., 1989b). However, the stimulation of adenylyl cyclase activity by CGRP in cultured rat juxtaglomerular cells was reported to be due to cross-reaction with calcitonin receptors and not specific CGRP receptors (Goltzman and Mitchell, 1985). In contrast to this observation, Geppetti et al. (1989b) demonstrated that CGRP, but not calcitonin, increased cAMP accumulation in the papilla of the rat kidney. As well, CGRP caused significantly longer elevation of renal cAMP elevation than did calcitonin (Zaidi et al., 1990c). These latter observations rule out the possibility that adenylyl cyclase stimulation by CGRP is mediated via calcitonin receptors.

1.10.3. Renal effects of CGRP

CGRP caused renal vasodilatation in dogs and rats (Villarreal et al., 1988; Gardiner et al., 1989, Amuchastegui et al., 1994), man and rabbits (Bauerfiend et al., 1985; Kurtz et al., 1989a). In the isolated perfused rat kidney, CGRP reduced peripheral vascular resistance and increased glomerular filtration rate as well as renal Na^+ excretion ((Kurtz et al., 1989b, Chin et al., 1994). In healthy human subjects, CGRP increased the fractional excretion of Na^+ and chloride but did not alter glomerular filtration rate (Gnaedinger et al., 1989). CGRP increased the secretion of renin *in vivo* (Itabashi et al., 1988; Kurtz et al., 1988; Murakami et al., 1991) and *in vitro* (Kurtz et al., 1988) and release of atrial natriuretic peptide, but inhibited the secretion

of aldosterone both *in vitro* and *in vivo* (Murakami et al., 1989; 1991).

CGRP also affects the motility of the lower urinary tract. It increases the motility of the urinary bladder but inhibits the motility of the rat isolated proximal urethra and ureters and counteracts the contractile response to neurokinins (Maggi et al., 1987).

The kidney is a major site for the clearance of exogenously-infused CGRP (Braslis et al., 1988). Rubinstein et al. (1993) showed that CGRP has a half-life of about 64 min in the filtering isolated perfused rat kidney. The blockade of filtration by elevating the osmolality of the perfusate abolishes CGRP degradation. These investigators concluded that renal CGRP degradation occurs in the renal tubules after glomerular filtration, and that there was no active CGRP degradation in the capillary endothelium.

1.11. Adrenomedullin

Adrenomedullin is a novel vasoactive peptide that was isolated, purified and identified from human pheochromocytoma arising from the adrenal medulla (Kitamura et al., 1993a,b). RNA blot analysis has shown that rat adrenomedullin mRNA is widely expressed in tissues from the adrenal glands, lungs, kidneys, heart, spleen, duodenum and submandibular glands (Sakata et al., 1993). Although the concentrations of adrenomedullin in the lungs, ventricles and kidneys were less than 3% of that in the adrenal medulla, the amount of peptide synthesized in these tissues was probably higher, since adrenomedullin biosynthesized in these tissues was rapidly released into the circulation instead of stored in granules, as in the adrenal medulla (Kitamura et al., 1993 b; Ichiki et al., 1994). Adrenomedullin is also present over a considerable

concentration (range from 3 to 19 fmol/ml) in human plasma (Kitamura et al., 1993a; 1994).

Adrenomedullin has been shown to produce a rapid-onset and long-lasting hypotension (Kitamura et al., 1993a; Sakata et al., 1993; Perret et al., 1993) due to reduced total peripheral resistance (Ishiyama et al., 1993). In methoxamine-precontracted perfused rat mesenteric artery pre-treated with guanethidine, adrenomedullin caused long-lasting vasodilatation and reduction in perfusion pressure through a non-cholinergic, non-adrenergic mechanism (Nuki et al., 1993). In conscious rats, adrenomedullin induced dose-dependent vasodilatation in renal, mesenteric and hindquarter vascular beds (Gardiner et al., 1995). However, cerebro-ventricular or cisternal injections of adrenomedullin elicited centrally-induced vasopressor response, tachycardia and increases in sympathetic outflow (Takahashi et al., 1994).

Adrenomedullin consists of 52 amino acids in human and 50 amino acids in rat and it shows homology in chemical structure with CGRP (Kitamura et al., 1993a,b). The intracellular mechanisms underlying the vasorelaxant effect of adrenomedullin and CGRP are similar, as both peptides increase cAMP concentration in vascular smooth muscle cells (Kubota et al., 1985; Eguchi et al., 1994; Ishizaka et al., 1994). The CGRP receptor antagonist CGRP(8-37) blocked the elevation by adrenomedullin of cAMP level in cultured rat vascular smooth muscle cells (Eguchi et al., 1994), attenuated the vasodilator effect of adrenomedullin in rat perfused mesenteric bed (Nuki et al., 1993), and inhibited its centrally-induced vasopressor response

(Takahashi et al., 1994). The aforementioned evidence suggests that both adrenomedullin and CGRP may be sharing the same receptor. However, Ishizaka et al., (1994) reported that the binding of [¹²⁵I]adrenomedullin in vascular smooth muscle cells was inhibited by adrenomedullin but not CGRP, suggesting that adrenomedullin is acting through its own specific receptors. Moreover, the vasodilatations induced by adrenomedullin in the renal, mesenteric and hindquarter beds were not affected by CGRP(8-37)(Gardiner et al., 1995).

1.12. Aims of the study

1.12.1. Renal vascular and tubular actions of CGRP

The existence of CGRP sensory nerve fibres and CGRP receptors in the kidney, and the coupling of the receptors to adenylyl cyclase suggest a role for CGRP in the regulation of renal microcirculation, electrolyte transport and water homeostasis. There is a lack of information on the effects of CGRP on renal haemodynamics and renal excretion. The initial goal of this study was to characterize the dose-renal vascular and tubular effects of CGRP.

1.12.2. Indirect mechanisms mediating renal actions of CGRP

CGRP has direct as well as indirect actions via modulation of the release of neurotransmitters and hormones. The effector mechanisms involved are likely dependent on the dose, route of administration and experimental conditions. We attempted to first elucidate the renal vascular and tubular effects of CGRP by renal arterial injection as well as infusion of CGRP, and second the mechanisms underlying the renal actions of CGRP via renal arterial injection of a specific antagonist or

inhibitor of various endogenous vasopressor and vasodepressor systems as follows:

- a. L-NAME to inhibit nitric oxide synthase
- b. Losartan to block angiotensin AT1 receptors
- c. Phenoxybenzamine and mecamylamine to block the sympathetic autonomic ganglia and α -adrenoceptors
- d. Indomethacin to block prostanoid synthesis

1.12.3. CGRP receptor-subtypes mediating renal actions of CGRP

Evidence suggests the existence of heterogeneous CGRP receptors in effector sites but subclassification of these receptors is hampered by a lack of selective antagonists to the putative CGRP2 receptors. The present study investigated the vascular and tubular effects of renal arterial administration of the yet uncharacterized CGRP antagonist [tyr⁰]CGRP(28-37) and the CGRP1 receptor antagonist CGRP(8-37).

1.12.4. Renal action of adrenomedullin

It has been suggested that adrenomedullin, a novel peptide which shares homology with CGRP, mediates its actions via the activation of CGRP receptors. Studies were undertaken to compare the renal vascular and tubular effects of renal arterial injections of adrenomedullin with those of CGRP. The effects of CGRP receptor antagonists [tyr⁰]CGRP(28-37) and CGRP(8-37) on renal vascular and tubular actions of renal arterially infused adrenomedullin were also examined.

2. MATERIALS AND METHODS

2.1. Rats

Male Sprague-Dawley rats (280-400 g) were used in this study. All the animals were from the Animal Care Center of the University of British Columbia. Rats were housed six rats per cage and allowed free access to Purina Rat Chow and water. The recommendations of the Canada Council of Animal Care and internationally accepted principles in the care and use of experimental animals were followed.

2.2. Surgical procedure

The rats were anaesthetized with Inactin (100 mg/kg, i.p.). A rectal thermometer and a heating pad connected to a Thermistemp Temperature Controller (Model 71; Yellow Springs Instrument Co. Inc., Ohio) were used to maintain body temperature at 37.5°C. The left femoral artery was cannulated for the continuous measurement of mean arterial pressure (MAP) with a Statham pressure transducer (Model P23 DB, Gould Statham, CA). Heart rate (HR) was derived electronically from the upstroke of the arterial pulse pressure with a tachograph (model 7P4G, Grass, MA). MAP and HR were monitored by a Grass polygraph (model RP57C8, Grass, MA). Cannulae were inserted into the left femoral vein for the administration of ⁵¹Cr-EDTA solution (i.v. bolus of 13.8 µCi in 1.75 ml over 2 min followed by infusion of 0.16 µCi/min at 20 µl/min) (Stacy and Thorburn, 1966; Leyssac et al., 1991) and into the right femoral artery for blood sampling (0.5 ml per sample, each sample replaced by

injection of 1 ml normal saline). The abdominal cavity was opened through a ventral midline incision. The right suprarenal artery was located and its origin from the renal artery verified. A tapered PE-10 tube was inserted retrogradely into the suprarenal artery as described by Smits et al. (1983), and connected to a syringe pump (SAGE 341A, TX) for the infusion of drugs. A transonic flow probe (Model 1RB630, Transonic) connected to a flowmeter (Model T206, Transonic, NY) was placed around the right renal artery for continuous measurement of renal blood flow. A piece of PE-10 cannula (<15 cm length or <10 μ l dead space) was inserted into the right ureter for the collection of urine at 10 min intervals in pre-weighed, closed vials which contained a small hole in the vial cap to allow the passage of the catheter. The rats were allowed a 1 h stabilization period after surgery before the study began. All studies involve six blood and urine collections periods at cycles of 15 min unless specified otherwise (as in Sections 2.4.1.2 and 2.4.8).

A blood sample was taken at the end of the stabilization period and after the completion of the study to monitor changes in haematocrit, plasma osmolality and levels of Na^+ and K^+ during the course of the study.

2.3. Treatment of samples

The samples were prepared immediately after collection to avoid evaporation. Blood samples were centrifuged for 5 min to separate the plasma from cells. Urine volume was measured gravimetrically. ^{51}Cr EDTA concentrations were determined using a gamma counter (1185 series dual channel, Nuclear-Chicago, IL)). Urine Na^+ and K^+ concentrations were measured by flame photometry (Model IL143, Fisher

Scientific, MA). Urine osmolality was measured with a vapour pressure osmometer (Model 5500, WESCOR, Utah).

2.4. Experimental protocol

2.4.1. Preliminary studies

2.4.1.1. Time and vehicle effect

Vehicle was continuously infused into the renal artery. Blood and urine samples were taken every 10 min for six consecutive sampling periods with five min interval between each sampling period to detect possible time-related changes in renal haemodynamics and renal excretion during the course of the study.

2.4.1.2. Time to attain steady state responses to CGRP

To ascertain that a steady state response could be attained following 10 min infusion of CGRP, either a low (0.3 pmol/kg/min) or a high (300 pmol/kg/min) dose of CGRP was infused for 20 min into two groups of rats ($n = 3$ per group). Following equilibration, two control samplings of 10 min duration each were taken. Afterwards, a single dose of CGRP was infused for 20 min. Blood and urine samples were again taken at 10 min intervals. After stopping CGRP infusion, responses were allowed to recover and blood and urine samplings were again taken immediately after renal blood flow returned to normal.

2.4.2. Determination of the renal actions of CGRP

Rats were randomly divided into two groups ($n = 8$ each). In the first group, after two control sampling periods, single doses of CGRP (0.3-300 pmol/kg/min) were

infused into the renal artery for 10 min, each dose being followed by a recovery period of 5 min. In the second group (vehicle-control), an equal volume of vehicle (0.45% NaCl) was continuously infused instead of CGRP. Blood was sampled at 10 min after the start of drug infusion whereas urine collection began from 3 till 13 min after the start of drug administration. The later collection time of urine allowed extra time for the equilibration of drug responses and the drainage of urine from the nephron to the ureter and collecting catheter. Blood and urine samples were also taken at the same time-points in the vehicle time-control rats.

2.4.3. Effect of L-NAME on renal actions of CGRP

Five groups of rats ($n = 6-8$ each) were used. A bolus dose of L-NAME ($2 \mu\text{mol/kg}$) was injected into the renal artery in two groups after the first sampling period. This was followed by infusion of CGRP ($0.3-300 \text{ pmol/kg/min}$; 10 min each dose) or an equal volume of vehicle (0.45% NaCl) after the second sampling. Another two groups were treated similarly but instead given a high dose of L-NAME ($20 \mu\text{mol/kg}$). In the last group, phenylephrine (50 nmol/kg/min) was continuously infused into the renal artery after the first sampling period and this group served as positive control for the low dose L-NAME group to find out if the L-NAME induced changes were due to renal vasoconstriction or the inhibition of nitric oxide synthase. In a previous study, phenylephrine constricted the renal vasculature (Elhawary et al., 1992) and reduced urine flow and urine Na^+ concentration by 55 and 44% of control, respectively (Elhawary and Pang, 1994), without altering MAP or HR. Single doses of CGRP ($0.3-300 \text{ pmol/kg/min}$) were infused into rats receiving phenylephrine as described for the

L-NAME-treated groups. Blood and urine samplings were also taken as described before (see Section 2.4.1.1).

2.4.4. Effect of losartan on renal actions of CGRP

Five groups of rats ($n = 6$ each) were used. A bolus dose of losartan ($0.3 \mu\text{mol/kg}$) was injected into the renal artery of two groups of rats after the first sampling period. This was followed by infusion of either CGRP ($0.3\text{--}300 \text{ pmol/kg/min}$; 10 min each dose) or an equal volume of vehicle ($0.45\% \text{ NaCl}$) after the second sampling. Another two groups were treated similarly but were instead given a high dose of losartan ($3 \mu\text{mol/kg}$). In the fifth group, sodium nitroprusside (50 nmol/kg/min) was continuously infused into the renal artery after the first sampling period and this group served as positive control for the losartan to find out if changes caused by losartan were due to reduced perfusion pressure or blockade of angiotensin AT1 receptors.

2.4.5. Effect of phenoxybenzamine and mecamylamine on renal actions of CGRP

Four groups of rats ($n=6$ each) were used. A bolus dose of phenoxybenzamine ($3 \mu\text{mol/kg}$) was injected into the renal artery of two groups after the first sampling period. This was followed by the infusion of CGRP ($0.3\text{--}300 \text{ pmol/kg/min}$; 10 min each dose) or an equal volume of vehicle ($0.45\% \text{ NaCl}$) after the second sampling. In radioligand binding assays, the dose of POB used was shown to abolish all α_1 -adrenoceptors and one third of α_2 -adrenoceptors (Smyth et al., 1984; Elhawary et al., 1991). Another two groups were treated similarly except that mecamylamine ($1 \mu\text{mol/kg}$) was injected in place of POB.

2.4.6. Effect of indomethacin on renal actions of CGRP

Two groups of rats ($n = 6$ each) were renal arterially injected with indomethacin ($3 \mu\text{mol/kg}$) at the beginning of the second sampling period followed by renal arterial infusion of CGRP ($0.3\text{--}300 \text{ pmol/kg/min}$) or an equal volume of vehicle at the third sampling period.

2.4.7. Effect of CGRP(8-37) and $[\text{tyr}^0]\text{CGRP}(28\text{--}37)$ on renal actions of CGRP

Rats were randomly divided into eight groups ($n = 6$ each). Two groups received renal injections of a low dose (1 nmol/kg) of CGRP(8-37) after the first sampling period and another two groups were given a high dose (10 mg/kg) of CGRP(8-37). The injections of the antagonists were followed by infusion of 20% of the bolus dose every hour ($15 \mu\text{l/min}$) for the remainder of the experiment. The two low dose CGRP(8-37) groups were infused with either CGRP ($0.3\text{--}300 \text{ pmol/kg/min}$) or an equal volume of vehicle ($0.45\% \text{ NaCl}$) after the second sampling period and likewise with the two high dose CGRP(8-37) groups.

Another four groups were treated similarly but were instead given $[\text{tyr}^0]\text{CGRP}(28\text{--}37)$ in doses of 3 or 30 nmol/kg . Blood and urine samples were taken at the same time-points as described before (see Section 2.4.1.1).

2.4.8. Renal actions of adrenomedullin

The rats were randomly divided into three groups ($n = 8$ each). In two groups, after two baseline periods of 10 min duration each, single doses (0.001 , 0.01 , 0.1 and 1 nmol/kg) of either adrenomedullin or CGRP were given as bolus injections into the renal artery at dose-intervals of 20 min. The third group (time and vehicle control)

received renal arterial injections of the vehicle (0.45% NaCl) at intervals of 20 min after two baseline periods of 10 min duration. Renal blood flow (RBF), MAP and HR measurements were taken at the time of peak RBF responses (0.5-1.5 min after i.a. bolus) to each drug. Urine samples were collected from 3 till 13 min after drug or vehicle injection. A blood sample was also taken at the end of the stabilization period and after the completion of the study to monitor possible changes in any renal or blood parameters during the course of the study.

2.4.9. Effect of CGRP receptor antagonists on renal actions of adrenomedullin

Rats were randomly divided into nine groups (n = 6 each). After two control sampling periods, single doses of adrenomedullin (0.001-1 nmol kg/ min) were infused into one group for 10 min each dose followed by a recovery period of 5 min. Another two groups each received renal arterial injections of either a low (1 nmol/kg) or a high dose (10 nmol/kg) of CGRP(8-37) after the first sampling period. This was followed by infusion of 20% of the bolus dose every hour (15 µ/min) for the remainder of the experiment. The two low dose CGRP(8-37) groups were infused with either adrenomedullin (0.001-1 nmol/kg/min) or an equal volume of vehicle (0.45% NaCl) after the second sampling period and likewise for the two high dose CGRP(8-37) groups. Four other groups were given either a low (3 nmol/kg) or a high (30 nmol/kg) dose of [tyr⁰]CGRP(28-37) instead of CGRP(8-37). Blood and urine samples were also taken at the same time-points as in the time and vehicle groups as described in Section 2.4.1.1.

2.5. Materials

Inactin (thiobarbituric acid) was obtained from BYK Gulden Konstanz (Germany). Adrenomedullin 11-50 (rat) and [tyr⁰]CGRP(28-37) were purchased from Peninsula Lab. Inc. (Belmont, CA). Rat α -CGRP, rat CGRP(8-37), phenylephrine, mecamylamine, indomethacin and L-NAME were obtained from the Sigma Chemical Co. (St. Louis, MO). Sodium nitroprusside was obtained from Fisher Scientific Co. (NJ). Losartan was a gift from Du Pont Pharmaceutical (Delaware). Phenoxybenzamine was from Research Biochemicals International (Natick, MA). ⁵¹Cr-EDTA was obtained from Amersham International (UK) and was solubilized in 0.9% NaCl solution. Indomethacin was dissolved in 80% ethanol then diluted in 0.45% NaCl solution. All other drugs were dissolved in 0.45% NaCl solution.

2.6. Calculations

Renal arterial conductance (RBF/MAP) was computed to normalize flow independent of changes in MAP. Glomerular filtration rate (GFR) was calculated as the ratio of urine to plasma concentration of ⁵¹Cr EDTA multiplied by urine flow rate. Urine Na⁺ and K⁺ excretion rates were estimated by the product of ionic concentration and urine flow. Fractional Na⁺ excretion was calculated by the percentage of ratio of urine excretion to plasma concentration of Na⁺ divided by GFR.

2.7. Statistical analysis

The animals were randomly assigned into groups within each experimental design. Experiments normally consist of n = 6 except when indicated otherwise. All

data are expressed as mean \pm S.E. The results were analyzed by the analysis of variance block design for the comparison of data within the same group and random design for the comparison of data among groups. This was followed by Duncan's multiple range test with $p < 0.05$ selected as the level of statistical significance (see Zar, 1984).

3. RESULTS

3.1. Time Effect

In the time-control group, continuous infusion of the vehicle caused insignificant changes in MAP, HR, RBF, GFR, urine flow, urinary excretion of Na^+ and K^+ and urine osmolality (Table 1).

3.2. Time-course of responses to CGRP

Following equilibration for 1h, all measured parameters were identical at 10 and 20 min prior to the renal arterial infusion of either a low or a high dose of CGRP in two groups of rats (Table 2). The low dose of CGRP increased RBF and GFR at 10 as well as 20 min after the start of infusion but did not significantly alter other variables. In contrast, the high dose of CGRP reduced RBF and increased urine flow, and the excretion rates of Na^+ and K^+ ; however, the increases in K^+ excretion were not statistically significant at either the 10 or 20 min post-infusion periods. No other parameters were altered by the high dose of CGRP. These responses elicited by the continuous infusion of the low or high dose of CGRP were not qualitatively different from the corresponding responses obtained with the infusion of single doses of CGRP in the main part of the study (see later). The increase and decrease in RBF elicited by the low and high dose of CGRP have rapid onsets and reached plateau values within 3-4 and 1-1.5 min, respectively. Since similar vascular and renal readings (SP1 and SP2) were obtained at 10 and 20 min following the start of infusion of either the low or high dose of CGRP, plateau responses could be attained after 10 min infusion of

CGRP. Recovery of the RBF response took 4-6 min and 12-15 min, respectively, after the termination of infusion of the low and the high dose of CGRP. At these recovery times, all other parameters also returned to control levels (Table 2).

	SP1	SP2	SP3	SP4	SP5	SP6
MAP	108±2	109±3	109±4	108±4	108±5	107±4
HR	400±19	402±21	403±20	408±22	427±26	433±27
RBF	11.1±1.1	11.3±1.2	11.2±1.3	10.8±1.5	10.8±1.5	10.4±1.7
GFR	0.97±0.09	0.94±0.13	0.86±0.08	0.87±0.06	0.84±0.07	0.86±0.08
V	4.6±0.5	4.7±0.4	4.8±0.6	5.3±0.7	5.2±0.5	4.9±0.6
U_{Na}V	239±84	284±97	348±115	408±110	411±127	422±153
U_KV	1045±160	1113±111	1140±179	1121±241	1046±205	956±238
U_{OSM}	1421±236	1424±227	1360±222	1363±216	1285±151	1237±130

Table 1: Effects (mean ± S.E.) of vehicle (0.45% NaCl) on mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), glomerular filtration rate (GFR, ml/min), urine flow (V, µl/min), urinary Na⁺ and K⁺ excretion (U_{Na}V and U_KV, nEq/min) and urine osmolality (U_{OSM}, mOsm/kg) during the six sampling periods (SP1 to SP6, 10 min) in actin-anaesthetized rats (n = 6).

	CGRP (0.3 pmol/kg/min)					CGRP (300 pmol/kg/min)				
	C1	C2	SP1	SP2	R	C1	C2	SP1	SP2	
MAP	104±3	103±3	102±4	103±3	104±5	102±3	102±3	97±8	92±9	1
HR	385±14	390±12	396±19	395±17	402±21	384±14	392±12	432±21	442±27	4
GFR	0.99±0.1	0.96±0.08	1.34±0.09*	1.23±0.11*	0.94±0.05	0.95±0.1	1.06±0.08	1.02±0.04	0.94±0.07	0.9
RBF	10.4±0.6	10.1±0.5	12.9±0.6*	12.5±0.7*	10.6±0.8	10.7±0.6	10.2±0.5	7.6±0.4*	6.8±1.5*	10
V	4.8±0.6	4.7±0.5	5.1±0.7	4.7±0.4	4.3±0.6	4.5±0.6	4.7±0.5	8.5±2.1*	8.2±1.5*	4
U_{Na}V	247±76	259±67	267±64	288±97	331±75	237±78	259±68	770±185*	711±127*	3
U_KV	1047±251	998±195	1159±150	1113±111	1009±254	1043±231	1088±192	1566±287	1646±205	10

Table 2: Values (mean ± S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), glomerular filtration rate (GFR, ml/min), urine flow (V, µl/min) and urinary Na⁺ and K⁺ excretion rate (U_{Na}V, µmol/min and U_KV, µmol/min) at baseline condition (C1 and C2), 10 and 20 min during infusion of CGRP (SP1, SP2) and after the renal blood flow response (R) to CGRP in Inactin-anaesthetized rats (n = 3 for each dose of CGRP). *Significant difference from the corresponding baseline reading C2.

3.3. Renal actions of CGRP

3.3.1. Effects of CGRP on MAP, HR, RBF, conductance and GFR

Table 3 shows the baseline values of MAP, HR, renal haemodynamics and electrolyte excretion prior to the infusion of CGRP, or vehicle in two groups. Baseline values at sampling period 1 (SP1) and sampling period 2 (SP2) were obtained after continuous infusion of the vehicle (0.45% NaCl).

The vehicle did not alter MAP or HR (Fig. 1A,B). Renal arterial infusion of CGRP caused insignificant changes in MAP and HR (Fig. 1A,B). The lowest two doses of CGRP increased RBF and renal arterial conductance indicating renal vasodilatation, whereas the highest dose reduced RBF and conductance indicating vasoconstriction (Fig. 2A,B). GFR was increased by the two lowest doses of CGRP but was not affected by the two highest doses (Fig. 2C).

3.3.2. Effects of CGRP on urinary flow, osmolality, Na⁺ and K⁺

CGRP did not affect urine osmolality but dose-dependently ($p < 0.05$) increased urine flow (Fig. 3A,B) and Na⁺ excretion (Fig. 4A), which were significantly different from the corresponding time-control readings at the highest two doses of CGRP. Fractional excretion of Na⁺ was also increased (baseline value 0.13% and 0.35, 0.45 and 0.57% after the second, third and fourth doses of CGRP, respectively). CGRP increased K⁺ excretion, which was significantly different from the time-control reading at the highest two doses of CGRP (Fig. 4B) but did not alter fractional excretion of K⁺ at any dose (results not shown).

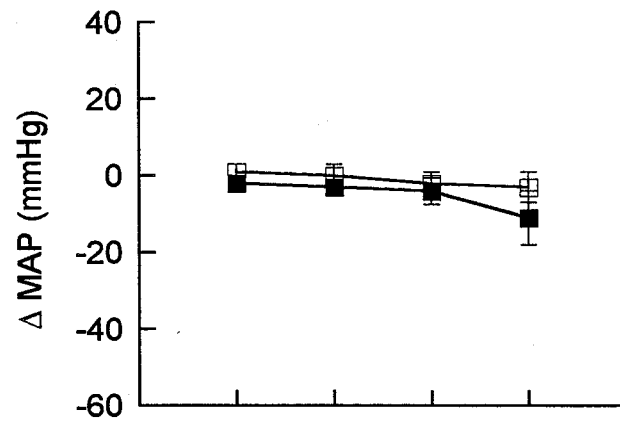
The haematocrit ($46 \pm 2\%$), plasma values of osmolality (295 ± 3 mOsm/kg), Na⁺

(136 ± 4 mEq/L) and K^+ (3.5 ± 0.4 mEq/L) at the end of the experiments were similar to the corresponding values at the end of the stabilization period.

GROUP	SP	MAP	HR	RBF	COND	GFR	V	U _{Na} V	U _K V
Veh.: veh.	SP1	108±2	400±19	11.1±1.1	0.102±0.009	0.97±0.09	4.6±0.5	239±84	1045±16
	SP2	109±3	402±21	11.3±1.0	0.103±0.009	0.94±0.13	4.7±0.4	283±97	1113±11
Veh.: CGRP	SP1	104±4	364±12	11.4±0.5	0.109±0.004	0.97±0.09	4.5±0.6	199±45	978±13
	SP2	103±3	373±13	11.2±0.6	0.109±0.005	0.94±0.10	4.9±0.5	187±57	1017±6

Table 3: Baseline values (mean ± S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine flow rate (V, ml/min), urine Na⁺ (U_{Na}V, nEq/min), urine K⁺ (U_KV, nEq/min) and urine osmolality (U_{OSM}, mOsm/kg) following the first and second sampling (SP2) periods which consist of 10 min each of *i.a.* infusion of vehicle (0.45% NaCl) in two groups (each) of Inactin-anaesthetized rats.

