Investigation of CGRP Receptors and Peptide Pharmacology in Human Coronary Arteries. Characterization with a Nonpeptide Antagonist

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Received April 22, 2002; accepted September 26, 2002

ABSTRACT

Calcitonin gene-related peptide (CGRP), adrenomedullin (AM), and amylin are structurally related peptides mediating vasorelaxation in the coronary circulation possibly via CGRP receptors (subtypes 1 or 2). Functional CGRP receptors appear to consist of at least three different kinds of proteins: the calcitonin receptor-like receptor (CRLR), receptor-activity-modifying proteins (RAMPs) and the receptor component protein (RCP). No CGRP receptor has yet been cloned. Using reverse transcriptase-polymerase chain reaction, the presence of mRNA sequences encoding CRLR, RCP and RAMPs was demonstrated in human coronary arteries. Relaxant responses were studied on isolated segments of coronary arteries after precontraction with U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F2α). The human peptides αCGRP, AM, and amylin induced relaxation with mean pEC50 values of 8.6, 6.8, and 6.3 M, respectively. Preincubation with αCGRP8–37 (10⁻⁷ -10⁻⁵ M) and a novel nonpeptide CGRP antagonist “Compound 1” (WO98/11128) (10⁻⁷ –10⁻⁵ M) caused a dose-dependent rightward shift of the concentration-response curves for αCGRP with pA2 values of 7.0 and 7.1, respectively. Preincubation with αCGRP8–37 (10⁻⁶ M) and Compound 1 (10⁻⁶ M) caused significant rightward shift of the concentration-response curves for AM and amylin as well with pKb values between 6.6 and 7.5. Preincubation with AM22–52 had no antagonistic effect on the AM and amylin response, neither did diacetoamidomethyl cysteine CGRP cause any concentration dependent (10⁻¹¹–10⁻⁶ M) dilatation. In conclusion, mRNA for the components forming CGRP, AM and receptor components formed CGRP receptors. AM receptors was detected in the human left anterior descending coronary arteries, AM receptors, and AM amylin mediated vasorelaxation via the CGRP receptor. Compound 1 acted as a nonpeptide antagonist at the CGRP receptor and could thus become a tool for the study of CGRP-mediated functional responses in human tissue.

Calcitonin gene-related peptide (CGRP), adrenomedullin (AM), and amylin are structurally related peptides with effect in the coronary circulation. The two isoforms of CGRP, α- and β-CGRP, have similar biological activities (Morris et al., 1984). CGRP is released from peripheral sensory nerves, and a rich supply of CGRP-immunoreactive nerve fibers has been demonstrated at the adventitial- medial border of human coronary arteries (Gulbenkian et al., 1993). CGRP potently relaxes human isolated coronary arteries (Gulbenkian et al., 1993) and has even been demonstrated to cause dilation of coronary arteries at the site of atheromatous stenoses and to delay the onset of myocardial ischemia during treadmill exercise in patients with chronic stable angina (Uren et al., 1993).

AM was originally discovered in a human pheochromocytoma (Kitamura et al., 1993). AM is a circulating vasodilator peptide expressed in a number of cell types including vascular cells (Kitamura et al., 1995). AM has been demonstrated to induce relaxation in different vascular beds and to in-

ABBREVIATIONS: CGRP, calcitonin gene-related peptide; AM, adrenomedullin; CRLR, calcitonin receptor-like receptor; [Cys(ACM)²⁻], diacetoamidomethyl cysteine; RAMP, receptor-activity-modifying protein; RCP, receptor component protein; U46619, (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F2α); Compound 1 (WO98/11128), (4-(2-oxo-2,3-dihydro-benzoimidazol-1-yl)-piperidine-1-carboxylic acid [1–3,5-dibromo-4-hydroxy-benzyl]-2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-amide; LAD, left anterior descending coronary artery; RT-PCR, reverse transcriptase-polymerase chain reaction; bp, base pair(s).
crease coronary blood flow in conscious sheep (Parkes, 1995). Furthermore, AM has important antiproliferative actions on vascular cells (Kano et al., 1996), and the basal production of AM in the human coronary circulation was attenuated in subjects with coronary atherosclerosis, possibly due to the atherosclerosis-induced endothelial dysfunction and thereby decreased AM production (Hojo et al., 2000).

