Direct evidence for the role of neuropeptide Y in sympathetic nerve stimulation-induced vasoconstriction

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Han, Songping, Chun-Lian Yang, Xiaoli Chen, Linda Naes, Bryan F. Cox, and Thomas Westfall. Direct evidence for the role of neuropeptide Y in sympathetic nerve stimulation-induced vasoconstriction. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H290–H294, 1998.—Neuropeptide Y (NPY) is a vasoconstrictor peptide and a cotransmitter with norepinephrine (NE) in sympathetic nerve terminals and is thought to be involved in sympathetic nerve stimulation (SNS)-induced vasoconstriction. Using BIBP-3226, a Y1 receptor selective antagonist, we examined this hypothesis in the isolated and perfused mesenteric vascular bed. SNS produced a frequency-dependent increase in perfusion pressure and concomitant overflow of NPY immunoreactivity in the perfusate. [Leu31,Pro34]NPY potentiated NE-induced and ATP-induced vasoconstriction, indicating the presence and biological action of Y1 receptors in this vascular bed. The potentiation effect of [Leu31,Pro34]NPY of the increase in perfusion pressure by NE, ATP, or SNS was prevented by BIBP-3226. In addition, SNS-induced vasoconstriction at both high and low frequencies was significantly attenuated by BIBP-3226 at a concentration that completely blocked the [Leu31,Pro34]NPY-induced potentiation of the NE- or ATP-induced vasoconstrictor effect. These results suggest that ~30% of vasoconstriction produced by SNS depends on NPY in the mesenteric vascular bed.

BIBP-3226: neuropeptide Y release; adenosine 5′-triphosphate; mesenteric vascular bed; rat

NOREPINEPHRINE (NE) is the classical neurotransmitter released from sympathetic nerve terminals during sympathetic discharge and produces vasoconstriction by activating α-adrenoceptors (22) located on vascular smooth muscles. However, complete α1-receptor blockade only attenuates rather than prevents nerve stimulation-induced vasoconstriction (14), indicating the presence of a nonadrenergic component. Pretreatment with 6-hydroxydopamine completely abolishes the vasoconstrictor response to nerve stimulation, implicating sympathetic origin of the nonadrenergic vasoconstriction.

In addition to NE, neuropeptide Y (NPY) is also found in sympathetic nerve fibers (17). Subcellular fractionation experiments suggest that NPY and NE are colocalized in large dense-core vesicles (5). In addition, NPY gene expression, storage, and release are all increased after sympathetic nerve activation such as seen during stress (9, 10). NPY elicits a pressor response after intravenous administration (14, 25) as well as producing vasoconstriction (4) or potentiation of the effect of other vasoconstrictor transmitters in vitro (24). In certain blood vessels, the vasoconstrictor potency of NPY is 25 times greater than NE on a molar basis (13). There is increasing evidence that the vascular response to NPY is mediated by Y1 subtype of NPY receptors, although some of the effect may also be mediated by Y2 receptors (8).

On the basis of these and other observations, it is hypothesized that NPY mediates nonadrenergic vasoconstriction during sympathetic nerve stimulation (SNS) (14). Recently, BIBP-3226, the first NPY Y1 receptor selective antagonist, was developed (2, 20, 26). We used it in the present study to examine the role of endogenous NPY in SNS-induced vasoconstriction in the isolated and perfused mesenteric vascular bed.

MATERIALS AND METHODS
Isolated and perfused mesenteric vascular bed preparation. Male Sprague-Dawley rats (325 g; Harlan) were anesthetized with pentobarbital sodium (50 mg/kg ip), and the mesenteric arterial bed was excised and perfused using a modified method as described previously (12). Briefly, the abdomen was opened, and heparin sodium (100 U/ml; 2 mg/kg) was administered via the inferior vena cava. The mesenteric arterial bed and associated intestine were removed after ligation of the descending colon proximal to the rectum and the duodenum proximal to the stomach, and the superior mesenteric artery then was cannulated with PE-90 polyvinyl tubing connected to a syringe and flushed with heparinized saline. The four main branches were ligated. The mesenteric vascular bed was dissected from the intestinal wall. The vascular bed was placed in an organ bath maintained at 37°C, perfused, and superfused with a modified Krebs-bicarbonate buffer using a Gilson mini-pump at a rate of 5 and 0.5 ml/min, respectively. The modified Krebs-bicarbonate buffer was composed of the following (in mM): 120 NaCl, 5.0 KCl, 1.2 MgSO4, 2.4 CaCl2, 11.1 dextrose, 25 NaHCO3, and 0.027 EDTA sodium. The perfusate was maintained at 37°C and aerated constantly with 95% O2-5% CO2. The tissue was allowed to equilibrate for 45–60 min.

