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Characterization of calcitonin gene-related peptide(CGRP) receptors in intramural coronary arteries from male and female Sprague Dawley rats

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1 In this study we characterized the CGRP-receptor subtype by Schild-plot analysis using the C-terminal fragment, human-αCGRP(8–37), a putative competitive $CGRP_1$-receptor selective antagonist. In addition, the effect of rat-αCGRP was compared with that of homologous peptides rat-βCGRP, rat-amylin, rat-adrenomedullin and [Cys(Acm)^{2,7}]-human-αCGRP, a putative selective $CGRP_2$-receptor agonist, in the left coronary arteries of 3 months old male and female Sprague Dawley rats.

2 Isolated rings from the distal, intramural part of the left anterior descending (LAD) coronary artery in both groups of rats were mounted on a double wire-myograph. The arteries were then stretched to their optimal lumen diameter for active tension development and precontracted with 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), after which agonists were added to the organ bath in a cumulative manner.

3 Rat-αCGRP induced endothelium-independent relaxations in male and female Sprague-Dawley rats. Rat-βCGRP concentration-response relations (10^{-11}–10^{-7} M) were similar to those of rat-αCGRP in either sex. The maximal relaxations induced by rat-amylin and rat-adrenomedullin, both at 10^{-6} M, were significantly ($P<0.05$) lower than those induced by rat-α- and rat-βCGRP. In contrast, the selective $CGRP_2$-receptor agonist [Cys(Acm)^{2,7}]-human-αCGRP failed to induce significant relaxations at the highest concentration used (10^{-7} M) in the coronary arteries of male and female rats.

5 The C-terminal fragment, human-αCGRP(8–37) blocked concentration-dependently (10^{-7}–10^{-6} M) the rat-αCGRP-induced relaxation in 10^{-5} M $PGF_{2\alpha}$-precontracted coronary arteries. The slopes of the regression lines of the Schild-plots for both male and female rats were not significantly ($P>0.05$) different from unity and the pA_2 values for human-αCGRP(8–37) were 6.93 and 6.98 in arteries from male and female rats, respectively. There was no significant ($P>0.05$) difference in estimated pK_B values for human-αCGRP(8–37) between male (6.99 ± 0.10, $n=13$) and female (6.95 ± 0.08, $n=13$) rats.

6 The concentration-response relationships for rat-α- and rat-βCGRP were similar in male and female Sprague Dawley rats. The predominant CGRP receptor subtype in small intramural coronary arteries appeared to belong to the $CGRP_1$-receptor subtype in both sexes.

Keywords: Calcitonin gene-related peptide receptor (CGRP); CGRP(8–37); [Cys(Acm)^{2,7}]-CGRP; amylin; adrenomedullin; coronary artery

Introduction

Calcitonin gene-related peptide (CGRP) is a 37 amino acid residue peptide generated by alternative splicing of the calcitonin gene transcripts and exists in two isoforms, designated α- and β-CGRP (Amara *et al*., 1982). As a neurotransmitter CGRP is found in sensory nerves, which primarily innervate the atrial muscle and the ventricular vasculature, indicating an important function of CGRP in the regulatory processes of coronary blood flow (Franco-Cereceda *et al*., 1987).

In vitro studies have shown that CGRP causes a positive inotropic action in the atria of guinea-pig, rat and man as well as potent vasodilatation in various mammalian coronary arteries (see reviews Poyner, 1992; Bell & McDermott, 1996; Wimalawansa, 1996). CGRP can be released during hypoxia and low pH levels in the myocardium (Franco-Cereceda *et al*., 1993), thus indicating a vasodilator role during ischaemic conditions. CGRP induces both endothelium-dependent and endothelium-independent relaxation of rat coronary arteries depending on the size and the location of the vessels (Prieto *et al*., 1991). Recently, two $CGRP_1$-like receptors have been cloned (Kapas & Clark, 1995; Aiyar *et al*., 1996). Northern blot analysis has revealed that the messenger RNA for this receptor is predominantly expressed in human lung and heart (Aiyar *et al*., 1996). The present information on the CGRP-binding sites indicates that it is monomeric, G-protein coupled (Chatterjee *et al*., 1992; Poyner *et al*., 1992; Stangl *et al*., 1993; Wimalawansa *et al*., 1993; Chatterjee & Fischer, 1995), with a M_r of 17–67 kDa, and possibly glycosylated (Chatterjee *et al*., 1992; Stangl *et al*., 1993; Wimalawansa *et al*., 1993). In hamster pancreatic β cells CGRP receptors are not coupled to the adenylate cyclase enzyme, indicating the existence of CGRP-receptor subtypes (Barakat *et al*., 1993). This is also indicated by the action of the CGRP(8–37) fragment, which is a competitive receptor antagonist (Dennis *et al*., 1990; Mimeault *et al*., 1992; Quirion *et al*., 1992). Several studies with this analogue indicate the existence of at least two subtypes of CGRP receptors, one type having a high affinity for the antagonist ($pA_2\geqslant7.0$) and another type having no or very low affinity ($pA_2<<7.0$) for this antagonist (Quirion *et al*., 1992; Poyner, 1995; Bell & McDermott, 1996). The receptors have been denominated $CGRP_1$- and $CGRP_2$-receptors, respectively (Quirion *et al*., 1992; Poyner, 1995; Wimalawansa, 1996). The receptor subtypes are also revealed by the actions of the agonists, CGRP and cys(ACM)^{2,7}-CGRP, of which the latter only has agonistic actions at the $CGRP_2$-

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receptor subtype (Quirion *et al*., 1992). Peptides such as amylin and adrenomedullin which have relatively high homology with CGRP can activate $CGRP_1$-receptors (Nuki *et al*., 1993; Entzeroth *et al*., 1995; Poyner, 1995; Westfall *et al*., 1995; Bell & McDermott, 1996; Wimalawansa, 1996), but both peptides also interact with specific receptors for amylin and adrenomedullin that cannot be antagonized by the CGRP(8–37) fragment, and these receptors have little affinity for CGRP (Muff *et al*., 1995; Poyner, 1995; Bell & McDermott, 1996; Tomlinson & Poyner, 1996).

