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Noradrenaline release evoked by a physiological irregular sympathetic discharge pattern is modulated by prejunctional α- and β-adrenoceptors *in vivo*

[1]Thomas Kahan, John Pernow, Jonas Schwieler, *B. Gunnar Wallin, Jan M. Lundberg & Paul Hjemdahl

Departments of Pharmacology, Karolinska Institutet, S-104 01 Stockholm, and *Clinical Neurophysiology, Sahlgren's Hospital, S-413 45 Göteborg, Sweden

1 Sympathetic nerve stimulation-evoked overflow of endogenous noradrenaline (NA) and vasoconstriction were studied in canine blood-perfused gracilis muscle *in situ*. Nerves were stimulated at an average frequency of 2 Hz (240 pulses, 120 s) with impulses derived from a recording of the normal irregular sympathetic discharge to human skeletal muscle, with regular bursts of impulses, or with the conventional continuous stimulus mode.

2 Variations in impulse activity were closely paralleled by changes in vascular tone. However, all stimulation patterns evoked the same integrated NA overflow and the same degree of vasoconstriction.

3 Blockade of β-adrenoceptors by propranolol (0.5 mg kg^{-1} i.v.) significantly reduced NA overflow and vasoconstriction evoked both by continuous and irregular nerve stimulation, by approximately 15–20%.

4 The enhancement of NA overflow following α-adrenoceptor blockade by phenoxybenzamine (0.5 mg kg^{-1} local i.a.) was significantly greater when evoked by continuous than by irregular nerve discharge (24 vs 14 fold). Effects were similar with irregular and regular burst activity. Half of this enhancement has been shown to be due to blockade of neuronal uptake of NA by phenoxybenzamine. Vasoconstrictor responses to all stimulation patterns were similarly reduced, but not abolished, by phenoxybenzamine.

5 The normal irregular sympathetic discharge seems to evoke a similar integrated NA release and equally pronounced vasoconstriction as stimulation with regular bursts or at constant frequency. We provide additional evidence for a physiological prejunctional α-adrenoceptor-mediated inhibition of NA release. This mechanism may be influenced by the discharge pattern. Also prejunctional β-adrenoceptors seem to modulate NA release under physiological conditions. However, the α-adrenoceptor-mediated mechanism is quantitatively more important.

Introduction

Sympathetic nerve stimulation evokes a frequency-dependent release of noradrenaline (NA) and vasoconstriction. Previous studies of postjunctional responses to continuous stimuli at constant frequencies have suggested that the average discharge rate in sympathetic vasomotor fibres rarely exceeds 10 Hz under physiological conditions (Folkow, 1952). However, direct recordings of sympathetic impulse traffic in animals and in man indicate that the normal pattern of firing in sympathetic neurones is highly irregular. The instantaneous impulse frequency, i.e. the frequency calculated from the interval between successive spikes, may thus reach quite high values also at a low average firing rate (see Wallin, 1987). Some observations *in vivo* suggest that effector responses may differ if nerves are stimulated continuously or by bursts of impulses (Andersson *et al.*, 1982; 1983; Lundberg *et al.*, 1986). *In vitro*, electrical field stimulation of small mesenteric arteries with the naturally occurring intermittent sympathetic nerve discharge at a low average frequency evokes a greater integrated contractile response than continuous stimulation at a constant rate (Nilsson *et al.*, 1985). However, the effects of naturally occurring

[1] Author for correspondence.

and other modes of sympathetic nerve discharge on the release of NA and on postjunctional responses do not seem to have been studied *in vivo*.

Prejunctional mechanisms can modulate the amount of NA released per nerve impulse. Experimental evidence favours the existence of physiologically important prejunctional inhibitory α-adrenoceptors (Langer, 1981; Starke, 1987). There are also prejunctional facilitatory β-adrenoceptors (Stjärne & Brundin, 1975; Langer, 1981). This facilitatory mechanism can be demonstrated *in vivo* (Dahlöf *et al.*, 1975; Schmidt *et al.*, 1984; Kahan *et al.*, 1987b), but the functional significance of these β-adrenoceptors has not been established (Cousineau *et al.*, 1984; Kahan & Hjemdahl, 1987a; Kahan *et al.*, 1987b).

