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VASODILATOR EFFECT OF ADRENOMEDULLIN AND CALCITONIN GENE-RELATED PEPTIDE RECEPTORS IN RAT MESENTERIC VASCULAR BEDS

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Summary; The effect of adrenomedullin, a novel peptide of 52 amino acids, on vascular tone was investigated in the perfused rat mesenteric vascular bed. In the vasculature contracted with methoxamine, perfusion of adrenomedullin (10^{-11}-10^{-7}M) caused a concentration-dependent decrease in perfusion pressure due to vasodilation. Additionally, a bolus infusion of adrenomedullin (300 and 500 pmol) produced a long-lasting vasodilator response, which was not affected in the presence of atropine (10^{-7}M) and propranolol(10^{-7}M). However, this response was inhibited in the presence of CGRP[8-37] (10^{-6}M), an antagonist for CGRP receptor. These results suggest that adrenomedullin induces nonadrenergic and noncholinergic vasodilation in which CGRP receptors may be involved.

Adrenomedullin, a peptide of 52 amino acids, originally discovered in human pheochromocytoma arising from adrenal medulla, is distributed in the peripheral tissues including adrenal medulla, lung and kidney (1). Amino acid sequence of adrenomedullin is homologous to those of calcitonin gene-related peptide (CGRP) (2,3) and amylin (4), by sharing a ring structure with disulfide bridge and the C-terminal amide

Abbreviations used are: CGRP, calcitonin gene-related peptide; CGRP[8-37], calcitonin gene-related peptide[8-37]; BSA, bovine serum albumin; ACh, acetylcholine; ISO, isoproterenol.

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structure (1). CGRP, a potent vasodilator peptide, has been shown to increase cAMP level in cultured rat aortic smooth muscle cells (5), and adrenomedullin also causes an increase in cAMP level in platelets to an extent similar to that due to CGRP (1). Furthermore, adrenomedullin, like CGRP, causes a hypotensive effect in anesthetized rats *in vivo* (1). However, the precise vascular effect of adrenomedullin remained unknown.

Therefore, we now investigated the vascular effect of adrenomedullin in the perfused rat mesenteric vascular bed. We also compared the vascular effect of adrenomedullin with that of CGRP to examine the possible mechanism of adrenomedullin for the regulation of vascular tone.

Materials and Methods

Materials : Acetylcholine (ACh) hydrochloride (Daiichi Pharmaceutical Co., Tokyo, Japan), human adrenomedullin (kindly supplied by Dr. S. Sakakibara, Peptide Institute, Osaka, Japan), atropine sulfate (Wako Jyunyaku, Osaka, Japan), BSA (Sigma, St. Louis MO, USA), human CGRP (Peptide Institute), human CGRP[8-37] (Peptide Institute), guanethidine sulfate (Tokyo Kasei, Tokyo, Japan), isoproterenol (ISO) hydrochloride (Sigma), methoxamine hydrochloride (Nihon Shinyaku, Kyoto, Japan), papaverine hydrochloride (Dainihon Seiyaku, Osaka, Japan), propranolol hydrochloride (Sigma).

Perfusion of mesenteric vascular beds : The mesenteric vascular bed isolated from male Wistar rats (350-400 g) under pentobarbital-Na (50 mg/Kg, i.p.) anesthesia was perfused as described previously (6). The preparation was placed in a water-jacketed organ bath maintained at 37℃ and perfused with an oxygenated modified Krebs-Ringer bicarbonate solution (KRB solution) [mM: NaCl, 120.0; KCl, 5.0; $CaCl_2$, 2.4; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; EDTA-2Na, 0.027; glucose, 11.0] at a constant flow rate of 5 ml/min by a peristaltic pump (SJ-1215, ATTO). The preparation was also superfused with the same solution to prevent drying. Changes in the perfusion pressure were measured by a pressure transducer (MPU-0.5A, Nihon Kohden) and recorded on an electronic recorder (R-62, Rikadenki).

After the basal perfusion pressure was stabilized, the preparation was perfused with KRB solution containing methoxamine (7×10^{-6}M) to produce an active tone, in the presence of guanethidine (5×10^{-6}M) to block adrenergic neurotransmission. The final concentration of each peptide, which was diluted with KRB solution containing methoxamine plus guanethidine, was perfused. The concentration of the peptides was switched to a higher one when the vasodilation had reached the steady state (about 10 min). Vasodilator activity of adrenomedullin and human CGRP was expressed as the pD_2 value that was the molar concentration producing 50% of maximum response (7).

