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Multiple receptors for calcitonin gene-related peptide and amylin on guinea-pig ileum and vas deferens

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1 The responses of the electrically stimulated guinea-pig ileum and vas deferens to human and rat calcitonin gene-related peptide (CGRP) and amylin were investigated.

2 The inhibition of contraction of the ileum produced by human αCGRP was antagonized by human $\alpha CGRP_{8-37}$ (apparent pA_2 estimated at 7.15 ± 0.23) > human $\alpha CGRP_{19-37}$ (apparent pA_2 estimated as 6.67 ± 0.33) > [Tyr^0]-human $\alpha CGRP_{28-37}$. The amylin antagonist, AC187, was three fold less potent than $CGRP_{8-37}$ in antagonizing human αCGRP.

3 Both human β- and rat αCGRP inhibited contractions of the ileum, but this was less sensitive to inhibition by $CGRP_{8-37}$ than the effect of human αCGRP. However, $CGRP_{19-37}$ was twenty times more effective in inhibiting the response to rat αCGRP (apparent pA_2 estimated as 8.0 ± 0.1) compared to human αCGRP.

4 Rat amylin inhibited contractions in about 10% of ileal preparations; this effect was not antagonized by any CGRP fragment. Human amylin had no action on this preparation.

5 Both human and rat αCGRP inhibited electrically stimulated contractions of the vas deferens, which were not antagonized by 3 μM $CGRP_{8-37}$ or 10 μM AC187.

6 Rat amylin inhibited the stimulated contractions of the vas deferens ($EC_{50} = 77 \pm 9$ nM); human amylin was less potent ($EC_{50} = 213 \pm 22$ nM). The response to rat amylin was antagonized by 10 μM $CGRP_{8-37}$ ($EC_{50} = 242 \pm 25$ nM) and 10 μM AC187 ($EC_{50} = 610 \pm 22$ nM).

7 It is concluded that human αCGRP relaxes the guinea-pig ileum via $CGRP_1$-like receptors, but that human βCGRP and rat αCGRP may use additional receptors. These are distinct $CGRP_2$-like and amylin receptors on guinea-pig vas deferens.

Keywords: CGRP receptors; amylin receptors; CGRP antagonists; amylin antagonists; guinea-pig ileum; guinea-pig vas deferens

Introduction

Calcitonin gene-related peptide (CGRP) is a widely distributed, 37 amino acid neuropeptide with multiple actions (Poyner, 1992). CGRP receptors have been divided into two classes, $CGRP_1$ and $CGRP_2$ by Quirion and co-workers (Dennis *et al.*, 1989; 1990; Mimeault *et al.*, 1991). This classification is largely based on the effects of a series of CGRP fragments, most notably human $\alpha CGRP_{8-37}$, which possess antagonistic activity, and which are significantly more potent at $CGRP_1$ than $CGRP_2$ receptors. It is also supported by results with the linear CGRP analogue, (Cys[ACM][2,7])-CGRP, which is an agonist selective for $CGRP_2$ receptors (Dennis *et al.*, 1989). This classification has met with some success in rationalizing apparent CGRP receptor heterogeneity; thus the guinea-pig atrium and ileum appear to have $CGRP_1$ receptors, whereas the rat vas deferens has $CGRP_2$ receptors (Dennis *et al.*, 1990; Mimeault *et al.*, 1991; Maggi *et al.*, 1991). However, the magnitude of pA_2 values for $CGRP_{8-37}$ within the same preparation shows considerable heterogeneity; for the $CGRP_1$ receptor mediating the positive inotropic effect on the left atrium these vary from 7.7 to 6.9 (Mimeault *et al.*, 1991; Maggi *et al.*, 1991). This latter value approaches the range of 6.8 to 6.5 reported for $CGRP_2$ receptors on the rat vas deferens. It has further been reported that after treatment with the peptide inhibitor thiorphan, the rat vas deferens increases in sensitivity to $CGRP_{8-37}$, a result which led the authors to question the concept of a simple division of CGRP receptors (Longmore *et al.*, 1994).

The issue of CGRP receptor classification is made more complex by the discovery of peptides with significant structural homology to CGRP, such as amylin. This is a 37 amino acid peptide with an N-terminal disulphide and C-terminal amide, and 47% sequence identity to CGRP (see Rink *et al.*, 1993 for review). Although amylin can cross react with CGRP receptors (Chantry *et al.*, 1991), there is a good evidence that it has its own specific receptors, albeit possessing significant affinity for CGRP (Giuliani *et al.*, 1991). $Amylin_{8-37}$ and acetyl-[Asn^{30},-Tyr^{32}] salmon $calcitonin_{8-32}$ (usually known as AC187) are reported to be antagonists at amylin receptors (Deems *et al.*, 1991; Young *et al.*, 1994). Giuliani and co-workers (1992) have suggested that the rat vas deferens contains receptors for both amylin and CGRP, whereas the guinea-pig atrium contains only CGRP receptors and the guinea-pig urinary bladder contains the amylin-preferring subtype.

