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Short communication

Adrenomedullin and calcitonin gene-related peptide in the rat isolated kidney and in the anaesthetised rat: in vitro and in vivo effects

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Abstract

The effects of adrenomedullin and calcitonin gene-related peptide (CGRP) were compared in the rat isolated perfused kidney and in the anaesthetised rat. Adrenomedullin and CGRP both elicited concentration-dependent vasodilator responses from perfused kidneys (adrenomedullin was less potent than CGRP). These responses were blocked by CGRP-(8–37) (1 μM). Adrenomedullin and CGRP elicited dose-dependent hypotension in anaesthetised rats (adrenomedullin was less potent than CGRP). The hypotensive responses to CGRP, but not those to adrenomedullin, were antagonized by CGRP-(8–37) (12 nmol/kg/min). These studies indicate that the in vivo response to adrenomedullin may be mediated through CGRP-(8–37) insensitive receptors.

Keywords: Adrenomedullin; Kidney, rat; CGRP receptor; Anesthetized rat

1. Introduction

Adrenomedullin is a novel 52 amino acid peptide present in human plasma (Ichiki et al., 1994). It is synthesised in the adrenal medulla, lung and kidney (Kitamura et al., 1993) and has marked hypotensive effects in both rats (Ishiyama et al., 1993) and cats (Hao et al., 1994). It has also been shown to stimulate the production of cAMP in cultures of vascular smooth muscle cells, possibly through specific adrenomedullin receptors (Eguchi et al., 1994).

Adrenomedullin has limited sequence homology with the 37 amino acid neuropeptide, calcitonin gene-related peptide (CGRP), although both peptides possess a six-membered ring structure (Kitamura et al., 1993). Although these peptides are structurally distinct, both have been shown to exhibit similar hypotensive activities (Claing et al., 1992; Ishiyama et al., 1993; Hao et al., 1994) and both stimulate adenylate cyclase activity (Eguchi et al., 1994; Edwards and Trizna, 1990). In this study we compared the effects of the antagonist fragment of CGRP, CGRP-(8–37), on both the vasodilator effects of adrenomedullin and CGRP in the rat isolated perfused kidney, a preparation containing vascular $CGRP_1$ receptors (Chin et al., 1994), and also on the haemodynamic effects of adrenomedullin and CGRP in the anaesthetised rat.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (9–12 weeks old) were housed with a photoperiod of 12 h light/12 h dark at a temperature of 20 ± 1° C. Animals were allowed access to water and food (Clark-King GR2 pellets) ad libitum. On the day of use the animals were either killed by decapitation or were anaesthetised (urethane 1.3 g/kg i.p.).

2.2. Isolated perfused kidneys

Following decapitation, a central midline incision was made and a polyethylene cannula (outer diameter 1.5 mm, internal diameter 0.8 mm) was inserted into

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each renal artery. Both kidneys were flushed with saline, containing 250 U/ml sodium heparin (Bull Laboratories), removed from the rat, decapsulated, and placed into 400 ml tissue baths prior to perfusion (Cole-Parmer masterflex pump, model 7521-35) with oxygenated (5% O_2 in CO_2) modified Krebs solution (of composition, mM: NaCl 118; KCl 4.7; $MgSO_4$ 0.45; KH_2PO_4 1.18; $NaHCO_3$ 25.0; $CaCl_2$ 2.5; glucose 11.7) at a rate of 4 ml/min (to give a final pressure of approximately 100 mm Hg). Following a 20 min equilibration period tissues were constricted with the α_1-adrenoceptor agonist phenylephrine (10 μM), and allowed a further 25 min equilibration. This concentration of phenylephrine induced a sustained increase in pressure of approximately 80 mm Hg. Cumulative concentration-response curves to CGRP and to adrenomedullin were constructed by perfusing agonists through the phenylephrine-preconstricted preparations for 1 min. The antagonist fragment of CGRP, CGRP-(8–37) (0.1 or 1 μM) was perfused through one of each pair of kidneys for 30 min prior to the addition of agonists. The remaining kidney was used as a time control. Changes in perfusion pressure were recorded through Gould-Statham (model P-23) pressure transducers coupled to a MacLab/8 data acquisition system (ADInstruments, N.S.W. Australia). At the end of each experiment forskolin (10 μM) was perfused through each preparation (1 min) to stimulate adenylate cyclase. The responses to CGRP and adrenomedullin are expressed as a percentage of the maximum vasodilatation elicited by forskolin (-81 ± 7 mm Hg, $n = 16$).

