Neuropeptide Y and Y1-receptor agonists increase blood flow through arteriovenous anastomoses in rat tail

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Heath, Martha E. Neuropeptide Y and Y1-receptor agonists increase blood flow through arteriovenous anastomoses in rat tail. J. Appl. Physiol. 85(1): 301–309, 1998.—The purpose of this study was to characterize neuropeptide Y (NPY)-induced vasodilation in the rat tail. Sterile surgical technique was used (with pentobarbital sodium anesthesia) to equip rats with a jugular catheter and a blind-ended thermocouple reentrant tube next to the carotid artery. Tail skin and core temperature were measured with thermocouples during experiments. Tail skin blood flow was monitored with a laser Doppler flowmeter, and tail total blood flow and volume were measured with plethysmography. After baseline data were collected, saline, NPY (16, 32, 64, and 128 µg/kg), [Leu31 Pro34]NPY (63.25 µg/kg), or NPY[13–36] (44.7 µg/kg) was administered intravenously. Tail total blood flow, volume, and tail skin temperature increased, whereas tail skin blood flow and core temperature decreased in response to both NPY- and the Y1-receptor agonist [Leu31 Pro34]NPY but not in response to saline or NPY[13–36]. Studies conducted with the use of color microspheres demonstrated that arteriovenous anastomoses are involved in this NPY-induced vasodilation.

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter that occurs, in separate vesicles, in the same sympathetic adrenergic fibers as does norepinephrine (NE) (8, 26). It also coexists with vasoactive intestinal peptide (VIP) in peripheral non-noradrenergic neurons (25), occurs in the adrenal medulla, and is one of the most abundant of all neuropeptides found in the brain (5, 39).

Six NPY-receptor subtypes have been identified (5, 40) thus far. Studies using C-terminal peptide fragments of NPY at the synaptic cleft revealed two recepto...

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Methods

Animals and surgical preparation. Twenty-one male Long-Evans rats (weighing 290–310 g) were studied. All rats were equipped with a cannula (S-26, ITT, Life Science) in a jugular vein, and some were equipped with a blind-ended reentrant tube sutured to the muscle adjacent to the carotid artery. A thermocouple was inserted into the reentrant tube for measures of body core temperature (Tc). These sterile surgical
procedures were done while the rats were fully anesthetized with ~50 mg/kg pentobarbital sodium. Measurements. The temperature of the air (Tₐ), Tₛ, and Tₛₘ, was measured with 40-gauge thermocouples. Tail BFₜₜ was measured in the tail by VOP. A mercury-in-Silastic strain gauge (model EC-4; D.E. Hokanson, see Ref. 21) was connected to a thermocouple (BSM 403A; TSI Laserflow; see Ref. 21). So that hair would not interfere with laser Doppler, VOP BF, or Tₛₘ measurements, the hair was removed from the tail by using a depilatory. Preparation for experiments. Rats were anesthetized with ~50 mg/kg pentobarbital sodium and were gently introduced into a cylindrical Plexiglas rat restrainer. The restrainer allowed free access to the hind legs and tail and to the end of the cannula and reentrant tube located between the shoulders. Tail BFₜₜ was measured by using a pneumatic venous occlusion cuff, which must be positioned at the base of the tail, and a mercury-in-Silastic strain gauge that measures changes in volume of the tail during the periodic occlusions. The latter had to be positioned midway along the tail (e.g., 7–10 cm from base of the tail) to avoid artifacts either from being too close to the cuff or from being in a region (tail tip) where the radius is too small. A laser Doppler flow probe and a thermocouple were positioned on the skin adjacent to each other and as near to the strain gauge as possible (e.g., 6–8 cm from the base of tail). A BFₕₛ reading of 5 ml·min⁻¹·100 ml tissue⁻¹ was used as the minimum acceptable baseline value. If BFₕₛ was lower than this, then the laser Doppler probe was repositioned until an acceptable reading was obtained. All instrumentation was connected to an IBM-compatible computer via an analog-to-digital converter (Keithley). Thermocouple and laser Doppler channels were sampled at 1-s intervals and averaged for 20 s. The VOP measure of tail BFₜₜ was done at 20-s intervals. The occlusion cuff was inflated to 50–55 mmHg for 5 s, and tail BFₜₜ was assessed between the 2nd and 4th s of the cuff inflation.