It is clear that much remains to be done to establish the classification of CGRP and amylin receptors. The extent of species differences is largely undocumented, despite the fact that the prototypic $CGRP_1$ and $CGRP_2$ expressing tissues are from guinea-pig and rat, respectively. Little work has been done on the guinea-pig vas deferens. Most of the receptor classification relies on studies with the single peptide fragment $CGRP_{3-37}$, although other fragments have also been reported to be useful antagonists, particularly [Tyr^0]-$CGRP_{28-37}$ (Chakder & Rattan, 1990). In this study we describe the effects of a series of CGRP fragments and AC187 against CGRP and amylin in the guinea-pig ileum and vas deferens, and present further evidence for distinct CGRP and amylin receptors. Preliminary accounts of these findings have been given elsewhere in abstract form (Tomlinson & Poyner, 1995a,b).

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Methods

Tissue preparation

Male Duncan-Hartley guinea-pigs (300–400 g) were killed by cervical dislocation, and the vas deferens and sections of the ileum (10 cm from the ileo-caecal valve) were removed and placed in Krebs solution (composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaCO_3$ 13.4, $MgSO_4$ 1.2, glucose 11.6, pH 7.2) gassed with 95% O_2, 5% CO_2. Tissues were set up longitudinally in organ baths (at 32°C) and allowed to equilibrate for an hour under 1 g load. The vas deferens was stimulated transmurally using a Grass S48 stimulator with 1 s trains of pulses of 0.5 ms width, 70 V amplitude at 20 Hz, repeated at 25 s intervals (Ellis & Burnstock, 1989). The ileum was stimulated transmurally using a Scientific and Research Instrument Ltd. (U.K.) stimulator with 0.1 ms pulses, 10 V amplitude at a frequency of 0.1 Hz (Kerr *et al.*, 1990). Cumulative concentration-response curves to agonist alone were constructed, allowing 5 min between each addition of drug. The agonist was then washed out, the tissue allowed to rest for 30 min and then the curves repeated in the presence of antagonist (added 5 min prior to the lowest concentration of agonist). Responses were expressed as percentage inhibition of contraction prior to addition of agonist. Control experiments established that there was no significant desensitization between dose-response curves to either CGRP or amylin. A single concentration of antagonist was normally tested on each preparation.

Data evaluation

All dose-response curves were fitted using EBDA-LIGAND to obtain EC_{50} values and Hill coefficients. Statistical significance of values was determined by one-way ANOVA followed by Dunnett's test.

Significance was accepted at the 0.05% level. Throughout the text, values are quoted as means ± standard errors. Apparent pA_2 values were estimated from calculated dose-ratios, using the equation $pA_2 = \log[(\text{dose ratio} - 1)/\text{antagonist concentration}]$. As data from multiple antagonist shifts were not normally available, it was assumed that interactions were competitive.

Drugs

Peptides were obtained from Sigma (rat and human amylin, $CGRP_{8-37}$ and rat αCGRP), Peninsula (α[Tyr^0]-$CGRP_{28-37}$) and Calbiochem (human αCGRP). In addition, batches of human $\alpha CGRP_{8-37}$, human $\alpha CGRP_{19-37}$, human α[Tyr^0]-$CGRP_{28-37}$ and AC187 were synthesized on an Applied Biosystems Synergy peptide synthesizer, using FMOC chemistry with cleavage in trifluoroacetic acid. Purity was confirmed by high performance liquid chromatography (h.p.l.c.). Concentrations were confirmed, where possible, by bioassay against commercial material. Precautions in handling peptides were as described previously (Poyner *et al.*, 1992).

Thiorphan was purchased from Sigma.