The CGRP receptor subtype has not yet been characterized in rat intramural coronary resistance arteries. The purpose of the present study was to characterize the calcitonin gene-related peptide (CGRP) receptors in intramural ring segments of the distal part of the left anterior descending coronary artery from 3 months old Sprague Dawley rats. We also compared male and female rats in this study since the level of CGRP-immunoreactivity is higher in females than in males (Valdemarsson *et al*., 1990), indicating a possible gender related difference in neuronal and CGRP-receptor function.

Methods

Intramural segments (1–2 mm long) of the distal part of the left anterior descending coronary artery (LAD) were isolated from the hearts of 3 months old made and female Sprague Dawley rats, as previously described (Nyborg & Mikkelsen, 1985). The arteries were mounted as rings on two 40 μm stainless steel wires connected to a force transducer and a micrometer, respectively, in the organ bath of a small vessel myograph (Mulvany & Nyborg, 1980), which allowed direct determination of the isometric wall tension, while the internal circumference of the vessels was controlled.

Experimental procedure

After being mounted, the arteries were equilibrated at 37°C for 30 min in oxygenated (95% O_2 and 5% CO_2) physiological salt solution (PSS) of the following composition in (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, $CaCl_2 \cdot 2H_2O$ 2.5, ethylene diamine tetraacetic acid (EDTA) 0.027 and glucose 5.5 with pH adjusted to 7.4. The vessels were then stretched to their optimal lumen diameter $1_1 = 0.9 \times 1_{100}$, where 1_{100} is an estimate of the diameter the vessel would have under a passive transmural pressure of 100 mmHg (13.3 kPa (Nm^{-2})) in order to obtain the optimal condition for active tension development (Nyborg *et al*., 1987). Each experiment was initiated by contracting the vessels repeatedly with K-PSS (similar to PSS except that NaCl was exchanged with KCl on an equimolar basis) until reproducible wall tensions were recorded. The maximal contractile response of the vessels (ΔT_{max}) was then determined by measuring the differences in vessel wall tension (N m^{-1} vessel wall) when the vessels were maximally contracted with K-PSS, to which 10^{-5} M 5-hydroxytryptamine (5-HT) and 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) had been added, and when maximally relaxed in Ca^{2+}-free PSS (Nyborg, 1991). Ca^{2+}-free PSS was similar to PSS except that $CaCl_2$ was replaced with 0.01 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA). Vessels were accepted only if the maximal active pressure (calculated according to the Laplace relation: $\Delta P_{max} = 2 \times \Delta T_{max}/1_1$) exceeded 9 kPa.

Four different series of experiments were used to characterize the CGRP receptors in 10^{-5} M $PGF_{2\alpha}$-precontracted coronary arteries. In the first series of experiments, the role of the endothelium was determined by use of two consecutive arterial segments; one was used as a control and the other segment had its endothelium mechanically removed by insertion of a human scalp hair into the vessel lumen, which then was gently rubbed by pushing the hair back and forth (Prieto *et al*., 1991). Removal of endothelium was considered effective if acetylcholine (10^{-5} M) no longer induced relaxation.

In the second series of experiments, the effect of rat-αCGRP was compared to that of rat-βCGRP, rat-amylin and rat-adrenomedullin. These experiments were performed by constructing four consecutive concentration-response curves for rat-βCGRP (10^{-11}–10^{-7} M), rat-amylin (10^{-11}–10^{-6} M), rat-adrenomedullin (10^{-11}–10^{-6} M) and rat-αCGRP (10^{-11}–10^{-7} M), respectively. The coronary arteries were activated twice for 3 min with KPSS (125 mM K^+) within 15 min washout period between each two concentration-response curves.

In the third series of experiments, two concentration-response curves (10^{-11}–10^{-7} M) were made, one with rat-αCGRP and the other one with [Cys(Acm)^{2,7}]-human-αCGRP. The arteries were also activated twice for 3 min with K-PSS (125 mM K^+), with a 15 min washout period between the first and second concentration-response curve.

In the fourth series of experiments, two cumulative rat-αCGRP concentration-response curves were made, the first (10^{-11}–10^{-7} M) was obtained in the absence of the C-terminal fragment antagonist, human-αCGRP(8–37). The coronary arteries were then activated twice for 3 min with K-PSS (125 mM K^+) to overcome tachyphylaxis to rat-αCGRP (data not shown). The arteries were incubated with the antagonist for 15 min before the second rat-αCGRP concentration-response curve was obtained. Three separate groups of coronary arteries were used for the three different concentrations (1×10^{-7}, 3×10^{-7}, 1×10^{-6} M) of the antagonist. In the second rat-αCGRP concentration-response curve, the last concentration of agonist was adjusted to that of antagonist in order for a maximum response to be achieved.

Drugs

Drugs used were: rat-αCGRP, human-αCGRP(8–37), 5-hydroxytryptamine HCl (Sigma, St Louis, MO, U.S.A.), [Cys(Acm)^{2,7}]-human-αCGRP, rat-βCGRP, rat-amylin, rat-adrenomedullin (Peninsula Laboratories, Inc., Belmont, California, U.S.A.) and prostaglandin $F_{2\alpha}$ (Dinoprost, UpJohn, Belgium). Rat-αCGRP, rat-βCGRP, rat-amylin, rat-adrenomedullin, human-αCGRP(8–37) and [Cys(Acm)^{2,7}]-human-αCGRP were dissolved in distilled water Stock solutions (10^{-3} or 10^{-4} M) were stored at −20°C and dilutions were made just before experimentation.

