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Release of [^{3}H]-noradrenaline from the sympathetic nerves to bovine mesenteric lymphatic vessels and its modification by α-agonists and antagonists

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1 Isolated segments of bovine mesenteric lymphatic vessels were loaded with [^{3}H]-noradrenaline and its efflux in response to field stimulation examined. Vessels were attached to an isometric force transducer for the simultaneous recording of mechanical activity.

2 Field stimulation at 1, 4 and 8 Hz (0.3 ms pulses, 1 min train) increased spontaneous contraction rate and evoked ^{3}H release up to a maximum of 4.5% of total tissue ^{3}H at 8 Hz. Output per pulse was maximal at 4 Hz.

3 Tetrodotoxin (3×10^{-6} M) blocked the release of ^{3}H in response to field stimulation although the drug did not attenuate release evoked by high K^+ (65 mM) solution. Field-evoked release of ^{3}H was also absent in Ca^{2+}-free solution containing EGTA (1 mM).

4 When vessels were preincubated with labelled transmitter plus cocaine (5×10^{-5} M) evoked release of ^{3}H was absent. After preloading with [^{3}H]-noradrenaline, cocaine (10^{-6} M) potentiated both the mechanical response to field stimulation and evoked ^{3}H release.

5 The relatively non selective α-adrenoceptor antagonist phentolamine (3×10^{-6} M) and the α_2-antagonists yohimbine (10^{-8} M) and rauwolscine (10^{-6} M) significantly increased evoked ^{3}H release at both of the frequencies examined (1 and 4 Hz). In contrast, the selective α_1-antagonist prazosin (10^{-6} M) failed to alter ^{3}H release to 4 Hz stimulation although release at 1 Hz was potentiated in the presence of the drug.

6 The postsynaptic excitatory response to field stimulation remained in the presence of prazosin (10^{-6} M), but was converted to an inhibitory effect in the presence of phentolamine (3×10^{-6} M), yohimbine (10^{-6} M) or rauwolscine (10^{-6} M).

7 Evoked ^{3}H efflux was significantly reduced by clonidine (10^{-6} M), xylazine (10^{-6} M) and exogenous noradrenaline (5×10^{-7} M), although phenylephrine (10^{-6} M) reduced release only at the lower of the two frequencies tested (1 Hz).

8 These findings suggest that release of ^{3}H by field stimulation reflects endogenous transmitter release and that this is subject to autoinhibition via feedback onto inhibitory prejunctional α_2-adrenoceptors. The postjunctional excitatory response is mediated via postjunctional α_2-adrenoceptors.

Introduction

Lymphatic vessels show regular spontaneous contractions which are known to be capable of propelling fluid in both isolated cannulated preparations and *in vivo* (Hall *et al.*, 1965; Campbell & Heath, 1973; McHale & Roddie, 1976; McGeown *et al.*, 1987). Histochemical, pharmacological and electrophysiological evidence suggests that these vessels are innervated by noradrenergic nerves which can modulate both the frequency and force of spontaneous contraction and thus lymph flow. For example, isolated bovine mesenteric lymphatics respond to selective excitation of their intramural nerves by an increase in the frequency and force of their spontaneous contraction. This excitatory effect

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is mediated via α-adrenoceptors nad is converted to a β-inhibitory response after α-adrenoceptor blockade with phentolamine or phenoxybenzamine (McHale *et al.*, 1980; Allen & McHale, 1986).

In recent years it has become evident that the quantity of transmitter released from adrenergic terminals is not determined solely by the frequency of impulse firing in the presynaptic axon. Evidence has accumulated from both *in vivo* and *in vitro* studies to suggest that there are a variety of receptors present on noradrenergic nerve endings to which the transmitter, hormones, other neurotransmitters and various locally produced substances such as prostaglandins may bind and modify the output of noradrenaline (for reviews see: Starke, 1977; Westfall, 1977; Langer, 1981). The physiological consequence of presynaptic receptor activation would be, it is assumed, a modification of the effector response as transmitter output is modulated. Whether or not such presynaptic regulation exists in the sympathetic supply to lymphatic vessels is unknown.

Noradrenaline release from sympathetic nerves may be examined by preloading the terminals with tritiated transmitter and monitoring its efflux in response to excitation of the intramural nerves (Su & Bevan, 1970a). Tritium is released in response to a variety of forms of stimulation, e.g. nerve (Alberts *et al.*, 1981), field (Su & Bevan, 1970a), sympathomimetic agents (Su & Bevan, 1970b), high K^+ Krebs (Stjarne, 1973) and low K^+ Krebs (Bonaccorsi *et al.*, 1977). The release of tritium is thought to parallel the release of endogenous noradrenaline. However, morphological evidence suggests that the sympathetic innervation to lymphatic vessels is sparse when compared to the accompanying artery or vein (Alessandrini *et al.*, 1981). It was the aim of the investigation presented here to establish the applicability of the [^{3}H]-noradrenaline technique to isolated mesenteric lymphatic vessels and to determine if noradrenaline release from these neurones is subject to negative feedback control via prejunctional α-adrenoceptors.

Methods

Techniques for the collection and isolation of bovine mesenteric lymphatic vessels have been described in detail previously (McHale & Roddie, 1976; Allen *et al.*, 1983). Vessel segments approx 3 cm in length and 2 mm in external diameter were sutured at both ends and mounted in a water-jacketed organ bath (1 ml capacity) maintained at 37°C. Krebs solution (in mM: NaCl 120, KCl 5.9, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5 and glucose 5.55) gassed with 95% O_2 plus 5% CO_2 was perfused through the organ bath at a rate of 1 ml min^{-1} by a Gilson Miniplus II flow inducer. The lower end of the vessel was fixed and the upper end attached to an isometric force transducer (Gould UC3) the output of which was displayed on a Gould 8000S chart recorder. Initially the lymphatics were placed under approx 2 mN tension and left to equilibrate for at least 45 min. After the onset of spontaneous activity the vessels were superfused for 45 min with oxygenated Krebs solution containing [^{3}H]-norandrenaline (50 μCi ml^{-1}) and ascorbic acid (1 mM). Following this period of incubation the vessels were washed by continuous perfusion with Krebs solution for a 2 h period.

