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Effects of calcitonin gene-related peptide receptor antagonists on renal actions of adrenomedullin

Abdelhamid M. Elhawary, Jason Poon & [1]Catherine C.Y. Pang

Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver B.C., Canada, V6T 1Z3

1 Adrenomedullin is a novel vasoactive peptide which is produced in the lungs, ventricle, kidneys, heart and adrenal medulla. Adrenomedullin shows homology to calcitonin gene-related peptide (CGRP) and has similar pharmacological actions to CGRP.

2 This study examined the dose-response effects of adrenomedullin (rat, 11-50) on mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF), glomerular filtration rate (GFR) and renal tubular electrolyte excretion in Inactin-anaesthetized Sprague Dawley rats. The possible involvement of CGRP receptors in actions of adrenomedullin was also examined via renal arterial injection of a CGRP receptor antagonist, CGRP (8-37) (1 or 10 nmol kg^{-1}) or [Tyr^0]CGRP(28-37) (3 or 30 nmol kg^{-1}), starting 15 min prior to the administration of adrenomedullin.

3 Renal arterial infusion (0.001 to 1 nmol kg^{-1}) of adrenomedullin did not alter MAP, HR and renal K^+ excretion but dose-dependently increased RBF and arterial conductance, GFR, urine flow and Na^+ excretion.

4 The renal actions of adrenomedullin were not blocked by either the low or the high dose of CGRP(8-37) or [Tyr^0]CGRP(28-37).

5 The results show that adrenomedullin causes renal vasodilatation, increments in GFR, diuresis and natriuresis. The renal actions of adrenomedullin are not mediated via the activation of $CGRP_1$ receptors.

Keywords: Adrenomedullin; calcitonin gene-related peptide; renal blood flow and conductance; sodium, potassium and water excretion; CGRP(8-37); [Tyr^0]CGRP(28-37)

Introduction

Adrenomedullin is a novel vasoactive peptide that was recently isolated and identified from human phaeochromocytoma arising from the adrenal medulla (Kitamura *et al.*, 1993a,b). It consists of 52 amino acids in man and 50 amino acids in the rat. Adrenomedullin is present in a considerable concentration (range from 3-19 fmol ml^{-1}) in human plasma (Kitamura *et al.*, 1993a; 1994) and its mRNA is expressed in tissues from the adrenal glands, lungs, kidneys, heart, spleen, duodenum and submandibular glands (Sakata *et al.*, 1993; Ichiki *et al.*, 1994). Adrenomedullin caused a rapid-onset and long-lasting depressor response in the anaesthetized rat (Kitamura *et al.*, 1993a; Sakata *et al.*, 1993; Perret *et al.*, 1993) due to peripheral vasodilatation (Ishiyama *et al.*, 1993). In methoxamine-preconstricted, perfused rat mesenteric artery pretreated with guanethidine, adrenomedullin caused long-lasting vasodilatation via a non-cholinergic, non-adrenergic mechanism (Nuki *et al.*, 1993).

Adrenomedullin shows homology in chemical structure with calcitonin gene-related peptide (CGRP) (Kitamura *et al.*, 1993a,b), a potent hypotensive agent (see review by Preibisz, 1993). There is evidence to suggest that adrenomedullin and CGRP may activate the same receptors in the vasculature and the central nervous system. The $CGRP_1$ receptor antagonist CGRP(8-37) blocked the adrenomedullin-induced elevation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) level in rat cultured vascular smooth muscle cells (Eguchi *et al.*, 1994; Ishizaka *et al.*, 1994). CGRP(8-37) also attenuated the vasodilator response to adrenomedullin in the perfused mesenteric artery of the rat (Nuki *et al.*, 1993) and inhibited centrally-induced vasopressor response to adrenomedullin in anaesthetized rats (Takahashi *et al.*, 1994). Thus, both adrenomedullin and CGRP seem to activate $CGRP_1$-receptors. However, the binding of [^{125}I]-adrenomedullin in vascular smooth muscle cells was inhibited by adrenomedullin but not by CGRP, suggesting that adrenomedullin does not activate CGRP receptors in vascular smooth muscles (Ishizaka *et al.*, 1994).

The first aim of this study was to investigate the effects of intra-renal arterial infusion of adrenomedullin on renal blood flow and conductance, glomerular filtration rate and renal tubular electrolytes excretion. The second aim was to discover if the renal effects of adrenomedullin are due to activation of CGRP receptors. Evidence suggests the existence of heterogeneous CGRP receptors but sub-classification of these receptors is imprecise due to a lack of selective antagonists for subtypes of CGRP receptors. CGRP binding sites are classified as $CGRP_1$ and $CGRP_2$ according to the high or low affinity, respectively, of the sites for the C-terminal fragment of CGRP(8-37) (Mimeault *et al.*, 1991; see Poyner, 1992). Similarly, CGRP receptors are believed to be of the $CGRP_1$ or $CGRP_2$ subtype on the basis of susceptibility of the responses to antagonism by CGRP(8-37) in isolated tissues (Dennis *et al.*, 1989; 1990; Mimeault *et al.*, 1991). Other related peptides such as [Tyr^0]CGRP(28-37) (Chakder & Rattan, 1990; Maton *et al.*, 1990), CGRP(23-37) and CGRP(19-37) (Rovero *et al.*, 1992) also antagonize responses to CGRP but their selectivities are unclear. In this study, two doses each of CGRP(8-37) and [Tyr^0]CGRP(28-37) were used to examine whether adrenomedullin acts via the activation of CGRP receptors.

