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Neuropeptide Y release and contractile properties: differences between canine veins and arteries

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Abstract

During intense sympathetic activation, as occurs during hemorrhage, veins constrict to a greater degree than do arteries. This study determined if differences in the amounts or actions of the sympathetic cotransmitter neuropeptide Y released from perivascular nerves could contribute to these differences. Strips of canine mesenteric and popliteal arteries and of saphenous and portal veins were superfused, and the releases of noradrenaline and neuropeptide Y evoked by transmural stimulation were assessed. Both compounds were released in greater amounts in the veins than in the arteries. In other experiments rings of each vessel were mounted in organ chambers for isometric-tension recording. Neuropeptide Y (up to 10^{-4} M) did not contract any vessel; however, at 3×10^{-7} M it shifted the frequency-response and concentration-response curves to noradrenaline in the arteries only. In the veins neuropeptide Y had no postsynaptic effect on strong contractions. These results suggest that neuropeptide Y functions locally to affect vasoconstriction of the arteries studied, and may have a different role in the veins. Further, processes involving neuropeptide Y do not appear to account for the differences in responsiveness of these arteries as compared to the veins during intense sympathetic stimulation.

Keywords: Neuropeptide Y; Noradrenaline; Peptide; Blood vessel; Vascular control; Hemorrhage

1. Introduction

Intense activation of the sympathetic nervous system as initiated by hemorrhage or strenuous exercise results in marked constriction of medium to large sized veins (Rothe, 1983). This redistributes venous blood and results in increases in central blood volume and in central venous pressure, factors important in increasing cardiac efficiency and output. However most arteries of comparable size constrict only slightly under these circumstances. Constriction initiated by sympathetic mechanisms is mediated largely by the neurotransmitter noradrenaline, although other vasoactive compounds, including the cotransmitter neuropeptide Y, may affect the responses. Neuropeptide Y is stored together with noradrenaline in postganglionic sympathetic nerve endings of blood vessels (Lundberg et al., 1982) and, significantly, is released during high-frequency firing of the nerves (Lundberg et al., 1986). Depending upon which pre- or post-junctional neuropeptide Y receptors are activated, neuropeptide Y may inhibit its own release as well as that of noradrenaline (Pernow et al., 1986b,1987), contract vascular smooth muscle (Lundberg and Tatemoto, 1982; Lundberg et al., 1982; Pernow et al., 1986b; Rudehill et al., 1986), or potentiate contractions caused by other agonists (Ekblad et al., 1984; Pernow et al., 1986b; Daly and Hieble, 1987; Hieble et al., 1989).

The aim of this study was to determine the degree to which the differences in contractile responses between arteries and veins could be due to differences in: (a) the amounts of neuropeptide Y released from vascular nerve endings, (b) the direct contractile effects of neuropeptide Y or (c) the potentiating effects of neuropeptide Y on contractile responses initiated by noradrenaline or by nerve depolarization.

Four canine blood vessels were studied; saphenous and portal veins (vessels expected to constrict markedly to intense sympathetic activation), and popliteal and mesenteric arteries (vessels anticipated to constrict less when stimulated). The rationale was to characterize, in each vessel, the releases of noradrenaline and neuropeptide Y from sympathetic nerve endings, and to relate these release

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characteristics to the capacities of the compounds to influence vasoconstriction.

Commonly in in vitro studies, the evoked *release* of noradrenaline, defined as exocytotic liberation of noradrenaline from sympathetic nerve endings, is determined by quantifying noradrenaline *overflow* (that noradrenaline which, after release, diffuses out of the vessel) in the presence of inhibitors of neuronal and extraneuronal noradrenaline uptake. The noradrenaline which overflows is then equated with that which is released since the released noradrenaline cannot be removed from the junctional cleft by uptake mechanisms. However, neuropeptide Y release is markedly reduced when amine uptake is inhibited (Haass et al., 1989; Lacroix et al., 1989), due largely to activation of presynaptic α_2-adrenoceptors by the increased levels of noradrenaline in the junctional cleft. Therefore in this study it was necessary to assess noradrenaline release (and neuropeptide Y release) in the absence of these inhibitors. We did so by using a method described recently whereby the overflows of evoked noradrenaline and of dihydroxyphenylglycol, the major metabolite of noradrenaline, were measured, then summated and used as an *index* of noradrenaline release (Tyce et al., 1995). While this index is not an absolute measure of noradrenaline release in that it does not account for the small amount of noradrenaline that is taken up by non-neuronal tissues after its release, it does take into account the much larger proportions of the released noradrenaline which are taken back up into the sympathetic nerve endings and deaminated as well as that which diffuses from the tissue.