Platinum ring electrodes positioned at the top and bottom of the organ bath were connected to a Grass S88 pulse generator for field stimulation of the preparation. For stimulation of the intramural nerves pulses of 0.3 ms duration at 40 V (nominal) were used. Previous work has shown pulses of this duration to stimulate selectively nerves in isolated lymphatic vessels (McHale *et al.*, 1980; Allen & McHale, 1986). Following incubation with [^{3}H]-noradrenaline all preparations were stimulated at 4 Hz for 1 min to displace extraneuronal transmitter which may have been released upon contraction of the vessel. Unless otherwise stated cocaine (10^{-6} M) and normetanephrine (10^{-6} M) were added at this time to block neuronal and extraneuronal uptake of noradrenaline and remained for the rest of the experiment.

Efflux of [^{3}H]-noradrenaline

Tritium efflux from the preparation was determined by counting the perfusate collected over a 1 or 2 min period i.e. 1 or 2 ml. To the fraction was added aqueous counting scintillant (Fluoran-HV, BDH; 7 ml to a 1 ml sample) and the samples were counted on an LKB Betarack Liquid Spectrometer with automatic channels ratio to determine efficiency. At the end of each experiment the vessels were removed from the organ bath and placed in scintillant for determination of total tissue ^{3}H.

Tritium efflux was expressed as either disintegrations per min (d.p.m.) or percentage of total ^{3}H in the tissue at the time of stimulation. The latter was calculated as:–

$$\frac{\text{evoked rise, over baseline, in efflux of } ^3\text{H}}{\text{total tissue } ^3\text{H at the time of stimulation}}$$

Data are expressed as mean ± s.e. Where indicated the statistical test applied was Student's t test for matched pairs. P values of <0.05 were considered significant.

High K^+ Krebs solution

In a number of experiments the $[K^+]$ of the Krebs solution was increased to 65 mM by isosmotic replacement of NaCl with KCl.

Calcium-free solution

Calcium-free Krebs solution was obtained by omission of $CaCl_2$ and the addition of EGTA (1 mM) to chelate residual calcium ions.

Drugs and radiochemicals

The drugs used and their sources were as follows: cocaine hydrochloride (Sigma), clonidine hydrochloride (Boehringer Ingelheim), rauwolscine hydrochloride (Carl Roth), prazosin hydrochloride (Pfizer), xylazine hydrochloride (Bayer), yohimbine hydrochloride (Sigma), phenylephrine hydrochloride (Sigma), phentolamine mesylate (Rogitine, CIBA), normetanephrine hydrochloride (Sigma), (−)-noradrenaline bitartrate (Levophed, Winthrop), tetrodotoxin (Sigma) and the radioisotope (−)-[7-8-^{3}H]-noradrenaline (Amersham, specific activity 30–50 Ci mmol^{-1}). Concentrations of noradrenaline refer to the base, all other concentrations to their respective salts. All drugs were present in the perfusate for approximately 15 min before their effect on evoked ^{3}H overflow was examined.

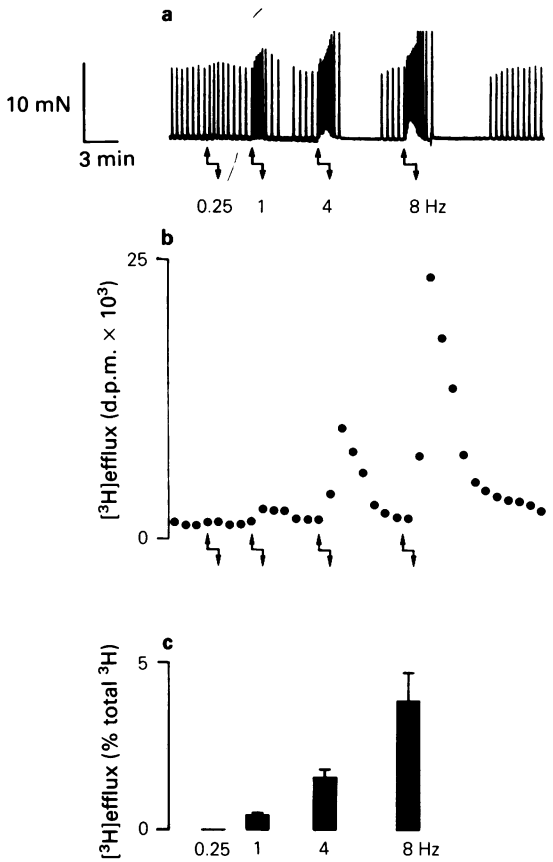

Figure 1 (a) The response of a spontaneously contracting lymphatic vessel to field stimulation at 0.25, 1, 4 and 8 Hz (0.3 ms pulses, 40 V nominal, 1 min train). Periods of stimulation are indicated by the arrows. The corresponding ^{3}H efflux (in d.p.m.) is plotted in (b) below the original record. Stimulation at 1, 4 and 8 Hz evoked an obvious rise in ^{3}H overflow above baseline, although this was not clear at 0.25 Hz. (c) Shows mean ^{3}H efflux from 6 experiments of this type. In the latter case efflux is expressed as a percentage of the total ^{3}H in the tissue at the time of stimulation. Vertical lines represent s.e.mean; $n = 6$.