2.3. In vivo studies

Following anaesthesia (urethane 1.3 g/kg i.p.) both the left jugular vein and the carotid artery were cannulated with polyethylene cannula (SP37, Dural Plastics, Dural, Australia). The jugular cannula was flushed with heparin (500 U/rat) and the preparations allowed at least 30 min to equilibrate. Agonists or vehicle (Haemaccel, Behring, Germany) were added, at approximately 15 min intervals, as a bolus (< 0.1 ml) into the jugular cannula. Changes in arterial pressure were monitored (10 samples/s) via Gould-Statham (model P-23) or disposable (model 43-212 Baxter Healthcare) pressure transducers coupled to a MacLab/8 data acquisition system (ADInstruments).

Antagonists or vehicle were infused at 7 μl/min (Razel model A-99 syringe pump) into the jugular cannula for 60 min prior to the addition of agonists. The animals were continuously infused with antagonists throughout the day. Each animal was administered agonists sequentially, with a minimum of 15 min between agonist additions. No animal received more than one agonist. Mean arterial blood pressure and mean heart rate were calculated over 10 s periods (100 samples). The maximal hypotensive effect and change in heart rate, recorded approximately 1 min after agonist addition, were used to generate dose-response curves.

2.4. Data analysis

Vasodilator and hypotensive responses were analysed using FLEXIFIT (Guardabasso et al., 1988), to provide estimates of IC_{50} and potency ratios, and were compared using an F-test. When complete concentration-response curves could not be generated the Student's t-test was used to compare maximal agonist effects. In all cases a P value less than 0.05 was considered statistically significant.

2.5. Drugs

Human adrenomedullin (Peptide Institute, Osaka, Japan); rat CGRP-(8–37) (Bachem, Torrance, USA); rat α-CGRP (Bachem, Torrance, USA); forskolin (Sigma Chemical Co., St. Louis, USA); phenylephrine (Sigma Chemical Co., St. Louis, USA).

Forskolin was dissolved as a stock solution in ethanol (99%), stored at $-20°C$ and diluted to volume with

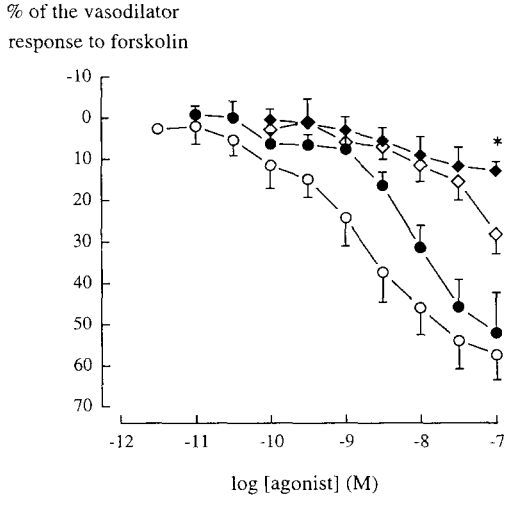

Fig. 1. Effects of CGRP-(8–37) (1 μM) on responses to CGRP (○) and adrenomedullin (◇) in preparations of rat isolated kidney. Filled symbols show responses in the presence of CGRP-(8–37). Preparations were perfused at a rate of 4 ml/min and preconstricted with phenylephrine (10 μM), giving a perfusion pressure of approximately 100 mm Hg. The horizontal axis shows the log (M) concentration of agonist, the vertical axis shows the responses to agonists, expressed as a percentage of the maximal response to forskolin (10 μM). Each symbol shows the mean and S.E.M. of 5–6 replicate experiments. * Significant ($P < 0.05$, Student's t-test, $df = 11$) difference from antagonist-free control.

buffer on the day of use. All other drugs were made up in vehicle on the day of use.