Methods

Male Sprague Dawley rats (350-400 g) were anaesthetized with Inactin (100 mg kg^{-1}, i.p.). A rectal thermometer and a heating pad connected to a Thermistemp Temperature Controller (Model 71; Yellow Springs Instrument Co. Inc., Ohio) was used to maintain body temperature at 37.5°C. Cannulae (PE50) were inserted into the left femoral artery, for the continuous measurement of mean arterial pressure (MAP) with a Statham pressure transducer (Model P23 DB, Gould Statham

[1] Author for correspondence.

CA), and into the right femoral artery for blood sampling (0.5 ml per sample, each sample replaced by injection of 1 ml normal saline). Heart rate (HR) was derived electronically from the upstroke of the arterial pulse pressure with a tachograph (model 7P4G, Grass, MA). The left femoral vein was cannulated for the administration of [^{51}Cr]-EDTA solution (i.v. bolus at 13.8 μCi in 1.75 ml over 2 min followed by infusion at 0.16 μCi min^{-1} at 20 μl min^{-1}) (Stacy & Thorburn, 1966; Leyssac *et al.*, 1991). 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 PE10 catheter 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 flowmeter (Model T206, Transonic, NY, USA) was placed around the right renal artery for continuous measurement of renal blood flow (RBF). Mean arterial pressure (MAP), heart rate (HR) and RBF were monitored by a Grass polygraph (model RP57C8, Grass, MA). A piece of PE10 cannula (<15 cm length or <10 μl dead space) was inserted into the right ureter for the collection of urine at 10 min intervals in a pre-weighed, closed vial which contained a small hole in the vial cap to allow the passage of the catheter. The samples were prepared immediately after collection to avoid evaporation. The rats were allowed 1 h for stabilization after surgery before the study began.

Experimental protocol

The rats were randomly divided into ten groups ($n=6$ each). One group was given renal arterial infusions of single doses of adrenomedullin (0.001 – 1 nmol kg^{-1} min^{-1}) after two control sampling periods (15 min each), with each dose infused for 10 min followed by a recovery period of 5 min. Another group was continuously infused i.v. with the vehicle (0.45% NaCl) for the duration of the experiments. In preliminary studies, stable renal vasodilatation and tubular responses were obtained 10 min after the start of adrenomedullin infusions. Two groups were given bolus renal arterial injections of a low dose (1 nmol kg^{-1}) of CGRP(8-37) and two other groups were given a high dose (10 nmol kg^{-1}) of CGRP(8-37) after the first sampling period. This was followed by continuous infusion of 20% of the bolus dose of the antagonist every hour (15 μl min^{-1}) until the end of the experiments. In preliminary studies, the low dose of CGRP(8-37) completely inhibited and the low dose of [Tyr^0]CGRP(28-37) reduced the vasodilatation response to low doses (0.3 and 3 pmol kg^{-1} min^{-1}) as well as the vasoconstrictor response to a high dose (300 pmol kg^{-1} · min^{-1}) of CGRP (unpublished observations). Single doses of adrenomedullin (0.001 – 1 nmol kg^{-1} min^{-1}) or an equal volume of vehicle (0.45% NaCl) were infused after the second sampling period in these four groups. Another two groups each were instead given either a low (3 nmol kg^{-1}) or a high (30 nmol kg^{-1}) dose of [Tyr^0]CGRP(28-37), as described for the CGRP(8-37) groups. Blood was sampled at 10 min after the start of drug administration, whereas urine collection was from 3 until 13 min after the start of drug administration. The later collection time for urine allowed extra time for the equilibration of drug responses in the kidney and 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 groups.

Urine volume was measured gravimetrically. [^{51}Cr]-EDTA concentrations were determined by a gamma counter (1185 series dual channel, Nuclear-Chicago, IL, U.S.A.). Urine Na^+ and K^+ concentrations were measured by flame photometry (Model IL143, Fisher Scientific, MA, U.S.A.). Urine osmolality was measured by a vapour pressure osmometer (Model 5500, WESCOR, Utah, U.S.A.). 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.

Materials

Inactin (thiobarbituric acid) was obtained from BYK Gulden Konstanz (Germany). Adrenomedullin 11-50 (rat) and [Tyr^0]CGRP(28-37) were purchased from Peninsula Lab. Inc. (Belmont, CA, U.S.A.) and rat CGRP(8-37) was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). These drugs were dissolved in 0.45% NaCl solution. [^{51}Cr]-EDTA was obtained from Amersham International (UK) and was dissolved in 0.9% NaCl solution.

