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Neuropeptide Y and reserpine-resistant vasoconstriction evoked by sympathetic nerve stimulation in the dog skeletal muscle

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1 The effects of sympathetic nerve stimulation (evoked by recordings of authentic irregular vasoconstrictor nerve fibre discharge with average frequencies of 0.59, 2.0 and 6.9 Hz) on the perfusion pressure and the overflow of noradrenaline (NA) and neuropeptide Y-like immunoreactivity (NPY-LI) were investigated in the blood-perfused gracilis muscle of the dog *in situ*.

2 Nerve stimulation in the untreated control group evoked a frequency-dependent increase in perfusion pressure and overflow of NA. A significant overflow of NPY-LI was found at the highest frequency only.

3 In a separate group of animals, the sympathetic supply was unilaterally interrupted by preganglionic decentralization before the administration of reserpine (1 mg kg^{-1} i.v.) 24 h before the experiment. Reserpine reduced the NA content of the intact and decentralized gracilis and gastrocnemius muscle by 98–99%. Reserpine also induced a marked (80%) reduction of the muscular content of NPY-LI. The depletion of NPY-LI was, in contrast to that of NA, prevented by the decentralization, suggesting that nerve impulse activity was of primary importance for the reserpine-induced depletion of NPY-LI.

4 A slowly developing and long-lasting perfusion pressure increase was evoked by nerve stimulation, at 2.0 and 6.9 Hz after reserpine treatment. These responses were larger in the decentralized, as compared to the intact gracilis muscle and correlated with the nerve stimulation evoked overflow of NPY-LI ($r = 0.79$, $P < 0.001$). Stimulation at 0.59 Hz caused vasoconstriction in the decentralized but not in the intact gracilis.

5 Administration of α,β-methylene adenosine triphosphate did not evoke an increase in perfusion pressure in the gracilis muscle of reserpine-treated animals.

6 In conclusion, a large perfusion pressure increase to sympathetic nerve stimulation occurs in the reserpine-pretreated skeletal muscle vasculature of the dog *in vivo*, providing that preganglionic decentralization has been performed. It is suggested that the released NPY-LI may mediate this vasoconstrictor response.

Introduction

Neuropeptide Y (NPY), a peptide with 36 amino acid residues (Tatemoto, 1982), is co-localized with the classical sympathetic neurotransmitter noradrenaline (NA) in perivascular sympathetic neurones (Lundberg *et al.*, 1982; 1983; Ekblad *et al.*, 1984). Sympathetic nerve stimulation evokes a co-release of NA and NPY-like immunoreactivity (NPY-LI) in the cat and pig spleen (Lundberg *et al.*, 1984a; 1986c). We recently demonstrated that sympathetic nerve stimulation at a high frequency (10 Hz) evokes an overflow of NPY-LI from the dog gracilis muscle (Pernow *et al.*, 1988). Furthermore, about 50% of the nerve stimulation-evoked increase in perfusion pressure remained after administration of α- and β-adrenoceptor antagonists, suggesting that it may be mediated by the release of NPY-LI (Pernow *et al.*, 1988; see also Lundberg *et al.*, 1984a; 1986c). However, the possibility remains that the residual vasoconstrictor response was due to insufficient γ-adrenoceptor blockade or because NA acti-

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vated adrenoceptors of a non-α subtype such as γ-adrenoceptors (Hirst & Nield, 1980; Nield & Zelcer, 1982).

A situation where the tissue is depleted of its neuronal content of NA but not of NPY would be more suitable for evaluating a possible role of NPY in sympathetic vascular control. Reserpine, which causes an almost total depletion of the tissue stores of NA (see Carlsson, 1965), also lowers the tissue content of NPY-LI (Lundberg *et al.*, 1984b; 1985b, c; 1986a; Allen *et al.*, 1986). Interestingly, the reserpine-induced depletion of NPY-LI, but not that of NA, appears to be highly dependent on intact nerve activity and can be prevented by ganglionic blockade (Lundberg *et al.*, 1985c; 1986a) or by surgical transection of the preganglionic nerves (Lundberg *et al.*, 1986b; 1987). In the present study we investigated the vasoconstrictor responses and the overflow of NA and NPY-LI evoked by sympathetic nerve stimulation after reserpine treatment in the dog gracilis muscle. In addition, the influence of preganglionic denervation on the reserpine-induced impairment of sympathetic transmission was studied.

