Alteration of Response to Neuropeptide Y in the Nucleus Tractus Solitarius of Spontaneously Hypertensive Rats

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Abstract In normotensive rats, microinjections of neuropeptide Y (2.5 to 25 pmol) into the unilateral nucleus tractus solitarius elicited dose-dependent vasodepressor and bradycardic responses accompanied by an inhibition of sympathetic nerve firing. After microinjections of the α2-adrenergic receptor antagonist yohimbine (100 ng) into the nucleus tractus solitarius, the depressor and bradycardic responses to the injection of neuropeptide Y (25 pmol) into the nucleus tractus solitarius were significantly attenuated. In contrast, pretreatment with the α2-adrenergic receptor antagonist doxazosin (200 ng) injected into the nucleus tractus solitarius did not alter these responses. In spontaneously hypertensive rats, microinjections of neuropeptide Y (25 pmol) into the nucleus tractus solitarius also elicited depressor and bradycardic responses that were significantly less than those of normotensive Wistar-Kyoto rats. However, pretreatment with yohimbine (100 ng) in the nucleus tractus solitarius did not diminish these depressor responses in spontaneously hypertensive rats. Depressor responses to neuropeptide Y, which was administered after yohimbine pretreatment, were also less in Wistar-Kyoto rats than in spontaneously hypertensive rats. The results suggest that the depressor and bradycardic responses elicited by neuropeptide Y were accompanied by the inhibition of sympathetic nerve activity. These responses may be mediated in part by α2-adrenergic receptor in the nucleus tractus solitarius. The impairment of α2-adrenergic receptor-mediated responses to neuropeptide Y in spontaneously hypertensive rats may contribute to the development of hypertension.

Key Words • neuropeptide Y • rats, inbred SHR • blood pressure • receptors, adrenergic, alpha

Neuropeptide Y (NPY) is colocalized with noradrenergic neurons of the nucleus tractus solitarius (NTS) and dopaminergic neurons of the hypothalamus,1,2 which are important in cardiovascular regulation. It has been reported that microinjections of NPY into the posterior hypothalamic nucleus produced pressor responses,3 whereas injections of NPY into the NTS elicited depressor and bradycardic responses.4 Thus, these findings suggest that NPY is involved in the central regulation of blood pressure. In in vitro studies, it has been demonstrated that NPY could interact with the α2-adrenergic receptor in the medulla oblongata and that this interaction could be impaired in spontaneously hypertensive rats (SHR).5 However, the underlying mechanism of action of NPY in the brain area has not been clarified.

The present study was designed to determine the role of the α2-adrenergic receptor in cardiovascular responses to NPY injected into the NTS in normotensive rats and SHR.

Methods

Rats used in this study were handled according to the guidelines of the American Physiological Society, and the experimental protocol was approved by the Experimental Animal Care Committee of the Kyoto Prefectural University of Medicine. Male Wistar rats (220 to 250 g) (n=49) and SHR (n=16) were age matched with male Wistar-Kyoto (WKY) rats (n=12) (all 9 weeks old). After rats had been anesthetized with urethane (4.2 g/kg IP), phasic blood pressure and heart rate were recorded through a cannula inserted into a femoral artery and connected to a low-volume-displacement pressure transducer (MPU-NEC-Sanei Co, Ltd, Tokyo, Japan) and a biotachometer (heart rate meter, NEC-Sanei Co, Ltd). To record sympathetic nerve activity (SNA), we exposed the left splanchnic nerve and placed a bipolar platinum electrode over it. To reduce noise during recording, spontaneous respiration was abolished by paralyzing the skeletal muscles with decamethonium bromide (2 mg/kg IV). The rats were then connected to a respirator (Ealing Co, Ltd, UK). Neurograms were amplified and filtered (from 30 to 3000 Hz) using two amplifiers (Bioelectric, Sanei 4124, and Extracellular, DPA-100E, Tokyo, Japan) and recorded on a data recorder (TEAC Co, Ltd, Tokyo, Japan). Individual spikes were converted to uniform pulses using an amplitude analyzer. Pulses were counted every 3 seconds using a rate analyzer (DSE-325P, DIA Medical System Co, Ltd, Tokyo, Japan). For microinjection into the NTS, each rat was mounted on a stereotaxic apparatus (Narisinge, Tokyo, Japan) with its head inclined 45°. The dorsal surface of the medulla was exposed by occipital craniotomy. A three-barreled glass micropipette was filled with drugs that were delivered in a volume of 100 nL over 30 seconds by computer-controlled air pressure. The micropipette tip was placed into the left NTS at the following coordinates: 0.5 mm anteroposterior, 0.5 mm mediolateral from the obex, and 0.5 mm vertical from the medullary surface. After experimental treatment, 100 nL of methylene blue was injected into the NTS as a marker for subsequent brain sectioning. After perfusion with 20% Formalin through the femoral artery cannula, the brain was carefully removed. The injection site was localized by histological examination of frozen brain sections. Injection sites were in the NTS. If an area outside of the NTS was stained, the data obtained in those rats were discarded.
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Results