Calculations and statistical analysis

Renal arterial conductance (RBF/MAP) was computed to normalize renal blood flow independently of changes in MAP.

Table 1 Baseline values (mean ± s.e.mean) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats min^{-1}), renal blood flow (RBF, ml min^{-1}), renal arterial conductance (Cond), glomerular filtration rate (GFR, ml min^{-1}), urine flow (UF, μl min^{-1}), urine Na^+ ($U_{Na}V$, nmol min^{-1}), urine K^+ (U_KV, nmol min^{-1}) and urine osmolality (U_{OSM}, mOsm kg^{-1}) in ten groups ($n=6$ each) of Inactin-anaesthetized rats, prior to the administration of the calcitonin gene-related peptide (CGRP) antagonists or an equal volume of the vehicle

Group	*MAP*	*HR*	*RBF*	*Cond*	*GFR*	*UF*	$U_{Na}V$	U_KV	U_{OSM}
Vehicle: vehicle	108 ± 2	360 ± 19	12.3 ± 0.4	0.118 ± 0.006	1.27 ± 0.14	4.6 ± 0.5	239 ± 84	1045 ± 160	1421 ± 236
Vehicle: AM	97 ± 5	363 ± 13	11.8 ± 0.7	0.123 ± 0.009	1.42 ± 0.10	4.4 ± 0.3	242 ± 36	897 ± 120	1303 ± 149
CGRP(8–37) 1 nmol kg^{-1}: Veh	110 ± 4	362 ± 10	13.9 ± 0.7	0.127 ± 0.011	1.36 ± 0.12	4.6 ± 0.4	223 ± 50	956 ± 133	1586 ± 207
CGRP(8–37) 1 nmol kg^{-1}: AM	99 ± 7	356 ± 12	13.6 ± 1.2	0.132 ± 0.010	1.41 ± 0.15	5.4 ± 0.6	203 ± 49	856 ± 168	1431 ± 259
CGRP(8–37) 10 nmol kg^{-1}: Veh	104 ± 3	378 ± 9	13.2 ± 0.9	0.116 ± 0.008	1.76 ± 0.19	4.9 ± 0.5	304 ± 72	782 ± 95	1662 ± 207
CGRP(8–37) 10 nmol kg^{-1}: AM	102 ± 7	355 ± 24	12.1 ± 1.5	0.122 ± 0.018	1.49 ± 0.22	5.5 ± 0.4	275 ± 53	969 ± 82	1640 ± 152
[Tyr^0]CGRP(28–37) 3 nmol kg^{-1}: Veh	104 ± 9	377 ± 14	13.1 ± 0.7	0.134 ± 0.011	1.39 ± 0.15	4.3 ± 0.4	213 ± 39	687 ± 93	1727 ± 237
[Tyr^0]CGRP(28–37) 3 nmol kg^{-1}: AM	100 ± 5	352 ± 16	12.5 ± 1.1	0.127 ± 0.016	1.46 ± 0.18	4.1 ± 0.4	227 ± 37	905 ± 111	1521 ± 184
[Tyr^0]CGRP(28–37) 30 nmol kg^{-1}: Veh	97 ± 4	340 ± 9	12.1 ± 1.1	0.128 ± 0.012	1.38 ± 0.19	5.2 ± 0.4	387 ± 56	1160 ± 82	1760 ± 162
[Tyr^0]CGRP(28–37) 30 nmol kg^{-1}: AM	100 ± 7	378 ± 14	13.4 ± 0.5	0.135 ± 0.008	1.20 ± 0.15	5.1 ± 0.4	364 ± 55	758 ± 87	1637 ± 164

AM = adrenomedullin; Veh = vehicle.

GFR was calculated as the ratio of urine to plasma concentration of [^{51}Cr]-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 the ratio of urine Na^+ excretion to plasma Na^+ concentration, divided by GFR. All data are expressed as mean ± s.e.mean and were analysed by analysis of variance/covariance followed by Duncan's multiple range test, with $P<0.05$ selected as the level of statistical significance.

Results

Table 1 shows the baseline values of MAP, HR, and renal haemodynamics and excretions prior to the infusion of adrenomedullin or the vehicle in the ten groups. Neither injection of the low or the high dose of CGRP(8-37) nor the two doses of [Tyr^0]CGRP(28-37) induced significant changes in any measured parameters.

Effects of adrenomedullin on MAP, HR, RBF, arterial conductance and GFR in the absence or presence of the CGRP receptor antagonists

The vehicle did not elicit significant changes in MAP, HR (Figure 1), RBF, renal arterial conductance (Figure 2), GFR, Na^+ excretion (Figure 3), urine flow or urine osmolality (Figure 4) either in the absence or presence of the CGRP receptor antagonists.