Methods

Surgical procedure

Mongrel dogs (10–20 kg body wt) of either sex were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v. followed by 3.5 mg kg^{-1} h^{-1} i.v.). A cannula was inserted in the trachea and catheters were placed in the carotid artery for systemic blood pressure measurement and arterial blood sampling, and in the brachial veins for the administration of drugs and fluid. The gracilis muscle of one (control animals) or both sides (reserpine-treated animals) was prepared according to Renkin & Rosell (1962) and Kahan *et al.* (1984). The muscle was isolated from the surrounding tissues and, after the administration of heparin (1000 iu kg^{-1} i.v. followed by 400 iu kg^{-1} h^{-1} i.v.), perfused with blood from the femoral artery at constant flow via a roller pump. Blood flow was adjusted to give a perfusion pressure of approximately 100 mmHg, as measured via a side arm of the arterial loop. Another side arm allowed local intra-arterial (i.a.) administration of drugs. The venous effluent from the muscle was returned into the femoral vein via a three-way connection for blood sampling. Blood pressures were measured with Statham P23Ac transducers and recorded on a Grass polygraph (model 7C). The tracheal cannula was connected to a respirator (Engström) for artificial ventilation, thereafter succinylcholine (4–500 mg h^{-1} i.v.) was administered to inhibit muscle contractions during nerve stimulation. The proximally cut gracilis nerve was placed on bipolar platinum electrodes. Tape recordings of the irregular discharge pattern derived from a few unit recordings of spontaneous human postganglionic sympathetic vasoconstrictor nerve activity to skeletal muscle (Sundlöf & Wallin, 1977; Wallin, 1981) were used to trigger a Grass stimulator (model S11; 15 V and 5 ms duration). Three average frequencies were studied: 0.59 (480 s duration), 2.0 (120 s) and 6.9 Hz (30 s). A continuous regular impulse activity (10 Hz for 120 s) was also studied. To prevent cholinergic influence, atropine (0.5 mg) was administered locally i.a.

One group of dogs was pretreated with reserpine (1 mg kg^{-1} i.v.) 24 h before the experiment. Before reserpine was administered, the left lumbar sympathetic chain was transected at the L3–L4 level via an abdominal incision under sodium pentobarbitone anaesthesia. This procedure is likely to result in a mainly preganglionic denervation, since the nerve supplying the gracilis muscle is a branch of the obturator nerve which leaves the sympathetic trunk at the L4–L6 level (see Bradley, 1959). The sympathetic innervation of the other side was left intact, and both muscles were prepared and used for experiments the following day. A separate control group received no pretreatment.

Experimental procedure

Nerve stimulation was performed once at 0.59 and at 2.0 Hz followed by two stimulations at 6.9 Hz. The irreversible α-adrenoceptor antagonist phenoxybenzamine (0.5 mg kg^{-1}) was then administered slowly i.a. to the reserpine-treated dogs, thereafter the nerves were stimulated at 6.9 and 10 Hz. Venous blood samples were collected just before nerve stimulation, continuously during, and at 30 s, 2, 5 and 15 min after each stimulation. Arterial blood was collected at the beginning and end of each sampling period. At the end of the experiment, biopsies of the gracilis muscle were frozen on dry ice and stored at −80°C until analysed for tissue content of NA and NPY-LI. For comparison, the tissue content of NA and NPY-LI in the gastrocnemius muscle, in which the sympathetic supply had not been stimulated during the experiment, was also determined.

