Clinical Investigation

Autoradiographic Mapping of Calcitonin Gene-Related Peptide Receptors in Human and Guinea Pig Hearts

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Calcitonin gene-related peptide (CGRP) is a 37–amino acid peptide that is a potent coronary vasodilator. Although CGRP is found in high concentrations around coronary arteries, its precise function in the control of coronary vasomotor tone remains unclear. We studied the distribution of specific receptors for CGRP in guinea pig and human hearts and found that the highest concentration of specific receptors for CGRP was in the major coronary arteries, which is consistent with the hypothesis that CGRP is implicated in control of coronary vasomotor tone. Areas of coronary artery with atheroma contained significantly decreased (158±35 grains/1,000 μm² tissue, n=3) binding sites compared with binding sites in normal arteries (266±10 grains/1,000 μm² tissue, n=11; p<0.001, t test). The decrease in receptors for CGRP around atheroma may predispose these vessels to coronary spasm. (Circulation 1990;81:741–747)

Calcitonin gene-related peptide (CGRP) is a 37–amino acid peptide, the existence of which was predicted by analysis of the calcitonin gene.1 CGRP has been detected in various tissues, including the thyroid gland,2 neuroendocrine cells,3 and peripheral sensory nerves, and its physiologic importance is unknown. CGRP is an extremely potent vasodilator in animal and human vessels,4 including coronary arteries.5 Immunohistochemical studies of animal and human tissues have shown that CGRP is localized to the nerves around the coronary arteries and arterioles.6 CGRP may be detected in the circulation in picomolar concentrations,4 and infusion of CGRP into the coronary arteries causes dilatation of large and small vessels.5,7 However, because CGRP almost certainly has its major effects by local release from nerves, the relevance of infusion studies may be questioned. Study of specific receptors, therefore, may provide a more accurate reflection of physiologic function.

Specific cell surface receptors for CGRP have been identified in a number of tissues by direct receptor-binding assay with [125I]-labeled CGRP.8 Because receptor-binding studies of membrane preparations do not give information about the localization of receptors, we investigated the distribution of CGRP receptors with an autoradiographic technique in human hearts removed at cardiac transplantation and in guinea pig hearts.

Methods

Tissue Preparation

Human coronary arteries and hearts were removed at cardiac transplantation. Five human hearts were studied, and all the patients were suffering from end-stage ischemic heart disease. The myocardial sections were taken from grossly noninfarcted tissue, and the arteries were a mixture of angiographically normal, stenosed, and occluded vessels. Guinea pig hearts were removed from freshly killed animals.

The tissues were snap-frozen in liquid dichlorodifluoromethane (Arcton 12, ICI, Macclesfield, UK) and cooled to −196°C by liquid nitrogen. Tissue sections (8–10 μm) were cut on a cryostat at −28°C and thaw-mounted onto glass microscope slides coated with poly-l-lysine. The sections were then stored at −80°C without loss of binding capacity.

Binding to Tissue Sections

Slides bearing tissue sections were preincubated in 50 mM Tris-HCl buffer (pH 7.4) for 5 minutes and then incubated with [125I]CGRP at 4°C. Saturation studies were performed with concentrations of CGRP ranging from 25 to 1,000 pM. Protein estima-
Autoradiography

Autoradiographs were generated by the method of Young and Kuhar. Slide-mounted sections were incubated as previously described, washed with ice-cold buffer (twice for 5 minutes each), and then rinsed in distilled water to remove residual salts, air dried rapidly, and stored dessicated overnight. Glass coverslips that had previously been coated with stripping film (AR 10 Kodak, London, UK) were then fixed to one end of the slide with cyanoacrylate adhesive and held in contact with the sections by butterfly clips. Slides were exposed to emulsion at 4°C for 5 days. The coverslips were then partially separated from the slides, and the sections and the emulsion were developed and fixed. The sections were stained with 1% cresyl violet and examined under a microscope equipped with light- and dark-field illumination.

Grains were counted on autoradiographs by using a calibrated eyepiece and were also counted on developed photographs. The relative binding to each site was compared by counting the individual grains with an oil emulsion lens (×1,000 magnification) in 12 separate areas of 7×7 μm² from each section studied, which yielded a total grain count area of nearly 600 μm² for each section. To minimize bias in the counting, the same squares in the graticule were assessed for each tissue, and the grains were counted by an individual who was not aware of which region was being counted. Similar results were obtained in separate tissue sections from the same and from separate guinea pigs. Grain counts in human tissue sections were made in a similar fashion.

Additional counts were made from developed photographs within a specified area, or in the case of small vessels, the total grains per vessel were counted, and the area of smooth muscle was calculated from a digitized image in the bright-field view. After correction for background counts, nonspecific binding was subtracted from the total binding in all cases.