Results

Field stimulated ^{3}H release

Isolated lymphatic vessels are normally spontaneously active and respond to field stimulation by increasing their contraction frequency, as illustrated in Figure 1a. At the beginning of the experiment the vessel was contracting spontaneously at a rate of approximately 3 per min. At the points indicated below the record the vessel was stimulated at frequencies of 0.25, 1, 4 and 8 Hz (0.3 ms pulses, 40 V nominal, 1 min train). With each pulse train spontaneous contraction rate and force were increased in proportion to stimulus frequency. At 1, 4 and 8 Hz excitation was followed by a short period of mechanical quiescence when the stimulation was switched off, after which activity returned at approximately the control frequency. This post-stimulatory inhibition is frequently observed and appears to be related to the duration and to the frequency of stimulation (McHale *et al.*, 1980; Allen & McHale, 1986).

Corresponding ^{3}H efflux (in d.p.m.) is plotted below the original record of mechanical activity in Figure 1. Stimulation at 1, 4 and 8 Hz evoked an obvious rise in ^{3}H overflow above baseline, although this was not clear at 0.25 Hz. Figure 1c shows mean ^{3}H efflux from 6 experiments of this type. In the latter case efflux is expressed as a percentage of the total ^{3}H in the tissue at the time of stimulation. Although stimulation at 0.25 Hz failed to elicit any measurable ^{3}H release, frequencies of 1, 4, and 8 Hz

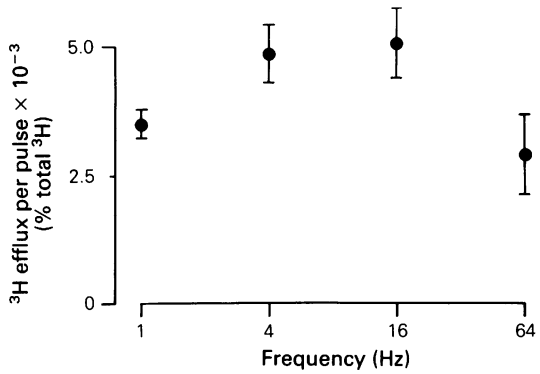

Figure 2 Effect of stimulus frequency on ^{3}H output per pulse plotted as a percentage of total tissue ^{3}H at the time of stimulation. Vertical lines represent s.e.mean; $n = 5$.

were effective in evoking release up to approximately 4.5% of total tissue ^{3}H at 8 Hz.

The effect of stimulus frequency on output per pulse

In this series of experiments ^{3}H output per pulse was determined in response to stimulation with 300 pulses delivered at frequencies of 1, 4, 16 and 64 Hz. Figure 2 summarizes the results from 5 experiments and shows output per pulse plotted as a percentage of total tissue ^{3}H at the time of stimulation. Between 1 and 4 Hz output per pulse rose significantly ($P < 0.05$) from a mean of 3.5×10^{-3}% to 4.9×10^{-3}%. Further increasing stimulus frequency to 16 Hz had no significant ($P > 0.05$) effect on ^{3}H output per pulse, although this was reduced at the highest frequency examined.

The effect of tetrodotoxin and Ca^{2+}-free solution

That the ^{3}H release evoked by field stimulation was produced by excitation of the intramural nerves is suggested by the fact that release was absent in the presence of the neurotoxin tetrodotoxin (TTX, 3×10^{-6} M) or after 30 min perfusion with Ca^{2+}-free solution containing EGTA (1 mM). In 5 experiments ^{3}H efflux in response to field stimulation at 8 Hz (1 min train) and to high K^+ (65 mM) Krebs (3 min perfusion) was examined under control conditions and again in the presence of TTX (3×10^{-6} M). Release in response to 8 Hz stimulation was totally abolished in the presence of TTX in all experiments, although that to 65 mM K^+ was not significantly altered from its control value of 2.68 ± 0.4% (mean ± s.e.mean, % total tissue ^{3}H) to 2.58 ± 0.9% in the presence of the drug. Both field stimulated ^{3}H release and that evoked by 65 mM K^+ were totally abolished in Ca^{2+}-free solution ($n = 5$).

The effect of cocaine

Vessels exposed to cocaine (5×10^{-5} M) before and during incubation with [^{3}H]-noradrenaline failed to release ^{3}H when stimulated at frequencies up to and including 16 Hz, although the mechanical response persisted. Further, after preloading with ^{3}H-noradrenaline, cocaine (10^{-6} M) potentiated field evoked ^{3}H release and the resultant mechanical response as illustrated in Figure 3. Figure 3a shows the mechanical response of one preparation to stimulation at 1 and 4 Hz before and after the addition of cocaine. Under control conditions field stimulation at these frequencies increased spontaneous contraction rate. After a 20 min incubation in Krebs containing cocaine (10^{-6} M), which itself had no effect on spontaneous activity, the mechanical response to stimulation was clearly potentiated. The latter observation is in agreement with the findings of McHale *et al.* (1980) who have previously shown potentiation of field-evoked responses by cocaine.

In Figure 3b the corresponding ^{3}H efflux from 9 similar experiments is plotted. At both 1 and 4 Hz field-evoked release of ^{3}H was significantly ($P < 0.05$) increased in the presence of the drug.

The effects of α-adrenoceptor agonists and antagonists

Phentolamine Phentolamine converted the normal excitatory effect of field stimulation on vessel mechanical activity to one of inhibition and significantly increased evoked ^{3}H release. Figure 4a shows the effect of field stimulation at 1 and 4 Hz (1 min trains) on spontaneous activity before and after α-adrenoceptor blockade with phentolamine (3×10^{-6} M). At the beginning of the experiment the vessel was contracting spontaneously at a rate of approx. 3 per min. Stimulation at 1 Hz increased contraction frequently to approx. 10 per min while 4 Hz caused a dramatic increase in vessel baseline tension. In the presence of phentolamine the β-adrenoceptor-mediated inhibitory response to field stimulation was unmasked (McHale *et al.*, 1980; Allen & McHale, 1986). At 1 Hz both frequency and force of contraction were reduced while 4 Hz abolished all spontaneous activity for just over 1 min.