Cardiovascular Responses to Microinjection of Neuropeptide Y Into the NTS of Normotensive Wistar Rats Anesthetized With Urethane

Microinjections of NPY (25 pmol) into the NTS elicited vasodepressor and bradycardic responses accompanied by inhibition of sympathetic nerve firing (Fig 1). These cardiovascular responses to NPY (25 pmol) were significantly larger than those elicited by a similar injection of the saline vehicle (percent change from baseline—blood pressure: saline, −5.1±1.1%; NPY, −37.5±5.6%; P<.01; heart rate: saline, −1.7±0.6%; NPY, −33.6±12.3%; P<.01; SNA: saline, 0.38±3.5%; NPY, −47.6±7.2%; P<.01).

Injections of increasing doses of NPY (2.5, 12.5, and 25 pmol) into the NTS produced dose-dependent decreases in blood pressure (2.5 pmol, −8.9±2.1%; 12.5 pmol, −14.0±1.4%; 25 pmol, −25.8±2.4%) (P<.05, F=25.18, analysis of variance and Duncan’s test) as well as in heart rate (2.5 pmol, −4.8±1.7%; 12.5 pmol, −8.0±2.1%; 25 pmol, −20.4±3.1%) (Fig 2).

Effects of Pretreatment With Yohimbine and Doxazosin on Cardiovascular Responses to Neuropeptide Y Injected Into the NTS of Normotensive Wistar Rats

Microinjections of yohimbine (100 ng) into the left NTS of normotensive Wistar rats produced pressor responses (percent change of blood pressure, 15.5±4.6% from baseline). Blood pressure returned to the preinjection level approximately 5 minutes later, and NPY (25 pmol) was microinjected into the left NTS. Depressor and bradycardic responses to NPY were significantly attenuated compared with the response of groups pretreated with saline (blood pressure: saline, −21±4.7%; yohimbine, −4.8±1.6%; P<.01; heart rate: saline, −15.0±4.6%; yohimbine, −1.5±0.8%; P<.01). Pretreatment with doxazosin (200 ng) administered into the NTS, which elicited depressor responses (percent change in blood pressure,
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17.4±4.2% from baseline), did not alter the depressor and bradycardic responses to the subsequent injection of NPY (25 pmol) (blood pressure: saline, -21±4.7%; doxazosin, -19.6±2.2%; P=NS; heart rate: saline, -15.0±4.6%; doxazosin, -10.5±1.7%; P=NS) (Fig 3).

Cardiovascular Responses to Microinjection of Neuropeptide Y Into the NTS of SHR

Microinjection of NPY (25 pmol) into the NTS of SHR also elicited vasodepressor and bradycardic responses. The depressor responses in SHR were significantly smaller than in normotensive WKY rats, although the change in heart rate did not differ between groups (blood pressure: WKY rats, -25.7±2.6%; SHR, -13.8±2.3%; P<.01; heart rate: WKY rats, -23.8±2.9%; SHR, -18.4±2.1%; P=NS). Pretreatment with yohimbine (100 ng) into the left NTS did not alter the depressor responses to NPY in SHR (blood pressure: saline, -17.7±2.5%; yohimbine, -19.4±3.2%; P=NS; heart rate: saline, -10.5±1.4%; yohimbine, -8.8±1.7%; P=NS). The depressor and bradycardic responses to NPY in yohimbine-pretreated rats were significantly smaller in WKY rats than in SHR (blood pressure: WKY rats, -7.2±0.4%; SHR, -19.4±3.2%; P<.01; heart rate, WKY rats, -2.4±1.3%; SHR, -8.8±1.7%; P<.05) (Fig 4).