Results

The binding of [125I]CGRP to guinea pig and human hearts was saturable and at equilibrium. Specific binding to heart sections reached a plateau at 60 minutes at 4°C with no further binding occurring in the next hour. Specific binding to guinea pig hearts was saturable and of high affinity. The binding analysis was run three times and gave Kd values of 600, 680, and 850 nM, and gave Bmax values of 77.8, 170, and 251.7 fmol/mg protein, respectively. A representative Scatchard plot is shown in Figure 1. A single human artery (microscopically normal) demonstrated saturable binding with a Kd of 670 nM and a Bmax of 58 fmol/mg protein. Unlabeled CGRP (1 μM) was effective in displacing the binding, whereas no displacement was seen with substance P or neurokinin A (1 μM).

As may be seen in the binding isotherm (Figure 1), there is a high level of nonspecific binding. However, as shown by the autoradiographs, nonspecific binding is attributable mainly to cardiac myocytes and associated interstitial stroma, which are compartments that have a low level of specific binding sites and account for the great bulk of the nonspecific binding seen in the isotherm. Thus, when studying sections of vessel, which contain the major concentration of specific binding sites, at a CGRP concentration of 250 μM, specific binding represented more than 50% of total binding in all sections studied.

High concentrations of CGRP were bound to coronary arteries (Figures 2 and 3), veins, and the cardiac valves (Figure 4). Arterioles, great vessels, and endocardium showed a lesser degree of specific binding. The large coronary arteries were more
densely labeled than small arteries (Figure 5), arterioles, and the great arteries. Myocardium, fibrous tissue, nerves, fat, and adventitia were not labeled. The results are expressed as autoradiographic grains/1,000 \( \mu m^2 \) tissue and are shown in Tables 1 and 2 (mean±SEM in all cases).

The high concentration of binding sites on the valves in guinea pig heart is localized to the endocardium rather than to the fibrous tissue. Labeling of the valve in the human heart was less dense but was again present in the endocardium rather than in the fibrous stroma.

Figure 2. Guinea pig aorta and coronary artery (CA). Panel A: Bright-field view. Panel B: Autoradiograph of the same area showing dense labeling in the major coronary artery.

Figure 3. Atrioventricular groove of guinea pig heart. Panel A: Bright-field view showing artery (A), veins (V), and myocardium (M). Panel B: Autoradiograph showing labeling in vessels. Panel C: Autoradiograph after incubation with excess nonradiolabeled CGRP. Note that the label has been displaced from the vessels but not from the myocardium (myo), indicating specific receptors in the vessels but not in the myocardium.

Figure 4. Guinea pig pulmonary valve. Panel A: Bright-field view showing pulmonary artery (PA) and valve (V). Panel B: Autoradiograph showing dense labeling in the endothelium of the valve but little in the connective tissue stroma.
In three patients with ischemic heart disease, we were able to compare normal and atheromatous sections of coronary artery. We studied 11 sections of normal artery from five patients, and there was a mean of 266±10 grains/1,000 μm² tissue. Mean counts from atheromatous sections of artery were 158±35 grains/1,000 μm² (p<0.001, t test). The smooth muscle at the site of, and adjacent to, the areas of atheroma contained fewer specific binding sites for CGRP than did the normal segments of either the same or different arteries (Figure 6). Detailed counts from the patients with diseased arteries are shown in Table 3. Occluded vessels containing thrombus had few specific binding sites over the smooth muscle. Atheroma itself takes up the ligand to a certain extent, presumably because of the endothelial proliferation that is a hallmark of the atheromatous process.

Detailed evaluation of a complex lesion is shown in Figure 7. The normal left anterior descending coronary artery and diagonal arteries contain significantly more binding sites for CGRP than did the diseased areas.

**Discussion**

We studied the distribution of CGRP receptors in human and guinea pig cardiac tissue. Cardiac vessels contain a high concentration of specific receptors for CGRP, which is consistent with a possible role for CGRP in the control of coronary vasomotor tone. A lower concentration of CGRP receptors was found in the endocardium and in the great vessels. No specific labeling was seen in the myocardium, nerve, or connective tissue.

Unlike receptor analysis on membrane preparations by radioligand binding, autoradiography allows assessment of the distribution of specific receptors.11 It is particularly useful for comparing receptor density between large, medium-sized, and small vessels, which is impossible with a membrane preparation.

The physiologic function of CGRP remains unclear. It is an extremely potent vasodilator in systemic and coronary arteries when infused at concentrations comparable to that found in the circulation.4,7 CGRP-containing nerves are found in close proximity to major coronary arteries, and CGRP is thought to act mainly by local rather than systemic release. The contribution of CGRP to regulation of coronary vasomotor tone in normal and diseased hearts remains to be established.

