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European Journal of Pharmacology 286 (1995) 255–263

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Receptor subtypes mediating renal actions of calcitonin gene-related peptide

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Received 13 April 1995; revised 18 July 1995; accepted 25 July 1995

Abstract

We previously reported that the renal arterial infusions of non-hypotensive doses of calcitonin gene-related peptide (CGRP) caused renal vasodilatation and increases in glomerular filtration rate at a low dose, but renal vasoconstriction, natriuresis and kaliuresis at a high dose. In the present study, we examined the effects of the specific $CGRP_1$ receptor antagonist CGRP-(8–37) (1 and 10 nmol/kg) and the putative CGRP receptor antagonist, [Tyr^0]CGRP-(28–37) (3 and 30 nmol/kg), on the renal vascular and tubular effects of CGRP in inactin-anaesthetized Sprague-Dawley rats. Renal arterial infusion of single doses of CGRP (0.3–300 pmol/kg per min) did not significantly alter mean arterial pressure or heart rate. However, during the continuous renal arterial infusion of either CGRP-(8–37) or [Tyr^0]CGRP-(28–37), a high dose of CGRP (300 pmol/kg per min) paradoxically reduced arterial pressure and increased heart rate. CGRP-(8–37) completely but [Tyr^0]CGRP-(28–37) incompletely inhibited the vasodilatation and increments in glomerular filtration rate elicited by low doses of CGRP. Both blockers abolished the renal vasoconstriction but did not inhibit diuresis, natriuresis and kaliuresis elicited by a high but non-hypotensive dose of CGRP. On the basis that CGRP-(8–37) is a competitive $CGRP_1$ receptor antagonist, our results suggest: (1) the renal vascular effect of CGRP is completely mediated via the activation of $CGRP_1$ receptors, (2) the renal tubular effects of CGRP are not mediated via $CGRP_1$ receptors, and (3) [Tyr^0]CGRP-(28–37) is a $CGRP_1$ receptor antagonist with potency and efficacy less than those of CGRP-(8–37).

Keywords: CGRP (calcitonin gene-related peptide); Renal blood flow; Renal conductance; Na^+ excretion; K^+ excretion; CGRP-(8–37); [Tyr^0]CGRP-(28–37)

1. Introduction

Calcitonin gene-related peptide (CGRP) is a neurotransmitter and a neuromodulator which is widely distributed in various tissues (see review by Ishida-Yamamoto and Tohyama, 1989). It exerts central and peripheral actions, which include vasodilatation (Brain et al., 1985; DiPette et al., 1987; Abdelrahman et al., 1992; Abdelrahman and Pang, 1992), cardiac acceleration (Ishikawa et al., 1987; Lappe et al., 1987; Miyauchi et al., 1988) and inhibition of gastric secretion (Lenz et al., 1985; Kraenzlin et al., 1985).

Within the kidney, a high density of CGRP-containing nerve fibers exists in the muscular layer of the renal pelvis, in the proximity of arteries and arterioles and in the periglomerular and peritubular space (Maggi et al., 1987; Geppetti et al., 1989a,b; Kurtz et al., 1989a). CGRP stimulated renal cortical and medullary adenylyl cyclase activity (Geppetti et al., 1989a,b; Aiyar et al., 1991) and caused renal vasodilatation in rats (Gardiner et al., 1989), dogs (Villarreal et al., 1988) and humans (Kurtz et al., 1989a). In the isolated perfused rat kidney, CGRP reduced vascular resistance (Kurtz et al., 1989a,b; Chin et al., 1994). CGRP also stimulated the release of renin (Kurtz et al., 1988) and atrial natriuretic peptide, and inhibited the secretion of aldosterone (Murakami et al., 1989,1991). We have shown that the renal effects of CGRP vary according to the dose. The infusion of low doses of CGRP caused renal vasodilatation and increments in glomerular filtration

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SSDI 0014-2999(95)00458-0

rate, whereas infusion of higher doses caused renal vasoconstriction, diuresis, natriuresis and kaliuresis (Elhawary and Pang, 1995).

Accumulating evidence suggests that there are at least two subtypes of CGRP receptors (Dennis et al., 1989, 1990; Mimeault et al., 1991; Giuliani et al., 1992; Stangl et al., 1993; see review by Poyner, 1992). $CGRP_1$ and $CGRP_2$ receptors were designated on the basis of affinity to the *C*-terminal fragment, CGRP-(8–37) (Dennis et al., 1989, 1990; Chiba et al., 1989). CGRP-(8–37) was proposed as a selective antagonist at the $CGRP_1$ receptor (Chiba et al., 1989; Maggi et al., 1991) and it has been shown to antagonize the vasodilatation response to αCGRP in the isolated, constant flow-perfused rat kidney (Chin et al., 1994). Other available CGRP fragments such as [Tyr^0]CGRP-(28–37) (Chakder and Rattan, 1990; Maton et al., 1990), CGRP-(23–37) and CGRP-(19–37) (Rovero et al., 1992) also possess antagonistic activity at CGRP receptors but their selectivities are unclear.

