ELSEVIER

European Journal of Pharmacology 314 (1996) 115–121

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Comparison of the in vitro and in vivo pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats

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Received 25 March 1996; revised 28 June 1996; accepted 5 July 1996

Abstract

Adrenomedullin has been reported to be structurally similar to a group of peptides that includes amylin, calcitonin and calcitonin gene-related peptide (CGRP). Human and rat adrenomedullin displaced [^{125}I]CGRP from membranes of SK-N-MC cells (CGRP receptors) with affinities intermediate between those of rat amylin and rat CGRPα (K_i values 0.12 ± 0.06, 0.017 ± 0.007, 3.83 ± 1.14 and 0.007 ± 0.001 nM, respectively). In contrast, K_i values for displacement of [^{125}I]rat amylin from nucleus accumbens membranes (amylin receptors), and [^{125}I]salmon calcitonin from T47D cells (calcitonin receptors) were lower than with rat amylin or rat CGRPα in these preparations (51 ± 5, 34 ± 2, 0.024 ± 0.002, 0.31 ± 0.07 nM, respectively, at amylin receptors; 33 ± 5, 69 ± 29, 2.7 ± 1.5 and 13 ± 3 nM, respectively, at calcitonin receptors). In anesthetized rats, the hypotensive potency of adrenomedullin was between that of amylin and CGRPα. In contrast, for amylin or calcitonin agonist actions (inhibition of [^{14}C]glycogen formation in soleus muscle, hyperlactemia, hypocalcemia and inhibition of gastric emptying), human adrenomedullin was without measurable effect. Thus, in its binding behaviour and in its biological actions, adrenomedullin appeared to behave as a potent CGRP agonist, but as a poor amylin or calcitonin agonist.

Keywords: Adrenomedullin; Amylin; CGRP (calcitonin gene-related peptide); (Rat); Lactate; Calcium; Gastric emptying

1. Introduction

Adrenomedullin, originally isolated from phaeochromocytoma tissue, is a 50-amino acid [rat (Sakata et al., 1993)] or 52-amino acid [human (Kitamura et al., 1993)] amidated peptide. Adrenomedullin shows some structural similarity to the calcitonins, to the pancreatic β-cell peptide, amylin (Rink et al., 1993), and to calcitonin gene-related peptide (CGRP). In addition to sharing some structural similarity, the ligands just mentioned share certain biological actions and cross-react to a varying extent with each other's receptors. For example, amylin interacts with CGRP receptors (Muff et al., 1995) and high doses of amylin are hypotensive (Brain et al., 1990). Also, amylin and CGRP are able to activate calcitonin receptors in vitro and produce hypocalcemic effects in vivo (Zaidi et al., 1990). In addition, CGRP is nearly as potent as amylin at modulating muscle glycogen metabolism and at competing for high-affinity amylin-binding sites in rat nucleus accumbens (Beaumont et al., 1993).

Like amylin, CGRP and calcitonins, adrenomedullin increases cAMP production in several tissues (Eguchi et al., 1994; Ishizaka et al., 1994; Kohno et al., 1995; Shimekake et al., 1995; Kawada and Inoue, 1994), consistent with its acting via G_s-coupled receptors. Several investigators have compared adrenomedullin's functional effects, which include hypotensive (Kitamura et al., 1993) and vasodilatory (Ishiyama et al., 1993) actions, to those of CGRP. However, the ability of adrenomedullin to produce the metabolic and hypocalcemic actions evoked by amylin and calcitonin, and to interact with the receptors for these peptides, has not been reported.

Our purpose in this work was to examine further the biologic actions of adrenomedullin and to compare these with the actions of CGRP and amylin. Therefore, we evaluated the ability of adrenomedullin to inhibit conversion of glucose to glycogen in insulin-stimulated isolated soleus muscle, and adrenomedullin's in vivo actions on plasma glucose, lactate and calcium concentrations, plasma renin activity, blood pressure and gastric emptying in the

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PII S0014-2999(96)00544-4

rat. We also assessed affinity of adrenomedullin for preparations (rat nucleus accumbens membranes, SK-N-MC cells and T47D cells) where binding appears to correlate with actions on glucose/lactate, blood pressure and calcium, respectively.

2. Materials and methods

2.1. Materials

Rat amylin (Cat. No. PCPE70) and rat CGRPα (Cat. No. PCAL60B) were obtained from Bachem (Torrance, CA, USA). Human and rat adrenomedullin was obtained from Peptide Institute (Osaka, Japan). Both species of adrenomedullin were used in binding studies. Human adrenomedullin was used in the in vivo experiments.

