Vasodilative Effect of Adrenomedullin in Isolated Arteries of the Dog

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ABSTRACT—Adrenomedullin is known to induce profound hypotension in vivo, but the direct effect of this peptide on isolated arteries has not been demonstrated. This study estimated the vasodilative effects of adrenomedullin in comparison with those of calcitonin gene-related peptide (CGRP) in basilar, mesenteric, coronary, renal and femoral arteries isolated from the dog. Adrenomedullin (3 to 100 nM) and CGRP (1 to 30 nM) induced concentration-dependent relaxation of these arteries with and without endothelium, and the relaxing effects were slightly greater in endothelium-intact arteries than in denuded ones. The vasodilative potency of adrenomedullin relative to CGRP was smaller in the femoral artery than in basilar, mesenteric, coronary and renal arteries.

Keywords: Adrenomedullin, Calcitonin gene-related peptide, Vasodilatation

Adrenomedullin was isolated from human pheochromocytoma cells as a peptide to increase cAMP levels in platelets (1), and it has been demonstrated to induce profound hypotension in vivo (1-4). Adrenomedullin shares structural homology with calcitonin gene-related peptide (CGRP) (1, 5), which is one of the most potent vasodilators known (6). Since the effect of adrenomedullin has been demonstrated to be inhibited by a CGRP-receptor antagonist, CGRP[8-37], it was first believed that adrenomedullin acts on CGRP receptors to exert its effects (2). However, recent investigations (7, 8) demonstrated adrenomedullin-specific binding sites in vascular smooth muscles, implying important roles of this peptide in the regulation of vascular tone. The findings that the adrenomedullin is secreted from the adrenal medulla (1, 9) and exists in considerable amount in plasma (1) suggested that this peptide works as a circulating hormone. Moreover, formation of adrenomedullin in vascular endothelial cell has been demonstrated recently (10), and it was suggested that this peptide may also act as one of the endothelium-derived regulators of vascular tone.

Ishiyama et al. (3), using anesthetized rats, have demonstrated that cardiovascular depression induced by adrenomedullin is mediated mainly by a decrease of peripheral vascular resistance. Experiments using rat mesenteric bed (2), cat hindlimb vascular bed (11) and cat pulmonary vascular bed (12) have also revealed the potent vasodilative effect of adrenomedullin. However, the effect of this peptide on isolated arteries has not been demonstrated. The present study was conducted to evaluate the direct effects of adrenomedullin on the tension of canine peripheral arteries, to compare the effects with those of CGRP. Since effects of vasoactive agents often exhibit differences depending on organs, peripheral arteries including mesenteric, basilar, femoral, renal and coronary arteries were used to test possible organ differences in the vascular effects of these peptides.

The experimental protocol was approved by the Kyoto University Animal Use Committee. Nine male mongrel dogs weighing 10-15 kg were anesthetized with ketamine (10 mg/kg, i.v.) and killed by bleeding from the common carotid arteries. Basilar (0.6-0.8 mm o.d.), mesenteric (0.6-1.2 mm o.d.), coronary (0.6-1.5 mm o.d.) and intrarenal renal arteries (0.6-1.0 mm o.d.), and the distal part of the femoral artery (0.8-1.5 mm o.d.) were isolated. They were cut into rings 2-4 mm long, and placed in 10-ml organ baths containing Krebs' bicarbonate solution of the following composition: 118.2 mM NaCl, 4.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.8 mM $NaHCO_3$ and 10 mM dextrose (pH 7.35-7.45). The bath fluid was aerated continuously with a mixture of 95% O_2 and 5% CO_2 and maintained at 37±0.5℃. Endothelia of some arterial rings were mechanically removed.