Analytical procedures

Blood samples were collected in test-tubes containing EDTA (final concentration 10 mM) and immediately put on ice. After centrifugation at +4°C, plasma was removed and stored at −80°C until analysed. NA was determined by cation exchange high performance liquid chromatography with electrochemical detection (Hjemdahl *et al.*, 1979; Hjemdahl, 1987). NPY-LI was determined by

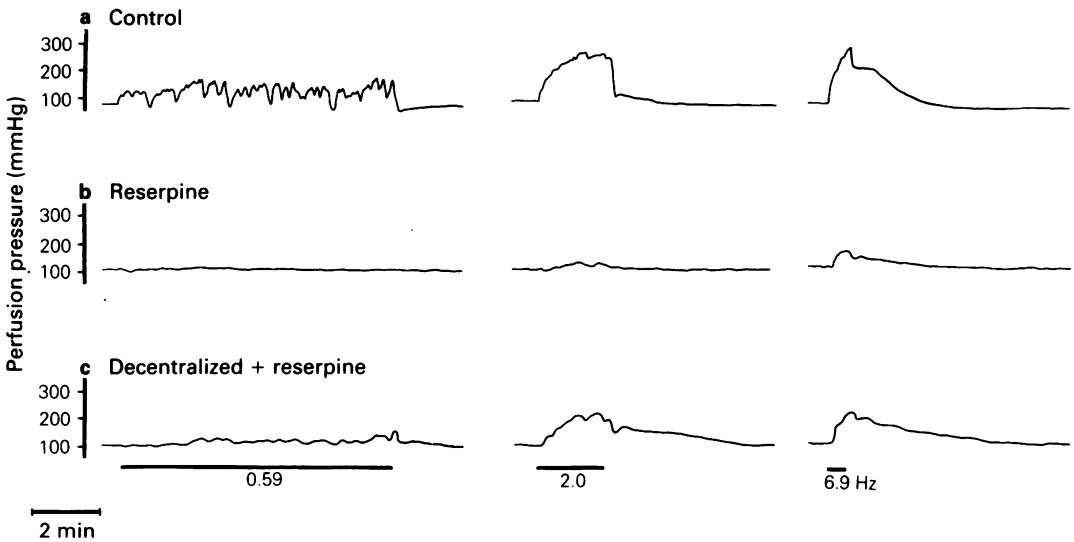

Figure 1 Original recordings of the perfusion pressure responses in the blood perfused dog gracilis muscle, evoked by stimulation of the sympathetic nerves with the irregular activity recorded from human postganglionic vasoconstrictor nerves to skeletal muscle, with a mean frequency of 0.59 (8 min duration), 2.0 (2 min) and 6.9 Hz (30 s). (a) Shows the responses in the control situation. (b and c) Show the responses in a dog pretreated with reserpine (1 mg kg^{-1} i.v.) 24 h before the experiment, where the preganglionic sympathetic fibres were left intact (b) or cut at the L3–L4 level before administration of reserpine (c).

radioimmunoassay using a specific antiserum (N1) which shows no (<0.1%) cross-reactivity with structurally related peptides such as pancreatic polypeptide or peptide YY (see Theodorsson-Norheim *et al.*, 1985).

Calculations

The nerve stimulation-evoked overflow of NA and NPY-LI is expressed as the total integrated increase in veno-arterial concentration difference multiplied by plasma flow. Data are presented as mean values ± s.e.mean and were statistically evaluated by use of the Wilcoxon signed rank test, the Mann-Whitney U-test or Kruskall Wallis analysis of variance (Theodorsson-Norheim, 1986) where appropriate. A probability (P) value <0.05 was considered statistically significant.

Drugs

Atropine sulphate, sodium pentobarbitone and succinylcholine chloride (ACO, Stockholm, Sweden), phenoxybenzamine hydrochloride (Smith, Kline & French Laboratories Ltd, Welwyn Garden City, Hertfordshire, U.K.), sodium heparin (Kabi Vitrum AB, Stockholm, Sweden), reserpine (Ciba, Basel, Switzerland), α,β-methylene adenosine 5'-triphosphate (mATP; Sigma, St. Louis, Mo, U.S.A.). Drugs were, when required, diluted in isotonic saline immediately before use.

