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Short communication

Comparison of responses to adrenomedullin and adrenomedullin analogs in the mesenteric vascular bed of the cat

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Abstract

Responses to adrenomedullin, a newly discovered hypotensive peptide isolated from human pheochromocytoma cells, and the carboxy terminal 15–52 (adrenomedullin-(15–52)) and 22–52 (adrenomedullin-(22–52)) amino acid fragments of adrenomedullin were investigated in the mesenteric vascular bed of the cat. Under constant flow conditions, injections of adrenomedullin, adrenomedullin-(15–52), and calcitonin gene-related peptide (CGRP) in doses of 0.003–1 nmol into the perfused superior mesenteric artery caused significant dose-related decreases in mesenteric arterial perfusion pressure. Mesenteric vasodilator responses to adrenomedullin and adrenomedullin-(15–52) were similar in magnitude and duration, while vasodilator responses to CGRP were greater in magnitude and longer in duration than those produced by adrenomedullin or adrenomedullin-(15–52) when these agents were injected in doses of 0.1–1 nmol. Adrenomedullin-(22–52) caused no significant change in mesenteric arterial perfusion pressure when injected in doses up to 10 nmol. These results suggest that amino acids 15–52 and the six-membered ring structure of adrenomedullin are important for the expression of vasodilator activity in the mesenteric vascular bed of the cat.

Keywords: Adrenomedullin; Vascular bed, intestinal; Vasoactive peptide; (Six-membered ring structure); CGRP (calcitonin gene-related peptide)

1. Introduction

Adrenomedullin is a recently discovered hypotensive peptide isolated from human pheochromocytoma cells (Kitamura et al., 1993). Human adrenomedullin is composed of 52 amino acids and a disulfide bond that forms a six-membered ring structure (Kitamura et al., 1993). The ring structure is similar to the ring structure found in calcitonin gene-related peptide (CGRP) and pancreatic amylin (Kitamura et al., 1993). Adrenomedullin is found in a number of organ systems and is present in human plasma and may serve as a circulating hormone that regulates systemic arterial pressure (Kitamura et al., 1993; Ichiki et al., 1994). This novel peptide decreases systemic arterial pressure and has potent vasodilator activity in the hindlimb and pulmonary vascular beds in the cat and in the systemic bed of the rat (Ishiyama et al., 1993; DeWitt et al., 1994; Santiago et al., 1994). The present study was undertaken to investigate responses to adrenomedullin and CGRP in the mesenteric vascular bed of the cat under constant-flow conditions and to ascertain which amino acid sequences in the peptide are required for the expression of vasodilator activity in the mesenteric vascular bed of the cat.

2. Materials and methods

Seventeen adult cats of either sex weighing 2.4–4.6 kg were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with pentobarbital sodium (30 mg/kg i.v.). Supplemental doses of pentobarbital were administered as needed to maintain a uniform level of anesthesia. The trachea was cannulated, and the animals breathed room air or were

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ventilated with a Harvard model 607 respirator at a volume of 40–50 ml at 15–22 breaths/min. An external jugular vein was cannulated for the i.v. administration of drugs, and a carotid artery was catheterized for the measurement of the systemic arterial pressure. For constant flow perfusion of the mesenteric (intestinal) vascular bed, the superior mesenteric artery was approached through a midline abdominal incision and, after administration of heparin sodium (1000 U/kg i.v.), the vessel was cannulated and connected to the outlet side of the perfusion circuit. The superior mesenteric vascular bed was perfused with a Sigmamotor perfusion pump model T-8 with blood withdrawn from a catheter in a femoral artery. Perfusion pressure measured from a side arm in the perfusion circuit between the pump and the superior mesenteric artery catheter was recorded using a Statham P23 transducer and Grass model 7 polygraph. Mean pressures were obtained by electronic averaging, and the flow rate was set so that mesenteric arterial perfusion pressure approximated systemic arterial pressure and was not changed during the experiment. The flow rate ranged from 22–33 ml/min, and the vasoactive peptides were injected directly into the perfusion circuit distal to the pump in small volumes (30 and 100 μl) in a random sequence.