2. Materials and methods

2.1. Tissue preparation

The protocols used in this study were approved by the Institutional Animal Care and Use Committee. Segments of popliteal and superior mesenteric arteries and of portal and saphenous veins were removed from anesthetized (30 mg/kg i.v. pentobarbital) adult mongrel dogs of either sex, then dissected free of perivascular tissue. Two types of experiments were performed: (1) superfusion of vessel segments which had been cut into helical strips, in order to quantify the releases of endogenous noradrenaline and neuropeptide Y, and (2) tissue bath studies using ring preparations of each vessel in order to measure contractile responses. The endothelium was intact in all vessels.

2.2. Experimental protocols

2.2.1. Releases and tissue contents of noradrenaline and neuropeptide Y in blood vessel strips

Strips of each vessel were suspended vertically in glass chambers (height 10 cm, diameter 1 cm) maintained at 37°C (Hunter et al., 1988, 1992). Two grams passive force was applied to each strip. Krebs-Ringer solution (Rorie et al., 1980) was pumped from a reservoir to the upper end of each strip of vessel and allowed to flow down over the strip at 2 ml/min. The Krebs-Ringer solution was maintained at 37°C and aerated with 95% O_2, 5% CO_2. After a 60-min stabilization period, superfusate was collected in successive 10-min intervals. Throughout the second interval, transmural stimulation (10 V, 0.2 ms) was applied to each strip via two platinum wire electrodes parallel to and in contact with the entirety of the strips. One strip of each pair was stimulated at 2 Hz, the other at 12 Hz. At the end of the second sampling interval stimulation was stopped but superfusate collection was continued for an additional three sampling intervals for the strips stimulated at 2 Hz, and for an additional eight intervals for the strips stimulated at 12 Hz.

The noradrenaline, dihydroxyphenylglycol and neuropeptide Y which overflowed from each tissue into the superfusate during each collection interval were adsorbed onto a Sep-Pak Plus C_{18} cartridge (Waters Associates, Milford, MA, USA) which was attached directly to the bottom of the chamber. The compounds were analyzed as described previously (Hunter et al., 1988, 1992). At the end of each experiment, the strips were immediately removed from the chambers, blotted dry, weighed, and frozen on crushed dry ice. The analytes were then extracted from the vessels in a mixture of 0.1 N HCl, 0.1 mM disodium ethylenediamine tetraacetate and separated on Sep-Pak cartridges as were superfusates (Hunter et al., 1988, 1992). Noradrenaline and dihydroxyphenylglycol were quantified by high-performance liquid chromatography with electrochemical detection (Hunter et al., 1992).

The amounts of noradrenaline, dihydroxyphenylglycol and neuropeptide Y which overflowed into the superfusate were expressed as fmol/min; the amounts in tissue were expressed as pmol. Data were corrected for average recovery. Recoveries in superfusate of 2 ng each of noradrenaline and dihydroxyphenylglycol were 97.9 ± 1.9 and 84.7 ± 2.7%, respectively (means ± S.E.M., $n = 14$). Noradrenaline recovery in tissue was 99.6 ± 2.1%. The limits of detection for noradrenaline and dihydroxyphenylglycol in superfusate, expressed per 100 mg of tissue, were 7.0 and 6.7 fmol/min. The detection limit for noradrenaline in tissue was 0.2 pmol. The average tissue weights after superfusion were 139.1 ± 6.0, 72.6 ± 3.7, 112.4 ± 4.9, and 157.0 ± 8.5 mg for the mesenteric artery, popliteal artery, saphenous vein, and portal vein, respectively. Data were expressed per 100 mg tissue. For each vessel the index of noradrenaline release was determined by combining the total overflows above basal levels of noradrenaline and dihydroxyphenylglycol which occurred during and after each stimulation.

The neuropeptide Y in each sample was quantified by radioimmunoassay, using a method that has been well characterized (Allen et al., 1984; Hunter et al., 1988; Yaksh et al., 1988). The average recovery in superfusate of

authentic neuropeptide Y standards of 0.4 and 1 ng was 94.1 ± 8.1%; average recovery in tissue was 90.0 ± 5.4% ($n = 14$ each). The limits of sensitivity were 0.2 fmol/min in superfusate and 90.0 fmol in tissue.

