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Inhibition by adrenomedullin of amine release from adrenergic nerves in dog mesenteric arteries

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Abstract

Adrenomedullin and calcitonin gene-related peptide (CGRP) inhibited the pressor response to transmural electrical stimulation in perfused isolated canine mesenteric arteries. The response was abolished by treatment with either prazosin or tetrodotoxin. Adrenomedullin-(22–52), an adrenomedullin receptor antagonist, reduced the inhibitory effect of adrenomedullin (10^{-10} to 10^{-8} mol/l), but did not alter the action of CGRP. CGRP-(8–37), a $CGRP_1$ receptor antagonist, did not affect the inhibition induced by adrenomedullin, but reversed the CGRP-induced inhibition. In helical strips of the arteries, adrenomedullin (up to 10^{-8} mol/l) did not influence the contraction induced by noradrenaline, whereas CGRP attenuated the response. Adrenomedullin decreased the release of noradrenaline from adrenergic nerves elicited by transmural electrical stimulation, but CGRP had no effect. Adrenomedullin-(22–52) reversed the decrease in noradrenaline release induced by adrenomedullin. The adrenomedullin-induced relaxation of vascular strips precontracted with prostaglandin $F_{2\alpha}$ was suppressed by CGRP-(8–37) but was unaffected by adrenomedullin-(22–52). These findings suggest that adrenomedullin impairs noradrenaline release from adrenergic nerves by acting on adrenomedullin receptors located in the nerve terminals, whereas arterial relaxation caused by adrenomedullin and CGRP is due to activation of $CGRP_1$ receptors in vascular smooth muscle.

Keywords: Adrenomedullin; CGRP (Calcitonin gene-related peptide); Noradrenaline; Mesenteric artery; Electrical stimulation

1. Introduction

Adrenomedullin, a peptide originally isolated from human pheochromocytoma, possesses an intense hypotensive action (Kitamura et al., 1993). Vasodilation induced by adrenomedullin has been demonstrated in anesthetized rats (Ishiyama et al., 1993) and in a variety of vascular preparations in vitro (Nakamura et al., 1995; Okamura et al., 1997a). Adrenomedullin lowers systemic blood pressure in conscious mammals (Fukuhara et al., 1995; Charles et al., 1997). Because of its structural homology, the vascular action of adrenomedullin has been compared with that of calcitonin gene-related peptide (CGRP), a potent endogenous vasodilator. Both compounds share the same receptor, $CGRP_1$ receptor, in producing vasodilation in canine central retinal artery (Okamura et al., 1997a), rat mesenteric artery (Nuki et al., 1993) and porcine coronary artery (Yoshimoto et al., 1998), but this is not the case for rat aorta (Yoshimoto et al., 1998) and the cat hindlimb vascular bed, where the adrenomedullin-induced vasodilation is not dependent on the activation of CGRP receptors (Champion et al., 1997). In anesthetized rats, adrenomedullin-induced hypotension is not inhibited by CGRP-(8–37), a $CGRP_1$ receptor antagonist (Nandha et al., 1996). A radioligand study suggested the presence of adrenomedullin-specific receptors (Eguchi et al., 1994a). A recent report suggests that adrenomedullin receptors and CGRP receptors share a common structure and that their expression is dependent on the function of receptor-activity-modifying proteins (McLatchie et al., 1998).

Previously, we have reported that adrenomedullin and proadrenomedullin NH_2-terminal 20 peptide (PAMP) inhibit the neurally induced elevation of perfusion pressure in isolated canine mesenteric arteries (Okamura et al., 1997b). Since a sympathosuppressive action of adrenomedullin has not been reported, in contrast to that of PAMP (Shimosawa et al., 1995), we further investigated the mechanism underlying the inhibition by adrenomedullin of the pressor response to adrenergic nerve stimulation in isolated perfused dog mesenteric arterial segments and

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arterial strips. The effects of CGRP on these preparations were also investigated for comparison.