Simultaneous measurement of field stimulated ^{3}H release showed that this was potentiated in the presence of the drug. In Figure 4b the results from at least 7 experiments are summarized. Mean ^{3}H efflux in response to 1 and 4 Hz stimulation in the absence and presence of phentolamine (3×10^{-6} M) is plotted as a percentage of total tissue ^{3}H at the time of

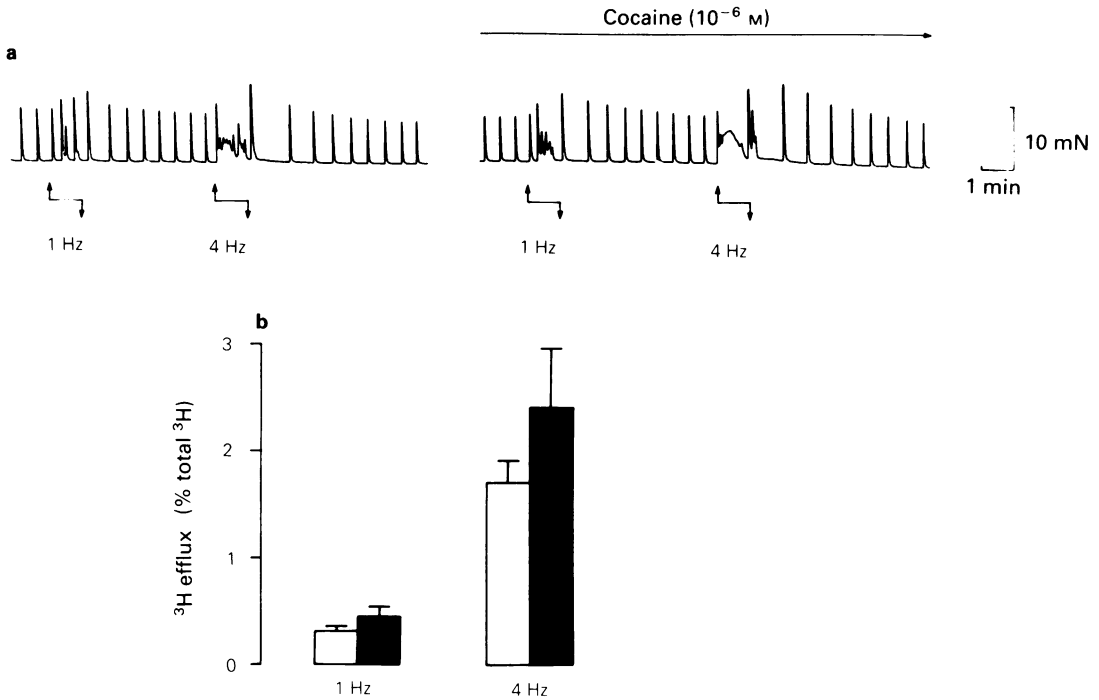

Figure 3 (a) The response of a spontaneously contracting lymphatic vessel to field stimulation at 1 and 4 Hz (0.3 ms pulses, 40 V nominal) before and after the addition of cocaine (10^{-6} M). Periods of stimulation are indicated by the arrows. The gap in the record represents a 20 min period during which time cocaine was added to the perfusate. (b) The corresponding ^{3}H efflux is plotted. Efflux is expressed as a percentage of total tissue ^{3}H at the time of stimulation in the absence (open columns) and presence of cocaine (10^{-6} M; solid columns). Vertical lines represent s.e.mean. Differences in mean values at each frequency are statistically significant ($P < 0.05$). $n = 9$.

stimulation. In the presence of phentolamine ^{3}H release was significantly increased from the control at both frequencies.

Rauwolscine Like phentolamine, the relatively selective α_2-antagonist rauwolscine (10^{-6} M) converted the postsynaptic response to field stimulation to one of inhibition and potentiated evoked ^{3}H release. Figure 5a shows the effect of field stimulation at 1 and 4 Hz on vessel spontaneous activity before and after the addition of rauwolscine (10^{-6} M). In the presence of the drug field stimulation at both frequencies abolished normal spontaneous contraction of the vessel, contrasting with the excitatory response evident during the control period. Figure 5b shows mean ^{3}H efflux from 4 similar experiments evoked by 1 and 4 Hz stimulation under control conditions and in the presence of rauwolscine (10^{-6} M). In the presence of the drug ^{3}H efflux was approximately doubled. The increase at both frequencies was significant ($P < 0.05$).

Yohimbine Yohimbine (10^{-6} M), another relatively selective α_2-antagonist, was also effective in blocking the postjunctional α-adrenoceptor in this preparation and unmasking β-adrenoceptor-mediated inhibition in response to field stimulation. However, at the lower concentration of 10^{-8} M, the postjunctional response remained largely unchanged by yohimbine as illustrated in Figure 6a. In Figure 6b mean ^{3}H efflux data from 5 similar experiments is shown. Yohimbine (10^{-8} M) significantly enhanced evoked tritium release ($P < 0.05$).

Prazosin In contrast to the action of the two relatively selective α_2-antagonists examined, the selective α_1-antagonist prazosin, at a concentration of 10^{-6} M, failed to block completely the excitatory response to field stimulation and was effective in potentiating evoked tritium release only at the lower frequency examined (1 Hz), as illustrated in Figure 7.