2.2.2. Contractile activity in blood vessel ring preparations

Isometric contractile activity was measured in rings 3–4 mm in width which were cut from vessel segments corresponding to those used in the superfusion studies. Each ring preparation, suspended between a force-displacement transducer and anchor, was submerged in 2 ml of Krebs-Ringer solution in a small jacketed tissue bath (Radnoti Glass Technology, Monrovia, CA, USA) maintained at 37°C. The Krebs-Ringer solution was constantly aerated with 95% O_2, 5% CO_2. After an initial 15-min period with no stretch applied, each ring was stretched in small increments until a basal pull, which had been determined by preliminary experiments to result in near-maximum contractile responsiveness, was reached. These were 3, 15, 2.5, and 3 g for the mesenteric artery, popliteal artery, saphenous vein, and portal vein, respectively.

Each ring was then equilibrated for 60 min, during which time the Krebs-Ringer solution was replaced every 10 min. Then the responsiveness of each ring was assessed by inducing a contraction with 40 mM KCl. For this determination 40 μl of Krebs-Ringer solution containing added KCl was pipetted into each chamber. After the contraction reached a plateau, the chamber was rinsed to remove the KCl, and the ring was equilibrated for a further 60 min while the contraction returned to the baseline level.

In experiments designed to determine if neuropeptide Y caused the vessels to contract, concentration-response curves to neuropeptide Y were performed. After the final 60-min equilibration period as indicated above, neuropeptide Y, in Krebs-Ringer solution, was pipetted into each chamber in 20 μl aliquots to give final concentrations from 10^{-9} to 10^{-4} M in half-log increments. Each ring was equilibrated with neuropeptide Y at each concentration for 5 min and if there was no contraction, the concentration was increased.

In another set of experiments with ring preparations, the possible capacity of neuropeptide Y to potentiate contractions caused by noradrenaline was assessed. The protocol was similar to that described above, except that response curves were generated with noradrenaline as the agonist instead of neuropeptide Y. Concurrent noradrenaline concentration-response curves were done with pairs of rings of each vessel (i.e., eight rings per experiment). One ring of each pair was a control, the other was exposed to neuropeptide Y (3×10^{-7} M); an aliquot of either Krebs-Ringer solution (control) or Krebs-Ringer solution containing neuropeptide Y was added to each chamber 3 min before the start of the curve and remained for the duration of the experiment. Neuropeptide Y in this concentration range has been shown to potentiate contractile responses of blood vessels (Hieble et al., 1989). For the response curves, noradrenaline dissolved in Krebs-Ringer solution, was added in 80 μl aliquots to each chamber to give incremental concentrations of 10^{-9} to 10^{-3} M. When each contraction reached a plateau (within several min) the concentration of noradrenaline was increased. These experiments were done using Krebs-Ringer solution which contained corticosterone, (4.3×10^{-7} M), desmethylimiprimine (10^{-6} M) and propranolol (10^{-6} M) to inhibit extraneuronal and neuronal uptakes of noradrenaline and β-adrenoceptors, respectively.

In additional tissue bath studies, the effects of neuropeptide Y on contractions induced by field stimulation were studied. This experimental protocol was similar to that described for the noradrenaline concentration-response curves except that the vessels were contracted by field stimulation at incremental frequencies from 0.2 to 12 Hz. Stimulation was applied via two small platinum plates positioned in each chamber on either side of the vessel ring.

Contractile responses were measured in g and expressed as the percentage of the contraction previously caused by the KCl.

2.3. Drugs

The drugs used were noradrenaline, corticosterone, desmethylimiprimine, and propranolol (Sigma Chemical Co., St. Louis, MO, USA) and human neuropeptide Y (Peninsula Labs, Belmont, CA, USA). Reagents were from Fisher Scientific, Minneapolis, MN, USA.

2.4. Statistics

2.4.1. Noradrenaline and neuropeptide Y releases

One-way analysis of variance with repeated measures was used to determine if significant differences existed between groups. When analysis of variance showed significant differences, the Student-Newman-Keuls test for paired observations was then performed to determine differences between individual mean values. Results were expressed as means ± S.E.M., and differences were considered significant at $P < 0.05$.

2.4.2. Contractile activity

A response curve was constructed for each ring preparation. Sensitivities to noradrenaline were expressed as an E_{max} value (maximum contraction) and a pD_2 value, where $pD_2 = -\log EC_{50}$, EC_{50} being the molar concentration of noradrenaline eliciting half-maximum contraction. EC_{50} values were obtained by logit/log regression analysis of the 20–80% response interval. Sensitivities to field stimulation were again expressed as an E_{max} but, in this case, with an FS_{50} value, where FS_{50} was the stimulation frequency, in Hz, which caused a contraction of half the maximum. The FS_{50} values were obtained by using a hyperbolic curve-fitting program.

The potentiating effects of exogenous neuropeptide Y on contractions to noradrenaline or to field stimulation were determined by comparing the E_{max} and pD_2 (or FS_{50}) values in paired rings. The differences were tested for significance ($P < 0.05$) by using the paired t-test. Results were expressed as means ± S.E.M.