Results

Effect of sympathetic nerve stimulation in control animals

Nerve stimulation in control dogs evoked a frequency-dependent increase in perfusion pressure of the gracilis muscle (Figures 1 and 2). The rapidly fluctuating response reflected the irregular nerve discharge pattern, particularly at the low frequency (average 0.59 Hz) stimulation, during which the perfusion pressure occasionally declined to sub-basal levels (Figure 1). A rapid decline was also observed at cessation of the nerve stimulation (Figure 1).

Sympathetic nerve stimulation was accompanied by an overflow of NA of 30 ± 5, 36 ± 7 and 38 ± 6 pmol 100 g^{-1} (corresponding to 0.11 ± 0.02, 0.15 ± 0.03 and 0.18 ± 0.03 pmol 100 g^{-1} per nerve impulse) at 0.59, 2.0 and 6.9 Hz, respectively ($n = 5$). There was a significant overflow of NPY-LI evoked

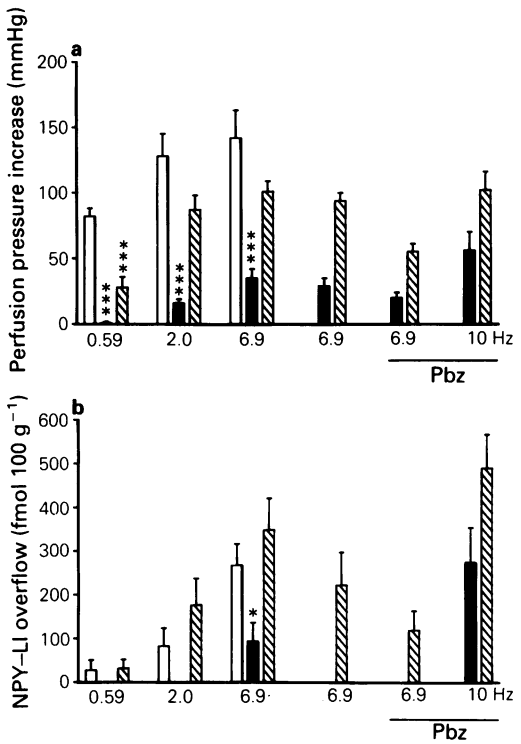

Figure 2 Effects of sympathetic nerve stimulation on (a) perfusion pressure and (b) the overflow of neuropeptide Y-like immunoreactivity (NPY-LI) in the blood perfused dog gracilis muscle. The sympathetic nerves were stimulated before and after (reserpine-treated dogs only) the administration of phenoxybenzamine (Pbz; 0.5 mg kg^{-1} i.a.) with mean frequencies of 0.59 (8 min duration), 2.0 (2 min), 6.9 (30 s) and 10 Hz (2 min). The effects obtained in control dogs (open columns) are compared with those obtained in the gracilis muscle of dogs pretreated with reserpine (1 mg kg^{-1} i.v.) 24 h before the experiment. In addition, the preganglionic sympathetic fibres were cut on one side at the L3–L4 level before reserpine was administered (hatched columns) while the nerves on the other side were left intact (solid columns). Mean values are given, with vertical lines indicating s.e.mean; $n = 5$–6 in each group. The increase in perfusion pressure and the overflow of NPY-LI were under all conditions significantly ($P < 0.05$) greater on the decentralized than on the intact side of reserpine-treated animals. Significant differences between the effects obtained in the control group and the reserpine-treated dogs are indicated, $^{*}P < 0.05$, $^{***}P < 0.001$.

by nerve stimulation at 6.9 Hz but not at the lower frequencies of stimulation ($n = 5$; Figure 2). Thus the overflow of NPY-LI per nerve impulse was 0.09 ± 0.06, 0.34 ± 0.17 and 1.31 ± 0.24 fmol 100 g^{-1} at 0.59, 2.0 and 6.9 Hz, respectively.