Renal arterial infusion of adrenomedullin caused insignif-

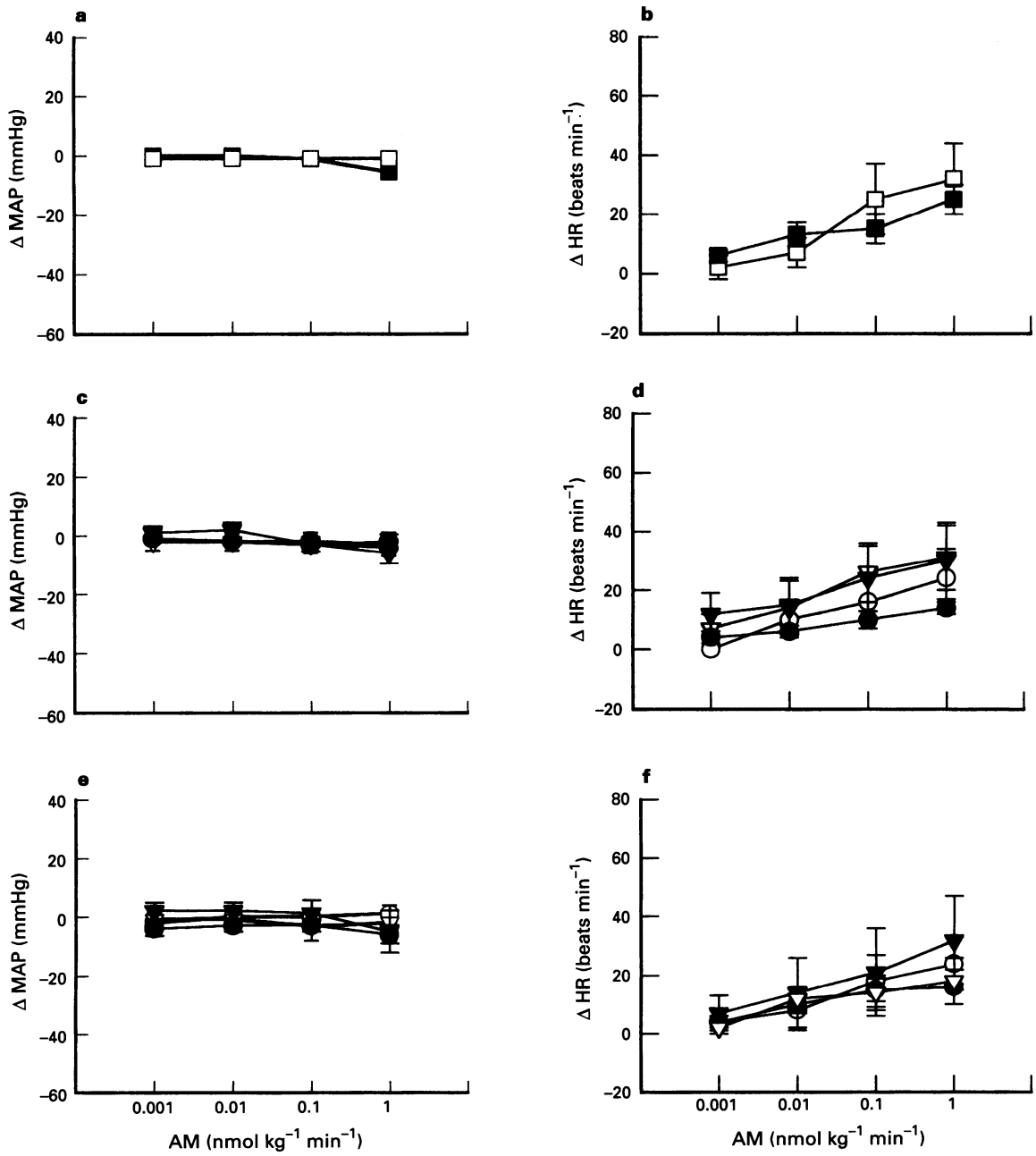

Figure 1 Dose-response effects of renal arterial infusion of adrenomedullin (AM, filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on mean arterial pressure (MAP) and heart rate (HR) in ten groups of Inactin-anaesthetized rats ($n=6$ each group) in the absence (■, □) of an antagonist (a, b), in the presence of a low (1 nmol kg^{-1}, ●, ○) or a high dose (10 nmol kg^{-1}, ▼, ▽) of CGRP(8-37) (c, d), and in the presence of a low (3 nmol kg^{-1}, ●, ○) or a high (30 nmol kg^{-1}, ▼, ▽) dose of [Tyr^0]CGRP(28-37) (e, f). Data are shown as mean ± s.e.mean.

icant changes in MAP and HR either in the absence (Figure 1a, b) or the presence of the low and high doses of CGRP(8-37) (Figure 1c, d) or [Tyr^0]CGRP(28-37) (Figure 1e, f).

Adrenomedullin, but not the vehicle, significantly and dose-dependently increased RBF and renal arterial conductance indicating vasodilatation (Figure 2a, b). Curve analyses show that neither of the two doses of CGRP(8-37) (Figure 2c, d) nor the two doses of [Tyr^0]CGRP(28-37) (Figure 2e, f) significantly inhibited the increases in RBF or arterial conductance elicited by adrenomedullin.

GFR was also increased by all doses of adrenomedullin (Figure 3a). The increases in GFR by adrenomedullin were not inhibited by either dose of CGRP(8-37) (Figure 3c) or by [Tyr^0]CGRP(28-37) (Figure 3e).