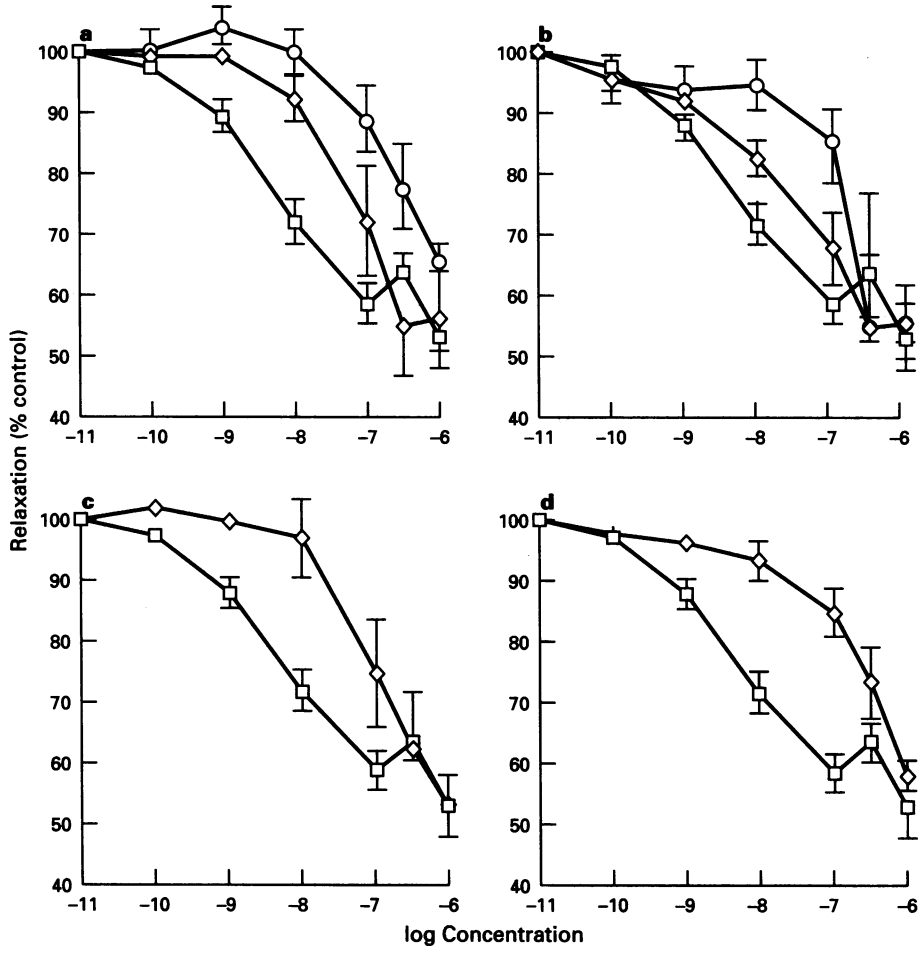

Figure 1 Effects of CGRP fragments on the human αCGRP-mediated relaxation of guinea-pig ileum (□; fitted $EC_{50} = 5.7 \pm 0.2$ nM). (a) $CGRP_{8-37}$; 1 μM (◇; $EC_{50} = 88.4 \pm 4.5$ nM, $n = 9$): 3 μM (○; $EC_{50} = 268 \pm 126$ nM, $n = 5$). (b) $CGRP_{19-37}$; 1 μM (◇; $EC_{50} = 26.5 \pm 14$, $n = 7$): 3 μM (○; $EC_{50} = 111 \pm 20$ nM, $n = 4$). (c) [Tyr^0]-$CGRP_{28-37}$; 10 μM (◇; $EC_{50} = 89 \pm 17$ nM, $n = 5$). (d) AC187; 10 μM (◇; $EC_{50} = 272 \pm 29$, $n = 3$).

Results

Pharmacology of CGRP on the guinea-pig ileum

In agreement with previous reports (Dennis *et al.*, 1990), human αCGRP caused a sustained inhibition of contraction of the stimulated guinea-pig ileum, with an EC_{50} of 5.7±0.2 nM ($n=37$), although a transient contraction was observed at concentrations above 100 nM (Figure 1). The inhibitory response was antagonized by human $\alpha CGRP_{8-37}$, which caused a parallel rightwards shift in the dose-response curve (Figure 1, Table 1). Using the two dose-ratios obtained from Figure 1a, an apparent pA_2 of 7.15±0.23 ($n=5$) was calculated. The experiments were repeated with human αCGRP fragments 19–37 and [Tyr^0]-(28–37) (Figure 1, Table 1). Both fragments antagonized human αCGRP, but with decreasing potency as the size of the fragment was reduced; using the two dose-ratios from Figure 1b, the apparent pA_2 for $CGRP_{19-37}$ was estimated as 6.67±0.33 ($n=4$). The putative amylin receptor antagonist, AC187, proved to be a very effective antagonist of human αCGRP in the ileum, being only three fold less potent than $CGRP_{8-37}$.

Broadly comparable results were obtained with rat αCGRP (Figure 2, Table 1). This was a little more potent than human αCGRP ($EC_{50}=1.7\pm0.1$ nM, $n=20$). It was less sensitive to most antagonists than human αCGRP. However, $CGRP_{19-37}$ was exceptional, in that it appeared to be much more effective against this agonist than against human αCGRP (apparent pA_2 of 8.0±0.1, $n=5$, Figure 2b).

Human βCGRP was also examined. This was less potent

Table 1 EC_{50} values of CGRP and amylin on guinea-pig ileum in the presence of antagonists

Antagonist	*hCGRP*	*rCGRP*	*rAmylin*
Control	5.7±0.2 (37)	1.7±0.1 (20)	119±12 (14)
$CGRP_{8-37}$ 1 μM	88.4±4.5* (9)	15.5±4 (10)	136±46 (3)
$CGRP_{19-37}$ 1 μM	26.5±9.7 (7)	142±19* (9)	246±50 (4)
[Tyr^0]-$CGRP_{28-37}$ 10 μM	89±17* (5)	19.4±4.0 (7)	312±107 ¶(4)
AC187 10 μM	272±29* (3)	141±21 (3)	ND

Values represent mean±s.e.mean of n determinations (n values in parentheses). r=rat, h=human. ND=not determined. ¶Concentration=1.5μM. *Significantly different from control, $P<0.05$.