Radioimmunoassay for NPY. The perfusate was collected for determination of NPY immunoreactivity (NPYir). A specific antiserum that was raised in rabbit against porcine NPY (1) was used in the assay. The antiserum showed no cross-reactivity to structurally related peptides such as peptide YY and rat pancreatic polypeptide (1). Radioimmunoassay was performed using a 5-day disequilibrium method. Samples were incubated with the NPY antiserum, Twenty-four hours later, 125I-labeled NPY was added to each tube. After a 72-h incubation at 4°C, antibody-bound NPY and free NPY were separated by a second antibody, and its radioactivity was determined in a gamma counter.

Statistical analysis of the data. The data are expressed as means ± SE. Two-way analysis of variance followed by Newman-Keuls test was used for statistical analysis of the data with multiple groups. Values were considered significantly different when P < 0.05.
RESULTS

Stimulation of the periarterial nerves (supramaximal voltage for 0.5 min) elicited a frequency-dependent increase in perfusion pressure in isolated and perfused mesenteric vascular bed of rat, indicative of vasoconstriction in this vascular bed (Fig. 1). It was observed that a maximal increase in perfusion pressure was produced by a frequency of 16 Hz. Because it has previously been reported that NPY release was more effectively seen at high frequencies of nerve stimulation (28), we measured the release of NPY in perfusate effluents before, during, and after SNS using a fraction collector, and NPY in perfusate was determined by radioimmunoassay. Figure 2 demonstrates that SNS at a frequency of 16 Hz resulted in a significant increase in NPY in perfusate. The SNS-induced vasoconstriction and NPY release occurred at the same time. The peaks of the vasoconstriction and NPY release both reside within 1 min after the stimulation. The vascular resistance and NPY release returned to prestimulation levels in 5 and 10 min, respectively, after the nerve stimulation.

The addition of NE or ATP to the perfusion buffer resulted in an increase in the perfusion pressure of the mesenteric arterial bed (Figs. 3 and 4). The NE-induced vasoconstriction was potentiated by the prior administration of the Y1 selective NPY agonist [Leu31,Pro34]NPY (LP-NPY) (Figs. 3 and 4). The potentiating effect of LP-NPY on NE-induced vasoconstriction was prevented by BIBP-3226 (100 nM, 5 min), implicating the presence and vascular action of Y1 receptors (n = 8–12). *P < 0.05 vs. NE.

BIBP-3226 was also employed to examine whether or not NPY released from sympathetic nerve terminals contributed to SNS-induced vasoconstriction. SNS (su-
pramaximal voltage, 16 Hz for 30 s) was induced three times at 15-min intervals (S1, S2, and S3). BIBP-3226 (100 nM) was added to the perfusate buffer 5 min before and during second stimulation (S2). Figure 5 depicts the results of this experiment and demonstrates that SNS-induced vasoconstriction was attenuated in the presence of BIBP-3226 (S2 vs. S1 and S3). BIBP-3226 alone did not produce any change in perfusion pressure (data not shown).

It is obvious that the maximal vasoconstriction produced by SNS of 16 Hz is only relevant for some extreme conditions in vivo such as during severe exercise. To examine whether NPY also plays a role regulating vascular tone at lower levels of sympathetic nerve activity, the effects of LP-NPY and BIBP-3226 on the vasoconstrictor response of the vascular bed to low frequency stimulation were also examined. According to the frequency-response curve, SNS of 8 Hz produced a modest vasoconstriction that was ~25% of the vascular tone produced by SNS of 16 Hz (Fig. 1).

LP-NPY (30 nM) alone did not produce any change in vascular tone (Fig. 6). However, a significantly greater vasoconstrictor response to SNS at a frequency of 8 Hz was observed after infusion of LP-NPY (Fig. 6). The potentiating effect of LP-NPY on low-frequency SNS-induced vasoconstriction was completely prevented by BIBP-3226 (100 nM) (Fig. 6). In addition, BIBP-3226 further partly blocked SNS-induced vasoconstriction (Fig. 6). These results suggest that endogenous NPY played a role in sympathetic nerve-mediated vasoconstriction at lower levels of sympathetic nerve activity as well.

