British Journal of Pharmacology (1995) 114, 592-597

Interaction of human adrenomedullin $_{13-52}$ with calcitonin gene-related peptide receptors in the microvasculature of the rat and hamster

[1]Judith M. Hall, Lynn Siney, *Howard Lippton, *Albert Hyman, †Jaw Kang-Chang & Susan D. Brain

Pharmacology Group, Biomedical Sciences Division, King's College London, Manresa Road, London, SW3 6LX, U.K; *Department of Surgery, Tulane Medical School, 1430 Tulane Avenue, New Orléans, LA 70112, U.S.A and †Pheonix Pharmaceuticals, 2462 Wyandotte Street, Moutainview, CA 94043, U.S.A.

1 Adrenomedullin (ADM), a recently discovered circulating hypotensive peptide, shares limited sequence homology with the sensory nerve-derived vasodilator, calcitonin gene-related peptide (CGRP). This study compared the vasodilator effect of sequence 13-52 of human adrenomedullin (ADM_{13-52}) with that of human α CGRP (CGRP), in the microvasculature of the hamster cheek pouch and rat skin *in vivo*.

2 Single arterioles (20–40 μm diameter) in the hamster cheek pouch were visualised by intravital microscopy and video recording, and measured by image analysis. Both ADM_{13-52} (1 pmol–0.4 nmol) and CGRP (0.1 pmol–1 nmol) evoked dose-related increases in the diameter of preconstricted arterioles ($n = 6$). ADM_{13-52} (ED_{50} 14 pmol) was 20 fold less active than CGRP (ED_{50} 0.71 pmol). The kinetics of onset and decline of vasodilator responses to both peptides were similar, with vasodilator responses to both peptides reaching a maximum at *ca.* 2 min, and reversing after 10–15 min ($n = 5-7$). The submaximal increase in blood flow evoked by ADM_{13-52} was significantly inhibited ($P<0.05$; $n = 6$) by the $CGRP_1$ receptor antagonist, $CGRP_{8-37}$, at a dose (300 nmol kg^{-1}, i.v.) that we have previously shown to inhibit significantly equivalent vasodilator responses to CGRP in this preparation.

3 In experiments measuring changes in local blood flow in rat skin by a ^{133}xenon clearance technique, intradermal injection of both ADM_{13-52} (3–300 pmol) and CGRP (0.1–30 pmol) evoked dose-related increases in local blood flow. ADM_{13-52} (ED_{50} 27 pmol) was 17 fold less potent than CGRP (ED_{50} 1.6 pmol) ($n = 6$). The submaximal increase in blood flow evoked by both peptides was significantly inhibited ($P<0.02$; $n = 5$) by $CGRP_{8-37}$ (100 nmol kg^{-1}, i.v.).

4 We conclude that ADM_{13-52} is a potent vasodilator in the microvasculature of the hamster and rat *in vivo*. It mediates its vasodilator effect by arteriolar dilatation and this effect is due, at least in part, to the stimulation of $CGRP_1$ receptors.

Keywords: Adrenomedullin; microvasculature; calcitonin gene-related peptide; $CGRP_{8-37}$; hamster cheek pouch; skin (rat); blood flow; vasodilatation; sensory neuropeptide

Introduction

Adrenomedullin (ADM) is a 52 amino acid peptide originally isolated from human phaeochromocytoma tissue (Kitamura *et al.*, 1993a). Human and rat ADM mRNA have subsequently been shown to be expressed in various peripheral tissues of rat and man, including the kidney, lung and adrenal medulla (Kitamura *et al.*, 1993b; Sakata *et al.*, 1993; Ichiki *et al.*, 1994). In man, ADM had been shown to be present at considerable concentrations in the blood (e.g. 3 fmol ml^{-1}, Kitamura *et al.*, 1994a), and in several species has been shown to have potent actions in the vasculature both *in vivo* and *in vitro*. For example, in the anaesthetized rat (Ischiyama *et al.*, 1993) and cat (Hao *et al.*, 1994), intravenous injection of ADM causes a potent and long-lasting hypotensive response, and when infused into the intralobar artery of the cat, evokes vasodilatation in the pulmonary circulation (DeWitt *et al.*, 1994; Lippton *et al.*, 1994). *In vitro*, low doses of ADM evoke vasodilatation in the isolated perfused mesentery of the rat (Nuki *et al.*, 1993). Recently, the 13–52 sequence of human ADM (ADM_{13-52}) has been shown to exhibit the full vasodilator properties of the present peptide, ADM_{1-52}, *in vivo* and *in vitro* (Perret *et al.*, 1993; Hao *et al.*, 1994; Lippton *et al.*, 1994), suggesting that biological activity largely resides in this C-terminal fragment.

ADM and ADM_{13-52} show some limited sequence homology with the sensory nerve-derived vasodilator calcitonin gene-related peptide (CGRP), sharing a six residue ring structure formed by an intramolecular disulphide linkage and a slight homology in the C-terminal amide sequence (Kitamura *et al.*, 1994b, Figure 1). It has recently been suggested that the vasodilator effect of ADM is due to its interaction with CGRP receptors, since vasodilator responses in the rat isolated perfused mesentery evoked by ADM *in vitro* were inhibited by the $CGRP_1$ receptor antagonist $CGRP_{8-37}$ (Nuki *et al.*, 1993). Further, in cultured vascular smooth muscle cells, CGRP displaced binding from specific sites labelled with [^{125}I]-ADM (Eguchi *et al.*, 1994), suggesting an interaction of CGRP and ADM with common receptor sites.