2. Methods

2.1. Change in perfusion pressure by electrical stimulation

Twenty five mongrel dogs of either sex, weighing 7 to 13 kg, were used for the experiments. The Animal Care and Use Committee of our university approved the use of dog blood vessels in this study. Dogs were anesthetized with intravenous injections of sodium thiopental (30 mg/kg) and killed by bleeding from the carotid arteries. Proximal portions of the superior mesenteric artery, 1.2- to 5.0-mm outside diameter, were isolated. The artery segment was placed in the bathing medium of a 40-ml organ bath and perfused luminally with modified Ringer–Locke solution maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2 at a constant rate of 1 ml/min with a pressure of 40 to 50 mm Hg (Zhang et al., 1996). Constituents of the solution were as follows: 120 mmol/l NaCl, 5.5 mmol/l KCl, 2.2 mmol/l $CaCl_2$, 1.0 mmol/l $MgCl_2$, 25.0 mmol/l $NaHCO_3$ and 5.6 mmol/l glucose. The pH of the solution was 7.36 to 7.43. The perfusion pressure was measured via a pressure transducer (Nihon–Kohden Kogyo, Tokyo) placed upstream of the artery segment. The perfused segment was placed between a pair of stimulating electrodes each made of a platinum plate. The gap between the segment and the electrodes was wide enough to allow undisturbed contractions and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals. Under resting conditions, electrical square pulses of supramaximal intensity (10 V, 0.2-ms duration) were applied transmurally at frequencies of 20 Hz for 10 s, every 10–15 min to stimulate the perivascular nerves innervating the arterial wall. Transmural electrical stimulation was applied repeatedly until steady responses were obtained, and then the agents, such as adrenomedullin or CGRP, were directly applied to the bathing media. At the end, tetrodotoxin was applied to determine whether the induced response was due to stimulation of perivascular nerves. In some experiments, antagonists, such as adrenomedullin-(22–52) or CGRP-(8–37), were applied at least 10 min before exposure to the agonists. Measurements in the presence or absence of the antagonists were obtained from the same preparations. In preliminary experiments, the stimulation-induced increase in perfusion pressure was observed to be steady over a 2-h period.

2.2. Noradrenaline release evoked by electrical stimulation

Canine mesenteric arteries (0.5–0.8 mm, outside diameter) were cut helically into strips approximately 20-mm long. The strips were placed between a pair of stimulating electrodes and superfused with the modified Ringer–Locke solution containing cocaine (3×10^{-5} mol/l) and corticosterone (4×10^{-5} mol/l) at a constant rate of 3 ml/min. The resting tension was adjusted to 1.5 g. Isometric contractions were recorded on an ink-writing oscillograph. The electrical stimulation was applied transmurally at a frequency of 5 Hz for 1 min. The stimulation-induced contractions were abolished by treatment with 10^{-5} mol/l prazosin or 3×10^{-7} mol/l tetrodotoxin as previously reported (Toda et al., 1988). Adrenomedullin or CGRP was applied just after the application of the first stimulation, and the second stimulation was applied 10 min after the application of the agonists. Antagonists, when used, were applied at least 10 min before the first stimulation until the end of the experiment. The superfusate was collected into tubes every 1 min and to this was added 60% $HClO_4$, 4% $NaHSO_3$ and 100 mmol/l Na_2EDTA at final concentrations of 1.5%, 0.1% and 3.3 mmol/l, respectively. The samples were kept in a freezer at −20°C until the time of assay. The sample was mixed with 0.1 pmol dihydroxy benzylamine as an internal standard and 1.5 ml of 2 mol/l Tris–HCl buffer (pH 8.7), and then noradrenaline was absorbed on 15 mg activated alumina by shaking for 20 min. The alumina was washed three times with 0.2 mol/l tris buffer containing 0.1 mmol/l Na_2EDTA and 0.1% $NaHSO_3$. Noradrenaline was eluted with 100 μl of 0.2 mol/l acetic acid containing 0.1 mmol/l Na_2EDTA and 0.1% $NaHSO_3$. After being filtered through a 0.4-μm membrane filter, 80 μl of eluted solution was injected into the high-performance liquid chromatography (HPLC) system (LC-6A, SIL-6A, SCL-6A, and C-R5A: Shimadzu, Kyoto, Japan) coupled to an octadecylsilane column (4.6×250 mm; Nacalai Tesque, Kyoto, Japan) and an electrochemical detector (E-502: Irica, Tokyo, Japan). The mobile phase was 25 mmol/l ammonium acetate buffer (pH 3.4) containing 0.01% sodium 1-octanesulphate, 10 μmol/l Na_2EDTA, and 6% (v/v) acetonitrile; the flow rate was 0.5 ml/min (modified the method described by Yamada and Kimura, 1991).