2.2. Binding to rat nucleus accumbens membranes (amylin receptors)

As previously described (Beaumont et al., 1993), membranes from the nucleus accumbens region in rat brain were obtained by homogenizing the brain region in cold Hepes, with 3 resuspension/centrifugation steps before storage at 70°C. Membranes suspended in 20 mM Hepes buffer were incubated with 15 pM [^{125}I]rat amylin (Bolton-Hunter labeled at the N-terminal lysine, specific activity 1950–2000 Ci/mmol) and with unlabeled peptides. Solutions were incubated for 60 min at 23°C and then filtered through polyethyleneimine-treated glass fiber filters. Filters were washed with cold phosphate-buffered saline and radioactivity assessed in a gamma-counter. Total binding varied from 2400–3000 cpm/0.2 ml, with non-specific binding, measured in the presence of 100 nM human amylin, accounting for 40–45% of total binding. Competition curves were generated by measuring binding of the radioligand in the presence of 10^{-11} to 10^{-6} M unlabeled peptide, and were fit to a 4-parameter logistic equation by a non-linear regression program to obtain IC_{50} values (Prism; GraphPad Software, San Diego, CA, USA). K_i was derived as $IC_{50}/(1 + ([L]/K_d))$ where $[L]$ was 15 pM and K_d was 27 pM.

2.3. Binding to membranes from SK-N-MC cells (CGRP receptors)

SK-N-MC cells were homogenized in 50 mM Hepes buffer, pH 7.4, and membranes were collected by centrifugation for 15 min at 48 000 × g. Membranes suspended at a concentration of 0.1–0.2 mg protein/0.2 ml aliquot were incubated in 50 mM Hepes, pH 7.4 containing bovine serum albumin, bacitracin, and 2 mM $MgCl_2$ with 15 pM [^{125}I]human-CGRP (labeled at ^{10}His, 2000 Ci/mmol) and unlabeled peptides. Additional methods were similar to those described for amylin receptor assays. K_i was derived as $IC_{50}/(1 + ([L]/K_d))$ where $[L]$ was 15 pM and K_d was 3 pM.

2.4. Binding to membranes from T47D cells (calcitonin receptors)

Membranes from human T47D breast carcinoma cells, previously shown to contain high densities of calcitonin receptors (Findlay et al., 1980). Membranes were prepared from confluent cultures of T47D cells as described for SK-N-MC cells. Membranes were incubated with 32 pM [^{125}I]salmon calcitonin (labeled at ^{22}Tyr, 2000 Ci/mmol), and with unlabeled peptides for 60 min at ambient temperature. Additional methods are similar to those described for CGRP receptor assays. K_i was derived as $IC_{50}/(1 + ([L]/K_d))$ where $[L]$ was 32 pM and K_d was 19 pM.

2.5. Glycogen metabolism in the isolated soleus muscle

This procedure has been described in detail (Young et al., 1992). Briefly, m. soleus was dissected from ≈ 200 g male Sprague-Dawley rats fasted for 4 h. Each muscle was split in half, and muscle strips then incubated at 37°C in 10ml of a Krebs-Ringer bicarbonate buffer containing (per l): NaCl 118.5 mmol (6.93 g), KCl 5.94 mmol (443 mg), $CaCl_2$ 2.54 mmol (283 mg), $MgSO_4$ 1.19 mmol (143 mg), KH_2PO_4 1.19 mmol (162 mg), $NaHCO_3$ 25 mmol (2.1 g), 5.5 mmol glucose (1.00 g) and recombinant human insulin (7.1 nmol), and peptides as specified. When gassed with 95% O_2: 5% CO_2, pH was between 7.1 and 7.4. Following a 30-min pre-incubation period, 0.5 μCi of [U-^{14}C]glucose was added to each flask, and the incubation was continued for a further 60 min. After incubation, muscle pieces were weighed and frozen for storage. [^{14}C]glycogen content in each muscle piece was measured by digesting the muscle in 60% KOH at 70°C for 45 min and precipitating the glycogen by adding 3 volumes of ethanol and cooling at −20°C overnight. After aspiration and washing with ethanol, glycogen pellets were dried under vacuum before being dissolved in 1 ml H_2O and 4 ml of scintillation fluid, and counted for [^{14}C]. Glucose incorporation into glycogen (expressed in μmol/g per h) was obtained from the specific activity of [^{14}C]glucose in the incubation medium and the total [^{14}C]glycogen extracted from each muscle.