Discussion

Tseng et al reported that microinjections of NPY into the NTS elicited dose-dependent depressor and bradycardic responses. The specific mechanism of NPY action in producing these cardiovascular responses has not been determined. Our results indicate that the depressor responses to the administration of NPY into the NTS could be produced by an inhibition of SNA. Martin et al reported that microinjections of NPY into the posterior hypothalamus in conscious rats produced pressor responses, with a magnitude similar to that found in anesthetized rats. It is therefore suggested that there may be little effect of anesthesia on the cardiovascular responses of NPY in the NTS. The NTS, which is the site of the termination of primary afferent fibers of arterial baroreceptors, is richly innervated by axons containing NPY colocalized with catecholamine-secreting neurons. Autoradiographic studies indicate that both α1- and α2-adrenergic receptors are located within the NTS. Kubo et al reported that the α2-adrenergic agonist phenylephrine injected into the NTS elicited pressor responses, whereas the α2-receptor agonist agonist α-methylnorepinephrine elicited depressor responses. In an in vitro study, Tsuda et al reported that NPY potentiated the inhibition of stimulation-evoked [3H]norepinephrine release by an α2-receptor agonist in slices of the medulla oblongata. This inhibitory effect of NPY was diminished by an α2-adrenergic receptor agonist, suggesting presynaptic receptor interaction of this peptide with α2-adrenergic receptors in the medulla oblongata. NPY may therefore...
produce cardiovascular responses by interacting with an \( \alpha_2 \)-adrenergic receptor in the NTS. This study demonstrated that pretreatment with the \( \alpha_2 \)-adrenergic receptor antagonist yohimbine injected into the NTS attenuated the depressor and bradycardic responses to NPY injected into the NTS. Pretreatment with the \( \alpha_2 \)-adrenergic receptor antagonist doxazosin, however, had no effect.

Our results suggest more than one possible explanation for the action of NPY on the sympathetic neurotransmitter junction of the NTS. First, NPY may have an agonistic action on the postsynaptic \( \alpha_2 \)-adrenergic receptor system. This is consistent with the finding that NPY and epinephrine coadministered intraventricularly to alert rats significantly antagonized each other's hypotensive effects. Second, NPY may enhance the action of the \( \alpha_2 \)-adrenergic receptor. This possibility is supported by a report that NPY could selectively increase the number of \( \alpha_2 \)-adrenergic binding sites in membranes of the rat medulla oblongata. The depressor responses to NPY injected into the NTS in the present study were not completely inhibited by pretreatment with an \( \alpha_2 \)-adrenergic receptor antagonist suggests a third possible action—that this peptide could produce cardiovascular responses by acting directly on the NPY receptor as a neurotransmitter.

In vivo studies in SHR indicate that the potency of the intracisternal injection of NPY on the vasodepressor response is significantly less than that of WKY rats. This is consistent with our finding that the depressor responses elicited by injecting NPY into the NTS of SHR were smaller than in WKY rats. There may be several explanations for the dysfunction of NPY in the NTS of SHR. Although MacAroone et al demonstrated that in SHR the content of this peptide in the NTS of SHR did not differ from that in WKY rats, it is possible that in SHR the sensitivity of the NPY receptor in the NTS may be lower than in WKY rats. Other in vivo studies have demonstrated an impaired interaction between NPY and the \( \alpha_2 \)-adrenergic receptor system in SHR. Tsuda et al reported that the ability of this peptide to enhance the inhibitory effects of the \( \alpha_2 \)-adrenergic receptor on the stimulation-evoked release of \([\text{H}]\)norepinephrine was reduced in synaptosomes isolated from the medulla oblongata of SHR compared with normotensive WKY rats. Agnati et al demonstrated that the number of \( \alpha_2 \)-adrenergic binding sites in medulla oblongata membrane was not altered by NPY in SHR compared with WKY rats. The lack of effect of pretreatment with an \( \alpha_2 \)-adrenergic receptor antagonist on the cardiovascular responses elicited by NPY further explains the smaller depressor responses to this peptide injected into the NTS of SHR. We observed that depressor responses to NPY injected into the NTS after yohimbine pretreatment were smaller in WKY rats than in SHR. In WKY rats, the depressor responses to NPY may be produced mainly by an interaction between its receptor and \( \alpha_2 \)-adrenergic receptors, although in SHR, this peptide may act on its receptor independent of \( \alpha_2 \)-adrenergic receptors.

Previous reports have discussed disorders of the catecholaminergic system in the medulla oblongata of SHR. Yao et al reported that the norepinephrine content in the NTS was lower in SHR than in WKY rats. Yamada et al demonstrated a specific loss of \( \alpha_2 \)-adrenergic receptors in the medulla oblongata of SHR. These findings suggest that the direct effect of NPY on its own receptor may compensate for the impairment of the catecholaminergic system in the NTS of SHR. Specific receptor antagonists for this neuropeptide are not available for further study of their role in the NTS.

In conclusion, the present study demonstrated that injection of NPY into the NTS of anesthetized rats elicited depressor and bradycardic responses accompanied by the inhibition of SNA. This effect, in part mediated by an \( \alpha_2 \)-adrenergic receptor and impaired in SHR, suggests an involvement of NPY in the hypertension of SHR.

References

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