Data analysis and statistics

Vessel responses are expressed as a percentage of the response induced by $PGF_{2\alpha}$. Sensitivity to agonists is expressed as pIC_{50} value, where $pIC_{50} = -\log(IC_{50}[M])$, and IC_{50} [M] is the molar concentration of agonist required to produce half-maximum relaxation. All concentration-response curves were analysed by iterative nonlinear regression analysis with the GraphPAD programme (GraphPAD corp, SanDiego, CA, U.S.A.). Each regression line was fitted to a sigmoid equation: $R/R_{max} = A[M]^{n_H}/(A[M]^{n_H} + IC_{50}[M]^{n_H})$, where R_{max} is the maximum response developed to the agonist, A[M] is the concentration of agonist and n_H is a curve-fitting parameter, the Hill coefficient (Kenakin 1986).

The effect of the C-terminal fragment antagonist human-αCGRP(8–37) on rat-αCGRP concentration-response curve was analysed by the Schild plot method (Arunlakshana & Schild, 1959). The plots of log(CR-1) against −log [antagonist (M)] were analysed by linear regression analysis. The pA_2-values were determined from the intercept of the regression lines with the x-axis on the Schild plots. If competitive antagonism is assumed, i.e. the slope of the Schild plot is equal to unity, the affinity of the antagonist expressed as pK_B-values have alternatively been estimated according to the equation: pK_B = log (CR-1)-log [antagonist (M)] (MacKay, 1978), where CR is the agonist concentration-ratio between the IC_{50} for rat-αCGRP in the presence and absence of the antagonist.

Results are given as mean ± s.e.mean (n = number of vessels). Differences between mean values were analysed by means of a two-tailed Student's t-test for paired or unpaired observations where appropriate. The level of significance was for all tests set to P values less than 0.05.

Results

Effect of endothelium-removal

Acetylcholine (10^{-5} M) relaxed endothelium-intact coronary arteries in male and female Sprague Dawley rats 71 ± 8% and 75 ± 5% (n = 7), respectively. In the coronary arteries of both male and female rats, 10^{-5} M acetylcholine produced a weak constrictor response after removal of the endothelium.

There was no significant difference in sensitivity and maximal response to rat-αCGRP between endothelium-intact and endothelium-denuded arteries in either sex (Figure 1). In male rats, pIC_{50} values were 8.35 ± 0.10 and 8.48 ± 0.04 (n = 7) and maximal relaxation 70 ± 9% and 70 ± 8% (n = 7), in endothelium-intact and endothelium-denuded arteries, respectively (Figure 1a). Mean lumen diameters (1_1) of the coronary arteries in male rats were 240 ± 16 μm vs 215 ± 15 μm (n = 7), in endothelium-intact and endothelium-denuded arteries, respectively. In female rats, pIC_{50} values were 8.48 ± 0.05 and 8.48 ± 0.08 (n = 7) and maximal relaxations 70 ± 8% and 72 ± 7% (n = 7), in endothelium-intact and endothelium-denuded arteries, respectively (Figure 1b). Mean lumen diameters (1_1) of the coronary arteries in female rats were 231 ± 11 μm and 210 ± 17 μm (n = 7), in endothelium-intact and endothelium-denuded arteries, respectively.