A



B

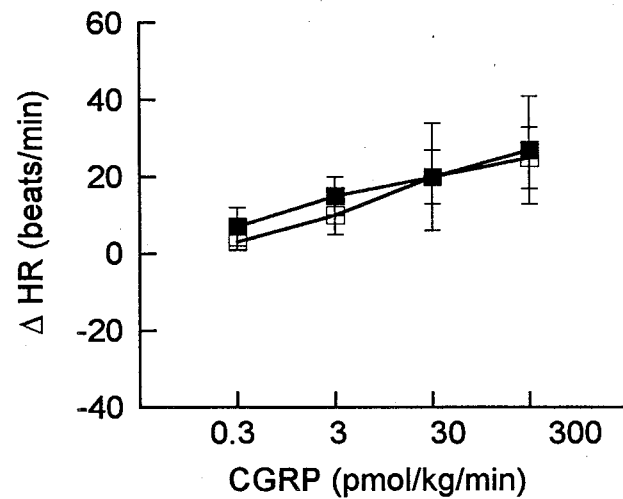


Figure 1: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) or equal volume of vehicle (0.45% NaCl solution, □) on mean arterial pressure (MAP, A) and heart rate (HR, B) in Inactin-anaesthetized rats (n = 8 per group). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

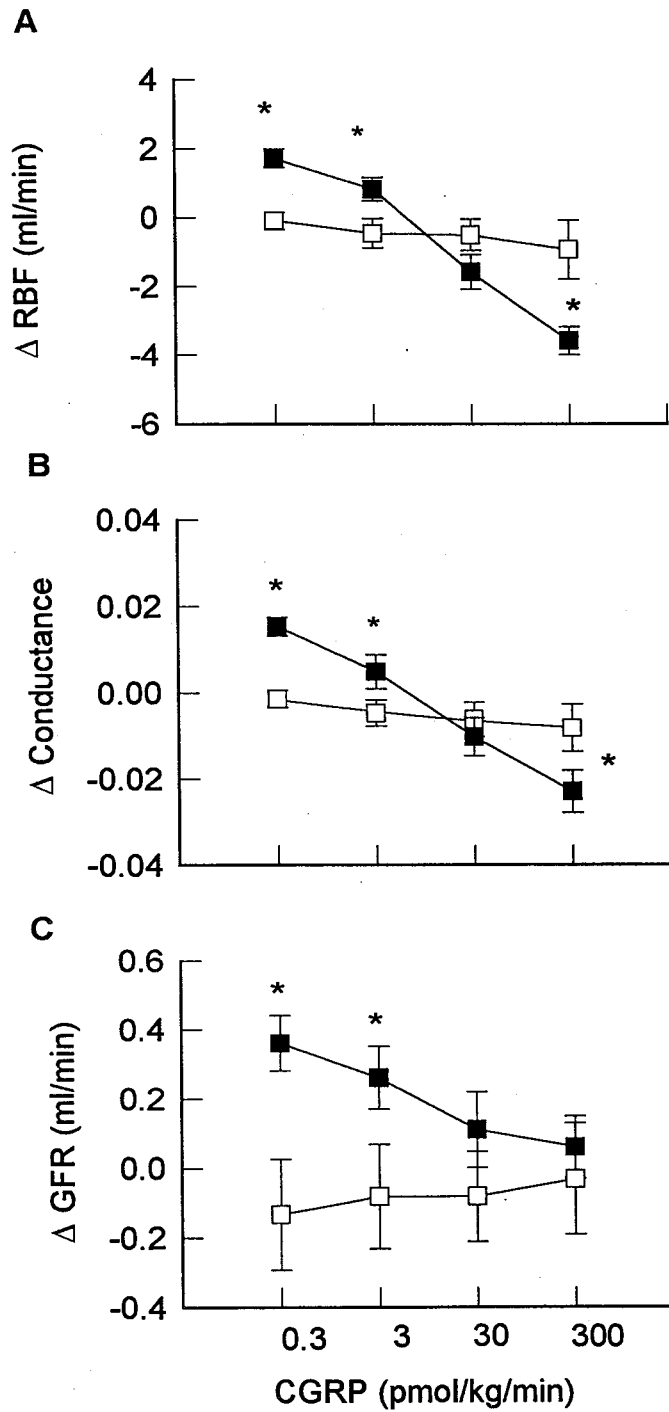


Figure 2: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, \blacksquare) and equal volume of vehicle (0.45% NaCl solution, \square) on renal blood flow (RBF, A), renal arterial conductance (B) and glomerular filtration rate (GFR, C) in Inactin-anaesthetized rats (n = 8 per group). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

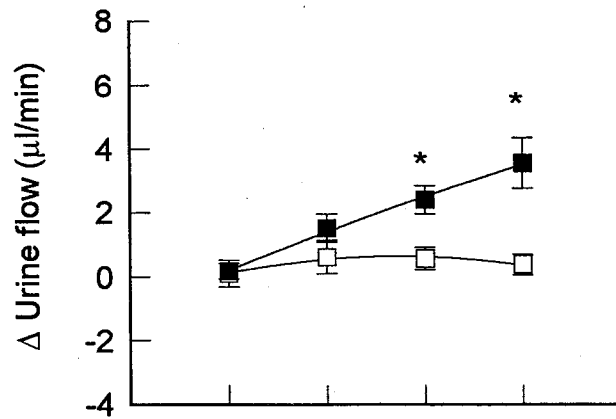
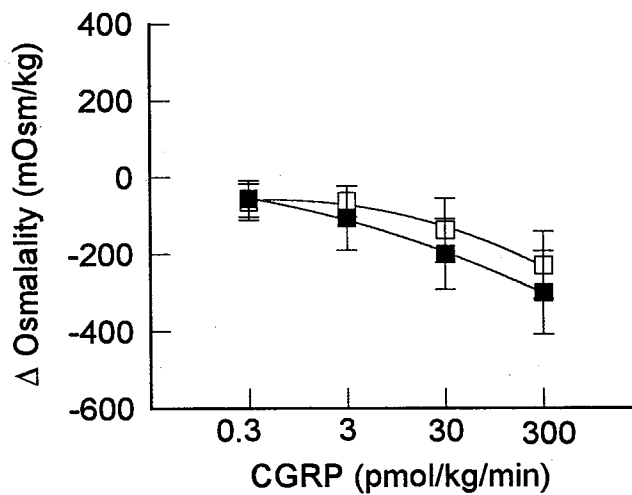
A**B**

Figure 3: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on urine flow (A) and urine osmolality (B) in Inactin-anaesthetized rats ($n = 8$ per group). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

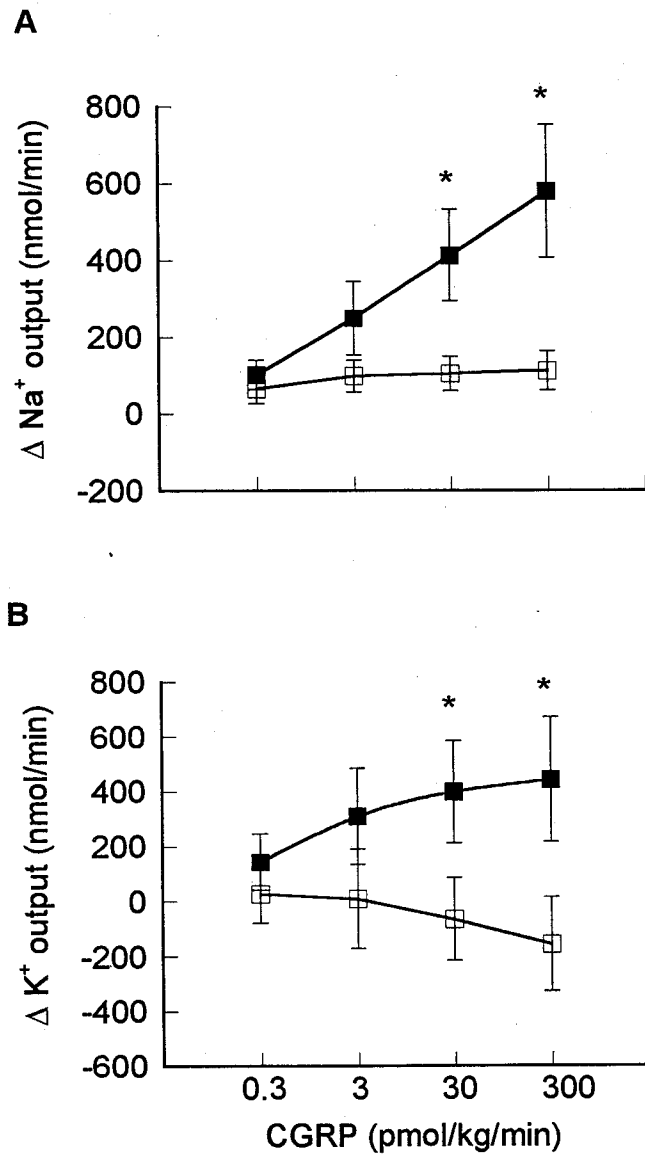


Figure 4: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on Na⁺ (A) and K⁺ (B) excretion in Inactin-anaesthetized rats (n = 6-8 per group). *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

3.4. Effect of L-NAME on renal actions of CGRP

Table 4 shows the baseline values of MAP, HR, renal haemodynamics and electrolyte excretion prior to the infusion of CGRP or the vehicle in four groups. Baseline values at sampling period 1 (SP1) were obtained after the infusion of the vehicle and sampling period 2 (SP2) values were obtained after the injection of the low or the high dose of L-NAME. Only the high dose of L-NAME significantly increased MAP. Both doses of L-NAME did not alter HR or urine osmolality but decreased RBF and conductance, GFR, urine flow, and Na⁺ and K⁺ excretion (Table 4).

Following treatment with the high (Fig. 5A,B) but not the low (Fig. 5C,D) dose of L-NAME, CGRP reduced MAP and increased HR at the highest dose. In the presence of both the low and the high dose of L-NAME, the renal vasodilatation effect of CGRP was completely abolished but the vasoconstriction effect was unchanged (Fig. 6B,D). The increases in GFR were attenuated by the low dose and abolished by the high dose of L-NAME (Fig. 7A,B).

In rats pre-treated with either the low or the high dose of L-NAME, CGRP had no effect on urine osmolality (Fig. 8B,D) but increased urine flow (Fig. 8A,C) and Na⁺ and K⁺ excretion (Fig. 9) suggesting that these effects are unrelated to NO biosynthesis. Fractional excretion of Na⁺ was increased from 0.11% (baseline) to 0.33, 0.47 and 0.50%, respectively, after the last three doses of CGRP in rats pre-treated with the low dose of L-NAME and from 0.16% to 0.71, 0.70 and 0.99% in rats pre-treated with the high dose of L-NAME. Fractional excretion of K⁺ was unaltered by CGRP in the presence of either the low or the high dose of L-NAME (results not

shown).

3.5. CGRP effects in phenylephrine-precontracted kidneys

Renal arterial infusion of phenylephrine (50 nmol/kg/min) did not alter MAP and HR but significantly reduced RBF, renal arterial conductance, urine flow and excretion of Na^+ and K^+ , and insignificantly reduced GFR (Table 5). These effects of phenylephrine are quantitatively but not qualitatively different from those of the low dose L-NAME.

In kidneys pre-constricted with phenylephrine, CGRP significantly increased RBF and GFR at the two lowest doses and increased renal arterial conductance at the lowest three doses. A comparison of these results with those of CGRP in control rats (Fig. 2) and those of the low dose L-NAME-treated rats (Fig. 6A,B; 7A) show that the renal vasodilatation effect of CGRP is suppressed by L-NAME but potentiated by phenylephrine.

In phenylephrine-treated rats, CGRP also significantly and dose-dependently increased urine flow and absolute renal excretion of Na^+ and K^+ at all doses (Tab. 5). Previously, the lowest dose of CGRP altered neither urine flow nor excretion of Na^+ or K^+ in kidneys pre-constricted with L-NAME (Fig. Fig. 8A, 9A, 9B). The highest dose of CGRP also caused significantly ($p < 0.05$) greater diuresis and excretion of Na^+ and K^+ in kidneys pre-constricted with phenylephrine than those constricted with L-NAME since urine flow and excretion of Na^+ and K^+ were increased to 3.7-, 6.9- and 3.5-fold of control values by the highest dose of CGRP in phenylephrine-treated rats and to 1.9-, 5.2- and 1.6-fold of controls by the highest dose of CGRP in L-NAME-treated

rats. Exaggerated diuresis and excretion of Na^+ and K^+ at low as well as high doses of CGRP in kidneys pre-constricted with phenylephrine and not L-NAME suggests that the inhibition of NO biosynthesis attenuates the diuretic, natriuretic and kaliuretic effects of CGRP.

GROUP	SP	MAP	HR	RBF	COND	GFR	V	U _{Na} V
L-NAME 2 μ mol/kg: Veh.	SP1	103 \pm 4	367 \pm 11	13.1 \pm 0.5	0.127 \pm 0.005	0.98 \pm 0.17	4.8 \pm 0.4	228 \pm 37
	SP2	106 \pm 5	368 \pm 13	9.3 \pm 0.5*	0.089 \pm 0.006*	0.63 \pm 0.14*	3.6 \pm 0.5*	137 \pm 22*
L-NAME 2 μ mol/kg: CGRP	SP1	102 \pm 4	353 \pm 8	11.8 \pm 0.8	0.117 \pm 0.007	1.04 \pm 0.05	3.9 \pm 0.4	189 \pm 37
	SP2	106 \pm 6	350 \pm 9	8.0 \pm 0.7*	0.076 \pm 0.007*	0.76 \pm 0.09*	3.0 \pm 0.2*	110 \pm 18*
L-NAME 20 μ mol/kg: Veh.	SP1	108 \pm 7	370 \pm 9	11.5 \pm 0.7	0.106 \pm 0.004	1.02 \pm 0.12	5.4 \pm 0.7	329 \pm 87
	SP2	136 \pm 6*	361 \pm 11	7.2 \pm 0.3*	0.053 \pm 0.003*	0.61 \pm 0.14*	3.4 \pm 0.5*	135 \pm 67*
L-NAME 20 μ mol/kg: CGRP	SP1	106 \pm 2	392 \pm 8	11.7 \pm 0.5	0.110 \pm 0.005	0.91 \pm 0.21	5.2 \pm 0.3	253 \pm 39
	SP2	143 \pm 5*	393 \pm 16	7.9 \pm 0.4*	0.056 \pm 0.005*	0.64 \pm 0.11*	3.5 \pm 0.3*	142 \pm 45*

Table 4: Baseline values (mean \pm S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine Na⁺ (U_{Na}V, nEq/min), urine K⁺ (U_KV, nEq/min) and urine osmolality (U_{OSM}, mOsm/kg) following first sampling period (SP1, 10 min of renal arterial infusion of vehicle, 0.45% NaCl) and the second sampling period (SP2, 10 min of renal arterial injection of L-NAME, 2 or 20 μ mol/kg) in four groups (n = 6-8 each) of Inactin-anaesthetized rats. * indicates significant difference from the corresponding control group.

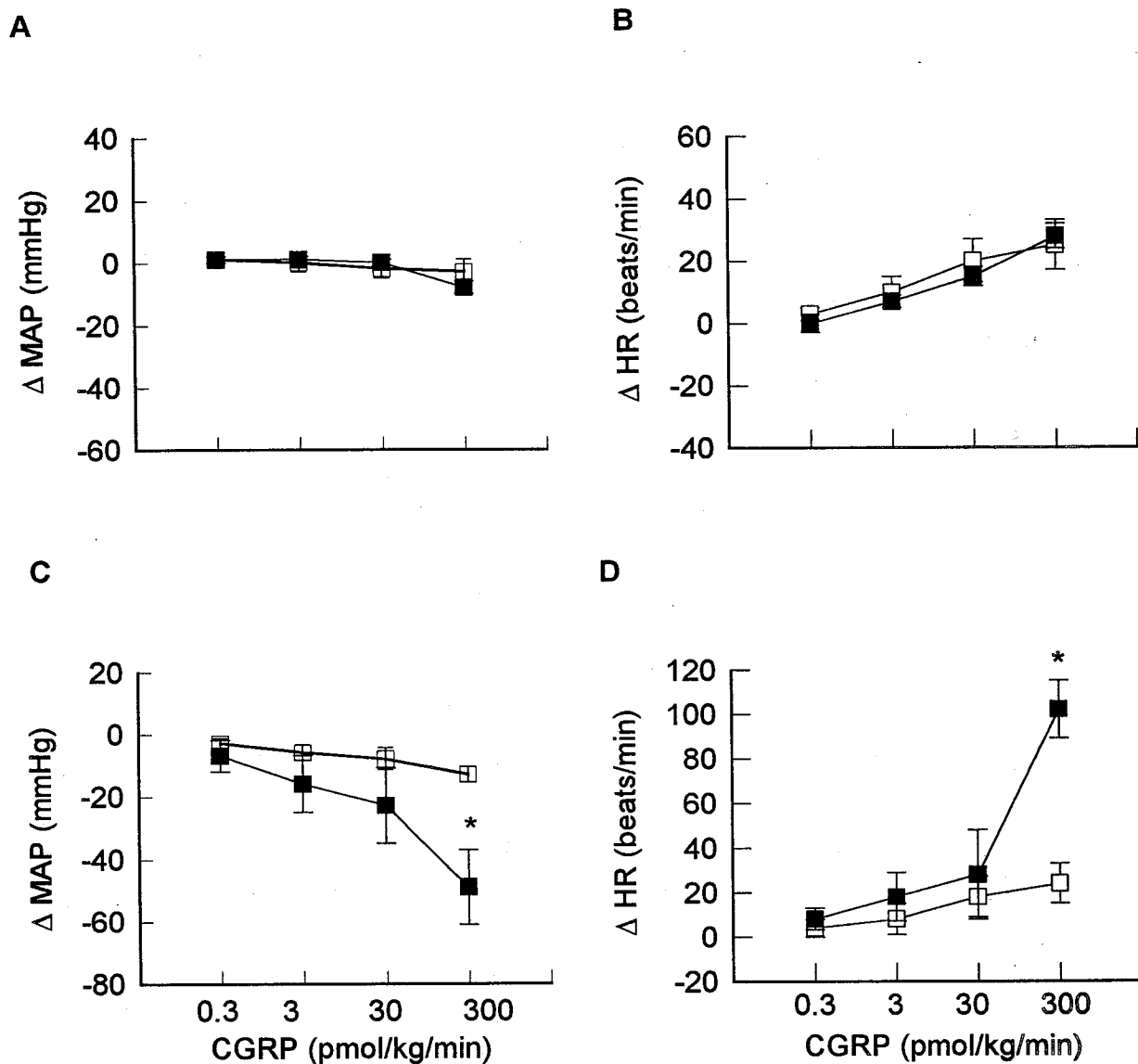


Figure 5: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) or equal volume of vehicle (0.45% NaCl solution, □) on mean arterial pressure (MAP) and heart rate (HR) in Inactin-anaesthetized rats ($n = 6-8$ per group) pre-treated with 2 μ mol/kg (A, B) or 20 μ mol/kg (C, D) of L-NAME. *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

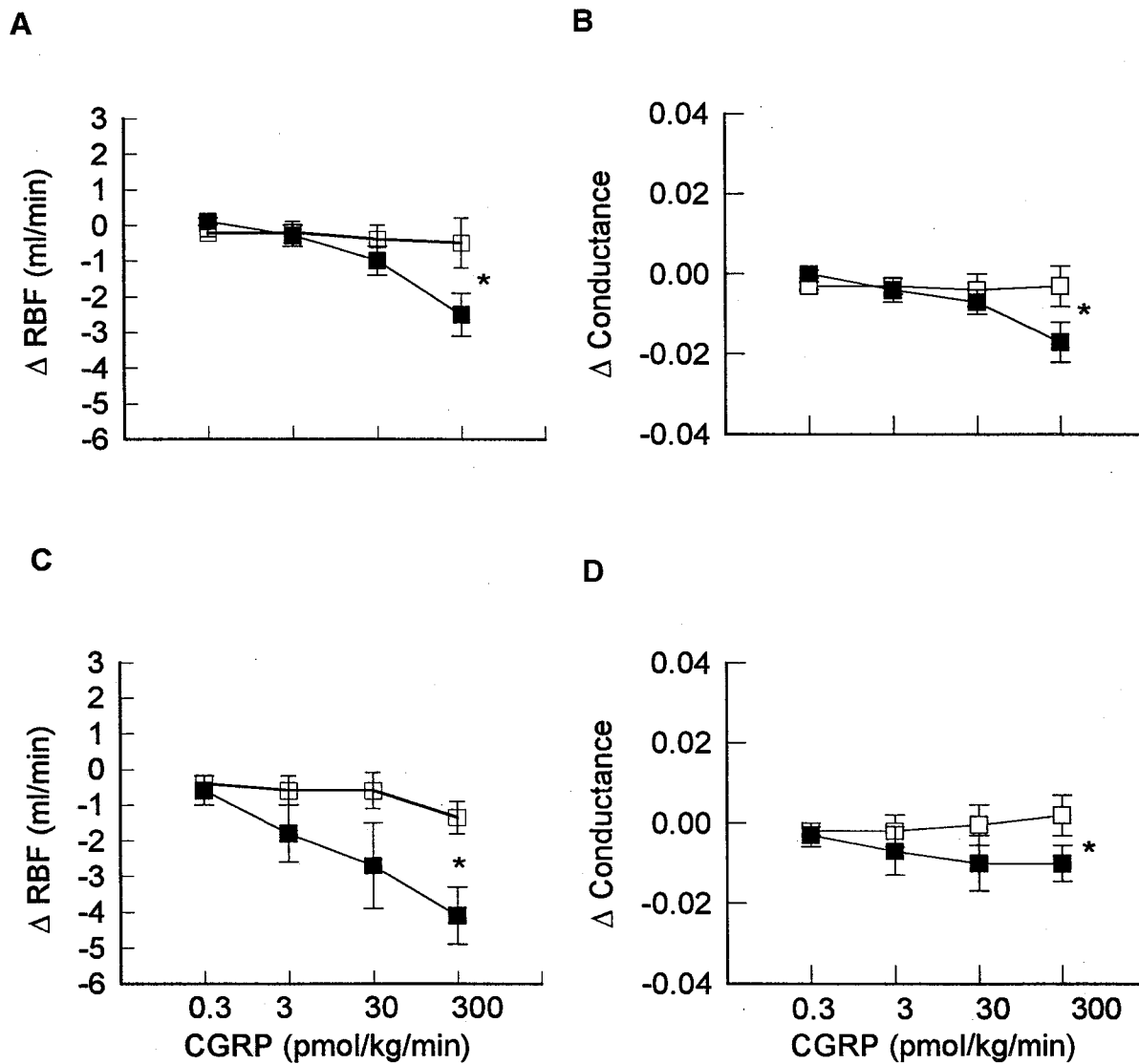


Figure 6: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on renal blood flow (RBF) and renal arterial conductance in Inactin-anesthetized rats (n = 6-8 per group) pre-treated with 2 μ mol/kg (A, B) or 20 μ mol/kg (C, D) of L-NAME. *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

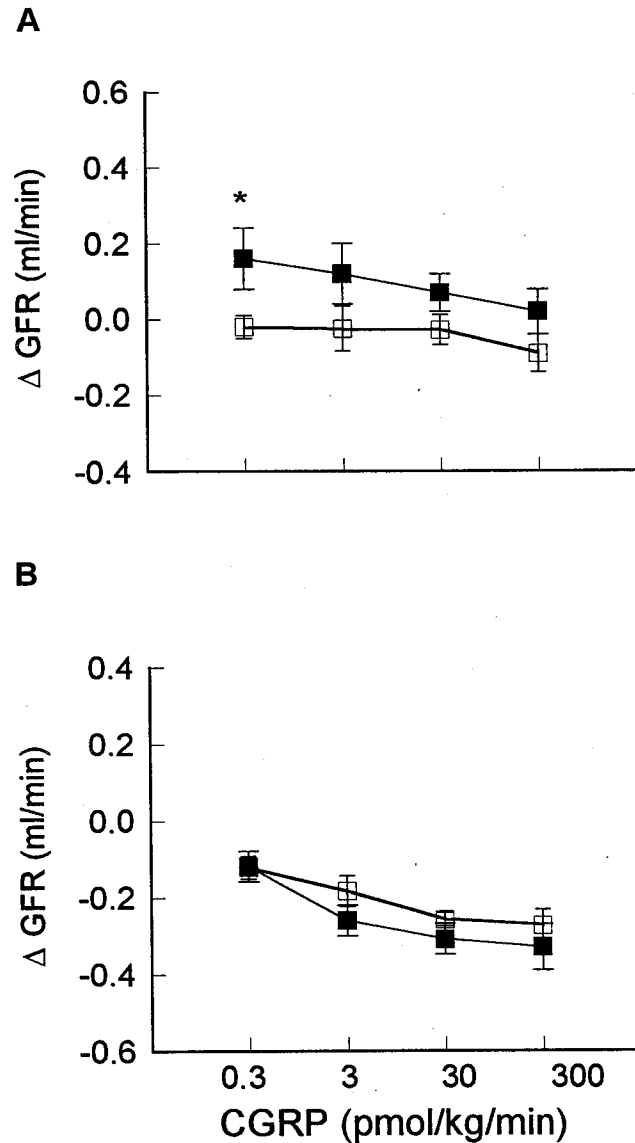


Figure 7: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on glomerular filtration rate (GFR) in Inactin-anaesthetized rats (n = 6-8 per group) pre-treated with 2 μ mol/kg (A) or 20 μ mol/kg (B) of L-NAME. *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