2.6. Studies in anesthetized rats

The preparation used for these in vivo studies has been previously described (Young et al., 1991). Male Harlan Sprague-Dawley rats were housed at 23 ± 1°C in a 12:12 h light:dark cycle (experiments being performed during the light cycle) and fed and watered ad libitum (Diet LM-485; Teklad, Madison, WI, USA). Animals weighing 300–350 g were fasted for ≈ 20 h prior to experimentation. Anesthesia was induced with 5% halothane, maintained at 2%

during surgery and at 0.7 to 1% thereafter. Tracheotomy, and cannulation of the right femoral artery and saphenous vein were performed. The arterial line, perfused with heparinized saline (2 U/ml) at 3 ml/h, was used for blood sampling and pressure measurement (Spectramed P23XL transducer, model 13-4615-58 amplifier; Gould, Cleveland, OH, USA). The venous line was used for drug injection as needed. Colonic temperature was measured and controlled using a thermistor probe/controller (model 73A; YSI, Yellow Springs, OH, USA) and a heated operating table. Signals for mean arterial pressure were periodically sampled at 1 Hz with 12 bit precision (DataTranslation DT2801A) and recorded (Labtech Notebook; AST Premium 386). Peptides were dissolved in 0.1 ml 0.15 M NaCl and given subcutaneously (s.c.) or intravenously (i.v.) at zero time, ≈ 2 h after surgery. Plasma glucose and lactate were measured immediately after sampling using immobilized enzyme chemistries (glucose oxidase, L-lactate oxidase; Analyzer model 2300-STAT, YSI, Yellow Springs, OH, USA). Total plasma calcium was measured using a dye-binding assay (*o*-cresolphthalein complexone, Sigma procedure 587; Sigma, St. Louis, MO, USA).

2.7. Measurement of gastric emptying

Gastric emptying was determined as previously reported (Young et al., 1995b). Male Sprague-Dawley (160–250 g) rats, fasted for ≈ 20 h, received 1.5 ml of 1.5% methyl cellulose containing 0.05% phenol red by gavage. Peptides, dissolved in 0.1 ml 0.15 M NaCl, were given s.c. 5 min before the gavage. After 20 min, stomachs of halothane-anesthetized rats were excised following clamping at the oesophagus and pylorus. Stomach contents were diluted to 100 ml with 0.1 M NaOH and the phenol red content determined by absorbance at 560 nm. To account for the small amount of dye (≈ 10%) that could not be recovered from the gastrointestinal tract by this method, gastric contents found 20 min after gavage were expressed as a fraction of those found immediately after gavage ($t = 0$ min) in parallel experiments: gastric contents (%) = (absorbance at $t = 20$ min)/(absorbance at $t = 0$ min).

2.8. Numerical methods

Dose-response curves were fitted to a 4-parameter logistic model using least-squares (Prism; Graphpad Software). Pairwise comparisons were performed using *t*-test functions in Instat (version 2.0; Graphpad Software).

3. Results

3.1. Receptor-binding studies

3.1.1. CGRP receptors

SK-N-MC human neuroblastoma cells have been shown to contain a high-affinity CGRP receptor that is coupled to adenylate cyclase and which has binding and specificity characteristics similar to CGRP receptors present in several other tissues (VanValen et al., 1990). K_i values derived from inhibition of [^{125}I]hCGRP binding to membranes from SK-N-MC cells are given in Table 1. K_i values for human and rat adrenomedullin of 0.12 and 0.017 nM were intermediate between the K_i values of rat CGRP and rat amylin, consistent with data below that suggest adrenomedullin is a $CGRP_1$ agonist.

Table 1
Binding of human and rat adrenomedullin, rat amylin and rat CGRPα at nucleus accumbens, SK-N-MC and T47D receptors

Peptide	Receptor		
	Nucleus accumbens (amylin)	SK-N-MC (CGRP)	T47D (calcitonin)
Human adrenomedullin	51 ± 5	0.12 ± 0.06	33 ± 5
Rat adrenomedullin	34 ± 2	0.017 ± 0.007	68 ± 20
Rat amylin	0.024 ± 0.002	3.8 ± 1.1	2.7 ± 1.5
Rat CGRP	0.31 ± 0.07	0.007 ± 0.001	13 ± 3

K_i values given (in nM) are means of 2–7 assays ± S.E.M.

3.1.2. Amylin receptors

Binding to rat nucleus accumbens was quantified by displacement of [^{125}I]rat amylin (Beaumont et al., 1993). Derived K_i values for rat and human adrenomedullin, CGRP and amylin binding to nucleus accumbens membranes are shown in Table 1. The adrenomedullins had the poorest binding of this group with affinities 1400–2100-