In the present study, we have compared the activity of ADM_{13-52} with human α CGRP (CGRP) in microvascular assays *in vivo*. In the hamster cheek pouch, we investigated the vasodilator effect of the two peptides on single arterioles viewed by intravital microscopy (see Hall & Brain, 1994), and in rat skin, we determined the effect of the two peptides on blood flow using a ^{133}xenon-clearance technique (see Lawrence & Brain, 1992). In both types of experiment, we used the $CGRP_1$ receptor antagonist, human α $CGRP_{8-37}$ (Dennis *et al.*, 1990) to investigate whether the effects of ADM_{13-52} were mediated via stimulation of $CGRP_1$ receptors.

[1] Author for correspondence.

Sequence homology of some peptides compared to α human calcitonin gene-related peptide (α-CGRPh)

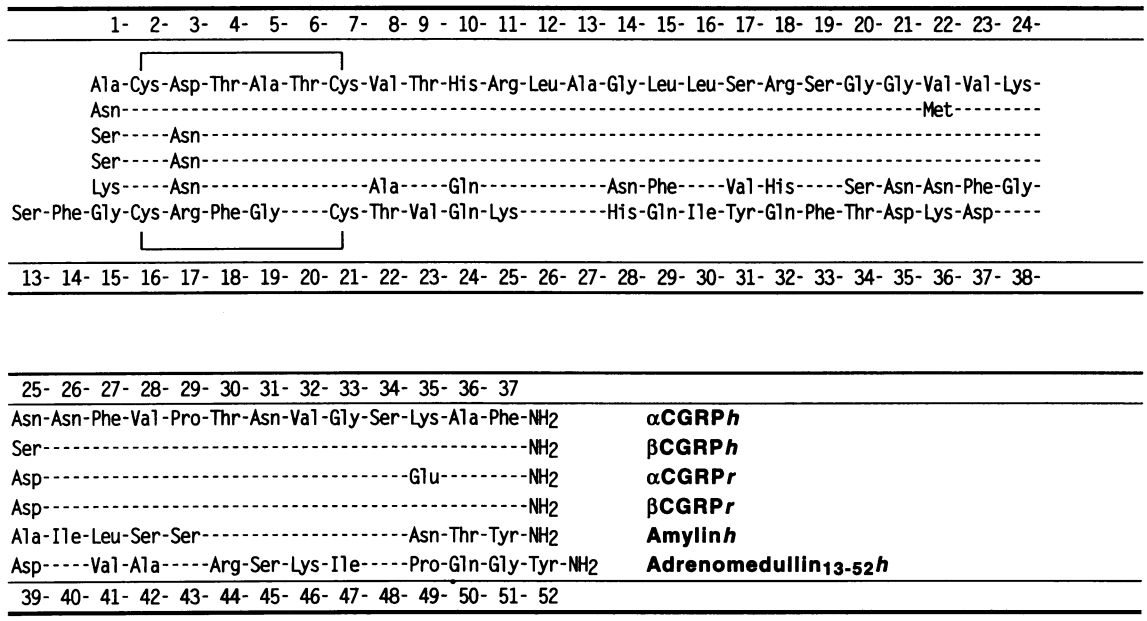

Figure 1 Comparison of amino acid sequences of calcitonin gene-related peptides, amylin, and adrenomedullin$_{13-52}$. Non-identical homologous residues, and the internal disulphide links, are indicated.

Preliminary accounts of this work have been presented to the 14th National Scientific Meeting of the Bayliss and Starling Society (Southampton, England) (Siney *et al.*, 1994a), and a Satellite Symposium of the XIIth IUPHAR Congress, The Second International Symposium of Calcitonin Gene-Related Peptide (Montréal, Canada) (Siney *et al.*, 1994b).