We have been unable to demonstrate specific receptors in myocardium and suggest that the receptors demonstrated previously in membrane homoge-
FIGURE 6. Human coronary artery. Panel A: Bright-field view showing a normal coronary artery. Panel B: Autoradiograph showing labeling in the smooth muscle of the artery. Panel C: Bright-field view showing diseased coronary artery (Ath, atheroma). Panel D: Autoradiograph showing labeling in the smooth muscle of the vessel and also the atheroma. The density of labeling in the smooth muscle is less than that in the normal artery (taken from patient 2, Table 3).

nates are likely to have come primarily from the vessels. This is supported by the observation that CGRP has no direct effect on papillary muscle. The discrepancy between the lack of demonstrable receptors in atrial myocardium in our study and the effects of CGRP on isolated atrial myocardium reported by Sigrist remain unexplained, although it is possible that a large amount of nonspecific binding to the myocardium may obscure a low density of receptors when assessed by our autoradiographic technique. The endocardium, in contrast, contained a moderate number of specific receptors.

The high concentration of CGRP found on valves is unexpected. We have shown, by the use of a trichrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Artery</th>
<th>Specific binding (silver grains/1,000 μm²)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Diagonal (normal)</td>
<td>263±26</td>
</tr>
<tr>
<td></td>
<td>Diagonal (diseased)</td>
<td>198±18</td>
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<td></td>
<td>LAD (occluded)</td>
<td>57±25</td>
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<tr>
<td>2</td>
<td>Lateral Cx (normal)</td>
<td>209±16</td>
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<tr>
<td></td>
<td>Lateral Cx (diseased)</td>
<td>174±20</td>
</tr>
<tr>
<td>3</td>
<td>LAD (normal)</td>
<td>291±16</td>
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<tr>
<td></td>
<td>LAD (diseased)</td>
<td>102±26</td>
</tr>
<tr>
<td></td>
<td>LAD (occluded)</td>
<td>58±16</td>
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<tr>
<td></td>
<td>Diagonal (normal)</td>
<td>254±21</td>
</tr>
<tr>
<td></td>
<td>Cx (normal)</td>
<td>239±17</td>
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Values are mean±SEM.
LAD, left anterior descending coronary artery; Cx, circumflex coronary artery.
Atheromatous sections of left anterior descending artery.

1. Normal LAD
2. LAD with atheroma
3. Atheromatous LAD with diagonal above
4. Branch of LAD and septal vessel
5. Thrombosed LAD

Transverse sections of left anterior descending artery.

291 ± 31 binding sites/1000 μm²
102 ± 26
106 ± 20
120 ± 22
57 ± 16

FIGURE 7. Left panel: *Left coronary angiogram in the left cranio-caudal projection showing a severe stenosis of the left anterior descending coronary artery before the diagonal and septal branches, with complete occlusion immediately after the bifurcation.* Right and bottom panels: *Schematic representation of the complex lesion as seen in left panel showing the site of five representative sections of the artery and schematic representation of the sections with specific binding found in each, given as number of silver grains/1000 μm² tissue. Specific binding in normal artery is greater than that in diseased artery and much greater than that in the thrombosed section.*

stain, that this binding is confined to the endothelium overlying the valve rather than to the underlying fibrous tissue. A possible explanation for the presence of these receptors may be that the endocardium and blood vessels have the same embryologic origin, in contrast to the myocardium.

Of interest, specific receptors are found in the endocardium. Whether the vasodilatation induced by CGRP is dependent on an intact endothelium remains controversial. It is clear, though, that there are both species differences and vessel differences within one species. Recent reports have stated that although the endothelium is not necessary for the relaxation of human pulmonary artery sections or for bovine coronary artery, it is necessary for the actions of CGRP on human coronary arteries.

Atheromatous sections of artery have a decreased number of specific binding sites for CGRP when compared with normal sections of the same artery and also distant arteries. It is not clear whether this reflects a decrease of CGRP binding sites per cell or a decrease in cellularity. CGRP is an extremely potent vasodilator, and this decrease in binding sites raises the possibility that sections of artery with a decreased density of CGRP receptors will be more prone to coronary spasm. Spasm of coronary arteries may occur in association with atheromatous disease, in isolation, or with so-called variant or Prinzmetal's angina. It is unlikely that the cause of the spasm in patients with Prinzmetal's angina is the same as that in patients with atheromatous disease. Coronary artery spasm occurring in association with atheromatous disease has been shown to occur at the site of the lesion. Therefore, we postulate that the decrease in receptors for CGRP found at the site of atheromatous disease will predispose that segment of artery to develop spasm. Infusion of CGRP into the coronary arteries of patients with Prinzmetal's angina had no effect on episodes of spasm, but the natural history of that condition is likely to be different. Because the major effects of CGRP are thought to be by local release from nerves, it is by no means certain that adequate concentrations will be reached by infusion down the vessel.
 Autoradiography allows detection and localization of specific receptors for calcitonin gene-related peptide. These receptors have been demonstrated in animal and human coronary vessels and support a role for CGRP in the regulation of coronary tone. The demonstrated reduction in receptor number in atheromatous arteries is likely to predispose the arteries to coronary spasm and may explain the spasm seen around atheromatous lesions.

References


KEY WORDS • heart • vasospasm • atheroma • vasodilation • autoradiography • calcitonin gene-related peptide receptors
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