Experimental studies of NA release evoked by recordings of authentic nerve impulse activity can now be performed, since the naturally occurring irregular discharge in few or single unit recordings of human sympathetic vasoconstrictor nerves are available. We have in a series of studies used the canine blood perfused skeletal muscle preparation *in situ* to investigate prejunctional modulation of sympathetic neurotransmission *in vivo* (see Kahan, 1987). Under these experimental conditions, the electrically-evoked overflow of endogenous NA and vasoconstriction represent pre- and post-junctional events, respectively. The overflow of NA seems to reflect NA release well in this model (Kahan *et al.*, 1984; 1985; Kahan, 1987). The first aim of the present study was to compare the effects of nerve stimulation at a regular frequency with those evoked by two types of irregular nerve discharge, i.e. intermittent bursts at a regular frequency, and bursts of irregular impulse frequencies from a human sympathetic neurogram. Secondly, we compared the α- and β-adrenoceptor-mediated prejunctional modulation of NA release elicited by the various nerve discharge patterns. To this end we studied the effects of sequential administration of propranolol and phenoxybenzamine on stimulation-evoked NA overflow and vascular responses.

Methods

Surgical procedure

Mongrel dogs of either sex (weighing 14–20 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v., followed by 3.5 mg kg^{-1} h^{-1} i.v.) and artifically ventilated. Catheters were placed in the carotid artery and in the brachial veins. The gracilis muscle (48–77 g) was isolated, heparin was given (1000 iu kg^{-1} i.v., followed by 400 iu kg^{-1} h^{-1}), and the tissue was perfused with blood from the femoral artery at constant flow by means of a roller pump, adjusted to give a perfusion pressure of approximately 100 mmHg (i.e. 4.3–8.0 ml min^{-1} 100 g^{-1}). Side arms in the arterial loop allowed measurement of the perfusion pressure and local intra-arterial (i.a.) administration of drugs. The venous effluent was returned to the femoral vein through polyethylene tubing with a three-way stop-cock for collection of blood samples. Blood pressures, recorded by Statham P23Ac transducers, were displayed on a Grass model 7C polygraph. For details, see Kahan *et al.* (1984).

Experimental procedure

Stimulation of the proximally cut gracilis nerve was achieved via bipolar platinum electrodes using a Grass model S11 stimulator (15 V, 5 ms duration) triggered by a tape recorder. Trains of 240 pulses were delivered for 120 s as (1) a continuous stimulus at constant frequency (2 Hz), (2) bursts of pulses (20 Hz for 1 s every 10 s), or (3) irregularly occurring impulses derived from a few unit recordings of spontaneous human sympathetic muscle nerve activity. These recordings were made with tungsten microelectrodes in the peroneal nerve of a healthy subject at rest and the vasoconstrictor impulses were transformed into standard pulses and stored on magnetic tape. For details of the recording technique, see Sundlöf & Wallin (1977). An infusion of succinylcholine chloride (400–500 mg h^{-1} i.v.) prevented muscle contractions. Venous blood was collected before, and at 0–3, 3–5, 7, 12 and 17 min following the start of a stimulation period. Arterial samples were obtained at the beginning and end of each collection period. The sequence of nerve stimulations was repeated after propranolol (0.5 mg kg^{-1} i.v.) and again following the administration of phenoxybenzamine (0.5 mg kg^{-1} locally, i.a., during which the venous effluent was discarded to reduce untoward systemic effects). Animals were pretreated with atropine (0.5 mg locally, i.a.) to inhibit muscarinic effects on sympathetic neurotransmission (Kahan *et al.*, 1985). Fluid losses were counteracted by a continuous infusion of isotonic saline.