Synthetic human adrenomedullin, CGRP (Peptides International, Belmont, CA, USA), the 15–52 (adrenomedullin-(15–52)) and the 22–52 (adrenomedullin-(22–52)) carboxy terminal fragments of the peptide (Peptide Laboratory, Tulane Medical School, New Orleans, LA, USA) were dissolved in normal saline. Adrenomedullin-(15–52) and adrenomedullin-(22–52) were synthesized using standard solid-phase chemistry, and the purity of the peptides was determined by reverse-phase, high-performance liquid chromatography. Arterial blood gases and pH were measured with a Corning model 178 blood gas analyzer and were in the normal range (pH 7.35, P_{O_2} 98 mm Hg, P_{CO_2} 35 mm Hg). Changes in mesenteric arterial perfusion pressure were expressed in absolute mm Hg and are presented as mean ± S.E. Responses were analyzed using a one-way analysis of variance and Scheffe's F test or a paired t-test (Snedecor and Cochran, 1980). A P value of less than 0.05 was used as the criterion for statistical significance.

3. Results

Responses to adrenomedullin, adrenomedullin-(15–52), adrenomedullin-(22–52), and CGRP were compared in the mesenteric vascular bed of the cat, and these results are summarized in Fig. 1. Under conditions of controlled blood flow, injections of adrenomedullin, adrenomedullin-(15–52), and CGRP in doses of 0.003–1 nmol into the perfused superior mesenteric artery caused dose-related decreases in mesenteric arterial perfusion pressure, whereas adrenomedullin-(22–52) caused no significant change in perfusion pressure when injected in doses up to 10 nmol (Fig. 1). Adrenomedullin, adrenomedullin-(15–52), and adrenomedullin-(22–52) produced no significant decrease in systemic arterial pressure when injected into the mesenteric arterial perfusion circuit, whereas CGRP in doses of 0.3 and 1 nmol decreased

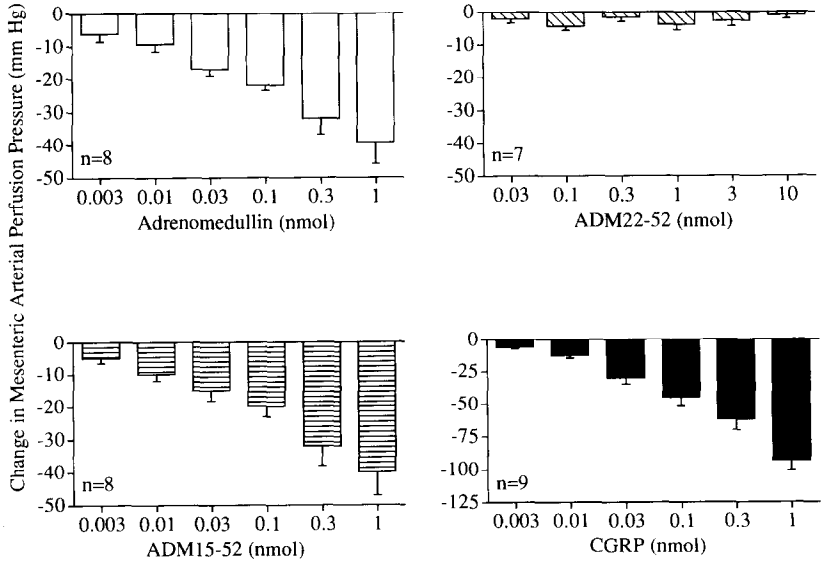

Fig. 1. Bar graph comparing the decreases in mesenteric arterial perfusion pressure in the cat in response to injections of adrenomedullin (top left), adrenomedullin-(15–52) (lower left), adrenomedullin-(22–52) (top right), and calcitonin gene-related peptide (CGRP; lower right). The peptides were injected into the mesenteric arterial perfusion circuit, and n indicates number of experiments.