Effect of reserpine on tissue levels of NA and NPY-LI

The tissue content of NA in both the intact and decentralized gracilis muscle was reduced by more than 99% 24 h after the administration of reserpine (Table 1). A similar reduction was also observed in the gastrocnemius muscle, i.e. a tissue not subjected to nerve stimulation (Table 1). The NA content of the decentralized gastrocnemius muscle was slightly higher than that of the intact side ($n = 6$; $P < 0.05$). The reserpine-induced reduction of NPY-LI in the gracilis muscle was not as complete as that of NA (Table 1). The NPY-LI content after reserpine was higher in the decentralized gracilis muscle than in the intact muscle (37 vs 17% of control values, respectively; $n = 6$; $P < 0.05$). In the decentralized gastrocnemius muscle, the tissue NPY-LI content was not significantly reduced from control levels by reserpine, while a similar reduction (84%) was observed in the intact gastrocnemius muscle as in the intact gracilis muscle (Table 1).

Effect of sympathetic nerve stimulation in reserpine-treated animals

Nerve stimulation at 2.0 and 6.9 Hz evoked small vasoconstrictor responses in the intact gracilis muscle of the reserpine-treated dogs (Figures 1 and 2), which were only 12 and 25%, respectively, of those in control animals (Figures 1 and 2). Stimulation at 0.59 Hz elicited no reproducible vascular response in the intact gracilis muscle (Figures 1 and 2). All stimulation frequencies evoked significantly greater ($P < 0.05$; $n = 6$) vasoconstrictor responses in the decentralized gracilis muscle than on the contralateral intact side (Figures 1 and 2). Thus, a clear-cut increase in perfusion pressure was observed at 0.59 Hz, although this response was smaller than in the control dogs. At nerve stimulation frequencies of 2.0 and 6.9 Hz on the decentralized side, the vascular responses were only slightly and insignificantly smaller than those observed in control dogs. Furthermore, the vasoconstrictor responses in the decentralized reserpine-treated gracilis muscle were slow in onset and long-lasting. Both the time for the perfusion pressure to increase to half of maximum during the stimulation ($t_{\frac{1}{2}i}$) and to decrease by half after stimulation ($t_{\frac{1}{2}d}$) were significantly longer in the decentralized reserpine-treated gracilis muscle than in controls both at 2.0 and 6.9 Hz (Figure 3). In addition, the perfusion pressure response to 0.59 Hz showed less fluctuation with no rapid decline to subbasal perfusion pressure levels (Figure 1).

There was a slight decline in the vasopressor response to repeated nerve stimulation at 6.9 Hz on both the intact and decentralized side, with a further

Table 1 Tissue content of noradrenaline (NA) and neuropeptide Y-like immunoreactivity (NPY-LI)

	NA (nmol $100\,g^{-1}$)		*NPY-LI* (pmol $100\,g^{-1}$)	
	Gracilis	*Gastrocnemius*	*Gracilis*	*Gastrocnemius*
Control	52.1 ± 2.4[a] (20)	57.9 ± 9.7 (4)	30.4 ± 4.2 (5)	18.4 ± 3.1 (4)
Reserpine	0.09 ± 0.04 (3)	0.21 ± 0.02 (6)	5.3 ± 1.0 (6)	3.0 ± 0.4 (6)
% reduction	>99***	>99***	83***	84***
Decent. + reserpine	0.34 ± 0.02 (3)	1.19 ± 0.21 (6)	11.1 ± 1.4 (6)	16.5 ± 0.2 (6)
% reduction	>99***	98***	63***	10

The tissue levels of NA and NPY-LI in the gracilis (stimulated) and gastrocnemius (unstimulated) muscle of control dogs, 24 h after reserpine ($1\,mg\,kg^{-1}$ i.v.) treatment (reserpine), and after preganglionic decentralization combined with reserpine (Decent. + reserpine) are presented as mean values ± s.e.mean. The number of observations are given in parentheses. Significant differences from the control values are indicated, ***$P < 0.001$. [a] From Kahan *et al.* (1984).