Clonidine In this series of experiments cocaine was omitted from the perfusate since this drug has previously been shown to reduce the presynaptic action

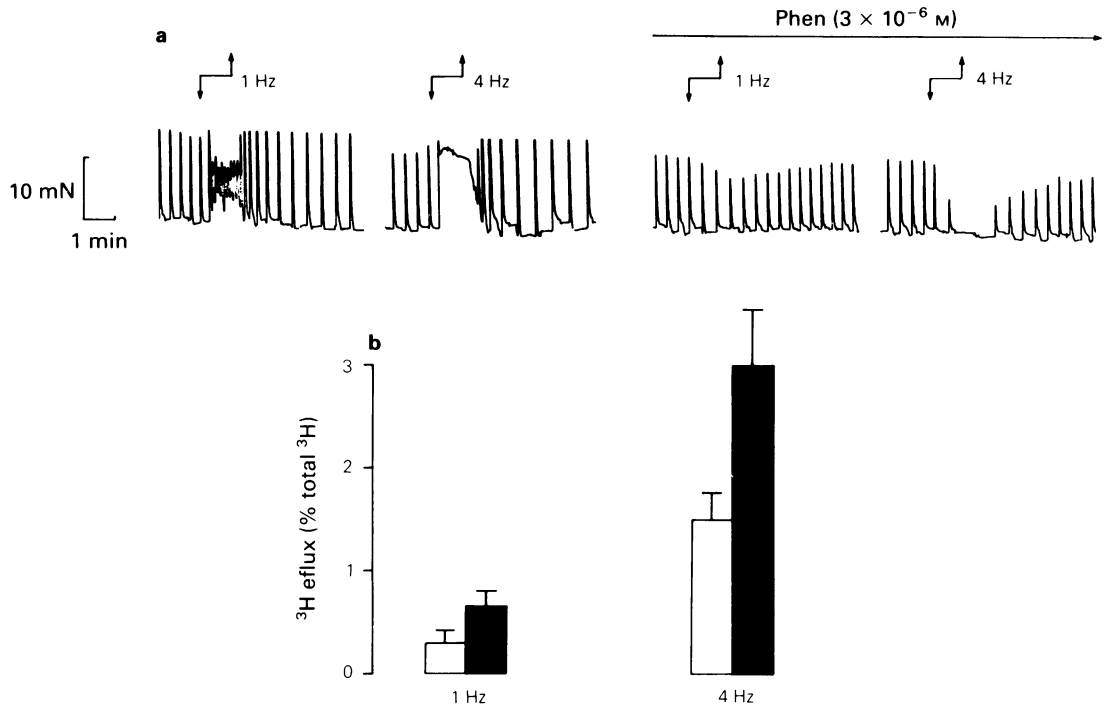

Figure 4 (a) The effect of field stimulation (1 min trains) at 1 and 4 Hz on lymphatic spontaneous activity before and after α-adrenoceptor blockade with phentolamine (Phen; 3×10^{-6} M). Periods of field stimulation and the presence of phentolamine are indicated by the arrows above the records. The gap in the record represents a 20 min period during which time phentolamine was present in the perfusate. (b) The columns represent mean ^{3}H efflux, as a percentage of total tissue ^{3}H at the time of stimulation, to 1 Hz ($n = 7$) and 4 Hz ($n = 9$) stimulation in the absence (open columns) and presence (solid columns) of phentolamine (3×10^{-6} M). Efflux was significantly increased in the presence of the α-blocker ($P < 0.05$, 1 Hz; $P < 0.01$, 4 Hz). Vertical lines represent s.e.mean.

of clonidine in other preparations (e.g. Sullivan & Drew, 1980). Figure 8a shows the effect of field stimulation at 1 and 4 Hz on spontaneous activity under control conditions and again in the presence of the α_2-agonist clonidine (10^{-6} M). Clonidine itself increased contraction frequency and raised baseline tension and this largely obscured any mechanical response to field stimulation which remained in the presence of the drug. In the presence of clonidine ^{3}H efflux was significantly ($P < 0.05$) reduced from the control (Figure 8b).

Xylazine The effect of another α_2-agonist, xylazine (10^{-6} M), on pre- and postsynaptic responses to field stimulation is shown in Figure 9. Like clonidine, xylazine alone increased spontaneous contraction rate. In the presence of the drug, the response to field stimulation remains evident, although reduced from the control. As shown in Figure 9b evoked ^{3}H efflux at both frequencies was significantly ($P < 0.05$) reduced from the control by xylazine (10^{-6} M).

Phenylephrine In contrast to the action of the α_2-agonists examined the α_1-agonist phenylephrine (10^{-6} M) failed to alter ^{3}H release evoked by field stimulation at 4 Hz. However, at 1 Hz ^{3}H release was significantly ($P < 0.05$) reduced from the control (Figure 10). Although phenylephrine increased spontaneous contraction rate the effect was slight and the postsynaptic response to field stimulation remained largely unaltered.

Noradrenaline The effects of the α-agonists and antagonists on evoked ^{3}H release described above are consistent with there being negative feedback of transmitter release mediated via prejunctional α-adrenoceptors. If this is so then exogenous noradrenaline should also act to reduce transmitter release to nerve stimulation. This was found to be the case. Figure 11 summarizes the results from 5 experiments in which ^{3}H release to 1 and 4 Hz stimulation was examined before and after the addition of noradrenaline (5×10^{-7} M) to the Krebs solution. The vessel initially responded to noradrenaline

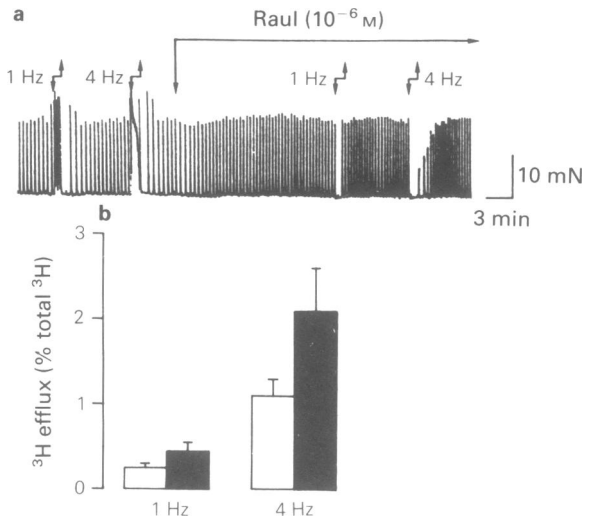

Figure 5 (a) The effect of field stimulation at 1 and 4 Hz (1 min trains) on lymphatic spontaneous activity before and after the addition of the α_2-antagonist rauwolscine (Raul; 10^{-6} M). Field stimulation and the presence of the drug are indicated as before. (b) The columns below the original record represent mean ^{3}H efflux ($n = 4$), as a percentage of total ^{3}H at the time of stimulation, to 1 and 4 Hz trains in the absence (open columns) and presence (solid columns) of rauwolscine (10^{-6} M).