Methods

Hamster cheek pouch

Male golden (Syrian) hamsters (85–106 g) were anaesthetized with sodium pentobarbitone (Sagatal 50 mg kg^{-1}; i.v.) and anaesthesia maintained with 15 mg kg^{-1} pentobarbitone as required. A single layer of vascular membrane was prepared as described by Duling (1973) with some modifications (Hall & Brain, 1994). Briefly, the hamster was placed on a specially designed stage with a central depressed well, the right cheek pouch was carefully everted and placed in the well and pinned to a silicon-rubber ring encircling the window. A single vascular layer was dissected out keeping an intact blood supply, and all connective tissue was removed. In some experiments, the left jugular vein was cannulated (Portex 18 gauge) for intravenous administration of CGRP$_{8-37}$. The tissue was superfused with Krebs-bicarbonate solution (composition mM: NaCl 120, KCl 4.7, $MgCl_2$ 0.12, $CaCl_2$ 0.18, KH_2PO_4 0.4, $NaHCO_3$ 23, glucose 10) at a rate of 4 ml min^{-1} and the Krebs solution, warmed to 35°C, was gassed with 5% CO_2 in air maintained at pH 7.4. An area of microvasculature was selected which allowed an arteriole and adjacent venule (each between 20 and 40 μm in diameter) to be viewed concomitantly. The microvessels were observed with a Leitz Dialux microscope having a 27 × salt-water dipping objective with 10 × eye pieces. Diameters of microvessels were measured with a computerised imaging system (Kompira Limited, Strathclyde, U.K.). In all experiments, arterioles were pre-constricted (by *ca.* 50%) with human endothelin-1 (usually 30–300 pM) superfused 20 min before, and throughout, the experiment. CGRP and ADM$_{13-52}$, dissolved in Krebs solution, were applied topically in 10 μl (or 20 μl for 0.4 nmol ADM$_{13-52}$ in view of the maximum concentration of the stock solution) using a Finn pipette. The CGRP receptor antagonist, CGRP$_{8-37}$ (300 nmol kg^{-1}), dissolved in 0.5 ml saline: bovine serum albumin (BSA;0.1%), was applied i.v. over 1 min, followed by i.v. 0.75 ml saline; BSA over 2 min, 5 min before administration of ADM$_{13-52}$. Following application of test agent, vessel diameter was measure at 15 s, 30 s, 45 s, 1 min, and every minute thereafter, until the dilator response had fully reversed and predilator vessel diameter was recovered.

In experiments comparing the vasodilator activities of CGRP (0.1 pmol–1 nmol) and ADM$_{13-52}$ (1 pmol–0.4 nmol), dose-response curves were obtained by applying single doses of the peptide every 15 min. In half the experiments, the dose-response curve to CGRP was obtained first followed by ADM$_{13-52}$, in the other half, *vice versa*. In all experiments, control responses to the peptide solvent (10 or 20 μl Krebs solution) were measured. In another series of experiments, the CGRP receptor antagonist, CGRP$_{8-37}$, was tested against submaximal vasodilator responses to ADM$_{13-52}$. The response to a submaximal dose of ADM$_{13-52}$ (0.1 nmol) was obtained and this was repeated 30 min later following i.v. administration of CGRP$_{8-37}$ (300 nmol kg^{-1}; i.v.). Reversal of the antagonist effect was determined by obtaining responses to ADM$_{13-52}$ 30 and 60 min after antagonist administration.

Rat skin

The effect of ADM$_{13-52}$ and CGRP on local blood flow was evaluated by local clearance of ^{133}xenon (^{133}Xe) in the dorsal skin of male Wistar rats (200–230 g) following intradermal (i.d.) injection of multiple test agents, as previously described (Williams, 1976; Lawrence & Brain, 1992). Briefly, the rats

were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) and the dorsal skin was shaved. Each test solution was made up in modified Tyrode solution (composition mM: NaCl 137, KCl 2.7, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6), and equal quantities of ^{133}Xe were mixed with 1 ml samples of each. A 100 µl sample of each test agent was rapidly injected i.d., in duplicate, in random order according to a balanced site pattern. On completion of injections, a 100 µl aliquot of each solution was rapidly injected into vials containing paraffin oil, and immediately capped: the total radioactivity present in 100 µl of each sample could then be counted. After a clearance period of 15 min, the rat was killed, the dorsal skin removed, and the sites punched out. Radioactivity was measured using an automated gamma-counter.

In experiments to compare the vasodilator activities of CGRP and ADM_{13-52}, dose-response curves were determined by the i.d. injection of five dose-levels of the two peptide agonists, at different randomized sites in each rat. In all experiments, control vehicle responses were measured. In a separate series of experiments, the CGRP receptor antagonist, $CGRP_{8-37}$, was tested against the vasodilator responses to ADM_{13-52} and CGRP. Some rats were treated with $CGRP_{8-37}$ (100 nmol kg^{-1}, i.v.) 10 min before i.d. injections, and responses compared to those in control rats also injected 10 min before with vehicle (0.9% saline). Dose-response curves for ADM_{13-52} and CGRP were then determined as before. In all cases, changes in local blood flow were expressed as percentage change in local clearance of ^{133}Xe at test sites, as compared to control sites injected with Tyrode solution (see Lawrence & Brain, 1992). A decrease in clearance indicates a decrease in local blood flow due to vasoconstriction of microvessels.

Source of drugs

Drugs were obtained as follows: pentobarbitone sodium (Sagatal; Rhône Mérieux Ltd, Essex, U.K.) endothelin-1, human αCGRP and human $\alpha CGRP_{8-37}$ (Bachem, Essex, U.K.). ADM_{13-52} was dissolved in saline, all other peptides were dissolved in distilled water and stored at −20°C, peptide dilutions were made up in Krebs or Tyrode solution, as appropriate. All salts were of analytical grade and were obtained from B.D.H, U.K. ADM_{13-52} was synthesized by Dr Jaw Kang-Chang, Pheonix Pharmaceuticals, California, U.S.A.