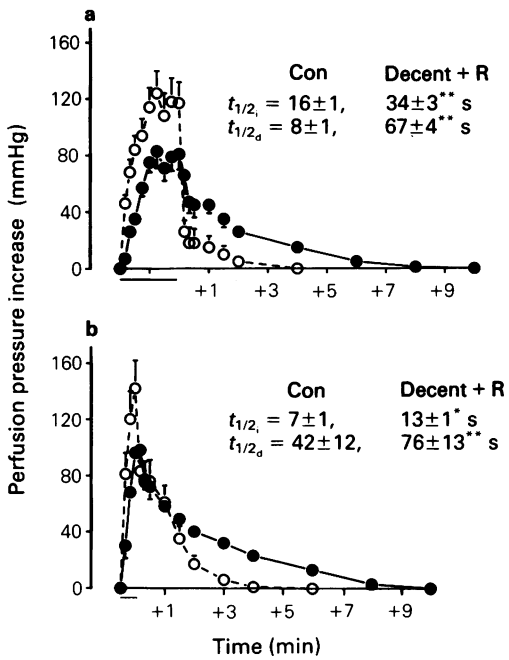

Figure 3 Effect on perfusion pressure during and up to 10 min following sympathetic nerve stimulation in the blood perfused gracilis muscle of control dogs (Con; O- - - -O) and of reserpine-treated ($1\,mg\,kg^{-1}$ i.v.) animals (R) in which the gracilis muscle was decentralized 24 h before the experiment (Decent + R; ●———●). The sympathetic nerves were stimulated with mean frequencies of (a) 2.0 (2 min duration) and (b) 6.9 Hz (30 s). The time for the perfusion pressure to increase to half of maximum ($t_{\frac{1}{2}i}$), and to decrease by half after cessation of the stimulation ($t_{\frac{1}{2}d}$) are also shown. Values are presented as mean values, $n = 6$ (vertical lines indicate s.e.mean). Significant differences between the $t_{\frac{1}{2}}$ of the groups are indicated, *$P < 0.05$, **$P < 0.01$.

reduction after the administration of phenoxybenzamine (Figure 2). Subsequent stimulation at 10 Hz evoked large vasoconstrictor responses in both muscles of the reserpine-treated dogs in the presence of phenoxybenzamine, the perfusion pressure increase in the decentralized gracilis being twice ($P < 0.05$) that in the intact muscle (Figure 2). These latter vascular responses were not maintained at a maximal level throughout the stimulation period.

Basal plasma levels of NPY-LI were 2–3 times higher in reserpine-treated dogs (arterial 134 ± 30, venous 130 ± 30 pM, $n = 6$) than in control animals (arterial 60 ± 11, venous 58 ± 11 pM, $n = 5$). In the decentralized reserpine-treated gracilis muscle, nerve stimulation at 2.0 and 6.9 Hz, but not at 0.59 Hz, evoked a significant overflow of NPY-LI (Figure 2). Upon repeated stimulation (6.9 Hz), the overflow tended to decrease in parallel with the attenuated vasoconstrictor response observed (Figure 2). The nerve stimulation-evoked overflow of NPY-LI correlated well with the increase in perfusion pressure ($r = 0.79$, $P < 0.001$; Figure 4). The overflow of NPY-LI from the intact reserpine-treated gracilis was only analysed in association with nerve stimulation at 6.9 Hz before, and 10 Hz after phenoxybenzamine administration. Both frequencies of nerve stimulation evoked an overflow of NPY-LI, but it was smaller (73 and 45%, respectively; $P < 0.05$; $n = 6$) than that evoked in the contralateral decentralized muscle (Figure 2).

Basal plasma levels of NA in reserpine-treated dogs were low, 0.14 ± 0.04 nM in arterial plasma and 0.17 ± 0.04 nM in the venous effluent from the gracilis muscles ($n = 6$). For comparison, basal arterial and venous plasma NA levels in control dogs were 0.73 ± 0.18 and 0.68 ± 0.14 nM, respectively ($n = 5$). Nerve stimulation at 6.9 Hz did not evoke a significant overflow of NA from either side