2.3. Change in isometric tension of arterial strips

The arterial strips were fixed vertically between hooks in a 20-ml bath filled with modified Ringer–Locke solution (37 ± 0.3°C) and aerated with a mixture of 95% O_2 and 5% CO_2. The upper end of the strip was connected to the lever of a force–displacement transducer (TB-611T: Nihon Kohden Kogyo, Tokyo, Japan). The isometric tension response was recorded with an ink-writing oscillograph (AP-621G: Nihon Kohden Kogyo; SR-6211: Graphtec, Tokyo, Japan). The strips were washed every 10 min until the resting tension, 1.5 g, had stabilized. The contractile response to 30-mmol/l KCl was first obtained, and the strips were washed several times every 10 min. For vasoconstricting agents such as noradrenaline, concentrated

solutions were successively applied to the bathing media and the contraction was cumulatively recorded. For vasorelaxing agents, the strips were partially contracted with prostaglandin $F_{2\alpha}$ (5×10^{-7}–2×10^{-6} mol/l), and the precontracted tension was about 20% of the 30-mM-KCl induced contraction and was considered as 0% relaxation. After relaxation was obtained by cumulative addition of the agents, papaverine (10^{-4} mol/l) was successively applied and the relaxation was considered as 100% relaxation. In some experiments, antagonists or drugs were applied at least 10 min before exposure to the agonists.

2.4. Statistics and drugs used

The results shown in the text and figures are expressed as means ± standard error of the mean. Statistical analyses were done by using Student's *t*-test (unpaired unless specified in the text), and $P < 0.05$ was considered as statistically significant. Chemicals used were as the followings: human adrenomedullin, human CGRP, adrenomedullin-(22–52) and CGRP-(8–37) (Peptide Institute, Minoh, Japan), prostaglandin $F_{2\alpha}$(Pharmacia and Upjohn, Tokyo, Japan), prazosin hydrochloride and activated alumina (Wako, Osaka, Japan), sodium thiopental (Tanabe Seiyaku, Osaka, Japan), sodium-1-octansulphate (Nacalai Tesque, Kyoto, Japan), papaverine hydrochloride (Dainippon Pharmaceutical, Osaka, Japan), DL-noradrenaline, cocaine hydrochloride and tetrodotoxin (Sankyo, Tokyo, Japan), dihydroxy benzylamine (Aldrich Chemical, Milwaukee, USA), corticosterone (Sigma, St. Louis, USA). Corticosterone was dissolved in ethanol and the concentrated solution (4×10^{-2} mol/l) was diluted with the superfusion media. Other drugs were dissolved in water and were diluted with the modified Ringer–Locke solution.

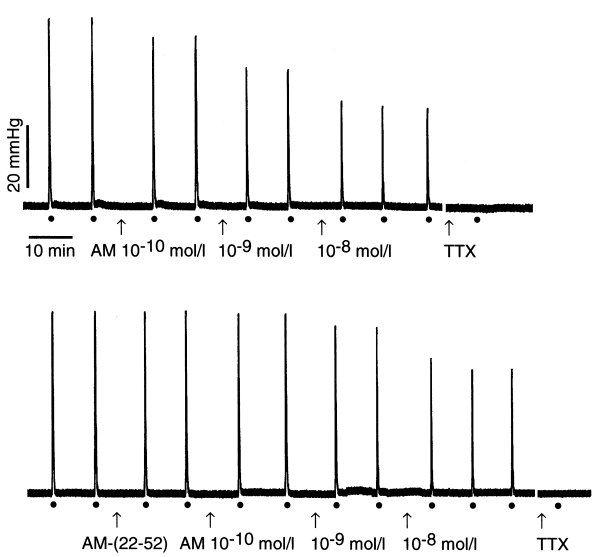

Fig. 1. Typical responses to transmural electrical stimulation (20 Hz for 10 s) of perfused isolated mesenteric arteries before and after treatment with adrenomedullin (AM; 10^{-10}, 10^{-9} and 10^{-8} mol/l) and tetrodotoxin (TTX, 3×10^{-7} mol/l) in the absence (upper) and presence (lower) of adrenomedullin-(22–52) (AM-(22–52); 10^{-6} mol/l). Adrenomedullin was cumulatively applied to the organ bath. Dots below the tracings indicate the application of electrical stimulation.