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

Adrenomedullin dose-dependently ($P<0.05$) increased Na^+ excretion (Figure 3b) and urine flow (Figure 4a) but did not affect urine osmolality (Figure 4b) or K^+ excretion (results not shown). Fractional excretion of Na^+ was not significantly changed by adrenomedullin (from 0.24% baseline to 0.25, 0.22, 0.28 and 0.25% after the four incremental doses of adrenomedullin).

In rats pretreated with either of the two doses of CGRP(8-37) or of [Tyr^0]CGRP(28-37), adrenomedullin increased Na^+

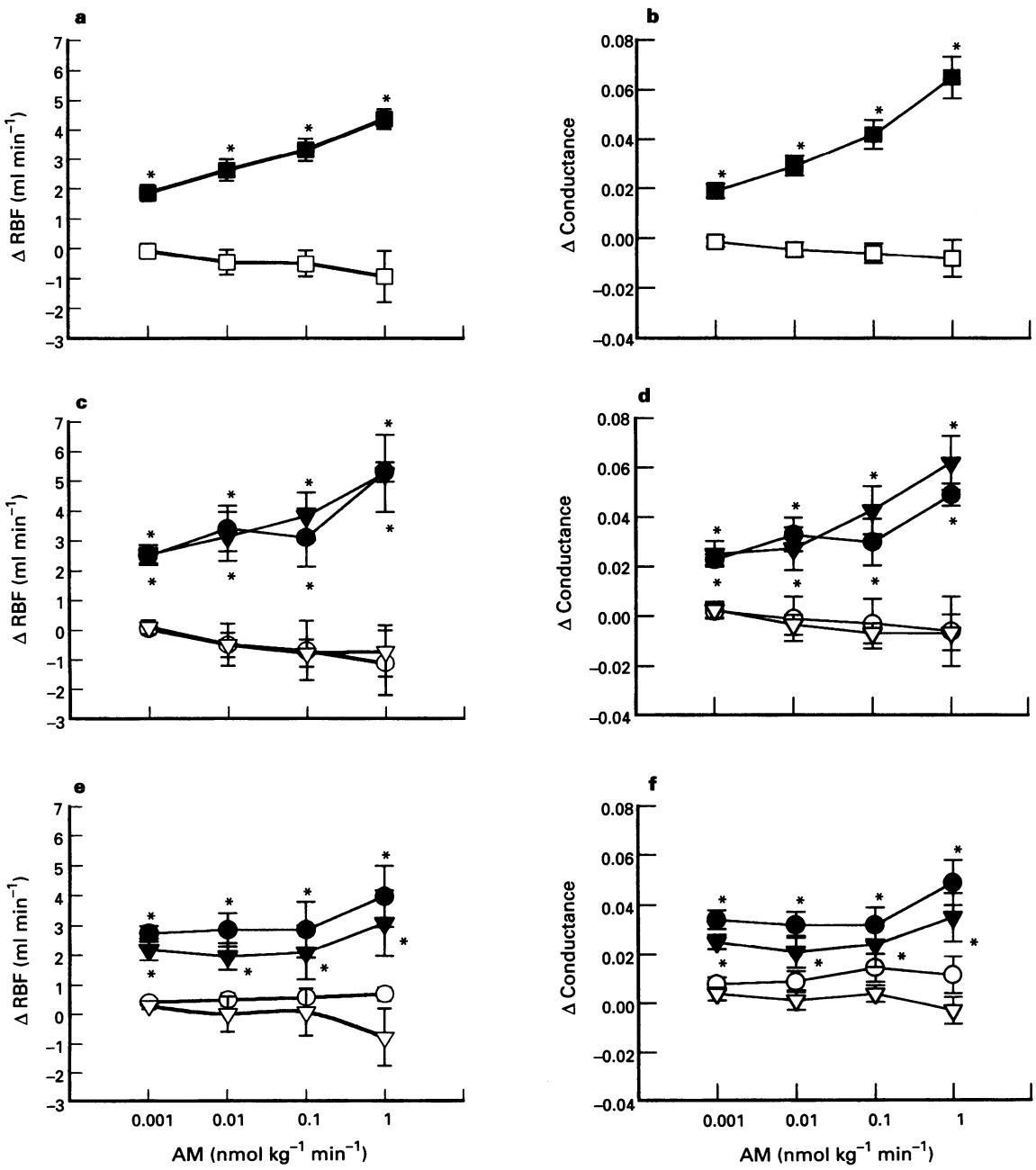

Figure 2 Dose-response effects of renal arterial infusion of adrenomedullin (AM, filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on renal blood flow (RBF) and renal arterial conductance in ten groups of Inactin-anaesthetized rats ($n=6$ each group) in the absence (■, □) of an antagonist (a, b), in the presence of a low (1 nmol kg^{-1}, ●, ○) or a high dose (10 nmol kg^{-1}, ▼, ▽) of CGRP(8-37) (c, d), and in the presence of a low (3 nmol kg^{-1}, ●, ○) or a high (30 nmol kg^{-1}, ▼, ▽) dose of [Tyr^0]CGRP(28-37). Data are shown as mean ± s.e.mean. *Significantly different from the corresponding vehicle-control readings.

excretion (Figure 3d, f) and urine flow (Figure 4c, e) and had no effect on urine osmolality (Figure 4d, f) or K^+ excretion (results not shown). Fractional excretion of Na^+ was also unaltered by adrenomedullin in the presence of either CGRP(8-37) or [Tyr^0]CGRP(28-37) (results not shown).