3. Results

3.1. Noradrenaline and neuropeptide Y releases

The overflow of noradrenaline during basal conditions was clearly measurable in each of the superfused vessels and it was significantly increased by transmural stimulation (Table 1). The overflows from the vessels stimulated at 12 Hz are shown in Fig. 1.

During basal conditions dihydroxyphenylglycol overflowed from each vessel in greater amounts (between 123.8 ± 15.4 fmol/min and 508.2 ± 15.7 fmol/min in the four vessels) than did noradrenaline. The overflow of dihydroxyphenylglycol was increased during 2 Hz stimulation (to between 209.0 ± 37.7 fmol/min and 800.5 ± 27.4 fmol/min), and further increased during 12 Hz stimulation (441.4 ± 50.9 fmol/min to 1307.8 ± 88.5 fmol/min).

During transmural stimulation, less noradrenaline overflowed from arteries than from veins (Table 1). For each vessel, the combined overflows of noradrenaline and dihydroxyphenylglycol evoked by the transmural stimulations were used to calculate the index of noradrenaline release (Table 2). The index was less in the arteries than in the veins.

During basal conditions, the overflow of neuropeptide Y was low (Table 1, Fig. 1) in all vessels and typically approached the limit of assay sensitivity. In the veins, neuropeptide Y overflow was increased by 12 Hz transmural stimulation (analysis of variance). The overflow in the arteries tended to increase above basal levels when they were stimulated at 12 Hz; however, the increases over time were too variable to attain significance (Table 1, Fig. 1).

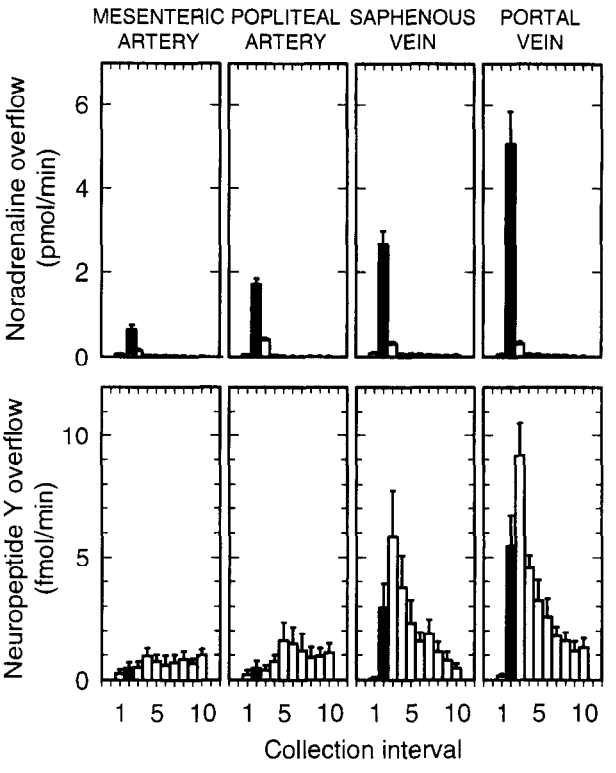

Fig. 1. Comparison of the concurrent overflows of noradrenaline and of neuropeptide Y from strips of four different blood vessels superfused in vitro and stimulated at 12 Hz (10 V, 0.2 ms). Each strip was superfused for 60 min under basal conditions, then superfusate was collected for 10 consecutive 10-min intervals. The vessels were stimulated during the second collection interval as indicated by the single dark bar in each graph. Data are expressed per 100 mg tissue and are means ± S.E.M. of six determinations each.

Table 1
Content of noradrenaline and neuropeptide Y in superfusate and tissue of four blood vessels

	Vessel			
	Mesenteric artery	Popliteal artery	Saphenous vein	Portal vein
Noradrenaline				
Tissue (pmol)	677.8 ± 69.5	278.1 ± 41.2 [c]	1021.6 ± 155.5 [c,d]	585.8 ± 52.8 [d,e]
Superfusate (fmol/min)				
Basal	73.5 ± 4.9	59.0 ± 5.7	100.7 ± 16.7 [d]	62.6 ± 12.5 [e]
2 Hz	106.5 ± 7.6 [a]	119.4 ± 12.2 [a]	210.5 ± 17.4 [a,c,d]	240.3 ± 20.4 [a,c,d]
12 Hz	343.7 ± 50.4 [a,b]	555.2 ± 42.3 [a–c]	777.6 ± 79.3 [a–d]	1384.8 ± 202.4 [a–e]
Neuropeptide Y				
Tissue (pmol)	3.46 ± 0.5	4.49 ± 0.9	6.03 ± 0.8 [c]	1.94 ± 0.3 [c–e]
Superfusate (fmol/min)				
Basal	0.29 ± 0.11	0.27 ± 0.12	0.04 ± 0.02 [c]	0.21 ± 0.09
2 Hz	0.27 ± 0.09	0.24 ± 0.11	0.61 ± 0.21 [a]	0.89 ± 0.47
12 Hz	0.68 ± 0.25	0.80 ± 0.26	3.72 ± 1.21 [a–d]	5.60 ± 0.91 [a–d]