Arterial rings obtained from each animal were randomly assigned to two groups, one of which was for the adrenomedullin study and the other for the CGRP study. Each arterial ring was fixed vertically between two hooks, and the hook anchoring the upper end was connected to the lever of a force-displacement transducer (Toyo Baldwin T7-240, Tokyo). Changes in isometric tension were recorded on an ink-writing oscillograph (Rectigraph 8K; Nihondenki Sanei Co., Tokyo). The resting tension was adjusted to the optimal tension that produced maximal contraction upon application of 20 mM KCl; the optimal tension was 2 g in basilar, mesenteric and renal arteries and 3 g in coronary and femoral arteries. The arterial rings were then allowed to equilibrate for 90–120 min in the control Krebs' solution, which was replaced every 15 min. The response to 30 mM KCl was evaluated in each arterial ring, and then the bath fluid was replaced three times and equilibrated. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.1 to 1 mM) was added to the bath to induce a contraction 40 to 70% of that induced by 30 mM KCl; and after stabilization of the tension, the rings were exposed to human adrenomedullin (1–100 nM) or human CGRP (0.3–30 nM) in cumulative concentrations. At the end of each cumulative exposure, papaverine (0.1 mM) was added to the bath fluid to induce maximal relaxation, and the response to each concentration of the peptides was expressed as a percentage of this maximal response.

To confirm the integrity or removal of the endothelium, after the responses to adrenomedullin or CGRP had been evaluated, the arteries were washed more than three times with fresh bathing fluid and equilibrated. Then they were again precontracted with $PGF_{2\alpha}$, and the basilar artery was exposed to substance P (0.1 μM) and other arteries to acetylcholine (10 μM), before exposure to papaverine (0.1 mM). In the present study, all arterial rings assigned as endothelium-denuded showed no significant response to these endothelium-dependent relaxants. Basilar arteries showing a response to substance P of more than 25% of the response to papaverine and mesenteric, coronary, renal and femoral arteries showing a response to acetylcholine exceeding 60% of that to papaverine were considered to have intact endothelium. Data obtained from arteries showing a lower response to substance P or acetylcholine were excluded from the study for endothelium-intact arteries.

Human adrenomedullin was synthesized by the solid phase method and purified by reverse-phase HPLC. Human CGRP was purchased from Peptide Institute, Inc., Osaka. The other drugs used were substance P (Sigma, St. Louis, MO, USA), $PGF_{2\alpha}$ (Ono Pharmaceutical Co., Osaka) and acetylcholine (Daiichi-Pharmaceutical, Tokyo). They were dissolved in 0.9% NaCl and added directly to the bath fluid; the volume added was less than 1.0% (v/v) of the bath fluid volume. Data are expressed as means±S.E. The difference between endothelium-intact and denuded arteries were analyzed by Student's *t*-test for unpaired data. Other data were analyzed by analysis of variance and Scheffe's test. Differences at $P<0.05$ were considered to be significant.

Figure 1 shows typical recordings of tension change in mesenteric and femoral arteries with intact endothelium, which were exposed to one of the two peptides in cumula-

