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PROADRENOMEDULLIN NH_2-TERMINAL PEPTIDE (PAMP)(12-20) HAS VASODEPRESSOR ACTIVITY IN THE RAT AND CAT

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Abstract. Decreases in systemic arterial pressure in response to human proadrenomedullin NH_2-terminal 20 peptide (hPAMP), a truncated analog, hPAMP(12-20), and human adrenomedullin (hADM) were compared in the rat and cat. The order of potency was hADM>hPAMP>hPAMP(12-20). hPAMP(12-20) was approximately 3-fold less potent than the full sequence peptide, hPAMP, and 10-fold less potent than the related peptide, hADM. The duration of the vasodepressor responses to hPAMP(12-20) and hPAMP were similar, and responses to both peptides were significantly shorter in duration than hADM. Vasodepressor responses to hPAMP(12-20), hPAMP, and hADM were greater in the rat when compared to responses to the peptides in the cat.

Key Words: **proadrenomedullin NH_2-terminal 20 peptide, adrenomedullin, vasodepressor responses, systemic vascular bed, species differences**

Introduction

Adrenomedullin (ADM) is a recently-discovered hypotensive peptide isolated from human pheochromocytoma cells (1). Human (h) ADM consists of 52 amino acids and a six-membered ring structure, which shares sequence homology with calcitonin gene-related peptide (CGRP) and pancreatic amylin (1). ADM is present in a number of organ systems, and plasma levels are increased in a number of pathological conditions (2-4). The DNA sequence encoding the ADM precursor, proadrenomedullin, has been determined in rat, porcine, and human tissue (5-7). Proadrenomedullin contains 185 amino acids, and cleavage of the signal peptide between amino acids Thr^{21} and Ala^{22} yields a shortened propeptide composed of 164 amino acids, which contains ADM. Proadrenomedullin contains three paired basic amino acids that can be sites for enzymatic cleavage (5). The $Gly^{42}Lys^{43}Arg^{44}$, which is the first group of basic amino acids, is a typical site for enzymatic cleavage to yield the peptide called proadrenomedullin NH_2-terminal peptide (PAMP) (5). Like ADM, PAMP is found in a number of organ systems, such as the adrenal medulla, heart, kidney, and brain, and is detectable in plasma (8,9). PAMP and ADM are distinct products of the ADM gene, and both ADM and hPAMP have hypotensive activity in the rat (1,7). Although ADM has been shown to decrease vascular resistance by a direct action on vascular smooth muscle or by releasing nitric oxide from the endothelium, PAMP has been shown to induce vasodilation by inhibiting the release of norepinephrine from adrenergic nerve endings in the mesenteric vascular bed of the rat (10-14). While the hypotensive effects of

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hADM and hPAMP have been investigated, the effects of the truncated form of hPAMP, hPAMP(12-20), which was synthesized in an attempt to define the minimal chain length needed for activity, have not been determined. Therefore, the present study was undertaken to investigate the systemic hemodynamic effects of hPAMP(12-20) in the rat.

Methods

Sprague-Dawley rats of either sex weighing 340-520 g were anesthetized with pentobarbital sodium (50 mg/kg ip). Adults cats, unselected as to sex, weighing 2.5-4.0 kg were sedated with 20-30 mg/kg im ketamine hydrochloride and anesthetized with pentobarbital sodium (30 mg/kg iv). Supplemental doses of pentobarbital were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated, and the rats breathed room air or were ventilated with a Harvard model 683 rodent ventilator at a tidal volume of 2.4-2.67 ml at a rate of 30-35 breaths/min. The cats breathed room air or were ventilated with a Harvard model 607 respirator at a tidal volume of 40-60 ml and a rate of 15-22 breaths/min. Catheters were inserted into the external jugular vein for the iv administration of drugs and into the carotid artery for the measurement of systemic arterial (aortic) pressure. Systemic arterial pressure was measured with Statham P23 pressure transducers and was recorded on a Grass model 7 polygraph. Mean pressures were derived by electronic averaging.

Proadrenomedullin NH_2-terminal peptide (hPAMP), hPAMP(12-20), and hADM (Peptide Research Labs, Tulane Medical School, New Orleans, LA) were dissolved in 0.9% NaCl; and each peptide was divided into aliquots and stored in 1 ml plastic tubes. The aliquots were stored frozen and thawed on the day of an experiment and kept on crushed ice during an experiment. The peptides were administered intravenously in small volumes (30-150 µl) in doses of 0.03-100 nmol/kg. Injections were made over a period of 10-15 seconds in a random sequence. Injections of the saline vehicle in this manner had no effect on systemic arterial pressure.