Analytical procedures and calculations

Two ml of blood was collected in test tubes containing EDTA (10 mM final concentration) on ice. Plasma was removed after centrifugation at +4°C, and analysed for endogenous NA and adrenaline by high performance cation exchange liquid chromatography with electrochemical detection (Hjemdahl *et al.*, 1979; Hjemdahl, 1987). Transmitter overflow was calculated as veno-arterial NA concentration difference multiplied by plasma flow. The integrated responses during and 15 min following nerve stimu-

lation are presented (i.e. pmol NA $100\,g^{-1}$). Stimulation-evoked vasoconstrictor responses are expressed as the percentage change in vascular conductance (i.e. ml blood flow $mmHg^{-1}$ $100\,g^{-1}$) and as absolute changes in perfusion pressure; both procedures gave similar results. Under the present experimental conditions the vasopressor responses are linearly related to nerve stimulation frequency (1–4 Hz) and are well below the maximum responses that can be attained (Kahan *et al.*, 1984). Reproducibility of the responses has been demonstrated previously (Kahan *et al.*, 1984; Kahan & Hjemdahl, 1987b) and moderate changes in vascular tone do not seem to influence the diffusion of released NA into the venous effluent (Kahan *et al.*, 1985).

Mean values ± s.e.mean are presented. Statistical evaluation was made with Student's *t* test (two-tailed) for paired observations or for two independent groups, or by the Walsh test (Siegel, 1956), as appropriate. A probability (P) <0.05 was considered statistically significant.

Materials

Phenoxybenzamine hydrochloride (SK&F, Welwyn Garden City, Hertfordshire, U.K.), (±)-propranolol hydrochloride (ICI, Macclesfield, Cheshire, U.K.), atropine sulphate, sodium pentobarbitone and succinylcholine chloride (ACO, Stockholm, Sweden), and sodium heparin (Kabi, Stockholm, Sweden) were used.

Results

Control conditions

Basal arterial plasma levels of NA and adrenaline were 1.09 ± 0.23 and 0.22 ± 0.05 nM, respectively ($n = 7$), and remained essentially unchanged throughout the experiments. The integrated overflow of NA elicited by stimulation of the sympathetic nerves was similar whether evoked by irregular nerve activity, stimulation with regular bursts, or continuous stimulation (Figure 1). As shown in Figure 2, variations in nerve discharge were reflected in corresponding rapid fluctuations in vascular tone. When vascular responses were integrated, the three modes of nerve stimulation evoked similar vasoconstrictor responses, measured either as reductions of vascular conductance or as increases in perfusion pressure (Figure 1). Also when transient peak vasoconstrictor responses to irregular or burst nerve activity were compared, no significant differences appeared (not shown).

Effects of propranolol

Propranolol reduced heart rate (from 161 ± 16 to 114 ± 9 beats min^{-1}, $P < 0.01$, $n = 6$) with essen-