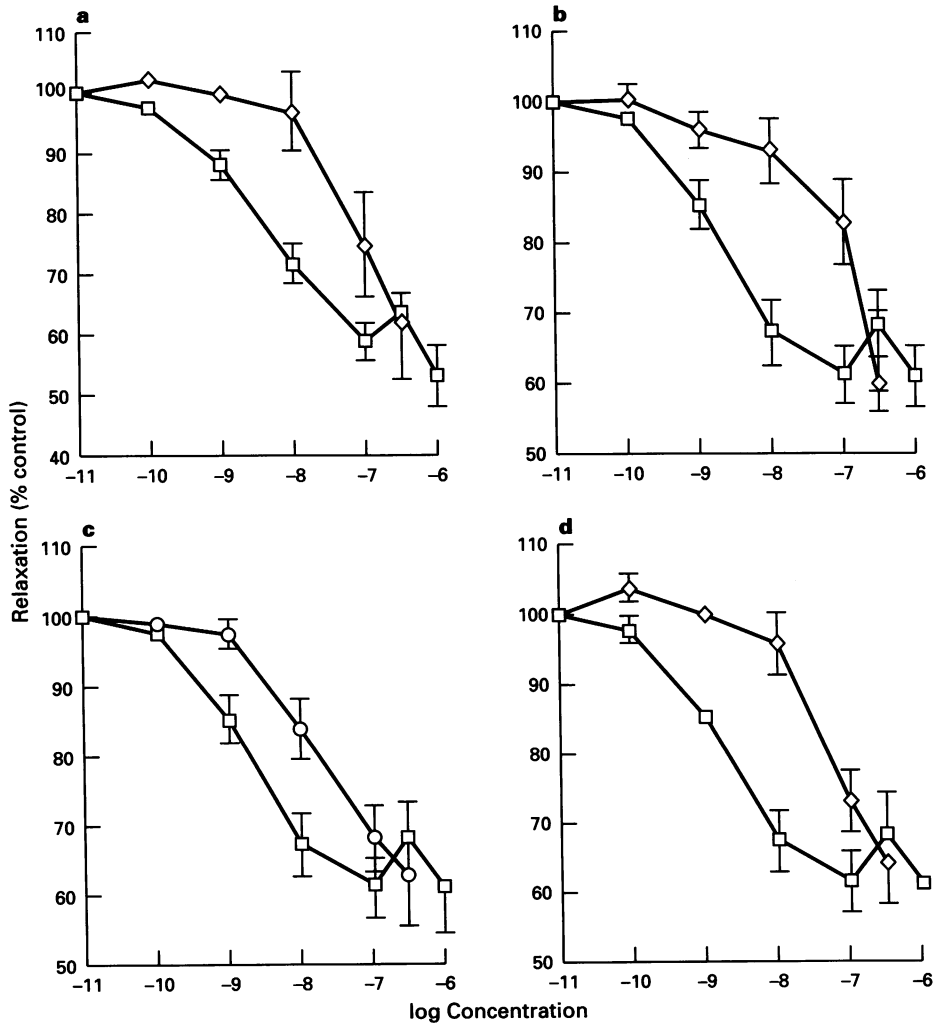

Figure 2 Effects of CGRP fragments on the rat αCGRP-mediated relaxation of guinea-pig ileum (□; $EC_{50}=1.7\pm0.1$ nM). (a) $CGRP_{8-37}$ 1 μM (◇; $EC_{50}=15\pm5$ nM, $n=10$). (b) $CGRP_{19-37}$ 1 μM (◇; $EC_{50}=165\pm21$ nM, $n=5$). (c) [Tyr^0]-$CGRP_{28-37}$ 10 μM (◇; $EC_{50}=19\pm4$ nM, $n=7$). (d) AC187 10 μM (◇; $EC_{50}=141\pm20$ nM, $n=3$).

than human αCGRP ($EC_{50}=20.0\pm5.3$ nM, $n=3$), and 1 μM $CGRP_{8-37}$ failed to produce a significant, parallel shift in its dose-response curve ($EC_{50}=84\pm54$ nM, $n=3$; Figure 3).

Pharmacology of amylin on the guinea-pig ileum

Rat amylin caused inhibition of stimulated contractions in 14 preparations (about 10% of the total examined), with an EC_{50} of 119 ± 11 nM ($n=14$). In these preparations, CGRP fragments at concentrations of 3 to 30 μM did not antagonize the amylin response (Table 1). Human amylin at concentrations of up to 1 μM never caused inhibition of stimulated contractions.