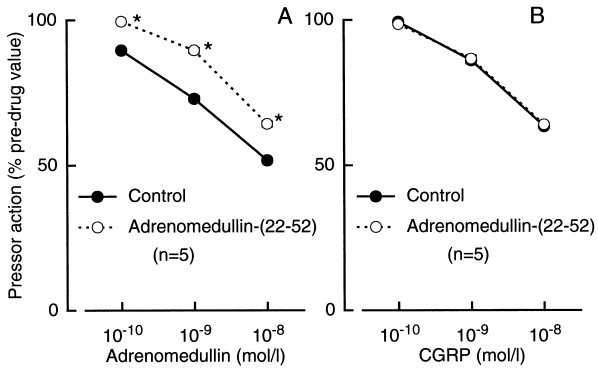

Fig. 2. Effect of adrenomedullin (A) and calcitonin gene-related peptide (CGRP; B) on the pressor response to transmural electrical stimulation (20 Hz, 10 s) in perfused mesenteric artery segments in the presence (○) and absence (●) of adrenomedullin-(22–52) (10^{-6} mol/l). The pressor responses are expressed as values relative to the control response (mean absolute value: 39.2 ± 4.3 mm Hg, $n = 10$). Significantly different from the corresponding value in the control media, $^{*}P < 0.05$.

3. Results

3.1. Change in perfusion pressure elicited by electrical stimulation

Transmural electrical stimulation increased the perfusion pressure of canine mesenteric arterial segments. The pressor response was abolished by treatment with either 10^{-5} mol/l prazosin ($n = 7$) or 3×10^{-7} mol/l tetrodotoxin (Fig. 1). Adrenomedullin (10^{-10}–10^{-8} mol/l) and CGRP (10^{-9}–10^{-8} mol/l) inhibited the response to the electrical stimulation in a dose-dependent manner (Figs. 2 and 3). The inhibitory effect of adrenomedullin was significantly greater than that of CGRP. Treatment with

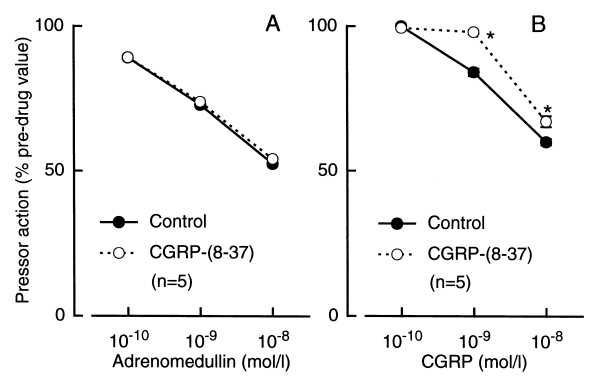

Fig. 3. Effect of adrenomedullin (A) and calcitonin gene-related peptide (CGRP; B) on the pressor response to transmural electrical stimulation (20 Hz, 10 s) in perfused mesenteric artery segments in the presence (○) and absence (●) of CGRP-(8–37) (3×10^{-7} mol/l). The pressor responses are expressed as values relative to the control response. Significantly different from the corresponding value in the control media, $^{*}P < 0.05$.