The present study investigated the effects of a low and a high dose of the $CGRP_1$ receptor antagonist CGRP-(8–37) and those of the putative CGRP receptor antagonist [Tyr^0]CGRP-(28–37) on the actions of CGRP on renal haemodynamics and excretions of water, sodium (Na^+) and potassium (K^+) in the anaesthetized rat.

2. Materials and methods

2.1. Surgical procedure

Male Sprague-Dawley rats (350–400 g) were anaesthetized with inactin (100 mg/kg, i.p.). Body temperature was maintained at 37.5°C using a rectal thermometer and a heating pad connected to a Thermistemp Temperature Controller (Model 71; Yellow Springs Instrument Co., OH, USA). Cannulae (PE-50) were inserted into the left femoral artery, for the continuous measurement of mean arterial pressure with a Statham pressure transducer (Model P23 DB, Gould Statham, CA, USA), and into the right femoral artery for blood sampling (0.5 ml per sample, each sample replaced by the injection of 1 ml normal saline). Heart rate was derived electronically from the upstroke of the arterial pulse pressure using a tachograph (model 7P4G, Grass, MA, USA). The left femoral vein was cannulated for the administration of ^{51}Cr-EDTA solution (infusion at 6.9 μCi/min at 0.8 ml/min for 2 min followed by 0.16 μCi/min at 20 μl/min) (Leyssac et al., 1991; Stacy and Thorburn, 1966). The abdominal cavity was opened through a ventral midline incision. The right suprarenal artery was located and its origin from the renal artery was verified. A tapered PE-10 tubing was inserted retrogradely into the suprarenal artery as described by Smits et al. (1983), and connected to a syringe pump (SAGE 341A, TX, USA) for the infusion of drugs. A transonic flow probe (Model 1RB630, Transonic) which was connected to a flowmeter (Model T206, Transonic, NY, USA) was placed around the right renal artery for the continuous measurement of renal blood flow. Mean arterial pressure, heart rate and renal blood flow were monitored by a Grass polygraph (model RP57C8, Grass, MA, USA). A piece of PE-10 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 contains a small hole in the vial cap to allow the passage of the catheter. The samples were prepared immediately after collection to avoid evaporation. A 1 h stabilization period was allowed after surgery before the study began.

2.2. Experimental protocol

Preliminary experiments ($n = 6$) were first conducted to observe the effect of continuous renal arterial infusion of the vehicle (0.45% NaCl) on haemodynamic and renal responses (time-control). Afterwards, rats were randomly divided into nine groups ($n = 6$ each). One group was renal arterially infused with single doses of CGRP (0.3–300 pmol/kg per min) after two control sampling periods; each dose of CGRP was infused for 10 min followed by a recovery period of 5 min. Two groups were pretreated with bolus renal arterial injections of a low dose (1 nmol/kg) of the CGRP receptor antagonist CGRP-(8–37) after the first sampling period and another two groups were pretreated with a high dose (10 nmol/kg) of CGRP-(8–37). The injections of the antagonists were followed by infusions of 20% of the bolus dose per hour (15 μl/min) for the remaining of the experiments. The two low dose CGRP-(8–37)-pretreated groups were infused with either CGRP (0.3–300 pmol/kg per min) or an equal volume of vehicle (0.45% NaCl) after the second sampling period, and likewise with the two high dose CGRP-(8–37)-pretreated groups. Another four groups were pretreated with the CGRP antagonist [Tyr^0]-CGRP-(28–37), with two groups given a low dose (3 nmol/kg) and two groups a high dose (30 nmol/kg) of the antagonist, followed by the infusion of CGRP or vehicle, as described for the CGRP-(8–37)-treated groups. Blood was sampled at 10 min after the start of drug infusion or injection whereas urine collection began from 3 till 13 min after the start of drug administration. The later collection time for 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 groups. The duration of infusion and urine

Table 1
Effects (means ± S.E.M.) of vehicle (0.45% NaCl) on mean arterial pressure (MAP, mm Hg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), glomerular filtration rate (GFR, ml/min), urine flow (UF, μl/min), urine osmolality (UOSM, mOsm/kg) and urinary Na^+ and K^+ excretion rate ($U_{Na}V$ and U_KV, nmol/min) during the six sampling periods (SP1 to SP6, 10 min each) in inactin-anaesthetized rat ($n = 6$)