All responses were analyzed using a one-way analysis of variance and Scheffe's F test or a paired t-test (15). A P value of less than 0.05 was used as the criterion for statistical significance.

Results

Responses to hPAMP(12-20)

Intravenous injections of hPAMP, hPAMP(12-20) in doses of 0.3-100 nmol/kg, and hADM in doses of 0.03-3 nmol/kg produced dose-related decreases in systemic arterial pressure, and these data are summarized in Figure 1. When compared to hADM, the dose-response curve for hPAMP was 0.3-1 log unit to the right of hADM, and hPAMP(12-20) was approximately 0.3 log unit to the right of the hPAMP curve (Fig. 1B).

Time-course of responses to hPAMP(12-20), hPAMP, and hADM

The time-course of the decreases in systemic arterial pressure to hPAMP(12-20), hPAMP, and hADM in doses of 30, 10, and 3 nmol/kg iv, respectively, are shown in Figure 2. The onset of the vasodepressor response to these peptides was similar (Fig. 2), and the duration of the vasodepressor responses to hPAMP(12-20) and hPAMP were similar (Fig. 2). However, the total duration of the vasodepressor response to hADM was significantly greater (Fig. 2C). The

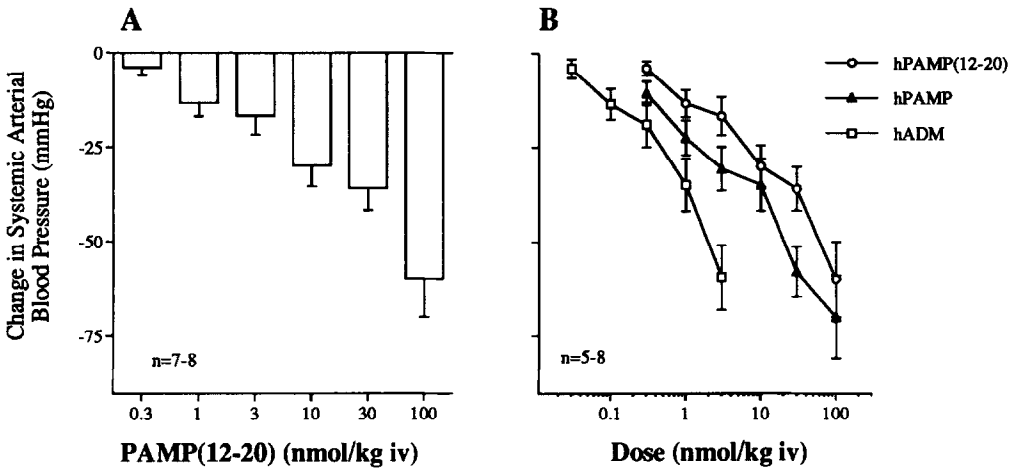

Fig. 1

A: Bar graph showing decreases in systemic arterial blood pressure in response to intravenous injections of PAMP(12-20) in the rat. B: Line graph comparing decreases in systemic arterial pressure in response to intravenous injections of human adrenomedullin (hADM), human proadrenomedullin NH_2-terminal 20 peptide (hPAMP), and hPAMP(12-20) in the rat. Doses of the peptides are expressed on a nmol basis to take molecular weight into account. n indicates number of experiments.

recovery half-times (T½) of the vasodepressor responses to hPAMP(12-20), hPAMP, and hADM were compared, and these data are shown in Figure 2. In terms of T½, vasodepressor responses to hPAMP(12-20) and hPAMP were not significantly different, while the T½ of the vasodepressor response to hADM was significantly longer than for hPAMP and hPAMP(12-20) (Fig. 2D).

Comparison of responses to hPAMP(12-20), hPAMP, and hADM in the rat and cat

Vasodepressor responses to intravenous injections of hPAMP(12-20), hPAMP, and hADM were compared in the rat and cat, and these results are summarized in Figure 3. Intravenous injections of hPAMP(12-20), hPAMP, and hADM induced dose-dependent decreases in systemic arterial pressure in both the rat and cat (Fig. 3). However, hPAMP(12-20), hPAMP, and hADM were 3-10 times more potent in the rat when compared to hypotensive responses obtained in the cat (Fig. 3).