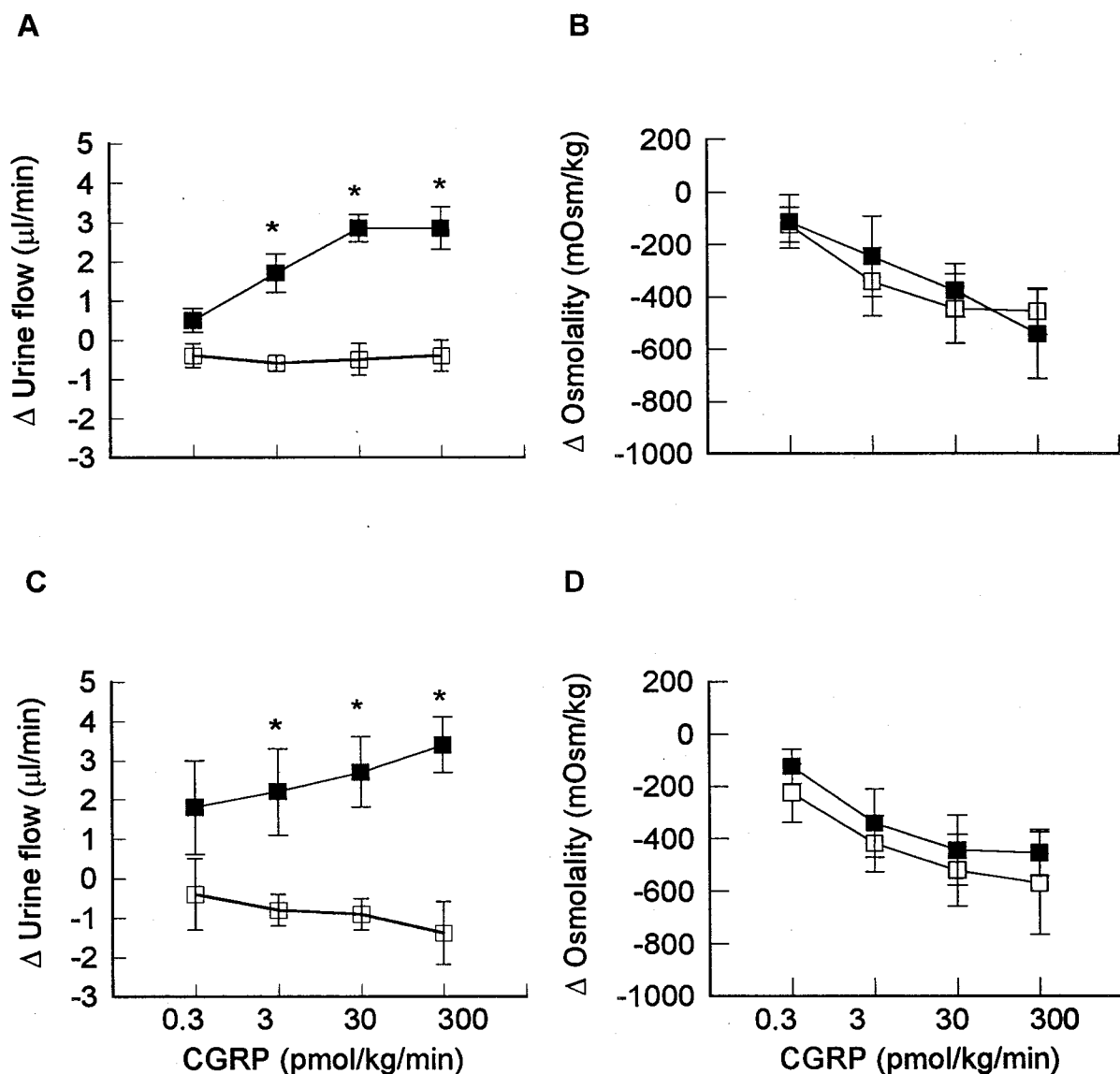


Figure 8: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, \blacksquare) and equal volume of vehicle (0.45% NaCl solution, \square) on urine flow and urine osmolality in Inactin-anaesthetized rats (n = 6-8 per group) pre-treated with 2 $\mu\text{mol}/\text{kg}$ (A, B) or 20 $\mu\text{mol}/\text{kg}$ (C, D) of L-NAME. *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

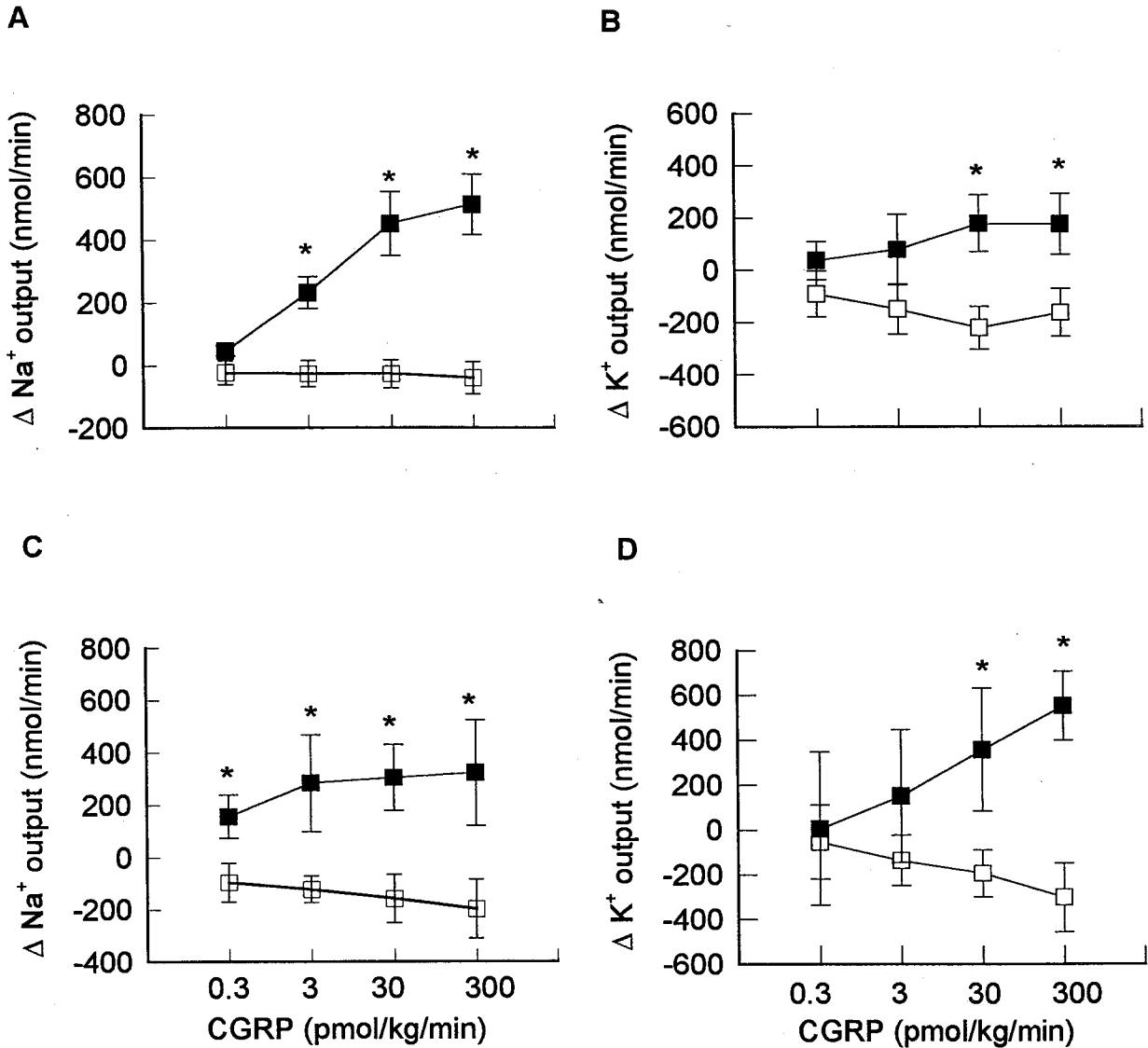


Figure 9: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on urinary Na^+ and urinary K^+ excretion rates in Inactin-anaesthetized rats ($n = 6-8$ each group) pre-treated with 2 $\mu\text{mol/kg}$ (A, B) or 20 $\mu\text{mol/kg}$ (C, D) of L-NAME. *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

	SP1	SP2	SP3	SP4	SP5	SP6
MAP	101±4	105±4	108±6	109±6	99±10	94±9
HR	372±26	374±30	378±29	386±26	408±34	422±38
RBF	11.5±0.5	8.4±1.3 ^a	13.4±1.1 ^b	12.4±1.7 ^b	10.6±1.9	7.9±1.7
COND	0.114±0.005	0.080±0.007 ^a	0.124±0.006 ^b	0.115±0.004 ^b	0.107±0.005 ^b	0.081±0.007
GFR	0.95±0.09	0.74±0.13	1.46±0.18 ^b	1.17±0.06 ^b	0.94±0.07	0.96±0.08
V	4.6±0.6	2.6±0.5 ^a	4.8±0.6 ^b	5.3±0.7 ^b	7.2±0.5 ^b	9.6±0.6 ^b
U_{Na}V	260±66	130±32 ^a	298±36 ^b	376±136 ^b	592±227 ^b	897±80 ^b
U_KV	989±204	484±152 ^a	946±140 ^b	1209±309 ^b	1027±376 ^b	1673±201 ^b

Table 5: Effects (mean ± S.E.) of renal arterial infusion of CGRP (0.3, 3, 30 and 300 pmol/kg/min, SP3-SP6) on arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine flow (V, µl/min) and urinary excretion rates for Na⁺ (U_{Na}V, U_KV, nEq/min) prior to (SP1) and 10 min after the start of continuous renal arterial infusion of phenylephrine (0.3, 3, 30 and 300 nmol/kg/min, SP2) in Inactin-anaesthetized rat n = 5). ^a Significantly different from SP1. ^b Significantly different from SP1.

3.6. Effect of losartan on renal actions of CGRP

Table 6 shows the baseline values of MAP, HR, and renal haemodynamics and excretion prior to the infusion of CGRP or the vehicle in four groups. Baseline values at sampling period 1 (SP1) were obtained after the infusion of the vehicle and sampling period 2 (SP2) values were obtained after the injection of the low dose or the high dose of losartan. Only the high dose of losartan significantly reduced MAP. Both doses of losartan did not alter HR, K^+ excretion or urine osmolality but increased RBF and conductance, GFR, urine flow and Na^+ excretion (Table 6).

Following treatment with both the low and high dose of losartan, high doses of CGRP increased HR (Fig. 10B,D) and reduced MAP (Fig. 10A,C). Neither the low nor the high dose of losartan inhibited the renal vasodilatation, vasoconstriction (Fig. 11) or increments in GFR effects of CGRP (Fig. 12).

CGRP had no effect on urine osmolality in losartan-treated rats (Fig. 13B,D) and these results are similar to those in control rats (fig. 3B). The increments in urine flow (Fig. 3A) and excretion of Na^+ and K^+ (Fig. 4) elicited by high doses of CGRP were markedly attenuated following treatment with the low dose of losartan (Fig. 13A, 14A,B). In the presence of the high dose of losartan, CGRP significantly decreased urine flow as well as excretion of Na^+ and K^+ (Fig. 13C; 14C,D).

3.7. CGRP effects in nitroprusside pre-dilated kidneys

Renal arterial infusion of sodium nitroprusside (50 nmol/kg/min) slightly but significantly reduced MAP without affecting HR. Sodium nitroprusside significantly increased RBF, renal arterial conductance, GFR, urine flow and

excretion of Na^+ and K^+ (Table 7) without changing urine osmolality (results not shown). These effects are quantitatively but not qualitatively different from those of the high dose losartan (Table 6).

In kidneys pre-dilated with sodium nitroprusside, CGRP caused slight renal vasodilatation at a low dose and vasoconstriction at the two highest doses but did not alter GFR. Urine flow and excretion of Na^+ and K^+ were increased by the third dose of CGRP but unchanged by the other doses (Tab. 7). A comparison of these results with those obtained in control rats (Fig. 3A, 4A,B) and those after pre-treatment with losartan (Fig. 13C, 14C,D) suggests that the inhibitory effects losartan and sodium nitroprusside on CGRP-induced excretion of urine, Na^+ and K^+ were due to reduced renal perfusion pressure and flow elicited by higher doses of CGRP in presence of the vasodilator agents, losartan and sodium nitroprusside.

GROUP	SP	MAP	HR	RBF	COND	GFR	V	U _{Na} V
LOS 0.3 $\mu\text{mol/kg}$: veh.	SP1	100 \pm 3	368 \pm 11	12.4 \pm 0.5	0.124 \pm 0.004	1.28 \pm 0.15	4.9 \pm 0.3	306 \pm 41
	SP2	102 \pm 3	370 \pm 12	13.9 \pm 0.4*	0.137 \pm 0.002*	1.67 \pm 0.12*	6.0 \pm 0.4*	478 \pm 63*
LOS 0.3 $\mu\text{mol/kg}$: CGRP	SP1	107 \pm 4	368 \pm 13	12.4 \pm 1.0	0.128 \pm 0.009	1.04 \pm 0.13	4.7 \pm 0.2	268 \pm 29
	SP2	105 \pm 5	381 \pm 11	14.9 \pm 0.8*	0.159 \pm 0.009*	1.38 \pm 0.14*	5.9 \pm 0.3*	377 \pm 36*
LOS 3 $\mu\text{mol/kg}$: veh.	SP1	110 \pm 3	380 \pm 6	11.6 \pm 0.8	0.106 \pm 0.008	0.97 \pm 0.09	4.4 \pm 0.7	283 \pm 57
	SP2	101 \pm 4*	375 \pm 7	13.2 \pm 0.3*	0.133 \pm 0.003*	1.33 \pm 0.14*	5.9 \pm 0.5*	501 \pm 97*
LOS 3 $\mu\text{mol/kg}$: CGRP	SP1	116 \pm 4	382 \pm 8	11.0 \pm 0.6	0.110 \pm 0.005	1.12 \pm 0.11	4.5 \pm 0.6	353 \pm 39
	SP2	106 \pm 3*	403 \pm 16	12.8 \pm 0.8*	0.126 \pm 0.005*	1.39 \pm 0.12*	5.6 \pm 0.3*	542 \pm 85*

Table 6: Baseline values (mean \pm S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), renal plasma flow (V, $\mu\text{l/min}$), urine Na⁺ (U_{Na}V, nEq/min), urine K⁺ (U_KV, nEq/min) and urine osmolality (U_{OSM}, mOsm/kg) for the first sampling period (SP1, 10 min of renal arterial infusion of vehicle, 0.45% NaCl) and the second sampling period (SP2, 10 min after renal arterial injection of losartan (LOS, 0.3 or 3 $\mu\text{mol/kg}$) in four groups (n = 6 each) of inactive rats. * indicates significant difference from the corresponding control group.

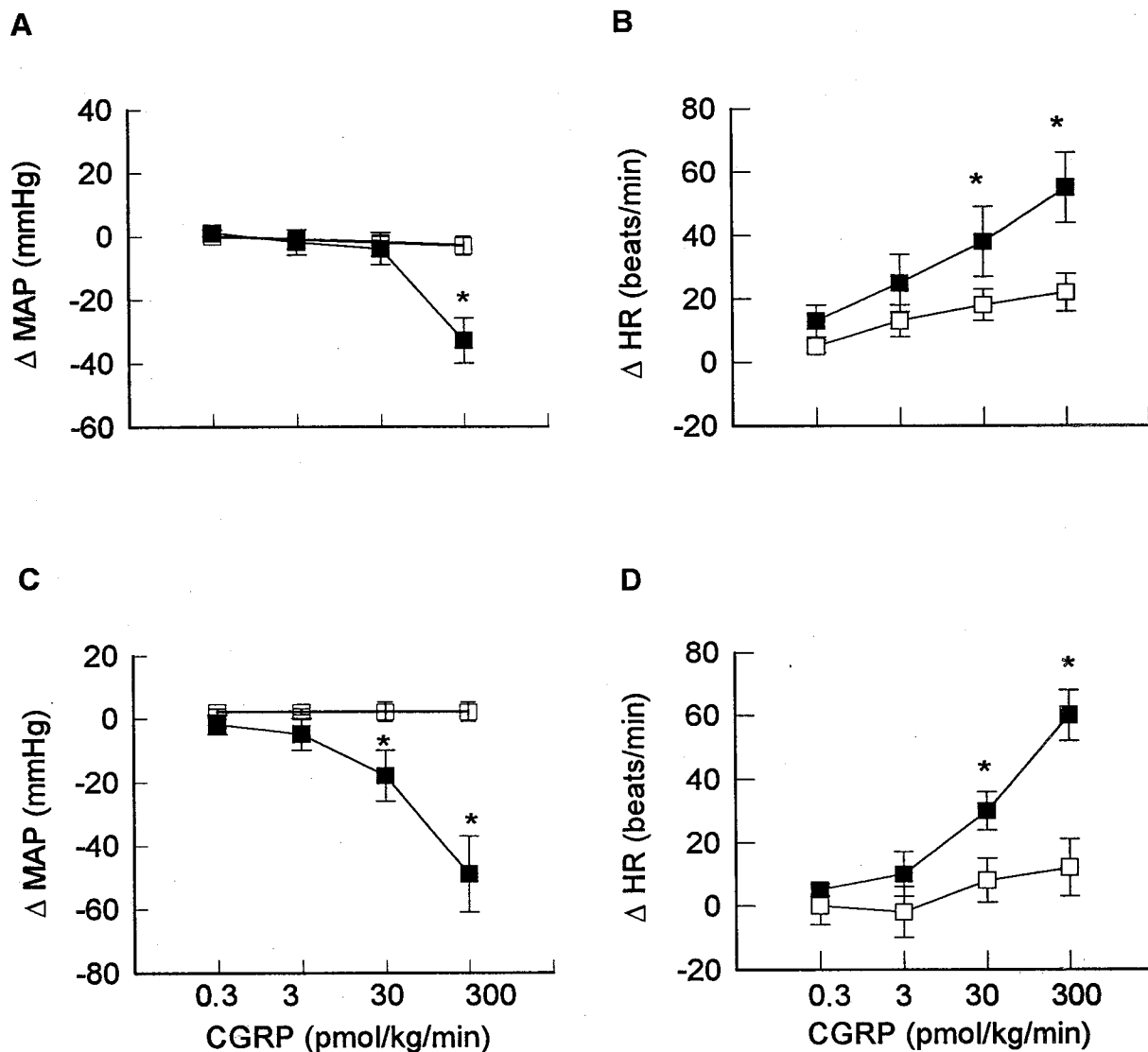


Figure 10: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) or equal volume of vehicle (0.45% NaCl solution, □) on mean arterial pressure (MAP) and heart rate (HR) in Inactin-anaesthetized rats (n = 6 per group) pre-treated with 0.3 μ mol/kg (A, B) or 3 μ mol/kg (C, D) of losartan. *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

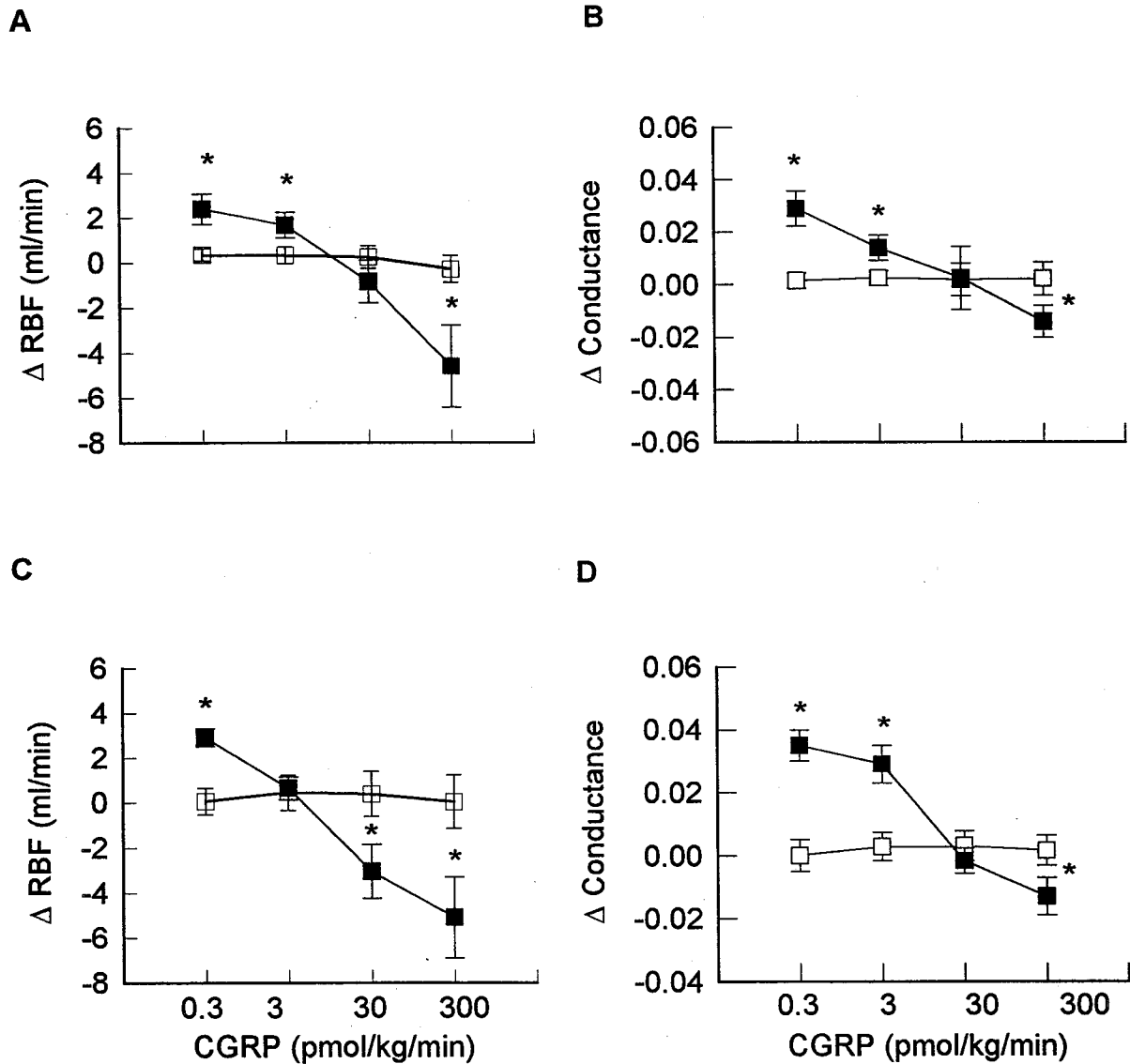


Figure 11: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on renal blood flow (RBF) and renal arterial conductance in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with 0.3 $\mu\text{mol/kg}$ (A, B) or 3 $\mu\text{mol/kg}$ (C, D) of losartan. *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

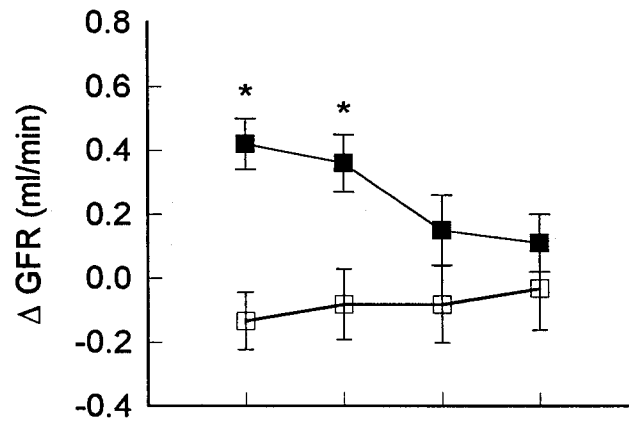
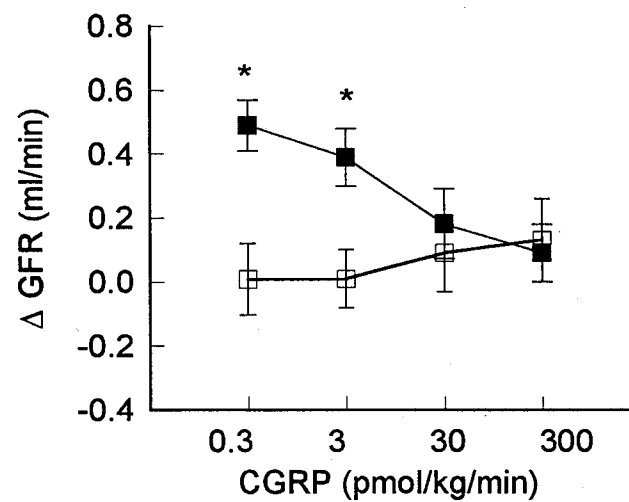
A**B**

Figure 12: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on glomerular filtration rate (GFR) in Inactin-anaesthetized rats (n = 6 per group) pre-treated with 0.3 μ mol/kg (A) or 3 μ mol/kg (B) of losartan. *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

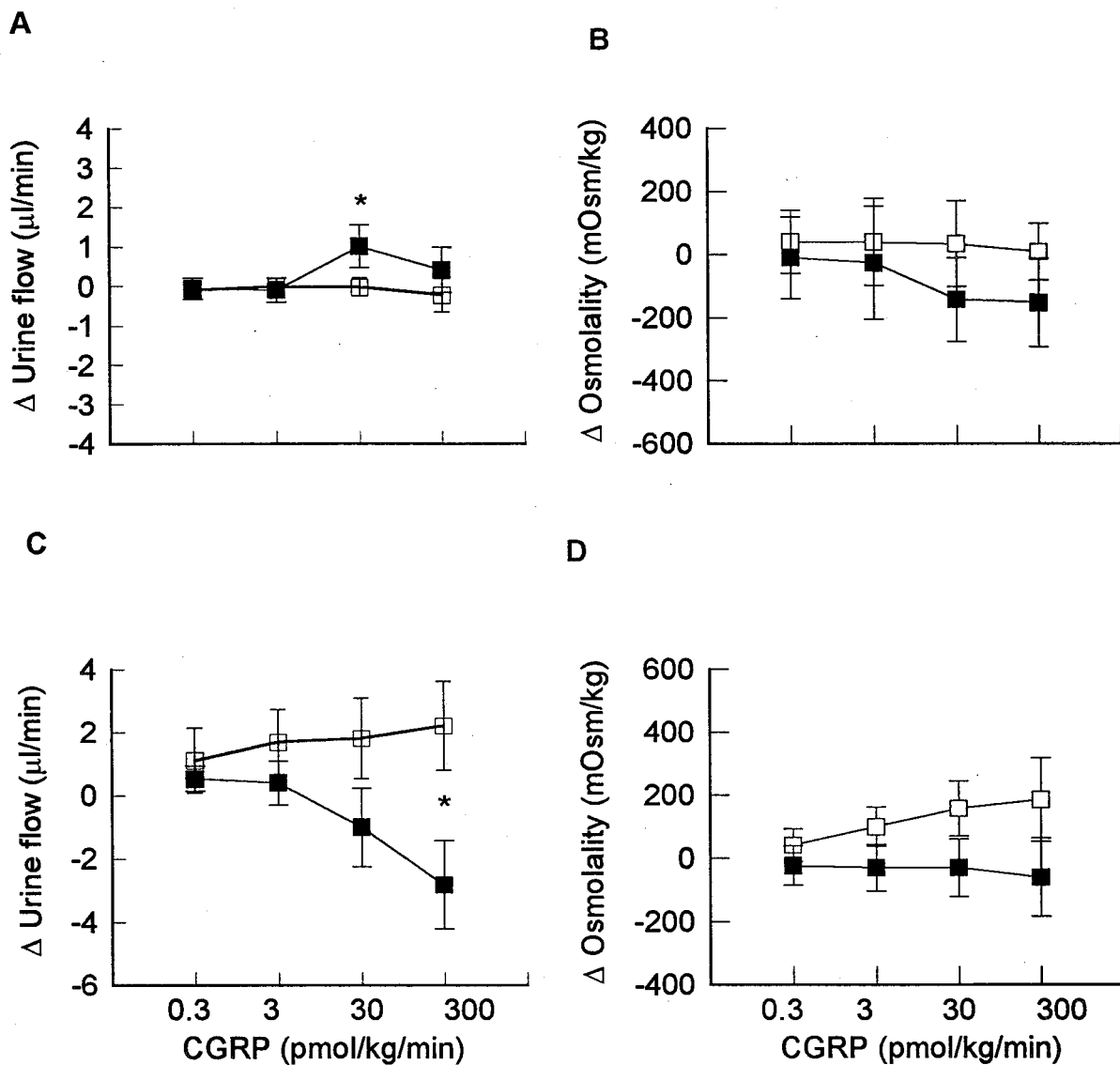


Figure 13: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on urine flow and urine osmolality in Inactin-anaesthetized rats (n = 6 per group) pre-treated with 0.3 μ mol/kg (A, B) or 3 μ mol/kg (C, D) of losartan. *Indicates significant difference from the corresponding value in the vehicle group (P < 0.05).