Increasing the dose of CGRP(8-37) to 30 nmol kg^{-1} ($n=2$) and [Tyr^0]CGRP(28-37) to 100 nmol kg^{-1} ($n=2$) also did not affect the renal vascular or tubular effects of adrenomedullin.

The haematocrit (44±2%), and blood values of osmolality (293±4 mOsmol kg^{-1}), Na^+ (134±4 mmol l^{-1}) and K^+ (3.4±0.4 mmol l^{-1}) at the end of the experiments were similar to the corresponding values at the end of the stabilization period (results not shown).

Discussion

Results from the present study show that renal arterial infusion of adrenomedullin, at doses which did not alter MAP, induced renal vasodilatation (increased arterial conductance), increased GFR, diuresis and natriuresis. The vascular and tubular actions of adrenomedullin were qualitatively different from those elicited by renal arterial infusion of αCGRP (0.3 to 300 pmol kg^{-1} min^{-1}) which caused a biphasic response in the renal bed: vasodilatation at a low dose (0.3 pmol kg^{-1} min^{-1}) and vasoconstriction at a high dose (300 pmol kg^{-1} min^{-1}) (Elhawary & Pang, 1995). The effects of adrenomedullin on GFR were also different from those of αCGRP. GFR was

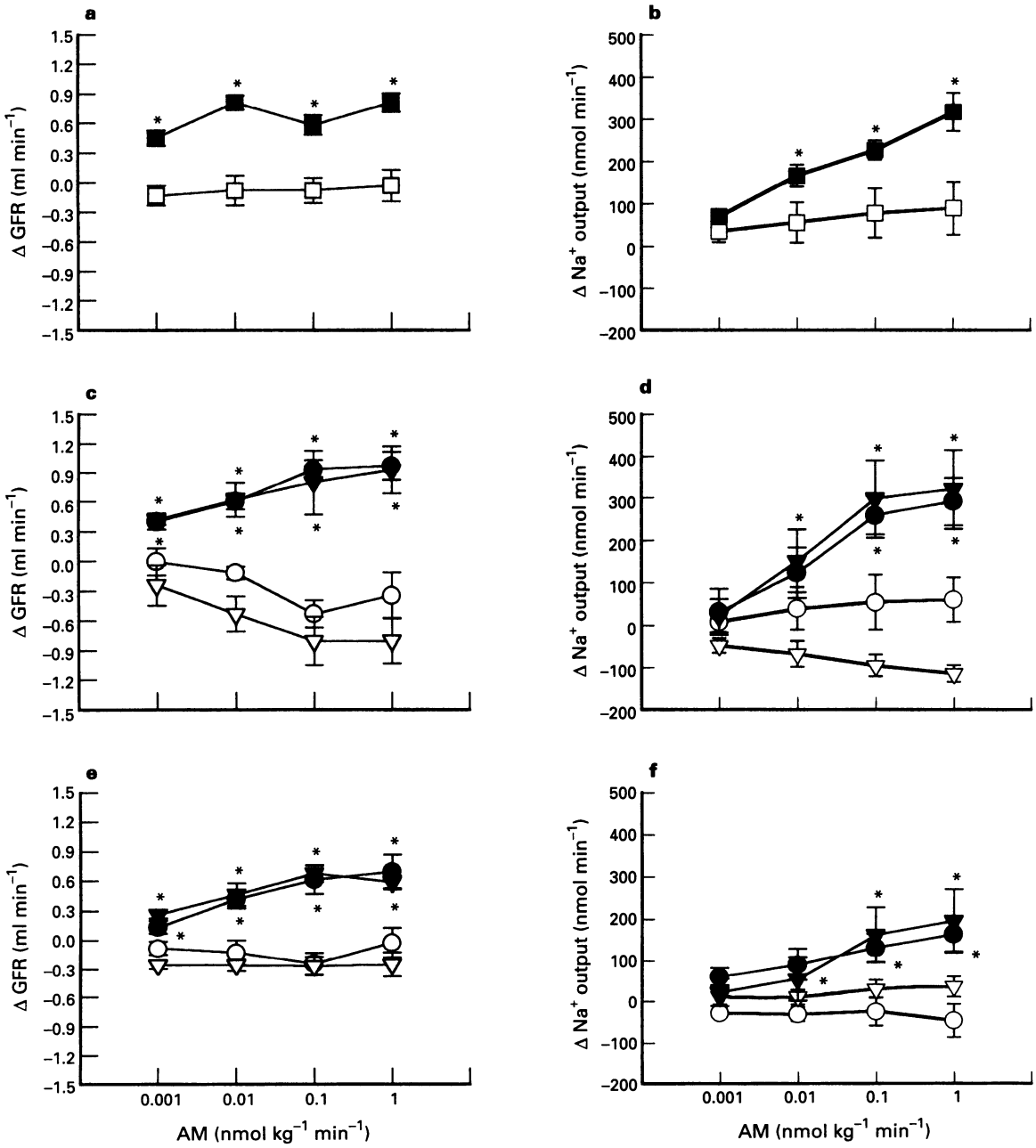

Figure 3 Dose-response effects of renal arterial infusion of adrenomedullin (AM, filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on glomerular filtration rate (GFR) and renal Na^+ excretion in ten groups of Inactin-anaesthetized rats ($n=6$ each group) in the absence (■, □) of an antagonist (a, b), in the presence of a low (1 nmol kg^{-1}, ●, ○) or a high dose (10 nmol kg^{-1}, ▼, ▽) of CGRP(8-37) (c, d) and in the presence of a low (3 nmol kg^{-1}, ●, ○) or a high (30 nmol kg^{-1}, ▼, ▽) dose of [Tyr^0]CGRP(28-37) (e, f). Data are shown as mean±s.e.mean. *Significantly different from the corresponding vehicle-control readings.

increased by only low doses (0.3 and 3 pmol kg^{-1} min^{-1}) of αCGRP (Elhawary & Pang, 1995) but increased by all doses (1 to 1,000 pmol kg^{-1} min^{-1}) of adrenomedullin in the present study. The increases in both GFR (+32 to +56%) and RBF (+15 to +38%) by adrenomedullin suggest that adrenomedullin vasodilates preferentially afferent rather than efferent arterioles.

Like αCGRP, adrenomedullin dose-dependently increased Na^+ excretion and urine flow. However, 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 αCGRP was associated with increased fractional excretion of Na^+ suggesting reduced tubular Na^+ reabsorption. Neither αCGRP (Elhawary & Pang, 1995) nor adrenomedullin altered urine osmolality; however, αCGRP but not adrenomedullin increased K^+ excretion (Elhawary & Pang, 1995). The lack of effect of adrenomedullin on urine osmolality and K^+ suggests that the release of antidiuretic hormone and aldosterone is unaltered; however, this assumption requires confirmation from measurements of the plasma levels of these hormones. Since adrenomedullin did not change urine osmolality or fractional excretion of Na^+ or K^+, its natriuretic and diuretic effects are probably indirectly mediated via renal vasodilatation and increments in GFR. There is as yet no report on the regional distribution of adrenomedullin binding sites or the expression of adrenomedullin mRNA within the kidney. Such studies may provide insight on the site and mechanism of renal action of adrenomedullin.