Discussion

The results of the present study demonstrate that hPAMP(12-20) decreases systemic arterial pressure in the rat and cat. In terms of relative vasodepressor activity, hPAMP(12-20) was approximately 3-fold less potent than the full-sequence peptide, hPAMP, and approximately 10-fold less potent than the related peptide, hADM. The duration of the vasodepressor response to hPAMP, and hPAMP(12-20) was similar, and responses to both peptides were significantly shorter in duration than were responses to hADM. hPAMP(12-20), hPAMP, and hADM were

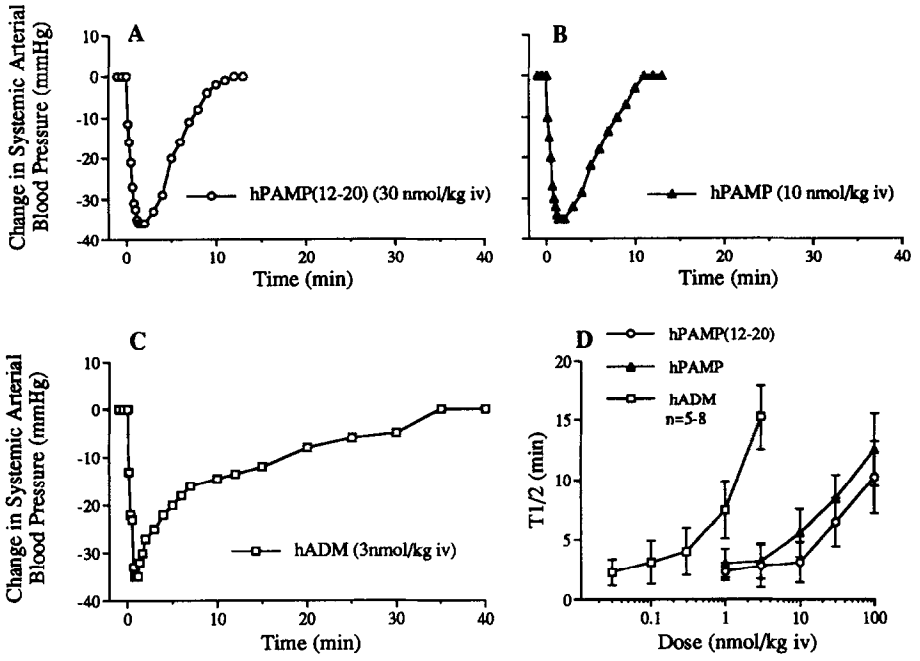

Fig. 2

Line graphs showing the time-course of vasodepressor responses to A) human proadrenomedullin NH_2-terminal 20 peptide [hPAMP(12-20)], B) hPAMP, and C) human adrenomedullin (hADM). D) Comparison of response duration as measured by the response half-recovery times (T½) for hPAMP(12-20), hPAMP, and hADM. All peptides were administered intravenously. n indicates number of experiments.

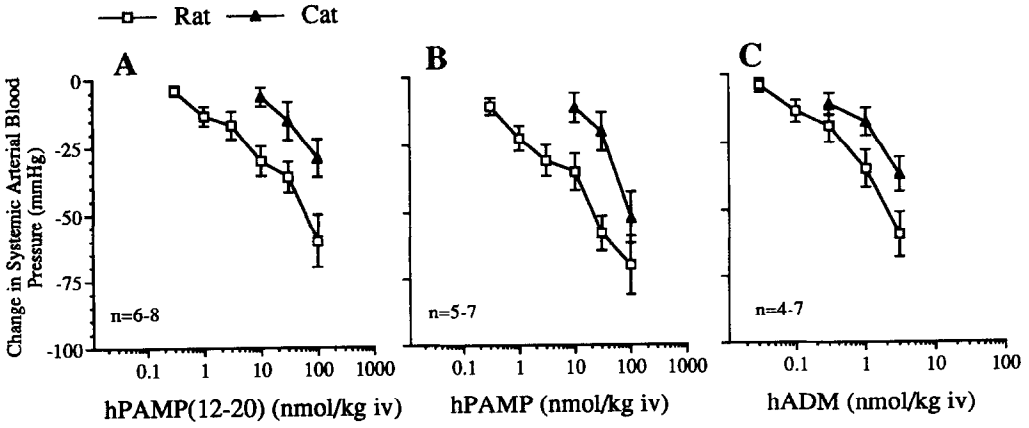

Fig. 3.