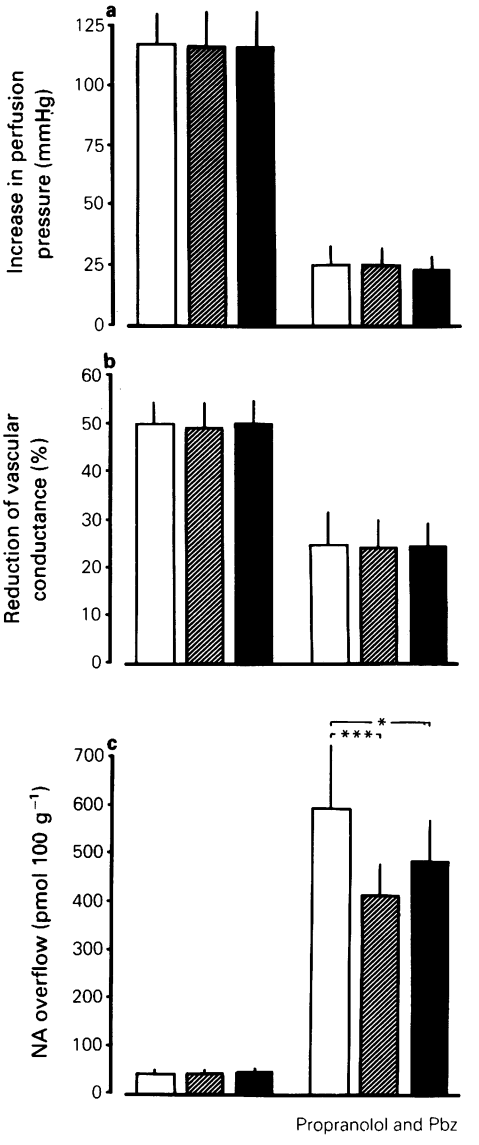

Figure 1 The effects of continuous nerve stimulation (open columns), irregular nerve activity (hatched columns) and regular burst discharge (solid columns) on vasoconstrictor responses (evaluated as increase in perfusion pressure (a) and reduction of vascular conductance (b) and the integrated noradrenaline (NA) overflow (c) before and after α- and β-adrenoceptor blockade with propranolol and phenoxybenzamine (Pbz). Nerve stimulation evoked significant overflows of NA ($P < 0.01$) and vasoconstrictor responses ($P < 0.05$) under all conditions studied. The overflow of NA differed with the mode of nerve stimulation, as indicated: * $P < 0.05$, *** $P < 0.001$. Mean values and s.e.mean from 6–7 experiments are shown.

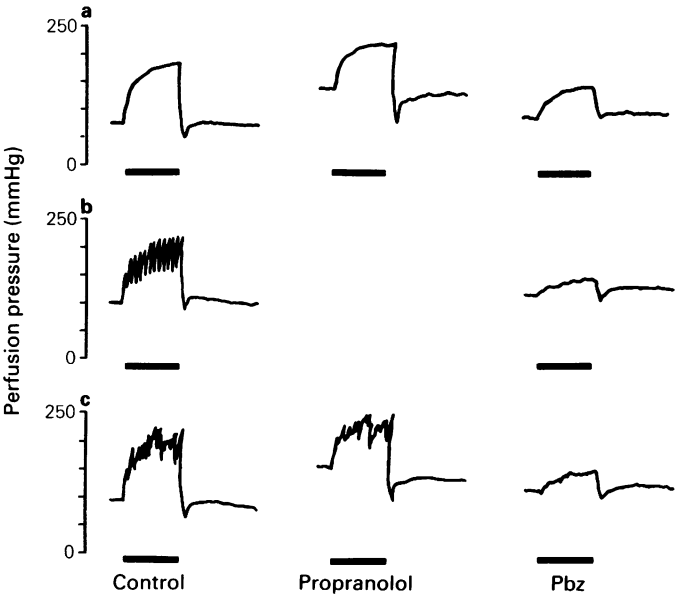

Figure 2 Original records of perfusion pressure during 2 min of nerve stimulation (indicated by horizontal bars) with (a) continuous stimulation frequency, (b) regular burst discharge and (c) irregular nerve activity in the control situation, following propranolol, and after the addition of phenoxybenzamine (Pbz). Regular burst stimulation was not tested after propranolol.

tially no effects on systemic mean arterial blood pressure. There was an elevation of basal perfusion pressure following propranolol (136 ± 17 vs 115 ± 11 mmHg, $P < 0.05$). Propranolol caused significant reductions in NA overflow and vasoconstrictor responses evoked by both continuous and irregular nerve activity (Figure 3, Table 1; regular burst activity was not studied following propranolol).

Effects of phenoxybenzamine

Local administration of the irreversible α-adrenoceptor antagonist phenoxybenzamine (after propranolol pretreatment) reduced basal perfusion pressure from 136 ± 17 to 78 ± 14 mmHg ($P < 0.05$, $n = 6$). There were no systemic haemodynamic effects. Phenoxybenzamine markedly enhanced nerve stimulation-evoked NA overflow (Figure 3, Table 1).