Our results are somewhat different from those of Ebara *et*

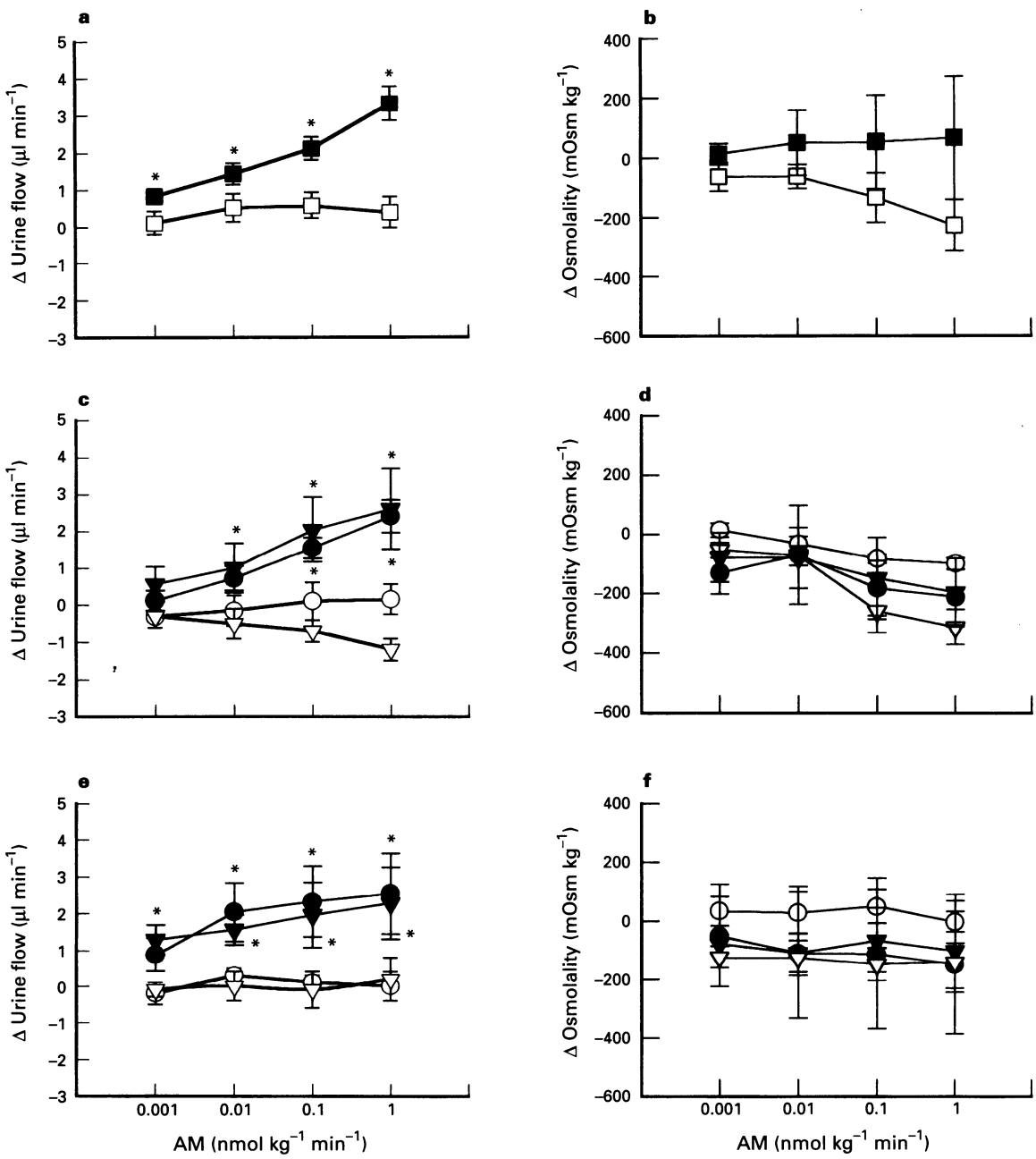

Figure 4 Dose-response effects of renal arterial infusion of adrenomedullin (AM, filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on urine flow and urine osmolality in ten groups of Inactin-anaesthetized rats ($n=6$ each group) in the absence (■, □) of an antagonist (a, b), in the presence of a low (1 nmol kg^{-1}, ●, ○) or a high dose (10 nmol kg^{-1}, ▼, ▽) of CGRP(8-37) (c, d), and in the presence of a low (3 nmol kg^{-1}, ●, ○) or a high (30 nmol kg^{-1}, ▼, ▽) dose of [Tyr^0]CGRP(28-37) (e, f). Data are shown as mean ± s.e.mean. *Significantly different from the corresponding vehicle-control readings.

al. (1994) who showed that renal arterial infusion of non-hypotensive doses of adrenomedullin into pentobarbitone-anaesthetized dogs caused dose-dependent renal vasodilatation and increases in urine flow and excretion of Na^+ as well as K^+, but increases in GFR only at high doses of adrenomedullin. The differences in the effects of adrenomedullin on K^+ excretion and GFR between the study by Ebara *et al.* and ours may be due to the use of different preparations, i.e., pentobarbitone-anaesthetized dogs vs Inactin-anaesthetized rats.

Vasodilator responses to CGRP are readily antagonized by CGRP(8-37). Thus, CGRP(8-37) inhibited relaxation responses to CGRP in the rat isolated perfused kidney (Castellucci *et al.*, 1993; Chin *et al.*, 1994), perfused rat mesenteric arterial bed (Han *et al.*, 1993) and porcine coronary artery (Franco-Cereceda, 1992). CGRP(8-37) also inhibited vasodilatation elicited by CGRP *in vivo* in the rabbit (Hughes & Brain, 1991) and rat (Escott & Brain, 1994) skin beds and in cat cerebral arterioles (Wei *et al.