Expression of results and statistical analysis

Arteriolar vasodilatation in the hamster cheek pouch was calculated as % maximal increase in diameter (compared to pre-constricted diameter). Changes in local blood flow in rat skin were expressed as % change in local clearance of ^{133}Xe at test sites, compared with control sites injected with Tyrode solution. Data from individual experiments were combined, and results are expressed as mean ± s.e.mean of experiments carried out in at least 5 animals for each study. Estimates of ED_{50} with 95% confidence limits, calculated as geometric means were made from individual log concentration-response curves, and are defined as the dose producing 50% of the maximal recorded increase in arteriolar diameter in the hamster cheek pouch, or 50% of maximum response in blood flow in rat skin. The maximum responses evoked by ADM_{13-52} and CGRP were similar in both assays at the highest doses tested. For the hamster cheek pouch experiments, tests for significant differences between log ED_{50}s were made using Student's paired or unpaired t tests, as appropriate. With the rat skin assay, differences between means were assessed by Bonferroni's modified t test, which uses the standard error estimate for analysis of variance, to allow comparison of multiple sites.

Results

Hamster cheek pouch

ADM_{13-52} (1 pmol–0.4 nmol) and CGRP (0.1 pmol-1 nmol) potently evoked arteriolar vasodilatation but had no consistent dilator effect on venules. The time-course of the vasodilatation evoked by both peptides was similar. Figure 2 shows the time-course of vasodilatation of equi-effective doses of ADM_{13-52} (0.1 nmol) and CGRP (10 pmol). The maximal vasodilatation was evoked 2 min after peptide application, and reversed 10–15 min after application. ADM_{13-52} was *ca.* 20 fold less potent than CGRP in evoking arteriolar vasodilatation (Figure 3a), ED_{50} estimates were 14 pmol (1.8–111) and 0.71 pmol (0.2–2.9), respectively. It was not possible to determine the maximal dilator response to ADM_{13-52} in view of the limited concentration of the stock solution; however, the maximal increase in vessel diameter recorded at the highest dose tested was similar for ADM_{13-52} and CGRP (140 ± 25% and 136 ± 28%, respectively). The CGRP receptor antagonist, $CGRP_{8-37}$ (300 nmol kg^{-1}; i.v.), in its own right produced a small constriction in arteriolar tone, which was maximal (at 10.5 ± 1.8) 1.75 min after i.v. injection, but which had essentially reversed after 5 min. $CGRP_{8-37}$ significantly inhibited ($P<0.05$) submaximal vasodilator responses to ADM_{13-52}. The antagonism was partially reversible 30 and 60 min after antagonist administration (Figure 2c). We have previously shown that the same dose of $CGRP_{8-37}$ significantly inhibits submaximal vasodilator responses to CGRP in this preparation (for comparison, a 178% increase in vasodilatation was reduced to 59% by $CGRP_{8-37}$; unpublished observations).

Rat skin

ADM_{15-52} caused a dose-related increase in blood from in the rat cutaneous microvasculature (Figure 4a). However, ADM_{13-52} was shown to be *ca.* 17 fold less potent than CGRP as compared in the same rats, yielding ED_{50} estimates of 27 (22–32) pmol and 1.6 (0.9–2.9) pmol, respectively (Figure 3a). In this system, there was no significant difference in the maximal increase in blood flow induced by the two peptides; 55 ± 6% and 46 ± 4%, for ADM_{13-52} and CGRP, respectively. The antagonist $CGRP_{8-37}$ (100 nmol kg^{-1}) significantly ($P< 0.05$) inhibited ADM_{13-52}-induced increases in blood flow (30–100 pmol/site). Vehicle alone had no significant effect on ADM_{13-52}-induced vasodilatation (Figure 4b). Further, $CGRP_{8-37}$ alone (100 nmol kg^{-1} i.v.), had no significant effect on the clearance at saline-injected sites.

Discussion

This study demonstrates that ADM_{13-52} is a potent vasodilator in the microvasculature of the rat and hamster. It mediates its vasodilator effect by arteriolar dilatation, and the vasodilatation is due, at least in part, to the stimulation of $CGRP_1$ receptors.

Interaction of ADM_{13-52} with CGRP receptors

When applied topically in the hamster cheek pouch, or intradermally in rat skin, microvasculature; both ADM_{13-52} and CGRP, in a dose-related manner, potently evoked arteriolar dilatation, or increased local blood flow, respectively. In both microvasculature preparations, ADM_{13-52} was *ca.* 20 fold less active than CGRP. These results are consistent with results of a recent *in vitro* studying using the full sequence ADM_{1-52} which was found to be *ca.* 10 fold less active than CGRP in the rat isolated perfused mesentery (pD_2 estimates were 8.3 and 9.2 respectively; Nuki *et al.*, 1993).

