Interaction of Neuropeptide Y and the Sympathetic Nervous System in Vascular Control in Man

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Background. There is increasing evidence that neuropeptide Y (NPY) contributes to the autonomic control of the circulation. NPY coexists with noradrenaline in perivascular nerve terminals, may be released during sympathetic stimulation, and is a potent constrictor of the human coronary circulation and other vascular beds. In vitro studies show that NPY can act either directly on vascular smooth muscle or indirectly by modulation of the presynaptic release or the postsynaptic actions of noradrenaline. It is unclear to what extent these mechanisms operate in vivo.

Methods and Results. The effect on forearm blood flow of intra-arterial NPY was studied in six volunteers during coinfusion of noradrenaline and during reflex sympathetic stimulation induced by lower-body negative pressure. NPY alone induced a dose-dependent reduction of forearm blood flow in all subjects studied, to a maximum of 49±6.1%. The reduction of flow during infusion of noradrenaline alone was 42±8%. The response to noradrenaline was unaffected by coinfusion of a threshold constrictor dose of NPY (50 pmol/min). Furthermore, the reflex sympathetic vasoconstrictor response to 20 cm H2O of lower-body negative pressure was similar in both the infused and control arms during the infusion of 50 pmol/min NPY. The response to noradrenaline was abolished by α-blockade with phentolamine, but the flow reduction induced by NPY was unaffected by α-blockade.

Conclusions. NPY is a potent constrictor of human forearm resistance vessels and has a direct effect independent of α-receptors. NPY has no detectable modulating effect in vivo on the action of endogenous or infused noradrenaline. (Circulation 1991;83:774–777)

Following the demonstration by Lundberg et al1 that sympathetic nerve terminals contain a peptide showing an immunoreactivity similar to that of avian polypeptide, Tatemoto et al2 isolated the structurally related peptide, neuropeptide Y (NPY), which was subsequently shown to be colocalized with noradrenaline in sympathetic nerve endings.3 Evidence has since accumulated implicating NPY in autonomic control of the circulation. NPY is a potent constrictor of human vascular tissue4 and is released with noradrenaline during sympathetic nerve stimulation.5 In vitro studies have shown that NPY acts directly, presumably through its own receptor, on vascular smooth muscle by a mechanism dependent on extracellular calcium.6 Experimental evidence suggests that NPY also has an indirect action at the neuroeffector junction. In a way similar to that of noradrenaline itself,7 NPY has been shown to inhibit the presynaptic release of noradrenaline4 and to potentiate the postsynaptic action of noradrenaline.8 We examined the effect of locally administered NPY on forearm blood flow and defined a dose that is just above the constrictor threshold. We then investigated whether this dose of NPY influences vasoconstriction resulting from reflex sympathetic activity and from exogenous noradrenaline infusion.

Methods

Six healthy male volunteers between 25 and 40 years of age participated in each of these studies, which were approved by the Ethics Committee of St. George’s Hospital. Informed consent was given by all volunteers. Forearm blood flow was measured in both arms simultaneously using venous occlusion.
plethysmography with temperature-compensated mercury-in-Silastic strain gauges. During measurement, blood flow to the hands was prevented by wrist cuffs inflated to 200 mm Hg. A collecting cuff pressure of 40 mm Hg was used. Flows were recorded for 10 of every 15 seconds. The mean of the final five measurements of each recording period was used for subsequent analysis.

A 27-gauge unmounted steel cannula was inserted into the nondonominant (left) brachial artery using local anesthesia (1% lidocaine hydrochloride). Solutions were infused at a constant rate of either 0.5 or 1.0 ml/min throughout the experiment by means of a constant-rate infusion pump (944A, Harvard Apparatus, South Natick, Mass.). When two drugs were infused simultaneously, a Y-connector delayed mixing until the solutions entered the cannula. The dominant arm was not cannulated and served as a control. Each study took place in the morning, after the volunteers had rested supine in a quiet clinical laboratory for a minimum of 30 minutes at a room temperature maintained between 26° and 28°C. Saline (0.9% NaCl, Travenol Laboratories, Deerfield, Ill.) was infused for 10 minutes, followed by infusion of three incremental doses of freshly reconstituted NPY (50, 200, and 1,000 pmol/min, Peninsula Laboratories, Inc., Belmont, Calif.), each given for 6 minutes. Forearm blood flow was measured during the last 3 minutes of each infusion, and measurements continued at intervals for up to 20 minutes after stopping the NPY infusion. The 10-minute saline infusion was repeated, followed by infusion of incremental doses of noradrenaline (125, 250, and 500 pmol/min), each given for 6 minutes. Forearm blood flow was measured during the final 3 minutes of each infusion. After 20 minutes, the infusions of noradrenaline were repeated during coinfusion of 50 pmol/min NPY.

In each subject a lower-body negative-pressure chamber constructed of polyethylene sheeting over a reinforced mesh wire framework was placed around the lower portion of the body from the iliac crest down. A seal was formed between the walls of the chamber and the subject using adhesive tape. An adjustable vacuum pump was connected to the sealed chamber, and the negative pressure achieved was measured continuously using a water manometer. Saline, 50 pmol/min NPY, and saline were sequentially infused during three consecutive 12-minute periods. During each period, forearm blood flow was measured continuously after the third minute. From the sixth to the ninth minutes lower-body negative pressure was applied to a degree (20 cm H2O) that has been shown to have no effect on blood pressure and heart rate.

In four subjects 500 pmol/min noradrenaline and then 1,000 pmol/min NPY were infused for 6 minutes each, followed by an interval of 10 minutes to allow blood flow to return to baseline. Phentolamine (300 nM/min) alone was then infused for 20 minutes, at which time noradrenaline was added to the phentol-

amine infusion. Following cessation of the noradrenaline infusion, NPY was infused during continued infusion of phentolamine. Blood flow was measured during the last 3 minutes of each infusion.

