Amylin: Localization, Effects on Cerebral Arteries and on Local Cerebral Blood Flow in the Cat

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Amylin and adrenomedullin are two peptides structurally related to calcitonin gene-related peptide (CGRP). We studied the occurrence of amylin in trigeminal ganglia and cerebral blood vessels of the cat with immunocytochemistry and evaluated the role of amylin and adrenomedullin in the cerebral circulation by in vitro and in vivo pharmacology. Immunocytochemistry revealed that numerous nerve cell bodies in the trigeminal ganglion contained CGRP immunoreactivity (-ir); some of these also expressed amylin-ir but none adrenomedullin-ir. There were numerous nerve fibres surrounding cerebral blood vessels that contained CGRP-ir. Occasional fibres contained amylin-ir while we observed no adrenomedullin-ir in the vessel walls. With RT-PCR and Real-Time–PCR we revealed the presence of mRNA for calcitonin receptor-like receptor (CLRL) and receptor-activity-modifying proteins (RAMPs) in cat cerebral arteries. In vitro studies revealed that amylin, adrenomedullin, and CGRP relaxed ring segments of the cat middle cerebral artery. CGRP and amylin caused concentration-dependent relaxations at low concentrations of PGF₂α-precontracted segment (with or without endothelium) whereas only at high concentration did adrenomedullin cause relaxation. CGRP₈₋₃₇ blocked the CGRP and amylin induced relaxations in a parallel fashion. In vivo studies of amylin, adrenomedullin, and CGRP showed a brisk reproducible increase in local cerebral blood flow as examined using laser Doppler flowmetry applied to the cerebral cortex of the α-chloralose–anesthetized cat. The responses to amylin and CGRP were blocked by CGRP₈₋₃₇. The studies suggest that there is a functional sub-set of amylin-containing trigeminal neurons which probably act via CGRP receptors.

KEY WORDS: amylin; adrenomedullin; CGRP; immunocytochemistry; vasomotor responses; cerebral blood flow

DOMAINS: neuroscience, cardiovascular biology, endocrinology, pharmacology, protein-protein interaction, intercellular communication, molecular pharmacology, membrane hormone action, biochemistry, molecular biology

INTRODUCTION

Amylin is a 37-amino acid peptide that shows 46% amino acid homology with calcitonin gene-related peptide (CGRP) and 15% homology with human calcitonin. Since the amylin and CGRP genes are located on chromosome 11 and 12, respectively, the two genes are probably the result of a duplication of a common ancestral gene. cDNA cloning has revealed that amylin is highly conserved among species. Amylin as well as CGRP exhibit vasodilator activity in peripheral and in cerebral blood vessels. By immunohistochemical determination amylin has been reported in the gastrointestinal tract. Immunocytochemistry has also revealed amylin-ir in neuroendocrine cells in the gastrointestinal tract. In pancreatic islet β-cells, where amylin is expressed in secretory granules, it is co-stored with insulin and co-secreted with insulin in response to stimulation.

Adrenomedullin is a fairly recently discovered 52-amino acid peptide, originally isolated from human pheochro-
Amyloid como en el caso de la amilina, que muestra un 24% de homología con el CGRP y muestra una homología con la amilina. El adrenomedullin relaja las arterias cerebrales y la adrenomedulina. 23-34. En algunas regiones del cerebro, la adrenomedulina se une a receptores G-Protein-coupled receptor familia de péptidos. Recientemente, McLaughlin y colaboradores 32 informaron que la adrenomedulina (AM) receptores, dependiendo en qué miembro de una nueva familia de proteínas transmembrana (AM) receptor actividad modificando proteínas, RAMPs) expresados. Co-exprésion de RAMP1 y CRLR revela un receptor CGRP, donde co-exprésion de RAMP2 o RAMP3 y CRLR forma un AM receptor 32. Similarmente, RAMPs pueden interactuar con el calcitonina (CT) receptor para inducir la expresión de distintos amilina receptor fenotipos 37. En el cerebro humano, CRLR y tres de los RAMPs 34. El estudio presentó para el examen de la distribución de la amilina, adrenomedulina, y CGRP en el cerebro del gato, utilizando inmunocitoquímica, para examinar con RT-PCR el presencia de CLRL y RAMPs, y a estudiar una posible función del role using in vitro and in vivo pharmacology.