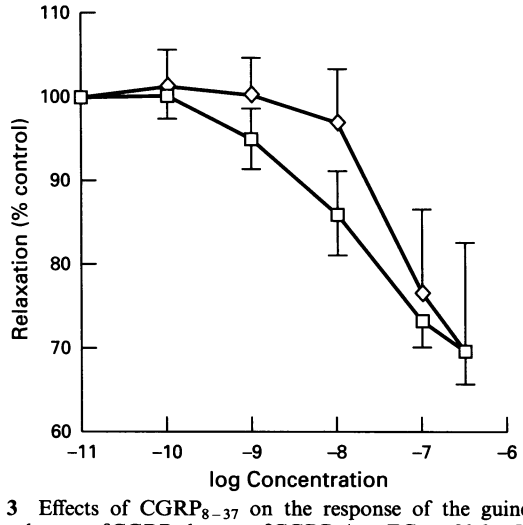

Figure 3 Effects of $CGRP_{8-37}$ on the response of the guinea-pig ileum to human βCGRP: human βCGRP (□; $EC_{50}=20.0\pm5.3$ nM, $n=3$); human βCGRP in the presence of 1 μM $CGRP_{8-37}$ (◇; $EC_{50}=84\pm54$ nM, $n=3$).

Pharmacology of CGRP on the guinea-pig vas deferens

The guinea-pig vas deferens resembled the rat vas deferens, in that stimulated contractions were inhibited by both human and rat αCGRP (EC_{50} values of 9 ± 3 nM, $n=47$, and 10.5 ± 0.5 nM, $n=33$, respectively). These responses were not antagonized by 3 μM $CGRP_{8-37}$ (Figure 4, Table 2). In contrast to the situation found for the rat vas deferens, the addition of thiorphan had only a small effect on the action of 1 μM $CGRP_{8-37}$ against human αCGRP ($EC_{50}=32.5\pm16.5$, $n=5$). $CGRP_{19-37}$ did have some antagonist activity, particularly against human αCGRP, when present at 10 μM; antagonism by [Tyr^0]-$CGRP_{28-37}$ was much weaker (Figure 4, Table 2). The putative amylin antagonist, AC187, had no action on the responses to either human or rat αCGRP (Figure 4, Table 2). To exclude the possibility that the failure of $CGRP_{8-37}$ and AC187 to antagonize human CGRP was due to an insufficient equilibration, pre-incubation with the antagonist was increased from 5 min to 30 min. However, even with 10 μM thiorphan present to guard against proteolysis, little or no antagonism was seen (EC_{50} for CGRP in the presence of 3 μM $CGRP_{8-37}=21\pm8$ nM, $n=3$; in the presence of 10 μM $AC187=12\pm3$ nM, $n=3$).

Pharmacology of amylin on the guinea-pig vas deferens

Both human and rat amylin caused inhibition of stimulated contractions of the vas deferens, although rat amylin was more potent than human amylin (EC_{50}'s of 77 ± 9 nM, $n=38$ and