NPY, NE, [Leu₁¹ Pro₃⁴]NPY, and NPY[¹³–₃⁶] protocols. In preliminary studies, doses of 32 and 64 µg/kg of NPY were shown to result in plasma levels of 2.0–2.5 pmol/ml of NPY in 300-g rats. The range of plasma levels observed in rats is shown to result in plasma levels of 2.0–2.5 pmol/ml of NPY in preliminary studies, doses of 32 and 64 µg/kg of NPY were equimolar to 64 µg/kg (44.7 µg/kg) and NPY[¹³–₃⁶] (44.7 µg/kg) administered at 1-min intervals are provided from one representative experiment in which NPY, NE, [Leu₁¹ Pro₃⁴]NPY, and NPY[¹³–₃⁶] were administered. To summarize the BFₜₜ and BFₕₛ responses to NPY, the mean values for each 5-min period, including baseline and up to 40 min postadministration, were calculated for each experiment. The mean ± SD of the responses for each dose of NPY and for physiological saline were then calculated. Analysis of variance (not repeated measures) and Dunnett’s multiple-comparison test were used to assess whether the BFₜₜ and BFₕₛ responses to different doses of NPY were significantly different from responses to saline (control) administration. Dunnett’s test is appropriately used when one data group is the control (saline) to which all other groups are compared. Paired t-tests were used to assess whether there were more microspheres in the lung than in the tail skin tissue after saline and NPY administration (n = 8).

RESULTS

Effect of NPY on BF and body temperatures. Figure 1 (left) illustrates the effects of 64 µg/kg exogenous NPY on (top to bottom) BFₜₜ, BFₕₛ, and Tₛₘ in the rat tail in one experiment. The effect of NPY has an immediate pronounced dynamic phase that is of short duration and is followed by a prolonged and less pronounced static phase. The dynamic phase included an immediate marked increase in tail BFₜₜ and tail volume that peaked within 1–3 min. Concurrent with the increase in BFₜₜ is an increase in tail Tₛₘ, and a consistent 1–3% increase in total tail volume (not shown) is observed as a pronounced upward shift in the baseline of the analog and digitized plethysmographic record. In contrast, BFₕₛ in the tail declined to <50% of baseline within 1–3 min, and Tₛₘ declined by up to 1.5°C. This dynamic
NPY AND AVA BLOOD FLOW IN RATS

**Fig. 1.** Representative experiments in which neuropeptide Y (NPY; left) and norepinephrine (NE; right) were administered to rats. NPY causes marked increase in total tail blood flow (BF$_{tot}$ (BT)), and skin temperature (T$_{tail skin}$), and a decrease in skin blood flow (BF$_{sk}$). Dotted line, mean baseline (BL); T$_{core}$, core body temperature. In contrast, NE causes an immediate decrease in all measures of blood flow.
phase ended as the values of all parameters moved toward baseline levels, albeit without fully achieving them. In the subsequent static phase, that continued throughout the rest of the experiment, BF_tot remained somewhat elevated and BF_sk remained somewhat depressed.

The effect of exogenous NE (400 µg/kg) on these same variables is presented in Fig. 1 (right) for comparison. NE invokes marked reductions in both BF_tot and BF_sk due to vasoconstriction and has no effect on tail T_sk. In comparison, the marked increase in BF_tot after NPY administration suggests that NPY dilates some vessels in the tail. However, the reduction in BF_sk at the same time indicates that this increase in BF does not involve the cutaneous microvascular bed.

Dose-response relationships. Figure 2A illustrates the dose-response relationship, including the time component, for NPY and BF_tot. Each bar represents the mean BF during a 5-min period of the experiment. The results represent the means ± SD from five (16 and 32 µg/kg) and seven rats (saline, 64 µg/kg), respectively. In the saline control experiments, there was normally a slight rise in BF_tot as the experiment progressed. Although a dose of 16 µg/kg caused an elevation in BF_tot during the dynamic phase (minutes 5–10), neither it nor the level during the static phase (compared at minutes 20–25) was significantly different from saline control (P > 0.05). Doses of 32 and 64 µg/kg caused larger, significant increases in BF_tot in both the dynamic (minutes 5–10; P < 0.05) and static phases (minutes 20–25; P < 0.05) of the response. A dose of 128 µg/kg (not shown) did not further increase BF_tot above that observed for 64 µg/kg.