In the hamster cheek pouch, the time-course of the arteriolar dilatation was similar for ADM_{13-52} and CGRP, with

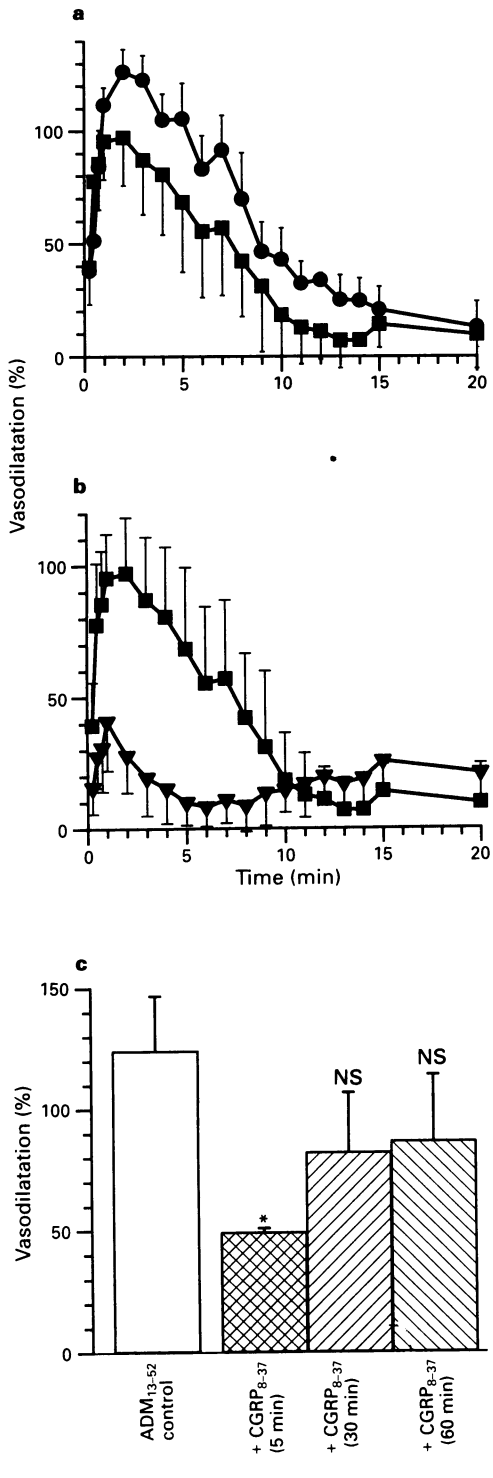

Figure 2 (a) Shows a comparison of time-courses of arteriolar vasodilatation evoked by human adrenomedullin $_{13-52}$ (ADM_{13-53}) (■) and human α calcitonin gene-related peptide (CGRP) (●) in the hamster cheek pouch. Vasodilatation evoked by ADM_{13-52} (0.1 nmol) and CGRP (10 pmol), is expressed as % increase in diameter of arterioles preconstricted (by *ca.* 50%) by human endothelin-1. Vessel diameter was measured 15, 30, 45, 60 s and then every minute up to 20 min after topical application of peptide (in 10 μl Krebs solution). (b) Shows inhibition of submaximal vasodilator responses evoked by ADM_{13-52} (0.1 nmol), Control (■), or after administration of the $CGRP_1$ receptor antagonist $CGRP_{8-37}$ (▼) (300 nmol kg^{-1}, i.v.). (c) Shows inhibition of the maximal vasodilator response evoked by ADM_{13-52} by $CGRP_{8-37}$. Control vasodilatation is shown by the open column, and dilatation recorded 5, 30 and 60 min following intravenous administration of $CGRP_{8-37}$ respectively. Responses are shown as the mean ± s.e.mean in 5–7 hamsters. Vehicle control (10 μl Krebs) produced an 18.6 ± 10.5% increase in arteriole diameter. $^*P<0.05$. NS not significant.

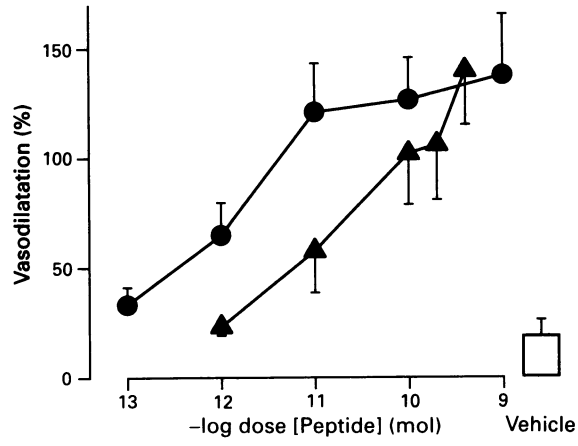

Figure 3 Dose-related vasodilatation in the hamster cheek pouch evoked by human adrenomedullin$_{13-52}$ (ADM_{13-52}) (▲) or human α calcitonin gene-related peptide (CGRP) (●) measured following topical application of peptide (in 10 or 20 μl Krebs solution). Vasodilatation is expressed as maximal % increase in diameter of arterioles preconstricted (by *ca.* 50%) by human endothelin-1, and mean responses, ± s.e.mean, are shown for experiments carried out in 6 hamsters. Estimates of ED_{50} were 14 pmol (1.8–111) and 0.72 (0.2–2.9) pmol, respectively.