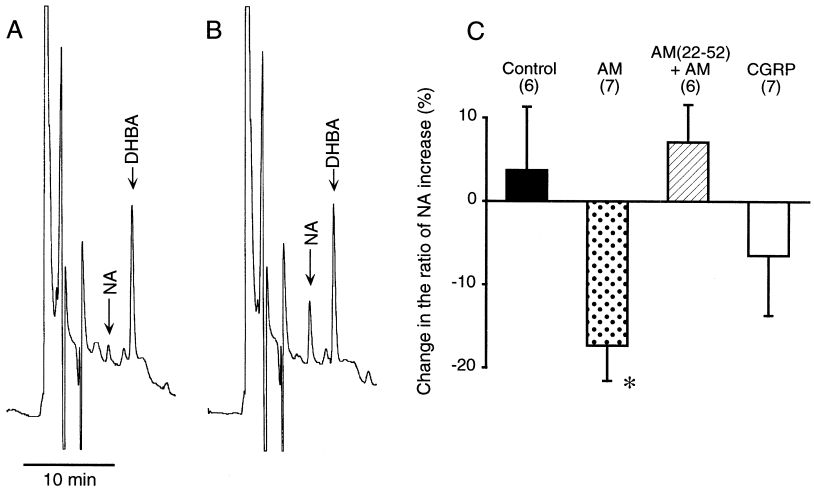

Fig. 4. (A and B) Chromatogram obtained from the superfusate sample of a canine mesenteric artery before (A) and after (B) transmural electrical stimulation. NA: noradrenaline, DHBA: 0.1 pmol dihydroxy benzylamine. (C) Change in the ratio of the increase in noradrenaline release from arterial strips elicited by the second to that elicited by the first electrical stimulation in the control media (closed column) and after treatment with adrenomedullin (AM; 10^{-8} mol/l; dotted column), adrenomedullin-(22–52) (10^{-6} mol/l) plus adrenomedullin (AM-(22–52) + AM; hatched column), or CGRP (10^{-8} mol/l; open column). Significantly different from the value before treatment, $^{*}P < 0.05$ (paired *t*-test).

10^{-6} mol/l adrenomedullin-(22–52), an adrenomedullin receptor antagonist (Eguchi et al., 1994b), significantly suppressed the inhibition evoked by adrenomedullin (Figs. 1 and 2A), but did not alter the CGRP action (Fig. 2B). In contrast, treatment with 3×10^{-7} mol/l CGRP-(8–37), a $CGRP_1$ receptor antagonist (Dennis et al., 1990), attenuated the inhibitory effect of CGRP, but the adrenomedullin-induced inhibition was not influenced (Fig. 3). Neither adrenomedullin-(22–52) (Fig. 1) nor CGRP-(8–37) affected the basal perfusion pressure or the response to electrical stimulation ($n = 5$).

3.2. Noradrenaline release elicited by transmural electrical stimulation

In order to assess the effect of adrenomedullin and CGRP on sympathetic nerve endings, we measured the amount of noradrenaline released from superfused mesenteric arterial strips by using HPLC. The basal release of noradrenaline was 2.2 ± 0.2 pmol/min/g tissue. Transmural electrical stimulation increased noradrenaline release by 3.7 ± 0.7 pmol/min/g tissue ($n = 6$, $P < 0.01$, paired *t*-test). Electrical stimulation was applied twice at an interval of more than 10 min, and the ratio (S_2/S_1) of the increase in noradrenaline release elicited by the first (S_1) and second (S_2) stimulation was calculated. The superfusion medium containing adrenomedullin or CGRP was applied just after the first stimulation. Treatment with 10^{-8} mol/l adrenomedullin reduced the stimulation-induced increase in noradrenaline release by $17 \pm 4\%$ ($P < 0.05$, paired *t*-test), while treatment with 10^{-8} mol/l CGRP did not significantly alter the response (Fig. 4). Adrenomedullin-(22–52) 10^{-6} M did not significantly alter the stimulation-induced noradrenaline release and reversed the

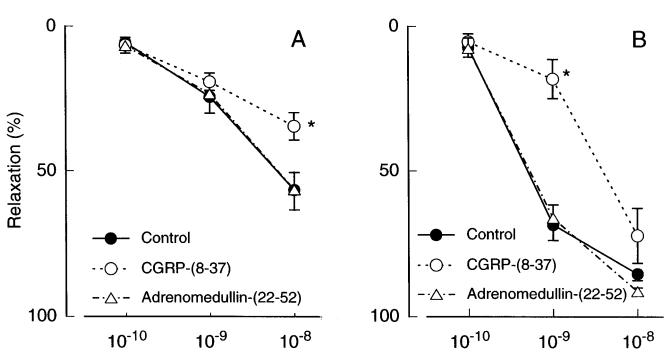

Fig. 5. Relaxation induced by adrenomedullin (A) and CGRP (B) in canine mesenteric artery strips precontracted with prostaglandin $F_{2\alpha}$ in the absence (●) or presence of CGRP-(8–37) (3×10^{-7} mol/l, ○) or adrenomedullin-(22–52) (10^{-6} mol/l, Δ). Significantly different from the corresponding value in the control media, $^{*}P < 0.05$.