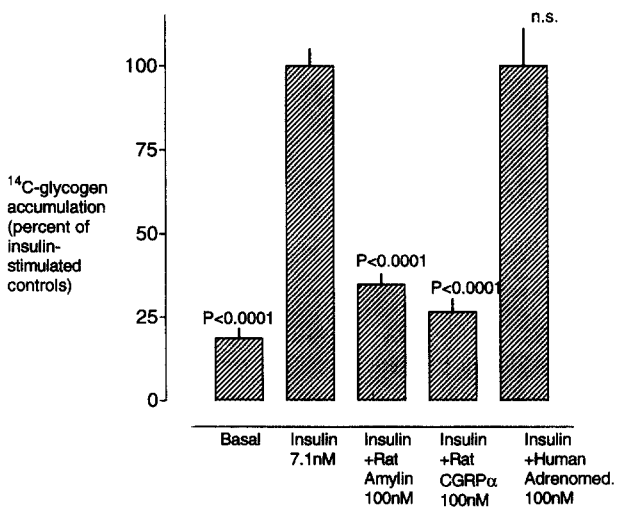

Fig. 1. Effects of amylin, CGRP and adrenomedullin on insulin-stimulated [^{14}C]glycogen accumulation in isolated rat soleus muscle. Insulin (7.1 nM, present in all experiments) increased the amount of label from [U-^{14}C]glucose incorporated over 1 h into muscle glycogen over 5-fold. Rat amylin and rat CGRPα (100 nM) markedly reduced rates of incorporation while human adrenomedullin was without measurable effect. Bars are means of 12–14 muscle strips. Error bars are S.E.M. and significance values are vs. insulin-stimulated controls (second bar).

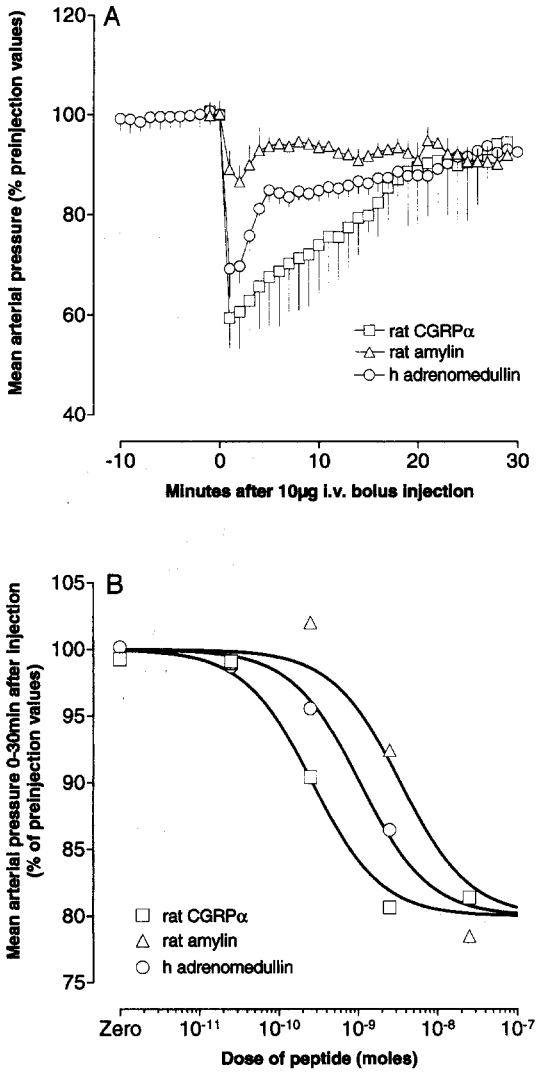

Fig. 2. (A) Acute effect of 10 μg i.v. bolus injections of rat amylin, rat CGRPα and human adrenomedullin on mean arterial pressure in anesthetized rats. (B) Dose-response for the hypotensive effects of i.v. doses of these peptides in anesthetized rats. ED_{50} values were: adrenomedullin, 1064 pmol ±0.05 log; amylin 3453 pmol ±0.17 log; CGRP 267 pmol ±0.08 log. Blood pressure responses represent means of values measured continuously from 0 to 30 min, expressed as a percentage of pre-treatment values ($n = 3$–10).

fold lower than that of amylin. The low affinity of adrenomedullin to nucleus accumbens receptors is consistent with data presented below, showing adrenomedullin to have little or none of the functional activity that has been associated with agonism at receptors of this type.

3.1.3. Calcitonin receptors

Binding to calcitonin receptors was quantified by displacement of [^{125}I]salmon calcitonin from membranes in human T47D carcinoma cells. The adrenomedullins showed ≈ 20-fold lower affinity for these receptors than did amylin, consistent with the low hypocalcemic activity of adrenomedullins, reported below.

3.2. Radioglucose incorporation into glycogen in isolated rat soleus muscle

As shown in Fig. 1, insulin stimulated incorporation of [U-^{14}C]glucose into glycogen in the isolated stripped soleus muscle and 100 nM rat amylin significantly reduced [^{14}C]glycogen formation ($P < 0.0001$). Human adrenomedullin, at a concentration of 100 nM, had no significant effect on insulin-stimulated [^{14}C]glycogen formation.

3.3. Actions in anesthetized rats

Adrenomedullin decreased blood pressure rapidly and dose-dependently when given either s.c. or i.v. Fig. 2A shows the time-course of the response to i.v. injections of 10 μg human adrenomedullin compared to the effects of the same dose of rat amylin and rat CGRP. Dose-responses for the hypotensive effects of the same peptides are shown in Fig. 2B. In its hypotensive actions, the potency of adrenomedullin (ED_{50} 1064 pmol/rat ±0.05 log) was greater than that of amylin (ED_{50} 3453 pmol ±0.17 log), and, consistent with previous reports (Feng et al., 1994; Baskaya et al., 1995), was less than that of CGRP (ED_{50} 267 pmol ±0.08 log).