In another series of experiments, after the perfusion pressure elevated by $7x10^{-6}$M methoxamine plus $5x10^{-6}$M guanethidine was stabilized, adrenomedullin (300 and 500 pmol), CGRP (50 pmol), ACh (2 nmol) and ISO (5 nmol) were directly infused into the perfusate proximal to the arterial cannula using an infusion pump (Model 975, Harvard) without or with perfusion of the final concentration of atropine (10^{-7}M), and propranolol (10^{-7}M), or atropine plus propranolol plus CGRP[8-37] (10^{-6}M), a CGRP receptor antagonist. The volume of infusion was 100 μ l/10 sec. All agents were diluted with KRB solution containing $7x10^{-6}$M methoxamine plus $5x10^{-6}$M guanethidine when infused in a bolus. At the end of each experiment, 10^{-4}M papaverine was perfused to produce a complete relaxation. Vasodilation is expressed as the percentage of the perfusion pressure at maximum relaxation induced by papaverine. Statistical analysis: All data were expressed as mean ± S.E.M. Statistical analysis was performed using Student's *t* test for paired group or one way analysis of variance followed by Dunnett's test. Significance was accepted for $P<0.05$.

Results

Vascular effects of adrenomedullin and CGRP

In the perfused mesenteric vascular bed with active tone produced by $7x10^{-6}$M methoxamine plus $5x10^{-6}$M guanethidine, perfusion of adrenomedullin caused a concentration-dependent decrease in perfusion pressure due to vasodilation (Fig. 1). Perfusion of human CGRP also produced a concentration-dependent vasodilation. The pD_2 values for adrenomedullin (8.32) (n=4) and human CGRP (9.20) (n=4) suggest that the vasodilator

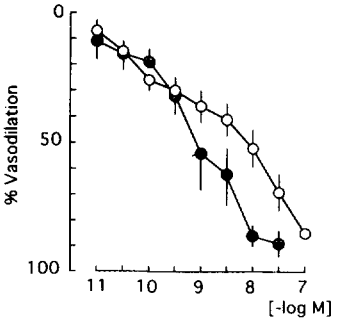

Figure 1. Vasodilator effects of perfusion of adrenomedullin (○)and CGRP(●) in the mesenteric vascular beds with active tone produced by methoxamine ($7x10^{-6}$M) plus guanethidine ($5x10^{-6}$M). Ordinate indicates percentage of vasorelaxation. Abscissa indicates concentration of peptides. Data represent mean ± S.E.M. (n=4).

activity of adrenomedullin was approximately 10 times less potent than that of human CGRP.

Effects of atropine (muscarinic receptor antagonist), propranolol (β -adrenoceptor antagonist) and CGRP[8-37] (CGRP receptor antagonist) on vasodilator responses to bolus infusion of adrenomedullin

As shown in Figs 2A and 3, bolus infusions of adrenomedullin (300 and 500 pmol) caused a concentration-dependent and long-lasting fall in perfusion pressure due to vasodilation. The bolus infusion of CGRP (50 pmol) also produced a long-lasting vasodilator response, of which time-

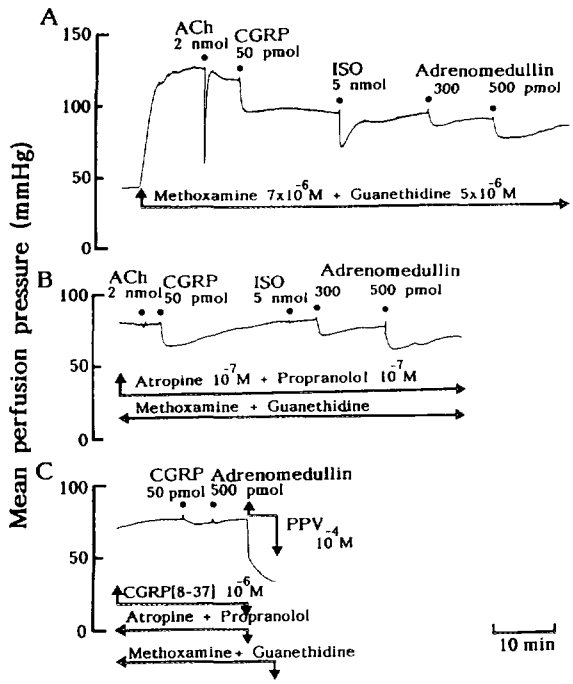

Figure 2. Typical recordings showing vasodilator effects induced by bolus infusions of adrenomedullin (300 and 500 pmol), ACh (2 nmol), ISO (5 nmol) and CGRP (50 pmol) in the (A) absence or presence of (B) atropine (10^{-7}M) plus propranolol (10^{-7}M), and (C) atropine, propranolol plus CGRP [8-37] (10^{-6}M) in the rat mesenteric vascular beds with active tone. ● , bolus infusion. PPV, perfusion of papaverine.