Figure 2B illustrates the dose-response relationship between NPY and BF_sk. NPY caused a clear and marked reduction in BF_sk, the magnitude of which was dose dependent. The reduction in BF_sk was not significant for 16 µg/kg (P > 0.05) but was significant for both 32 and 64 µg/kg (P < 0.01) when values were compared at minutes 20–25 of the experiment. It should be noted

![Fig. 2. Dose-response relationship for NPY and BF_tot (A) and BF_sk (B). Each bar represents average response during 5 min. Effect of dose on both short-term dynamic phase and more prolonged static phase of NPY is demonstrated.](http://jap.physiology.org/Downloaded from 10.220.33.4 on August 15, 2017)
NPY AND AVA BLOOD FLOW IN RATS

that the laser Doppler flowmeter used for measuring microvascular BF is limited to making that measurement at only one small area. However, given the consistency of the response during multiple experiments, this measurement is likely to be representative of what is occurring throughout the skin.

Microsphere studies. Microspheres were used to test the hypothesis that AVAs dilate and participate in the NPY-induced increased BFtot. Figure 3 shows the number (mean ± SD) of microspheres/g tissue reaching the tail skin (left) and lung (right) after normal administration of saline (300 µl iv; control) or 64 µg/kg NPY in a 300-µl injection. After saline, 14,080 ± 10,420 microspheres/g tissue were found in the lung. Significantly more microspheres (40,571 ± 26,790/g tissue; P < 0.01) were found in the lung after administration of NPY. Furthermore, the number of microspheres trapped in the tail skin tissue is actually higher (P < 0.005) after saline (2,988 ± 2,010/g tissue) than after NPY (1,496 ± 1,223/g tissue) administration. This is as expected, considering the reduced microvascular BF measured by laser Doppler flowmeters. These results demonstrate the participation of AVAs in the NPY-induced increase in rat tail BFtot.

Effect of \([\text{Leu}^{31}\text{Pro}^{34}]\text{NPY}\) and NPY[13–36] on BF and body temperatures. Figure 4 illustrates the effects of \(\text{[Leu}^{31}\text{Pro}^{34}]\text{NPY}\) (the \(Y_1\)-receptor agonist) and of NPY[13–36] (the \(Y_2\)-receptor agonist), respectively, on tail BFtot and BFsk in the tail. Clearly, the \(Y_1\)-receptor agonist has a similar effect to that of NPY (compare with Fig. 1). In marked contrast, the \(Y_2\)-receptor agonist has no effect on either tail BFtot or BFsk in the tail. These results suggest that \(Y_1\) receptors, or receptors responsive to \(Y_1\) agonist, participate in the NPY-induced increase in BFtot and concurrent decreases in BFsk. The results also indicate that \(Y_2\) receptors are not involved in this increased BF.

DISCUSSION

The results of these studies demonstrate that exogenous NPY, administered at physiological levels, consistently invokes in the rat tail an increase in BFtot, tail blood volume, and tail Tsk, and a decrease in tail BFsk. It is further demonstrated that there is a dose-response relationship for both BFtot and BFsk, and that the increase in BFtot is caused by a pronounced vasodilation in which AVAs play a role. Finally, this NPY-induced vasodilation is also invoked by the \(Y_1\)-receptor agonist, [\text{Leu}^{31}\text{Pro}^{34}]\text{NPY}\), but not by a \(Y_2\)-receptor agonist, NPY[13–36]. The discussion that follows examines these observations and conclusions in some detail.

Clear evidence of NPY-induced increase in BFtot. The present study provides unrefutable evidence that physiological levels of NPY induce increases in BFtot in the rat tail. This is because the method used to measure BFtot (VOP) is a direct, quantitative, and frequently used measure that cannot provide a false indication of such increases in BFtot. The observation is supported further by the concurrent increase in tail (blood) volume and tail Tsk, notwithstanding the reduction in superficial microvascular tail BFsk. It should be noted that such decreases in microvascular or capillary BF have often been observed when blood flows by another path of lesser resistance (20, 22). The conclusion that some vessels in the tail dilate in response to exogenous NPY is supported by the observed simultaneous increase in tail blood volume and BFtot.

AVAs. AVAs are relatively large-diameter connections between arteries and veins that allow an increase in non-nutrient thermoregulatory BF through the skin (12). They are opened during heat stress (19). They are also thought to open, periodically, during exposure to extreme cold to prevent tissue freezing (commonly referred to as cold-induced vasodilation) (10).