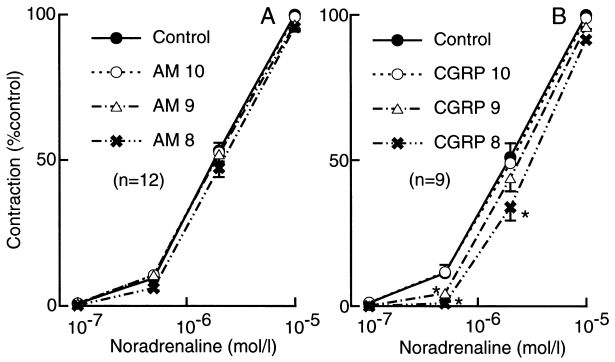

Fig. 6. Contraction induced by noradrenaline of mesenteric arterial strips before (●) and after treatment with adrenomedullin (AM, 10^{-10}–10^{-8} mol/l; A) or CGRP (10^{-10}–10^{-8} mol/l; B). The ordinal value represents relative values to the maximal contraction induced by 10^{-5} mol/l noradrenaline. Significantly different from the corresponding value in the control media, $^{*}P < 0.05$.

inhibitory effect of adrenomedullin (Fig. 4). In this series of experiments, contractions induced by electrical stimulation were suppressed by adrenomedullin (10^{-8} mol/l) and CGRP (10^{-8} mol/l), the suppression being $20 \pm 5\%$ ($n = 7$, $P < 0.05$, unpaired *t*-test, compared with control) and $69 \pm 4\%$ ($n = 7$, $P < 0.01$), respectively.

3.3. Change in isometric tension of arterial strips

In canine mesenteric artery strips precontracted with prostaglandin $F_{2\alpha}$, both adrenomedullin and CGRP (10^{-9} and 10^{-8} mol/l) produced relaxation in a concentration–relation manner (Fig. 5). Treatment with CGRP-(8–37) (3×10^{-7} mol/l) significantly inhibited the relaxations caused by adrenomedullin and CGRP, whereas adrenomedullin-(22–52) (10^{-6} mol/l) did not affect the responses. The contraction induced by noradrenaline (5×10^{-7}–2×10^{-6} mol/l) was inhibited by the treatment with CGRP (10^{-9}–10^{-8} mol/l), while adrenomedullin (up to 10^{-8} mol/l) did not affect tthe contraction (Fig. 6). Note that the contraction induced by 5×10^{-7} mol/l noradrenaline was comparable to the precontraction induced by prostaglandin $F_{2\alpha}$.

4. Discussion

The pressor response to transmural electrical stimulation of perfused dog mesenteric arterial segments was abolished by prazosin or tetrodotoxin, suggesting that the response is solely mediated by α_1 adrenoceptor activation due to noradrenaline released from perivascular nerve and that vasoconstricting neurotransmitters other than noradrenaline, if any, are not involved in the response. Although electrical stimulation may activate non-adrenergic nerve terminals, CGRP nerves, if present, are not activated in this preparation, since CGRP-(8–37) did not alter the neurogenic pressor response. Adrenomedullin inhibited the pressor response and the release of noradrenaline elicited by electrical stimulation. Adrenomedullin reportedly decreases the plasma noradrenaline level of conscious sheep (Charles et al., 1997). In contrast, Shimosawa et al. (1995) have reported that PAMP, but not adrenomedullin, inhibits noradrenaline overflow from peripheral sympathetic nerve in the rat mesenteric artery. The reason for this discrepancy is not known, but it may be due to differences in animal species (dog vs. rat), artery size (proximal vs. distal) and/or the experimental conditions used (presence vs. absence of neuronal and extraneuronal noradrenaline uptake inhibitors, or superfusion vs. perfusion for noradrenaline measurement). In our dog preparations, the inhibitory action of adrenomedullin on the perfusion pressure elevated by sympathetic nerve stimulation is more potent than that of PAMP (Okamura et al., 1997b).

The inhibitory effect of adrenomedullin on the neurogenic pressor response was significantly suppressed by adrenomedullin-(22–52) but not by CGRP-(8–37). Adrenomedullin reduced the release of noradrenaline elicited by electrical stimulation, and the reduction by adrenomedullin was reversed by adrenomedullin-(22–52). Adrenomedullin in the doses used, however, did not significantly alter the contraction induced by exogenous noradrenaline. These results suggest that adrenomedullin mainly acts on the adrenomedullin-specific receptor of the perivascular nerve terminals and inhibits noradrenaline release. The inhibition by adrenomedullin at high concentrations of the neurogenic pressor response was not fully restored by 10^{-6} M adrenomedullin-(22–52); higher concentrations of the adrenomedullin antagonist could not be tested in this study. Whether or not a mechanism other than that associated with adrenomedullin-specific receptors is involved in this inhibition remains to be determined. In anesthetized rats, the hypotensive effect of adrenomedullin is not inhibited by CGRP-(8–37) in a dose sufficient to completely inhibit the hypotension induced by CGRP (Nandha et al., 1996). The specific binding of adrenomedullin in the human brain is not inhibited by either CGRP or adrenomedullin-(22–52) (Sone et al., 1997). However, Saita et al. (1998) recently reported that the central action of adrenomedullin to inhibit peripheral sympathetic nerve activity is markedly suppressed by adrenomedullin-(22–52), indicating the presence of functioning adrenomedullin-specific receptors in the rat brain.