	MAP	HR	RBF	GFR	UF	UOSM	$U_{Na}V$	U_KV
SP1	108 ± 2	360 ± 19	10.9 ± 0.9	0.97 ± 0.04	4.6 ± 0.5	1421 ± 236	239 ± 84	1045 ± 160
SP2	109 ± 3	362 ± 21	11.3 ± 1.2	0.97 ± 0.03	4.7 ± 0.4	1424 ± 227	283 ± 97	1112 ± 111
SP3	108 ± 4	365 ± 20	11.2 ± 1.3	0.84 ± 0.06	4.8 ± 0.6	1360 ± 222	348 ± 115	1140 ± 179
SP4	108 ± 4	369 ± 23	10.8 ± 1.5	0.89 ± 0.03	5.3 ± 0.7	1362 ± 216	408 ± 110	1121 ± 241
SP5	108 ± 5	386 ± 26	10.8 ± 1.5	0.89 ± 0.02	5.2 ± 0.5	1286 ± 151	411 ± 127	1046 ± 205
SP6	106 ± 4	393 ± 27	10.4 ± 1.7	0.94 ± 0.04	5.0 ± 0.6	1195 ± 159	422 ± 153	956 ± 238

collection for CGRP were found to be sufficient to give steady state responses (Elhawary and Pang, 1995).

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 by a vapor pressure osmometer (Model 5500, WESCOR, Utah). 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 the levels of Na^+ and K^+ during the course of the study.

2.3. Materials

Inactin (thiobarbituric acid) was obtained from BYK Gulden Konstanz (Germany). α-CGRP (rat) and rat CGRP-(8–37) was from Sigma Chemical Co. (St. Louis, MO, USA). [Tyr^0]CGRP-(28–37) was purchased from Peninsula Lab. (Belmont, CA, USA). The drugs were dissolved in 0.45% NaCl solution. ^{51}Cr-EDTA was obtained from Amersham International (UK) and was solubilized in 0.9% NaCl solution.

2.4. Calculations and statistical analysis

Renal arterial conductance (renal blood flow/mean arterial pressure) was computed to normalize renal blood flow independent of changes in mean arterial pressure. Glomerular filtration rate 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 respective product of ionic concentration and urine flow. Electrolyte fractional excretion was calculated by the percentage of the ratio of urinary excretion rate to the product of plasma concentration and glomerular filtration rate. All data are expressed as means ± S.E.M. The results were analyzed by the analysis of variance, block design for the comparison of data within the same group and random design for comparison among groups, followed by Duncan's multiple range test, with $P < 0.05$ selected as the level of statistical significance.

Table 2
Baseline values (means ± S.E.M.) of mean arterial pressure (MAP, mm Hg), heart rate (HR, beats/min), renal blood flow (RBF, ml/min), renal arterial conductance (COND), glomerular filtration rate (GFR, ml/min), urine flow (UF, μl/min), urine Na^+ ($U_{Na}V$, nmol/min), urine K^+ (U_KV, nmol/min) and urine osmolality (UOSM, mOsm/kg) following the first sampling period of i.v. infusion of vehicle (0.45% NaCl) in nine groups ($n = 6$ each) of inactin-anaesthetized rats, prior to administration of the CGRP antagonists or equal volume of the vehicle (see full description of treatments for each group in text)

Group	MAP	HR	RBF	COND	GFR	UF	$U_{Na}V$	U_KV	UOSM
Vehicle									
CGRP	99 ± 5	366 ± 13	11.5 ± 0.7	0.116 ± 0.009	1.52 ± 0.10	5.4 ± 0.3	264 ± 36	857 ± 121	1303 ± 148
CGRP-(8–37) (1 nmol / kg)									
Vehicle	107 ± 4	369 ± 10	12.9 ± 0.7	0.124 ± 0.011	1.46 ± 0.14	4.6 ± 0.4	253 ± 50	916 ± 133	1566 ± 207
CGRP	102 ± 7	358 ± 12	12.6 ± 1.2	0.122 ± 0.010	1.47 ± 0.18	5.4 ± 0.6	243 ± 49	866 ± 168	1431 ± 252
CGRP-(8–37) (10 nmol / kg)									
Vehicle	105 ± 3	368 ± 19	13.2 ± 0.9	0.126 ± 0.008	1.56 ± 0.18	4.9 ± 0.5	304 ± 72	789 ± 95	1762 ± 205
CGRP	107 ± 7	355 ± 24	12.1 ± 1.5	0.115 ± 0.018	1.48 ± 0.23	5.7 ± 0.4	295 ± 53	949 ± 82	1640 ± 252
[Tyr^0]CGRP-(28–37) (3 nmol / kg)									
Vehicle	99 ± 9	367 ± 14	13.2 ± 0.7	0.133 ± 0.011	1.39 ± 0.18	4.8 ± 0.4	243 ± 39	697 ± 93	1747 ± 239
CGRP	100 ± 5	357 ± 16	12.5 ± 1.1	0.125 ± 0.016	1.46 ± 0.18	4.6 ± 0.4	267 ± 37	805 ± 111	1521 ± 189
[Tyr^0]CGRP-(28–37) (30 nmol / kg)									
Vehicle	98 ± 8	349 ± 19	12.1 ± 1.1	0.128 ± 0.012	1.37 ± 0.19	5.2 ± 0.4	387 ± 56	960 ± 82	1757 ± 169
CGRP	100 ± 7	378 ± 14	13.4 ± 0.5	0.134 ± 0.007	1.40 ± 0.15	5.3 ± 0.4	362 ± 55	758 ± 87	1647 ± 169