Basal overflow values are for one 10 min pre-stimulation superfusion interval; 2 Hz and 12 Hz values are average overflows for the respective 10 min stimulation intervals and the three immediate post-stimulation intervals for the tissues stimulated at 2 Hz and for eight post-stimulation intervals for the tissues stimulated at 12 Hz. Data are corrected to 100 mg tissue weight and are means ± S.E.M. of 5–10 determinations each. Significant differences, $P < 0.05$. [a] Significantly different from basal value in the same vessel; [b] significantly different from 2 Hz value in the same vessel. Significantly different from corresponding value in: [c] mesenteric artery; [d] popliteal artery; [e] saphenous vein.

Table 2
Index of noradrenaline release (pmol/min) in four blood vessels

Stimulation frequency	Vessel			
	Mesenteric artery	Popliteal artery	Saphenous vein	Portal vein
2 Hz	0.75 ± 0.08	0.46 ± 0.06 [b]	0.66 ± 0.06 [c]	1.22 ± 0.17 [b-d]
12 Hz	2.38 ± 0.31 [a]	2.48 ± 0.14 [a]	3.64 ± 0.46 [a-c]	7.13 ± 0.88 [a-d]

The index of noradrenaline release was determined by summating the overflows above basal levels of noradrenaline and dihydroxyphenylglycol which occurred during a 10-min interval of transmural stimulation and a 10-min interval immediately afterwards. Data are corrected to 100 mg tissue weight. Significantly different ($P < 0.05$) from: [a] the same vessel at 2 Hz; [b] mesenteric artery at the same frequency; [c] popliteal artery at the same frequency; [d] saphenous vein at the same frequency.

When stimulated at 12 Hz, more neuropeptide Y was released from the veins than from the arteries, and the overflow decreased rapidly, to less than one-half the peak levels within 20 min. In the subsequent collection intervals overflow decreased more slowly but was still elevated above basal levels at the end of the superfusion.

3.2. Contractile activity

The average contractions generated by added KCl in each vessel ring preparation were 15.8 ± 0.9, 9.9 ± 0.6, 11.4 ± 1.2, and 10.1 ± 0.9 g ($n = 12$ to 14) in mesenteric artery, popliteal artery, saphenous vein and portal vein, respectively. In experiments designed to examine the effect of neuropeptide Y alone on contractile response, exogenous neuropeptide Y in cumulative concentrations from 10^{-9} to 10^{-4} M failed to elicit contractions in unstimulated rings of any of the vessels studied (not shown).

Another set of experiments was aimed at determining the capacity of neuropeptide Y to modify contractions induced by noradrenaline. Rings of all vessels contracted vigorously when exposed to cumulative concentrations of noradrenaline up to 10^{-3} M (Fig. 2). Pretreatment of the arteries with neuropeptide Y (3×10^{-7} M) caused a significant leftward shift in their noradrenaline concentration-response curves, but had no effect on the maximum responses (Fig. 2, Table 3). Neuropeptide Y also potentiated the contractions to noradrenaline in the saphenous vein, but only at the lower end of the response curve. Consequently, although the EC_{50} value (as described by the pD_2) was unchanged by neuropeptide Y (Table 2), the EC_{25} (the concentration of noradrenaline which resulted in 25% of the maximum contraction) was, however, shifted from $10^{-7.50} \pm 10^{-0.09}$ M in control preparations to $10^{-7.85} \pm 10^{-0.13}$ M in preparations exposed to neuropeptide Y, a more than three-fold increase in sensitivity to low concentrations of noradrenaline. Neuropeptide Y, added 3 min before the noradrenaline concentration-response curves were initiated, caused a weak (< 3% of maximum) contractile response in several of the saphenous vein ring

Table 3
Effects of neuropeptide Y on noradrenaline concentration-response curves and frequency-response curves in four blood vessels