MATERIALS AND METHODS

Tissue Processing

Ocho gatos fueron anestesiados y muertos por una sobredosis de pentobarbital (50 mg/kg, i.p.). El encéfalo se retiró de cada el trigémino ganglio, secciones de la cortical medial membra, y los vasos desde el círculo de Willis fueron disecados. Los especímenes fueron fijados por inmersión en un fluido de 2% formaldehído y 0.2% ácido o ácido buferado a pH 7.2 con 0.1 M buffer fosfato. Fueron lavados en una solución de Tyrode y se conservaron a 10% solución de sacarosa. Para la inmunocitoquímica el los especímenes fueron congelados en seco y seccionaron en un corte a 10 μm. Algunos de ellos fueron congelados en seco y secados en un corte a 10 μm, se liberaron en un buffer fosfato, y se expusieron a caldo de cromocromóide de corte como secciones, y se prepararon como corte de microscopía.
design primers to recognize human RAMP1 mRNA sequences. The nucleic acid sequence of the forward primer was CCT GCC CCA TCA CCT CTT C, and of the reverse primer CCG TAG TTA GCC TCC TGG CA.

The primers were chosen to form minimal internal structure such as primer-dimer and/or hairpin structures. 1 μM of the forward and reverse primers were 59° and 58.9°C, respectively, and the length of the resulting cDNA amplicon was 51 bp.

A 50-μl reaction mixture for each well was used for the Real-Time PCR (GeneAMp 5a700, PE Biosystems, Perkin Elmer, USA). The composition of the final solution was as follows: MgCl₂, 3 mM; Syber green PCR-buffer, 1X, dNTP (dATP+dCTP+dGTP+dTTP) mixture, 0.2 mM; Amplitaq gold, 0.025 U/μl; forward primer of RAMP1, 0.9 μM; reverse primer of RAMP1, 0.9 μM; and 6 μl cDNA with arbitrary concentrations of 1 (no dilution), 0.4 (2.5 times diluted), and 0.08 (12.5 times diluted).

In Vitro Pharmacology

The middle cerebral artery (MCA) was removed from adult cats (n = 6) of either sex weighing between 3 and 5 kg sacrificed by exsanguination under pentobarbitone anesthesia. Ring segments, 2 mm long and 300-400 μm in diameter with intact endothelium were carefully prepared and suspended between two L-shaped metal prongs in tissue baths containing 2.5 ml of the buffer solution which was continuously gassed with 5% CO₂ in O₂ resulting in a pH of 7.4. The solutions were kept at 37°C. Isometric circular contractions were recorded through FT 03 C Grass transducers and recorded using a MacLab unit with the Chart® software. The vessels were given a passive load of 2 mN and allowed to stabilize at this level of tension for 1 ½ hr. The contractile capacities of the preparations were first tested by exposure to a buffer solution containing 60 mM potassium. This resulted in marked contractions 8.3 ± 1.4 mN. After two reproducible 60 mM potassium contractions had been obtained, prostaglandin F₂₀ (10⁻⁴ M) was applied. This produced a steady level of contraction during which the peptides under study were applied. In parallel experiments the ability to relax to acetylcholine (10⁻⁴ - 10⁻⁵ M) were taken as evidence for intact functional endothelium (data not shown). In separate tests the endothelium was removed by 10 sec of intraluminal perfusion with Triton X100 to study the role of the endothelium for relaxing by the peptides.

In order to examine if CGRP₈₋₃⁷ acted as antagonist it was given 15 min before agonist application. During all tests 4 or 8 ring segments were run in parallel tissue baths; in blockade experiments they were exposed to the antago-

nist or solvent only. As analyzed in separate experiments the responses were seen to remain stable throughout the test period. The influence of prostaglandin synthesis and nitric oxide on the responses to the CGRP family of peptides using indometacin (10⁻⁴ M) and L-NMMA (10⁻⁴ M) treatment respectively, were used.

