β -adrenoceptor-mediated facilitation of [^3H]-noradrenaline release from the intramural nerves of bovine mesenteric lymphatic vessels

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1 Isolated segments of bovine mesenteric lymphatic vessels were loaded with [^3H]-noradrenaline and its efflux in response to field stimulation at 2 Hz (0.3 ms pulses, 1 min train) examined.

2 Isoprenaline (10^{-6} M) increased evoked fractional ^3H efflux (^3H released as a percentage of total tissue ^3H at the onset of stimulation) from its control value of 0.67 ± 0.09 to $1.2 \pm 0.18\%$ (s.e.mean; $n = 5$).

3 Propranolol (10^{-6} M) alone had no effect on evoked fractional ^3H efflux but blocked the increase in transmitter overflow induced by isoprenaline (10^{-6} M).

4 In the presence of the α -adrenoceptor antagonist phentolamine (10^{-6} M) the facilitatory effect of isoprenaline (10^{-6} M) on transmitter efflux was enhanced. In 5 experiments isoprenaline increased evoked fractional ^3H efflux from its control value of 1.07 ± 0.17 to $2.5 \pm 0.37\%$ when phentolamine was present.

5 Adrenaline (10^{-8} M) increased evoked fractional ^3H efflux from its control value of 0.76 ± 0.09 to $1.13 \pm 0.17\%$ ($n = 7$).

6 These findings are consistent with the presence of presynaptic β -adrenoceptors in this preparation. There is no evidence that these receptors play any role in feedback regulation of transmitter release.

Introduction

Lymphatic vessels show regular spontaneous contractions which are capable of propelling fluid in both isolated cannulated preparations and *in vivo* (Hall *et al.*, 1965; Campbell & Heath, 1973; McHale & Roddie, 1976; Olszewski & Engeset, 1985; McGeown *et al.*, 1987a). Histochemical, pharmacological and electrophysiological evidence suggests that these vessels are innervated by adrenergic nerves which can modulate both the frequency and force of spontaneous contraction and thus lymph flow. For example, isolated bovine mesenteric lymphatics respond to field stimulation by an increase in the frequency and force of their spontaneous contraction. This excitatory effect is mediated via α -adrenoceptors and is converted to a β -adrenoceptor

inhibitory response after α -adrenoceptor blockade with phentolamine or phenoxybenzamine (McHale *et al.*, 1980; Allen & McHale, 1986). Further, *in vivo* studies on the sheep suggest that sympathetic stimulation increases lymph flow as a consequence of a direct effect on lymphatic pumping (McGeown *et al.*, 1987b).

There is now a considerable body of evidence which supports the postulate that total transmitter efflux from sympathetic nerves is not simply under the control of the rate of discharge from the CNS. For example, the nerve terminals may possess an array of receptor types sensitive to neurally liberated or circulating substances. Stimulation of these receptors can modify the amount of transmitter released per impulse (for reviews see: Westfall, 1977; Langer, 1981). The presence of these receptors may give the

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nerve terminals which possess them a means for local regulation of transmitter release and thus the postsynaptic response.

Transmitter release from adrenergic neurones may be examined by preloading terminals with [^3H]-noradrenaline and monitoring its efflux in response to field stimulation (Su & Bevan, 1970). Despite their relatively sparse innervation when compared to accompanying arteries or veins (Alessandrini *et al.*, 1981) this technique has been applied successfully to isolated lymphatic vessels (Allen *et al.*, 1988). In the latter study the α -adrenoceptor agonists clonidine, xylazine and noradrenaline were shown to depress field evoked release of labelled transmitter while the α -adrenoceptor antagonists phentolamine, yohimbine and rauwolscine increased field stimulated release. These findings were interpreted as evidence for the existence of presynaptic α -adrenoceptors to which the endogenous transmitter could bind and limit further noradrenaline release.

In addition to α -adrenoceptor-mediated negative feedback control of transmitter release, evidence for the existence of presynaptic β -adrenoceptors has also been obtained in a number of preparations (for review see: Majewski, 1983). It has been suggested that in those terminals possessing β -adrenoceptors the endogenous transmitter may bind to this receptor and provide positive (facilitatory) control for the release process (Adler-Graschinsky & Langer, 1975). It was the purpose of this investigation to examine the innervation to mesenteric lymphatic vessels for the presence of β -adrenoceptor-mediated facilitatory control of transmitter release.

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 approximately 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 4.7, NaHCO_3 25, NaH_2PO_4 1.2, MgCl_2 1.2, CaCl_2 2.5 and glucose 5.55) gassed with 5% CO_2 in O_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 approximately 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 [^3H]-noradrenaline ($50 \mu\text{Ci ml}^{-1}$) and ascorbic acid (10 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 and the standard stimulus consisted of trains of pulses of 0.3 ms duration at 2 Hz and 40 V (nominal) applied for 1 min. Previous work has shown pulses of this duration to stimulate selectively intramural nerves in isolated lymphatic vessels (McHale *et al.*, 1980; Allen & McHale, 1986). Following incubation with [^3H]-noradrenaline all preparations were stimulated initially at 4 Hz for 1 min to displace extraneuronal transmitter which may have been released upon contraction of the vessel. Under these conditions subsequent field stimulation-evoked ^3H -efflux reflects endogenous transmitter release (Allen *et al.*, 1988).