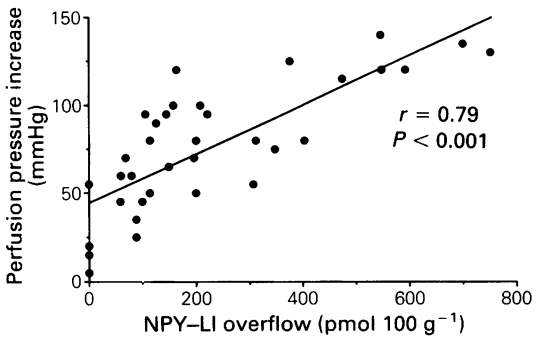

Figure 4 The relationship between increase in perfusion pressure and overflow of neuropeptide Y-like immunoreactivity (NPY-LI) evoked by sympathetic nerve stimulation (0.59, 2.0, 6.9 and 10 Hz) in the blood perfused gracilis muscle of dogs pretreated with reserpine (1 mg kg^{-1} i.v.) in combination with decentralization of the gracilis muscle 24 h before the experiments. The data were obtained from 6 separate experiments.

of the reserpine-treated dogs (0.52 ± 0.46 and 0.58 ± 0.36 pmol 100 g^{-1} in the intact and decentralized muscles, respectively; $n = 6$).

Local i.a. injections of mATP (giving an estimated arterial plasma concentration of 0.1–10 μM) failed to increase the perfusion pressure (105 ± 14 before vs 108 ± 11 mmHg after mATP) in the decentralized gracilis muscle of reserpine-treated dogs ($n = 5$).

Discussion

The present data show that the NA content of the gracilis and gastrocnemius muscle was reduced by 98–99% 24 h after the administration of reserpine. The NA content in the muscle which had been preganglionically decentralized before reserpine administration was only slightly higher, indicating that intact nerve activity is of minor importance for the reserpine-induced depletion of NA during the present time interval (24 h), as compared to shorter treatment periods (Hertting *et al.*, 1962; Weiner *et al.*, 1962). Reserpine also induced a marked, although less complete, reduction of the content of NPY-LI in the muscles with intact sympathetic input. The reserpine-induced depletion of NPY-LI in the gastrocnemius muscle was prevented by decentralization, in agreement with results obtained in other tissues with pharmacological blockade of NPY release (Lundberg *et al.*, 1985c; 1986a) or surgical denervation (Lundberg *et al.*, 1986b; 1987). These findings suggest that reserpine depletes NPY-LI from the perivascular nerves by a mechanism depending entirely on intact sympathetic nerve activity, in contrast to what is known for the depletion of monoamines (see Carlsson, 1965). The content of NPY-LI in the gracilis muscle used for nerve stimulation was, however, markedly reduced despite decentralization in combination with reserpine treatment. This reduction is likely to be related to release of NPY, in excess of resupply by axonal transport, evoked by repeated nerve stimulation during the experiment. This suggestion is supported by the finding that nerve stimulation-evoked overflow of NPY-LI from the gracilis muscle gradually decreased upon repeated stimulation.

The nerve stimulation-evoked reserpine-resistant vasoconstrictor responses were considerably larger in the decentralized gracilis than in the contralateral muscle with intact preganglionic input. Almost similar results were obtained by Rosell & Sedvall (1962) who studied sympathetic control of blood flow in cat skeletal muscle. This was interpreted as being due to incomplete depletion of the NA stores by reserpine after decentralization. However, Sedvall & Thorson (1965) subsequently showed that the functional response to sympathetic nerve stimulation was almost abolished after reserpine with as much as 30% of the NA content still present. In combination with decentralization before reserpine administration, they found a virtually intact vasoconstrictor response to nerve stimulation despite tissue NA levels as low as 10% of control values (Sedvall & Thorson, 1965). In the present study nerve stimulation evoked marked vascular responses despite an almost complete (99%) depletion of tissue NA stores and an undetectable nerve stimulation-evoked overflow of NA. Since NA seems to inhibit NPY release via prejunctional α-adrenoceptors in this experimental model (Pernow *et al.*, 1988), the finding that phenoxybenzamine did not enhance the stimulation-evoked overflow of NPY-LI further indicates that very little or no NA was released by the nerve stimulation. Moreover, the vasopressor response remained after the administration of phenoxybenzamine in a dose which completely blocks the vasoconstrictor effect of exogenous NA (Pernow *et al.*, 1988). These findings argue against the idea that the reserpine-resistant vascular responses to nerve stimulation were caused by NA acting on either α-adrenoceptors or other adrenoceptors, such as the proposed γ-receptors (Hirst & Nield, 1980).