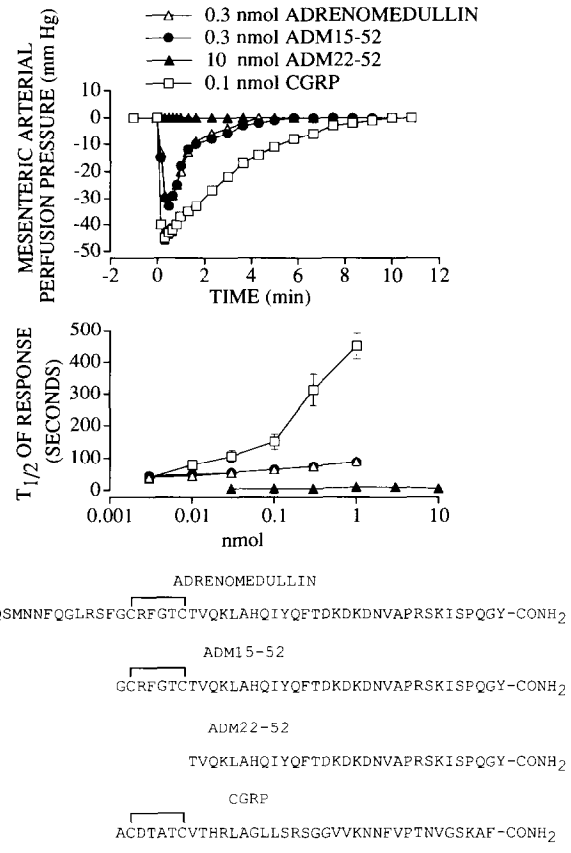

Fig. 2. Top: Line-graph showing the time-course of vasodilator responses to adrenomedullin, adrenomedullin-(15–52), adrenomedullin-(22–52), and CGRP in the mesenteric vascular bed of the cat. Middle: Comparison of response duration as measured by the response half-recovery times ($T_{1/2}$) for adrenomedullin, adrenomedullin-(15–52), adrenomedullin-(22–52), and CGRP in the mesenteric vascular bed of the cat. $n = 7–9$ experiments. Bottom: Comparison of the peptide sequences of adrenomedullin, adrenomedullin-(15–52), adrenomedullin-(22–52), and CGRP.

systemic arterial pressure 27 ± 4 and 42 ± 6 mm Hg, respectively ($P < 0.05$). In terms of relative vasodilator activity, responses to adrenomedullin and adrenomedullin-(15–52) were very similar in magnitude and duration when doses of the peptides are compared on a nmol basis (Fig. 1; Fig. 2, middle panel). Mesenteric vasodilator responses to adrenomedullin and adrenomedullin-(15–52) were rapid in onset, and perfusion pressure returned to baseline value over a 150–200 s period, depending on the dose of these peptides injected (Fig. 2, top panel). In terms of relative duration of response, the recovery half-times ($T_{1/2}$) of the vasodilator responses to adrenomedullin and adrenomedullin-(15–52) were very similar in the mesenteric vascular bed of the cat (Fig. 2, middle panel). Decreases in mesenteric arterial perfusion pressure in response to CGRP were greater in magnitude and duration than were responses to adrenomedullin and adrenomedullin-(15–52) (Fig. 1; Fig. 2, middle panel). The structures of adrenomedullin, adrenomedullin-(15–52), adrenomedullin-(22–52), and CGRP are compared in the bottom panel of Fig. 2.

4. Discussion

Results of the present investigation in the mesenteric (intestinal) vascular bed of the cat show that adrenomedullin and adrenomedullin-(15–52) caused significant dose-related decreases in mesenteric arterial perfusion pressure when the peptides were injected directly into the perfused mesenteric artery in doses of 0.003–1 nmol. Inasmuch as blood flow was maintained constant, the decreases in mesenteric arterial perfusion pressure in response to the two peptides reflect decreases in mesenteric vascular resistance. In terms of relative vasodilator activity in the mesenteric vascular bed, dose-response curves for adrenomedullin and adrenomedullin-(15–52) were very similar, and both peptides were less potent than CGRP in dilating the mesenteric vascular bed of the cat. Mesenteric vasodilator responses to adrenomedullin and adrenomedullin-(15–52) were very similar in duration and responses to the two peptides lasted for 150–200 s, depending on the dose of the peptide injected. The duration of vasodilator responses to CGRP was significantly greater than the duration of responses to adrenomedullin or adrenomedullin-(15–52) when the duration of the response to equidepressor doses was compared.