The data are expressed as pEC₅₀ which is the concentration of agonist eliciting half maximum relaxation. The differences between the values were tested for statistical significance using Student’s t-test. p < 0.05 is considered statistically significant. All data are presented as the mean ± S.D. The dissociation constant, pKᵦ, was calculated according to Tallarida et al².

Solutions

The standard buffer solution used was of the following composition (mM): NaCl 119, KCl 4.6, CaCl₂ 1.5, MgCl₂ 1.2, NaHCO₃ 15, Na H₂ PO₄ 1.2, and glucose 5.5. The elevated potassium buffer was obtained by a partial (50%) equimolar substitution of NaCl for KCl resulting in a potassium concentration of 60 mM.

Drugs

The following agents were used in the experiments: Human α-calcitonin gene-related peptide (hCGRP); hCGRP₁₋₇; amylin; adrenomedullin (all obtained from Auspep, Australia); acetylcholine, L-NMMA, indometacin, prostaglandin F₂₀ (Sigma, USA). All drugs were dissolved and further diluted in 0.9% saline. The concentrations are expressed as the final molar concentration in the tissue bath.

Physiological Studies

All studies reported were carried out under a project license issued by the UK Home Office under the Animals (Scientific Procedures) Act of 1986. Eight cats (3.8 ± 0.3 kg, mean ± SD) were anesthetized with α-chloralose (60 mg/kg, i.p.) after halothane induction and ventilated with a mixture of 60% air-40% O₂ and placed in a stereotaxic device (David Kopf Instruments, Tujunga, CA). End-expiratory CO₂ was continuously monitored and adjusted by altering the stroke volume of a ventilator (Hugo-Basille); the fractional concentration of O₂ in inspired gas (FIO₂) was continuously observed (DATEX Instruments, Finland). Arterial blood gases were measured during the study. Polyethylene catheters were placed in the femoral artery and vein bilaterally
for measurement of blood pressure (CWE Instruments) and administration of drugs or fluids, respectively. The level of anesthesia was monitored by observing cardiovascular responses to noxious stimuli and supplemental doses (15–20 mg/kg a-chloralose, i.v.) administered at two hourly intervals.

**Blood Flow Measurements**

Cerebral cortical perfusion was measured continuously using laser Doppler flowmetry (LDF). The principles of operation of LDF have been thoroughly described and its use in this laboratory has been described in detail. Briefly, infrared light from a laser diode with a wavelength of 780 mm is directed to the surface of the brain via a fiber optic cable. The cable contains two further fibers that detect backscatter from a tissue area of approximately 1 mm². Analysis of the Doppler shift caused by that portion of light scattered by the red blood cells gives the velocity of flow for those cells, while the proportion of light backscattered is directly related to the volume of moving red cells in the brain sampled. A flow index can thus be derived from these signals. In these experiments the Moor Instruments (UK) device was used with a probe mounted in a stereotaxic manipulator (David Kopf Instruments, CA), and the signals for flow and volume continuously monitored.

Biparietal craniotomies were carried out with a low speed dental drill that was cooled with saline to prevent underlying thermal injury and the dura left intact under the probe although it was cut adjacent for drug administration. The probe was positioned over the superior aspect of the posterior parietal cortex to ensure adequate flow responses. Cerebrovascular reactivity to a brief period of hypercapnia (6–8%) was tested before collecting data and during the experiment as part of the protocol. Solutions of vehicle, amylarin or adrenomedullin, were injected directly into the cortex in a volume of 1 μl using a pressure-driven glass micropipette. Doses of 10⁻⁷ M, 10⁻⁸ M, or 10⁻⁹ M of each of amylarin or adrenomedullin, or CGRP (10⁻⁸ M) were injected into separate cortical areas. Preparation of the cortex bilaterally for injection effectively doubled the possible injection sites without additional use of animals.