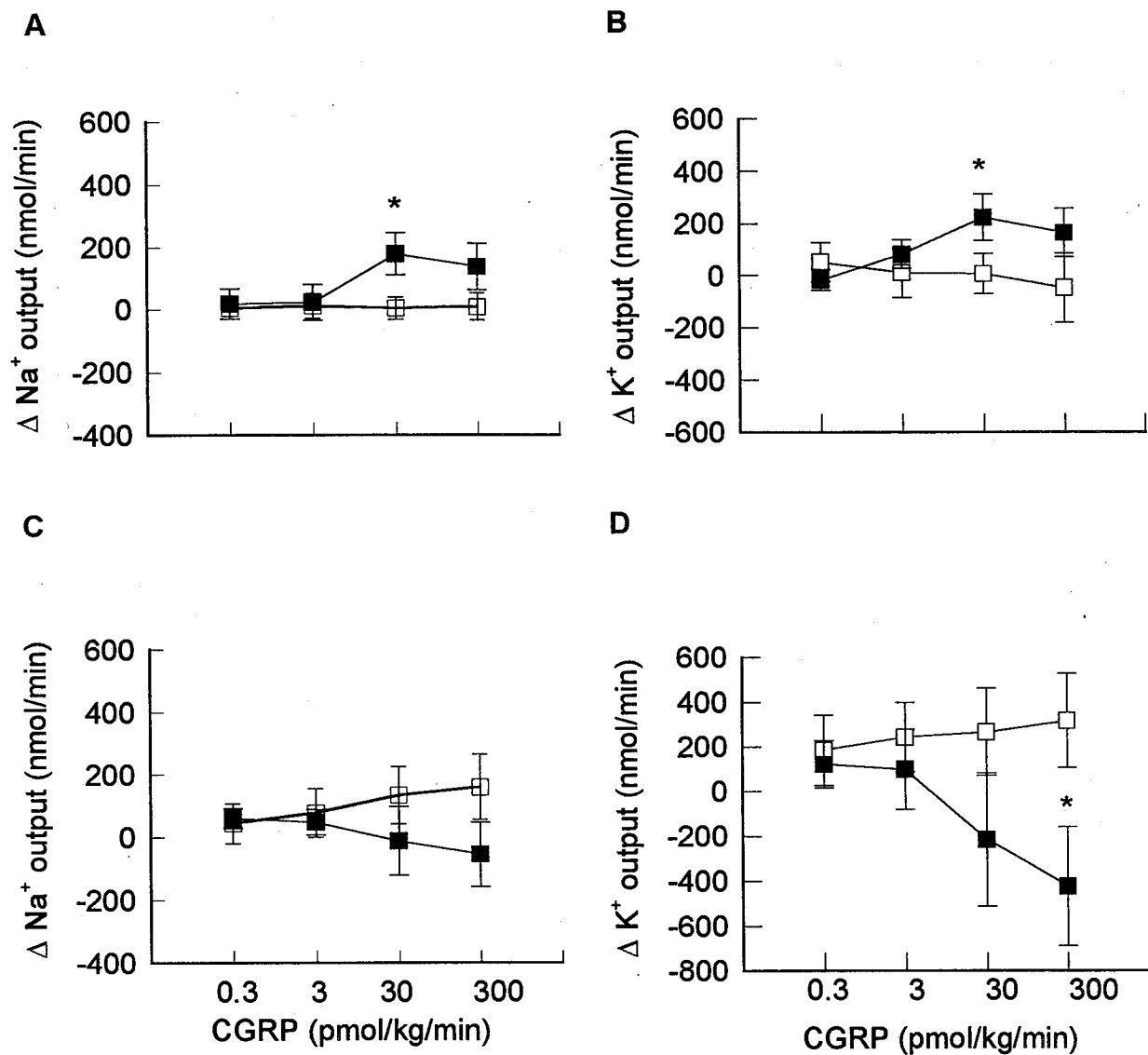


Figure 14: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on urinary Na⁺ and urinary K⁺ excretion rates in Inactin-anaesthetized rats (n = 6 each group) pre-treated with 0.3 μ mol/kg (A, B) or 3 μ mol/kg (C, D) of losartan. *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

	SP1	SP2	SP3	SP4	SP5	SP6
MAP	103±3	96±4 ^a	93±4	91±6	89±5	87±7
HR	372±26	375±30	379±29	389±26	407±34	425±38
RBF	11.5±0.5	13.4±0.6 ^a	14.4±0.8	11.4±1.7	9.6±1.3 ^b	7.9±1.7 ^b
COND	0.118±0.006	0.138±0.008 ^a	0.151±0.006 ^b	0.125±0.004	0.107±0.005 ^b	0.081±0.007
GFR	0.95±0.09	1.24±0.13 ^a	1.36±0.16	1.17±0.06	0.94±0.07	0.96±0.08
V	4.6±0.4	5.9±0.5 ^a	5.6±0.6	5.3±0.7	6.7±0.5 ^b	4.6±0.6
U_{Na}V	260±66	430±52 ^a	498±36	476±126	692±127 ^b	397±80
U_KV	789±104	1084±152 ^a	946±160	1009±159	1427±176 ^b	1073±201

Table 7: Effects (mean ± S.E.) of i.v. infusion of CGRP (0.3, 3, 30 and 300 pmol/kg/min, SP3-SP6) on mean pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine flow (V, µl/min) and urinary excretion rates for Na⁺ (U_{Na}V, U_KV, nEq/min) prior to (SP1) and 10 min after the start of continuous renal arterial infusion of sodium nitroprusside (SP2, 50 nmol/kg/min) in Inactin-anaesthetized rats (n=5). ^a Significantly different from SP1. ^b Significantly different from SP2.

3.8. Effect of phenoxybenzamine and mecamlamine on renal actions of CGRP

Table 8 shows the baseline values of MAP, HR, and renal haemodynamics and excretion prior to the infusion of CGRP or the vehicle in four groups. Baseline values at sampling period 1 (SP1) were obtained after the infusion of the vehicle and sampling period 2 (SP2) values were obtained after the injection of phenoxybenzamine or mecamlamine. Mecamlamine but not phenoxybenzamine significantly reduced MAP. Neither one of the antagonists altered HR, renal haemodynamics and renal excretion (Table 8).

Following treatment with phenoxybenzamine, CGRP decreased MAP and HR at the highest dose (Fig. 15A,B). After pre-treatment with mecamlamine, the highest two doses of CGRP significantly reduced MAP without altering HR (Fig. 15C,D). Neither phenoxybenzamine nor mecamlamine affected the vasodilator effect or increases in GFR elicited by low doses of CGRP (Fig. 16B,D; 17A,B) when these responses are compared with those elicited by CGRP in control rats (Fig. 2 B,C). The vasoconstrictor effect of the high doses of CGRP (Fig. 2B) was blocked by pre-treatment with phenoxybenzamine but not mecamlamine (Fig. 16B,D) indicating that it was not due to stimulation of sympathetic ganglia but due to release of noradrenaline from the sympathetic nerve terminals and the activation of α -adrenoceptors. After pre-treatment with phenoxybenzamine, CGRP increased urine flow at the second and third infused doses (Fig. 18A) and urine Na^+ and K^+ excretion in the third dose (Fig. 19A,B) without affecting urine osmolality (Fig. 18B). After mecamlamine, CGRP did not increase urinary flow, osmolality or Na^+ and K^+ excretion at any dose (Fig. 18C, D;

19C, D).

GROUP	SP	MAP	HR	RBF	COND	GFR	V	U _{Na} V	U _K V
POB: veh.	SP1	104±4	369±11	13.1±0.5	0.129±0.005	1.08±0.17	4.7±0.4	288±47	101
	SP2	101±5	366±13	12.3±0.5	0.121±0.006	1.03±0.14	3.8±0.5	237±32	957
POB : CGRP	SP1	106±4	357±8	11.8±0.8	0.117±0.007	1.19±0.05	4.9±0.6	349±57	952
	SP2	100±6	351±9	10.6±0.7	0.106±0.007	1.06±0.09	4.0±0.5	290±48	805
Mecamylamine : veh	SP1	108±5	372±9	11.5±0.7	0.106±0.004	1.21±0.12	5.4±0.7	329±87	101
	SP2	96±4*	371±11	10.6±0.6	0.111±0.006	1.06±0.14	4.4±0.5	235±67	917
Mecamylamine : CGRP	SP1	106±6	372±8	11.7±0.5	0.110±0.005	1.11±0.21	5.1±0.5	353±49	113
	SP2	93±5*	383±12	10.9±0.6	0.118±0.007	1.04±0.11	4.2±0.6	249±55	964

Table 8: Baseline values (mean ± S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine flow rate (V, ml/min), urine Na⁺ (U_{Na}V, nEq/min), urine K⁺ (U_KV, nEq/min) and urine osmolality (U_{OSM}, mOsm/kg) following the first sampling period [SP1, 10 min of renal arterial infusion of vehicle, 0.45% NaCl] and the second sampling period [SP2, 10 min after intravenous injection of 3 µmol/kg of phenoxybenzamine (POB) or 1 µmol/kg of mecamylamine] in four groups (n = 6 per group) of anaesthetized rats. * indicates significant difference from the corresponding control group.

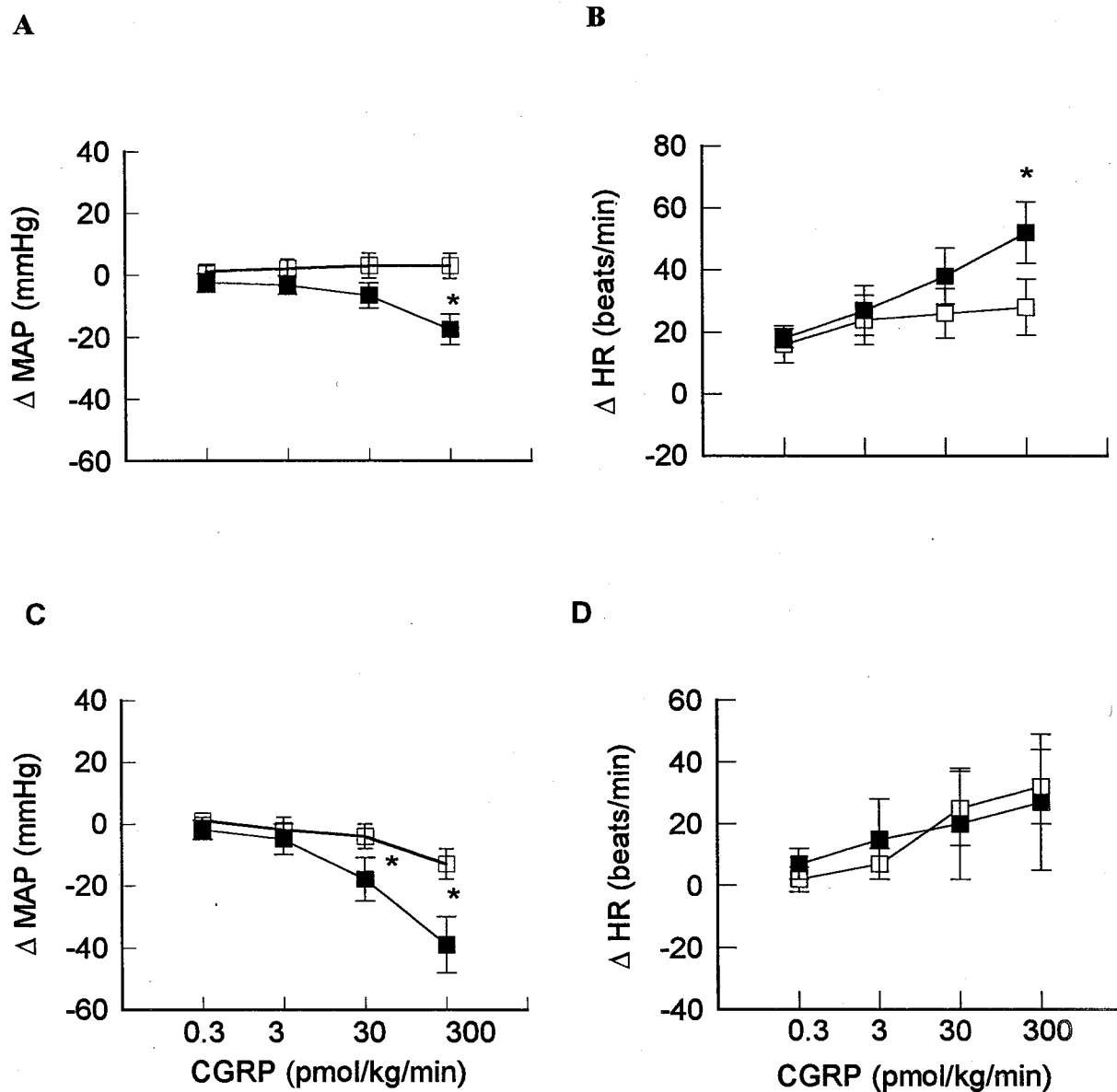


Figure 15: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) or equal volume of vehicle (0.45% NaCl solution, □) on mean arterial pressure (MAP) and heart rate (HR) in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with phenoxybenzamine (POB, 3 μ mol/kg, A, B) or mecamlamine (1 μ mol/kg, C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

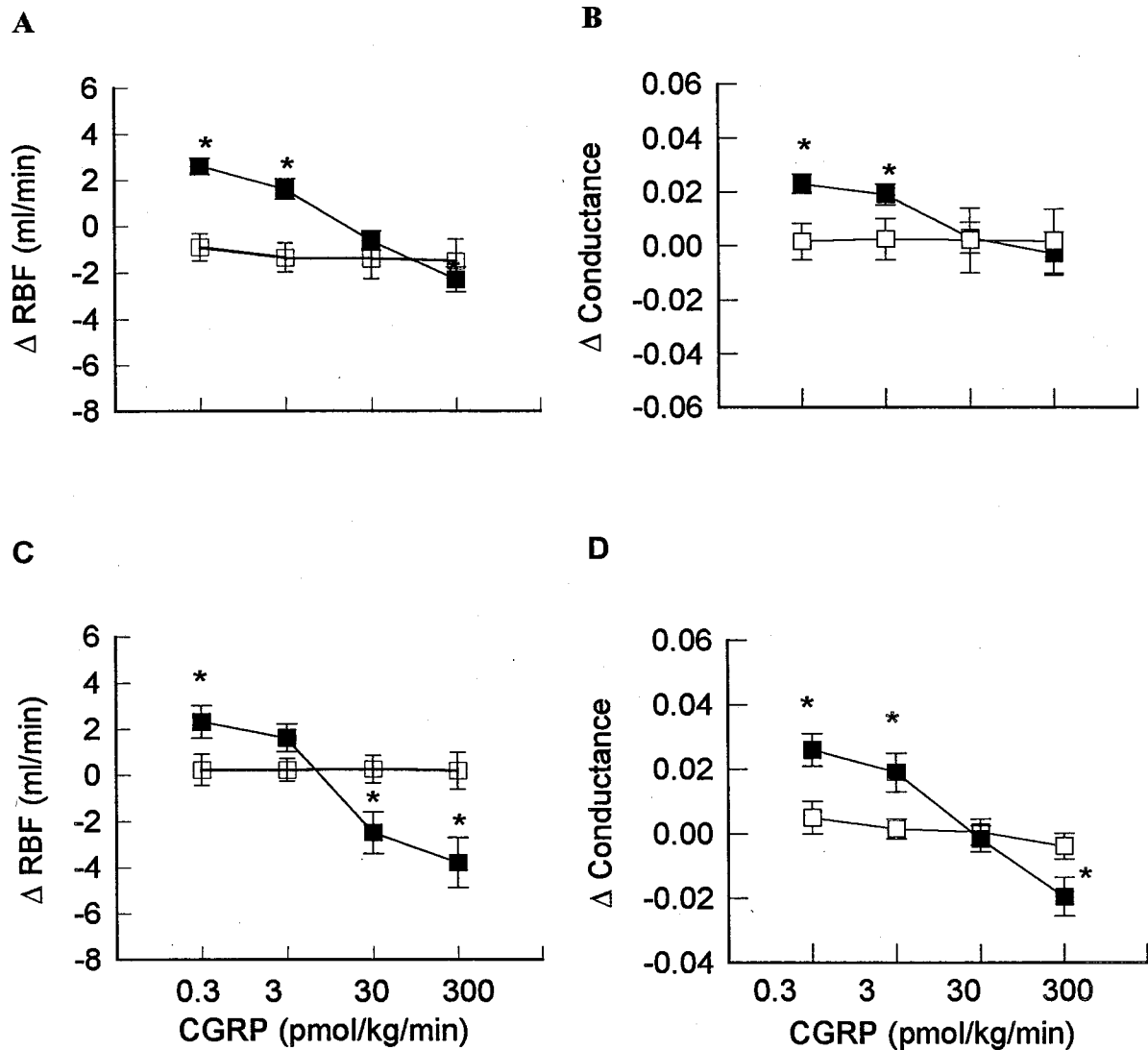


Figure 16: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on renal blood flow (RBF) and renal arterial conductance in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with phenoxybenzamine (POB, $3 \mu\text{mol/kg}$, A, B) or mecamylamine ($1 \mu\text{mol/kg}$, C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

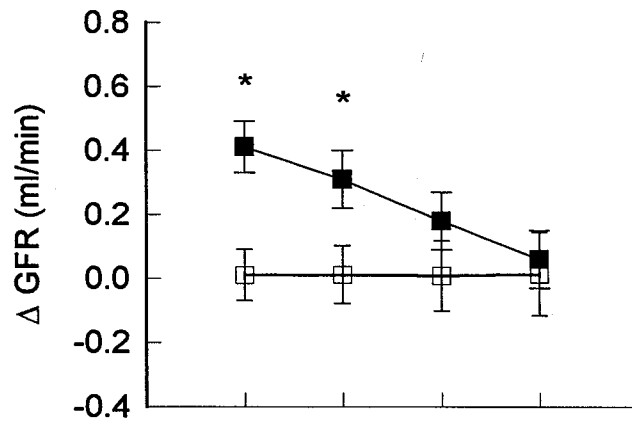
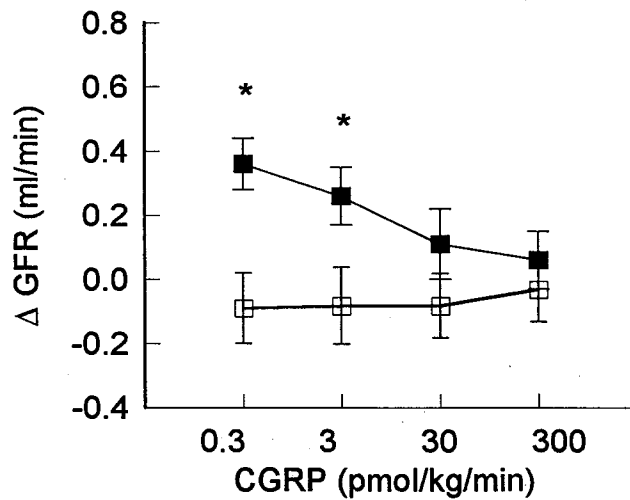
A**B**

Figure 17: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on glomerular filtration rate (GFR) in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with phenoxybenzamine (POB, 3 μ mol/kg, A) or mecamlamine (1 μ mol/kg, B). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

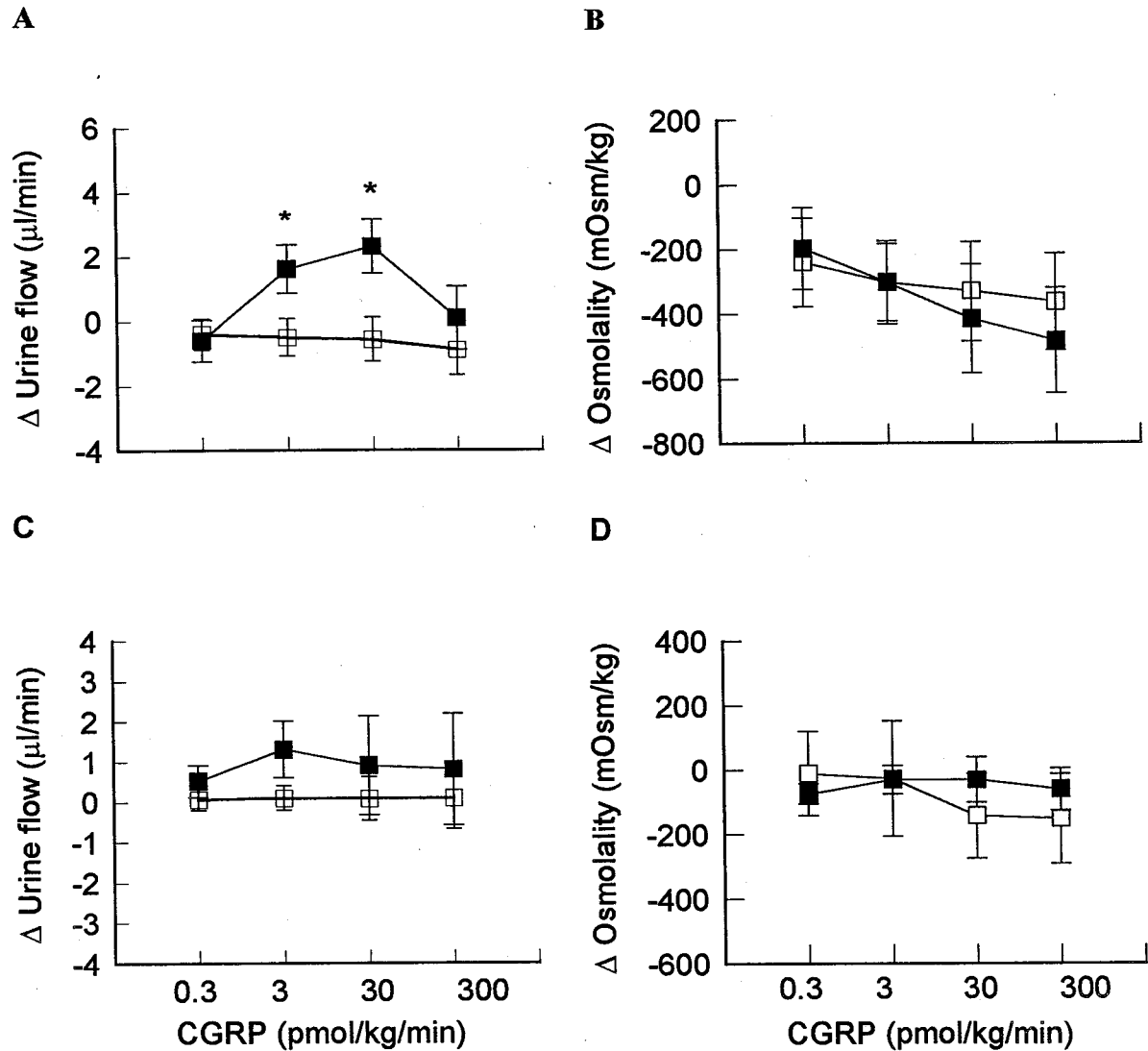


Figure 18: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on urine flow and urine osmolality in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with phenoxybenzamine (POB, 3 μ mol/kg, A, B) or mecamlamine (1 μ mol/kg, C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

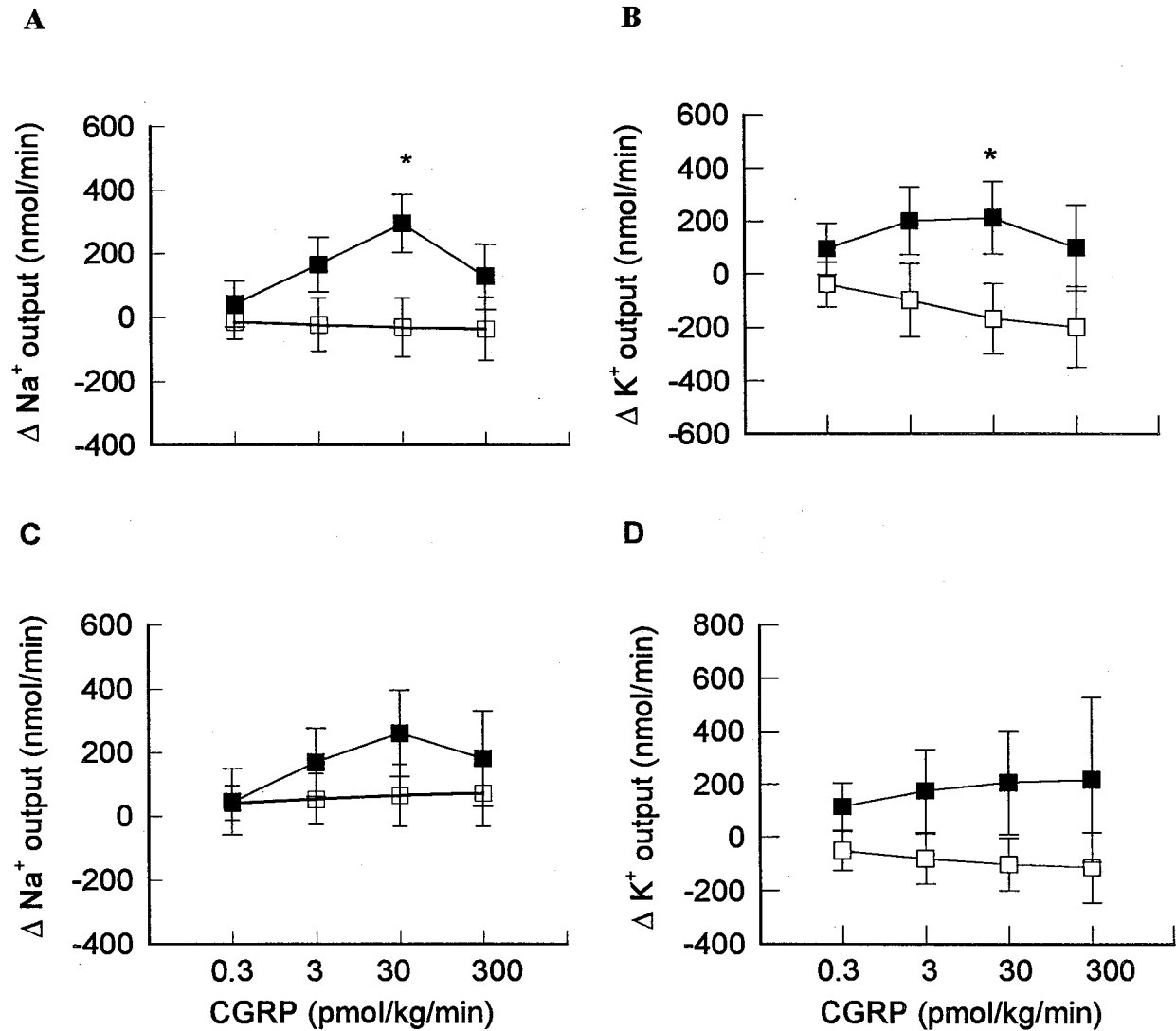


Figure 19: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, \blacksquare) and equal volume of vehicle (0.45% NaCl, \square) on urinary Na^+ and urinary K^+ excretion rates in Inactin-anaesthetized rats ($n = 6$ each group) pre-treated with phenoxybenzamine (POB, 3 $\mu\text{mol/kg}$, A, B) or mecamylamine (1 $\mu\text{mol/kg}$, C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

3.9. Effect of indomethacin on renal actions of CGRP

Renal arterial injection of indomethacin (3 $\mu\text{mol/kg}$) did not alter MAP, HR, renal haemodynamics or renal excretion. (Tab. 9). Pre-treatment with indomethacin did not affect CGRP actions on MAP, HR, RBF, conductance or GFR (Fig. 20; 21) when these effects are compared with those of CGRP in control rats (Fig. 1, 2). The effects of CGRP on urine flow, osmolality and Na^+ excretion (Fig. 3, 4) were also unaffected by pre-treatment with indomethacin (Fig. 22, 23A). However, the increases in K^+ excretion by CGRP were abolished by indomethacin (Fig. 23B).