3. Results

The vehicle (time-control group) caused insignificant changes in mean arterial pressure, heart rate, renal blood flow, glomerular filtration rate, urine flow, urine osmolality and urinary excretions of Na^+ and K^+ (Table 1). Table 2 shows the baseline values of mean arterial pressure, heart rate, renal haemodynamics and renal excretion prior to the infusion of CGRP or the vehicle in the remaining nine groups of rats. Neither the low nor the high dose of CGRP-(8–37) or [Tyr^0]CGRP-(28–37) induced any significant changes in all parameters measured at the end of the sampling periods (vehicle groups in the figures).

3.1. Effects of CGRP on mean arterial pressure, heart rate, renal blood flow, arterial conductance and glomerular filtration rate in the absence and presence of CGRP-(8–37) or [Tyr^0]CGRP-(28–37)

Renal arterial infusion of CGRP caused insignificant changes in mean arterial pressure and heart rate (Fig. 1A,B). However, in the presence of both the low and high dose of CGRP-(8–37), a high dose CGRP decreased mean arterial pressure significantly and increased heart rate, but the heart rate effect was significant only in the presence of the low dose of CGRP-(8–37) (Fig. 1C,D). The highest dose of CGRP also significantly reduced mean arterial pressure and increased heart rate in the presence of either the low or the high dose of [Tyr^0]CGRP-(28–37) (Fig. 1E,F).

Low doses of CGRP (0.3 and 3 pmol/kg per min) significantly increased renal blood flow and renal arterial conductance indicating vasodilatation (Fig. 2A,B) but a high dose (300 pmol/kg per min) decreased renal blood flow and arterial conductance indicating vasoconstriction. The increases in renal blood flow and conductance by CGRP (0.3 pmol/kg per min) were completely abolished by both doses of CGRP-(8–37) (Fig. 2C,D) but incompletely though significantly ($P < 0.05$ relative to the respective CGRP responses in untreated rats) inhibited by both doses of [Tyr^0]-CGRP-(28–37) (Fig. 2E,F). Whereas only the lower dose of CGRP-(8–37) and none of the doses of