	Vessel			
	Mesenteric artery	Popliteal artery	Saphenous vein	Portal vein
Norepinephrine				
pD_2				
Noradrenaline	5.59 ± 0.10	6.00 ± 0.07 [b]	7.05 ± 0.13 [b,c]	6.18 ± 0.06 [b,d]
Noradrenaline + neuropeptide Y	6.00 ± 0.12 [a]	6.34 ± 0.10 [a,b]	7.24 ± 0.13 [b,c]	6.22 ± 0.10 [d]
E_{max}				
Noradrenaline	146.95 ± 16.14	151.23 ± 6.25	164.39 ± 20.38	169.48 ± 7.35
Noradrenaline + neuropeptide Y	157.86 ± 15.41	168.10 ± 14.50	170.05 ± 18.54	179.11 ± 10.70
Field stimulation				
FS_{50}				
Stimulation	3.54 ± 0.55	1.75 ± 0.17 [b]	1.16 ± 0.17 [b,c]	1.37 ± 0.20 [b]
Stimulation + neuropeptide Y	3.60 ± 0.21	1.90 ± 0.24 [b]	1.04 ± 0.11 [b,c]	1.38 ± 0.18 [b,c]
E_{max}				
Stimulation	11.06 ± 2.41	19.86 ± 3.41 [b]	153.73 ± 15.63 [b,c]	95.77 ± 14.19 [b-d]
Stimulation + neuropeptide Y	24.34 ± 4.70 [a]	41.18 ± 9.80 [a]	176.86 ± 11.69 [b,c]	107.71 ± 18.04 [b]

pD_2, −log noradrenaline concentration (M) eliciting half-maximum contraction; FS_{50}, frequency (Hz) which resulted in half-maximum contraction; E_{max}, maximum contraction expressed as a percentage of contraction to KCl. Data are expressed as mean ± S.E.M. of 5–9 determinations each. Curves were done with paired rings, either in the absence or presence of 3×10^{-7} M neuropeptide Y. Significant differences, $P < 0.05$. [a] Significant difference due to neuropeptide Y in same vessel. Significantly different from corresponding value in: [b] mesenteric artery; [c] popliteal artery; [d] saphenous vein.

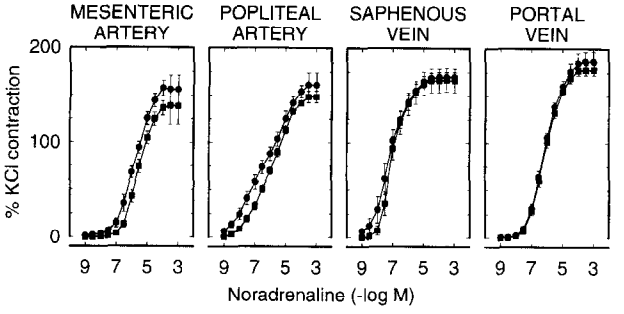

Fig. 2. Effects of neuropeptide Y (3×10^{-7} M) on noradrenaline concentration-response curves in isolated rings of four different blood vessels. Squares, control; circles, neuropeptide Y. The curves of the mesenteric artery and popliteal artery were shifted to the left by neuropeptide Y as determined by significant ($P < 0.05$) changes in the pD_2 values. Values for pD_2 and E_{max} are reported in Table 2. Data are expressed as the percentage of the response to 40 mM K^+ and are means ± S.E.M. of 6 or 7 determinations each.

segments which were pre-exposed to corticosterone, desmethylimiprimine, and propranolol. In the portal vein, neuropeptide Y had no significant effect on the noradrenaline concentration-response curve (Fig. 2, Table 3).

Field stimulation in step-wise fashion resulted in frequency-dependent contractions in all vessels (Fig. 3). Both arteries responded to field stimulation to a similar degree, and the maximum contractions were much less in the arteries than in the veins. In the arteries also the maximum responses to field stimulation were substantially less than those caused in the same vessels by the highest concentration of noradrenaline (Table 3, Figs. 2 and 3). Neuropeptide Y (3×10^{-7} M) potentiated these contractions throughout the range of frequencies tested as indicated by significant shifts in the E_{max} but not the FS_{50} values. By contrast, the veins contracted vigorously to field stimulation, but neuropeptide Y was without significant effect on these contractions.