GROUP	SP	MAP	HR	RBF	COND	GFR	V	U _{Na} V	U _K V
Indomethacin: veh.	SP1	103±4	377±12	13.1±0.5	0.127±0.005	1.28±0.17	4.8±0.4	268±37	1019±
	SP2	106±5	358±13	12.3±0.4	0.119±0.006	1.33±0.14	3.6±0.5	337±42	771±
Indomethacin: CGRP	SP1	102±4	373±8	11.8±0.8	0.117±0.007	1.44±0.15	3.9±0.4	389±37	952±
	SP2	106±6	359±11	12.2±0.7	0.116±0.007	1.36±0.09	3.0±0.2	410±68	675±

Table 9: Baseline values (mean ± S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine flow (V, ml/min), urine Na⁺ (U_{Na}V, nEq/min), urine K⁺ (U_KV, nEq/min) and urine osmolality (U_{OSM}, mOsm/kg) following the first sampling period (SP1, 10 min of i.v. infusion of vehicle, 0.45% NaCl) and the second sampling period (SP2, 10 min after renal artery infusion of indomethacin (3 µmol/kg) in two groups (n = 6 each) of Inactin-anaesthetized rats. * indicates significant difference from the corresponding control group.

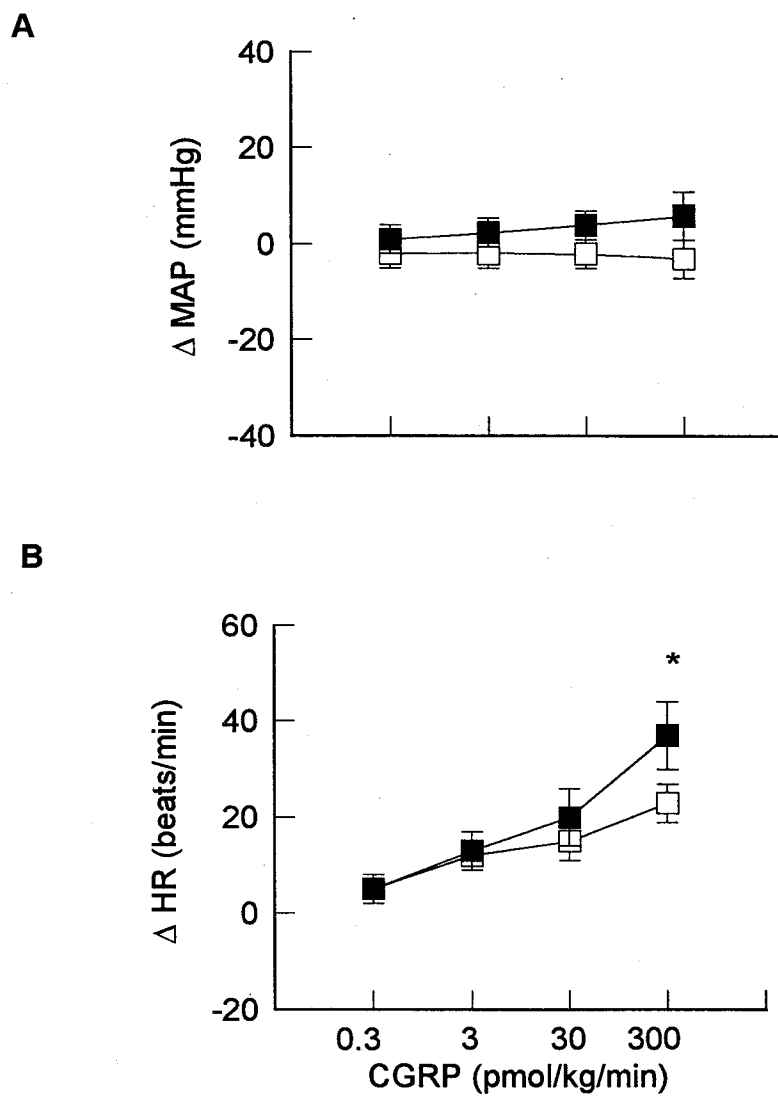


Figure 20: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) or equal volume of vehicle (0.45% NaCl solution, □) on mean arterial pressure (MAP, A) and heart rate (HR, B) in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with indomethacin ($3 \mu\text{mol/kg}$). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

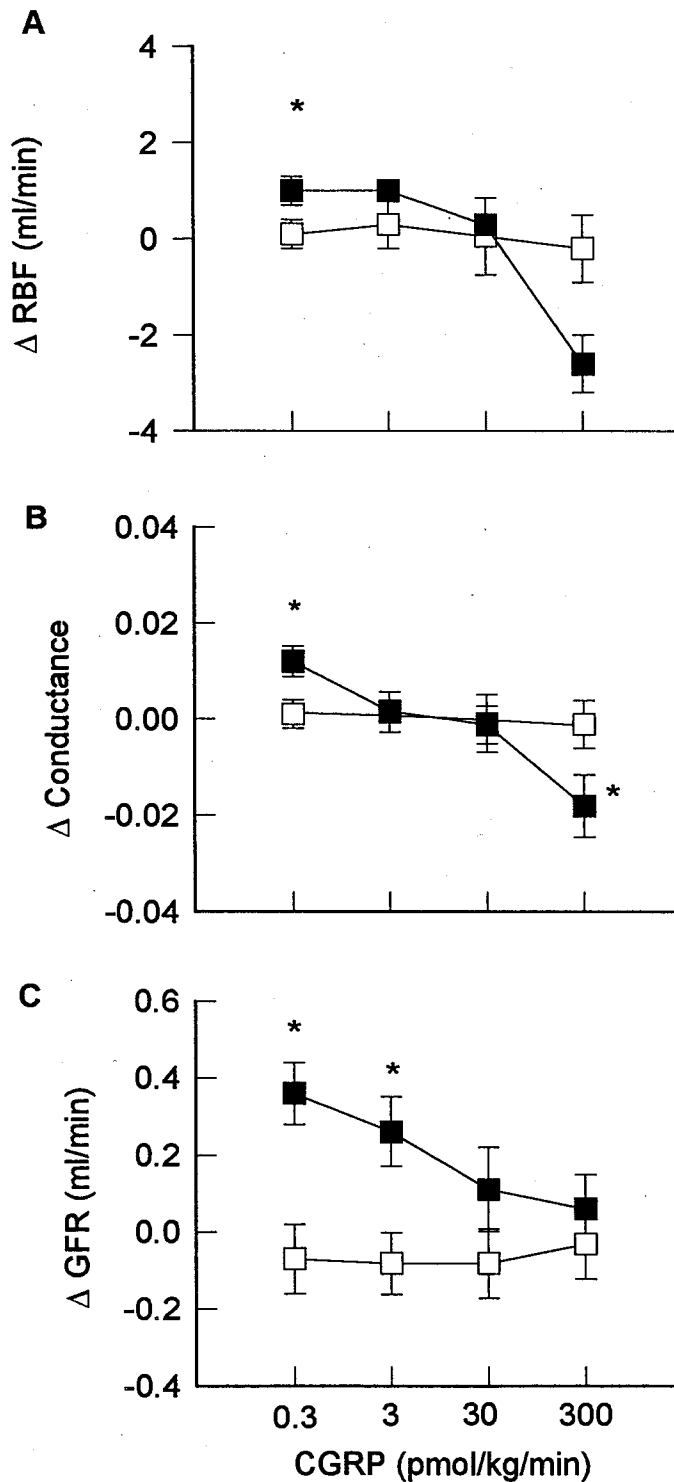


Figure 21: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, \blacksquare) and equal volume of vehicle (0.45% NaCl solution, \square) on renal blood flow (RBF, A), renal arterial conductance (B) and GFR (C) in Inactin-anaesthetized rats (n = 6 per group) pre-treated with indomethacin (3 μ mol/mg). *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

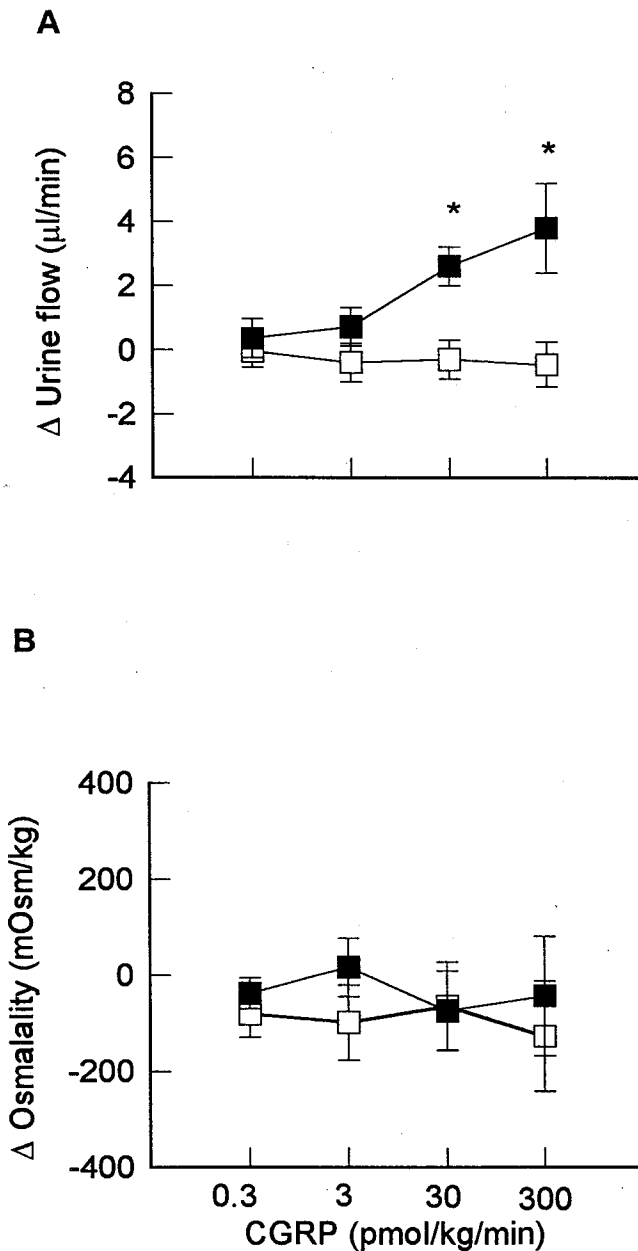


Figure 22: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl solution, □) on urine flow (A) and urine osmolality (B) in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with the indomethacin ($3 \mu\text{mol/kg}$). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

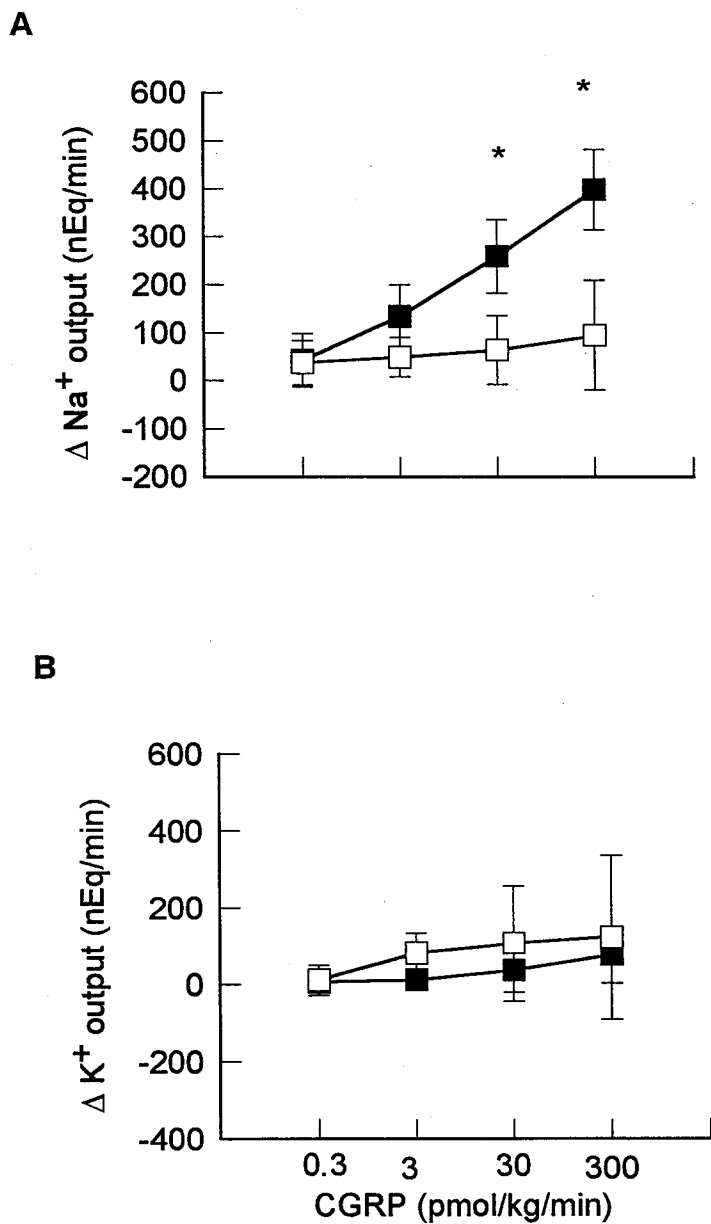


Figure 23: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, ■) and equal volume of vehicle (0.45% NaCl, □) on urinary Na⁺ (A) and urinary K⁺ (B) excretion rates in Inactin-anaesthetized rats (n = 6 each group) pre-treated with indomethacin (3 μ mol/kg). *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

3.10. Effect of CGRP(8-37) and [tyr⁰]CGRP(28-37)

Table 10 shows the baseline values of MAP, HR, and renal haemodynamics and excretion prior to the infusion of CGRP or the vehicle in eight groups of rat. Neither renal arterial injection of CGRP(8-37) nor [tyr⁰]CGRP(28-37) induced any significant changes in all parameters (results not shown).

3.10.1. Effects of CGRP on MAP, HR, RBF, arterial conductance and GFR in the absence or presence of CGRP(8-37) and [tyr⁰]CGRP(28-37)

In the presence of both the low and high dose of CGRP(8-37), a high dose CGRP decreased MAP and increased HR, but the HR effect was significant only in the presence of the low dose of CGRP(8-37) (Fig. 24A,B). In the presence of either the low or the high dose of [tyr⁰]CGRP(28-37), the highest dose of CGRP also significantly reduced MAP and increased HR (Fig. 24C,D).

The increases in RBF and conductance by CGRP (0.3 pmol/kg/min) were completely abolished by both doses of CGRP(8-37) (Fig. 25A,B) but incompletely though significantly ($p < 0.05$ relative to the respective CGRP responses in untreated rats in Fig. 2B) inhibited by both doses of [tyr⁰]CGRP(28-37) (Fig. 25C,D). Whereas only the lower dose of CGRP(8-37) and none of the doses of [tyr⁰]CGRP(28-37) attenuated the decrease in RBF induced by the highest dose of CGRP, both doses of CGRP(8-37) and [tyr⁰]CGRP(28-37) inhibited the reduction of renal arterial conductance elicited by the highest dose of CGRP. This suggests that the reduction in RBF by the high dose of CGRP in the presence of either one of the antagonists was secondary to hypotension.

The increases in GFR by low doses of CGRP (Fig. 2C) were inhibited by both doses of CGRP(8-37) completely (Fig. 26A) and by both doses of [tyr⁰]CGRP(28-37) incompletely (Fig. 26B).

3.10.2. Effects of CGRP on urinary flow, osmolality and excretion of Na⁺ and K⁺ in the absence or presence of the CGRP antagonists

Relative to the responses in control rats (Fig. 3A and 4), the increments in urine flow (fig. 27A, C) and excretion of Na⁺ (fig. 28A, C) and K⁺ (fig. 28B, D) by 300 pmol/kg/min of CGRP were similarly blocked by both the low and high doses of CGRP(8-37) or [tyr⁰]CGRP(28-37).

CGRP did not affect urine osmolality either before (Fig. 3B) or after treatment with CGRP(8-37) or [tyr⁰]CGRP(28-37) (Fig. 27B,D).

Higher doses of CGRP(8-37) (30 nmol/kg) (n=2) and [tyr⁰]CGRP(28-37) (100 nmol, n=2) did not elicit greater blockade of the renal tubular effects of CGRP (results not shown).

Group	MAP	HR	RBF	COND	GFR	V	U _{Na} V
CGRP(8-37) 1 nmol/kg: veh.	107±4	369±10	12.9±0.7	0.124±0.011	1.46±0.14	4.6±0.4	253±50
CGRP(8-37) 1 nmol/kg: CGRP	102±7	358±12	12.6±1.2	0.122±0.010	1.47±0.18	5.4±0.6	243±49
CGRP(8-37) 10 nmol/kg: veh.	105±3	368±19	13.2±0.9	0.126±0.008	1.56±0.18	4.9±0.5	304±72
CGRP(8-37) 10 nmol/kg: CGRP	107±7	355±24	12.1±1.5	0.115±0.018	1.48±0.23	5.7±0.4	295±53
[tyr⁰]CGRP(28-37) 3 nmol/kg: veh.	99±9	367±14	13.2±0.7	0.133±0.011	1.39±0.18	4.8±0.4	243±39
[tyr⁰]CGRP(28-37) 3 nmol/kg: CGRP	100±5	357±16	12.5±1.1	0.125±0.016	1.46±0.18	4.6±0.4	267±37
[tyr⁰]CGRP(28-37) 30 nmol/kg: veh.	98±8	349±19	12.1±1.1	0.128±0.012	1.37±0.19	5.2±0.4	387±56
[tyr⁰]CGRP(28-37) 30 nmol/kg: CGRP	100±7	378±14	13.4±0.5	0.134±0.007	1.40±0.15	5.3±0.4	362±55

Table 10: Baseline values (mean ± S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine Na⁺ (U_{Na}V, nmol/min), urine K⁺ (U_KV, nmol/min) and urine osmolality (U_{OSM}, mOsm/kg) at the first sampling renal arterial infusion of vehicle (0.45% NaCl) in eight groups (n = 6 each) of Inactin-anaesthetized rats, prior of the CGRP antagonists or an equal volume of the vehicle.

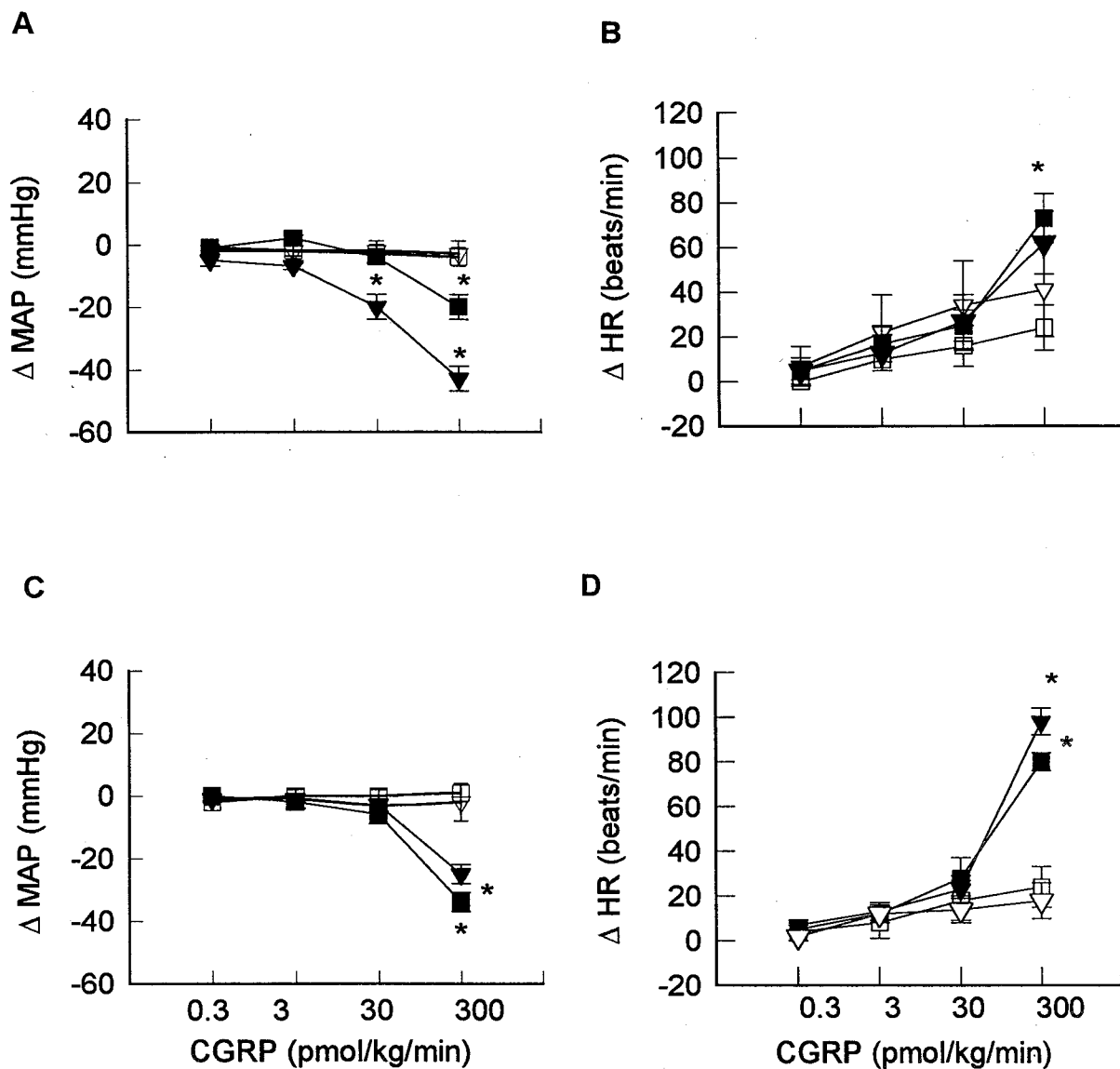


Figure 24: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, filled symbols) or equal volume of vehicle (0.45% NaCl solution, open symbols) on mean arterial pressure (MAP) and heart rate (HR) in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with CGRP (8-37) at 1 nmol/kg (\blacksquare , \square) or 10 nmol/kg (\blacktriangledown , \triangledown) (A, B) or [tyr^0]CGRP(28-37) at 3 nmol/kg (\blacksquare , \square) or 30 nmol/kg (\blacktriangledown , \triangledown) (C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

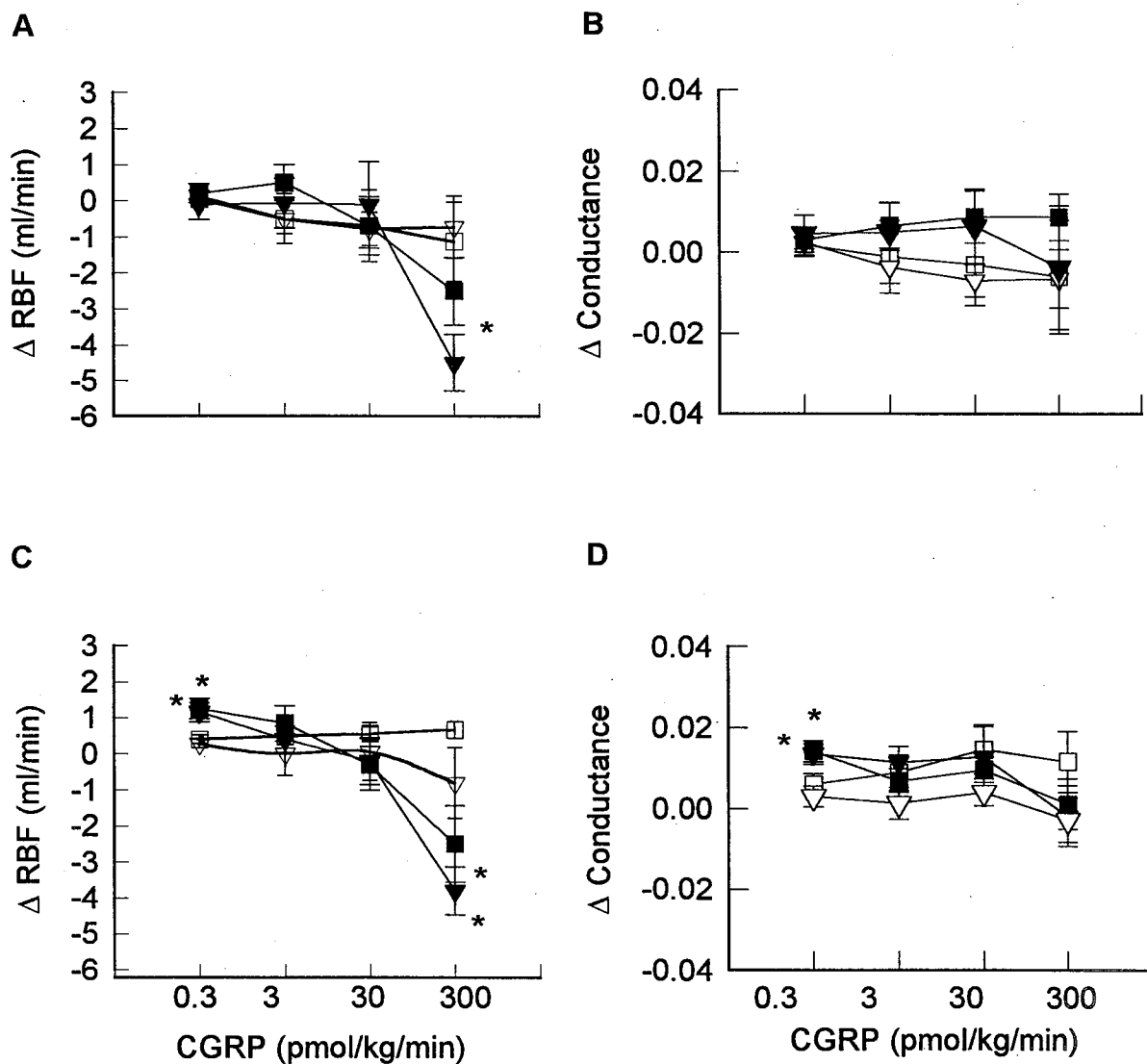
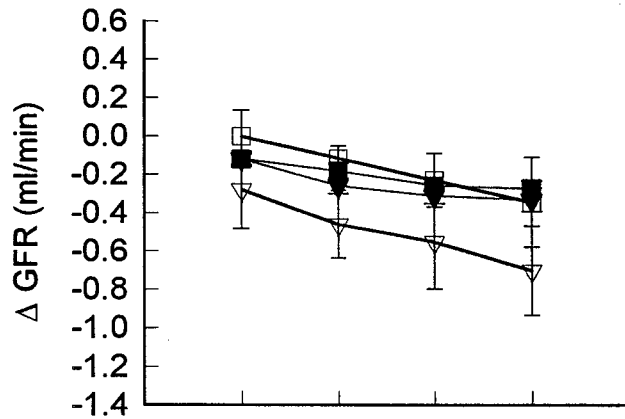


Figure 25: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, filled symbols) or equal volume of vehicle (0.45% NaCl solution, open symbols) on renal blood flow (RBF) and renal arterial conductance in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with CGRP (8-37) at 1 nmol/kg (\blacksquare , \square) or 10 nmol/kg (\blacktriangledown , \triangledown) (A, B) or [tyr⁰]CGRP(28-37) at 3 nmol/kg (\blacksquare , \square) or 30 nmol/kg (\blacktriangledown , \triangledown) (C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

A



B

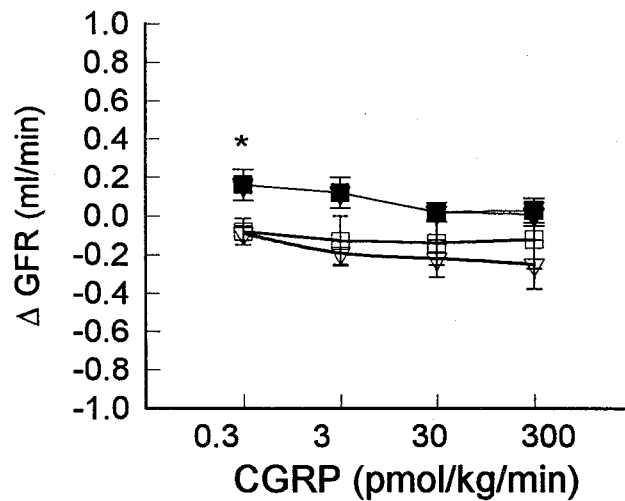


Figure 26: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, filled symbols) or equal volume of vehicle (0.45% NaCl solution, open symbols) on glomerular filtration rate (GFR) in Inactin-anaesthetized rats (n = 6 per group) pre-treated with CGRP (8-37) at 1 nmol/kg (■, □) or 10 nmol/kg (▼, ▽) (A) or [tyr⁰]CGRP(28-37) at 3 nmol/kg (■, □) or 30 nmol/kg (▼, ▽) (B). *Indicates significant difference from the corresponding value in the vehicle group (P<0.05).