Table 1 The effects of blockade of α- and β-adrenoceptors on nerve stimulation-evoked overflow of noradrenaline (NA) and vasoconstrictor responses

		Vasoconstriction		
	NA overflow	*Vascular conductance*	*Perfusion pressure*	n
Propanolol				
Continuous	−22 ± 8*	−15 ± 5*	−17 ± 4**	6
Irregular	−15 ± 4*	−14 ± 5*	−14 ± 3**	6
Phenoxybenzamine				
Continuous	+2358 ± 747*	−34 ± 15	−69 ± 10**	5
Irregular	+1391 ± 327*	−32 ± 11*	−70 ± 6***	5

The relative changes in nerve stimulation-evoked NA overflow and vascular responses following β-adrenoceptor blockade with propranolol and α-adrenoceptor blockade with phenoxybenzamine (in propranolol pretreated animals) are expressed as % changes from control responses. Nerves were activated by an irregular nerve discharge pattern or by continuous stimulation. Vasoconstrictor responses were evaluated as reduction of vascular conductance and as increase in perfusion pressure. Mean values ± s.e.mean are shown, n indicates number of experiments. Significant changes are shown: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

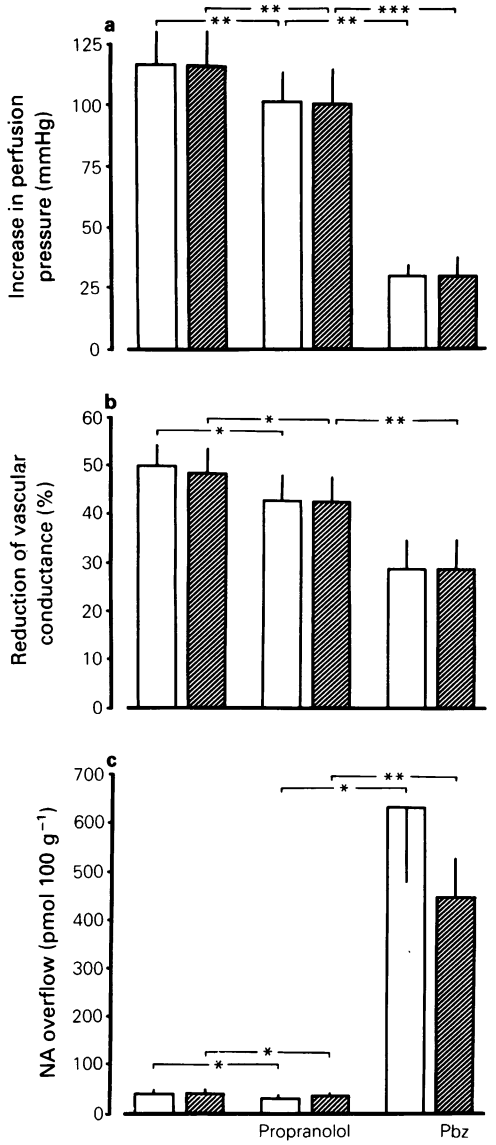

Figure 3 The effects of β-adrenoceptor blockade by propranolol and α-adrenoceptor blockade by phenoxybenzamine (Pbz; in propranolol pretreated animals) on the vasoconstrictor responses (evaluated as increase in perfusion pressure (a) and reduction of vascular conductance (b)) and the integrated noradrenaline (NA) overflow (c) elicited by continuous stimulation (open columns) and irregular nerve activity (hatched columns). Nerve stimulation evoked significant overflow of NA ($P < 0.01$) and vasoconstrictor responses ($P < 0.05$) under all conditions studied. Drug-induced changes are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The relative drug-induced changes are given in Table 1. Mean values and s.e.mean from 5–6 experiments are shown.

Irregular nerve activity and regular burst activity elicited smaller transmitter overflow responses ($-24 \pm 7\%$, $P < 0.05$ and $-20 \pm 3\%$, $P < 0.001$, respectively, $n = 5$–6) than continuous stimulation (Figure 1). Thus, as shown in Table 1 the relative enhancement of NA overflow in the presence of phenoxybenzamine was smaller when evoked by irregular nerve activity than by stimulation at a constant rate (14 vs 24 fold, $P < 0.05$). When compared to the untreated control situation, the NA overflow elicited by continuous stimulation, irregular nerve activity and regular burst stimuli following propranolol and phenoxybenzamine was enhanced by 1999 ± 650, 1222 ± 332 and $985 \pm 258\%$, respectively ($P < 0.01$ for continuous vs regular bursts, $n = 5$). Phenoxybenzamine markedly reduced the vasoconstrictor responses to nerve stimulation (Figure 2, Table 1). However, there was a significant ($P < 0.05$) residual response during all conditions studied (Figure 1). There were no differences between nerve discharge patterns in this respect.