Plasma renin activity increased after s.c. injection of 10 μg (but not lower doses) of adrenomedullin (data not shown). The 10 μg dose of adrenomedullin was also associated with a fall in blood pressure. CGRP dose-dependently stimulated plasma renin activity in association with decreases in blood pressure. Amylin increased plasma renin activity at a dose that did not decrease blood pressure.

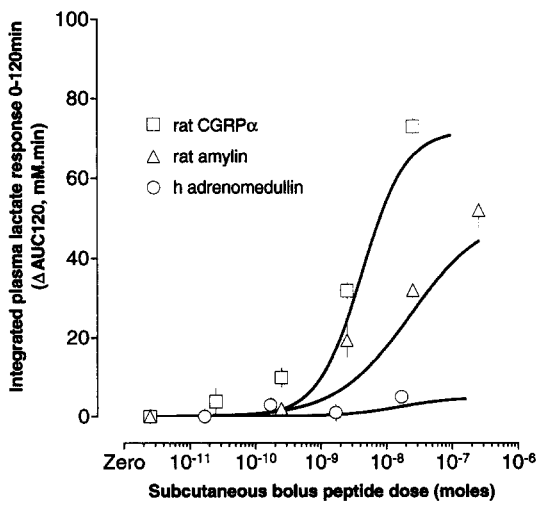

Fig. 3. Dose-response curves for the effects of rat amylin, rat CGRPα and human adrenomedullin on plasma lactate. Lactate response is quantified as the increment over pre-treatment values, integrated for 120 min after s.c. peptide administration. Data are presented as mean ± S.E.M. ($n = 3$–9).

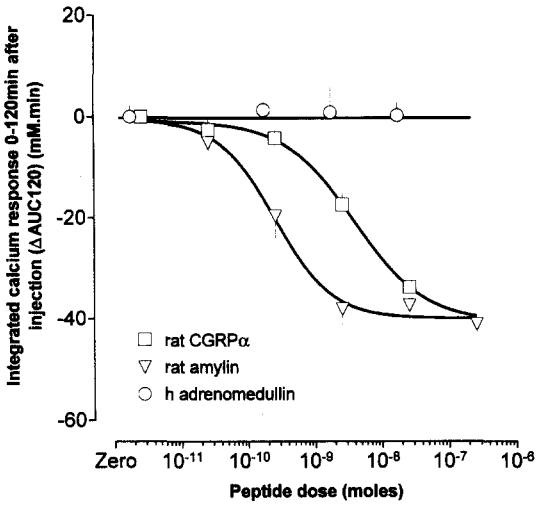

Fig. 4. Dose-response curves for the effects of rat amylin, rat CGRPα and human adrenomedullin on total plasma calcium. The calcium response is quantified as the increment over pre-treatment values, integrated for 120 min after s.c. peptide administration. Data are presented as mean ± S.E.M. ($n = 3$–9).

Amylin and CGRP dose-dependently increased plasma lactate, significant integrated responses being observed with s.c. doses of each between 1 and 10 μg. In contrast, adrenomedullin did not increase plasma lactate at doses up to 100 μg, the highest used, as shown in Fig. 3. The pattern of glucose responses (not shown) was similar to those for lactate; at doses of adrenomedullin below 100 μg, there was no change relative to saline-injected controls in integrated glycemic response; with 100 μg s.c. adrenomedullin, the integrated glycemic response was 24 and 11%, respectively, of that observed with the same doses of amylin and CGRP.

Amylin and CGRP dose-dependently reduced 2 h-integrated plasma calcium, rat amylin being somewhat more potent than rat CGRP (ED_{50} 0.25 nmol ±0.03 log units vs. 3.2 nmol ±0.02 log units, respectively). There was no change in plasma calcium with any dose of adrenomedullin (Fig. 4).

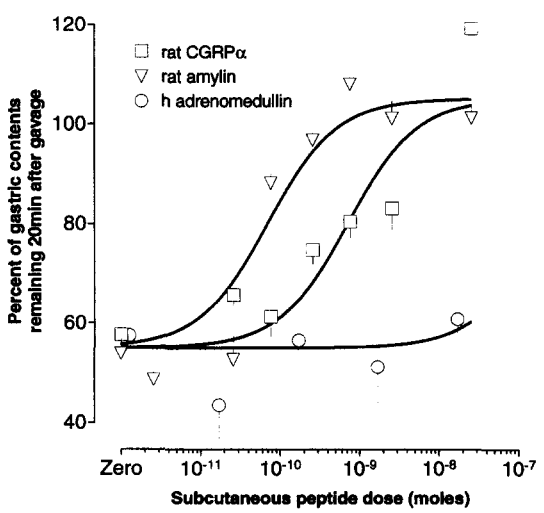

Fig. 5. Dose-response curves for the effects of rat amylin, rat CGRPα and human adrenomedullin on gastric emptying. Gastric emptying is the amount of a Phenol-red-labeled methyl-cellulose gel remaining in the stomach 20 min after gavage, expressed as a percentage of the amount recoverable immediately after gavage. Data are presented as mean ± S.E.M. $n = 3$–9 except for basal (zero peptide) where $n = 17$.