Both adrenomedullin and CGRP relaxed mesenteric arterial strips precontracted with prostaglandin $F_{2\alpha}$, and the relaxation induced by CGRP was greater than that induced by adrenomedullin. CGRP-(8–37) inhibited the relaxation induced by adrenomedullin or CGRP, whereas adrenomedullin-(22–52) at the same concentration which inhibited the prejunctional action of adrenomedullin was without effect, suggesting that both adrenomedullin and CGRP relax vascular smooth muscle directly via $CGRP_1$ receptors. The inhibition by CGRP-(8–37) at 3×10^{-7} M of the relaxation elicited by adrenomedullin was not more

potent than that eicited by CGRP. In our previous study (Okamura et al., 1997a), we reported that 10^{-7} M CGRP-(8–37) almost abolished the adrenomedullin-induced relaxation in isolated canine retinal arteries. However, this was not the case for canine mesenteric arteries, because the adrenomedullin-induced relaxations were significantly inhibited, but were not abolished, by the CGRP receptor antagonist even at 3×10^{-7} M. Since concentrations higher than 3×10^{-7} M of the CGRP antagonist could not be tested in this study, whether or not the adrenomedullin-induced relaxations totally depend on the CGRP receptors in this particular artery could not be determined. The inhibitory effect of CGRP on the pressor response to electrical stimulation was reduced by CGRP-(8–37). CGRP in a dose sufficient to inhibit the pressor response did not alter the release of noradrenaline induced by electrical stimulation but inhibited the contraction induced by noradrenaline up to 2×10^{-6} M. These findings suggest that CGRP acts on the $CGRP_1$ receptor of vascular smooth muscle and inhibits the neurogenic contraction. The lack of a prejunctional inhibitory effect of CGRP on noradrenaline release in the rabbit ear artery has been described (Maynard and Burnstock, 1994). One may ask whether the CGRP-induced relaxation in arterial strips precontracted with prostaglandin $F_{2\alpha}$ was more potent than the inhibitory effects of CGRP on the noradrenaline-induced contraction in the strips and on the neurogenic pressor response in the perfusion experiment. However, the magnitude of the precontraction elicited by prostaglandin $F_{2\alpha}$ was similar to that elicited by 5×10^{-7} M noradrenaline in the same strips. Therefore, the potency of the CGRP-induced relaxation was comparable against these two vasoconstrictors. The degree of inhibition by CGRP in the isometric tension experiment cannot be compared to that in the perfusion experiment. The differences in the method (tension vs. perfusion pressure) and in the size of the arteries used (distal vs. proximal) may explain these differences. It is reported that adrenomedullin and CGRP share the same receptors in vascular smooth muscle to cause the vasorelaxant action (Nuki et al., 1993; Mori et al., 1997; Okamura et al., 1997a; Yoshimoto et al., 1998). However, this does not seem to be always true (Champion et al., 1997; Yoshimoto et al., 1998).

Receptors responsible for the adrenomedullin-induced hemodynamic and vascular actions have been diversely reported and they may vary with the tissues and/or animal species used. The presence of adrenomedullin-specific receptors and their relationships to CGRP receptors has still to be determined. Recently, McLatchie et al. (1998) reported that both receptors consist of the same structure and that their expression is dependent on the function of receptor-activity-modifying proteins, $RAMP_1$ for CGRP receptors and $RAMP_2$ for adrenomedullin receptors. This interesting hypothesis may explain the diversity of the receptors involved in the actions of adrenomedullin in different tissues.

In summary, the adrenomedullin-specific receptor of adrenergic nerve endings appears to be responsible for the inhibition of noradrenaline release from adrenergic nerves and for the inhibition of neurogenic vasoconstriction in canine mesenteric arteries. The hypotensive action induced by adrenomedullin in vivo may be associated not only with vascular smooth muscle relaxation but also with inhibition of noradrenaline release.

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