Discussion

Sympathetic neurotransmission has almost invariably been studied experimentally by means of continuous sympathetic nerve stimulation *in vivo* or field stimulation *in vitro* at constant impulse frequencies. In the present investigation, stimulation triggered by authentic recordings of the normal irregular sympathetic discharge to human skeletal muscle at a relatively low average frequency evoked a substantial overflow of endogenous NA and marked vasoconstriction in canine skeletal muscle. A similar NA overflow was also elicited by nerve discharge at constant frequency and by stimulation with regular bursts. Previously, we have found a frequency-dependent overflow of NA from this tissue, the fractional overflow of NA being positively related to the stimulation frequency (Kahan *et al.*, 1984). The present findings of similar integrated overflows of NA with the different stimulation patterns suggest that the release of NA per nerve impulse is fairly constant when there are variations in the sympathetic discharge pattern at a given average frequency.

The variations in nerve impulse activity were closely paralleled by changes in vascular tone, as previously observed *in vitro* (Nilsson *et al.*, 1985). This illustrates the continuous and rapid modulation of the effector organ response by variations in nerve activity. When integrated over time to obtain average responses, the vasoconstrictor responses evoked by irregular, continuous and regular burst discharges were all of a similar magnitude. This is in agreement with results obtained in the feline hind limb, when vasoconstrictor responses to continuous and burst stimulation of the sympathetic nerves were

compared (Andersson, 1983). The average discharge frequency of the resting sympathetic neurone is very low. It is therefore of interest that the naturally occurring irregular nerve activity evoked a similar integrated vasoconstrictor response and NA overflow as continuous nerve stimulation at an average frequency of 0.6 Hz under the present experimental conditions (unpublished). Hence, the control of the skeletal muscle vasculature does not seem to be critically dependent on the pattern of sympathetic nerve discharge. Other vascular beds may be different, as stimulation evokes greater vasoconstriction in the spleen (Lundberg *et al.*, 1986) and greater parasympathetic vasodilatation in the salivary gland and colon (Andersson *et al.*, 1982; 1983) than discharge at a constant rate. This has been attributed to the release of neuropeptide Y (Lundberg *et al.*, 1986) and vasoactive intestinal polypeptide (Andersson *et al.*, 1982; 1983), respectively, and may be related to a preferential release of the coexisting peptide at high stimulation frequencies (see Lundberg *et al.*, 1986). In addition, small rat mesenteric arteries, but not veins, show a greater integrated contractile response *in vitro* to authentic cutaneous vasoconstrictor nerve impulses than to continuous stimulation (Nilsson *et al.*, 1985). The findings in skeletal muscle *in situ* thus differ from previous observations of impulse discharge pattern-dependent responses.

The physiological significance of prejunctional β-adrenoceptors has not been clearly demonstrated as, for example, β-adrenoceptor blockade failed to reduce nerve stimulation-evoked NA overflow significantly in the presence of neuronal uptake inhibition (Kahan & Hjemdahl, 1987a), unless α-adrenoceptors were blocked and NA overflow enhanced by pretreatment with phenoxybenzamine (Dahlöf *et al.*, 1987). In the present investigation propranolol reduced the overflow of NA elicited by both irregular and continuous nerve stimulation when neuronal uptake mechanisms were intact. The apparent discrepancy between these results may be explained if neuronal uptake inhibition by augmentation of junctional NA concentrations activates prejunctional inhibitory α-adrenoceptors more effectively, and that this offsets the β-adrenoceptor-mediated facilitation. Prejunctional β-adrenoceptors in canine skeletal muscle vasculature are of the β_2-subtype (Dahlöf *et al.*, 1987; Kahan & Hjemdahl, 1987a; Kahan *et al.*, 1987b), and propranolol in all probability reduced nerve stimulation-evoked NA overflow by blockade of prejunctional β_2-adrenoceptors. A local anaesthetic effect of this dose of propranolol is less likely (Fitzgerald, 1984). In agreement with observations by others (Schmidt *et al.*, 1984), the present results thus suggest that prejunctional β-adrenoceptors can facilitate sympathetic neurotransmission under physiological conditions. The β-adrenoceptor-mediated influence on sympathetic neurotransmission seems relatively small (see Dahlöf, 1981; Kahan, 1987). Hence, this β-adrenoceptor operated mechanism appears to be quantitatively much less important than the prejunctional α-adrenoceptor-mediated control (present results; Kahan & Hjemdahl, 1987b; Kahan *et al.*, 1987a,b).