In order to determine accurately the changes in cerebrovascular dynamics measured with the laser Doppler the physiological variables were monitored on-line by a microcomputer. The blood pressure, heart rate, end-expiratory CO₂, and laser Doppler volume and flow signals were passed to a signal conditioning device and then to an analog-to-digital converter (Data Translation DT2839, UK) in an 80486-based microcomputer. Signals were all continuously monitored and displayed on the computer screen using locally written software in C. All data were stored on disk for later analysis and plotting.

**Statistics**

In each animal, control data were collected prior to drug treatment and re-test. The CBF₁ DF data were converted to percentage change from the previous baseline level for the calculations. The data were analyzed as a complete cohort using a single way analysis of variance (SPSS version 8) – the Dunnett’s test for comparison to the vehicle injection and Scheffe’s test for comparison with ha-CGRP. Significance was assessed at the p < 0.05 level.

**RESULTS**

**Immunocytochemistry**

In the trigeminal ganglion numerous nerve cell bodies of varying size contained CGRP immunoreactivity (about 40 to 50% of all neurons). Occasional amylarin-ir nerve cell bodies (Fig. 1) were seen. Double immunostaining revealed that some of these co-localised with CGRP-ir while we saw no adrenomedullin-ir at all. A network of CGRP-ir nerve fibers was evident around cerebral blood vessels. Only few nerve fibers that contained amylarin-ir could be visualized around the vessels (Fig. 2) while nerve fibers containing adrenomedullin-ir were not seen.

**Molecular Biology**

The expression of CRLR, RAMP1, RAMP2, and RAMP3 was examined in MCA by RT-PCR. As positive control, expression of CRLR and RAMPs was found to be present in the human atrium (Fig. 3). In the cat MCA, strong signals to CRLR and RAMP3 were seen, while only traces could be detected upon UV examination of the agarose gel of RAMP1 and RAMP2. Consequently, we analysed RAMP1 with Real-Time PCR (Fig. 4). The primer pairs designed for recognition of the human RAMP1 appeared to recognize the mRNA from cat MCA with Real-Time PCR. Comparison of the peaks of the dissociation plot for the cat MCA with the human atrium, which was used as a reference, showed no difference in Tm for the two tissues, indicating no major differences in nucleic acid sequence of the amplicons.

The Cₐ-value (cut-off value) for the cat was 32.5 at a threshold of 0.5, which was much higher than the Cₐ-value of 23.2 (threshold 0.5) for the human atrial tissue at a comparable concentration (0.4), indicating a very low concen-
tation of RAMP1 mRNA in cat MCA. A comparable C<sub>t</sub>-value of the human atrial tissue was seen at a relative concentration of 0.00064 (C<sub>t</sub> = 32.2), i.e., at a concentration approximately 1000 times lower. However, these C<sub>t</sub>-values are outside the linear range of the standard curve and can only be used as a very rough guideline.

**In Vitro Pharmacology**

CGRP, amylin, and adrenomedullin induced relaxation of the ring segments of the cat middle cerebral artery. However, there were marked differences in reactivity to the peptides (Fig. 5). The cat MCA relaxed by 85 ± 5% (n = 10) and had a pIC<sub>50</sub> value of 8.3 ± 0.2 upon CGRP administration. This response was shifted toward higher a-CGRP concentrations in the presence of the antagonist ha-CGRP<sub>8-37</sub> (10⁻⁶ M) without any significant change in maximum effect. Amylin relaxed the MCA in a concentration-dependent manner within the same dose range but with less effect (Fig. 5). Also, the amylin response was antagonized by ha-CGRP<sub>8-37</sub> (10⁻⁶ M) in the same manner. Calculation of the pKₐ for CGRP<sub>8-37</sub> using only one concentration (10⁻⁴ M) resulted in a value of 7.0 ± 0.2 and 6.9 ± 0.3 with CGRP and amylin as agonists, respectively. Only in the highest dose tested (3 × 10⁻⁷ M) was a weak relaxation seen by adrenomedullin (Fig. 5). Removal of the endothelium in vitro or treatment with indomethacin (10⁻⁴ M) or L-NMMA (10⁻⁴ M) did not alter the vasodilator responses to CGRP, adrenomedullin, or amylin (data not shown).