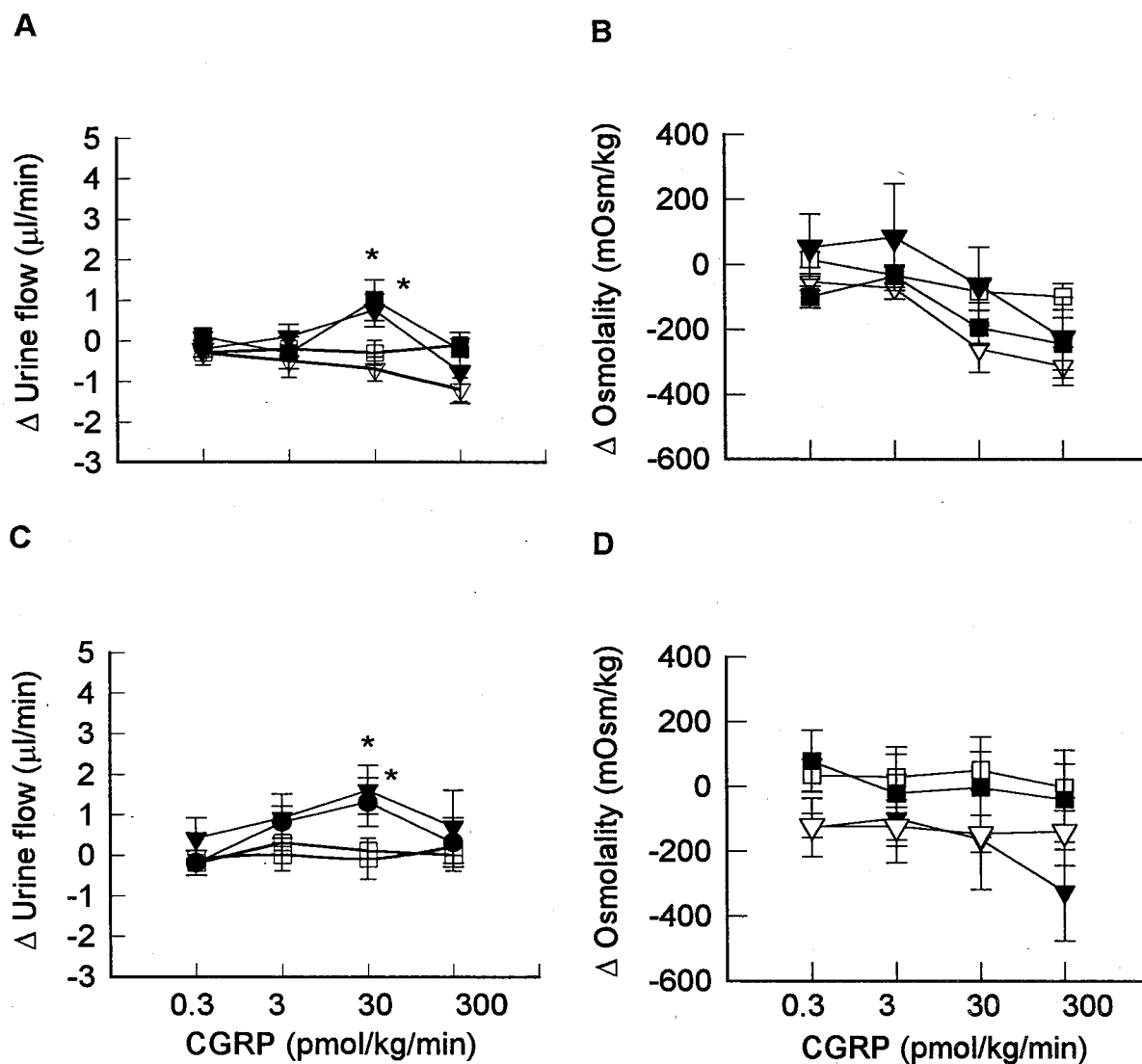


Figure 27: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, filled symbols) or equal volume of vehicle (0.45% NaCl solution, open symbols) on urine flow and urine osmolality in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with CGRP (8-37) at 1 nmol/kg (\blacksquare , \square) or 10 nmol/kg (\blacktriangledown , \triangledown) (A, B) or [tyr^0]CGRP(28-37) at 3 nmol/kg (\blacksquare , \square) or 30 nmol/kg (\blacktriangledown , \triangledown) (C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

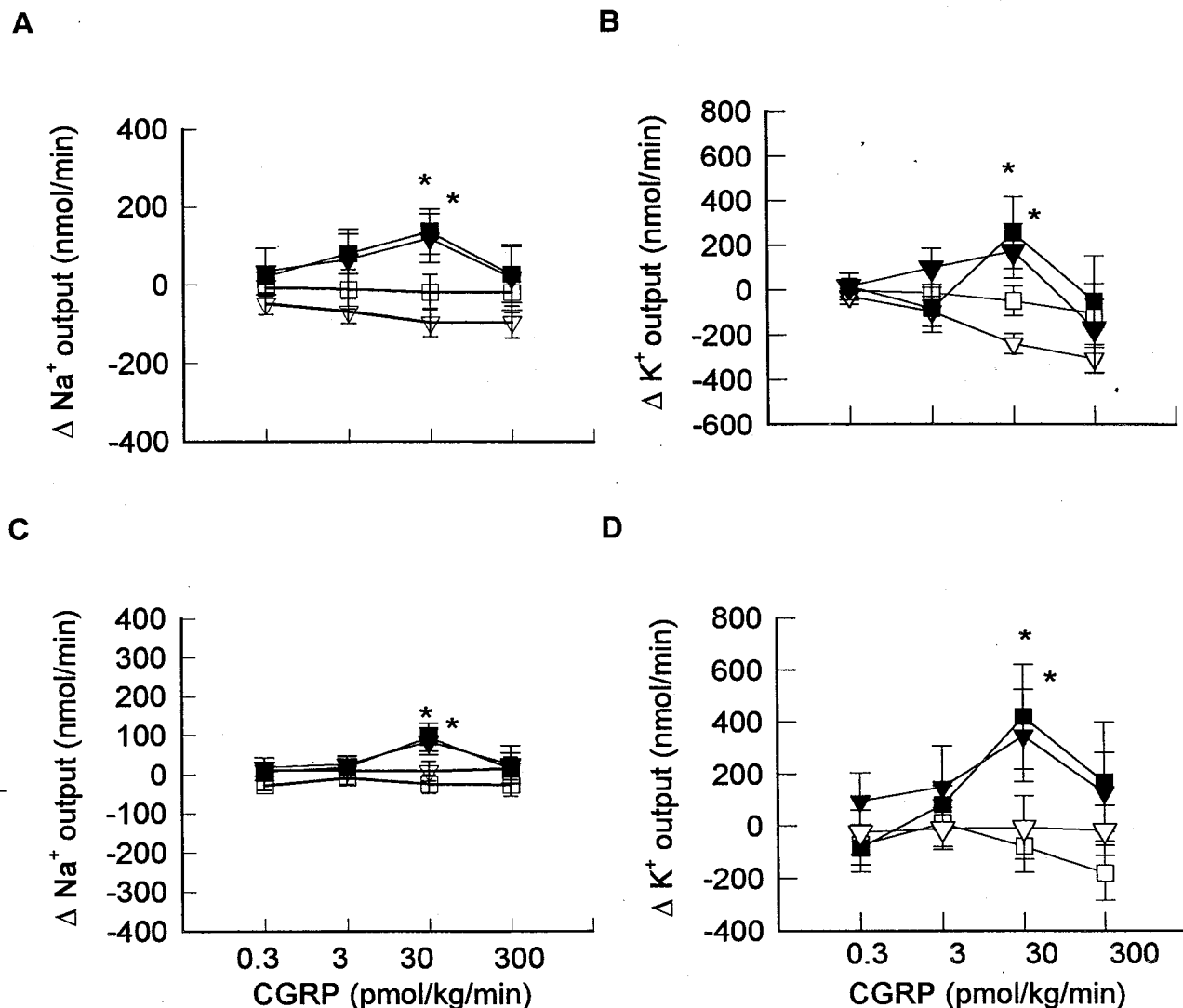


Figure 28: Effects (means \pm S.E.) of calcitonin gene-related peptide (CGRP, filled symbols) or equal volume of vehicle (0.45% NaCl solution, open symbols) on urinary Na^+ and urinary K^+ excretion rates in Inactin-anaesthetized rats ($n = 6$ per group) pre-treated with CGRP (8-37) at 1 nmol/kg (■, □) or 10 nmol/kg (▼, ▽) (A, B) or [tyr^0]CGRP(28-37) at 3 nmol/kg (■, □) or 30 nmol/kg (▼, ▽) (C, D). *Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

3.11. Renal effects of adrenomedullin

Control rats and rats to be given CGRP or adrenomedullin had similar baseline haemodynamic and renal measurements (Table 11). The renal arterial injection of the vehicle caused insignificant changes in MAP, HR, RBF and conductance, urine flow, urine Na^+/K^+ and urine osmolality (Figs. 29 to 32).

Intrarenal arterial injections of low doses of adrenomedullin and CGRP did not alter MAP or HR. However, MAP was decreased by the highest dose of adrenomedullin (-6%) and the highest two doses of CGRP (-7% and -11%) (Fig. 29A). The highest dose of CGRP also increased HR (Fig. 29B).

Adrenomedullin dose-dependently increased RBF and renal arterial conductance at all doses (Fig. 30A,B). CGRP also increased RBF at the lowest three doses and increased conductance at all doses (Fig. 30A,B). Whereas the highest three doses of adrenomedullin caused significantly greater increments in RBF than the corresponding doses of CGRP, only the highest dose of adrenomedullin evoked greater increase in arterial conductance. At 10 and 20 min following injections of the highest dose of each peptide, renal arterial conductances were 42% and 23% of the corresponding peak vasodilatation responses for adrenomedullin and 42% and 25% respectively, of the peak responses for CGRP. These results suggest that both peptides have similar durations of renal vasodilatation.

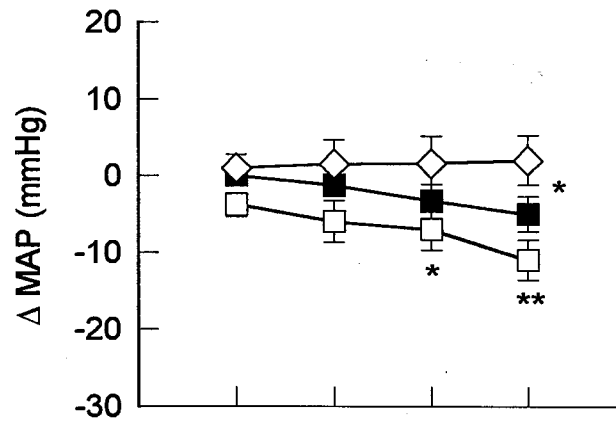
Both peptides dose-dependently increased urine flow (Fig. 31A) and Na^+ excretion (Fig. 32A); the diuretic and natriuretic effects of the highest three doses of CGRP were significantly greater than those of adrenomedullin. CGRP, but not

adrenomedullin, dose-dependently reduced urine osmolality (Fig. 31B) and increased K^+ excretion (Fig. 32B).

Group	MAP	HR	RBF	Cond	V	U _{Na} V	U _K V	U _{OSM}	HC%	PL Na
Vehicle	99±2	376±21	10.9±0.9	0.101±0.009	4.9±0.5	239±34	944±159	1374±124	44±2%	139±4
AM	98±3	378±13	11.6±0.5	0.121±0.008	4.2±0.3	216±28	883±104	1298±136	45±2%	138±6
CGRP	93±3	379±13	10.4±0.8	0.111±0.005	4.4±0.2	211±22	739±119	1311±119	43±1%	136±5

Table 11: Baseline values (mean ± S.E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (cond, ml/min/mmHg), urine flow (V, µl/min), urine Na⁺ excretion (U_{Na}V, nEq/min), urine K⁺ excretion (U_K, nEq/min), urine osmolality (U_{OSM}, mOsm/kg), haematocrit (HC%), plasma Na⁺ (PL Na, mEq/l) and plasma osmolality (PL_{OSM}, mOsm/kg) in three groups of Inactin-anaesthetized rats prior to adrenomedullin, CGRP or vehicle (n = 8 per group).

A



B

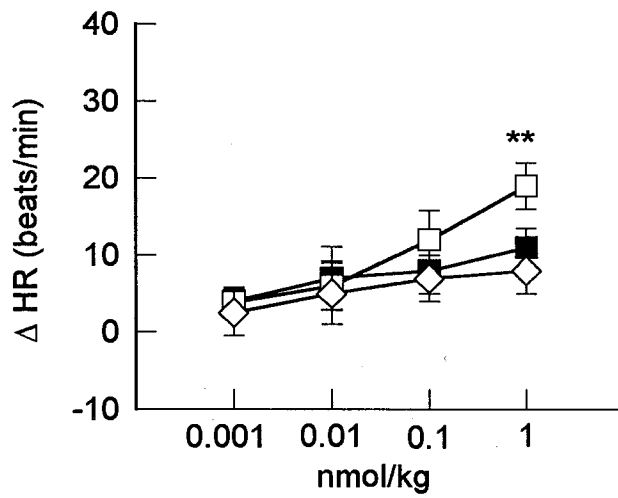


Figure 29: Dose-response effects (mean \pm S.E.) on mean arterial pressure (MAP, A) and heart rate (HR, B) following renal arterial injections of adrenomedullin (■), calcitonin gene-related peptide (□) or an equal volume of vehicle (0.45% NaCl; ◇) in three groups ($n = 8$ each) of Inactin-anaesthetized rats. *significantly different from the time-control; **significantly different from the other two groups.

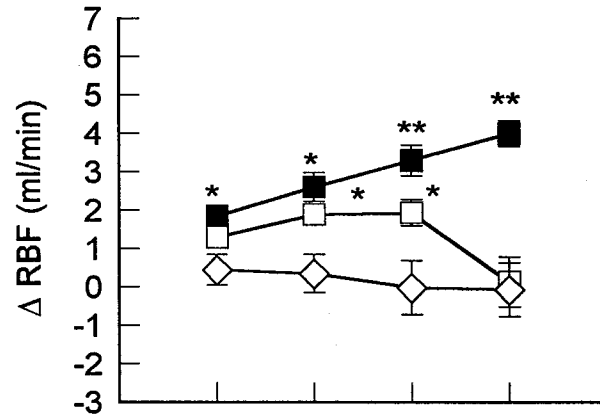
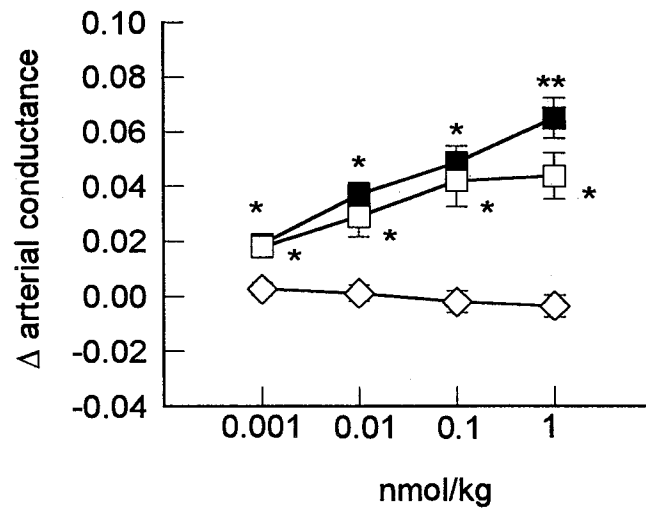
A**B**

Figure 30: Dose-response effects (mean \pm S.E.) on renal blood flow (RBF, A) and renal arterial conductance (B) following renal arterial injections of adrenomedullin (■), calcitonin gene-related peptide (□) or an equal volume of vehicle (0.45% NaCl; ◇) in three groups (n = 8 each) of Inactin-anaesthetized rats. *significantly different from the time-control; **significantly different from the other two groups.

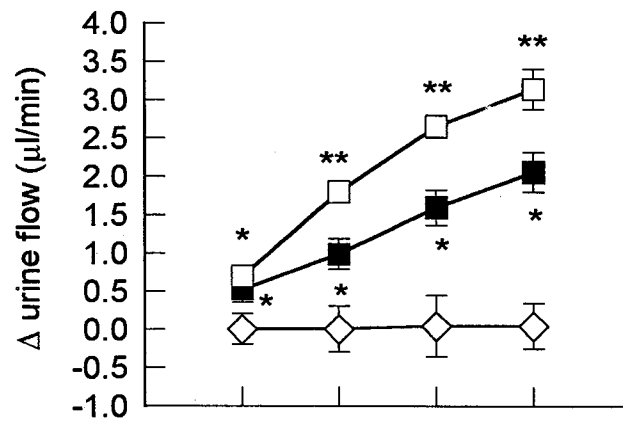
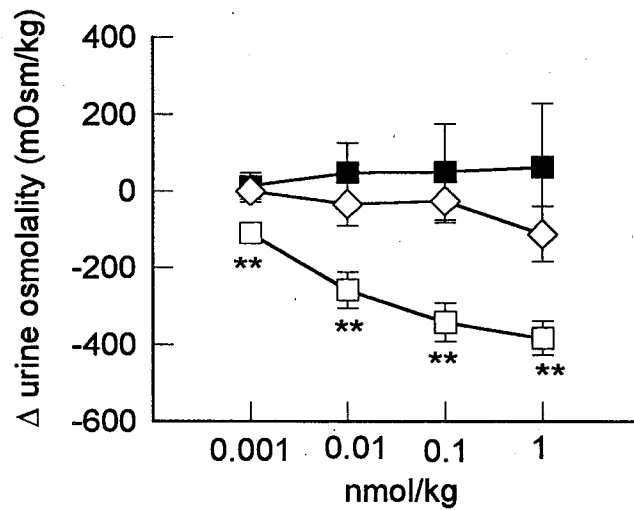
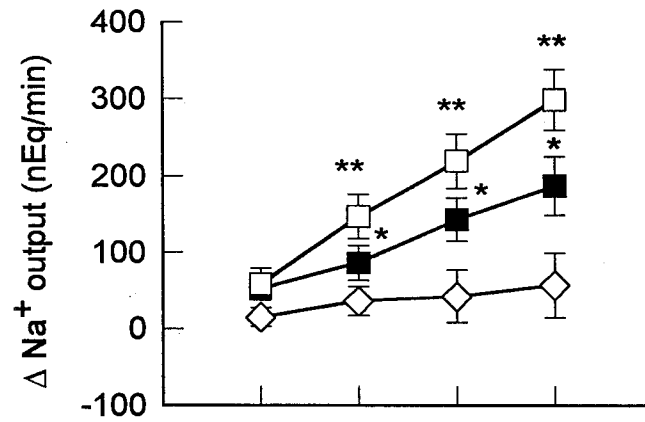
A**B**

Figure 31: Dose-response effects (mean \pm S.E.) on urine flow (A) and urine osmolality (B) following renal arterial injections of adrenomedullin (■), calcitonin gene-related peptide (□) or an equal volume of vehicle (0.45% NaCl; ◇) in three groups (n = 8 each) of Inactin-anaesthetized rats. *significantly different from the time-control; **significantly different from the other two groups.

A



B

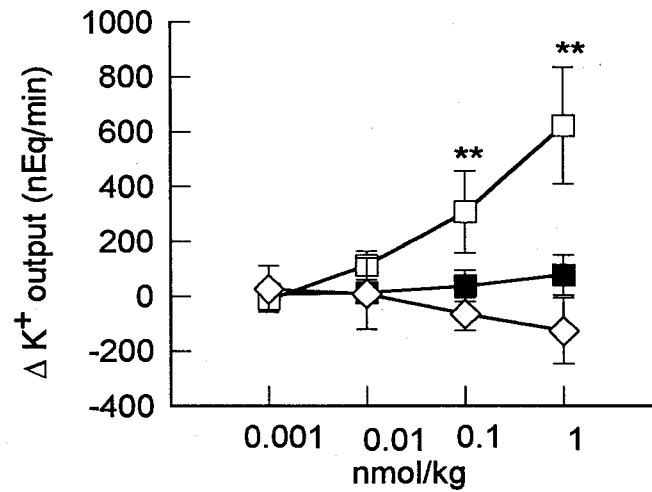


Figure 32: Dose-response effects (mean \pm S.E.) on urinary Na^+ (A) and K^+ excretion rate (B) following renal arterial injection of adrenomedullin (■), calcitonin gene-related peptide (□) or an equal volume of vehicle (0.45% NaCl; ◇) in three groups ($n = 8$ each) of Inactin-anaesthetized rats. *significantly different from the time-control; **significantly different from the other two groups.

3.12. Effect of CGRP(8-37) and [tyr⁰]CGRP(28-37) on renal actions of adrenomedullin

Table 12 shows the baseline values of MAP, HR, and renal haemodynamics and excretion prior to the infusion of adrenomedullin or the vehicle in eight groups.

3.12.1. Effects of adrenomedullin on MAP, HR, RBF and GFR in the absence or presence of CGRP receptor antagonists:

Renal arterial infusion of adrenomedullin caused insignificant changes in MAP and HR either in the absence (Fig. 33A,B) or the presence of the low and high doses of CGRP(8-37) (Fig. 33C,D) or [tyr⁰]CGRP(28-37) (Fig. 33E,F).

Adrenomedullin but not the vehicle, significantly and dose-dependently, increased RBF and renal arterial conductance indicating renal vasodilatation (Fig. 34A,B). Curve analyses show that neither one of the two doses of CGRP(8-37) (Fig. 34C,D) nor the two doses of [tyr⁰]CGRP(28-37) (Fig. 34E,F) significantly inhibited the increases in RBF or arterial conductances elicited by adrenomedullin.

GFR was also increased by all doses of adrenomedullin (Fig. 35A). The increases in GFR by adrenomedullin were not inhibited by both doses of CGRP(8-37) (Fig. 35B) or [tyr⁰]CGRP(28-37) (Fig. 35C).

3.12.2. Effects of adrenomedullin on urinary flow, osmolality, Na⁺ and K⁺ excretion in the absence or presence of the CGRP receptor antagonists

Adrenomedullin did not affect urine osmolality or K⁺ excretion (Figs. 36B; 37B) but dose-dependently ($p < 0.05$) increased urine flow and Na⁺ excretion (Figs. 36A; 37A), which were significantly different from the corresponding time-control readings at

all doses for urine flow and the highest three doses for Na^+ excretion. Fractional excretion of Na^+ was not significantly changed by any dose of adrenomedullin (0.24% baseline value, and 0.25, 0.22, 0.28, 0.25% after the four incremental doses of adrenomedullin).

In rats pre-treated with either the low or the high doses of CGRP(8-37) or $[\text{tyr}^0]$ CGRP(28-37), adrenomedullin similarly had no effect on urine osmolality (Fig. 36D,F), or absolute or fractional K^+ excretion (Fig. 37D,F) but significantly increased urine flow and Na^+ excretion (Fig. 36C,E; 37C,E). Fractional excretion of Na^+ was also not increased by CGRP following administrations of either of the two doses of CGRP(8-37) or $[\text{tyr}^0]$ CGRP(8-37) (results not shown).

Increasing the dose of CGRP(8-37) to 30 nmol/kg (n=2) and the dose of $[\text{tyr}^0]$ CGRP(28-37) to 100 nmol (n=2) similarly did not block either the vascular or tubular effects of adrenomedullin (results not shown).

GROUP	MAP	HR	RBF	COND	GFR	V	U _{Na} V	U
CGRP(8-37) 1 nmol/kg: veh.	110±4	362±10	13.9±0.7	0.127±0.011	1.36±0.12	4.6±0.4	223±50	956
CGRP(8-37) 1 nmol/kg: AM	99±7	356±12	13.6±1.2	0.132±0.010	1.41±0.15	5.4±0.6	203±49	856
CGRP(8-37) 10 nmol/kg: veh.	104±3	378±9	13.2±0.9	0.116±0.008	1.76±0.19	4.9±0.5	304±72	78
CGRP(8-37) 10 nmol/kg: AM	102±7	355±24	12.1±1.5	0.122±0.018	1.49±0.22	5.5±0.4	275±53	96
[tyr⁰]CGRP(28-37) 3 nmol/kg: veh.	104±9	377±14	13.1±0.7	0.134±0.011	1.39±0.15	4.3±0.4	213±39	68
[tyr⁰]CGRP(28-37) 3 nmol/kg: AM	100±5	352±16	12.5±1.1	0.127±0.016	1.46±0.18	4.1±0.4	227±37	905
[tyr⁰]CGRP(28-37) 30 nmol/kg: veh.	97±4	340±9	12.1±1.1	0.128±0.012	1.38±0.19	5.2±0.4	387±56	116
[tyr⁰]CGRP(28-37) 30 nmol/kg: AM	100±7	378±14	13.4±0.5	0.135±0.008	1.20±0.15	5.1±0.4	364±55	75

Table 12: Baseline values (mean ± S E.) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND, ml/min/mmHg), glomerular filtration rate (GFR, ml/min), urine Na⁺ (U_{Na}V, nmol/min), urine K⁺ (U_KV, nmol/min) and urine osmolality (U_{OSM}, mOsm/kg) at the first sample following renal arterial infusion of vehicle (0.45% NaCl) in eight groups (n = 6 each) of inactin-anaesthetized rats. AM, administration of the CGRP antagonists or an equal volume of the vehicle.