Unless otherwise stated cocaine (10^{-6} M) and normetanephrine (10^{-6} M) were added to the perfusing solution at this time to block neuronal and extra-neuronal uptake of noradrenaline and remained present for the rest of the experiment.

Drugs and radiochemicals

The drugs used and their sources were as follows: cocaine hydrochloride (Sigma); isoprenaline sulphate (Saventrine, Pharmax); propranolol hydrochloride (Inderal, ICI); adrenaline ((-)-epinephrine, Sigma); phentolamine mesylate (Rogitine, CIBA); normetanephrine hydrochloride (Sigma); (-)-noradrenaline bitartrate (Levophed, Winthrop) and the radioisotope (-)-[7-8- ^3H]-noradrenaline (Amersham, specific activity 30–50 Ci mmol $^{-1}$). All drugs were present in the perfusate for approximately 15 min before their effect on evoked ^3H overflow was examined.

Efflux of [^3H]-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 (Flouran-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 a 48 h period. At the end of this time vessels had disintegrated and total tissue ^3H was determined.

Fractional ^3H efflux represents tritium efflux expressed as a percentage of total ^3H in the tissue at

Figure 1 Tritium efflux (in d.p.m.) from a single lymphatic vessel in response to field stimulation on two consecutive occasions (S1 and S2). Each point represents efflux over a 1 min period.

the time of stimulation (Farnebo & Hamberger, 1971) calculated as:-

$$\frac{\text{evoked rise, above baseline, in efflux of } ^3\text{H}}{\text{total tissue } ^3\text{H at the onset of stimulation}} \times 100$$

Mean data are expressed together with s.e.mean. Where indicated the statistical test applied was a Student's *t* test for matched pairs. *P* values of <0.05 were considered significant.

Results

Field stimulation applied to lymphatic vessels on two occasions (S1 and S2) with a 25 min interval between trains evoked ^3H efflux (d.p.m.) to a similar extent on each occasion (Figure 1). Results from 5 such experiments showed no significant difference in fractional ^3H efflux between S1 ($0.72 \pm 0.07\%$) and S2 ($0.76 \pm 0.11\%$) with an S2/S1 ratio of 1.05.

Isoprenaline

The β -adrenoceptor agonist isoprenaline (10^{-6} M) enhanced stimulation-induced overflow of ^3H without affecting basal ^3H efflux (d.p.m. Figure 2). Figure 2b summarizes the results from 5 such experiments and shows isoprenaline to increase significantly ($P < 0.05$) field-stimulated fractional ^3H efflux ($\text{S2/S1} = 1.8$).

In 6 experiments control (S1) evoked fractional ^3H efflux in the presence of propranolol (10^{-6} M) was $0.73 \pm 0.1\%$ and after the inclusion of isoprenaline (S2) was not significantly different at $0.72 \pm 0.08\%$.

The α -adrenoceptor antagonist phentolamine is known to increase evoked ^3H overflow by blocking presynaptic α -adrenoceptors (Allen *et al.*, 1988). In

Figure 2 (a) ^3H efflux (in d.p.m.) from a single lymphatic vessel: the vessel was stimulated on two consecutive occasions (S1 and S2) as before. Each point represents efflux over a 1 min period. Periods of stimulation are indicated below the graph. At the time indicated above the graph, isoprenaline (Iso; 10^{-6} M) was added and remained for the rest of the experiment. In (b) mean fractional ^3H effluxes from 5 similar experiments are presented. Vertical lines represent s.e.mean.

the present study, phentolamine (10^{-6} M) potentiated the effect of isoprenaline on field stimulated release of ^3H . In 5 experiments in which phentolamine was present, isoprenaline increased evoked fractional ^3H efflux from its control (S1) value of $1.07 \pm 0.17\%$ to $2.5 \pm 0.37\%$ ($\text{S2/S1} = 2.34$).

Propranolol

The β -adrenoceptor antagonist propranolol (10^{-6} M) did not affect field evoked fractional ^3H efflux. In 5 experiments, fractional ^3H efflux was $0.56 \pm 0.07\%$ before (S1) propranolol and was not significantly altered ($0.52 \pm 0.08\%$) after (S2) drug addition ($\text{S2/S1} = 0.93$). Similarly, propranolol (10^{-6} M) was without effect on evoked fractional ^3H release after α -adrenoceptor blockade with phentolamine

(10^{-6} M). In 4 experiments, evoked fractional ^3H efflux in the presence of phentolamine was $1.56 \pm 0.28\%$ and after the inclusion of propranolol was not significantly different at $1.4 \pm 0.25\%$ ($S_2/S_1 = 0.90$).

