INHIBITORY ACTION ON ALPHA-HUMAN ATRIAL NATRIURETIC POLYPEPTIDE ON VASCULAR ADRENERGIC NEUROTRANSMISSION IS ATTENUATED IN SPONTANEOUSLY HYPERTENSIVE RATS

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The purpose of the present study is twofold, firstly to investigate the effects of alpha-human atrial natriuretic polypeptide (α-hANP) on norepinephrine overflow from sympathetic nerve endings, and secondly to compare vascular responsiveness in perfused mesenteric preparations in spontaneously hypertensive rats (SHR, Okamoto and Aoki, 7–9 weeks old) and a cohort of Wistar Kyoto rats (WKY).

In preliminary studies using normotensive Wistar rats, the pressor responses to electrical nerve stimulation or exogenous norepinephrine application were inhibited by α-hANP. Norepinephrine overflow was also suppressed by α-hANP, during nerve stimulation.

The pressor responses and norepinephrine overflow during nerve stimulation were significantly greater in SHR than in WKY rats. The inhibitory effect of α-hANP on these responses was reduced in SHR.

These results indicate that α-hANP could affect both pre- and postsynaptic sites of the resistance vessels. Further, the reduced inhibition of pressor responses and norepinephrine overflow by α-hANP in SHR suggests an insufficient regulation of adrenergic transmission by α-hANP in hypertension.

Recent studies have succeeded in sequencing endogenous natriuretic polypeptides isolated from human and rat hearts. Some natriuretic polypeptides such as α-human atrial natriuretic polypeptide (α-hANP) are now chemically synthesized. The biological function of α-hANP is considered to be plasma volume regulation through natriuresis. It has been demonstrated experimentally that α-hANP has potent diuretic activities in anesthetized rats and an acute hypotensive action when administered intravenously. Moreover, Ishihara et al. reported that α-hANP showed a direct vasodilating action suggesting the existence of specific ANP-receptors on vascular smooth muscle cells. Atarashi et al. found that aldosterone secretion from adrenal cells was inhibited by atrial extracts in vivo.

Results from these previous studies suggest that α-hANP has multiple sites of actions, and that the abnormal regulatory function of ANP might contribute to the pathogenesis of hypertension.

The aim of this study was to investigate hypotensive mechanisms of α-hANP. Effects of α-hANP on vascular responsiveness and on norepinephrine release from adrenergic nerve

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endoings in rat mesenteric vasculatures were examined. Alpha-hANP responsiveness in SHR and a cohort of WKY rats was also compared in an attempt to investigate whether or not the response to α-hANP is altered under conditions of hypertension.

MATERIALS AND METHODS

Male Wistar rats were used for initial investigation of α-hANP. Male spontaneously hypertensive rats (SHR, Okamoto and Aoki) (7–9 weeks old) were examined in comparison with a cohort of Wistar Kyoto rats (WKY). The systolic pressures were 168.0 ± 3.6 mmHg in SHR (n = 7), and 120.0 ± 3.5 mmHg in WKY (n = 6), respectively. Systolic pressure was measured by the tail cuff method.

The isolated mesenteric vasculatures were prepared for perfusion by the modified method of Castellucci et al. The superior mesenteric artery was cannulated and four main branches from the mesenteric arterial trunk were isolated. The preparations were perfused with modified Ringer-Locke solution (mmol/L: NaCl 120.7, KCl 5.9, CaCl₂ 2.5, MgCl₂ 1.3, NaHCO₃ 15.5, NaH₂PO₄ 1.2 and glucose 11.5, pH 7.4, 37°C) at a constant flow rate (0.8 ml/min.) using a peristaltic pump. The perfusion pressure was recorded with a pressure transducer connected to a polygraph (Nihon Kohden Model CP-620G).

A pair of platinum electrodes was placed around the proximal end of the mesenteric artery and intramural sympathetic nerves were stimulated. The stimulation was applied at supramaximal voltage (40 volts) with biphasic rectangular pulses of 5 milliseconds duration for 1 min at 10 and 15 Hz (Nihon Kohden Model SEN-3201).