AVAs are the most likely vascular tissue to be involved in the NPY-induced increase in BFtot, because of the several reasons already enumerated in the introduction and the following additional reasons. The possibility that the dilation occurs in arteries, arterioles, venules, and veins is rejected, because the subsequent flow through capillaries would be a limiting factor and because it has already been demonstrated that capillary BF in the skin is reduced rather than increased. The observed increase in blood volume could be explained by pooling of blood in compliant veins, but that cannot explain the increased tail BF. AVAs are known to occur in large numbers in the rat tail (12). In addition, the rat tail is the only one of many peripheral tissues studied where NPY causes increases in BFtot associated with vasodilation, and it is the only one of these tissues that is known to have abundant AVAs.

Evidence supporting the hypothesis that AVAs participate in the NPY-induced increase in BFtot in the rat tail. That AVAs are involved in the NPY-induced increase in BFtot in the rat tail is convincingly demonstrated by the results of the experiments using color microspheres (Fig. 3). Blood flowing through the arterial vessels of the tail will end up in the veins by passing either
through capillaries that are 2–3 µm in diameter or through AVAs that are 20–50 µm in diameter. Whereas 15-µm-diameter microspheres easily pass through AVAs, they are trapped in the capillary beds. The fact that the number of microspheres ending up in the lung tissue is severalfold greater after NPY administration than after the administration of saline clearly indicates that AVAs participate in the NPY-induced increase in BFtot in the rat tail. Furthermore, the decline in the number of color microspheres trapped in the capillaries of the tail skin after NPY administration, compared with after saline administration, reaffirms the decline in microcirculatory BFsk after NPY administration, as measured locally by the laser Doppler flowmeter.

Y1-receptor agonist invokes NPY-induced vasodilation. The protocol used for observing the effects of NPY was repeated with [Leu31 Pro34]NPY (a Y1-receptor agonist) and NPY[13–36] (a Y2-receptor agonist). Figure 4 presents and compares the effects in the tail of [Leu31 Pro34]NPY and NPY[13–36] on BFtot and BFsk. The Y1-receptor agonist (Fig. 4, left) has an effect that is essentially identical to that of NPY (compare with Fig. 1, left). In marked contrast, the Y2-receptor agonist (Fig. 4, right) has no apparent effect on tail BFtot or BFsk. Thus the present study eliminates the possible involvement of Y2 and related receptors and implicates the involvement of Y1 or [Leu31 Pro34]NPY-responsive receptors. It should be noted that Y2 receptors show a similar level of affinity for [Leu31 Pro34]NPY as Y1 receptors (5) and thus could potentially be involved in the response. In contrast, Y4 and Y5 receptors show much lesser affinity (1/8 and 1/50) for [Leu31 Pro34]NPY than do Y1 receptors (13). Because NPY and [Leu31 Pro34]NPY in equimolar doses had similar effects on BFtot, it is unlikely that Y4 and Y5 receptors are involved.

Finding an NPY-induced increase in BFtot in the rat tail was initially unexpected in light of the numerous reports that NPY causes either vasoconstriction or no change in vascular tone in blood vessels and that NPY reduced BF in the organs and tissues heretofore studied (8, 11, 34). NPY has also been shown to cause systemic hypertension (7). Thus one plausible explanation for the increased BFtot is that it is due to an elevation in blood pressure that is caused by vasoconstriction in other tissues. Although the present study did not measure blood pressure, two observations con-
flict with this explanation. First, microvascular BF_{stk} should have increased rather than decreased with elevations in blood pressure. Second, although an increase in blood pressure can increase BF through vessels, it cannot increase BF through AVAs unless the AVAs are already open. Thus we return to the conclusion that NPY, either directly or indirectly, is affecting AVA tone. In addition, it should be noted that high doses (>3 nM/kg) of NPY are reported to decrease mean arterial pressure because of release of histamine from mast cells (17). The timing of the reported hypotension response (1- to 12-min postinjection) correlates well with the timing of the increased tail BF_{tot} observed in the present study.