*, 1992). In conscious rats, CGRP(8-37) inhibited vasodilator responses to CGRP in the renal and hindquarter beds and vasoconstrictor response to CGRP in the mesenteric bed (Gardiner *et al.*, 1990; 1995). The ability of CGRP(8-37) to block vascular actions of CGRP but inability to block vasodilator effects of adrenomedullin in the present study suggest that the renal vasodilator effect of adrenomedullin is not mediated via $CGRP_1$ receptors. More recently, Gardiner *et al.* (1995) reported that CGRP(8-37), at a dose which blocks vascular responses to CGRP, did not block the vasodilator effects of adrenomedullin in the renal, mesenteric and hindquarter beds in the conscious rat. These data are consistent with our present findings.

[Tyr^0]CGRP(28-37) has been shown to have no effect on resting tension but to cause a rightward shift in the concentration-response curve to CGRP in the opossum internal anal sphincter smooth muscle (Chakder & Rattan, 1990). [Tyr^0]CGRP(8-37) also caused a rightward displacement of the CGRP-induced amylase secretion dose-response curve in guinea-pig pancreatic acini (Maton *et al.*, 1990). There is as yet no published information on the selectivity of [Tyr^0]CGRP(28-37) on CGRP receptors. The present results show that like CGRP(8-37), [Tyr^0]CGRP(28-37) did not significantly alter the renal 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 into Inactin-anaesthetized rats 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 [Tyr^0]CGRP(28-37) inhibited the renal actions of adrenomedullin, suggesting that these effects are not mediated via the activation of $CGRP_1$ receptors.

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