The nerve stimulation-evoked overflow of NPY-LI was, on the other hand, closely related to the vasoconstrictor response in the reserpine-treated gracilis muscle, suggesting that this peptide may be involved in the reserpine-resistant increase in perfusion pressure. The marked differences in tissue content and overflow of NPY-LI between the intact and decentralized muscles agree well with the corre-

sponding vasopressor responses to nerve stimulation. Furthermore, the reserpine-resistant increase in perfusion pressure was slow in onset and long lasting, which resembles the vasoconstriction evoked by i.a. infusions of exogenous NPY in this (Pernow *et al.*, 1988) and other experimental models (Lundberg & Tatemoto, 1982; Lundberg *et al.*, 1985a; 1987; Pernow *et al.*, 1987). It is interesting to note that Folkow (1952) obtained markedly prolonged vasoconstrictor responses to sympathetic nerve stimulation in cat skeletal muscle, when high stimulation frequencies were used as compared to low frequencies. Such a prolongation may suggest release of an additional transmitter, such as NPY, together with NA during high frequency stimulation. Taken together, these findings suggest that the vasoconstriction in skeletal muscle vasculature evoked by high frequency stimulation may, in part, be mediated by the release of NPY. However, from the present investigations, the possibility that other substances may also contribute to the vascular response cannot be excluded.

Studies of large isolated arteries *in vitro*, like the rat tail artery (Sneddon & Burnstock, 1984), the rabbit saphenous artery (Burnstock & Warland, 1987) and the dog mesenteric artery (Muramatsu, 1987), have suggested that ATP may act as a co-transmitter with NA in synpathetic periarterial nerves. However, ATP does not appear to be a mediator of the reserpine-resistant nerve stimulation-evoked vascular response under the present *in vivo* conditions. Firstly, high doses of the metabolically stable analogue mATP were without vasoconstrictor effects in the dog gracilis muscle. Also, mATP does not appear to affect the nerve stimulation-evoked vasoconstrictor response which is resistant to adrenoceptor blockade in this vascular bed (Pernow *et al.*, 1988). Secondly, ATP-mediated effects, contrary to the present results, seem relatively more important at low frequencies of stimulation (Burnstock & Warland, 1987). Finally, ATP mechanisms are uninfluenced by reserpine treatment (Muramatsu, 1987).

The vasoconstrictor responses to repeated nerve stimulation at 6.9 Hz in the reserpine-treated dogs tended to be attenuated and were further reduced after administration of phenoxybenzamine. The parallel gradual decline in the overflow of NPY-LI is a likely explanation for the reduction of the vasoconstrictor response, in agreement with observations in the spleen (Lundberg *et al.*, 1986b; 1987). A sign of fatigue in the vascular response was also obtained during nerve stimulation at 10 Hz, as the vasoconstrictor response was not maintained throughout the stimulation period. This may be related to a large release of the peptide in excess of sufficient resupply of terminal stores by axonal transport, or that only a certain fraction of terminal NPY is available for release. Accordingly, the tissue content of NPY-LI was relatively lower in the decentralized reserpine-treated gracilis than in the unstimulated ipsilateral gastrocnemius muscle.

The rapid changes in perfusion pressure and the sharp fall in vascular tone after cessation of nerve stimulation observed in control dogs was absent after reserpine treatment. This vasodilatator component during control conditions might be due to activation of postjunctional β-adrenoceptors (Dahlöf *et al.*, 1987) or, possibly, via the production of adenosine (Fredholm, 1976; Sollevi & Fredholm, 1983).

In conclusion, sympathetic nerve stimulation evokes vasoconstrictor responses in the skeletal muscle vasculature of the dog *in vivo*, after treatment with reserpine and an α-adrenoceptor antagonist. The frequency-dependent functional responses were slow in onset and long-lasting, and correlated well with the overflow of NPY-LI, suggesting that they may be evoked by the released NPY. To evaluate further the physiological importance of NPY in sympathetic control of skeletal muscle vasculature suggested by the present and previous (Pernow *et al.*, 1988) findings, specific NPY antagonists are required.

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