3.4. Gastric emptying in conscious rats

Amylin and CGRP fully inhibited gastric emptying with ED_{50} values of 69 pmol ±0.19 log and 705 pmol ±0.22 log, respectively. In contrast, s.c. adrenomedullin did not significantly affect gastric emptying at doses up to 100 μg (16 600 pmol) (Fig. 5).

Thus, in vivo, adrenomedullin is at least 100-fold less potent than CGRP or amylin for effects on plasma glucose, lactate, calcium and gastric emptying. In contrast, it is slightly less potent than CGRP, and is more potent than amylin, in evoking hypotension.

4. Discussion

Adrenomedullin bound with higher affinity to CGRP receptors than it did to amylin or calcitonin receptors. Adrenomedullin evoked hypotensive actions, but had no effect, or only weak effects, on plasma glucose, plasma lactate, plasma calcium, soleus muscle glycogen or gastric emptying. The pattern of receptor binding and spectrum of actions seen in these experiments is consistent with adrenomedullin being a CGRP receptor agonist, but not an amylin or calcitonin agonist. This interpretation is exemplified in Table 2 where relative affinities of the natural ligands CGRP, adrenomedullin and amylin for CGRP, calcitonin and amylin receptors are shown in juxtaposition with relative potency in evoking responses presumptively linked to those receptors.

SK-N-MC cell membranes (CGRP receptors) bound CGRP with highest affinity, followed by adrenomedullin, and then amylin. This pattern of affinities mirrors the systemic hypotensive potencies of these ligands, supporting the interpretation that such responses are mediated, at least partly, via CGRP receptors. The relative hypotensive

Table 2
Correlation of potencies of biologic actions of CGRP, amylin and adrenomedullin to affinity to cognate receptors

Binding/action	Rank order of potency
SK-N-MC binding	CGRP > adrenomedullin > amylin
Hypotension	CGRP > adrenomedullin > amylin
T47D binding	Amylin > CGRP > adrenomedullin
Hypocalcemia	Amylin > CGRP ≫ adrenomedullin
Nucleus accumbens binding	Amylin ≥ CGRP ≫ adrenomedullin
Soleus muscle glycogen	Amylin ≥ CGRP ≫ adrenomedullin
Plasma glucose	CGRP ≥ amylin ≫ adrenomedullin
Plasma lactate	CGRP ≥ amylin ≫ adrenomedullin
Gastric emptying	Amylin > CGRP ≫ adrenomedullin
Plasma renin activity	Amylin > CGRP ≫ adrenomedullin

potencies of CGRP, adrenomedullin and amylin seen here are consistent with previous reports where adrenomedullin was ≈ 1 order of magnitude less potent than CGRP (Feng et al., 1994; Hall et al., 1995), and amylin ≈ 2 orders less potent (Brain et al., 1990) in evoking vasodilation. A similar relationship was also seen in the cerebral vasodilator activities of adrenomedullin, CGRP and amylin in the dog (Baskaya et al., 1995). In that tissue bed, the CGRP receptor antagonist CGRP-(8-37) (Chiba et al., 1989) blocked the vasodilator actions of both CGRP and adrenomedullin (Baskaya et al., 1995).

However, in certain other vascular beds (Gardiner et al., 1995; Heaton et al., 1995), adrenomedullin-induced vasodilation was not blocked by CGRP-(8-37). Also, adrenomedullin was more potent than CGRP in stimulating adenylyl cyclase activity in cultured human endothelial cells, and this activity was also not inhibited by CGRP-(8-37) (Kato et al., 1995). The implication from these data that there exists a population of adrenomedullin receptors distinct from CGRP receptors was affirmed by our discovery of a cell line (bovine pulmonary artery endothelium) expressing adrenomedullin but not CGRP receptors (Beaumont et al., in submission). An adrenomedullin receptor showing similar binding behaviour to those in the cell line has recently been cloned (Kapas et al., 1995).

In contrast to its relatively high affinity for CGRP receptors, adrenomedullin had a very low potency in competing for [^{125}I]salmon calcitonin binding to calcitonin receptors in T47D carcinoma cell membranes. Calcitonin receptors had highest affinity for amylin, followed by CGRP, then adrenomedullin. Consistent with that pattern, amylin was most hypocalcemic, followed by CGRP, while adrenomedullin evoked no detectable hypocalcemic activity.