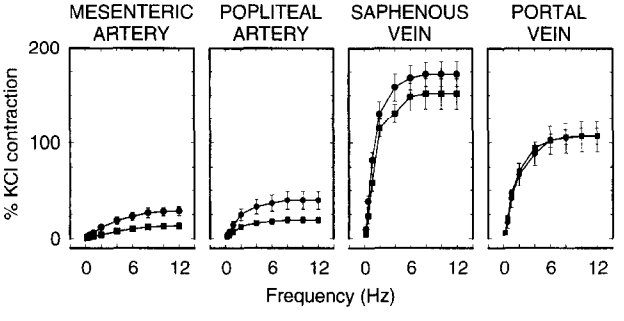

Fig. 3. Effects of neuropeptide Y (3×10^{-7} M) on frequency-response curves in isolated rings of four different blood vessels. Squares, control; circles, neuropeptide Y. The data are expressed as the percentage of the response to 40 mM K^+, and are means ± S.E.M. of 5 or 6 determinations each. The maximum response (E_{max}) was increased significantly ($P < 0.05$) in the mesenteric artery, and popliteal artery. Individual values for E_{max} and FS_{50} (the frequency which resulted in half-maximum contraction) are reported in Table 3. Data are means ± S.E.M. of 4 to 8 determinations each.

4. Discussion

4.1. Differences in the release characteristics of noradrenaline and neuropeptide Y

Previous studies showed that the releases of noradrenaline and neuropeptide Y from canine blood vessels and other sympathetically innervated tissues were abolished by tetrodotoxin and guanethidine, indicating their neuronal origin (Rorie et al., 1981; Lundberg et al., 1984). Dihydroxyphenylglycol is also of neuronal origin; once formed, it rapidly diffuses through the neuronal membrane and into the superfusate. During high-frequency transmural stimulation, noradrenaline release was greater in the veins than in the arteries, which could contribute to observed differences in contractility. After cessation of the stimulation, noradrenaline overflow quickly returned to pre-stimulation levels in all vessels studied, reflecting its rapid removal from the junctional clefts via reuptake. The arteries released similar amounts of noradrenaline, although less overflowed from the mesenteric artery, undoubtedly because of its avid neuronal reuptake system (Tyce et al., 1995). The overflows of noradrenaline from each vessel give an indication of their relative contribution to plasma noradrenaline levels during intense sympathetic stimulation in vivo. This suggests that the veins are more likely to be a significant source of plasma noradrenaline than are the arteries, with the portal vein contributing three to four times as much noradrenaline as the arteries, relative to the amounts of vascular tissue.

The long latency of neuropeptide Y overflow after its release is consistent with in vitro (Hunter et al., 1988; Rorie et al., 1991) and in vivo (Pernow et al., 1986a; Lacroix et al., 1989) studies and is characteristic of peptides. Neuropeptide Y was released in greater amounts in the veins than in the arteries. In the veins, neuropeptide Y release was evoked even at 2 Hz stimulation. Thus, if neuropeptide Y receptors are located on smooth muscle of the veins which serve a direct or a potentiating role in vasoconstriction, the larger amounts of neuropeptide Y released might explain their greater contractility.

Relating the amounts of the compounds measured in superfusate and in the vessels to the tissue weight allows comparisons to be made between the different vessels. Veins are generally thinner-walled than arteries which, on a weight basis, might result in overestimation of the degree of innervation. However, the vessels used in this study were chosen partly for their comparable thickness and diameter in an effort to reduce the possibility of error relating to weight.

4.2. Differences in contractile responses

The differences between the arteries and veins in contractile responses to field stimulation were, on a relative

basis, comparable to those expected to occur during sympathetic activation in vivo. However, it appears that neuropeptide Y does not contribute to these differences.

The lack of effect of neuropeptide Y per se on contraction in unstimulated vessels is consistent with the concepts that: (1) there are no neuropeptide Y receptors on the endothelium of these vessels which promote or inhibit directly the release or effects of vasoactive compounds and (2) there are no neuropeptide Y receptors on the smooth muscle which, when activated, cause vasoconstriction. These findings underscore the heterogenous nature in the responsiveness to neuropeptide Y seen among different vascular beds as well as among species. For example, neuropeptide Y directly constricts the rabbit saphenous vein (Cadieux et al., 1993); human, rat and guinea pig femoral vein (Ekblad et al., 1984); and veins of the human forearm (Pernow et al., 1987). Neuropeptide Y also constricts arteries, including the rat basilar artery (Pernow et al., 1986b), pig coronary artery (Rudehill et al., 1986), and feline pial artery (Edvinsson et al., 1984), as well as forearm arteries of the human (Pernow et al., 1987).

The possibility cannot be excluded that neuropeptide Y receptors which mediate direct vasoconstriction are, in fact, located on the smooth muscle of these vessels but were not demonstrable by the neuropeptide Y concentration-response curves because the receptors became desensitized during each experiment (Tessel et al., 1993). This likely was not the case, however, since exogenous neuropeptide Y in concentrations up to 10^{-4} M failed to elicit even slight contractions in any vessel. Furthermore, the single application of neuropeptide Y (3×10^{-7} M) before the frequency-response curves and the noradrenaline concentration-response curves did not contract the vessels (see below regarding saphenous vein).