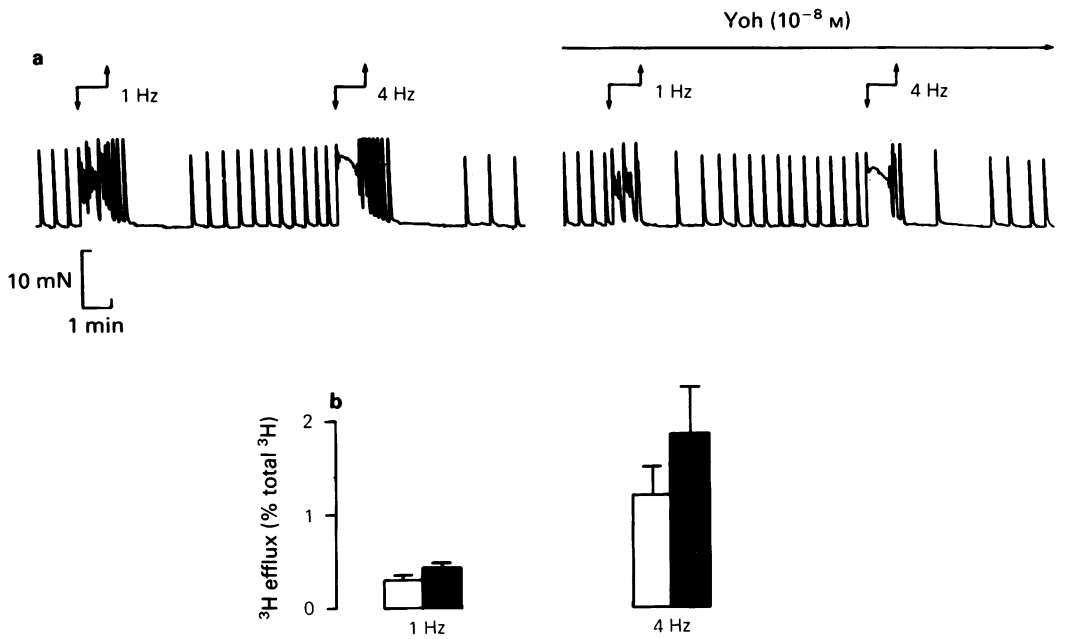

Figure 6 (a) The effect of field stimulation (1 min trains) at 1 and 4 Hz on lymphatic spontaneous activity in the absence and presence of the α_2-antagonist yohimbine (Yoh; 10^{-8} M). Periods of field stimulation and the presence of yohimbine are indicated by the arrows as before. The gap in the record represents a 20 min period during which time yohimbine was added to the perfusate. (b) Mean ^{3}H efflux ($n = 5$), as a percentage of total tissue ^{3}H at the time of stimulation, to 1 and 4 Hz trains in the absence (open columns) and presence (solid columns) of yohimbine (10^{-8} M). Vertical lines represent s.e.mean.

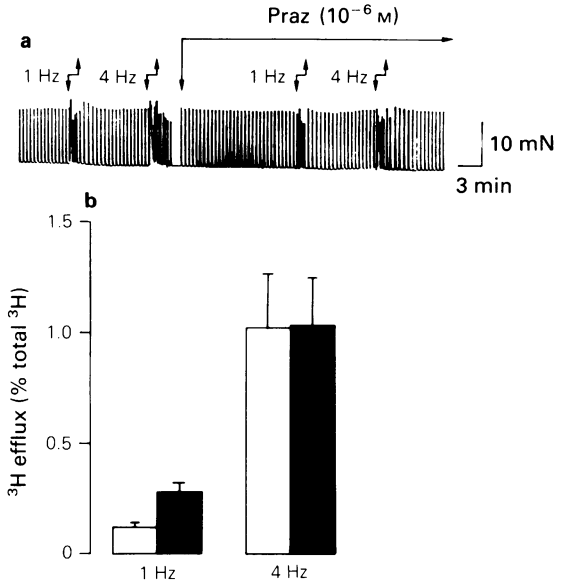

Figure 7 (a) The mechanical response of a single vessel to field stimulation at 1 and 4 Hz before and after the addition of the α_1-adrenoceptor antagonist prazosin (Praz; 10^{-6} M). Periods of field stimulation and drug addition are indicated in the usual way. (b) Mean ^{3}H efflux ($n = 4$), as a percentage of total tissue ^{3}H, in the absence (open columns) and presence (solid columns) of the drug are shown. Vertical lines represent s.e.mean.

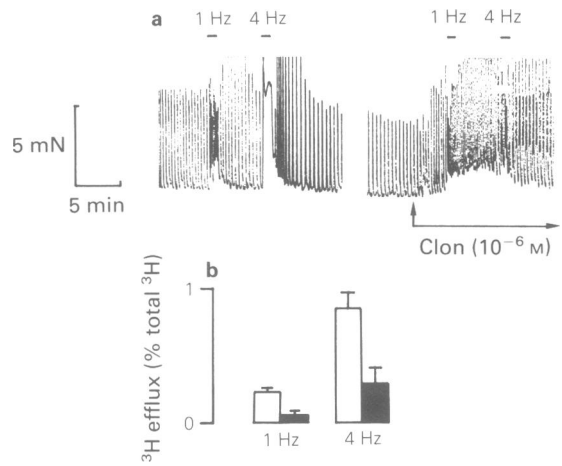

Figure 8 (a) The effect of field stimulation (1 min trains) at 1 and 4 Hz on lymphatic spontaneous activity before and after addition of clonidine (Clon; 10^{-6} M). Periods of field stimulation are indicated above the record. Clonidine addition is indicated by the arrows. The gap in the record represents a 10 min period. (b) Mean ^{3}H efflux ($n = 5$), as a percentage of total tissue ^{3}H at the time of stimulation, to 1 and 4 Hz stimulation in the absence (open columns) and presence (solid columns) of clonidine (10^{-6} M). Vertical lines represent s.e.mean.

with an approximately four fold increase in contraction frequency, although this was not maintained in the continued presence of the drug. Relatively rapid postsynaptic desensitization to noradrenaline is a characteristic of these vessels and has been described previously (McHale *et al.*, 1988). In the presence of the drug, evoked ^{3}H efflux ($P < 0.05$) was significantly decreased at both frequencies and the postsynaptic response was obviously reduced at 1 Hz.