Amylin was originally found in the islet β-cells of the pancreas (Cooper et al., 1987). It is co-secreted from the β-cells with insulin in response to glucose. The expression of amylin has been demonstrated in other tissues but never in vascular cells. Amylin is a well known vasodilator, although its most important effect probably is to reduce the tissue-glucose response to insulin (Feuerstein et al., 1995).

Based on functional studies, two receptor subtypes for CGRP were originally proposed by Quirion and coworkers, termed CGRP1 and CGRP2 (Dennis et al., 1989). Until now the C-terminal fragment of αCGRP, αCGRP 8–37 and diacept-amidomethyl cysteine, [Cys(ACM) 2,7]CGRP have been the tools used in the classification of CGRP receptors. Thus, αCGRP 8–37 has high affinity (pA2 = 7–8) for the CGRP1 receptor but low affinity (pA2 = 5.5–6.5) for the CGRP2 receptor, whereas [Cys(ACM) 2,7]CGRP has high affinity for the CGRP2 receptor but low affinity for the CGRP1 receptor (Juanaeda et al., 2000). This classification represents a good framework but is likely to be a simplification of the real situation. Thus, a wide variety of pA2 values for αCGRP 8–37 have been reported, from below 6 (Giuliani et al., 1992) to above 9 (Longmore et al., 1994). Some of the variation may reflect species or experimental variation, but the spread remains remarkably wide. Different studies have indicated the presence of CGRP1 receptors in the coronary arteries in different species (Sheykhzade and Nyborg, 1998). In the porcine coronary arteries, CGRP, AM, and amylin mediate vasorelaxant effect via the CGRP receptor, probably the CGRP1 receptor (Hasbak et al., 2001). Different studies have speculated in the presence of CGRP2 receptors in large (external diameter >1 mm) coronary arteries based on functional studies using αCGRP 8–37 and [Cys(ACM) 2,7]CGRP (Waugh et al., 1999). But recently, the functional CGRP2 receptor in the porcine coronary artery was identified as a CGRP1 receptor by radioligand binding and RT-PCR (Rorabaugh et al., 2001).

No molecular cloning strategies have yet succeeded in isolating a CGRP2 receptor. Novel nonpeptide CGRP receptor antagonists have been introduced, and they may be promising tools for future studies of the complicated CGRP pharmacology (Doods et al., 2000; Aiyar et al., 2001; Edvinsson et al., 2001).

Investigations indicate that the calcitonin receptor-like receptor (CRLR) form the basis of the receptors for CGRP and AM. Thus, CGRP and AM bind to the same receptor, the calcitonin receptor-like receptor (CRLR), with receptor specificity being determined by receptor-activity-modifying proteins (RAMPs). Three different RAMPs have been described in human tissue, RAMP1, RAMP2, and RAMP3. Coexpression of RAMP1 and CRLR reveals a CGRP receptor, whereas coexpression of RAMP2 or RAMP3 and CRLR form an AM receptor (McLatchie et al., 1998). In addition to the RAMPs, the CGRP receptor complex might require another accessory protein to function optimally. The CGRP receptor component protein (RCP) is expressed in CGRP responsive tissues, and RCP protein expression correlates with the biological efficacy of CGRP in vivo (Evans et al., 2000).

The purpose of the present study was to detect mRNA encoding the human CRLR, RAMP1 to 3, and RCP in the human coronary arteries. Using the classic tools, αCGRP 8–37 and [Cys(ACM) 2,7]CGRP, we wanted to determine the CGRP receptor subtype by which αCGRP, AM, and amylin mediated vasorelaxation and further to test the antagonistic properties of the novel nonpeptide CGRP antagonist Compound 1 (WO 98/11128) and AM 22–52.

Materials and Methods

The investigations conform to the principles outlined in the World Medical Association Declaration of Helsinki (1997). The collection of human tissue was in accordance with institutional guidelines, and the local ethics committee at each institution approved the project. (Ethical committee of Copenhagen, Denmark; registration number K99046m.)

Vessels. Explanted human hearts were obtained from seventeen patients with dilated cardiomyopathy (eight women and nine men; age = 44.7 ± 7.9, mean ± S.E.M) undergoing heart transplantation at Rigshospital, Copenhagen, Denmark or University Hospital, Lund, Sweden. The left anterior descending (LAD) coronary artery was isolated near the apex of the heart, and fat and connecting tissue were removed under a microscope. All vessels were without any macroscopic atheromatous plaques. The artery, approximately 1 to 2 mm in external diameter, was cut into ring segments, 2 mm long. The vessels were then transported to our laboratory in ice-cold physiological salt solution (154 mM NaCl; DAKO, Glostrup, Denmark). Approximately 5 h (4.7 ± 2.4, mean ± S.E.M) elapsed between the removal of the vessels and the time when the tissue was received in the laboratory.