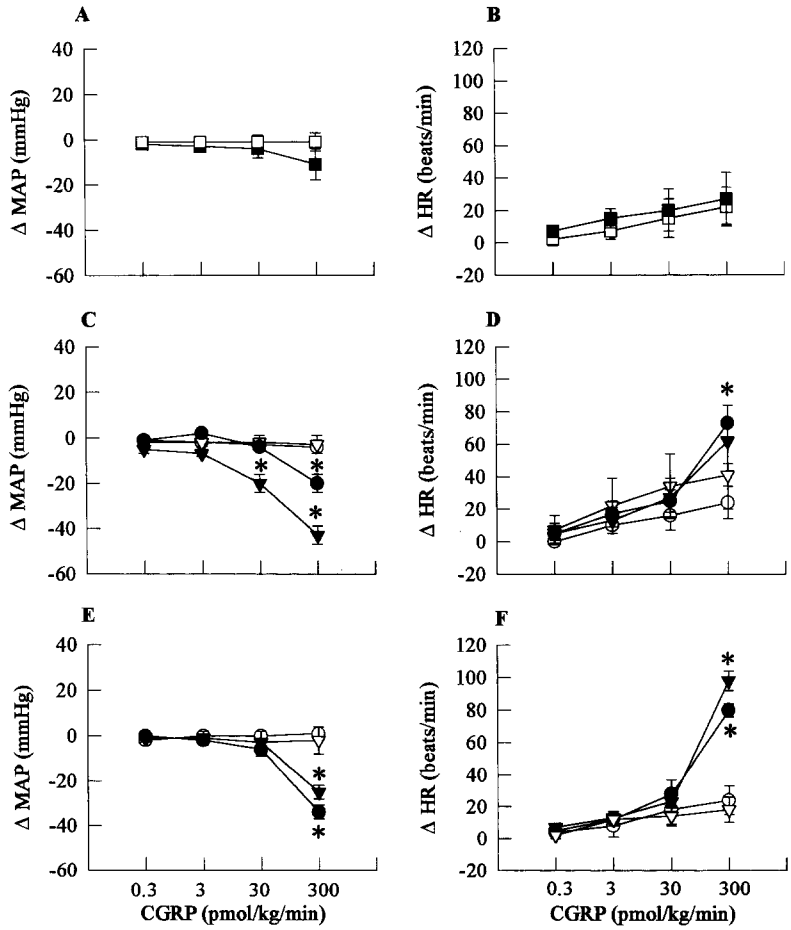

Fig. 1. Dose-response effects of renal arterial infusion of CGRP (filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on mean arterial pressure (MAP) and heart rate (HR) in ten groups of inactin-anaesthetized rats ($n = 6$ each group) in the absence (■,□) of an antagonist (A,B), in the presence of a low (1 nmol/kg, ●,○) or a high (10 nmol/kg, ▼,▽) dose of CGRP-(8–37) (C,D), and in the presence of a low (3 nmol/kg, ●,○) or a high (30 nmol/kg, ▼,▽) dose of [Tyr^0]CGRP-(28–37) (E,F). Data are shown as means ± S.E.M. * Significantly different from the corresponding vehicle control.

[Tyr^0]CGRP-(28–37) attenuated the decrease in renal blood flow induced by the highest dose of CGRP, both doses of CGRP-(8–37) and [Tyr^0]CGRP-(28–37) inhibited the reduction of renal arterial conductance elicited by the highest dose of CGRP. This suggests that the reduction in renal blood flow by the high dose of CGRP in the presence of either one of the receptor antagonists was secondary to hypotension.

Glomerular filtration rate was increased by low doses (0.3 and 3 pmol/kg per min) of CGRP but unchanged by the higher doses (Fig. 3A). The increases in glomerular filtration rate by low doses of CGRP were inhibited by both doses of CGRP-(8–37) completely (Fig. 3B) and by both doses of [Tyr^0]CGRP-(28–37) incompletely (Fig. 3C).

3.2. Effects of CGRP on urinary flow, osmolality, and Na^+ and K^+ excretion in the absence and presence of CGRP-(8–37) or [Tyr^0]CGRP-(28–37)

CGRP dose dependently increased urine flow (Fig. 4A), and excretions of Na^+ (Fig. 5A) and K^+ (Fig. 5B), which were significantly different from the corresponding time-control readings at the highest two doses. Fractional excretion of Na^+ was increased by CGRP from a baseline value of 0.15% to 0.35, 0.43 and 0.55% at the second, third and fourth dose of CGRP. The increments in urine flow (Fig. 4C,E) and excretions of Na^+ (Fig. 5C,E) and K^+ (Fig. 5E,F) by 300 but not 30 pmol/kg per min of CGRP were similarly blocked by both the low and high doses of CGRP-(8–37) or [Tyr^0]CGRP-(28–37).

CGRP did not affect urine osmolality either before or after treatment with CGRP-(8–37) or [Tyr^0]CGRP-(28–37) (Fig. 4B,D,F).

The haematocrit (43 ± 3%), and blood values of osmolality (290 ± 3 mOsm/kg), Na^+ (141 ± 5 mEq/l) and K^+ (3.3 ± 0.4 mEq/l) at the end of the experiments were similar to the corresponding values at the end of the stabilization period (results not shown).