Discussion

In the present study isolated lymphatic vessels were incubated with [^{3}H]-noradrenaline and the stimulation-evoked release of ^{3}H measured. This measurement includes both [^{3}H]-noradrenaline and subsequently formed metabolites and should reflect endogenous transmitter release (Langer, 1974; Starke, 1977). Initial experiments demonstrated measurable ^{3}H release to field stimulation at 1, 4 and 8 Hz. That the evoked ^{3}H overflow did indeed reflect release of endogenous transmitter stores and not release from some extraneuronal pool of ^{3}H is suggested by the following findings:

(a) Field-evoked release of ^{3}H was absent in the presence of tetrodotoxin (TTX). TTX is known to block action potential firing in nerve axons (Narahashi *et al.*, 1964) suggesting that evoked ^{3}H release was a consequence of impulses fired in the preterminal axons invading terminal varicosities. TTX did not itself interfere with transmitter release since this could still be evoked by high K^+ solution.

(b) Field-evoked release of ^{3}H was also absent when vessels were preincubated with labelled transmitter plus cocaine. Cocaine is known to inhibit uptake of transmitter into adrenergic nerve terminals (Iversen, 1967) and was also effective in potentiating evoked ^{3}H release when added after preloading with labelled transmitter.

(c) Neurotransmitter release evoked by potassium and electrical stimulation is known to be a calcium-dependent process (e.g. Kirpekar & Wakade, 1968) and when incubated in Ca^{2+}-free solution containing EGTA (1 mM) vessels failed to release ^{3}H in response to either field stimulation or 65 mM K^+ Krebs solution.

(d) Repetitive stimulation of noradrenergic neurones induces a progressive increase in the amount of transmitter released per pulse. This was first shown by electrophysiological techniques (Burnstock & Holman, 1961: Burnstock *et al.*, 1964) and subse-

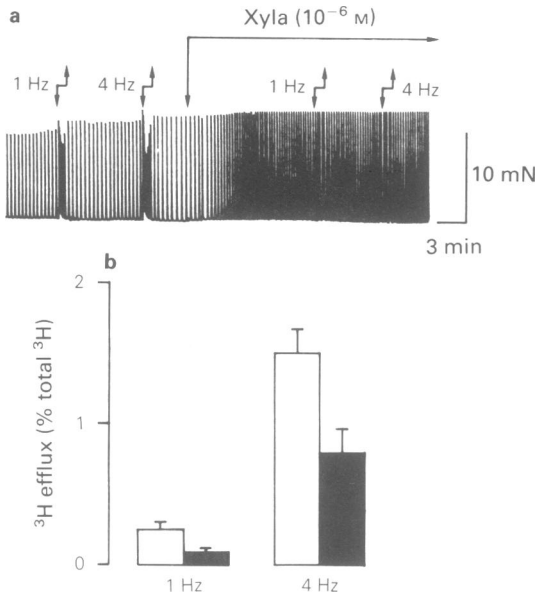

Figure 9 (a) The mechanical response of a single vessel to field stimulation at 1 and 4 Hz before and after the addition of the α_2-adrenoceptor agonist xylazine (Xyla; 10^{-6} M). Periods of field stimulation and drug addition are indicated in the usual way. (b) Mean ^{3}H efflux ($n = 5$), as a percentage of total tissue ^{3}H, in the absence (open columns) and presence (solid columns) of the drug. Vertical lines represent s.e.mean.

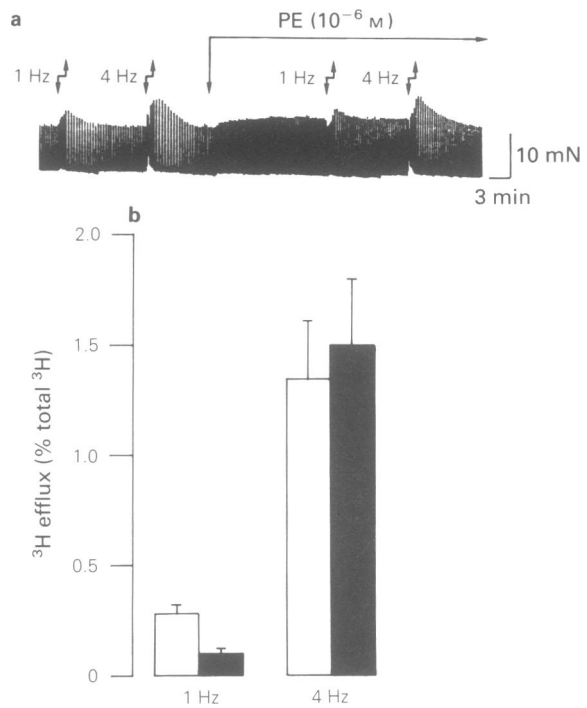

Figure 10 (a) The mechanical response of a single vessel to field stimulation at 1 and 4 Hz before and after the addition of the α_1-adrenoceptor agonist phenylephrine (PE; 10^{-6} M). Periods of field stimulation and drug addition are indicated as before. (b) Mean ^{3}H efflux ($n = 4$), as a percentage of total tissue ^{3}H, in the absence (open columns) and presence (solid columns) of the drug. Vertical lines represent s.e.mean.