3. Results

3.1. Isolated perfused kidneys

Adrenomedullin and CGRP elicited concentration-dependent vasodilatation from preparations of rat isolated kidney preconstricted with phenylephrine (10 μM); CGRP was approximately 100-fold more potent than adrenomedullin (Fig. 1). The CGRP receptor antagonist CGRP-(8–37) (0.1 μM) did not shift concentration-response curves to CGRP ($n = 6$, data not shown). Increasing the concentration of CGRP-(8–37) (to 1 μM) significantly ($P < 0.05$, F-test, $df = 32,33$) shifted responses to CGRP (4.4-fold; Fig. 1) and significantly ($P < 0.05$, Student's t-test, $df = 11$) attenuated the vasodilator response to adrenomedullin (Fig. 1).

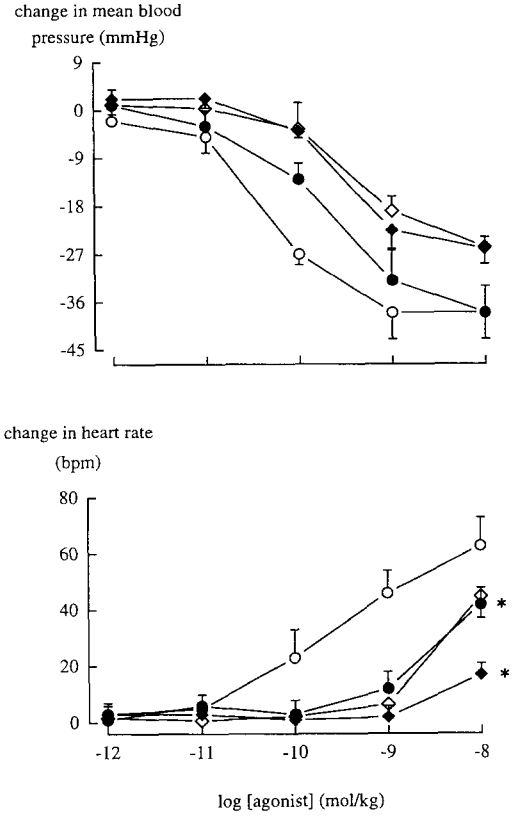

Fig. 2. Effects of CGRP-(8–37) (12 nmol/kg/min) on CGRP (○) and adrenomedullin (◇) induced changes in mean arterial pressure (MAP), upper panel, and heart rate (bpm), lower panel in anaesthetised rats. Filled symbols show responses in the presence of CGRP-(8–37). The vertical axes of the upper and lower panels show the changes in MAP (mm Hg) and changes in heart rate respectively. The horizontal axes show the log concentration of agonist (mol/kg). Each symbol shows the mean and S.E.M. of 6–8 replicate experiments. * Significant ($P < 0.05$, Student's t-test, $df = 8$ and 11 respectively) difference from vehicle only (antagonist-free) group.

3.2. In vivo studies

Following anaesthesia with urethane (1.3 g/kg i.p.) preparations were stable for approximately 3.5 h with a mean arterial blood pressure of 90 ± 4 mm Hg and a mean heart rate of 344 ± 4 beats/min ($n = 32$).

Adrenomedullin and CGRP both elicited concentration-dependent hypotension in anaesthetised rats (mean ± S.E. neg log IC_{50} values were 9.4 ± 0.19 and 10.3 ± 0.18 mol/kg respectively, Fig. 2, upper panel). The maximal hypotension elicited by CGRP was significantly ($P < 0.05$, F-test, $df = 53,54$) greater than that elicited by adrenomedullin (Fig. 2, upper panel).

Responses to CGRP were not affected by CGRP-(8–37) (3 nmol/kg/min; $n = 6$, data not shown), but were significantly ($P < 0.05$, F-test, $df = 45,46$) shifted by CGRP-(8–37) (12 nmol/kg/min, Fig. 2, upper panel). Dose-response curves to adrenomedullin were not affected by CGRP-(8–37) (12 nmol/kg/min, Fig. 2, upper panel).