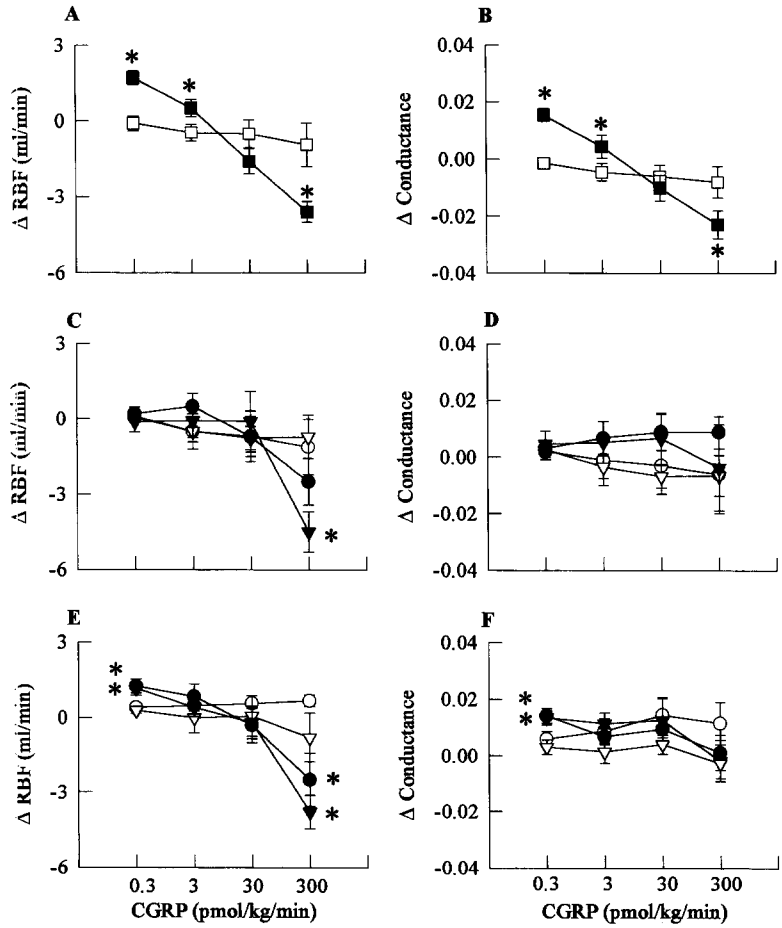

Fig. 2. Dose-response effects (means ± S.E.M.) of renal arterial infusion of CGRP (filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on renal blood flow (RBF) and renal arterial conductance in ten groups of inactin-anaesthetized rats (n = 6 each group) in the absence (A,B) or presence of CGRP-(8–37) (C,D) or [Tyr^0]CGRP-(28–37) (E,F). See legend to Fig. 1 for explanation of symbols. * Significantly different from the corresponding vehicle control.

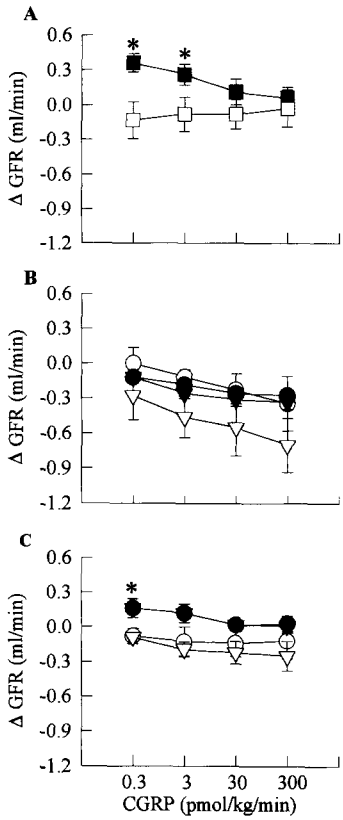

Fig. 3. Dose-response effects (means ± S.E.M.) of renal arterial infusion of CGRP (filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on glomerular filtration rate (GFR) in ten groups of inactin-anaesthetized rats ($n = 6$ each group) in the absence (A) or presence of CGRP-(8–37) (B) or [Tyr^0]CGRP-(28–37) (C). See legend to Fig. 1 for explanation of symbols. * Significantly different from the corresponding vehicle control.

4. Discussion

The present results showed that renal arterial infusion of CGRP, at doses that did not significantly alter mean arterial pressure and heart rate, induced a biphasic renal vascular response: increased renal blood flow and renal vasodilatation at low doses and reduced renal blood flow and vasoconstriction at high doses. Low doses of CGRP also increased glomerular filtration rate and this was likely secondary to the increase in renal blood flow. These results were similar to those reported previously (Elhawary and Pang, 1995). The renal vasodilatation and increased glomerular filtration responses 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 [Tyr^0]CGRP-(28–37). Both antagonists blocked the renal vasoconstrictor responses to CGRP.

CGRP binding sites are classified as $CGRP_1$ and $CGRP_2$ according to the high or low affinity, respectively, of the sites to the *C*-terminal fragment of CGRP-(8–37) (Mimeault et al., 1991). Similarly, CGRP receptors are postulated to be of $CGRP_1$ or $CGRP_2$ subtype 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). There is, however, insufficient information to indicate if the $CGRP_2$ receptors are homogeneous or heterogeneous. The ability of CGRP-(8–37) to block the vasodilator effect of CGRP in the present study suggests that this is mediated via the activation of $CGRP_1$ receptors. We have previously shown that the renal vasodilator response to low doses of CGRP is blocked by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (Elhawary and Pang, 1995). Therefore, the renal vasodilator response to CGRP is likely due to the activation of renal vascular $CGRP_1$ receptors causing the release of endothelium-dependent relaxing factor/nitric oxide. The renal vasoconstrictor effect of CGRP was likely reflex-mediated in response to slight, though insignificant, hypotension and this was also blocked by CGRP-(8–37).