Vasomotor Responses. Each vessel segment with intact endothelium (confirmed by histological examination and by substance P-induced vasorelaxation) was mounted in a temperature-controlled tissue bath (37°C) containing a buffer solution (119 mM NaCl, 15 mM NaHCO3, 4.6 mM KCl, 1.5 mM CaCl2, 1.2 mM NaH2PO4, 1.2 mM MgCl2, and 5.5 mM glucose). The bath was continuously bubbled with a mixture of 95% O2 and 5% CO2, giving a pH of approximately 7.4. The vessels were equilibrated for 30 min before beginning the experiments.

Fig. 1. Demonstration of mRNA encoding the calcitonin receptor-like receptor (CRLR) (size 445 bp), receptor-activity-modifying protein 1 (RAMP1) (size 445 bp), RAMP2 (size 283 bp), RAMP3 (159 bp), and RCP (size 392 bp) in the human left anterior descending coronary artery by RT-PCR. Ladder, 100-base pair ladder; blind, negative controls without the reverse transcriptase enzyme.
7.4. To measure the isometric circular wall tension of the vessels, each segment was suspended between two L-shaped metal pins (0.2 mm in diameter) in a myograph (model 610M; Danish Myo Technology, Aarhus, Denmark). As previously described (Mulvany and Halpenny, 1977) and to achieve maximal active force development, the vessels were initially stretched to equalize 90% of L_{100} (L_{100} equals the distance between the pins if the vessel is exposed to a passive transmural pressure of 13.3 kPa). After approximately 1 h, the vessels were depolarized when exposed to a buffer solution containing 60 mM KCl, obtained by substituting equimolar concentrations of NaCl for KCl in the previously described buffer solution. Only vessel segments responding with a reproducible potassium-induced relaxation response in the same vessel segment was compared. When testing the effect of potential antagonists, the compound was added to the tissue bath 30 min before adding cumulative concentrations of agonist. To prevent tachyphylaxis, only one concentration-response experiment was allowed on each artery segment.

**Drugs.** The human forms of the peptides αCGRP, αCGRP_{8-37}, [Cys(ACM){\textsuperscript{6,7}}]CGRP, AM, AM\textsubscript{22-52}, and amylin were obtained from Bachem AG, Bubendorf, Switzerland. U46619 was purchased from Sigma-Aldrich (St. Louis, MO) Compound 1 (4-(2-oxo-2,3-dihydrobenzoimidazol-1-yl)-piperidine-1-carboxylic acid [1-3,5-dibromo-4-hydroxybenzyl]-2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-amide, Compound 1, Karl Thomaes GmbH, WO 98/11128) was synthesized by Medicinal Chemistry, Merck Research Laboratories (Rahway, NJ). All peptides were dissolved in distilled water, U46619 was synthesized by Karl Thomae GmbH, WO 98/11128.