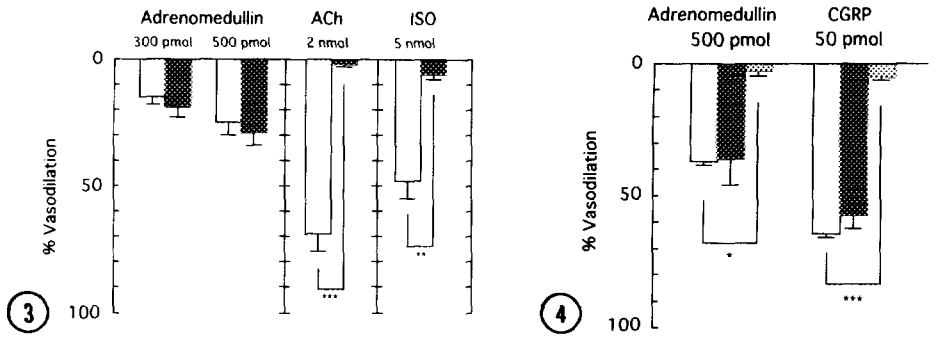

Figure 3. Effects of atropine plus propranolol on the vasodilator responses to bolus infusions of adrenomedullin, ACh and ISO in the rat mesenteric vascular beds with active tone. Responses in the absence (open column) and presence (closed column) of atropine (10^{-7}M) plus propranolol (10^{-7}M). Ordinate indicates percentage of vasodilation. Data represent mean ± S.E.M. (n=5). **P<0.01 and ***P<0.001 compared with control as indicated by the thin lines.

Figure 4. Effect of CGRP[8-37] on the vasodilator response to bolus infusions of adrenomedullin (500 pmol) and CGRP (50 pmol) in mesenteric vascular beds with active tone. Open column: control response. Closed column: responses in the presence of atropine (10^{-7}M) and propranolol (10^{-7}M). Hatched column: responses in the presence of atropine (10^{-7}M), propranolol (10^{-7}M) and CGRP[8-37] (10^{-6}M). Data represent mean ± S.E.M. (n=3). *P<0.05 compared with control.

course was similar to that caused by adrenomedullin, but quite distinct from a transient fall in perfusion pressure caused by a bolus infusion of ACh (2 nmol) or ISO (5 nmol).

The vasodilator response to adrenomedullin was not affected by simultaneous perfusion of propranolol (10^{-7}M) and atropine (10^{-7}M), at their concentrations that effectively antagonized the vasodilator response to ACh (2 nmol) or ISO (5 nmol) (Figs. 2B and 3). The vasodilator responses to adrenomedullin (500 pmol) as well as to CGRP (50 pmol), on the other hand, were markedly inhibited by the application of CGRP[8-37] (10^{-6}M), a competitive antagonist for CGRP receptor (Figs. 2C and 4).

Discussion

In the perfused rat mesenteric vascular bed with active tone, we demonstrated that perfusion of adrenomedullin, a novel

peptide isolated from human pheochromocytoma, caused a concentration-dependent decrease in perfusion pressure due to vasodilation. In contrast to the transient vasodilation due to ACh or ISO, the adrenomedullin-induced vasodilator response lasted for approximately 10 min in a similar manner to that induced by CGRP, the most potent vasodilator peptide known (8). In *in vivo* experiments using anesthetized rats, adrenomedullin has been shown to induce a hypotensive effect, which was as potent as human CGRP (1). In the present study, the vasodilator activity of adrenomedullin is only 10 times less potent than human CGRP, when compared their pD_2 values. Kitamura et al (1) reported that adrenomedullin, like CGRP, increased cAMP level in rat platelets, of which potency was 3 times less than that of human CGRP. It can be inferred that adrenomedullin induces vasodilation by increasing cAMP level in the vascular smooth muscle.

We also found that adrenomedullin, when injected in a bolus, caused a long-lasting vasodilator response, while bolus infusions of ACh and ISO induced transient vasodilations. Furthermore, the vasodilator response to adrenomedullin was not affected by atropine and propranolol, at their concentrations that abolished the vasodilation caused by ACh and ISO, suggesting that the adrenomedullin-induced vasodilation is nonadrenergic and noncholinergic in nature.

In the present study, the vasodilator response to bolus infusion of adrenomedullin was markedly inhibited by the CGRP receptor antagonist, CGRP[8-37], at its concentration that inhibited the vasodilation induced by CGRP. In amino acid sequences, adrenomedullin has additional 14 residues in amino terminal as compared with human CGRP, having a disulfide bridge between position 16 and 21 which are corresponding to position 2 and 7 (1) of amino acid sequence of human CGRP, respectively.

Adrenomedullin and human CGRP also share the C-terminal amide structure (1). The structure-activity relationship of CGRP has shown that the disulfide bridge is important to produce vasodilator activity, and that the C-terminal amide is requried for the binding of CGRP to their receptors (9). Therefore, it is possible that CGRP receptors may be involved, at least in part, in the vasodilator response due to adrenomedullin in the mesenteric resistance blood vessel.

In conclusion, the present study suggests that adrenomedullin causes a potent and long-lasting as well as nonadrenergic and noncholinergic vasodilator response, in which process CGRP receptors seem to be involved.

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