The ability of CGRP-(8–37) to inhibit vascular responses to CGRP has been reported. CGRP-(8–37) inhibited mesenteric vasodilatation elicited by CGRP or peri-arterial nerve stimulation in vitro (Han et al., 1990), the microvascular dilator response induced by CGRP and capsaicin in the skin in situ (Hughes and Brain, 1991), and the vasodilator response to CGRP in the isolated perfused kidney (Chin et al., 1994). This antagonist also inhibited the vasodilator response to CGRP in the renal and hindquarter beds as well as the vasoconstrictor response to CGRP in the mesenteric bed of conscious rats (Gardiner et al., 1990). There is a paradox in the ability of CGRP-(8–37) to block the vasodilatation response to CGRP and yet potentiate the hypotensive effect to a high dose of CGRP. This potentiation was observed in every experiment involving CGRP-(8–37) and [Tyr^0]CGRP-(28–37). In contrast to our findings, i.v. administered CGRP-(8–37) either attenuated or blocked the hypotensive effect to i.v. infused CGRP (Gardiner et al., 1990; Donoso et al., 1990). The differences in the dose and mode of administration of CGRP-(8–37) and CGRP may account for the discrepancy in the results of ours and others. The mechanism by which CGRP reduced mean arterial pressure in the presence of either one of the CGRP receptor antagonists is unclear but is obviously not mediated via the activation of $CGRP_1$ receptor.

The selectivity of [Tyr^0]CGRP-(8–37) on subtypes of CGRP receptors is unclear. [Tyr^0]CGRP-(8–37) induced a rightward shift of the concentration-effect curves of CGRP analogs on the opossum internal anal sphincter smooth muscle without affecting the resting tension (Chakder and Rattan, 1990). This blocker also inhibited CGRP-induced amylase secretion in the isolated acini from the guinea pig pancreas (Maton et al.,

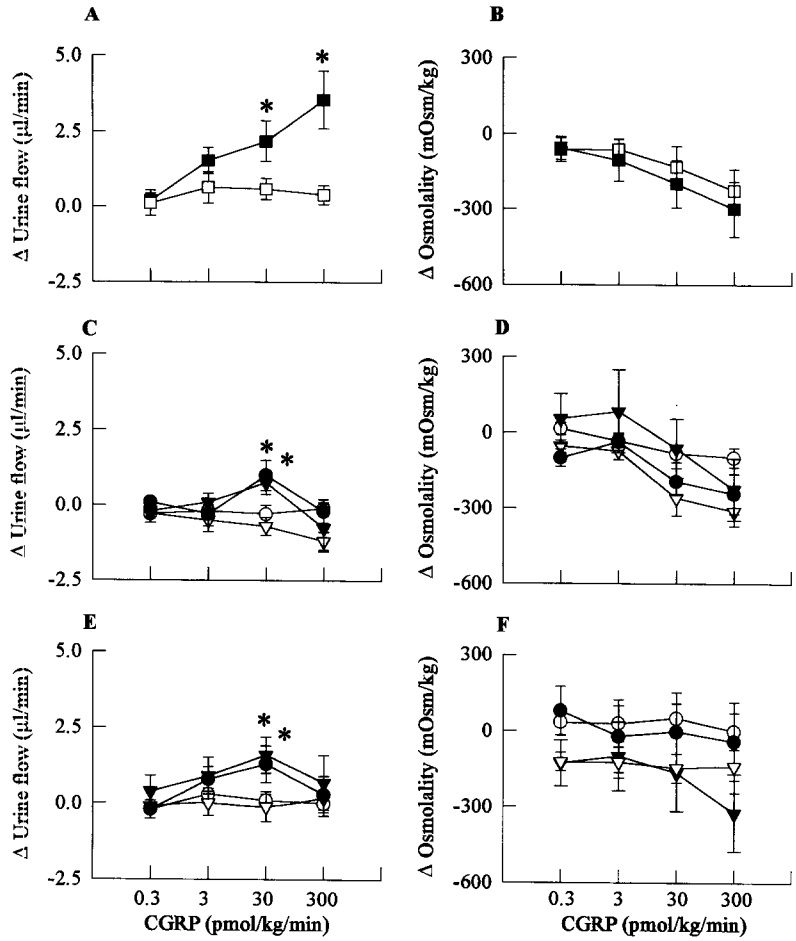

Fig. 4. Dose-response effects (means ± S.E.M) of renal arterial infusion of CGRP (filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on urine flow and urine osmolality in ten groups of inactin-anaesthetized rats ($n = 6$ each group) in the absence (A,B) or presence of CGRP-(8–37) (C,D) or [Tyr^0]CGRP-(28–37) (E,F). See legend to Fig. 1 for explanation of symbols. * Significantly different from the corresponding vehicle control.

1990). The ability of [Tyr^0]CGRP-(28–37) to inhibit the renal vasodilator and vasoconstrictor effects of CGRP in the present study suggests that [Tyr^0]CGRP-(28–37) is also a $CGRP_1$ receptor antagonist, though its potency and efficacy appear to be less than those of CGRP-(8–37).