**Table 1**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist (log M)</th>
<th>n_P</th>
<th>pEC_{50}</th>
<th>pK_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>αCGRP</td>
<td>αCGRP_{8-37} (-7)</td>
<td>11</td>
<td>8.6 ± 0.04</td>
<td>7.3 (7.2–7.3)</td>
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<tr>
<td></td>
<td>αCGRP_{8-37} (-6)</td>
<td>11</td>
<td>8.2 ± 0.02</td>
<td>6.9 (6.9–7.0)</td>
</tr>
<tr>
<td></td>
<td>αCGRP_{8-37} (-5.5)</td>
<td>6</td>
<td>7.0 ± 0.03</td>
<td>7.0 (6.8–7.2)</td>
</tr>
<tr>
<td>AM</td>
<td>Compound 1 (-7)</td>
<td>11</td>
<td>8.2 ± 0.02</td>
<td>7.2 (7.2–7.3)</td>
</tr>
<tr>
<td>AM</td>
<td>Compound 1 (-6)</td>
<td>11</td>
<td>7.6 ± 0.04</td>
<td>6.9 (6.9–7.1)</td>
</tr>
<tr>
<td></td>
<td>Compound 1 (-5.5)</td>
<td>6</td>
<td>7.2 ± 0.04</td>
<td>6.8 (6.5–7.1)</td>
</tr>
<tr>
<td></td>
<td>Compound 1 (-5)</td>
<td>6</td>
<td>6.7 ± 0.04</td>
<td>6.8 (6.5–7.0)</td>
</tr>
<tr>
<td></td>
<td>AM_{22-52} (-6)</td>
<td>7</td>
<td>6.3 ± 0.04</td>
<td>6.8 (6.5–7.0)</td>
</tr>
<tr>
<td></td>
<td>Compound 1 (-6)</td>
<td>9</td>
<td>5.3 ± 0.13</td>
<td>7.5 (7.2–7.7)</td>
</tr>
<tr>
<td></td>
<td>AM_{22-52} (-6)</td>
<td>9</td>
<td>6.7 ± 0.02</td>
<td>N.E.</td>
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<tr>
<td>Amylin</td>
<td>αCGRP_{8-37} (-6)</td>
<td>9</td>
<td>5.5 ± 0.16</td>
<td>6.6 (6.2–7.0)</td>
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<td></td>
<td>Compound 1 (-6)</td>
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<td>5.4 ± 0.15</td>
<td>6.8 (6.4–7.1)</td>
</tr>
<tr>
<td></td>
<td>AM_{22-52} (-6)</td>
<td>10</td>
<td>6.2 ± 0.04</td>
<td>N.E.</td>
</tr>
</tbody>
</table>

N.E., not estimated.

\( ^{a} \) Significant difference between values compared with the αCGRP response.

\( ^{b} \) Significant difference between values compared with the AM response.

\( ^{c} \) Apparent pEC_{50} or pK_B value.

\( ^{d} \) Significant difference between values compared with the amylin response.

**Fig. 2.** Concentration-response curves for αCGRP, AM, and amylin in human coronary arteries. Relative responses are given as percentage of fraction of the initial vessel response to U46619 (10^{-7.5} M) just before they were challenged with the relaxant peptides. Points represent mean values, and vertical lines indicate S.E.M.
dissolved in ethanol, and Compound 1 was dissolved in dimethyl sulfoxide. Human serum albumin (0.2%) was added to the final concentration of all reagents in the tissue bath.

**Molecular Experiments.** Primer pairs were designed to detect mRNA for human RCP (forward: 5'-H11032-GTC AAG GAT GCC AAT TCT GC-3' and reverse: 5'-H11032-TTC TTC TGC TCA GCC TCT GG-3'). The isolation of mRNA and RT-PCR assay for CRLR, RAMP1, RAMP2, and RAMP3 mRNA were performed using the primers and method previously described (Sams and Jansen-Olesen, 1998).

**Data Analysis and Statistics.** The concentration-response curves for αCGRP, AM, and amylin were analyzed by iterative non-linear regression analysis and the sensitivity to agonists expressed as pEC50 (log of EC50; concentration of the agonist that produced 50% of the maximal response), using GraphPad Prism 3.02 (GraphPad Software, Inc., San Diego, CA). A “true” pEC50/pKb value could not be estimated for every concentration-response curve due to the limitation of agonist in higher doses. However, assuming an S-shaped concentration-response curve, an apparent pEC50/pKb value was calculated using GraphPad Prism. The relaxant responses of each peptide are expressed as a percentage of the contraction induced by U46619 (10⁻⁷ M). Results are given as mean ± S.E.M (n), where n is the number vessels, each vessel from a different patient. The effects of agonists and antagonists were examined by comparing responses before and after antagonist treatment by means of one-way analysis of variance followed by Dunnett’s test (Winer, 1971). With a single concentration of antagonist, an apparent pKb value was calculated using the Gaddum equation: pKb = log(DR-1) - log[B], where DR is the concentration ratio of the EC50 values in the presence and absence of the antagonist and [B] is the molar concentration of the agonist.

Where multiple concentrations of antagonists were used, a Schild plot of log(DR-1) against log[B] was plotted using the estimated
Results

Molecular Experiments. The presence of mRNA encoding CRLR, RAMP1, RAMP2, RAMP3, and RCP in human coronary arteries was demonstrated by RT-PCR (n = 3) (Fig. 1).

Vasomotor Responses. Compound 1 and the human fragments αCGRP8–37, AM22–52 had no significant effect on the vasoconstriction induced by U46619 when added in doses up to 10^{-5} M. Data not shown.