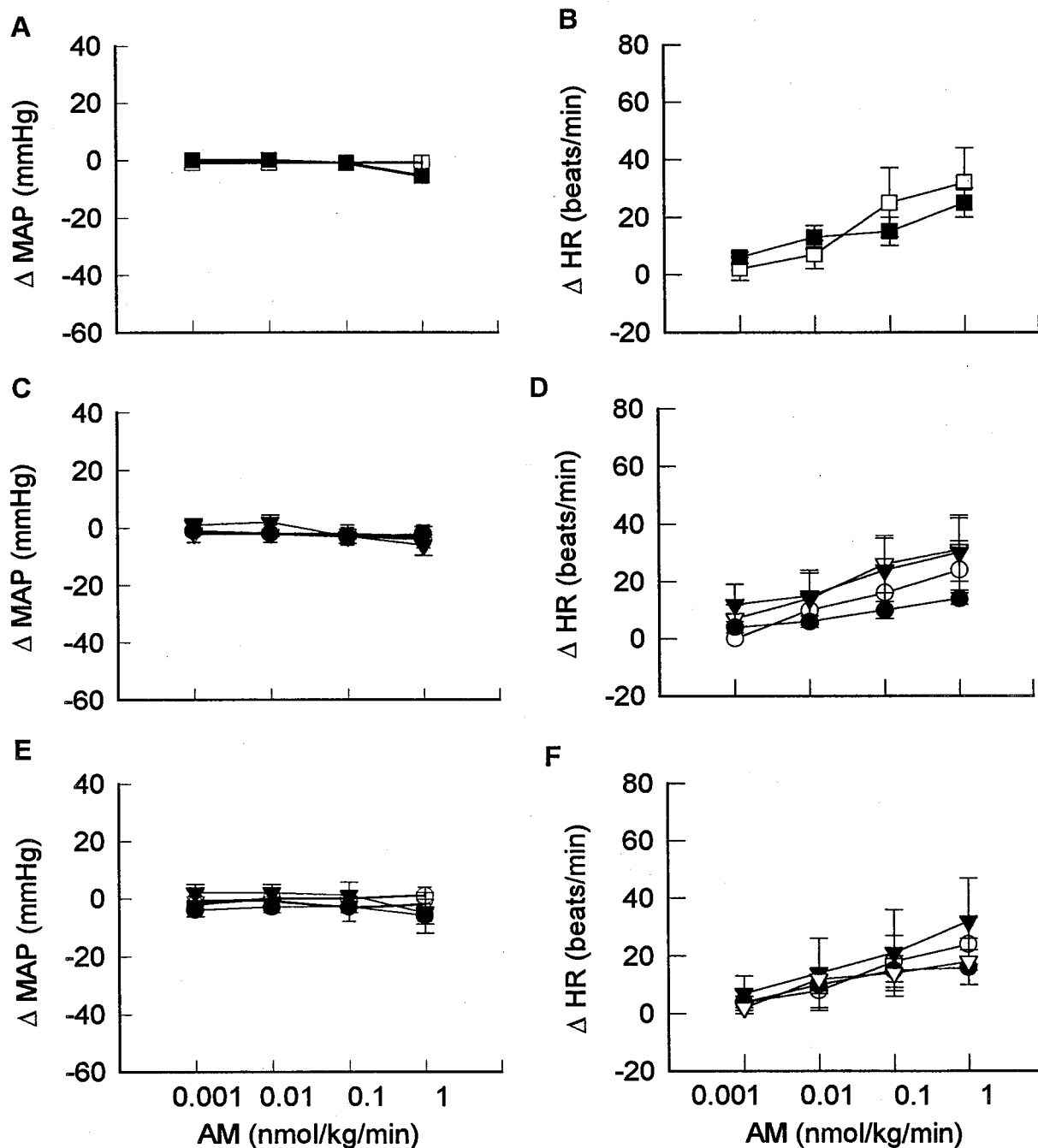


Figure 33: Effects (means \pm S.E.) of adrenomedullin (AM, ■) or equal volume of vehicle (0.45% NaCl solution; □) on mean arterial pressure (MAP) and heart rate (HR) in Inactin-anaesthetized rats ($n = 6$ per group) in the absence (A, B) or presence of CGRP (8-37) at 1 nmol/kg (●, ○) or 10 nmol/kg (▼, ▽) (C, D) or [tyr^0]CGRP(28-37) at 3 nmol/kg (●, ○) or 10 nmol/kg (▼, ▽) (E, F). * Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

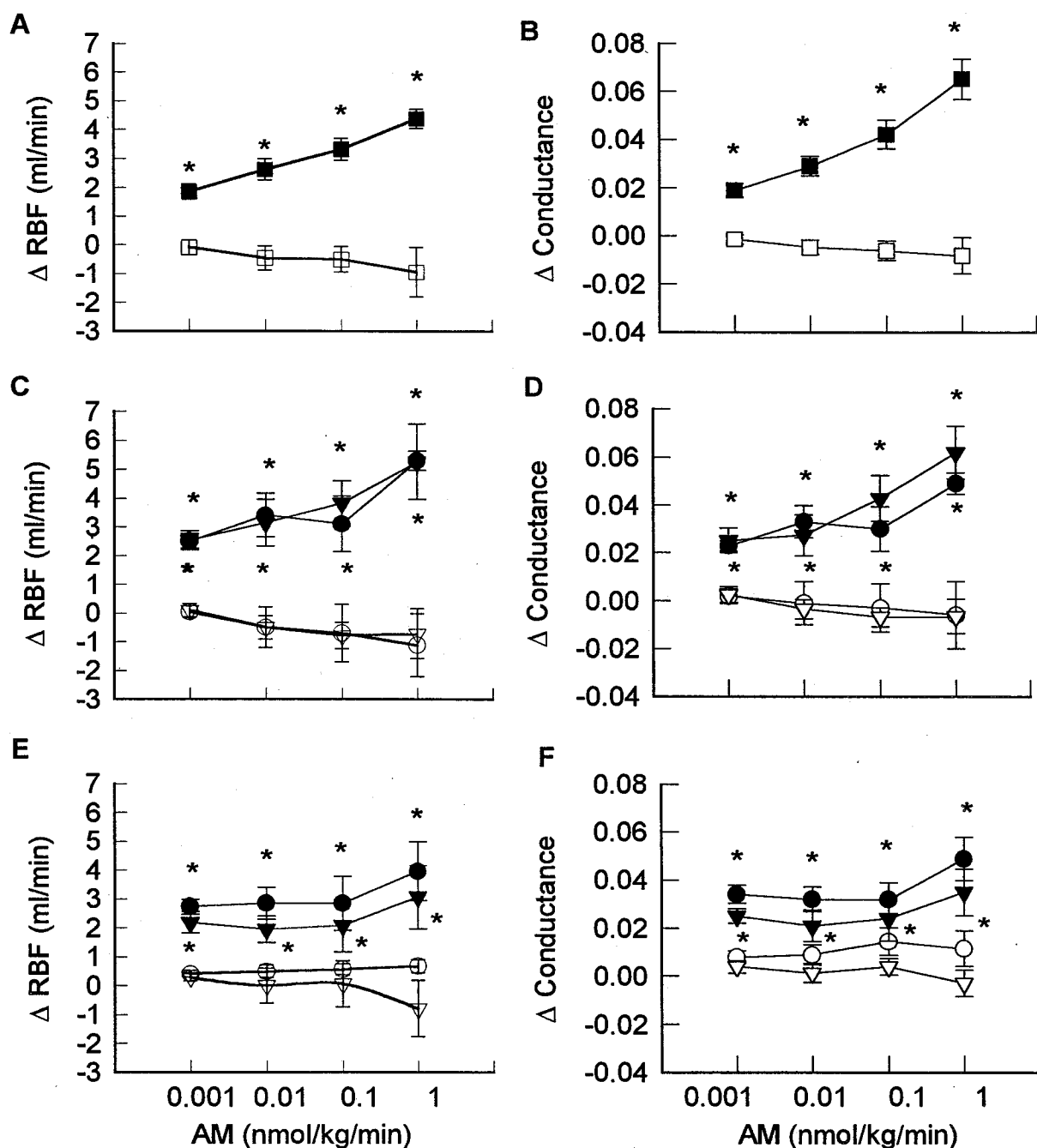


Figure 34: Effects (means \pm S.E.) of adrenomedullin (AM, ■) and equal volume of vehicle (0.45% NaCl solution, □) on renal blood flow (RBF) and renal arterial conductance in Inactin-anaesthetized rats ($n = 6$ per group) in the absence (A, B) or presence of CGRP (8-37) at 1 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (C, D) or [tyr⁰]CGRP(28-37) at 3 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (E, F). * Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

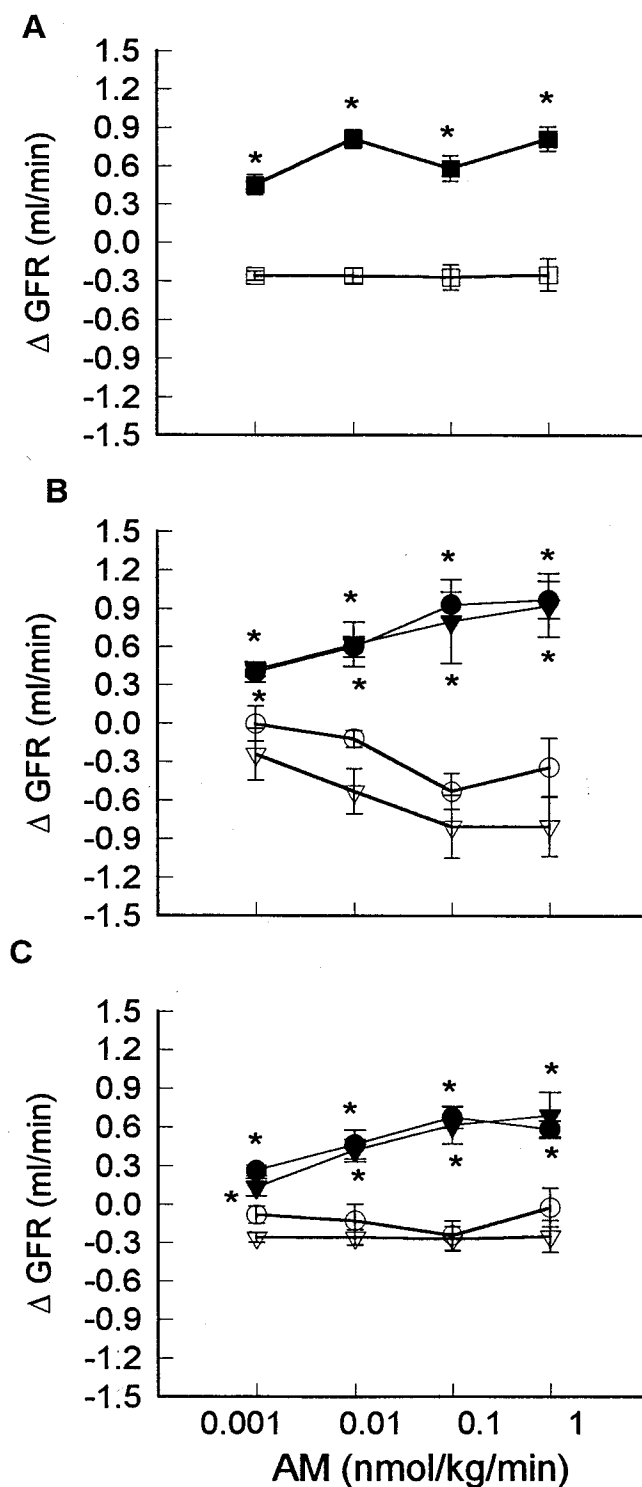


Figure 35: Effects (means \pm S.E.) of adrenomedullin (AM, ■) and equal volume of vehicle (0.45% NaCl, □) on glomerular filtration rate (GFR) in Inactin-anaesthetized rats ($n = 6$ per group) in the absence (A) or presence of CGRP (8-37) at 1 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (B) or [tyr⁰]CGRP(28-37) at 3 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (C). * Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

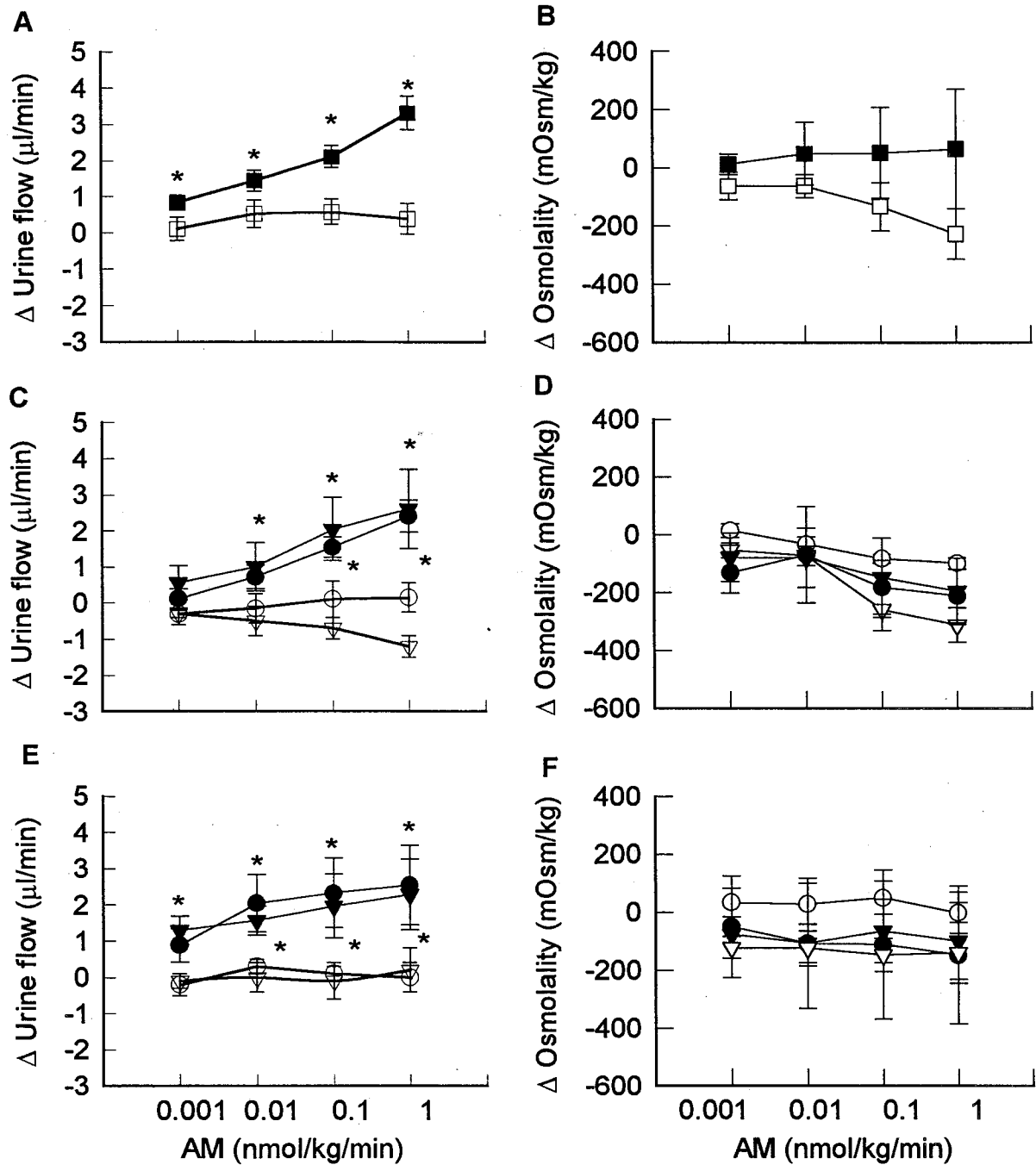


Figure 36: Effects (means \pm S.E.) of adrenomedullin (AM, ■) and equal volume of vehicle (0.45% NaCl solution, □) on urine flow and urine osmolality in Inactin-anesthetized rats ($n = 6$ per group) in the absence (A, B) or presence of CGRP (8-37) at 1 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (C, D) or [tyr⁰]CGRP(28-37) at 3 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (E, F). * Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

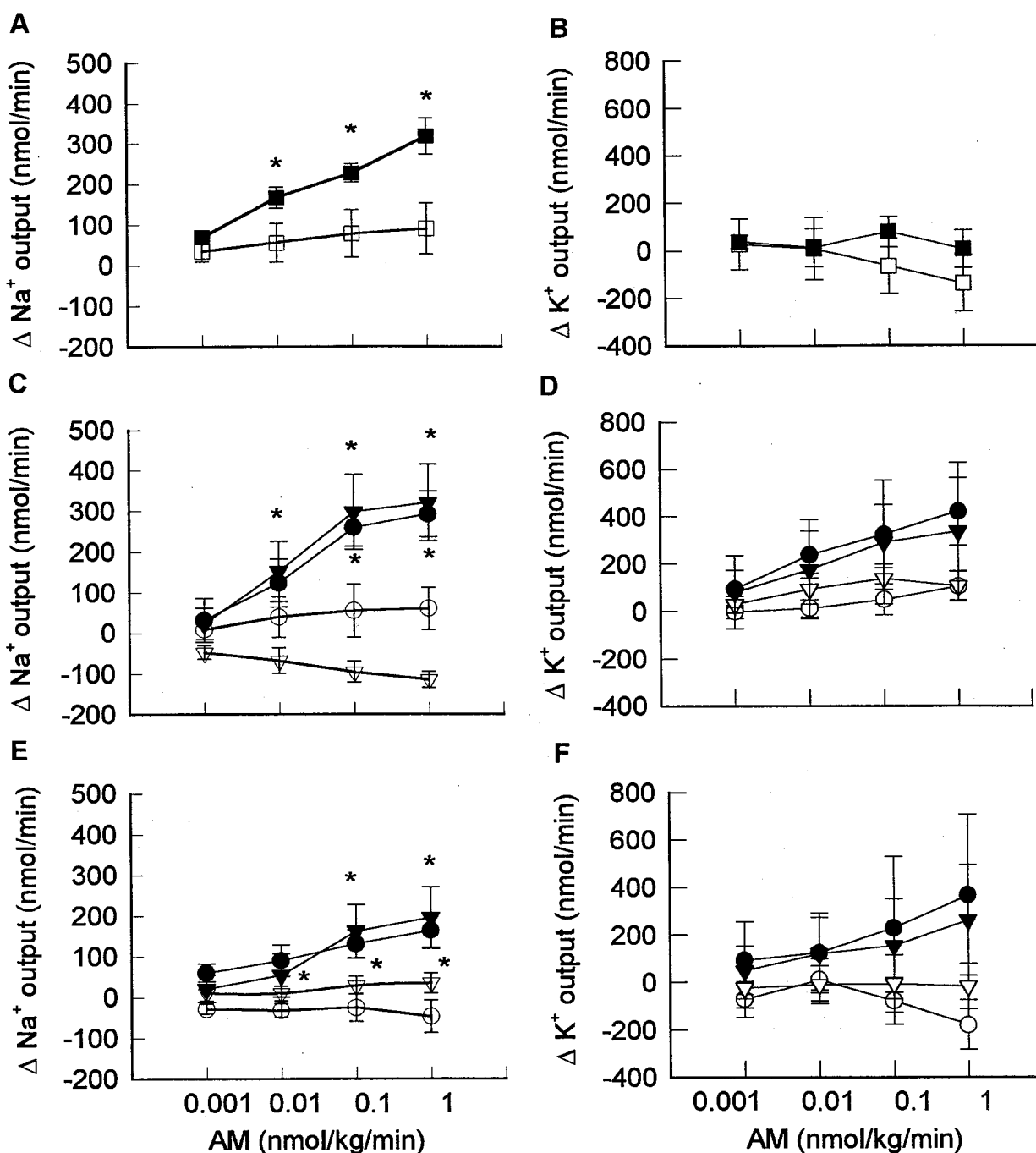


Figure 37: Effects (means \pm S.E.) of adrenomedullin (AM, ■) and equal volume of vehicle (0.45% NaCl, □) on urinary Na^+ and urinary K^+ excretion rates in Inactin-anaesthetized rats ($n = 6$ each group) in the absence (A, B) or presence of CGRP (8-37) at 1 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (C, D) or [tyr^0]CGRP(28-37) at 3 nmol/kg (●, O) or 10 nmol/kg (▼, ▽) (E, F). * Indicates significant difference from the corresponding value in the vehicle group ($P < 0.05$).

4. DISCUSSION

4.1. Renal actions of CGRP

The presence of CGRP nerve fibers and CGRP receptors in the kidney and the coupling of the receptors to adenylyl cyclase suggest that CGRP participates in the regulation of renal function. One of our goals was to characterize the dose-response relationship of CGRP on renal haemodynamics and renal excretion. Our results show that the renal arterial infusion of CGRP, at doses which did not alter systemic MAP or HR, caused a biphasic vascular response. At low doses (0.3, 3 pmol/kg/min), CGRP increased RBF and GFR, and elicited renal vasodilatation. At a high dose (300 pmol/kg/min), CGRP decreased RBF and produced vasoconstriction without changing GFR. The biphasic effect of CGRP on renal arterial conductance suggests the involvement of more than one effector mechanism/receptor subtype. The renal vasodilator as well as the vasoconstrictor effects of CGRP occurred in the absence of a change in MAP and therefore, were neither secondary to, nor modulated by, alterations in sinoaortic baroreflex activities.

The renal vasodilator, but not the vasoconstrictor, response to i.v. infused CGRP has been reported previously. In anesthetized rats and dogs, CGRP caused depressor responses and renal vasodilatation (Villarreal et al., 1988, Gardiner et al, 1989, Amuchastegui et al., 1994). In rabbits, CGRP reduced MAP and RBF but did not affect renal vascular conductance (Bauerfiend et al., 1989). In humans, CGRP reduced blood pressure, stimulated diuresis and induced two-fold increase in fractional

excretion of sodium without altering GFR. It also raised the plasma levels of noradrenaline, adrenaline and dopamine and increased renin activity (Gnaedinger et al., 1989). In the isolated, constant pressure-perfused rat kidney, CGRP reduced vascular resistance and increased GFR and urine flow (Kurtz et al., 1989b, Chin et al., 1994). The i.v. infusion of CGRP in conscious rats caused renal and hindquarters vasodilatation but mesenteric vasoconstriction (Gardiner et al., 1989).

Whereas low doses of CGRP did not affect urine flow, excretion of Na^+ and K^+ and urine osmolality, high doses increased urine flow and absolute Na^+ and K^+ excretion but did not affect urine osmolality. The fractional excretion of Na^+ was also increased by high doses of CGRP suggesting reduced tubular reabsorption of Na^+ rather than increased filtration load. The tubular actions of CGRP were independent of CGRP's vascular actions since RBF and GFR were not increased by high doses of CGRP. CGRP tubular actions are mediated either via a direct effect of CGRP on specific receptors in the renal tubules or an indirect mechanism which may involve the release of atrial natriuretic peptide or the inhibition of aldosterone release (Murakami et al., 1989, 1990). Surgical stress is also known to reduce the renal excretion of Na^+ (Maddox et al., 1977) but whether or not anesthesia and surgery affected the natriuretic and kaliuretic responses to CGRP is unclear.

To summarize, renal arterial infusion of CGRP caused renal vasodilatation and increased GFR at low doses, and vasoconstriction, diuresis, natriuresis and kaliuresis at high doses.

4.2. Effect of L-NAME on renal actions of CGRP

The involvement of EDRF/NO in the vasodilator response of CGRP was also investigated via the renal intra-arterial injection of L-NAME. L-NAME was reported to increase MAP and decrease RBF, renal plasma flow, renal arterial conductance, GFR, urine flow and Na⁺ excretion (Baylis et al., 1990; Lahera et al., 1991; Baumann et al., 1992; Granger et al., 1992). In our study, two doses of L-NAME were used. The low dose (2 µmol/kg) did not affect MAP and the high dose (20 µmol/kg) increased MAP by about 30%. In spite of causing drastically different systemic effects, both doses of L-NAME similarly reduced RBF, renal arterial conductance, GFR, urine flow, and Na⁺ and K⁺ excretion. Therefore, our results of L-NAME are in accordance with those reported previously.

The renal arterial infusion of CGRP also did not alter MAP or HR following the intra-arterial injection of the low, non-hypertensive dose of L-NAME. However, following pretreatment with the high dose of L-NAME which markedly raised MAP, CGRP dose-dependently reduced MAP and increased HR. Since the renal vasodilator effect of CGRP was inhibited by L-NAME (see later), the depressor response of CGRP was independent of EDRF/NO (L-NAME-resistant) and was likely due to vasodilatation of systemic (other than renal) resistance and/or capacitance vessels. Greater depressor response to CGRP during L-NAME-induced hypertension is in accordance to the well-known observations of augmented drug-induced vasodilatation of resistance and capacitance vessels in the presence of elevated vasomotor tone. The venodilator action of CGRP has been also reported previously (Abdelrahman and

Pang, 1992).

Pretreatment with L-NAME dose-dependently attenuated the renal vasodilator effect and increments in GFR elicited by low doses of CGRP but did not affect the vasoconstrictor effect. The ability of the non-hypertensive dose of L-NAME to abolish the renal vasodilatation effect of CGRP suggests that the renal vasodilatation response to CGRP was due to the local biosynthesis of EDRF/NO. To ensure that CGRP was capable of vasodilating a pre-constricted kidney, a CGRP dose-response curve was also constructed in a separate group of rats given continuous renal arterial infusion of phenylephrine. In contrast to L-NAME, precontraction of the kidney with phenylephrine potentiated the renal vasodilator and the increase in GFR effects of CGRP.

Our findings are different from those of Gardiner et al., (1991) which showed that i.v. infusion of a single dose of CGRP (15 nmol/kg/hr) into rats pretreated with L-NAME (10 mg/kg) caused sustained depressor response, tachycardia and transient renal vasodilatation suggesting that the renal vasodilatation response of CGRP was not due to the release of EDRF/NO. A comparison of results between this and our study is difficult, since all drugs were administered systemically in the study of Gardiner et al., (1991) but infused or injected directly into the renal artery in the present study to minimize systemic baroreflex activation. In agreement with our data, Amuchastegui et al. (1994) reported that L-NAME completely blocked the increase in renal plasma flow and glomerular filtration rate induced by i.v. infusion of CGRP in anaesthetized rats. L-arginine infusion in L-NAME treated rats restored the renal

responses to CGRP (Amuchastegui et al., 1994).

Neither doses of L-NAME inhibited the diuretic, natriuretic and kaliuretic effects of CGRP. The absence of inhibition by L-NAME suggests a lack of involvement of EDRF/NO in the tubular actions of CGRP. The diuretic, natriuretic and kaliuretic effects of CGRP were greater in kidneys pretreated with phenylephrine than those pretreated with the vehicle or L-NAME. The potentiation by phenylephrine could be related to enhanced vasodilatation and glomerular filtration in a precontracted kidney, effects which are not seen in the L-NAME-precontracted kidney due to the blockade of EDRF/NO biosynthesis.

Thus, the renal vasodilatation effect of CGRP and the increment in GFR by CGRP were inhibited by L-NAME indicating the involvement of EDRF/NO. In contrast, the vasoconstrictor, diuretic, natriuretic and kaliuretic effects of CGRP were unaffected by L-NAME.

4.3. Effect of losartan on renal actions of CGRP

The mechanism by which renal arterially-infused CGRP caused vasoconstriction is unclear but is unlikely due to baroreflex modulation since MAP was unaltered. The involvement of the renin-angiotensin system was a possibility since CGRP increased renin release (Kurtz et al., 1988) and captopril (angiotensin converting enzyme inhibitor) enhanced the renal vasodilator effect of CGRP (Bennett et al., 1989; Gardiner et al., 1990b). To examine this possibility, rats were pretreated with renal arterial injection of either a low, non-hypotensive dose or a high, hypotensive dose of losartan, the angiotensin II (AT1) receptor antagonist. Both doses

of losartan increased RBF, renal arterial conductance, GFR, urine flow and Na^+ excretion without changing HR, urine osmolality or K^+ excretion. Zhuo et al. (1992) reported that i.v. administration of losartan in anaesthetized rats slightly reduced MAP and increased GFR, urine flow and Na^+ excretion without changing renal plasma flow, renal vascular resistance or K^+ excretion (Zhuo et al., 1992). As well, intra-renal infusion of losartan in anesthetized dogs increased RBF, GFR, urine flow and urinary Na^+ output (Chan et al., 1992). Our results of the renal effects of losartan are therefore similar to those previously reported. The increases in RBF and GFR by losartan may indicate that endogenously released angiotensin II has a role in the physiological regulation of renal haemodynamics.