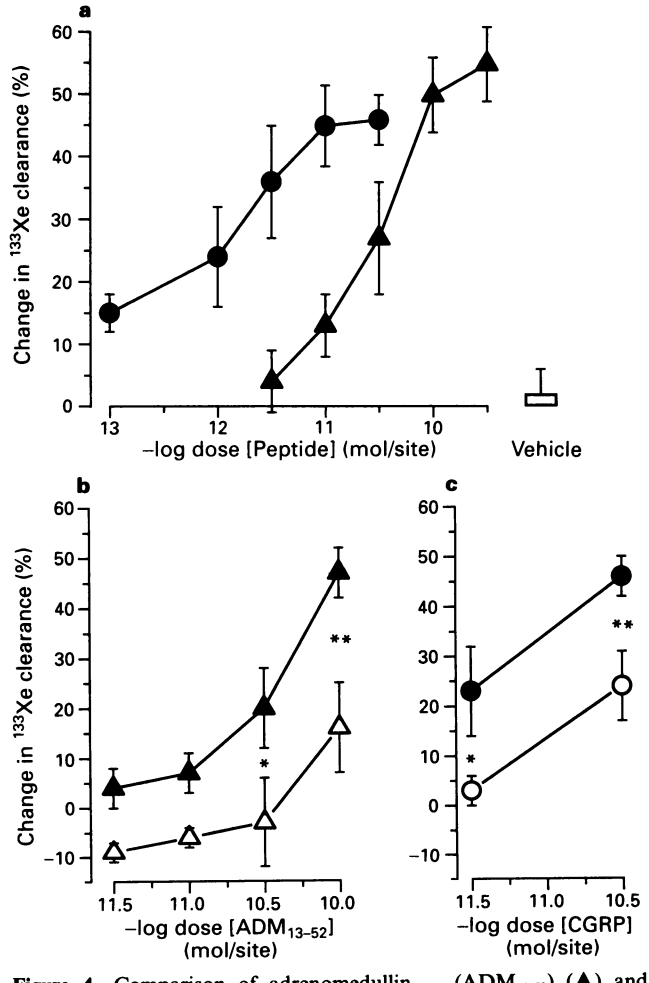

Figure 4 Comparison of adrenomedullin$_{13-52}$ (ADM_{13-52}) (▲) and human α calcitonin gene-related peptide (CGRP) (●) on local blood flow in rat dorsal skin (a). The effect of the antagonist $CGRP_{8-37}$ (100 nmol kg^{-1}, i.v.) against (b) ADM_{13-52}-induced changes in blood flow (test △; vehicle-treated animals/control ▲), and (c) against CGRP (test ○, and control ●). Effects are expressed as percentage change in ^{133}Xe clearance, as indicator of local blood flow, at test sites compared to Tyrode solution injected sites (mean ± s.e.mean; $n = 5$ rats). Dashed line represents blood flow at control Tyrode injected sites. $^*P<0.05$; $^{**}P<0.01$ antagonist *cf.* vehicle control.

dilatation reaching a maximum at 2 min and reversing after 10–15 min. The rate of onset and decline of responses to the two peptides is relatively slow when compared to certain other vasodilators such as substance P (see Hall & Brain, 1993; 1994). CGRP is well known to produce long-lasting microvascular vasodilatation, characterized by a pronounced and well maintained erythema in human skin (Brain *et al.*, 1985), maintained increases in blood flow in rabbit skin (Brain *et al.*, 1985), and prolonged decreases in perfusion pressure in isolated preparations such as the rat mesentery (Claing *et al.*, 1992) and rat kidney (Chin *et al.*, 1994). The similarity in time-course of vasodilator responses to CGRP and ADM_{13-52} suggests a similar mechanism of vasodilatation.

In the microvasculature of both the rat skin and the hamster cheek pouch, the $CGRP_1$ receptor antagonist, $CGRP_{8-37}$, significantly inhibited the vascular effects of both ADM_{13-52} and CGRP. These results with the CGRP receptor antagonist $CGRP_{8-37}$ are consistent with an interaction of ADM_{13-52} with $CGRP_1$ receptors. The doses of $CGRP_{8-37}$ we used to show inhibition of vasodilatation, 100 nmol kg^{-1} and 300 nmol kg^{-1} in the rat skin and hamster cheek pouch, respectively, are comparable with those used previously to exhibit a selective inhibition of CGRP-induced vasodilator responses (Escott & Brain, 1993). $CGRP_{8-37}$ did not inhibit submaximal vasodilator responses to substance P ($n = 5$; unpublished data) in the hamster cheek pouch, again indicating selective block of CGRP receptors. Inhibition of the vasodilator effects of full sequence ADM_{1-52} by $CGRP_{8-37}$ has recently been reported *in vitro* (Nuki *et al.*, 1993; Eguchi *et al.*, 1993); however, our study is the first demonstration of inhibition of ADM_{13-52}-evoked vasodilator responses in the microvasculature *in vivo* by $CGRP_{8-37}$. Further evidence also suggests that ADM, or an ADM-related peptide, can interact with the same receptor sites as CGRP. Thus, in vascular smooth muscle cells in culture, CGRP displaced specific binding sites labelled with ^{125}I-ADM_{1-52} (Eguchi *et al.*, 1994), and in several cell types it has been shown that both ADM and CGRP stimulate the generation of cyclic AMP (Kitamura *et al.*, 1993a; Eguchi *et al.*, 1994).