Comparison of αCGRP, AM, and Amylin. All peptides induced concentration-dependent relaxation of the human coronary arteries (Fig. 2, Table 1). For αCGRP the pEC_{50} value was 8.6 ± 0.04 and the maximal relaxation 100 ± 0% (αCGRP 10^{-6} M), mean ± S.E.M., calculated as percentage of the precontraction induced by U46619 (10^{-7.5} M). The pEC_{50} values for AM and amylin were 6.8 ± 0.05 and 6.3 ± 0.04, respectively. Since the highest dose (10^{-6} M) of AM and amylin only caused 87.2 ± 6.4 and 68.1 ± 6.4% relaxation, it is not clear whether or not maximal relaxation was achieved.

Comparison of αCGRP8–37 versus Compound 1. Pre-incubation with αCGRP8–37 (10^{-7}–10^{-5} M) and Compound 1 (10^{-7}–10^{-5} M) both induced concentration-dependent rightward shift of the αCGRP concentration-response curve (Figs. 3, top panel and 4, top panel and Table 1). Using the pEC_{50} values the Schild plot analysis revealed pA_{2} values of 7.0 (6.9–7.2) and 7.1 (6.9–7.4) for
αCGRP8–37 and Compound 1 (Figs. 3, bottom panel and 4, bottom panel). Preincubation with αCGRP8–37 (10^{-6} M) and Compound 1 (10^{-6} M) also caused significant rightward shifts of the concentration-response curves for AM and amylin (Figs. 5 and 6; Table 1) with estimated pK_i values between 6.6–7.5 (Table 1).

**Effect of AM22–52 and [Cys(ACM)2,7]CGRP.** Preincubation with AM22–52 (10^{-6} M) caused no significant rightward shift and did not affect the maximal response of the concentration-response curves for AM and amylin (Figs. 5 and 6; Table 1). Only a very weak vasorelaxant effect of [Cys(ACM)2,7]CGRP was observed with maximal relaxation of 3.8 ± 0.3% ([Cys(ACM)2,7]CGRP 10^{-6} M) on human coronary arteries (n_T = 5) (Table 1).

**Discussion**

Recently Compound 1, a novel nonpeptide CGRP receptor antagonist was introduced (Edvinsson et al., 2001) as a functional CGRP receptor blocker in human SK-N-MC cells with a pK_i value of 7.8 and in human cerebral arteries with a pA_2 values of 7.7 (a study performed with vessels from only three patients). In the human coronary and cerebral arteries the pA_2 values of αCGRP8–37 and Compound 1 were almost similar indicating that these receptor antagonists block the same receptor mediating the vasorelaxant effects of CGRP. But in the present study the pA_2 value of Compound 1/αCGRP8–37 was around 7 suggesting that the affinity of CGRP to the receptor site are lower in the coronary circulation compared with the cerebral circulation. Furthermore, αCGRP itself seems to be 10-fold more potent in cerebral arteries (pEC_50 = 9.6 ± 0.1) (Edvinsson et al., 2001) compared with coronary arteries (pEC_50 = 8.6 ± 0.04), which might be explained by the difference in artery diameter and/or maybe different receptor concentrations in the two studies. The coronary arteries used in this study were obtained from patients with dilated cardiomyopathy and although no atheromatous plaques were identified in the arteries, we do not know to what extent these arteries can be considered “normal” and representative of the human coronary circulation.