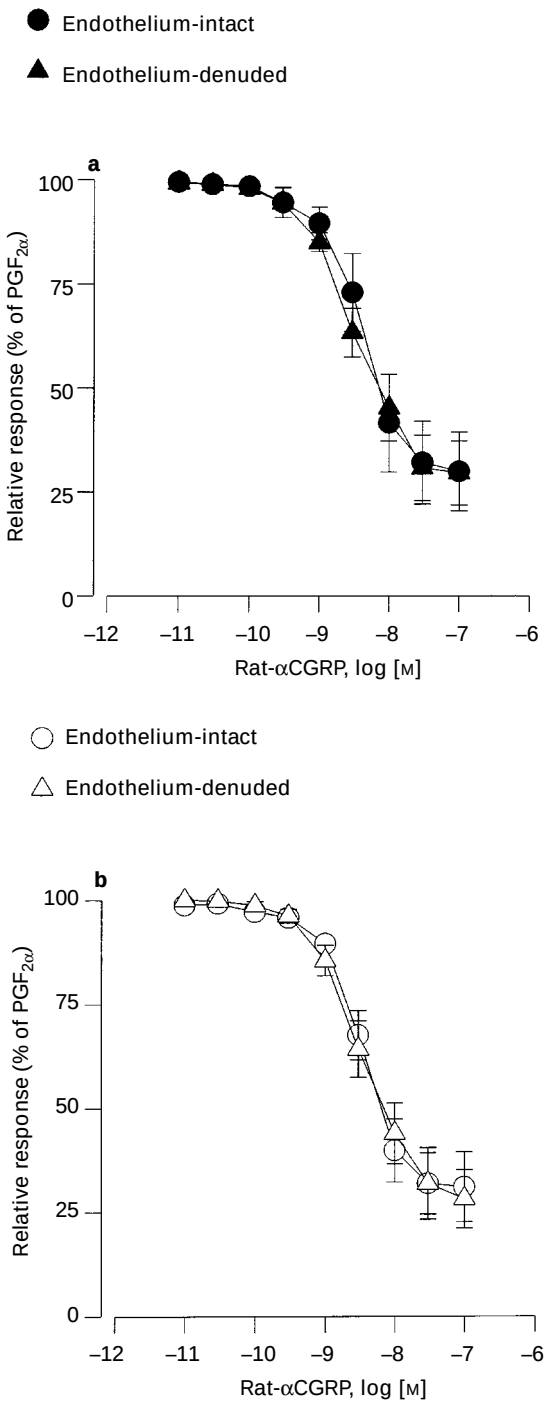

Figure 1 Rat-αCGRP concentration-response relationships (10^{-11}–10^{-7} M) in endothelium-intact and endothelium-denuded coronary arteries from (a) male and (b) female Sprague Dawley rats. Points represent mean values of 7 vessels and vertical lines indicate s.e.mean where this value exceeds the size of the symbol. Relative responses are given as percentage fraction of the initial vessel response to $PGF_{2\alpha}$ (10^{-5} M) just before they were challenged with rat-αCGRP.

Comparison of rat-αCGRP, rat-βCGRP, rat-amylin and rat-adrenomedullin

Rat-αCGRP and rat-βCGRP produced similar concentration-dependent responses in the coronary arteries from male and female Sprague Dawley rats (Figure 2). pIC_{50} values being 8.65 ± 0.08 vs 8.55 ± 0.06 (n = 6) and maximal relaxation 80 ± 6% vs 75 ± 9% (n = 6), for ratα- and ratβ-CGRP, respectively, in male rats. In female rats, the pIC_{50} values were 8.64 ± 0.05 vs 8.59 ± 0.07 (n = 6) and maximal relaxation 72 ± 7% vs 71 ± 6% (n = 6), for rat-αCGRP and rat-βCGRP, respectively. Mean lumen diameters (1_1) of the coronary arteries were 211 ± 15 μm and 200 ± 10 μm (n = 6), in male and female rats, respectively.

In male rats, the maximal relaxations induced by rat-amylin and rat-adrenomedullin, both at 10^{-6} M, were 19 ± 4% and 28 ± 5% (n = 6) (P < 0.05), respectively (Figure 2a). In female rats, the responses were 13 ± 2% and 29 ± 3% (n = 6) (P < 0.05), respectively (Figure 2b). Because of the low potency of rat-amylin and rat-adrenomedullin and their incomplete concentration-response curves it was not possible to calculate their pIC_{50} values.

Effect of the selective $CGRP_2$-receptor agonist [Cys(Acm)^{2,7}]-human-αCGRP

Comparative concentration-response relationships (10^{-11}–10^{-7} M) for rat-αCGRP and [Cys(Acm)^{2,7}]-human-αCGRP in

the coronary arteries from male and female Sprague Dawley rats are shown in Figure 3. The response to the selective $CGRP_2$-receptor agonist [Cys(Acm)^{2,7}]-human-αCGRP was not significant in the coronary arteries of both male and female rats as the maximal relaxations induced at 10^{-7} M were $9 \pm 5\%$ ($n=6$) and $9 \pm 4\%$ ($n=6$), respectively. The maximal

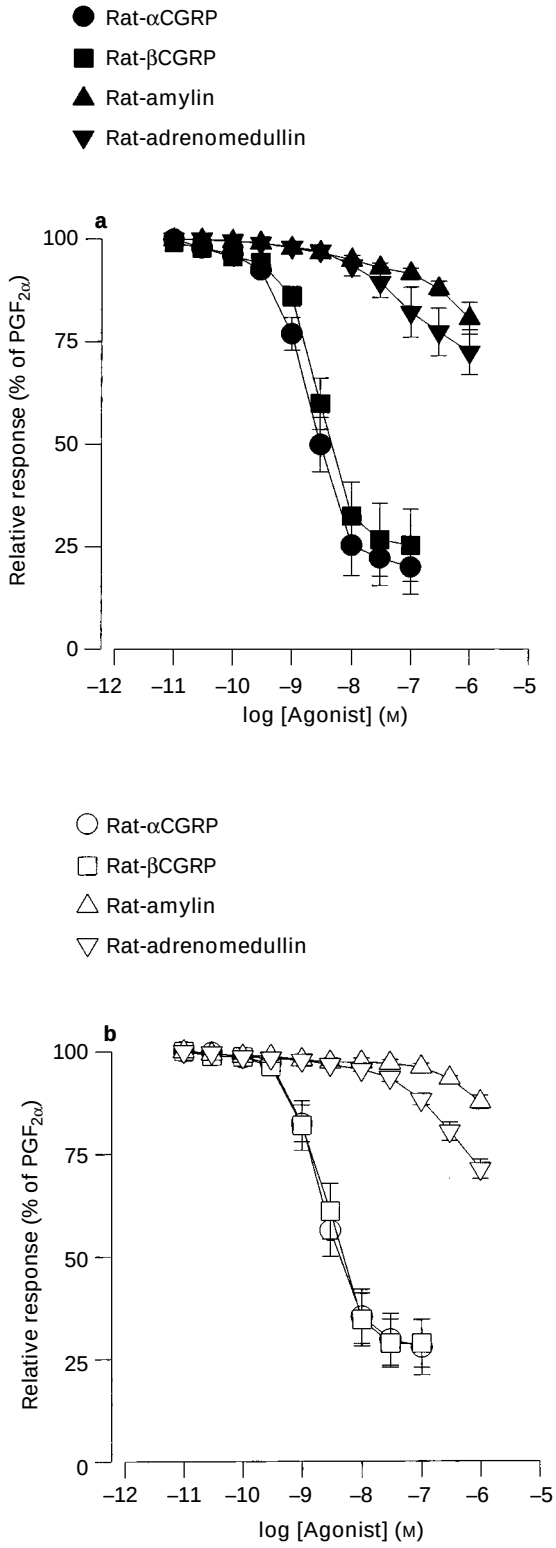

Figure 2 Concentration-response relationships for rat-α and rat-βCGRP ($10^{-11}-10^{-7}$ M), rat-adrenomedullin and rat-amylin ($10^{-11}-10^{-6}$ M) in $PGF_{2\alpha}$-precontracted coronary arteries from (a) male and (b) female Sprague Dawley rats. Points represent mean values of 6 vessels and vertical lines indicate s.e.mean where this value exceeds the size of the symbol. Relative responses are given as percentage fraction of the initial vessel response to $PGF_{2\alpha}$ (10^{-5} M) just before they were challenged with agonists.