As seen with calcitonin receptors, adrenomedullin also had a relatively low potency in competing for [^{125}I]Bolton-Hunter-amylin binding to receptors in rat nucleus accumbens membranes (Table 1). By contrast, CGRP was relatively potent at binding to these sites (Beaumont et al., 1993). Likewise, CGRP was nearly as potent as amylin at inhibiting radiolabeled glucose incorporation into soleus muscle glycogen (Beaumont et al., 1995b) and at stimulating increases in plasma lactate and glucose in rats (Young et al., 1993). Amylin receptor antagonists had a similar ability to inhibit nucleus accumbens binding, soleus muscle functional responses to amylin, and amylin-induced increases in plasma lactate and glucose (Beaumont et al., 1995a,b). Consistent with the interpretation that adrenomedullin is a CGRP agonist, but not an amylin agonist, adrenomedullin was either weak or totally inactive in its effects on muscle glycogen or plasma glucose and lactate.

The findings that adrenomedullin is a moderately active CGRP agonist but ineffective as an amylin or calcitonin agonist, and that adrenomedullin is without action on gastric emptying, suggest that CGRP receptors do not mediate this gastric action. In support of this idea, amylin-mediated inhibition of gastric emptying can be blocked by antagonists selective for amylin and calcitonin receptors over CGRP receptors (Gedulin et al., 1995), while salmon calcitonin, a very poor CGRP agonist, but a potent amylin and calcitonin agonist (Young et al., 1995a), inhibits gastric emptying (Jonderko et al., 1988).

In its binding and in its actions, adrenomedullin behaved in these experiments as a moderately active CGRP receptor agonist but not as an amylin or calcitonin agonist. In addition, adrenomedullin appears to bind to and activate distinct adrenomedullin receptors. Through this spectrum of actions, adrenomedullin may be a useful pharmacological and physiological tool for distinguishing responses mediated by adrenomedullin or CGRP receptors from those mediated by amylin or calcitonin receptors.

Acknowledgements

We gratefully acknowledge the technical assistance of Pamela Smith, Leslie Crocker, Deborah Green and Carolyn Jodka, and the secretarial assistance of Nancy Gallaher.

References

Baskaya, M.K., Y. Suzuki, M. Anzai, Y. Seki, K. Saito, M. Takayasu, M. Shibuya and K. Sugita, 1995, Effects of adrenomedullin, calcitonin gene-related peptide, and amylin on cerebral circulation in dogs, J. Cereb. Blood Flow Metab. 15, 827.

Beaumont, K., M.A. Kenney, A.A. Young and T.J. Rink, 1993, High affinity amylin binding sites in rat brain, Mol. Pharmacol. 44, 493.

Beaumont, K., C.X. Moore, K.S. Prickett and M.A. Kenney, Adrenomedullin selectively stimulates cyclic AMP accumulation in a pulmonary artery endothelial cell line (in submission).

Beaumont, K., C.X. Moore, R.A. Pittner, K.S. Prickett, L.S.L. Gaeta, T.J. Rink and A.A. Young, 1995a, Differential antagonism of amylin's metabolic and vascular actions with amylin receptor antagonists, Can. J. Physiol. Pharmacol. 73, 1025.

Beaumont, K., R.A. Pittner, C.X. Moore, D. Wolfe-Lopez, K.S. Prickett, A.A. Young and T.J. Rink, 1995b, Regulation of muscle glycogen metabolism by CGRP and amylin: CGRP receptors not involved, Br. J. Pharmacol. 115, 713.

Brain, S.D., S. Wimalawansa, I. MacIntyre and T.J. Williams, 1990, The demonstration of vasodilator activity of pancreatic amylin amide in the rabbit, Am. J. Pathol. 136, 487.

Chiba, T., A. Yamaguchi, T. Yamatani, A. Nakamura, T. Morishita, T. Inui, M. Fukase, T. Noda and T. Fujita, 1989, Calcitonin gene-related peptide receptor antagonist human CGRP-(8-37), Am. J. Physiol. 256, E331.

Eguchi, S., Y. Hirata, H. Kano, K. Sato, Y. Watanabe, T.X. Watanabe, K. Nakajima, S. Sakakibara and F. Marumo, 1994, Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells, FEBS Lett. 340, 226.

Feng, C.J., B. Kang, A.D. Kaye, P.J. Kadowitz and B.D. Nossaman, 1994, L-NAME modulates responses to adrenomedullin in the hindquarters vascular bed of the rat, Life Sci. 55, PL433.

Findlay, D.M., V.P. Michelangeli, J.A. Eisman, R.J. Frampton, J.M. Moseley, I. MacIntyre, R. Whitehead and T.J. Martin, 1980, Calci-

tonin and 1,25-dihydroxyvitamin D3 receptors in human breast cancer cell lines, Cancer Res. 40, 4764.