It appears likely, however, that neuropeptide Y has an active role in modulating vasoconstriction in the arteries in vivo even though the amounts released are small compared to release in the veins. Exogenous neuropeptide Y shifted the noradrenaline concentration-response curves and frequency-response curves to the left in the arteries, which indicates that neuropeptide Y receptors are located postsynaptically in these vessels and serve a potentiating role in vasoconstriction.

Since, in the veins, exogenous neuropeptide Y did not increase contractions to noradrenaline or to field stimulation in their E_{max} ranges, the robust contractions of veins during intense sympathetic activity appear not to be due to neuropeptide Y. The veins were very responsive to noradrenaline; the saphenous vein was, by far, the most sensitive vessel, being a full order of magnitude more sensitive to noradrenaline than were the arteries. Moreover, the cooperative effect of neuropeptide Y made the saphenous vein a further three-fold more sensitive, but only to noradrenaline in lower concentrations as indicated by the leftward shift of the EC_{25} value. This pattern of potentiation may have resulted from desensitization of neuropeptide Y receptors; however, related findings of this study suggest otherwise. There were weak contractions of some of the saphenous veins (and not of any of the other vessels studied) to the single application of exogenous neuropeptide Y (3×10^{-7} M) prior to the noradrenaline concentration-response curves. Significantly, no contractions to neuropeptide Y occurred in saphenous vein preparations not pre-exposed to corticosterone, desmethylimipramine, and propranolol. Thus it appears likely that noradrenaline was present in increased amounts in the synaptic clefts due to inhibition of the noradrenaline uptake processes by these drugs.

Contraction of the portal vein in vivo is also involved in the redistribution of blood during intense sympathetic activation. In the present study, neuropeptide Y, at a concentration which enhanced contractions to noradrenaline or to field stimulation in the arteries, did not do so in the portal vein. These data, taken together with the observation of the lack of effect of neuropeptide Y (up to 10^{-4} M) on the unstimulated veins, suggest that the canine portal vein is devoid of postsynaptic neuropeptide Y receptors which modulate vasoconstriction. The function of the large amounts of neuropeptide Y released in the portal vein thus remains unclear. However, neuropeptide Y likely has a local role in modulating vascular tone, if only indirectly, by activating presynaptic neuropeptide Y receptors which inhibit catecholamine release. The comparatively large amounts of neuropeptide Y that overflowed from both veins during high-frequency transmural stimulation suggest that these vessels are a source of plasma neuropeptide Y during periods of intense sympathetic activity in vivo (Lundberg et al., 1985; Pernow et al., 1986a; Takiyyuddin et al., 1994).

The finding that field stimulation, even at the highest frequencies, contracted the arteries to only a small degree of their capacity suggests reduced responsiveness of the arteries to sympathetic stimulation and may reflect the activation of different populations of adrenoceptors by the noradrenaline liberated by nerve depolarization versus that by exogenous noradrenaline. Noradrenaline liberated locally by sympathetic nerve stimulation is more likely to activate junctional adrenoceptors on the smooth muscle than is exogenous noradrenaline which additionally activates extrajunctional adrenoceptors (Langer et al., 1980). Factors other than direct sympathetic control can influence contractile behavior in vivo. For example, regulation of vessel tone by increased levels of noradrenaline in plasma during strenuous exercise (Pernow et al., 1986a; Devalon et al., 1989) or in patients with pheochromocytoma (Cryer, 1980) would be capable of contracting the arteries considerably more than would the noradrenaline liberated locally into junctional clefts.

The vessels examined in this study, like most blood vessels, are innervated by postganglionic sympathetic nerve

fibers, as exemplified by their contents of noradrenaline and neuropeptide Y. The larger amounts of noradrenaline released from the sympathetic nerve endings in the veins could account for their greater contractility, particularly since the veins are more sensitive to noradrenaline than are the arteries. Another factor may be ATP which is co-released with noradrenaline from some sympathetic perivascular nerves and causes vasoconstriction via postsynaptic P_{2X} purinoceptors. Of the vessels studied, only the mesenteric artery and portal vein are innervated by parasympathetic neurons. Therefore, the actions of vasoactive substances which might have originated from these neurons (primarily acetylcholine and vasoactive intestinal peptide) did not directly account for differences in responsiveness between the arteries and veins. The relationships between neurotransmitters released from other neurons, e.g., nonadrenergic-noncholinergic (NANC) or sensory-motor neurons, as well as endothelial-derived substances such as nitric oxide and endothelin may also be involved.

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