**Cortical Blood Flow**

The cardiovascular and blood gas data for the animals were within the normal range for the anesthetized cat. The respective core physiological data (n = 8, mean ± SD) were as follows: pH 7.3 ± 0.03, pCO₂ 4.74 ± 0.52 kPa, blood pressure 97 ± 8 mmHg. All animals reported had intact hypercapnic vasodilatation prior to data collection. Administration
FIGURE 2. Perivascular amylin-immunoreactive nerve fibres in the cat. Whole mount of pial artery (×200).

FIGURE 3. Expression of CRLR and RAMP1, -2, and -3 in cat MCA and human atrium demonstrated by RT-PCR. Ladder shows 100 base-pair steps. Bands corresponding to the presence of mRNA encoding CRLR as well as RAMP1, -2, and -3 (length of products were 497, 445, 283, and 159 bp, respectively) are evident for both preparations. No bands are seen in the negative control (Blank) where mRNA was not reverse transcribed to cDNA prior to amplification (lack of RT enzyme).
locally of CGRP<sub>8-37</sub> did not alter the cerebral vasodilator response to hypercapnia. At the resting state CGRP (10<sup>-8</sup>M) increased the CBF<sub>LDF</sub> by 42 ± 6% and the vasodilatation was substantially blocked by CGRP<sub>8-37</sub>.

Administration of the vehicle in the amylin series of cortices demonstrated a change of 7 ± 1% (n = 5) for CBF<sub>LDF</sub> (Fig. 6). The maximum response seen with local injection of amylin (10<sup>-7</sup>M) was a 32 ± 4% (n = 5) increase in CBF<sub>LDF</sub>. The responses to amylin were significant as a cohort (F<sub>5,26</sub> = 24, p < 0.05). In turn, both doses of 10<sup>-7</sup>M and 10<sup>-8</sup>M (p < 0.05, Dunnett’s test) were significantly different from vehicle while 10<sup>-9</sup>M (p = 0.11, NS) was not (Table 1).

The dose of 10<sup>-7</sup>M was re-tested to produce a 30 ± 3% (n = 5) change which was reduced to 19 ± 2% after local administration of CGRP<sub>8-37</sub> 10<sup>-5</sup>M (p < 0.05).

Administration of vehicle in the adrenomedullin series of cortices demonstrated a change on 7 ± 2% (n = 5) for CBF<sub>LDF</sub>. The mean maximum response seen with local injection of adrenomedullin (10<sup>-7</sup>M) was 15 ± 2% (n = 5; p < 0.05). No effect significant from vehicle was seen with adrenomedullin 10<sup>-5</sup> or 10<sup>-4</sup>M (n = 5, respectively). After administration of CGRP<sub>8-37</sub> 10<sup>-5</sup>M the response to adrenomedullin 10<sup>-7</sup>M was 14 ± 2% (n = 5), which was not different to the pretreatment level.

**DISCUSSION**

Amylin, CGRP, and adrenomedullin belong to a family of structurally related peptides. They have in common a six or seven amino acid ring structure linked by a disulfide bridge as well as being C-terminally amidated. The C-terminal part of adrenomedullin exhibits approximately 25% homology with CGRP and amylin. Amylin mRNA has been demonstrated in extracts from rat dorsal root ganglia. Recently, using *in situ* hybridization and immunocytochemistry,
amylin was found in a population of small to medium sized nerve cell bodies in sensory ganglia in the rat\cite{note}. In the present study, we observed in the cat a subpopulation of amylin-ir nerve cell bodies in the trigeminal ganglion. Also it was seen that the amylin-containing nerve cell bodies constituted a subpopulation of those expressing CGRP. Amylin-ir containing nerve fibers have been seen in the dorsal horns of the spinal cord and, to a lesser extent, in peripheral tissues receiving sensory innervation\cite{note}. We found amylin-ir fibers in cerebral vessels, and it is reasonable to
FIGURE 6. Response of local cortical blood flow to injection of amylin. There is a concentration dependent increase in cortical blood flow measured by laser Doppler flowmetry (CBF<sub>LDF</sub>) with a maximum after amylin 10<sup>-7</sup> M. This effect is antagonised by the CGRP receptor blocker CGRP<sub>8-37</sub>.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Concentration (M)</th>
<th>%ΔCBF&lt;sub&gt;LDF&lt;/sub&gt; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylin</td>
<td>10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>32 ± 3</td>
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<tr>
<td></td>
<td>10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>28 ± 3</td>
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<td></td>
<td>10&lt;sup&gt;-9&lt;/sup&gt;</td>
<td>13 ± 2</td>
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<tr>
<td>Adrenomedullin</td>
<td>10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>15 ± 2</td>
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<td></td>
<td>10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>10 ± 2</td>
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<tr>
<td></td>
<td>10&lt;sup&gt;-9&lt;/sup&gt;</td>
<td>9 ± 2</td>
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presume that they originate in the trigeminal ganglion, since studies of other cranial ganglia have been negative (unpublished data).