Adrenomedullin and CGRP both increased heart rate (Fig. 2, lower panel); the effects of these peptides were significantly ($P < 0.05$, Student's t-test, $df = 11$ and 8 respectively) reduced by CGRP-(8–37) (12 nmol/kg/min, Fig. 2, lower panel).

4. Discussion

We have demonstrated that CGRP is both a more potent vasodilator and a more potent hypotensive agent than is adrenomedullin, findings consistent with the reported effects of these peptides both in vitro and in vivo (Claing et al., 1992; Ishiyama et al., 1993; Nuki et al., 1993; Chin et al., 1994; Hao et al., 1994). Our finding, that the in vitro responses to both adrenomedullin and to CGRP were blocked by CGRP-(8–37), is consistent with both the action of adrenomedullin at $CGRP_1$ receptors (Entzeroth et al., 1995) and also with a recent report indicating that the rat perfused renal vasculature contains vascular $CGRP_1$ receptors (Chin et al., 1994).

Although CGRP-(8–37) had no effect on the adrenomedullin-mediated changes in mean arterial pressure, it did inhibit both the CGRP- and adrenomedullin-stimulated increases in heart rate. This later finding is not consistent with recent reports showing that adrenomedullin did not elicit tachycardia in anaesthetised rats (Ishiyama et al., 1993) or that the tachycardic effect of adrenomedullin was not blocked by CGRP-(8–37) in conscious rats (Gardiner et al., 1995). The contrasting findings obtained in this study and from those of Ishiyama et al. (1993) and Gardiner et al. (1995) may reflect the influence of different anaesthesia/animal models on the in vivo responses to adrenomedullin. It is possible that, in this study, the effects of CGRP-(8–37) upon the CGRP- and

adrenomedullin-stimulated increases in heart rate are due to the direct action of these peptides at cardiac CGRP receptors (Chatterjee et al., 1991; Entzeroth et al., 1995) rather than as a result of baroreflex.

There is evidence indicating that CGRP-(8–37) can block the hypotensive responses to adrenomedullin in vivo (Takahashi et al., 1994). We, however, were unable to repeat this finding, using a concentration of antagonist which blocks the in vivo responses to CGRP. Our finding that CGRP-(8–37) does not block the hypotensive response to adrenomedullin in vivo is, however, consistent with recent reports showing that the haemodynamic responses to adrenomedullin, in conscious rats, were resistant to the effects of CGRP-(8–37) (Gardiner et al., 1995) and that adrenomedullin and CGRP elicit pharmacologically distinct responses from the rat perfused hindquarters (Feng et al., 1994).

The results of the present study are consistent with recent evidence showing that, in vascular cell culture preparations, adrenomedullin was more potent than CGRP in stimulating cAMP production and that the responses to adrenomedullin were less susceptible to blockade by CGRP-(8–37) than were responses to CGRP (Eguchi et al., 1994). This group concluded that adrenomedullin interacted with specific adrenomedullin receptors to elevate cAMP in rat cultured aortic cells (Eguchi et al., 1994).

In contrast with our finding that the effects of adrenomedullin on the rat isolated perfused kidney were blocked by CGRP-(8–37), but consistent with our finding that the in vivo responses to adrenomedullin were insensitive to CGRP-(8–37), Gardiner et al. (1995) did not show any effect of CGRP-(8–37) on adrenomedullin-stimulated increases in renal flow, in vivo, and concluded that the actions of adrenomedullin were not mediated through $CGRP_1$ receptors. Since we have demonstrated a marked disparity between the in vitro and in vivo effects of adrenomedullin and CGRP we conclude that, in the rat isolated perfused kidney, both adrenomedullin and CGRP act through CGRP-(8–37)-sensitive receptors, but that in the anaesthetised rat, adrenomedullin may also act at CGRP-(8–37)-insensitive receptors to elicit the majority of its hypotensive effect.

Acknowledgements

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