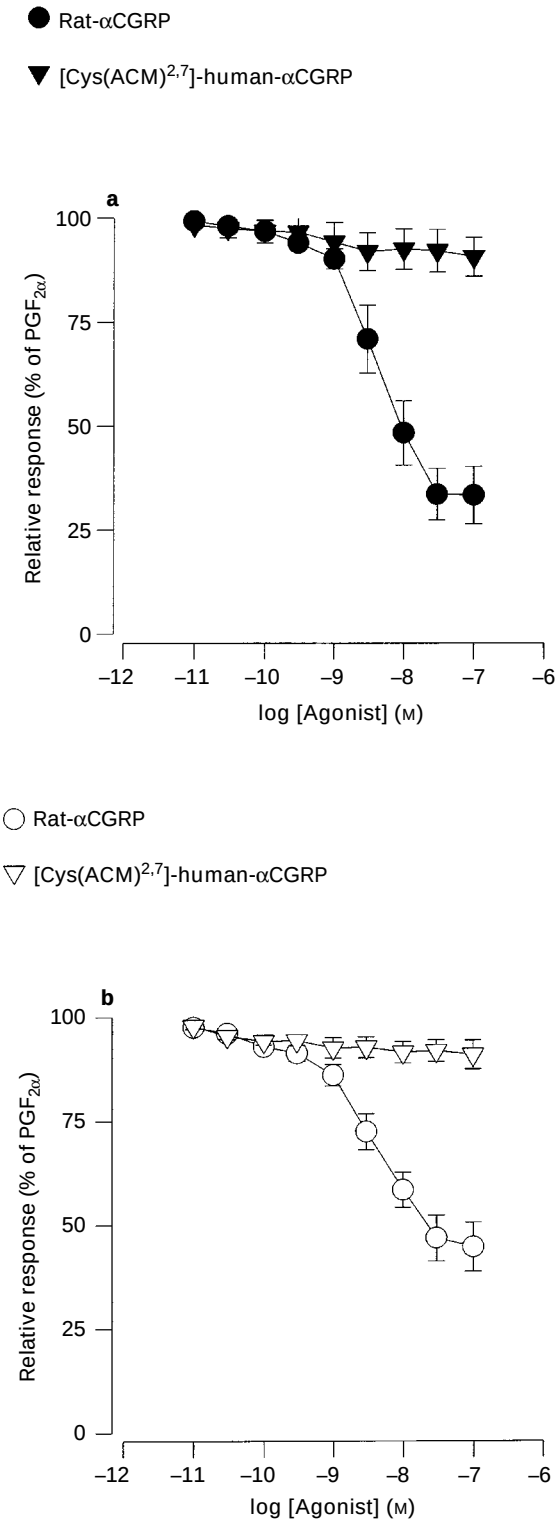

Figure 3 Concentration-response relationships ($10^{-11}-10^{-7}$ M) for [Cys(Acm)^{2,7}]-human-αCGRP, a selective $CGRP_2$-receptor agonist and rat-αCGRP in $PGF_{2\alpha}$-precontracted coronary arteries from (a) male and (b) female Sprague Dawley rats. Points represent mean values of 6 vessels and vertical lines indicate s.e.mean where this value exceeds the size of the symbol. Relative responses are given as percentage fraction of the initial vessel response to $PGF_{2\alpha}$ (10^{-5} M) just before they were challenged with rat-αCGRP or [Cys(Acm)^{2,7}]-human-αCGRP.

relaxations induced by rat-αCGRP were 67±7% ($n=6$) and 55±6% ($n=6$) in coronary arteries from male and female rats, respectively. Mean lumen diameters (1_1) were 216±23 μm ($n=6$) and 215±21 μm ($n=6$) in male and female rats, respectively.

Effect of the selective CGRP$_1$-receptor antagonist, human-αCGRP(8–37)

The $CGRP_1$-receptor antagonist, human-αCGRP(8–37), induced concentration-dependent (1×10^{-7}, 3×10^{-7} and 1×10^{-6} M) rightward shift in the sensitivity to rat-αCGRP in the coronary arteries from both male and female rats. The slopes of the Schild plots in both groups were not significantly different from unity (Figure 4), being equal to 1.14±0.20 ($r=0.83$, $n=13$) and 0.92±0.19 ($r=0.80$, $n=13$), in male and female rats, respectively. The pA_2 values obtained from the Schild plots were 6.93 and 6.98, in male and female Sprague Dawley rats, respectively. There was no significant difference in the estimated pK_B values between male and female rats either; $pK_B=6.99\pm0.10$ ($n=13$) and 6.95±0.08 ($n=13$) for male and female rats, respectively. Mean lumen diameters (1_1) were 213±11 μm ($n=13$) and 202±9 μm ($n=13$), for male and female rats, respectively.