In terms of structure-activity relationships, it is interesting that peptides with such limited sequence-homology can interact, and stimulate, the same receptors. Indeed, this 'cross-talk' is not limited to CGRP, ADM and its fragments. For example, the 37 amino acid hormone amylin, which exhibits *ca.* 50% sequence homology with CGRP, has also been shown to evoke various biological effects including vasodilatation in the rat isolated perfused kidney (Chin *et al.*, 1994), and inhibition of nerve or spasmogen-evoked contractions of non-vascular smooth muscle (e.g. Giuliani *et al.*, 1992), via an interaction with $CGRP_1$ receptors. The main structural similarity between CGRP, ADM and amylin is the presence of six-residue ring structure formed by an intramolecular disulphide linkage, and a C-terminal amide structure (see Figure 1). It has been previously shown that the N-terminal sequence of CGRP is important for agonist activity of CGRP, in as much as opening or modification of the disulphide bond leads to a considerable loss in vasodilator activity (Tippins *et al.*, 1986; Maggi *et al.*, 1990; Zaidi *et al.*, 1990). This suggests that the disulphide bond may well also be of importance for agonist activity of amylin and adrenomedullin at CGRP receptors. Further, the ability of the N-terminally deleted CGRP fragment, $CGRP_{8-37}$, to act as a receptor antagonist suggests that the C-terminal sequences of amylin and adrenomedullin are important in determining affinity, rather than intrinsic efficacy at the CGRP receptor. This is interesting since Figure 1 shows that the structural homology in the C-terminus sequences of the various naturally-occurring homologues of CGRP is very limited.

In conclusion, we have demonstrated for the first time that ADM_{13-52} is a potent arteriolar vasodilator in the microvasculature, and that its effects in both rat skin and the hamster cheek pouch vasculature are mediated, at least in part, via an interaction with $CGRP_1$ receptors. Currently, it is not established whether the whole of the ADM_{13-52} sequence is required for full biological activity, nor whether ADM_{1-52}, ADM_{13-52}, or another fragment, is the biologically active species secreted *in vivo*. The role of ADM in physiology or pathophysiology remains to be determined, though it is tempting to speculate that, in view of the interaction of ADM and ADM_{13-52} with CGRP receptors, that ADM or an ADM fragment, may have a role as a circulating activator of CGRP receptors and modulator of vascular tone.

We thank the Wellcome Trust and the British Heart Foundation for support. We are grateful to Dr I.K.M. Morton for analysis and preparation of the figures, and Dr U. Ney, Celltech Berkshire, for a gift of CGRP.

References

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.

CHIN, S.Y., HALL, J.M., BRAIN, S.D. & MORTON, I.K.M. (1994). Vasodilator responses to calcitonin gene-related peptide (CGRP) and amylin in the rat isolated perfused kidney are mediated via $CGRP_1$ receptors. *J. Pharmacol. Exp. Ther.*, **269**, 989–992.

CLAING, A., TÉLÉMAQUE, CADIEUX, A., FOURNIER, A., REGOLI, D. & D'ORLÉANS-JUSTE, P. (1992). Nonadrenergic and noncholinergic arterial dilatation and venoconstriction are mediated by calcitonin gene-related $peptide_1$ and neurokinin-1 receptors, respectively, in the mesenteric vasculature of the rat after perivascular nerve stimulation. *J. Pharmacol. Exp. Ther.*, **263**, 1226–1232.

DENNIS, T., FOURNIER, A., CADIEUX, A., POMERLEAU, F., JOLICOEUR, F.B., ST.PIERRE, S. & QUIRION, R. (1990). $hCGRP_{8-37}$, a calcitonin gene-related peptide receptor heterogeneity in brain and periphery. *J. Pharmacol. Exp. Ther.*, **245**, 123–128.

DEWITT, B.J., CHENG, D.Y., CAMINITI, G.N., NOSSAMAN, B.D., COY, D.H., MURPHY, W.A. & KADOWITZ, P.J. (1994). Comparison of responses to adrenomedullin and calcitonin gene-related peptide in the pulmonary vascular bed of the cat. *Eur. J. Pharmacol.*, **257**, 303–306.

DULING, B.R. (1973). The preparation and use of the hamster cheek pouch for studies of the microcirculation. *Microvasc. Res.*, **5**, 423–429,

EGUCHI, S., HIRATA, Y., KANO, H., SATO, K., WATANABE, Y., WATANABE, T.X., NAKAJIMA, K., SAKAKIBARA, S. & MARUMO, F. (1994). Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. *FEBS Lett.*, **340**, 226–230.

ESCOTT, K.J. & BRAIN, S.D. (1993). Effect of a calcitonin gene-related peptide antagonist ($CGRP_{8-37}$) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve. *Br. J. Pharmacol.*, **110**, 772–776.

GIULIANI, S., WIMILAWANSKI, S.J. & MAGGI, C.A. (1992). Involvement of multiple receptors in the biological effects of calcitonin gene-related peptide and amylin in rat and guinea-pig preparations. *Br. J. Pharmacol.*, **107**, 510–514.

HALL, J.M. & BRAIN, S.D. (1993). Quantification of the vasodilator effects of CGRP, amylin and $[Cys(ACM)^{2,7}]$-hCGRP on the microvasculature of the hamster cheek pouch *in vivo*. *Neuropeptides*, **24**, 207.