High doses of CGRP increased urine flow, absolute Na^+ and K^+ excretions and fractional Na^+ excretion, but did not affect urine osmolality. Both CGRP-(8–37) and [Tyr^0]CGRP-(28–37) did not affect the diuretic, natriuretic and kaliuretic effects elicited by 30 pmol/kg per min of CGRP but significantly attenuated ($P < 0.05$) the effects elicited by 300 pmol/kg per min of CGRP. The absence of blockade of tubular effects elicited by 30 pmol/kg per min of CGRP suggests that these effects are not mediated via the activation of $CGRP_1$ receptors. The attenuation of the diuretic, natriuretic and kaliuretic effects elicited by 300 pmol/kg per min of CGRP may be indirect: the results of systemic hypotension, reduced renal perfusion pressure and decreased renal blood flow.

To summarize, CGRP infused intra-arterially into the kidney caused vasodilatation and increased glomerular filtration rate in low doses, and vasoconstriction, diuresis, natriuresis and kaliuresis in high doses. CGRP-(8–37) abolished the vasodilatation, vasoconstriction and increments in glomerular filtration rate elicited by CGRP suggesting the predominance of $CGRP_1$ receptors in the vasculature. The diuresis, natriuresis and kaliuresis responses elicited by non-hypotensive doses of CGRP were not affected by CGRP-(8–37) suggesting the lack of involvement of $CGRP_1$ receptors. The profile of antagonistic actions of [Tyr^0]CGRP-(28–37) and CGRP-(8–37) were simi-

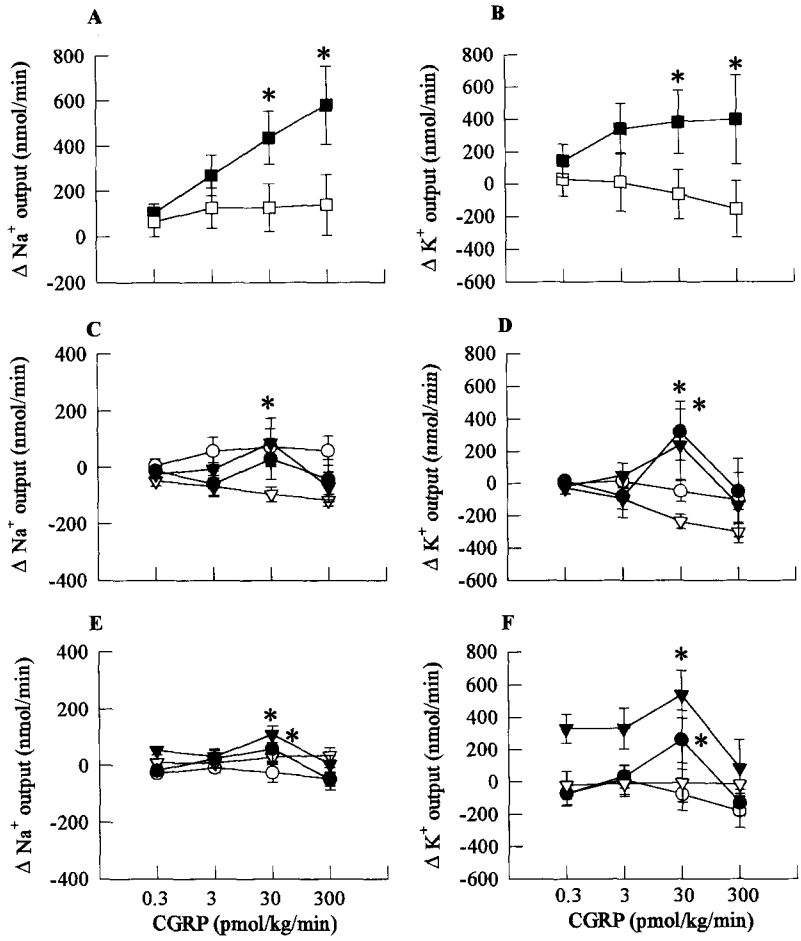

Fig. 5. Dose-response effects (means ± S.E.M.) of renal arterial infusion of CGRP (filled symbols) or an equal volume of vehicle (0.45% NaCl, open symbols) on renal Na^+ and K^+ excretions in ten groups of inactin-anaesthetized rats ($n = 6$ each group) in the absence (A,B) or presence of CGRP-(8–37) (C,D) or [Tyr^0]CGRP-(28–37) (E,F). See legend to Fig. 1 for explanation of symbols. * Significantly different from the corresponding vehicle control.

lar, and this indicates that [Tyr^0]CGRP-(28–37) is also an antagonist of $CGRP_1$ receptors, though its potency and efficacy are less than those of CGRP-(8–37).

Acknowledgements

This work was supported by the Heart and Stroke Foundation of B.C. and Yukon. J. Poon was a recipient of a summer research scholarship award from the Pharmaceutical Manufacturers Association of Canada.

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