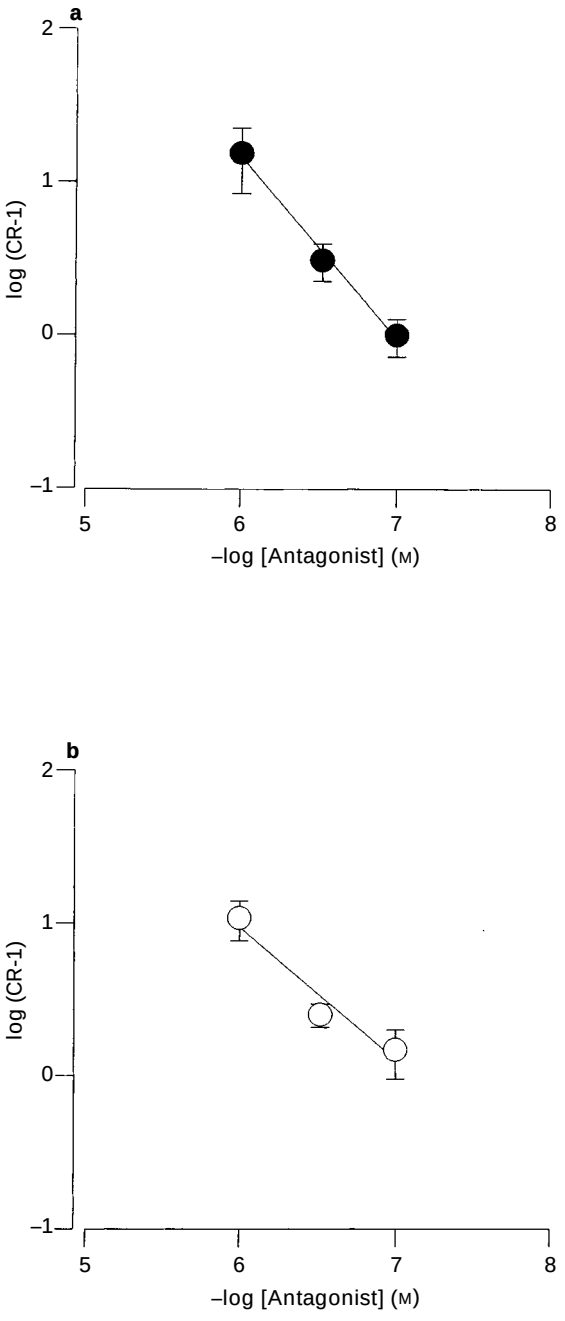

Figure 4 Schild plots for human-αCGRP)(8–37) in the concentrations of 1×10^{-7}, 3×10^{-7} and 1×10^{-6} M with rat-αCGRP (10^{-11}–10^{-6} M) as agonist in isolated coronary arteries from (a) male and (b) female Sprague Dawley rats. Each point represents mean of 4 or 5 separate experiments and vertical lines indicate s.e.mean where this value exceeds the size of the symbol.

Discussion

Our study shows that rat-αCGRP induces an endothelium-independent vasorelaxation in the intramural coronary arteries of both male and female Sprague Dawley rats. This is in good agreement with the results obtained in a previous study on coronary arteries from male Wistar rats (Prieto *et al*., 1991). However, CGRP induces endothelium-dependent relaxation in the proximal portion of the left coronary artery (Prieto *et al*., 1991) indicating heterogeneity in vasodilator mechanisms in different segments of the coronary circulation.

The two isoforms of rat-CGRP, rat-αCGRP and rat-βCGRP, produced similar concentration-response relations and were equipotent in the coronary arteries of male and female rats. Rat-amylin and rat-adrenomedullin, having 46% and 24% homology with CGRP and sharing the Cys-Cys ring structure (Wimsalawansa, 1996), produced significantly smaller relaxations with lower potencies compared to those of rat-α- and rat-βCCRP. In intramural coronary arteries, the general potency order seems to be rat-αCGRP ≈ rat-βCGRP > > rat-adenomedullin > rat-amylin. These data indicate that vasodilatation induced by these peptides is mediated by the CGRP-receptor, and that specific adenomedullin/amylin receptors are of insignificant importance for the overall responses.

Characterization of receptors relies generally on the estimation of antagonist affinities but a number of studies have shown heterogeneity of pA_2/pK_B values for human-αCGRP(8–37) with regard to species, tissue and regional variation within the same organ (Dennis *et al*., 1990; Foulkes *et al*., 1991; Poyner, 1992; 1995; Bell & McDermott, 1996) making CGRP-receptor characterization less stringent. The pK_B-values for human-αCGRP(8–37) which is accepted as a $CGRP_1$-receptor antagonist, cover a considerable range from about 6 to 8.5 in the rat (see review by Poyner, 1995). In large diameter pig coronary artery a pK_B value has been estimated of 5.7, whereas that for small diameter pig coronary artery was close to 7.0 (Foulkes *et al*., 1991). Another study showed that human-αCGRP(8–37) has competitive antagonistic activity in pig coronary arteries with a pK_B value of 6.7 (Gray *et al*., 1991). In rat mesenteric small artery, the pK_B value falls within the range 7.2 to 7.6 (Foulkes *et al*., 1991; Lei *et al*., 1994; Nuki *et al*., 1994). In rat thoracic aorta, human-αCGRP(8–37) seems to be a non-competitive antagonist of human-αCGRP (Gray *et al*., 1991) adding to the complexity of CGRP-receptor characterization.

Other factors not directly related to the receptor itself may also contribute to the variability in the antagonist affinity estimation. The presence of a peptidase metabolizing CGRP has been shown to interfere with the effects of two truncated forms of human-CGRP in rat vas deferens, where thiorphan (a peptidase inhibitor) was shown to potentiate the effect of human-αCGRP and human-αCGRP(8–37), but not that of human-βCGRP (8–37) leading to the conclusion that

differences in the effect of the truncated CGRP analogues may reflect differences in enzyme distribution rather than the existence of CGRP receptor subtypes (Longmore *et al*., 1994). Available data also suggest that the specific binding of human-αCGRP in rat L6 myocytes is sensitive to both ion concentration of Na^+ and Mg^{2+} and temperature (Poyner *et al*., 1992).