The molecular biology experiments demonstrated the presence of mRNA for CLRL and all three RAMPs in the cat MCA; however, RAMP1 and RAMP2 showed low signals. This might be due to a difference between feline and human RAMPs or low levels of these RAMPs in the feline tissue. With Real-Time PCR we observed the presence of RAMP1 in feline MCA but in comparison with the human atrium the level was low. The latter observation agrees well with data obtained from human brain vessels<sup>44</sup>. Furthermore, there are studies that have demonstrated the expression of CGRP<sup>8</sup> and adrenomedullin receptors mRNA<sup>33</sup> in...
the cerebral circulation. However, the next question we addressed was if the mRNA is translated into functional receptors.

Amylin, adrenomedullin, and CGRP exert their effects by increasing the tissue level of cAMP\(^{35}\). It has been suggested that most of the reported effects of amylin can be explained through its interaction with CGRP receptors\(^{22}\). There are some indications that the CGRP blocker CGRP\(_{8-37}\) can antagonize the responses to amylin, adrenomedullin, and CGRP\(^{43}\). In rat cerebral arterioles adrenomedullin has been shown to induce a concentration-dependent vasodilatation, probably acting through CGRP receptors since this dilation was supressed by pretreatment with the CGRP receptor antagonist CGRP\(_{8-37}\). In contrast, amylin did not cause vasodilatation at concentrations up to 10°\(^{-6}\) M\(^{44}\).

In the cat MCA we observed that amylin, adrenomedullin, and CGRP caused relaxation in the presence or absence of the endothelium. The relaxations induced by amylin and CGRP were not significantly attenuated by L-NMMA or by indomethacin. This is in agreement with previous studies of cerebral vessels for CGRP where the relaxation has been shown to occur in parallel with activation of adenyl cyclase\(^{5,10}\). In other vascular regions the situation may differ. Inhibition of nitric oxide synthase reduced the adrenomedullin-induced hypotension and the hindquarter perfusion pressure of the rat\(^{12}\). Furthermore, the relaxant effect of CGRP on the rat thoracic aorta was dependent on the presence of the endothelium\(^3\) and could be blocked by haemoglobin, methylene blue\(^{13}\) or L-arginine analogues\(^{17,20}\), but not by indomethacin\(^{15}\). The results suggest that adrenomedullin and CGRP have similar inhibitory effects in rat aorta and that the effects are mediated by nitric oxide. On the other hand, the responses to amylin and CGRP in cat cerebral arteries is independent of the endothelium\(^10\). This agrees well with studies of adrenomedullin and CGRP in the porcine coronary artery\(^{45,48}\). Previously, we have shown that CGRP relaxes cerebral vessels via the accumulation of cAMP\(^{30}\). This is in concert with two reports on pig coronary artery demonstrating that adrenomedullin and CGRP decrease intracellular Ca\(^{2+}\) and Ca\(^{2+}\) sensitivity\(^{14,30}\).