Exogenous 1-norepinephrine (3.3 μg) was given as a bolus injection (in 0.1 ml of the buffer) into the preparations through the arterial cannula. Pressor responses to the electrical nerve stimulation or exogenous norepinephrine were determined as an increase in the perfusion pressure. A thirty minute equilibration period was allowed for the basal perfusion pressure to stabilize.

In order to measure norepinephrine release from the sympathetic nerve terminals in the vascular beds, the perfusate through the mesenteric preparation was collected into tubes containing a mixture of EGTA and glutathione. Collection was carried out for 3 minutes before and 3 minutes after electrical nerve stimulation. The collecting period following electrical nerve stimulation consisted of the 1 minute-stimulation period and 2 minutes subsequent to electrical nerve stimulation. Norepinephrine in the perfusate was absorbed on alumina and extracted in 200 μl of 0.1 N perchloric acid. Norepinephrine was assayed by high pressure liquid chromatography using an electrochemical detector. The norepinephrine overflow due to electrical nerve stimulation was defined as the difference between the norepinephrine released prior to the nerve stimulation and that released after the nerve stimulation. (calculated per g of wet tissue weight for each mesenteric preparation).

In the first series, the experiments were performed in normotensive Wistar rats, weighing 260–340g, to evaluate the pressor responses and endogenous norepinephrine overflow during the electrical nerve stimulation with α-hANP present in the perfusion medium. In the second series of the experiments, a comparison of pressor responses and norepinephrine overflow during the nerve stimulation was made between SHR and the cohort of WKY. The effect of α-hANP on the pressor responses and norepinephrine overflow was also compared between SHR and WKY.

The mesenteric vascular beds were perfused with buffer containing α-hANP, 9 min prior to the electrical nerve stimulation or the application of exogenous norepinephrine.

Values were presented as mean ± SEM. The effect of α-hANP was expressed as the percentage ratio of the control value without α-hANP. Statistical significance was determined by the Student's t-test (paired or unpaired). A difference of p < 0.05 was considered significant.

Alpha-hANP was obtained from the Protein Research Foundation (Japan), and 1-norepinephrine was obtained from Sigma Chemical Co., Ltd. (U.S.A.).

RESULTS

1) Preliminary studies of the effects of α-hANP on adrenergic transmission in mesenteric vasculatures from normotensive Wistar rats

In the investigation of normotensive Wistar rats (weighing from 260–340g), the initial perfusion pressure was maintained at 21.5 ± 0.8 mmHg (n = 11). Seven preparations were used for electrical nerve stimulation, and 4 preparations were used for exogenous norepinephrine

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application. The pressor responses and norepinephrine overflow during the electrical nerve stimulation (15 Hz) were 62.3 ± 3.0 mmHg (n = 7), and 0.88 ± 0.05 ng/g of wet tissue weight (n = 7), respectively. The vasoconstrictor response to exogenous norepinephrine (3.3 μg) was 64.2 ± 10.3 mmHg (n = 4). As shown in our previous report, the periaxial stimulation was neuronal, and the responses were stable for at least 7 repeated stimuli.

The pressor response to either electrical nerve stimulation or exogenous norepinephrine was inhibited by α-hANP present in the perfusion medium, although no changes were shown in the resting perfusion pressure. The inhibition of the contractile responses was more marked in the contractions evoked by the electrical nerve stimulation than by exogenous norepinephrine application (electrical nerve stimulation; 3.4 × 10^{-7} M of α-hANP: 49.7 ± 5.2% of the control value without α-hANP, n = 7, p < 0.001, 1.0 × 10^{-6} M of α-hANP, 16.8 ± 5.3%, n = 7, p < 0.001, exogenous norepinephrine; 1.0 × 10^{-6} M of α-hANP, 86.9 ± 1.7% of the control, n = 4, p < 0.01).

The changes in the norepinephrine overflow during the electrical nerve stimulation are shown in Fig. 1. Alpha-hANP reduced the norepinephrine overflow during the nerve stimulation in a dose-dependent manner in addition to reducing pressor responses.

2) Effects of α-hANP on pressor responses and norepinephrine overflow in mesenteric vasculatures from SHR and WKY

There were no significant differences in the resting perfusion pressure between SHR and WKY (SHR 26.5 ± 1.8 mmHg, n = 7, WKY 25.9 ± 1.3 mmHg, n = 6).