quently by the use of labelled transmitter (Hughes & Roth, 1974). In agreement with this, ^{3}H output per pulse from mesenteric lymphatics showed facilitation between 1 and 4 Hz, Although further increasing stimulus frequency led to a reduction in transmitter output. Decline in the amount of transmitter released per shock at frequencies > 10 Hz is a relatively common observation (Haefely *et al.*, 1965; Kirpekar & Misu, 1967; Davies & Withrington, 1968; Stjarne & Brundin, 1977), although the reason for this remains obscure. It has been suggested that, at relatively high stimulus frequenceis, there is a decline in the number or size of impulses reaching the terminals (Kirpekar, 1975). Alternatively, as stimulus frequency is increased there may be a reduction in the amount of transmitter released per pulse (Stjarne & Brundin, 1977).

Thus it would appear that release of tritium by field stimulation after loading with [^{3}H]-noradrenaline reflects endogenous transmitter release and so provides a method for assessing the regulation of transmitter efflux at these peripheral sympathetic neurones.

The sympathetic nervous system is ultimately controlled by the central nervous system. However, in recent years it has become evident that peripheral neurones are susceptible to control by drugs, naturally occurring substances and the neurotransmitter itself. For example, noradrenaline released upon nerve stimulation is thought to combine with prejunctional α-adrenoceptors located on the nerve terminal to limit further release of transmitter. Thus one might expect that blockade of the prejunctional α-adrenoceptors would result in an increase in transmitter release evoked by a train of nerve impulses, while prejunctional α-adrenoceptor agonists should substantially reduce evoked release. This has been confirmed in a number of different tissues (for reviews see: Starke, 1977; Westfall, 1977; Langer, 1981).

In our experiments field-evoked ^{3}H efflux was affected by α-adrenoceptor agonists and antagonists in a manner consistent with the evoked release being subject to negative feed-back control via prejunctional α-adrenoceptors. Thus both the non-selective α-antagonist phentolamine and the more selective

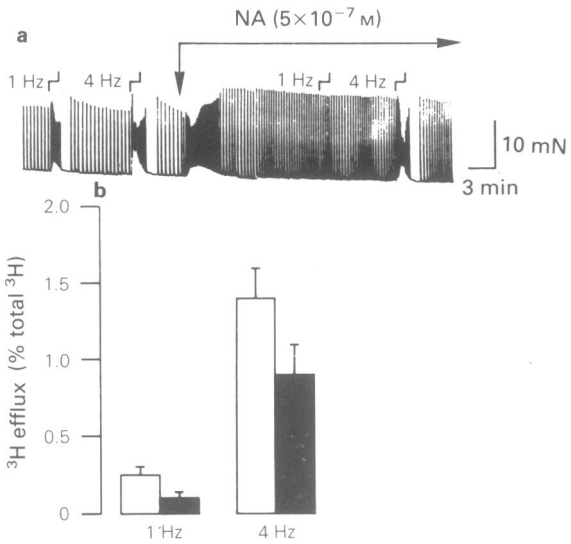

Figure 11 (a) The mechanical response of a single vessel to field stimulation at 1 and 4 Hz before and after the addition of noradrenaline (NA; 5×10^{-7} M). Periods of field stimulation and drug addition are indicated as before. (b) Mean ^{3}H efflux ($n = 5$), as a percentage of total tissue ^{3}H, in the absence (open columns) and presence (solid columns) of NA. Vertical lines represent s.e.mean.

α_2-antagonists yohimbine and rauwolscine were effective in potentiating evoked release of ^{3}H, while release was significantly reduced by clonidine, xylazine and noradrenaline itself. Both clonidine and xylazine are considered to be relatively selective α_2-agonists (Langer, 1981), although some doubt has recently been expressed concerning the specificity of clonidine (Baker *et al.*, 1984; Kalsner, 1985). Similarly, prazosin and phenylephrine were also effective in altering evoked release, but only at the lower of the two frequencies used. The latter two drugs are considered to be relatively selective for α_1-adrenoceptors (Langer, 1981).

Prejunctional α-adrenoceptors are usually capable of being classified within the α_2-subgrouping on the basis of relative potencies of selective α_1- and α_2-adrenoceptor agonists and antagonists although, more recently, evidence has accumulated for the existence of prejunctional α_1-adrenoceptors in a number of preparations (Kobinger & Pichler, 1982; Docherty, 1983). Nevertheless, the findings of the present study indicate that the prejunctional receptors in lymphatic vessels have more the properties of the α_2- than the α_1-subtype.

In recent years it has become clear that the α_2-adrenoceptor should not be considered completely synonymous with the prejunctional receptor. Drew & Whiting (1979) first suggested the possibility of a postjunctional α_2-adrenoceptor and since that time a substantial body of evidence has accumulated for the existence of postjunctional α_2-adrenoceptors in a number of preparations (e.g. Docherty & McGrath, 1980; Docherty & Hyland, 1985a,b). The findings of the present work also suggest that the postjunctional α-adrenoceptor on lymphatic vessels is of the α_2 subtype. Thus, the α_2-adrenoceptor antagonists yohimbine and rauwolscine were clearly more potent than prazosin in blocking stimulus-evoked excitation of isolated vessels, although a difference in vessel sensitivity to exogenous α_1- and α_2-adrenoceptor agonists was less obvious.

In conclusion, it would appear that field-evoked release of [^{3}H]-noradrenaline from the sympathetic nerves to these mesenteric lymphatic vessels is subject to autoinhibition via feedback of released transmitter onto inhibitory prejunctional α-adrenoceptors. The present study suggests that the prejunctional receptor is of the α_2 subtype and that the postjunctional response to the released transmitter is also mediated via combination with α_2-adrenoceptors on the postjunctional membrane.

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