Gardiner, S.M., P.A. Kemp, J.E. March and T. Bennett, 1995, Regional haemodynamic effects of human and rat adrenomedullin in conscious rats, Br. J. Pharmacol. 114, 584.

Gedulin, B., D. Green, L. Jodka and A. Young, 1995, Endogenous amylin and gastric emptying in rats: comparison with GLP-1 and CCK-8, Diabetologia 38, A244.

Hall, J.M., L. Siney, H. Lippton, A. Hyman, K.C. Jaw and S.D. Brain, 1995, Interaction of human adrenomedullin (13-52) with calcitonin gene-related peptide receptors in the microvasculature of the rat and hamster, Br. J. Pharmacol. 114, 592.

Heaton, J., B. Lin, J.K. Chang, S. Steinberg, A. Hyman and H. Lippton, 1995, Pulmonary vasodilation to adrenomedullin: a novel peptide in humans, Am. J. Physiol. 37, H2211.

Ishiyama, Y., K. Kitamura, Y. Ichiki, S. Nakamura, O. Kida, K. Kangawa and T. Eto, 1993, Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats, Eur. J. Pharmacol. 241, 271.

Ishizaka, Y., Y. Ishizaka, M. Tanaka, K. Kitamura, K. Kangawa, N. Minamino, H. Matsuo and T. Eto, 1994, Adrenomedullin stimulates cyclic AMP formation in rat vascular smooth muscle cells, Biochem. Biophys. Res. Commun. 200, 642.

Jonderko, G., T. Golab and K. Jonderko, 1988, Effect of calcitonin on gastric emptying, Digestion 40, 191.

Kapas, S., K.J. Catt and A.J.L. Clark, 1995, Cloning and expression of cDNA encoding a rat adrenomedullin receptor, J. Biol. Chem. 270, 25344.

Kato, J., K. Kitamura, K. Kangawa and T. Eto, 1995, Receptors for adrenomedullin in human vascular endothelial cells, Eur. J. Pharmacol. 289, 383.

Kawada, N. and M. Inoue, 1994, Effect of adrenomedullin on hepatic pericytes (stellate cells) of the rat, FEBS Lett. 356, 109.

Kitamura, K., K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakamura, H. Matsuo and T. Eto, 1993, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma, Biochem. Biophys. Res. Commun. 192, 553.

Kohno, M., K. Yokokawa, K. Yasunari, H. Kano, T. Horio and T. Takeda, 1995, Stimulation of cyclic adenosine monophosphate formation by the novel vasorelaxant peptide adrenomedullin in cultured rat mesangial cells, Metabolism 44, 10.

Muff, R., W. Born and J.A. Fischer, 1995, Receptors for calcitonin, calcitonin gene related peptide, amylin, and adrenomedullin, Can. J. Physiol. Pharmacol. 73, 963.

Rink, T.J., K. Beaumont, J. Koda and A. Young, 1993, Structure and biology of amylin, Trends Pharmacol. Sci. 14, 113.

Sakata, J., T. Shimokubo, K. Kitamura, S. Nakamura, K. Kangawa, H. Matsuo and T. Eto, 1993, Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide, Biochem. Biophys. Res. Commun. 195, 921.

Shimekake, Y., K. Nagata, S. Ohta, Y. Kambayashi, H. Teraoka, K. Kitamura, T. Eto, K. Kangawa and H. Matsuo, 1995, Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and $Ca2^{+}$ mobilization, in bovine aortic endothelial cells, J. Biol. Chem. 270, 4412.

VanValen, F., G. Piechot and H. Jürgens, 1990, Calcitonin gene-related peptide (CGRP) receptors are linked to cyclic adenosine monophosphate production in SK-N-MC human neuroblastoma cells, Neurosci. Lett. 119, 195.

Young, A.A., B. Gedulin, D. Wolfe-Lopez, H.E. Greene, T.J. Rink and G.J.S. Cooper, 1992, Amylin and insulin in rat soleus muscle: dose responses for cosecreted noncompetitive antagonists, Am. J. Physiol. 263, E274.

Young, A.A., B.R. Gedulin, T.J. Rink, R. Pittner and K. Beaumont, 1995a, Diabetogenic effect of salmon calcitonin attributed to amylin-like activity, Metabolism 44, 1581.

Young, A.A., B. Gedulin, W. Vine, A. Percy and T.J. Rink, 1995b, Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin, Diabetologia 38, 642.

Young, A.A., T.J. Rink and M.W. Wang, 1993, Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRP-alpha) in the fasted, anaesthetized rat, Life Sci. 52, 1717.

Young, A.A., M.W. Wang and G.J.S. Cooper, 1991, Amylin injection causes elevated plasma lactate and glucose in the rat, FEBS Lett. 291, 101.

Zaidi, M., H.K. Datta, P.J.R. Bevis, S.J. Wimalawansa and I. Macintyre, 1990, Amylin-amide: a new bone-conserving peptide from the pancreas, Exp. Physiol. 75, 529.