**DISCUSSION**

We utilized the isolated perfused mesenteric arterial bed as a model to study the release of NPY and postjunctional actions of NPY at the sympathetic vascular neuroeffector junction. We have previously observed that periarterial nerve stimulation of this preparation results in a frequency-dependent increase in perfusion pressure and NE release (23), indicating activation of sympathetic nerves. In addition to NE, the sympathetic nerves innervating many vascular beds are also thought to contain and release NPY as well as ATP and are thought to be sympathetic cotransmitters/comodulators (19). In the present study, we reconfirm that periarterial nerve stimulation of the mesenteric arterial bed resulted in a frequency-dependent increase in perfusion pressure. In addition, we observed that high-frequency nerve stimulation (16 Hz) resulted in a release of NPY-

NPY is known to be a potent vasoconstrictor (direct effect) as well as to potentiate the contractile effect of a variety of vasoactive agents, particularly in vitro (indirect effect). Most direct and indirect vascular effects of NPY are thought to be due to activation of NPY Y1 receptors, although Y2 receptors may be responsible in some vascular beds (7, 21, 28).

Recently, several compounds, such as BIBP-3226, have been developed and shown to antagonize the effects of NPY (20). BIBP-3226 has been shown to be a selective antagonist for NPY-Y1 receptors (26) and has been shown to block both the direct and indirect vascular effects of NPY (2, 3, 6, 15). In the present study, we utilized BIBP-3226 and demonstrated that it prevented the potentiating effect of the Y1 agonist LP-NPY on NE- and ATP-induced increase in perfusion pressure in the mesenteric arterial bed. The SNS-induced vasoconstriction was reduced by ~30% in the presence of BIBP-3226 at a concentration that effectively antagonized the LP-NPY-induced potentiation of the contractile effect of NE and ATP.

A low-frequency SNS (8 Hz) was also applied to the mesenteric vascular bed to produce a moderate vasoconstriction, representing the condition of neurotransmission at lower level of sympathetic nerve activity. LP-NPY did not produce any significant change in perfusion
pressure, although it significantly potentiated SNS-induced vasoconstriction. The potentiating effect of LP-NPY on SNS-induced vasoconstriction indicates that the vasoconstrictor response mediated by endogenous NE from sympathetic nerves can also be modulated by NPY. Although the Y5 subtype of NPY receptors can also be activated by LP-NPY, the prevention of the potentiating effect of LP-NPY by BIBP-3226 suggests that it is mediated by Y1 receptors. The further attenuation of low-frequency SNS-induced vasoconstriction strongly indicates that endogenous NPY and Y1 receptors play a modulatory role on vascular tone at both high and low levels of sympathetic nerve activity.

It is thought that differential release of cotransmitters can occur at different levels of sympathetic nerve activity. Peptide transmitters may be released preferentially over classic transmitters at higher levels of sympathetic nerve activity (28). If this is true for NE/NPY transmission in the mesenteric vascular bed, we would expect to see a smaller antagonistic effect of BIBP-3226 on SNS-induced vasoconstriction when a low-frequency stimulation is used, since NPY/NE may lower. In contrast, similar percent changes in SNS-induced vasoconstriction were produced by BIBP-3226 after both high- and low-frequency stimulation. The results seem to suggest that NPY plays a similar role modulating vascular tone at both high and low levels of sympathetic nerve activity.

Our results are consistent with those of Malström and Lundberg (18), who observed that BIBP-3226 blocked the vasoconstrictor effect of NPY as well as inhibiting the slow, long-lasting contraction evoked by high-frequency electrical nerve stimulation of the guinea pig vena cava. BIBP-3226 was also observed to antagonize the slow, long-lasting increase in blood pressure after in vivo SNS of the pig (15) as well as the stress-induced increase in mesenteric vascular resistance of the rat (27). Our results and those of others therefore support the idea that NPY is an endogenous neurotransmitter/neuromodulator with NE and ATP in sympathetic nerves.

In summary, our results demonstrated that SNS incited concomitant vasoconstriction and NPY release in the isolated and perfused mesenteric vascular bed. SNS-induced vasoconstriction was potentiated and attenuated by selective Y1 receptor agonists and antagonists, respectively. Our results suggest that NPY mediates SNS-induced nonadrenergic vasoconstriction in the mesenteric vascular bed.

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