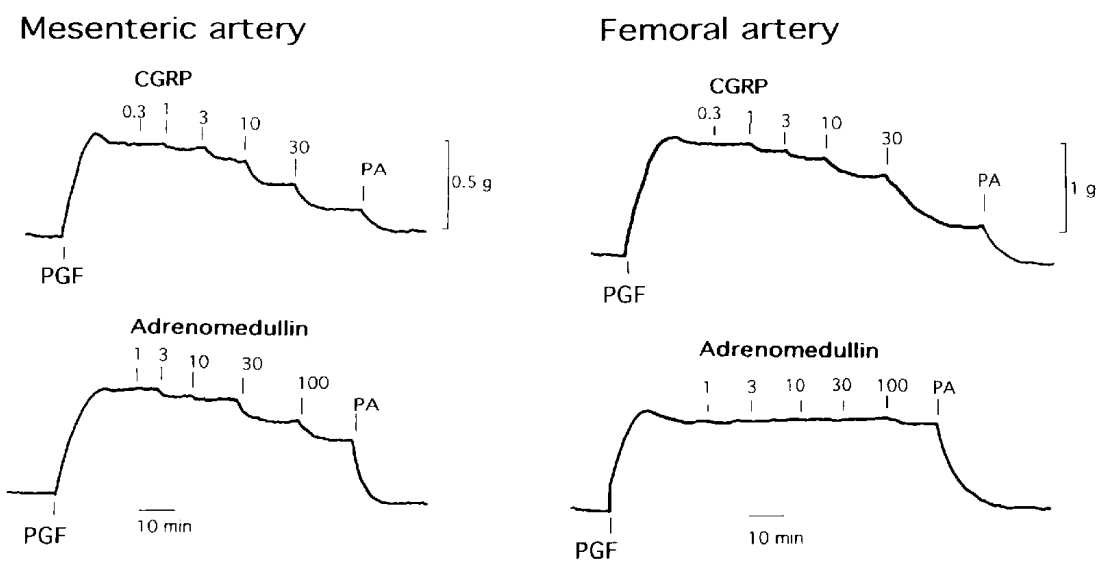

Fig. 1. Actual recordings of tension changes induced by cumulative exposure to CGRP (0.3–30 nM) and adrenomedullin (1–100 nM) in isolated mesenteric arteries (left) and femoral arteries (right) with intact endothelium. PGF, prostaglandin $F_{2\alpha}$; PA, papaverine (0.1 mM). Values over the tracing indicate concentrations of the peptides in nM.

tive concentrations. When the endothelia of the arteries were intact, adrenomedullin at concentrations greater than 1–3 nM relaxed the mesenteric and basilar arteries; at concentrations greater 10 nM, it relaxed coronary and renal arteries; and only at 100 nM, it relaxed the femoral artery; the relaxing effect of adrenomedullin (100 nM) was significantly smaller in the femoral, coronary and renal arteries than in the mesenteric and basilar arteries ($P<0.05-0.01$, Fig. 2). CGRP (1–30 nM) induced concentration-dependent relaxation of the arteries tested, and the dilative effect of CGRP (30 nM) was smaller in the coronary artery than in the mesenteric, basilar and femoral arteries ($P<0.05-0.01$, Fig. 3).

The relaxing responses to lower concentrations of adrenomudullin and CGRP were slightly smaller in endothelium-denuded arteries than in intact ones (Fig. 2, right and Fig. 3, right). The involvement of endothelium-derived factors in CGRP-induced relaxations had been demonstrated by previous investigators (13, 14), and the present study further suggested that a minor component of adrenomedullin-induced relaxation may be also mediated by endothelium-derived factors.

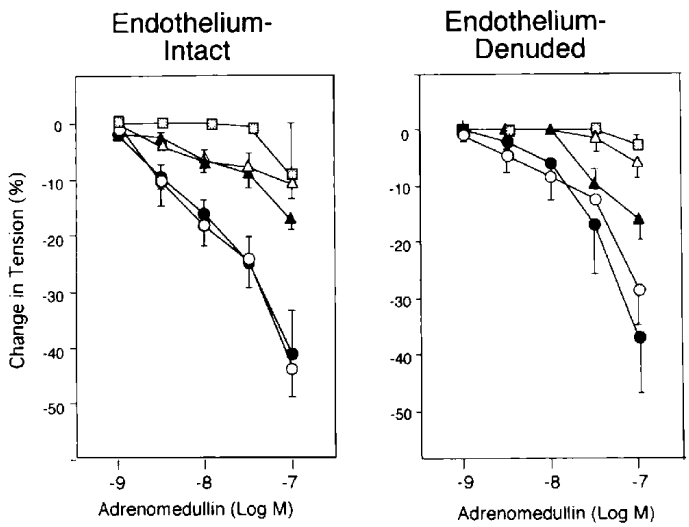

Fig. 2. Tension changes induced by cumulative exposure to adrenomedullin in endothelium-intact (left) and denuded (right) arterial rings of basilar (○), mesenteric (●), renal (▲), coronary (△) and femoral (□) arteries. n=6–10, each. The relaxing response to adrenomedullin (3 nM) was significantly smaller in endothelium-denuded mesenteric artery, and that to 3–10 nM was significantly smaller in endothelium-denuded renal artery, as compared to the responses in endothelium-intact ones ($P<0.05$).