HALL, J.M. & BRAIN, S.D. (1994). Inhibition of NK_1 tachykinin receptor mediated, nitric oxide dependent vasodilatation by SR140333 in the hamster cheek pouch microvasculature *in vivo*. *Br. J. Pharmacol.*, **113**, 522–526.

HAO, Q., CHANG, J-K., GHARAVI, H., FORTENBERRY, Y., HYMAN, A. & LIPPTON, H. (1994). An adrenomedullin (ADM) fragment retains the systemic vasodilator activity of human ADM. *Life Sci.*, **54**, PL265–PL270.

ICHIKI, Y., KITAMURA, K., KANGAWA, K., KAWAMOTO, M., MATSUO, H. & ETO, T. (1994). Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. *FEBS Lett.*, **338,** 6–10.

ISCHIYAMA, Y., KITAMURA, K., ICHIKI, Y., NAKAMURA, S., KIDA, O., KANGAWA, K. & ETO, T. (1993). Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats. *Eur. J. Pharmacol.*, **241,** 271–273.

KITAMURA, K., KANGAWA, K., KAWAMOTO, M., ICHIKI, Y., NAKAMURA, S., MATSUO, H. & ETO, T. (1993a). Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.*, **192,** 553–560.

KITAMURA, K., SAKATA, J., KANGAWA, K., KOJIMA, M., MATSUO, H. & ETO, T. (1993b). Cloning and characterization of cDNA encloding a precursor for human adrenomedullin. *Biochem. Biophys. Res. Commun.*, **194,** 720–725.

KITAMURA, K., ICHIKI, Y., TANAKA, M., KAWAMOTO, M., EMURA, J., SAKAKIBARA, S., KANGAWA, K., MATSUO, H. & ETO, T. (1994a). Immunoreactive adrenomedullin in human plasma. *FEBS Lett.*, **341,** 288–290.

KITAMURA, K., KANGAWA, K., KOJIMA, M., ICHIKI, Y., MATSUO, H. & ETO, T. (1994b). Complete amino acid sequence of porcine adrenomedullin and cloning of cDNA encoding its precursor. *FEBS Lett.*, **338,** 306–310.

LAWRENCE, E.J. & BRAIN, S.D. (1992). Responses to endothelins in the rat cutaneous microvasculture: A modulatory role of locally produced nitric oxide. *Br. J. Pharmacol.*, **106,** 733–738.

LIPPTON, H., CHANG, J-K., HAO, Q., SUMMER, W. & HYMAN, A.L. (1994). Adrenomedullin dilates the pulmonary vascular bed *in vivo*. *J. Appl. Physiol.*, **76,** 2154–2156.

MAGGI, C.A., ROVERO, P., GIULIANI, S., EVANGELISTA, S., REGOLI, D. & MELI, A. (1990). Biological activity of N-terminal fragments of calcitonin gene-related peptide. *Eur. J. Pharmarcol.*, **179,** 217–219.

NUKI, C., KAWASAKI, H., KITAMURA, K., TAKENAGA, M., KANGAWA, K., ETO, T. & WADA, A. (1993). Vasodilator effect of adrenomedullin and calcitonin gene-related peptide receptors in rat mesenteric vascular beds. *Biochem. Biophys. Res. Commun.*, **196,** 245–251.

PERRET, M., BROUSSARD, H., LEGROS, T., BURNS, A., CHANG, J-K, SUMMER, W., HYMAN, A. & LIPPTON, H. (1993). The effect of adrenomedullin on the isolated heart. *Phamacol. Lett.*, **53,** PL377-PL379.

SAKATA, J., SHIMOKUBO, T., KITAMURA, K., NAKAMURA, S., KANGAWA, K., MATSUO, H. & ETO, T. (1993). Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. *Biochem. Biophys. Res. Commun.*, **195,** 921–927.

SINEY, L., HALL, J.M. & BRAIN, S.D. (1994a). Comparison of the vasodilator peptides adrenomedullin and calcitonin gene-related peptide in the rat cutaneous microvasculature. *Regul. Pept.*, **51,** 292.

SINEY, L., HALL, J.M. & BRAIN, S.D. (1994b). Vasodilator effects of adrenomedullin in the rat cutaneous microvasculature. *Can. J. Physiol. Pharmacol.*, **72,** 51.

TIPPINS, J.R., DI MARZO, V.M., PANICO, M., MORRIS, H.R. & MACINTYRE, I. (1986). Investigation of the structure-activity relationship in human calcitonin gene-related peptide (CGRP). *Biochem. Biophys. Res. Commun.*, **134,** 1306–1311.

WILLIAMS, T.J. (1976). Simultaneous measurement of local plasma exudation and blood flow changes induced by intradermal injection of vasoactive substances using [^{131}I] albumin and [^{133}Xe]. *J. Physiol.*, **254,** 4–5P.

ZAIDI, M., BRAIN, S.D., TIPPINS, J.R., DI MARZO, MOONGA, B.S., CHAMBERS, T.J., MORRIS, H.R. & MACINTYRE, I. (1990). Structure-activity relationships of human gene-related peptide. *Biochem. J.*, **269,** 775–780.

(Received July 15, 1994
Revised September 20, 1994
Accepted October 6, 1994)