Dose-response curves comparing decreases in systemic arterial pressure in the rat and cat in response to intravenous injections of A) human proadrenomedullin NH_2-terminal 20 peptide [hPAMP(12-20)], B) hPAMP, and C) human adrenomedullin (hADM). n indicates number of experiments.

more potent in decreasing systemic arterial pressure in the rat when compared with decreases in pressure in response to the peptides in the cat.

hADM

H_2N-Thr-Arg-Gln-Ser-Met-Asn-Asn-Phe-Gln-Gly-Leu-Arg-Ser-Phe-Gly-Cys-Arg-Phe-Gly-Thr-Cys-Val-Gln-Lys-Leu-Ala-His-Gln-Ile-Tyr-Gln-Phe-Thr-Asp-Lys-Asp-Lys-Asp-Asn-Val-Ala-Pro-Arg-Ser-Lys-Ile-Ser-Pro-Gln-Gly-Tyr-$CONH_2$

hPAMP

H_2N-Ala-Arg-Leu-Asp-Val-Ala-Ser-Glu-Phe-Arg-Lys-Lys-Trp-Asn-Lys-Trp-Ala-Leu-Ser-Arg-$CONH_2$

hPAMP(12-20)

H_2N-Lys-Trp-Asn-Lys-Trp-Ala-Leu-Ser-Arg-$CONH_2$

Fig. 4
Comparison of peptide structures of human adrenomedullin (hADM), human proadrenomedullin NH_2-terminal 20 peptide (hPAMP), and hPAMP(12-20).

hADM and hPAMP produced dose-dependent decreases in systemic arterial pressure in the rat. These data are consistent with previous studies. hPAMP was approximately 10-fold less potent than hADM, and in an attempt to elucidate the minimal peptide length required for the expression of vasodilator activity of PAMP in the rat, hPAMP(12-20) was synthesized. Vasodilator responses to hPAMP(12-20) were similar to the full-sequence form of the peptide, providing support for the hypothesis that amino acids 1-11 are not necessary for the expression of the vasodepressor activity of the hPAMP molecule in the systemic vascular bed of the rat.

The time-course of the vasodepressor responses to hPAMP and hPAMP(12-20) were compared in the systemic vascular bed of the rat, and the duration of the time-course was similar. The duration of responses to hPAMP and hPAMP(12-20), as defined by the T½ of the vasodepressor response, ranged from 2 to 10 minutes, depending on the dose administered. The T½ of vasodepressor responses to hPAMP and hPAMP(12-20) was shorter in duration than with hADM with regard to all doses administered. In addition, the total duration of vasodepressor response was significantly shorter for hPAMP and hPAMP(12-20) than for hADM. The reason for the differences in T½ of vasodilator response and total duration of response are uncertain but may reflect differences in the mechanisms of action or of the metabolism of the peptides. It has recently been reported that PAMP is rapidly cleaved by neutral endopeptidase, and that the activity may result in the termination of action of the peptide (16). Although it has been reported that ADM is inactivated in the lung, the mechanism of inactivation of the peptide is uncertain in the present studies. It is possible that PAMP is metabolized more rapidly than ADM, thus exhibiting a shorter T½ and shorter total duration of action.

hPAMP(12-20), hPAMP, and hADM were more potent in their ability to decrease systemic arterial pressure in the rat when compared with vasodepressor responses to the peptides in the cat. The vasodepressor responses to these peptides were significantly longer in duration in the rat when compared to responses in the cat (data not shown). These data provide support for the hypothesis that vasodilator responses to these peptides differ with regard to species and vascular bed studied (12).

The mechanism by which hPAMP and hADM induce vasodilation is uncertain. Although ADM has been shown to decrease vascular resistance by a direct action on vascular smooth muscle or by releasing nitric oxide from the endothelium, PAMP has been shown to induce vasodilation by inhibiting the release of norepinephrine from adrenergic nerve endings in the mesenteric vascular bed of the rat (10-14). However, the mechanism by which hPAMP and hADM decreases systemic arterial pressure in the rat is uncertain.

The broad implications of the present study and the physiologic role of PAMP, PAMP(12-20), and ADM in the regulation of cardiovscular function are uncertain. However, since plasma levels of ADM and PAMP are elevated in many disease states, such as essential hypertension, renal failure, and congestive heart failure, the peptides may play a role as direct vasodilators in both physiologic and pathophysiologic conditions.

In summary, the results of the present investigation show that an N-terminally truncated analog of hPAMP, hPAMP(12-20), decreases systemic arterial pressure in the rat and cat. Vasodepressor responses to hPAMP(12-20) and hPAMP were similar in duration and both peptides were less potent than hADM in the systemic vascular bed of the rat and cat. hPAMP, hPAMP(12-20), and hADM were more potent in the rat when compared with responses in the cat. These data suggest that vasodepressor responses to these peptides differ with regard to species and vascular bed studied.

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