Also, the present observations seem to be in direct conflict with the finding by Neild (31) that NPY causes vasoconstriction in the isolated-vessel preparations of the rat tail artery. There are, however, several plausible reasons why the vasoconstriction observed in that isolated rat tail artery preparation was not observed in the present study and why those results should not be extrapolated to the whole animal. First, the plasma levels of NPY (1.5–2.5 pmol/kg) used in the present study were much lower than the 20–30 nM doses used to perfuse the isolated rat tail artery (31) and may not, therefore, have been great enough to cause constriction in the artery. Second, it should be understood that, although isolated vessel preparations can provide much insight about the pharmacology of a given vessel, such preparations do not fully mimic the conditions in the whole animal. Third, the vasculature of the rat tail is complex, with not just one, but several, arteries traversing its length, and there are numerous anastomoses between those arteries (31). Thus the actual route of arterial BF can be shifted between different arteries. It is possible that the ventral medial artery studied by Neild (31) responds differently to NPY than do the several lateral or collateral arteries or the anastomose connecting them. Finally, because AVAs are involved and because they are highly specialized vascular structures, unlike any of the other vascular tissues in which the response to NPY has been studied, it is not surprising that they would have unique responses to NPY or other pharmacological agents. Indeed, AVAs could not perform their unique function of altering the route of BF to increase both the flow and volume of blood in the skin in response to the specific conditions of heat stress if they were pharmacologically identical to arteries. The pharmacological mechanism involved in the control of BF through AVAs is not fully understood, although one report suggests the involvement of \( \alpha_1 \)-adrenergic receptors in pig AVAs (4).

The most obvious explanation for the observed increase in BF_{tot} via AVAs in response to NPY is that NPY acts at the presynaptic level to inhibit NE release, which subsequently results in relaxation of AVA tone. This conclusion is based on the following several observations. First, there is evidence that AVA tone is maintained by the sympathetic nervous system via action of NE (19, 30) on adrenergic receptors, as demonstrated in studies on dogs and sheep (18, 23). In addition, a more recent study has more specifically implicated the involvement of \( \alpha_1 \)-adrenergic receptors in regulating BF through AVAs in pigs (4). Second, NPY has been shown both to inhibit NE release by its presynaptic action on \( Y_2 \) receptors and also to postsynaptically potentiate the vasoconstriction induced by NE by action on \( Y_1 \) receptors (34). One problem with this hypothesis is that our results indicate that NPY acts on \( Y_1 \) receptors, which were previously reported to be postsynaptic (38), rather than \( Y_2 \) receptors, which were reported to be presynaptic (38). However, more recent studies have demonstrated that \( Y_1 \) receptors occur presynaptically and that \( Y_2 \) receptors occur postsynaptically (16, 29). Thus it is possible that \( Y_1 \) receptors could be presynaptic in a specialized tissue, such as AVAs, where NPY causes dilatation rather than vasoconstriction.

In more recent studies, it was shown that both \( Y_1 \) and \( Y_2 \) receptors occur in arterioles in intestinal submucosa of guinea pigs (32) and in the microvascular tree in the hamster cheek pouch (2). Neild and Lewis (32) observed that a \( Y_2 \)-receptor agonist caused a greater potentiation of a vasoconstriction response to short pulses of \( K^+ \) than to NPY per se, and, furthermore, that a \( Y_2 \)-receptor agonist reduced the magnitude of vasoconstriction. This differs from the inhibition of NE release presynaptically. Rather, the \( Y_2 \)-agonist PYY-(13–36) is acting on \( Y_2 \) receptors on the smooth muscle to modulate the opening of the \( Ca^{2+} \) channels, just as they do in nerve terminals (9).

Clearly, further study is needed to characterize definitively the receptors involved, to ascertain whether they are presynaptic or postsynaptic, and to understand the pharmacological mechanism responsible for the observed NPY-induced increase in tail BF_{tot}. On the basis of other reports in the literature, the possibility clearly remains that other receptor types and vasoactive substances are involved in the pharmacology of this mechanism. For example, NPY has also been found to be costored with ACh in parasympathetic nerves (35) and possibly to modulate ACh secretion (24). This is relevant, because ACh is a known vasodilator substance. Also, as mentioned above, larger doses of NPY have caused release of histamine from mast cells, which, due to the vasodilatory effects of histamine, resulted in hypotension (17). Finally, the potentiation of the vasoconstriction response induced by NE (34) is dependent on both endothelium and \( Ca^{2+} \) (3) and independent of adrenergic receptors (28). This opens the possible involvement of \( Ca^{2+} \) channels and endothelium-derived relaxing factor, which some investigators believe is nitric oxide (33) or nitrosothiols (41). Thus there are several plausible mechanisms by which NPY could invoke vasodilation in the rat tail, and it is clear that other neurotransmitters in addition to NPY and other receptors in addition to \( Y_1 \) receptors could be involved in this response.

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REFERENCES


32. Suzuki, N., J. E. Hardebo, J. Kahrstrom, and C. Owman. Neuropeptide Y co-exists with vasoactive intestinal polypeptide...