Experimental procedures can also affect estimation of affinity data for human-αCGRP. Foulkes *et al*. (1991) found that the affinity of small diameter pig coronary artery to human-αCGRP(8–37) was markedly altered by the experimental protocol. The pA_2 value for human-αCGRP(8–37) was around 7.02 when the vessel exposure to human-αCGRP(8–37) was minimized by using one concentration of human-αCGRP(8–37) per preparation, while it increased to 8.67 as the exposure time to human-αCGRP(8–37) was extended when three different increasing antagonist concentrations were used per vessel. The slope of the Schild-plot was also influenced by the exposure time, being markedly flatter (slope = 0.67) compared to that under minimized exposure conditions (slope = 0.84) (Foulkes *et al*., 1991). Foulkes *et al*. (1991) also showed that administration of human-αCGRP(8–37) itself to porcine coronary arteries precontracted with acetylcholine, elicited a further contraction in a third of the preparations. Finally, the $CGRP_2$-agonist [$Cys(Acm)^{2,7}$]-human-αCGRP also induces contractions in cerebral arteries at concentrations lower than 10^{-11} M (Jensen-Olesen, 1997). We did not observe any contractile effect of either the CGRP-antagonist or the selective $CGRP_2$-agonist in the coronary arteries from male and female rats. Thus, because a number of factors can modify the pA_2/pK_B value for human-αCGRP(8–37), caution must be taken when comparing these values between experiments, and characterization of $CGRP_1$-receptors then relies more on qualitative characteristics in terms of sensitivity to antagonists and agonists than exact affinity data.

In summary, our results showed that CGRP induces endothelium-independent relaxation which is antagonized by a selective $CGRP_1$-receptor antagonist, exhibiting unitary receptor characteristics in Schild plots, the selective $CGRP_2$-receptor agonist failed to induced significant relaxations, and finally, rat-adrenomedullin and rat-amylin, which both have relatively high homology with CGRP was much less potent than CGRP itself. All these observations strongly indicate that CGRP mediates vasodilatation through the $CGRP_1$-receptor subtype in rat isolated coronary small arteries. The reactivity of rat coronary arteries to CGRP as well as the receptor characteristics were not influenced by gender.

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References

AIYAR, N., RAND, K., ELSHOURBAGY, N.A., ZENG, Z., ADAMOU, J.E., BERGSMA, D.J. & LI, Y. (1996). A cDNA encoding the calcitonin gene-related peptide type 1 receptor. *J. Biol. Chem.,* **271,** 11325–11329.

AMARA, S.G., JONAS, V., ROSENFELD, M.G., ONG, E.S. & EVANS, R.M. (1982). Alternatively RNA processing in calcitonin gene expression mRNAs encoding different polypeptide products. *Nature,* **298,** 240–244.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.,* **14,** 48–58.

BARAKAT, A., ROSSELIN, G. & MARIE, J.C. (1993). Characterization of specific calcitonin gene-related peptide receptors present in hamster pancreatic β cells. *Biosci. Rep.,* **13,** 221–229.

BELL, D. & MCDERMOTT, B.J. (1996). Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. *Pharmacol. Rev.,* **48,** 253–288.

CHATTERJEE, T.K. & FISHER, R.A. (1995). Multiple affinity and guanine nucleotide sensitive forms of the calcitonin gene-related peptide (CGRP) receptor. *Can. J. Physiol. Pharmacol.,* **73,** 968–973.

CHATTERJEE, T.K., MOY, J.A., CAI, J.J., LEE, H.C. & FISHER, R.A. (1992). Solubilization and characterization of a guanine nucleotide-sensitive form of the calcitonin gene-related peptide receptor. *Mol. Pharmacol.,* **43,** 167–175.

DENNIS, T., FOURNIER, A., CADIEUX, A., POMERLEAU, F., JOLICOEUR, F.B., ST-PIERRE, S. & QUIRION, R. (1990). hCGRP8–37, a calcitonin gene-related peptide antagonist revealing calcitonin gene-related peptide receptor heterogeneity in brain and periphery. *J. Pharmacol. Exp. Ther.,* **254,** 123–128.

ENTZEROTH, M., DOODS, H.N., WIELAND, H.A. & WIENEN, W. (1995). Adrenomedullin mediates vasodilation via $CGRP_1$ receptors. *Life Sci.,* **56,** PL19–PL25.

FOULKES, R., SHAW, N., BOSE, C. & HUGHES, B. (1991). Differential vasodilator profile of calcitonin gene-related peptide in porcine large and small diameter coronary artery rings. *Eur. J. Pharmacol.,* **201,** 143–149.

FRANCO-CERECEDA, A., HENKE, H., LUNDBERG, J.M., PETERMANN, J.B., HÖKFELT, T. & FISCHER, J.A. (1987). Calcitonin gene-related peptide(CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. *Peptides,* **8,** 399–410.

FRANCO-CERECEDA, A., KALLNER, G. & LUNDBERG, J.M. (1993). Capsazepine-sensitive release of calcitonin gene-related peptide from C-fibre afferents in guinea-pig heart by low pH and lactic acid. *Eur. J. Pharmacol.,* **238,** 311–316.

GRAY, D.W., MARSHALL, I., BOSE, C., FOULKES, R. & HUGHES, B. (1991). Subtypes of the calcitonin gene-related peptide (CGRP) receptor in vascular tissues. *Br. J. Pharmacol.,* **102,** 189P.