In the presence of losartan, the high dose of CGRP reduced MAP. Unexpectedly, the vasoconstrictor effect to the high dose of CGRP was not affected by either the low or the high dose of losartan indicating that it was not due to activation of the renin-angiotensin system. Our results are somewhat different from those of Gardiner et al., (1990b) which showed that continuous i.v. infusion of CGRP into conscious rats elicited transient renal vasodilatation, then a return of renal vascular resistance to baseline values and renal vasoconstriction following the cessation of CGRP infusion. These investigators suggested that the termination and reversal of the renal vasodilatation effect of CGRP was due to renin release. This interpretation was supported by results which show that captopril abolished vasoconstriction that occurred after stopping CGRP infusion (Gardiner et al., 1990b). Since CGRP was infused into the renal artery in the present study, the vasoconstrictor response elicited

by a high dose of CGRP was not secondary to the adjustment of cardiovascular reflex systems in response to systemic hypotension. The lack of effect of losartan in inhibiting renal vasoconstrictor response to CGRP shows the lack of involvement of the renin-angiotensin system.

The diuretic, natriuretic and kaliuretic effects of CGRP were inhibited by both doses of losartan. Since renal arterial infusion of a hypotensive dose of sodium nitroprusside also inhibited CGRP-induced diuresis, natriuresis and kaliuresis, it is evident that the inhibition by losartan of tubular effects of CGRP was likely non-specific, due to reduced MAP, renal arterial pressure and RBF following the infusion of a high doses of CGRP in the presence of losartan.

To summarize, pre-treatment with losartan did not affect the vasoconstrictor effect to a high dose of CGRP. Losartan's inhibition of diuresis, natriuresis and kaliuresis elicited by a high dose of CGRP was primarily due to systemic hypotension and reduced RBF.

4.4. Effect of phenoxybenzamine and mecamylamine on renal actions of CGRP

Renal sympathetic nerves are well-known to actively participate in the regulation of renal function. Stimulation of renal sympathetic nerves or the administration of α -adrenoceptor agonists induces renal vasoconstriction and increased tubular reabsorption of water and Na^+ (DiBona 1985; Jeffries and Pettinger, 1989) via the activation of α_1 -adrenoceptors (DiBona 1985, DiBona and Sawin 1987) or α_2 -adrenoceptors (Smyth et al., 1984, 1986; Pettinger 1987; Wolff et al., 1989). It has also been shown that renal vasoconstriction is primarily mediated via α_{1A} -

adrenoceptor subtype (Elhawary et al., 1992), whereas the antidiuresis and increased renal Na^+ reabsorption primarily involve α_{1B} -adrenoceptors (Elhawary and Pang, 1994).

To examine if the renal vasoconstrictor effect of CGRP were due to renal sympathetic activation, the rats were pretreated with renal arterial injection of either the ganglion blocker mecamylamine or the irreversible α_1 -adrenoceptor antagonist phenoxybenzamine. Phenoxybenzamine in a dose similar to the dose used in our study blocked all renal α_1 -adrenoceptors and also one third of α_2 -adrenoceptors in radioligand binding assays (Smyth et al., 1984; Elhawary et al., 1991).

Mecamylamine but not phenoxybenzamine reduced MAP but neither blocker altered renal haemodynamics and renal excretion. MAP was reduced by the highest dose of CGRP in phenoxybenzamine-treated rats and the two highest doses of CGRP in mecamylamine-treated rats. This may have been due to partial systemic ganglionic blockade elicited by mecamylamine thereby interfering with cardiovascular compensation mechanisms. HR was increased by the highest dose of CGRP in rats treated with phenoxybenzamine but unaltered by any dose of CGRP in rats treated with mecamylamine suggesting that the tachycardiac effect of CGRP was reflexly mediated. Phenoxybenzamine did not affect the renal vasodilatation and the increase of GFR effects elicited by low doses of CGRP but inhibited the renal vasoconstrictor effect elicited by a high dose of CGRP suggesting that the vasoconstriction was mediated via α -adrenoceptors. Pre-treatment with mecamylamine did not affect the vasodilator, vasoconstrictor or increase in GFR effects of CGRP suggesting that these

actions are not mediated via reflex. The blockade by phenoxybenzamine, but not mecamylamine, of the vasoconstrictor effect elicited by a high dose of CGRP suggests that the renal vasoconstriction was due to the release of noradrenaline from sympathetic nerve terminals. Therefore, we suggest that CGRP has a complex vascular effect, it causes direct renal vasodilatation at a low dose and renal vasoconstriction at a high dose; the latter action is possibly mediated via the release of noradrenaline from sympathetic nerve terminals.

The diuretic, natriuretic and kaliuretic effects of CGRP were inhibited by pre-treatment with both phenoxybenzamine and mecamylamine despite the different levels of sympathetic blockade. The inhibition is likely non-specific, a result of reduced MAP, renal perfusion pressure and RBF, since the tubular effects of CGRP were also inhibited by losartan and sodium nitroprusside.

Possible interaction between CGRP and sympathetic nerve function has been suggested. CGRP is present in autonomic ganglia (Yamamoto and Tohyama, 1989). Central administration of CGRP increased sympathetic outflow and plasma noradrenaline level and induced a dose-related elevation of MAP and HR (Fisher et al., 1983; Hasegawa et al., 1993; Messmer et al., 1993; Kuo et al., 1994). The CGRP-induced central sympathetic activation was inhibited by central injection of the CGRP receptor antagonist CGRP (8-37), phenoxybenzamine and by chemical sympathectomy with 6-hydroxydopamine (Kuo et al., 1994), indicating that it was mediated via CGRP receptors, sympathetic activation and the release of catecholamines. Additionally, Hasegawa et al., (1993) reported that both electrical

stimulation or injection of CGRP into the hypothalamic paraventricular nucleus, which is the higher center for central regulation of sympathetic function, elicited both sympathetic noradrenergic and adrenomedullary outflows. Pre-treatment with the ganglion blocker chlorisondamine or the adrenergic neuron blocker bretylium or the α -adrenoceptor antagonist phentolamine abolished exocrine pancreatic secretion effects mediated by central administration of CGRP (Messmer et al., 1993). Peripherally, pretreatment with hexamethonium did not alter the cardiovascular responses to i.v. infused CGRP in anaesthetized rabbits (Andersson 1989). On the other hand, it has been suggested that adrenergic and CGRP nerves interact to cause reciprocal control of arteriolar tone at the level of sympathetic nerve terminals (Kawasaki et al., 1990a and b). CGRP released interferes with adrenergic vasoconstriction through a direct effect on vascular smooth muscle (Kawasaki et al., 1990a) and released noradrenaline modulates CGRP-induced vasodilatation by inhibiting CGRP release via presynaptic α_2 -adrenoceptors located on CGRP nerves (Kawasaki et al., 1990b).

To summarize, ganglionic blockade and the blockade of α -adrenoceptors potentiated the hypotensive and tachycardiac effects but did not affect the renal vasodilatation and the increase in RBF and GFR effects of CGRP. The vasoconstrictor effect elicited by a high dose of CGRP was inhibited by α_1 -adrenoceptor blockade but not ganglionic blockade suggesting that the vasoconstriction induced by a high dose of CGRP was due to the release of noradrenaline from the nerve terminals. Renal excretory effects elicited by high, hypotensive doses of CGRP were inhibited by sympathetic blockade and these effects

were likely mediated via CGRP-induced hypotension, reduced renal perfusion pressure and decreased RBF.

4.5. Effect of indomethacin on renal actions of CGRP

The possible contribution of renal prostanoids to the renal actions of CGRP was also investigated using the cyclo-oxygenase inhibitor indomethacin. Indomethacin has been shown to attenuate the *in vitro* relaxant effect of CGRP in precontracted rat aortic strips (Brain et al., 1985). Aspirin, another cyclo-oxygenase inhibitor, also abolished the flare elicited by intradermal injection of CGRP in man (Barnes et al., 1987). In cultured endothelial cells of the umbilical vein, CGRP induced concentration-dependent release of prostacyclin and activation of adenylyl cyclase (Crossman et al., 1987). These studies suggest a possible role of prostanoids in mediating CGRP's vascular effects. In contrast, *in vivo* administration of indomethacin had no effect on vasodilatation induced by CGRP in rabbit skin (Brain et al., 1985). Pre-treatment with indomethacin also did not affect cardiovascular responses to i.v. infused CGRP in rabbits (Andresson 1989).

It is generally believed that non-steroidal anti-inflammatory drugs such as indomethacin promote renal water and Na⁺ retention without affecting renal haemodynamics (Clive and Stoff, 1984). Antikaliuresis in response to indomethacin has been observed (Roman and Kauker, 1978; Dusing et al., 1982). In man, indomethacin markedly reduced Na⁺ but not K⁺ excretion (Van Buren et al., 1992) and had no effect on renal haemodynamics (Passmore et al., 1989). In anaesthetized rats, indomethacin did not affect RBF or urine flow rate but markedly inhibited absolute and

fractional excretion of K^+ (Kaufman and Hampurger, 1992). In intact rats, indomethacin decreased GFR and urinary excretion but increased fractional reabsorption of Na^+ . (Rubinger et al., 1990). However, indomethacin attenuated the contractile response to noradrenaline and potassium chloride in rat renal arcuate arteries (Wu et al., 1994).

Our results show that renal arterial injection of indomethacin has no effect on systemic blood pressure or renal function. Indomethacin also did not alter renal haemodynamic changes induced by CGRP thereby excluding the participation of prostanoids in CGRP's actions on RBF, renal arterial conductance and GFR. Similarly, the diuretic and natriuretic effects of CGRP were not affected by indomethacin. However, indomethacin abolished the increase in urinary K^+ excretion elicited by CGRP. This may be due to possible additive effect by indomethacin to CGRP's inhibitory effect on aldosterone release since both CGRP and indomethacin have been reported to inhibit aldosterone release (Murakami et al., 1989; 1991; Goldszer et al., 1981; Kutyrina et al., 1979).

In summary, pre-treatment with indomethacin did not affect CGRP actions on renal haemodynamics, diuresis and natriuresis but blocked the increase in K^+ excretion elicited by high doses of CGRP.

4.6. Receptor subtypes mediating renal actions of CGRP

Evidence suggest the existence of heterogeneous CGRP receptors (Dennis et al., 1989, 1990; Mimeault et al., 1991; Giuliani et al., 1992; Stengl et al., 1993; for review see Poyner, 1992a). The precise classification of these receptors has been

hindered by a lack of selective antagonists to subtypes of CGRP receptors. In the present study, we attempted to characterize subtypes of CGRP receptor mediating the renal tubular and vascular effects of CGRP through using the CGRP1 selective antagonist, CGRP(8-37) and the putative antagonist [tyr⁰]CGRP(28-37). CGRP dose-response curves were constructed by renal intra-arterial infusion of the drug in the absence and presence of either a low or a high dose of an antagonist.

The results show that the renal vasodilatation and increased GFR responses of CGRP were inhibited completely by both doses (1 and 10 nmol/kg) of CGRP(8-37) and incompletely inhibited by both doses (3 and 30 nmol/kg) of [tyr⁰]CGRP(28-37). Both antagonists also blocked the renal vasoconstrictor responses to CGRP. The ability of CGRP(8-37) to inhibit vascular responses to CGRP has been reported. CGRP(8-37) inhibited relaxation responses to CGRP in the isolated perfused rat kidney (Castellucci et al., 1993; Chin et al., 1994), rat perfused mesenteric arterial bed (Han et al., 1993), porcine coronary artery (Franco-Cereceda, 1992). CGRP (8-37) also inhibited vasodilatation elicited by CGRP *in vivo* in the rabbit (Hughes and Brain, 1991) and rat (Escott and Brain, 1994), skin beds and cat cerebral arterioles (Wei et al., 1992). In conscious rats, CGRP(8-37) inhibited vasodilatation responses to CGRP in the renal and hindquarter beds and vasoconstrictor response to CGRP in the mesenteric bed (Gardiner et al., 1990a).

The presence of molecular forms of CGRP with different potencies and biological activities (Poyner, 1992a) suggests the existence of multiple subtypes of CGRP receptors. Radioligand binding and pharmacological studies supported the

existence of at least two subtypes of CGRP receptors. CGRP binding sites are classified as CGRP1 and CGRP2 according to the high or low affinity, respectively, of these sites to the C-terminal fragment CGRP(8-37) (Mimeault et al., 1991). Similarly, CGRP receptors are postulated to be of the CGRP1 or CGRP2 subtypes on the basis of susceptibility of the response to antagonism by CGRP(8-37) in isolated tissues (Dennis et al., 1990, Mimeault et al., 1991). However, there is as yet insufficient information to indicate if the CGRP2 receptors are homogeneous or heterogeneous. The ability of CGRP(8-37) to block the renal vasodilatation as well as the vasoconstriction effects of CGRP in the present study suggests that both renal vasodilatation and vasoconstriction are mediated via the activation of CGRP1 receptor subtype. Since the renal vasodilatation response elicited by low doses of CGRP was also blocked by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (Section 4.2), our results suggest that renal vasodilatation in response to low doses of CGRP is due to the activation of renal vascular CGRP1 receptors causing the release of endothelium-dependent relaxing factor/nitric oxide.

The selectivity of [tyr⁰]CGRP(8-37) on subtypes of CGRP receptors is unclear. [Tyr⁰]CGRP(8-37) induced a rightward shift of the concentration-effect curves to CGRP analogs on the opossum internal anal sphincter smooth muscle without affecting the resting tension (Chakder and Rattan, 1990). This blocker also inhibited the CGRP-induced amylase secretion in the isolated acini from the guinea pig pancreas (Manton et al., 1990) and blocked the stimulatory effect of CGRP on cAMP production in neuroblastoma cells (van Vlen et al., 1989). The ability of [tyr⁰]CGRP(28-37) to inhibit

the renal vasodilator and vasoconstrictor effects of CGRP in the present study suggests that [tyr⁰]CGRP(28-37) is also a CGRP1 receptor antagonist, although its potency and efficacy are less than those of CGRP(8-37).

It is unexpected that the highest dose of CGRP, which had no effect on MAP and HR when given alone, caused a depressor response and tachycardia in the presence of either the low or the high dose of CGRP(8-37) or [tyr⁰]CGRP(28-37). The mechanism by which CGRP reduced MAP in the presence of either one of the CGRP receptor antagonist is unclear but it is obviously not mediated via the activation of CGRP1 receptor. The depressor response may be due to either reduced peripheral vascular resistance or reduced cardiac output due to venodilatation. Reduced cardiac output seems more plausible on the basis that CGRP(8-37) blocked the vasodilator actions of CGRP in several vascular beds which include the mesenteric, hindquarter and renal beds (Chin et al., 1994; Han et al., 1992; hughes et al., 1991; Gardiner et al., 1990) and that CGRP lowers venomotor tone in rats (Abdelrahman and Pang, 1992). Whether or not CGRP (8-37) inhibits the venodilator effects of CGRP has never been tested.

Both CGRP(8-37) and [tyr⁰]CGRP(28-37) did not affect the diuretic, natriuretic and kaliuretic effects elicited by 30 pmol/kg/min of CGRP but significantly attenuated ($p < 0.05$) the effects elicited by 300 pmol/kg/min of CGRP. The absence of blockade of tubular effects elicited by 30 pmol/kg/min of CGRP suggests that these effects are not mediated via the activation of CGRP1 receptors. The attenuation of the diuretic, natriuretic and kaliuretic effects elicited by 300 pmol/kg/min of CGRP may be indirect,

the result of systemic hypotension, reduced renal perfusion pressure and decreased RBF.

To summarize, CGRP(8-37) abolished the renal vasodilatation, renal vasoconstriction and increments in GFR elicited by low doses of CGRP suggesting the predominance of CGRP1 receptors in the vasculature. The diuretic, natriuretic and kaliuretic responses elicited by non-hypotensive doses of CGRP were not affected by CGRP(8-37) suggesting the renal tubular actions of CGRP are not mediated via CGRP1 receptors. The profile of antagonistic actions of [Tyr⁰]CGRP(28-37) was similar to that of CGRP(8-37) indicating that [Tyr⁰]CGRP(28-37) is also an antagonist of CGRP1-receptors, although its potency are less than those of CGRP(8-37).

4.7. Renal actions adrenomedullin

There is as yet no information on the renal actions of adrenomedullin, a peptide which is synthesized in large quantities in the kidney, ventricle and adrenal medulla (Kitamura et al., 1993a,b). The pharmacological actions of adrenomedullin may be similar to those of CGRP since adrenomedullin is of similar homology to CGRP (Kitamura et al., 1993a) and its vasodilator effect is blocked by the CGRP receptor antagonist CGRP (8-37) (Nuki et al., 1993; Eguchi et al., 1994).

In the present study we compared the renal actions of adrenomedullin with those of CGRP. Both peptides were injected directly into the renal artery to minimize systemic effects. However, the highest dose (0.1 nmol/kg) of adrenomedullin and the highest two doses (0.01-0.1 nmol/kg) of CGRP significantly lowered MAP. Our results also show that renal arterial bolus injection of either adrenomedullin or CGRP caused

renal vasodilatation (increased renal arterial conductance), diuresis and natriuresis. Neither drug caused renal vasoconstriction when given via bolus renal arterial injection. Only CGRP caused kaliuresis and decreased urine osmolality.

There are similarities as well as differences between the physiological and pharmacological actions of adrenomedullin and CGRP. Adrenomedullin is present at a considerable concentration (range from 3 to 19 fmol/ml) in the blood (Kitamura et al., 1993a; 1994). CGRP is released locally from perivascular nerves (Diez Guerra et al., 1988) and it has a plasma half-life of around 6-8 min in human, as measured by radioimmunoassay (Kraenzlin et al., 1985; Struthers et al., 1986). According to Zaidi et al. (1990a), the plasma half-life of CGRP may be considerably less than 6-8 min, since radioimmunoassay measures intact as well as fragments of CGRP. In the present study, CGRP caused greater depressor responses than did adrenomedullin. HR was significantly increased at the highest dose of CGRP and this may be mediated via reflex modulation of baroreflex activity. All doses of CGRP caused less increases in RBF than adrenomedullin did and these effects were largely secondary to the greater hypotension and therefore lowered renal perfusion pressure in response to CGRP than adrenomedullin. However, the highest dose of adrenomedullin did cause significantly greater renal vasodilatation (increased renal arterial conductance) than CGRP did. These results suggest that adrenomedullin is a more selective renal vasodilator than CGRP. The duration of the renal vasodilatation responses to adrenomedullin and CGRP appear to be similar since the percents of peak vasodilatation remaining at 10 and 20 min following injections of the highest doses of

both peptides were almost identical.

The last three doses of CGRP caused markedly greater increases in urine flow and Na^+ excretion than adrenomedullin did which suggests that adrenomedullin is a less potent and less efficacious (assuming near maximum responses were attained) diuretic and natriuretic peptide than CGRP. CGRP caused greater depressor responses and tachycardia than adrenomedullin did, although significant differences were only obtained at the highest dose. Hypotension elicited by CGRP would likely cause reflex increase in sympathetic nerve activity, and the tachycardia observed is consistent with this interpretation. Increase in renal sympathetic nerve activity is known to reduce renal Na^+ and water excretion, mediated predominantly by renal tubular α_1 -adrenoceptors (DiBona, 1985; 1989). Therefore, the reflex increase in renal sympathetic drive by CGRP might have attenuated its diuretic and natriuretic effects.

CGRP but not adrenomedullin increased K^+ excretion and reduced urinary osmolality. Kaliuresis occurred at hypotensive doses of CGRP. Therefore, it is unclear if the kaliuretic effect of CGRP is a direct action on renal tubules or indirectly mediated via modulation of the release of neurohumoral agents which may affect renal K^+ transport. All doses of CGRP reduced osmolality. This might be a consequence of reduced overall solute excretion, the particular solutes involved are clearly not Na^+ and K^+ .

To summarize, adrenomedullin is a more efficacious as a renal vasodilator than

CGRP. Its diuretic and natriuretic actions are less than those of CGRP. In contrast to CGRP, adrenomedullin did not affect K^+ excretion and urinary osmolality.

4.8. Effects of CGRP receptor antagonist on renal actions of adrenomedullin

The renal effects of renal arterially infused adrenomedullin was also investigated in the absence and presence of the CGRP antagonists, CGRP(8-37) and [tyr⁰]CGRP(28-37) to find out if adrenomedullin acts via CGRP receptors. Renal arterial infusion of adrenomedullin, at doses that did not alter systemic arterial pressure, induced renal vasodilatation (increased arterial conductance), increased glomerular filtration, diuresis and natriuresis. The vascular actions of adrenomedullin are qualitatively different from those elicited by renal arterial infusion of CGRP (0.3 to 300 pmol/kg/min) as CGRP caused a biphasic vascular response in the renal bed, vasodilatation at a low dose (0.3 pmol kg/min) and vasoconstriction at a high dose (300 pmol/kg/min). The effects of adrenomedullin on GFR were also different from those of CGRP. GFR was increased by only low doses (0.3 and 3 pmol/kg/min) of CGRP but increased by all doses (1 to 1,000 pmol/kg/min) of adrenomedullin. Similar to CGRP, adrenomedullin dose-dependently increased Na^+ excretion and urine flow. The natriuretic effect of adrenomedullin was due to increased filtration load rather than reduced tubular reabsorption as fractional Na^+ excretion was unchanged. In contrast, the natriuretic effect of CGRP was associated with increased fractional excretion of Na^+ suggesting reduced tubular Na^+ reabsorption. Adrenomedulin did not alter urine osmolality or K^+ excretion which may exclude the involvements of antidiuretic hormone or aldosterone. CGRP, on the other hand, did not alter urine osmolality but increased

K⁺ excretion at a high dose.

The ability of the CGRP antagonist, CGRP(8-37) to block actions of adrenomedullin has been reported. CGRP(8-37) inhibited the centrally induced vasopressor response to adrenomedullin in anesthetized rats (Takahashi et al., 1994). It also attenuated the vasodilatation induced by adrenomedullin *in vitro* in isolated perfused mesenteric bed (Nuki et al., 1993) and *in vivo* in hamster and rat cheek pouch microvasculature (Hall et al., 1995). The stimulatory effect of adrenomedullin on cAMP production in cultured rat vascular smooth muscle cells was also inhibited by CGRP(8-37) (Eguchi et al., 1994, Ishizaka et al., 1994). The ability of a CGRP antagonist to block adrenomedullin actions in the central nervous system and in the vasculature raised the possibility that both peptides are either sharing the same receptor at these sites or at least cross-interact at their own specific receptors similar to the interaction reported for CGRP and calcitonin (Goltzman and Mitchel, 1983), and CGRP and amylin (Kreutter et al., 1993). However, there is evidence to suggest that adrenomedullin and CGRP bind to different receptors. Ishizaka et al. (1994) reported that the binding of [¹²⁵I]adrenomedullin in vascular smooth muscle cells was inhibited by adrenomedullin but not CGRP, suggesting that adrenomedullin may not activate CGRP receptors in vascular smooth muscles. Additionally, Gardiner et al., (1995) reported that the vasodilatation induced by adrenomedullin in the renal, mesenteric and hindquarter beds of conscious rats was not affected by i.v. infusion of CGRP(8-37).

The renal vascular and tubular actions of adrenomedullin were unaltered by

pretreatment with CGRP-(8-37), at doses which abolished the renal vasodilatation and vasoconstriction responses to both a low (0.3 pmol/kg/min) and a high (300 pmol/kg/min) dose, respectively, of CGRP. These results show that the renal actions of adrenomedullin were not mediated via CGRP1 receptors. Similarly, [tyr⁰]CGRP(8-37) did not significantly alter the vascular or tubular actions of adrenomedullin. These results again suggest that the renal actions of adrenomedullin are not mediated via CGRP receptors.

To summarize, renal arterial infusion of non-hypotensive doses of adrenomedullin induced renal vasodilatation, increased glomerular filtration, diuresis and increased absolute but not fractional Na⁺ excretion. Adrenomedullin did not affect K⁺ excretion or urine osmolality. Neither CGRP(8-37) nor [tyr⁰]CGRP(28-37) inhibited the renal vascular and tubular effects of adrenomedullin. The results show that neither the renal vascular nor tubular action of adrenomedullin is mediated via the activation of CGRP1 receptors.

5. SUMMARY AND CONCLUSIONS

Renal intra-arterial infusion of non-hypotensive doses of CGRP caused renal vasodilatation and increased GFR at a low dose, and vasoconstriction, diuresis, natriuresis and kaliuresis at a high dose. The renal vasodilatation effect of CGRP and the increments in GFR by CGRP were inhibited by L-NAME suggesting the involvement of EDRF/NO. In contrast, the vasoconstrictor, diuretic, natriuretic and kaliuretic effects of CGRP were unaffected by L-NAME. Pre-treatment with losartan slightly potentiated the renal vasodilatation effect of low doses of CGRP and the increase in GFR. However, it did not affect the vasoconstrictor effect of a high dose of CGRP. Losartan partially inhibited CGRP induced increases in urine, Na⁺ and K⁺ excretion primarily due to reduced renal perfusion pressure and RBF caused by systemic hypotension. The blockade of renal autonomic ganglia and α_1 -adrenoceptors by renal arterial injection of mecamylamine and phenoxybenzamine, respectively, potentiated the hypotensive and tachycardiac effect to high doses of CGRP. The vasodilatation and the increases in RBF and GFR effects of CGRP were not affected by mecamylamine or phenoxybenzamine. The vasoconstrictor effect of the high dose of CGRP was inhibited by phenoxybenzamine but not by mecamylamine, suggesting that the vasoconstriction was due to the release of noradrenaline from the sympathetic nerve terminals. The increase in tubular urine, Na⁺ and K⁺ excretion elicited by high doses of CGRP were inhibited by both sympathetic blockers primarily due to hypotension and low renal perfusion. Indomethacin did not affect the CGRP actions

on renal haemodynamics, or excretion of urine or Na^+ but abolished the increase in K^+ excretion elicited by a high dose of CGRP.

CGRP(8-37) abolished the vasodilator, vasoconstrictor and increments in GFR elicited by low doses of CGRP suggesting the predominance of CGRP1 receptors in the vasculature. The diuretic, natriuretic and kaliuretic responses to non-hypotensive doses of CGRP were not affected by CGRP(8-37), indicating the lack of involvement of CGRP1 receptors for the renal tubular actions of CGRP. The profile of antagonistic actions of $[\text{Tyr}^0]\text{CGRP}(28-37)$ was similar to that of CGRP(8-37) indicating that $[\text{Tyr}^0]\text{CGRP}(28-37)$ is also an antagonist of CGRP1-receptors, although its potency and efficacy are less than those of CGRP(8-37).

Adrenomedullin, a novel vasoactive peptide with homology to CGRP was a more efficacious renal vasodilator than CGRP. Its diuretic and natriuretic actions are less than those of CGRP. Renal arterial injection of adrenomedullin, in contrast to CGRP, did not affect K^+ excretion or urinary osmolality. Renal arterial infusion of non-hypotensive doses of adrenomedullin induced renal vasodilatation, increased glomerular filtration, diuresis and increased absolute but not fractional Na^+ excretion. Adrenomedullin did not affect K^+ excretion or urine osmolality. Unlike CGRP, renal arterial infusion of a high dose of adrenomedullin did not cause renal vasoconstriction. Neither CGRP(8-37) nor $[\text{tyr}^0]\text{CGRP}(28-37)$ inhibited the renal vascular and tubular effects of adrenomedullin. The results show that neither the renal vascular nor tubular action of adrenomedullin is mediated via CGRP1 receptors.

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