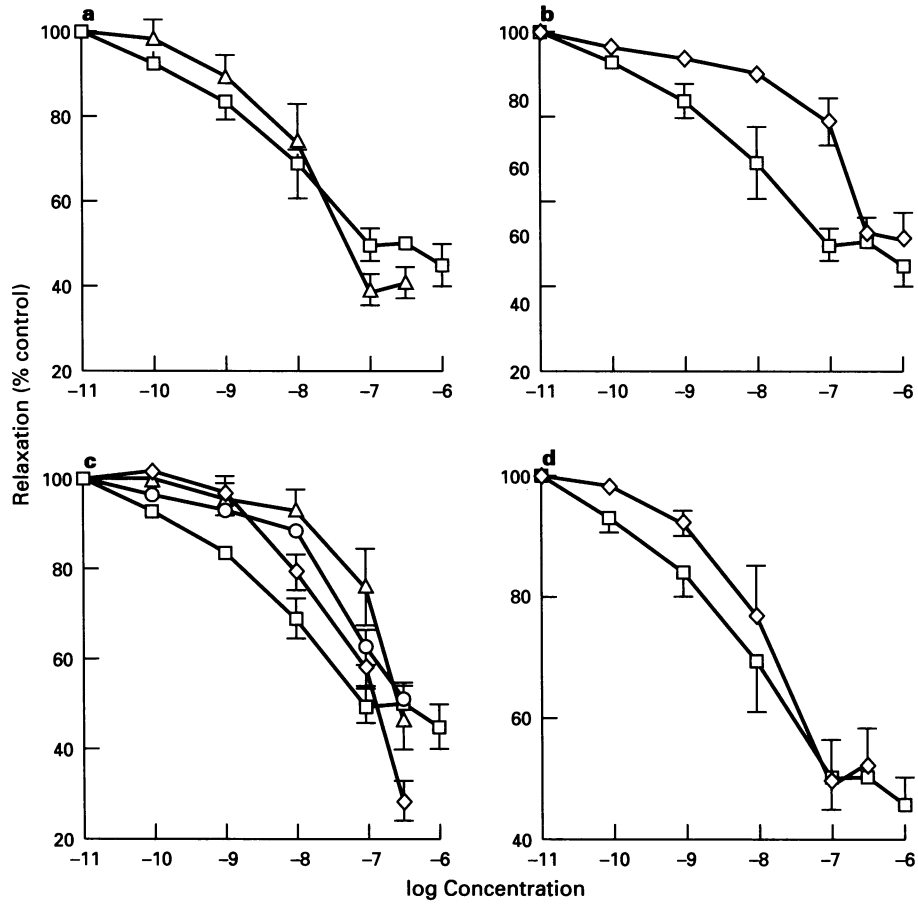

Figure 4 Effects of CGRP fragments on the human αCGRP mediated relaxation of the guinea-pig vas deferens (□; $EC_{50}=9\pm3$ nM). (a) $CGRP_{8-37}$ 3 μM (△; $EC_{50}=13.6\pm3.4$ nM, $n=5$). (b) $CGRP_{19-37}$ 10 μM (◇; $EC_{50}=119\pm12$ nM, $n=5$). (c) [Tyr^0]-$CGRP_{28-37}$ 1.5 μM (◇); 10 μM (○; $EC_{50}=70\pm42$ nM, $n=5$); 30 μM (△; $EC_{50}=124\pm10$ nM, $n=5$), (d) AC187 10 μM (◇; $EC_{50}=13.6\pm2.5$ nM, $n=3$).

Table 2 EC_{50} values for CGRP and amylin on guinea-pig vas deferens in the presence of antagonists

Antagonist	*hCGRP*	*rCGRP*	*rAmylin*	*hAmylin*
Control	9.0±3.0 (47)	10.5±4.6 (33)	77±9 (38)	213±22 (24)
$CGRP_{8-37}$ 3 μM	13.6±3.4 (5)	11.0±6.8 (3)	242±25¶* (6)	625±59*(5)
$CGRP_{19-37}$ 10 μM	119±12* (5)	3.8±1.7 (6)	180±92 (4)	91.3±23 (4)
[Tyr^0]-$CGRP_{28-37}$ 10 μM	70±42* (5)	15±5.2 (4)	164±10 (4)	355±66 (3)
AC187 10 μM	13.6±2.5 (3)	50±32 (4)	610±22* (5)	ND

Values represent means±s.e.means of *n* determinations (*n* values in parentheses). r = rat, h = human, ND = not determined ¶Concentration = 10 μM *Significantly different from control, $P<0.05$.