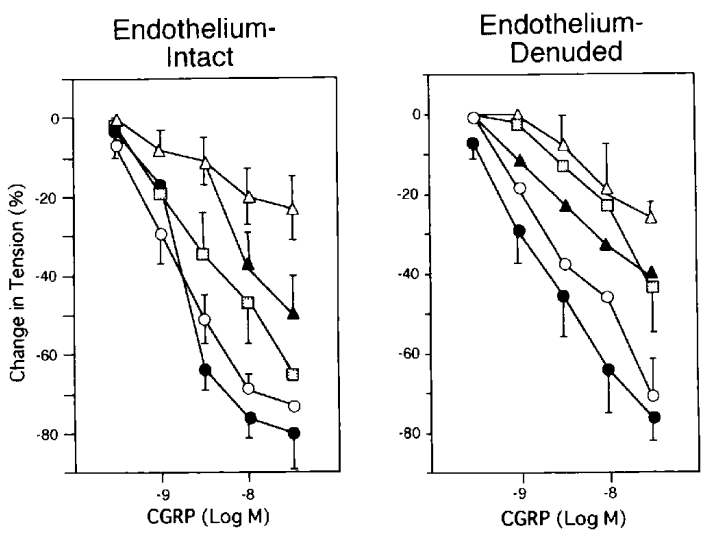

Fig. 3. Tension changes induced by cumulative exposure to CGRP in endothelium-intact (left) and denuded (right) arterial rings of basilar (○), mesenteric (●), renal (▲), coronary (△) and femoral (□) arteries. n=6–10, each. The relaxing response to adrenomedullin (1 nM) was significantly smaller in endothelium-denuded mesenteric artery, and that to 10 nM was significantly smaller in endothelium-denuded femoral artery, as compared to the responses in endothelium-intact ones ($P<0.05$).

Nuki et al. (2) demonstrated that the perfused mesenteric vascular bed of the rat was dilated nearly maximally with 0.1 μM adrenomedullin. In the present study, however, canine mesenteric artery was relaxed by this concentration of adrenomedullin only to 40% of the papaverine-induced maximal relaxation. The different degrees of relaxation may have been due to differences in species or experimental conditions or the lower susceptibility of the arteries used in the present study compared with resistance vessels.

The present study also demonstrated that the vasodilative potencies of CGRP and adrenomedullin differed among the five arteries tested. The rank order of susceptibility to the dilative effect of adrenomedullin was basilar, mesenteric $\geq$ renal, coronary $>$ femoral arteries; and that for susceptibility to CGRP was basilar, mesenteric $\geq$ femoral $>$ renal $\geq$ coronary arteries. The potency of adrenomedullin relative to CGRP was much lower in the femoral artery than in other arteries. In the cat pulmonary vascular bed, adrenomedullin has been demonstrated to be even more potent than CGRP (12), although it is less potent than CGRP in the rat mesenteric vascular bed (2), indicating differences according to the species or the sites of vessels.

The difference in the potency of adrenomedullin relative to CGRP depending on the origin of the arteries is difficult to interpret under an assumption that adrenomedullin exerts its vasodilative effect by binding to the CGRP receptor with uniform affinity. One possible explanation is that CGRP receptors show differences in affinity to adrenomedullin depending on the arterial sites; this is not unlikely, since CGRP receptors are known to have several subtypes (15). These results are also consistent with the assumption that adrenomedullin exerts its effect, at least partly, in a manner independent from the interaction with CGRP receptors. Recently, Ishizaka et al. (8) and Eguchi et al. (7) demonstrated that cultured vascular smooth muscle cells have adenyl cyclase-coupled high-affinity binding sites for adrenomedullin, although interaction of CGRP with these receptors is still controversial.

In conclusion, this is the first study to demonstrate direct vasodilative effects of adrenomedullin in isolated arteries. Adrenomedullin at 3 to 100 nM produced concentration-dependent relaxation of the mesenteric, basilar, femoral, renal and coronary arteries of the dog, and the femoral artery was less susceptible to adrenomedullin than the other arteries.

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