We observed that amylin and CGRP caused a concentration-dependent increase in CBF, and that the effect was blocked by CGRP\(_{8-37}\). The maximum responses to amylin and CGRP were 32 ± 4% and 42 ± 6%, respectively, while adrenomedullin only increased the blood flow by 15 ± 2% (vehicle caused 7 ± 2%). CGRP\(_{8-37}\) had no effect on the adrenomedullin response. The lack of effect of CGRP\(_{8-37}\) on the adrenomedullin response agrees well with that seen in other vascular beds\(^{15,21}\) and in cultured human endothelial cells\(^{28}\), suggesting the existence of a population of adrenomedullin receptors that are distinct from the CGRP receptors\(^{43}\).

There are few previous studies examining the effects of amylin and adrenomedullin on CBF. Baskaya et al\(^2\) performed a comprehensive study in the dog showing similar potency of adrenomedullin and CGRP while amylin was 100 times less potent. This stands in contrast to our findings in the cat which had the following potency order, CGRP = amylin > adrenomedullin. Clearly, this suggests species differences in the CGRP receptor profile for these peptides. In vivo studies\(^2\) have shown that NOS inhibition is ineffective, while cAMP increases and that pretreatment with CGRP\(_{8-37}\) abolishes the adrenomedullin response. This is in agreement with our studies of CGRP in vitro\(^{10,24}\). The lack of effect of CGRP\(_{8-37}\) on adrenomedullin responses in the cat (present data) as compared to the dog\(^2\) may be due to a difference within the CGRP receptor population. Adrenomedullin had only weak dilator effects in our study. Since the amylin response was blocked by CGRP\(_{8-37}\) it further indicates that the cerebrovascular effects that have been observed are likely to be mediated via activation of CGRP receptors. This issue will not be resolved until specific agonist or antagonist compounds have been developed.

In the present study, we found that CGRP\(_{8-37}\) antagonized the relaxant effects of amylin and CGRP both in vitro and in vivo, but not adrenomedullin in vivo. The responses to adrenomedullin were very weak and seen only at the highest concentrations tested. CGRP\(_{8-37}\) is a competitive antagonist of CGRP-induced relaxant responses, providing a pK\(_a\) value of around 7.0. This agrees well with the present observations. Amylin also relaxed the cat middle cerebral artery in a concentration-dependent manner and CGRP\(_{8-37}\) caused a comparable pK\(_a\) value. Thus, it is likely that CGRP and amylin act at the same CGRP receptor in cat cerebral arteries.

In the porcine coronary artery adrenomedullin caused relaxation, but this was not antagonized by CGRP\(_{8-37}\)\(^{48}\). However, in isolated rat heart the adrenomedullin-induced vasodilation was blocked by CGRP\(_{8-37}\) suggesting activation of CGRP receptors\(^{31}\). In human vascular endothelial cells adrenomedullin increases the accumulation of cAMP and this was antagonized by CGRP\(_{8-37}\).\(^{28}\) The weak relaxant effect of adrenomedullin in cat middle cerebral arteries is supported by a recent study in which adrenomedullin receptor mRNA could not be detected in human brain vessels\(^{33}\). However, in spite of the lack of expression of adrenomedullin receptor mRNA, the peptide exerted activation of adenyl cyclase, probably through activation of CGRP receptors\(^{33}\). Thus, the parallelism with the present data suggests the presence of CGRP receptors in cat MCA, and that both amylin and adrenomedullin may be able to interact with the CGRP receptors to induce the vascular responses.

In conclusion, the study has documented the presence of amylin-ir in the cerebral circulation and in a subset of
cells in the trigeminal ganglion where it is co-localised with CGRP-ir. In isolated cerebral arteries amylin caused dilatation and in vivo amylin increased cerebral flow, each indicating a functional effect for amylin. The response seems, however to be mediated via the CGRP receptor since the available antagonist had the same effect on both amylin and CGRP responses. Further dissection of any role for amylin will require better selective agonists and antagonists to allow the complex relationships to be disentangled. Certainly, amylin containing trigeminal neurons must be included in any complete formulation of the physiology of the neural innervation of the cerebral circulation.

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