Although vasodilator responses to adrenomedullin and adrenomedullin-(15–52) were very similar in magnitude and duration in the mesenteric vascular bed of the cat, adrenomedullin-(22–52) had little if any vasodilator activity when injected into the perfused mesenteric artery in doses up to 10 nmol. The results of the present study suggest that amino acids 15–52 in the peptide are important for the expression of vasodilator activity in the mesenteric vascular bed of the cat. The observation that adrenomedullin-(22–52) had little if any vasodilator activity suggests that the presence of the ring structure in the peptide is essential for the expression of vasodilator activity in the mesenteric vascular bed of the cat.

The mechanism by which adrenomedullin or adrenomedullin-(15–52) decreases mesenteric vascular resistance, the nature of the receptors to which the peptides interact, or the enzymatic pathways by which adrenomedullin is inactivated are uncertain. It has, however, been reported that adrenomedullin increases cAMP levels in platelets and in cultured aortic vascular smooth muscle cells from the rat (Eguchi et al., 1994; Ishizaka et al., 1994). It is, therefore, possible that adrenomedullin and adrenomedullin-(15–52) may dilate the mesenteric vascular bed by increasing cAMP

levels in vascular smooth muscle cells in resistance vessel elements in the mesenteric vascular bed; however, additional studies are required to determine the mechanism by which these peptides dilate the mesenteric vascular bed in the cat. It has been shown in pilot studies that vasodilator responses to adrenomedullin are reduced by inhibitors of nitric oxide synthetase in the pulmonary and hindlimb vascular beds of the rat, suggesting that responses to adrenomedullin may be dependent on the release of nitric oxide from the endothelium (Feng et al., 1994). It has recently been shown that adrenomedullin is synthesized by cultured endothelial cells and thus may be a new endothelium-derived relaxing factor (Sugo et al., 1994). In regard to the importance of the ring structure in CGRP, pilot data in our laboratory with the putative CGRP receptor antagonist CGRP 8–37, which does not contain the disulfide ring structure, show that this peptide has partial agonist activity (Santiago et al., 1994; unpublished observations). This observation may suggest that the ring structure is not essential for the expression of vasodilator activity of CGRP; however, more data are needed before a more definite statement can be made.

The results of the present study showing that CGRP had greater vasodilator activity than adrenomedullin in the mesenteric vascular bed of the cat are similar to results in the isolated rat mesenteric vascular bed in which the superior mesenteric artery and the mesentery is separated from the small intestine (McGregor, 1965; Nuki et al., 1993). The results of the present study extend previous results by showing that adrenomedullin and adrenomedullin-(15–52) have significant vasodilator activity in resistance vessels in the small intestine itself. Although CGRP was more potent than adrenomedullin in the isolated mesentery of the rat and in the mesenteric (intestinal) vascular bed of the cat, adrenomedullin had greater vasodilator activity than CGRP in the pulmonary vascular bed of the cat (DeWitt et al., 1994). These data indicate that relative vasodilator activity of adrenomedullin and CGRP is dependent on the specific vascular bed studied and may suggest that the lung has a greater number of adrenomedullin receptors relative to CGRP receptors than does the mesenteric vascular bed.

The pathways and the enzyme involved in the metabolism of adrenomedullin are unknown at the present time. However, the observation that adrenomedullin is present in a number of organ systems and in plasma and is synthesized by endothelial cells is consistent with the hypothesis that adrenomedullin may have an important role in the regulation of the cardiovascular system.

In conclusion, the results of the present investigation demonstrate that adrenomedullin and adrenomedullin-(15–52) have potent but short-lasting vasodilator activity in the mesenteric (intestinal) vascular bed of the cat. The observation that adrenomedullin-(22–52) has little if any vasodilator activity suggests that amino acids 15–52 and the six-membered ring structure are important for the expression of vasodilator activity in the mesenteric vascular bed of the cat.

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