Two different nonpeptide compounds with proposed antagonistic effect at the CGRP receptor have recently been reported; BIBN 4096BS (Doods et al., 2000) and SB-273779 (Aiyar et al., 2001). BIBN 4096BS demonstrated picomolar affinity for [125I]CGRP binding to SK-N-MC cell membranes and was characterized as a human-selective antagonist (Doods et al., 2000), whereas SB-273779 demonstrated an antagonist effect only at pharmacological (submicromolar) concentrations at the CGRP receptor but on the other hand served as a “cross-species” (human, porcine, rat) antagonist. Compound 1 like BIBN 4096BS probably has human-selective antagonistic properties as no antagonistic effect were found in the guinea pig basilaris arteries (Edvinsson et al., 2001) or in the porcine coronary arteries (Hasbak et al., 2001). However, the pA_2 for αCGRP8–37 of 7.0 (6.9–7.2) is considerably below the pK_i value of around 9 found for human CRLR and RAMP1 in the SK-N-MC cell expression system (Edvinsson et al., 2001). But differences between pA_2/pK_i values for αCGRP8–37 in cell lines and in whole tissue are also seen with other species and one explanation could be that the receptors are better exposed in cell cultures than in whole tissue with different diffusion gradient barriers. Previously pA_2 values of 7.9 (Saetrum and Edvinsson, 1996) and 7.3 (Edvinsson et al., 2002) were reported for αCGRP8–37 in human coronary arteries, but relatively little CGRP pharmacology has been carried out in humans to establish which pA_2 values would be expected for CGRP_1 and CGRP_2 receptors. Using the common criteria to distinguish CGRP receptor subtypes the antagonist affinity for CGRP8–37 is consistent with a CGRP_1 receptor (Juaneda et al., 2000). Considering that both CGRP8–37 and Compound 1 blocked the vasorelaxant effect of AM and amylin with pK_i values of 6.6–7.5, it is likely to conclude that these peptides act via the CGRP_1 receptors as well.

The human AM fragment, AM22–52, has been used as a specific AM receptor antagonist in several studies but with conflicting results (for review Hinson et al., 2000). In some studies it has been proposed that AM22–52 is not a very potent antagonist at the AM receptor, and its specificity has been questioned (Hinson et al., 2000). CRLR has a higher affinity for RAMP1 than RAMP2 (Buhlmann et al., 1999), which

![Fig. 5.](https://example.com/fig5.png)
might explain the failure to detect a functional response to AM via the AM receptor in some tissues. In the present study, we found no significant inhibition of the vasorelaxant effects of AM and amylin after preincubation with AM22–52. Interestingly and in contrast to the present study, Terata et al. (2000) showed a significant antagonistic effect of AM22–52 and no blocking effect of αCGRP8–37 on the vasoconstriction to AM in human coronary arterioles (50–150 μm in diameter). Perhaps the receptor density is different in the arterioles in the human coronary circulation compared with coronary arteries with greater diameter. Another explanation could be that the pharmacology in this study is based on the use of peptides, which could be subject to enzyme attack. Maybe, AM22–52 could be metabolized and therefore not active.

RAMPs are proteins identified within the last few years (McLatchie et al., 1998), and they are present in various tissues such as human myocardium (Saetrum et al., 2000). They interact and modify the phenotype of at least two families of receptors, the CGRP and calcitonin receptor (Sexton, 1999). Three potential consequences of RAMP interaction with its associated receptors have been described: transport of the receptor to the cell surface, modification of the receptor glycosylation, and direct and indirect modification of the ligand binding site through association with the receptor at the cell surface (Foord and Marshall, 1999). Our results demonstrate the presence of mRNA sequences encoding the RAMP and CRLR in the human LAD coronary arteries. Expression and formation of CGRP and AM receptors are therefore possible (McLatchie et al., 1998). The RCP fragment represents a new class of proteins (Luebke et al., 1996) that couples the receptor to the cellular signal transduction pathway and facilitates signal transduction at G protein‐coupled receptors. Thus, functional CGRP and AM receptors require CRLR, RAMPs, and RCP (Evans et al., 2000).

In summary, mRNAs for the components of a CGRP receptor (CRLR + RCP + RAMP1) are present in human coronary arteries. The antagonist affinity for αCGRP8–37 is consistent with the CGRP1 receptor and the vasorelaxant effect of αCGRP, AM, and amylin in the human coronary arteries can solely be explained by interaction with the CGRP1 receptors. AM receptor mRNAs (CRLR + RCP + RAMP2 or 3) was also demonstrated, but AM did not appear to mediate any vasorelaxant effect via the AM receptor. This may be due to lack of specificity and potency of AM22–52 or the fact that CRLR has a higher affinity for RAMP1 than RAMP2. Compound 1, a novel nonpeptide CGRP receptor antagonist with human‐selective properties had almost identical effect compared with αCGRP8–37 and should thus be promising as a tool for future studies of human CGRP pharmacology.

References


Fig. 6. Concentration‐response curves for amylin in the presence or absence of blockers: αCGRP8–37 (10–6 M), Compound 1 (10–6 M), and AM22–52 (10–6 M) on relaxation induced by cumulative concentrations of amylin (10–11–10–6 M). Relaxant responses are given as percentage of precontraction induced by U46619 (10–5–10–4 M). Points represent mean values, and vertical lines indicate S.E.M.


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