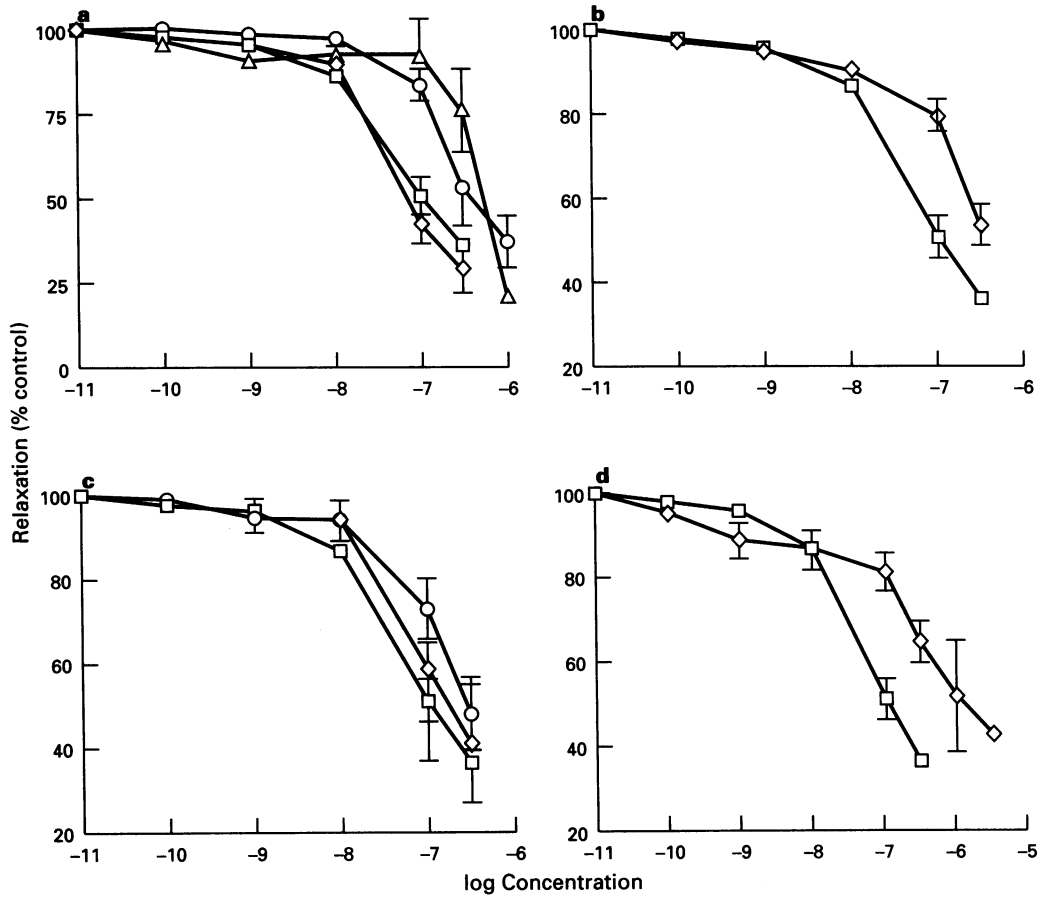

Figure 5 Effects of antagonists on the rat amylin-mediated relaxation of guinea pig-vas deferens (□, $EC_{50}=77\pm9$ nM). (a) $CGRP_{8-37}$ 0.3 μM, (◇; $EC_{50}=47\pm4$ nM, $n=5$), 1 μM (○; $EC_{50}=174\pm22$ nM, $n=10$), 10 μM (△; $EC_{50}=242\pm25$ nM $n=6$). (b) $CGRP_{19-37}$ 10 μM (◇; $EC_{50}=180\pm92$ nM, $n=4$). (c) [Tyr^0]-$CGRP_{28-37}$ 1 μM (◇; $EC_{50}=87\pm7.5$ nM, $n=4$) 10 μM (○; $EC_{50}=164\pm10$ nM, $n=4$). (d) AC187 10 μM, (◇; $EC_{50}=610\pm22$ nM, $n=5$).

213±22 nM, $n=24$, respectively). The rat and human amylin responses were sensitive to antagonism by $CGRP_{8-37}$ (Figure 5, Table 2). Addition of thiorphan failed to modify this antagonism (EC_{50} for rat amylin in the presence of 1 μM $CGRP_{8-37}$; 174±22 nM, $n=4$: in the presence of 10 μM thiorphan and 1 μM $CGRP_{8-37}$; 204±33 nM, $n=4$). Unlike the situation seen with CGRP, 10 μM AC187 did antagonize the response to rat amylin (Figure 5, Table 2).

Discussion

This study extends the known pharmacology of CGRP and amylin, and supports both the subdivision of CGRP receptors and the existence of distinct receptors which can be stimulated by amylin. As such, it is in broad agreement with other published studies (Dennis *et al.*, 1989; 1990; Giuliani *et al.*, 1992).

The CGRP receptors in the guinea-pig ileum have been examined in several previous reports. The receptors mediating relaxation are located directly on the smooth muscle and are sensitive to $CGRP_{8-37}$, with reported pA_2 values against human αCGRP in the range of 7.0 to 7.5 (see Poyner, 1992 for review). The apparent pA_2 of 7.15 observed for $CGRP_{8-37}$ in this study is in good agreement with the literature, and confirms the presence of a $CGRP_1$-like receptor. There are also pre-junctional receptors, insensitive to $CGRP_{8-37}$ and which cause contraction (Sun & Benishin, 1991). Although there were transient contractions of our preparations in response to CGRP at concentrations greater than 100 nM, only the inhibitory effects were examined in this study.

Limited work has been done on the structure-activity relationship of CGRP fragments on $CGRP_1$-like receptors. Mimeault *et al.* (1991) concluded that residues 9–11 were important in conferring high affinity binding to $CGRP_{8-37}$ and Rovero *et al.* (1992) showed that $CGRP_{19-37}$ had a pA_2 of 5.4 against human αCGRP on guinea-pig atrium. However, a

study with [Tyr^0]-$CGRP_{28-37}$ on the opossum internal anal sphincter suggested that it had an apparent pA_2 of 7.3 against human αCGRP; as high as is often found for $CGRP_{8-37}$ (Chakder & Rattan, 1991). Thus it was of interest to examine this compound in the ileum. Our results indicate that [Tyr^0]-$CGRP_{28-37}$ does have a lower affinity for the human αCGRP receptor than the full 8–37 fragment indicating that residues 9–27 are important in conferring high affinity binding, and that the high pA_2 in the opossum preparation may reflect a species variation. Jansen (1992) reported a similarly low affinity for this compound in guinea-pig basilar artery. $CGRP_{19-37}$ is more potent in antagonizing human αCGRP than [Tyr^0]-$CGRP_{28-37}$ in the ileum, suggesting an approximate relationship between fragment length and affinity. The apparent high affinity of AC187 against human and rat CGRP in the ileum was unexpected, as this is a salmon calcitonin derivative, with a high affinity for amylin receptors on rat skeletal muscle and a lower affinity for the rat, L6 skeletal myocyte $CGRP_1$-like receptor (Beaumont *et al.*, 1995). Human αCGRP is clearly not acting at an amylin receptor on guinea-pig ileum, and so the high affinity of AC187 against CGRP on the ileum must reflect a peculiarity of the CGRP receptors present on this preparation.