The pressor responses to the electrical nerve stimulation were significantly greater in SHR than in WKY (Table I). The norepinephrine overflow during the electrical nerve stimulation was also enhanced in SHR compared with WKY (Table I).

Figure 2 shows the effects of α-hANP on the pressor responses in SHR and WKY. The pressor responses to the electrical nerve stimulation were inhibited both in SHR and WKY, although the basal perfusion pressures were not affected by these concentrations of α-hANP. As shown in Table II, the inhibitory magnitudes of the pressor responses by α-hANP were significantly smaller in SHR than in WKY.

The norepinephrine overflow during the electrical nerve stimulation was also suppressed in both SHR and WKY, and the neurosuppressive action was less in SHR than in WKY (Table II).

DISCUSSION

The present study demonstrates that α-hANP has an inhibitory effect on norepinephrine
overflows from the adrenergic nerve endings as well as on vascular smooth muscle contractions.

It has been reported that α-hANP has a relaxing effect on the potassium-induced contractions of dog vascular beds. Garcia et al. observed that partially purified atrial natriuretic peptide obtained from rat atrias produced a potent, dose-dependent relaxing effect on rabbit and rat contracted atrial strips whether contraction was caused by norepinephrine or angiotensin II. However, the effect of α-hANP on sympathetic transmission in resistance vessels still remains unclear. In our study, direct measurement of endogenous norepinephrine overflow from the nerve terminals showed that α-hANP reduced electrically stimulated norepinephrine overflow in rat mesenteric vasculatures. This result suggests that ANP-receptors are located on sympathetic neurons. This conclusion is compatible with a previous study that identified ANP-binding sites in neuronal tissues.

Volpe et al. observed that acute administration of synthetic atrial peptide (auriculin A) to Goldblatt hypertensive rats showed a sufficient fall in blood pressure without a responsive increase in heart rate. This might be due to the suppressive action of ANP on sympathetic nerve activity by reducing norepinephrine output from nerve endings.

In other humoral tissues, aldosterone-secretion was inhibited by atrial extracts both in the basal state and following stimulation by angiotensin II or ACTH. Maack et al. found that auriculin depressed the plasma renin activity in dogs. Thus, it is likely that these cardiac peptides could affect several humoral systems, including vascular adrenergic transmission. However, the mechanism of these effects is not clear. Garcia et al. proposed that the vasodilating action of purified atrial natriuretic factor was not due to competition for α and β-adrenergic or muscarinic receptors. They also excluded the possible involvement of prostaglandin since indomethacin had no effects on the relaxation induced by the atrial factor. One suggestion is that the action of ANP is due to an increase in intracellular cGMP by inhibition of cGMP phosphodiesterase. Alternatively the natriuretic effects and the haemodynamic effects (renal vasoconstriction and dilation) may be calcium dependent. ANP would then mediate these responses by influencing the availability of calcium at the cellular level. However, experimental investigation has shown that the action of ANP is not related to calcium influx into the cells, since the relaxing effect of ANP is not reduced on norepinephrine-induced contractions in a calcium-free medium. Further investigation is required to understand the molecular mechanisms by which ANP mediates its actions.

Regarding the role of ANP in hypertension,

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Effects of α-hANP on Adrenergic Transmission in SHR

... has been reported elsewhere in SHR rats, aortas were less sensitive to the relaxing effects of ANP than those from WKY, while atrial content of ANP was increased in SHR. Our study shows that the electrically stimulated pressor responses and norepinephrine overflow from the nerve endings in mesenteric vasculatures were less inhibited by ANP in SHR than in WKY. An interpretation is that the activities of ANP receptors on nervous and vascular smooth muscle cells are reduced in SHR. The reduced sensitivity of ANP receptors as measured by a vasodilatory response might be due to the down-regulation of receptor [concentration/activity?] by increased circulating levels of ANP. The details of this mechanism, however, are still unknown.

In summary, the present study demonstrates that α-hANP could inhibit the vasoconstriction by decreasing the vascular norepinephrine release from the adrenergic nerve endings, the effects being significantly more attenuated in SHR than in WKY. It is suggested that α-hANP is central to the modulation of vascular tone, and that the regulatory disturbances of this polypeptide on adrenergic transmission may result in increased norepinephrine release from the nerve terminals and enhance the vascular responsiveness in hypertension.

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