Student’s t test for paired observations was used for statistical analysis, and the data are presented as mean±SEM.

Results

NPY reduced forearm blood flow in a dose-dependent fashion in all six subjects (Figure 1). The lowest dose (50 pmol/min) induced slight reduction in some subjects, but the overall effect was not significant. At the peak dose, blood flow was reduced from 3.8±0.6 to 1.8±0.2 ml/100 ml/min. At this dose, the effect of NPY was detectable within 2 minutes of commencing the infusion and persisted for up to 15 minutes after stopping the infusion. Blood flow in the control arm showed no consistent change during the study.

Noradrenaline also reduced forearm blood flow in all subjects in a dose-dependent fashion (Figure 2, left), from 4.8±1.1 to 2.5±0.4 ml/100 ml/min. This response was unaffected by coinfusion of 50 pmol/min NPY (Figure 2, right), blood flow falling from 5.2±1.2 to 2.3±0.4 ml/100 ml/min. The onset of the response to noradrenaline occurred in <1 minute,
and blood flow returned to basal values within 5 minutes of stopping the infusion.

Lower-body negative pressure reduced forearm blood flow in both arms, by 14.0±3% during saline infusions and by 20.3±3.0% during NPY infusion (differences not significant, Figure 3). While there was a trend toward enhanced reflex flow reduction during NPY infusion in both arms, this did not reach the level of significance.

A single dose of 1,000 pmol/min NPY resulted in a reduction in blood flow similar to that induced by 500 pmol/min noradrenaline. Coinfusion of phentolamine caused an increase in basal forearm blood flow and abolished the response to noradrenaline, but the response to NPY remained unchanged and was unaltered (Figure 4).

**Figure 3.** Effect of neuropeptide Y (NPY) on mean±SEM constrictor response to 20 cm H₂O lower-body negative pressure (LBNP) in six men. LBNP produced similar reductions in forearm blood flow in both arms during saline infusion. Infusion of 50 pmol/min NPY failed to alter ratio of constriction in the two arms, as shown by ratio of infused arm flow/control arm flow. □, control arm; ■, infused (nondominant) arm.

**Figure 4.** Effect of phentolamine on mean±SEM constrictor effect of noradrenaline (NA) and neuropeptide Y (NPY) in four men. Infusion of 500 pmol/min NA and 100 pmol/min NPY result in similar reductions in forearm blood flow. During coinfusion of phentolamine, which increases resting blood flow, response to NA is abolished while maximum response to NPY is unaltered.

**Discussion**

We examined the effects on the human forearm vascular bed of intra-arterial administration of NPY. At low concentrations, this peptide caused a large dose-dependent reduction in blood flow very similar to that demonstrated by Pernow et al. Previous studies have shown three possibly distinct actions of NPY in vitro using animal tissue: presynaptic inhibition of noradrenaline release, postsynaptic potentiation of noradrenaline's action, and a direct action independent of α-adrenergic receptors. The failure of the nonselective α-blocking agent phentolamine in these circumstances to diminish or abolish the constrictor action of NPY is clear evidence of the existence of a direct mechanism of action in vivo in man.

While no significant enhancement of reflex constriction to lower-body negative pressure was found during NPY infusion, a small decrease in blood flow was observed in both arms, suggestive of a central effect of the small increment in the circulating NPY concentration.

The similarity of the pressor responses in the two arms to lower-body negative pressure suggests that the much higher local concentration of NPY was unable to directly modify sympathetic constriction in the peripheral circulation. This lack of potentiation of constriction, induced by either reflex or by infusion of noradrenaline, suggests that in the human forearm NPY acts directly rather than through presynaptic or postsynaptic augmentation of noradrenergic sympathetic function. This is in contrast to many in vitro studies that clearly demonstrate an indirect constrictor effect of NPY. It is possible that with intraluminal administration, the concentration of the peptide is relatively less on the adventitial side of the vessel, where the majority of the neuroeffector junctions are located. However, an enhanced sympathetic response to lower-body negative pressure has been documented with intraluminal administration of angiotension II and, therefore, accessibility of binding sites does not appear to be a limiting factor.

Compared with noradrenaline, NPY is slower to act but the constriction is longer-lasting. The response in the forearm was quite reproducible and similar in all subjects studied. This is in contrast to our and other observations in the coronary bed and in vitro, where responses are variable and tend to be “all or nothing.”

The reduction in forearm blood flow demonstrates the ability of NPY to produce constriction of resistance vessels, as has been demonstrated in the coronary circulation in dogs and humans and is consistent with the observation of more NPY-containing nerve vesicles in smaller arteries.

The observation that phentolamine fails to abolish the constrictor response to NPY is consistent with the documented failure of α-blockade to completely abolish the vascular effects of sympathetic stimulation in other models. Indeed, α-blockade actually increases the overflow of NPY during nerve stimula-
tion. In view of the fact that varying stimulation frequencies of sympathetic nerves may cause a preferential release of NPY from the nerve terminal, it is conceivable that there are conditions in which sympathetic stimulation (central or otherwise) may provoke NPY release alone. It is therefore possible that this peptide plays an important part in constrictor responses resulting from sympathetic stimulation.

References

KEY WORDS • neuropeptide Y • sympathetic nervous system • vascular control
Interaction of neuropeptide Y and the sympathetic nervous system in vascular control in man.
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Circulation. 1991;83:774-777
doi: 10.1161/01.CIR.83.3.774
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/83/3/774

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