The majority of quantitative data available for $CGRP_{8-37}$ has been obtained against human αCGRP (Dennis *et al.*, 1989; 1990; Maggi *et al.*, 1991; Longmore *et al.*, 1994). A few studies have examined other forms of CGRP. In the opossum internal anal sphincter, [Tyr^0]-$CGRP_{8-37}$ was found to be an order of magnitude more potent against human αCGRP than rat αCGRP (Chakder & Rattan, 1990). Giuliani *et al.* (1992) obtained a pA_2 value of 6.66 for $CGRP_{8-37}$ against rat αCGRP on guinea-pig atria, lower than the previously reported range for human αCGRP of 6.89 to 7.66 (Maggi *et al.*, 1991; Dennis *et al.*, 1990). Hughes & Brain (1992), Chakder & Rattan (1991) and Jansen (1992) all found that CGRP antagonists were more effective in blocking the actions of human αCGRP than human βCGRP. In the light of these observations, it is interesting to note that this study has also shown differences between human αCGRP and other CGRP forms. Both human βCGRP and rat αCGRP appeared less sensitive to $CGRP_{8-37}$, and rat αCGRP was particularly sensitive to $CGRP_{19-37}$. Taken together, these data are consistent with the idea that human βCGRP and rat αCGRP may act on additional receptors to human αCGRP in some tissues, which show a distinct pharmacology to classical $CGRP_1$. It is possible that they could act on a variety of peptide-recognising, pre-junctional neuronal receptors to cause release of endogenous dilators (Sun & Benishin, 1991; Nuki *et al.*, 1991). Neuronal $CGRP_1$-like receptors present in the guinea-pig ileum have so far only been implicated in contractile responses; if they are involved in the responses to human βCGRP, then their role may be more complicated than previously supposed.

Little work has been done on the CGRP receptor of the guinea-pig vas deferens; most workers have used the rat tissue, which expresses $CGRP_2$ receptors, with a low affinity for CGRP fragments. In view of potential complications of species differences, it was of interest to examine the guinea-pig tissue. This appears broadly similar to the rat, in that $CGRP_{8-37}$ was at least an order of magnitude less potent antagonist than on the ileum. Although a detailed analysis was not pursued, it seems likely that $CGRP_{19-37}$ is roughly equipotent to $CGRP_{8-37}$ on the vas deferens, suggesting that residues 8–18 contribute little to overall binding of antagonists to the $CGRP_2$ receptor subtype.

Giuliani and co-workers (1992) described the presence of high-affinity amylin receptors on the rat vas deferens (in addition to those for CGRP), and the guinea-pig urinary bladder. Here, amylin-recognising receptors have also shown to be present on the guinea-pig vas deferens and, to a limited extent, on the ileum. The erratic responsiveness of the ileum to amylin precluded a detailed examination of the receptors mediating this response, although their lack of responsiveness to $CGRP_{8-37}$ and other fragments indicated that they were distinct from $CGRP_1$-like receptors. The receptors on the vas deferens could be examined in more detail. Like their counterparts in the rat, they were of low sensitivity to antagonism by CGRP fragments. More significantly, the guinea-pig receptors activated by amylin were clearly distinct from the $CGRP_2$ receptors, as demonstrated by the effects of AC187, which was selective for the amylin receptors, albeit with a lower affinity than against CGRP on the ileum.

There are a number of unresolved issues regarding the receptors which mediate the amylin response in the vas deferens. Although they respond to amylin, it remains to be established whether this is their physiological stimulus. It is unlikely they are the same as the amylin receptors found on rat soleus, where AC187 appears to be 300 fold more potent than here. A similar consideration suggests that a close relationship with the 'C3' amylin binding site of rat nucleus accumbens is unlikely (Beaumont *et al.*, 1993; 1995). The receptors do superficially resemble those found on guinea-pig urinary bladder and trachea (Giuliani *et al.*, 1992; Bhogal *et al.*, 1994), although these studies did not use AC187 which appears to discriminate between $CGRP_2$ and amylin receptors. Further work is required to establish whether the differences between rat skeletal muscle and guinea-pig smooth muscle with regard to amylin simply reflect species variation, or heterogeneity of amylin-recognising receptors.

Throughout this study, apparent pA_2 values have been derived from one or two measurements of dose-ratios, and so must be interpreted with caution as competitive behaviour has had to be assumed. In addition, peptidase inhibitors were not routinely employed and a short time period was used for antagonist pre-incubation. Accordingly, EC_{50} values may be underestimated. In spite of these limitations, our estimated pA_2 value of 7.15 for $CGRP_{8-37}$ in the ileum is in good agreement with the literature data. Where thiorphan and longer incubations were used in the vas deferens, the results for amylin or CGRP were not changed. Thus there are grounds for assuming these factors have not greatly influenced our overall results.

In conclusion, this study provides a detailed characterization of the CGRP-recognising receptors in the guinea-pig ileum and vas deferens. It also demonstrates the existence of distinct amylin receptors, sensitive to AC187. The CGRP receptors can be distinguished by their sensitivity to $CGRP_{8-37}$ and so fall broadly within the confines of the $CGRP_1$/$CGRP_2$ classification. However, the antagonism of rat αCGRP by $CGRP_{19-37}$ in the ileum suggests that the details of CGRP pharmacology are complicated with the possibility of the peptide cross-reacting at multiple receptors for CGRP, amylin and other related peptides.

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