JENSEN-OLESEN, I. (1997). The $CGRP_2$-receptor agonist, [$Cys(Acm)^{2,7}$]-CGRP, is a vasoconstrictor of guinea pig and human cerebral arteries. *Cephalalgia,* **17,** 387.

KAPAS, S. & CLARK, A.J. (1995). Identification of an orphan receptor gene as a type 1 calcitonin gene-related peptide receptor. *Biochem. Biophys. Res. Commun.,* **217,** 832–838.

KENAKIN, T.P. (1986). Analysis of dose-response data. In *Pharmacological Analysis of Drug-Receptor Interaction.* ed. Kenakin, T.P. pp. 31–51. New York: Raven Press.

LEI, S., MULVANY, M.J. & NYBORG, N.C. (1994). Characterization of the CGRP receptor and mechanisms of action in rat mesenteric small arteries. *Pharmacol. Toxicol.,* **74,** 130–135.

LONGMORE, J., HOGG, J.E., HUTSON, P.H. & HILL, R.G. (1994). Effect of two truncated forms of human calcitonin gene-related peptide: implications for receptor classification. *Eur. J. Pharmacol.,* **265,** 53–59.

MACKAY, D. (1978). How should values of pA_2 and affinity constants for pharmacological competitive antagonists be estimated? *J. Pharm. Pharmacol.,* **30,** 312–313.

MIMEAULT, M., QUIRION, R., DUMONT, Y., ST-PIERRE, S. & FOURNIER, A. (1992). Structure-activity study of $hCGRP_{8-37}$, calcitonin gene-related peptide receptor antagonist. *J. Med. Chem.,* **35,** 2163–2168.

MUFF, R., BORN, W. & FISCHER, J.A. (1995). Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions. *Eur. J. Endocrinol.,* **133,** 17–20.

MULVANY, M.J. & NYBORG, N. (1980). An increased calcium sensitivity of mesenteric resistance vessels in young and adult spontaneously hypertensive rats. *Br. J. Pharmacol.,* **71,** 585–596.

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,** 254–252.

NUKI, C., KAWASAKI, H., TAKASAKI, K. & WADA, A. (1994). Structure-activity study of chicken calcitonin gene-related peptide (CGRP) on vasorelaxation in rat mesenteric resistance vessels. *Jpn. J. Pharmacol.,* **65,** 99–105.

NYBORG, N.C.B. (1991). Ageing is associated with increased 5-HT_2-receptor affinity and decreased receptor reserve in rat isolated coronary arteries. *Br. J. Pharmacol.,* **102,** 282–286.

NYBORG, N.C.B., BAANDRUP, U., MIKKELSEN, E.O. & MULVANY, M.J. (1987). Active, passive and myogenic characteristics of isolated rat intramural coronary resistance arteries. *Pflugers Arch./Eur. J. Physiol.,* **410,** 664–670.

NYBORG, N.C.B. & MIKKELSEN, E.O. (1985). Characterization of β-adrenoceptors subtype in isolated ring preparations of intramural rat coronary small arteries. *J. Cardiovasc. Pharmacol.,* **7,** 1113–1117.

POYNER, D. (1992). Calcitonin gene-related peptide: multiple actions, multiple receptors. *Pharmacol. Ther.,* **56,** 23–51.

POYNER, D. (1995). Pharmacology of receptors for calcitonin gene-related peptide and amylin. *Trends Pharmacol. Sci.,* **16,** 424–428.

POYNER, D.R., ANDREW, D.P., BROWN, D., BOSE, C. & HANELY, M.R. (1992). Pharmacological characterization of a receptor for calcitonin gene-related peptide on rat, L6 myocytes. *Br. J. Pharmacol.,* **105,** 441–447.

PRIETO, D., BENEDITO, S. & NYBORG, N.C.B. (1991). Heterogeneous involvement of endothelium in calcitonin gene-related peptide-induced relaxation in coronary arteries from rat. *Br. J. Pharmacol,* **103,** 1764–1768.

QUIRION, R., VAN ROSSUM, D., DUMONT, Y., ST-PIERRE, S. & FOURNIER, A. (1992). Characterization of CGRP1 and CGRP2 receptor subtypes. *Ann. New York Acad. Sci.,* **657,** 88–105.

STANGL, D., MUFF, R., SCHMOLCK, C. & FISCHER, J.A. (1993). Photoaffinity labeling of rat calcitonin gene-related peptide receptors and adenylate cyclase activation: identification of receptor subtypes. *Endocrinology,* **132,** 744–750.

TOMLINSON, A.E. & POYNER, D.R. (1996). Multiple receptors for calcitonin gene-related peptide and amylin on guinea-pig ileum and vas deferens. *Br. J. Pharmacol.,* **117,** 1362–1368.

VALDEMARSSON, S., EDVINSSON, L., HEDNER, P. & EKMAN, R. (1990). Hormonal influence on calcitonin gene-related peptide in man: effects of sex difference and contraceptive pills. *Scand. J. Clin. Lab. Invest.,* **50,** 385–388.

WESTFALL, T.C. & CURFMAN-FALVEY, M. (1995). Amylin-induced relaxation of the perfused mesenteric arterial bed: meditation by calcitonin gene-related peptide receptors. *J. Cardiovasc. Pharmacol.,* **26,** 932–936.

WIMALAWANSA, S.J. (1996). Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology and therapeutic potentials. *Endocrinol. Rev.,* **17,** 533–585.

WIMALAWANSA, S.J., GUNASEKERA, R.D. & ZHANG, F. (1993). Isolation, purification, and characterization of calcitonin gene-related peptide receptor. *Peptides,* **14,** 691–699.

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