It may be argued that inhibition of neuronal uptake by phenoxybenzamine (see e.g. Langer, 1981) enhances nerve stimulation-evoked NA overflow. A substantial amount of the increase in transmitter overflow can, however, be attributed to blockade of prejunctional α-adrenoceptors, as inhibition of neuronal uptake by desipramine (Kahan *et al.*, 1984) or phenoxybenzamine (Kahan *et al.*, 1987a) increases nerve stimulation-evoked NA overflow only two to four fold. The present results obtained with the naturally occurring irregular nerve discharge thus confirm previous evidence obtained with constant frequency stimulation for prejunctional α-adrenoceptor-mediated inhibition of NA release (Langer, 1981; Starke, 1987; Kahan & Hjemdahl, 1987b; Kahan *et al.*, 1987a). Prejunctional α-adrenoceptors in canine gracilis muscle vasculature are predominantly of the α_2-subtype, but there may also be a small population of prejunctional α_1-adrenoceptors (Kahan & Hjemdahl, 1987b; Kahan *et al.*, 1987a). It is of interest that the overflow of NA was most markedly enhanced by phenoxybenzamine during continuous stimulation. The mechanism behind this difference in the effect of phenoxybenzamine is not fully understood, but the results may suggest that the α-adrenoceptor-mediated feed-back regulation of NA release is more effective with continuous than with irregular stimuli.

The reduced vasoconstrictor responses following propranolol may reflect a β-adrenoceptor-mediated reduction of nerve stimulation-evoked NA release. Altered vasoconstrictor responses may to some extent be related to changes in basal perfusion pressure (Kahan *et al.*, 1985). However, results were similar whether expressed as reduction of vascular conductance or absolute increases in perfusion pressure, indicating a true reduction of the vasoconstrictor response following propranolol.

There was a significant residual vasoconstrictor response following the irreversible α-adrenoceptor antagonist phenoxybenzamine. This dose of phenoxybenzamine has been shown to abolish the vasoconstriction elicited by i.a. administration of high doses of NA, suggesting a high degree of α-adrenoceptor blockade (Pernow *et al.*, 1988b). Neuropeptide Y (Lundberg & Tatemoto, 1982) and adenosine 5'-triphosphate (ATP, Burnstock & Kennedy, 1986) have been suggested as co-transmitters with NA in sympathetic nerve endings

and may mediate non-adrenergic vasoconstrictor responses elicited by nerve stimulation. Recent results suggest that the residual vasoconstrictor response in canine skeletal muscle vasculature may be due to the release of neuropeptide Y rather than to ATP (Pernow *et al.*, 1988a,b).

In summary, nerve stimulation with the naturally occurring irregular sympathetic discharge pattern evokes similar integrated NA overflows and vasoconstrictor responses as stimulation with regular bursts or at a constant frequency in an *in situ* preparation of canine skeletal muscle vasculature. Rapid changes in vascular tone closely reflect variations in nerve activity. Additional evidence for a significant prejunctional α-adrenoceptor-mediated inhibition of sympathetic neurotransmission was obtained. This mechanism may operate most effectively with regular nerve impulse activity. Prejunctional β-adrenoceptor-mediated facilitation also seems to modulate NA release under physiological conditions, but this is quantitatively less important.

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