Adrenaline

Adrenaline (10^{-8} M), like isoprenaline, potentiated the evoked release of ^3H . In 7 experiments fractional ^3H efflux to field stimulation was $0.76 \pm 0.09\%$ before drug addition (S_1) and was increased significantly to $1.13 \pm 0.1\%$ 15 min after (S_2) the addition of adrenaline ($S_2/S_1 = 1.49$).

Discussion

The selective β -adrenoceptor agonist isoprenaline (10^{-6} M) increased field stimulation-evoked fractional ^3H efflux from isolated mesenteric lymphatic vessels by approximately 80%; an effect abolished by the β -adrenoceptor antagonist propranolol (10^{-6} M). This observation suggests that the sympathetic nerve fibres to these vessels are endowed with facilitatory β -adrenoceptors.

Exogenously applied β -adrenoceptor agonists have been shown to increase evoked release of [^3H]-noradrenaline in many preparations including guinea-pig atria (Adler-Graschinsky & Langer, 1975) and vasa deferentia (Stjarne, 1975); cat nictitating membrane and aortic strips (Langer *et al.*, 1975); human omental blood vessels (Stjarne & Brundin, 1975) and oviduct (Hedqvist & Moawad, 1975); mouse atria and rabbit pulmonary artery (Johnston & Majewski, 1986). However, an effect of β -adrenoceptor agonists on transmitter release is not always easily demonstrated. Starke *et al.* (1975) and Endo *et al.* (1977) failed to observe an effect of isoprenaline on evoked [^3H]-noradrenaline release in the rabbit pulmonary artery. Similarly, early work with mouse atria suggested that prejunctional β -adrenoceptors were absent from this tissue (Farnebo & Hamberger, 1974). Nevertheless, in a more recent study of both these tissues (Johnston & Majewski, 1986) isoprenaline was shown to be effective in enhancing transmitter release when the α -adrenoceptor antagonist phentolamine was present.

The ability to demonstrate a presynaptic effect of isoprenaline only in the presence of an appropriate α -adrenoceptor antagonist has been reported for a number of different preparations including rabbit ear artery (Majewski & Rand 1981) and rat portal vein (Dahlof *et al.*, 1978). Moreover, in the rat atria the

facilitatory action of isoprenaline on endogenous noradrenaline release is inhibited by exogenous noradrenaline and enhanced after α -adrenoceptor blockade by phentolamine (Majewski & Rand, 1981). In agreement with this, Kalsner (1982) found noradrenaline prevented isoprenaline increasing transmitter release from the guinea-pig atria. The explanation for these findings is still a matter of debate although Majewski & Rand (1981) have proposed that activation of the α -adrenoceptor-mediated inhibitory feedback loop normally predominates over any β -adrenoceptor-mediated facilitation of transmitter release. Such an explanation is not consistent with all the available evidence (Johnston & Majewski, 1986) but interaction between these two opposing systems might explain the failure of isoprenaline to enhance noradrenaline release, at least in some preparations. It would also help to explain the finding of the present study that phentolamine was effective in potentiating the action of isoprenaline on evoked overflow of radio-labelled noradrenaline.

If presynaptic β -adrenoceptors form part of an on-going positive feedback loop it might be expected that the introduction of a β -adrenoceptor antagonist, such as propranolol, would result in a depression of evoked transmitter release. In some studies this has been found to be the case. Nevertheless, the operation of on-going β -facilitation of transmitter release is not always easily demonstrated and there are many studies reporting the failure of β -adrenoceptor antagonists to alter noradrenaline release (see Majewski, 1983); such was the case in the current investigation. Propranolol (10^{-6} M) did not alter evoked release of [^3H]-noradrenaline even after blockade of prejunctional α -adrenoceptors with phentolamine. This would imply the absence of any functional β -adrenoceptor-mediated feedback, at least under the conditions of the present experiments. However, Adler-Graschinsky & Carrara (1982) have demonstrated that the neuronal uptake blocker desipramine can prevent propranolol decreasing evoked noradrenaline release in guinea-pig isolated atria. It was suggested that the latter finding might help to explain the failure of several authors to observe a decrease in the overflow of noradrenaline with β -adrenoceptor antagonists, since in all of these studies neuronal uptake blockers were employed. This might also explain the findings of the present work. However, in a more recent examination of prejunctional β -adrenoceptors in the rabbit pulmonary artery and mouse atria, in which neuronal uptake blockers were excluded, no effect of propranolol on transmitter release could be demonstrated (Johnston & Majewski, 1986). It seems that neuronally released noradrenaline does not normally activate the prejunctional β -adrenoceptor.

The absence of a physiological role for noradrenaline in activating the presynaptic β -adrenoceptor does not exclude a functional role for β -facilitation of transmitter release. Adrenaline is known to enhance noradrenaline release in a number of different preparations and to do so even when noradrenaline is without effect (see Majewski, 1983). In our preparation, a low concentration of adrenaline (10^{-8} M) significantly enhanced transmitter release.

Thus, it may be that adrenaline is the normal physiological activator of the prejunctional β -adrenoceptors.

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