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FURTHER EVIDENCE THAT PROSTAGLANDINS INHIBIT THE RELEASE OF NORADRENALINE FROM ADRENERGIC NERVE TERMINALS BY RESTRICTION OF AVAILABILITY OF CALCIUM

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1 Guinea-pig vasa deferentia were continuously superfused after labelling the transmitter stores with [^{3}H]-(–)-noradrenaline. Release of [^{3}H]-(–)-noradrenaline was induced by transmural nerve stimulation.

2 Prostaglandin E_2 (14 nM) drastically reduced the release of [^{3}H]-(–)-noradrenaline, while tetraethylammonium (2 mM), rubidium (6 mM), phenoxybenzamine (3 μM) each in the presence or absence of Uptake $_1$ or $_2$ blockade, and prolonged pulse duration (from 0.5 to 2.0 ms) all significantly increased the release of [^{3}H]-(–)-noradrenaline per nerve impulse.

3 The inhibitory effect of prostaglandin E_2 on evoked release of [^{3}H]-(–)-noradrenaline was significantly reduced by tetraethylammonium, rubidium and prolonged pulse duration, whilst it was actually enhanced by phenoxybenzamine. This indicates that increased release of noradrenaline per nerve impulse does not *per se* counteract the inhibitory effect of prostaglandin E_2.

4 It is concluded that tetraethylammonium, rubidium and prolonged pulse duration counteracted the inhibitory effect of prostaglandin E_2 on [^{3}H]-(–)-noradrenaline release by promoting calcium influx during the nerve action potential. The results are consistent with, and add more weight to the view that prostaglandins inhibit the release of noradrenaline by restriction of calcium availability.

Introduction

Ample experimental evidence indicates that prostaglandins of the E series may control adrenergic neuroeffector transmission by inhibiting noradrenaline (NA) release evoked by nerve action potentials. Such an action has been regularly observed in a large number of isolated tissues from different animal species and from man, and several *in vivo* studies point to the same mechanism of action (for references see, Hedqvist, 1970, 1976a; Wennmalm, 1971).

NA release by nerve action potentials is critically dependent upon calcium ions, and it is believed that depolarization of the nerve terminal causes an inward movement of calcium which in turn promotes focal extrusion of NA into the junctional cleft (*cf.* Simpson, 1968; Hubbard, 1970). Apparently calcium interacts with the inhibitory effect of prostaglandin E. Thus, increasing the calcium concentration in the perfusion medium to the cat spleen counteracts the inhibitory action of prostaglandin E on NA release and restores normal output (Hedqvist, 1970). The same interaction between calcium ions and prostaglandin E is also seen in the guinea-pig vas deferens, and in fact the inhibitory effect of prostaglandin E on NA release is progressively increased when the ambient calcium concentration is reduced (Hedqvist, 1973, 1974a; Stjärne, 1973a, b). Notably, NA release by tyramine, which is a calcium-independent process, is not affected by prostaglandin E (Hedqvist, 1970). In view of the decisive role of calcium in the process of stimulus secretion coupling these observations suggest that prostaglandin E inhibits NA release actually by restriction of calcium availability.

The present investigation was undertaken to probe further into the mechanism of action of prostaglandin E_2 on adrenergic neurotransmission. Experiments were carried out to determine the effect of increased pulse duration and of administration of tetraethylammonium (TEA) and rubidium ions on the inhibitory action of prostaglandin E_2 on NA release evoked by nerve stimulation in the guinea-pig vas deferens. The results show that any one of these treatments, considered to increase calcium influx during the nerve action potential, significantly reduces the inhibitory effect of prostaglandin E_2 on NA release. A preliminary account of part of the results has been published (Hedqvist, 1976b).

Methods

Guinea-pigs, weighing 500–700 g, were killed by a blow on the head and the vasa deferentia were isolated

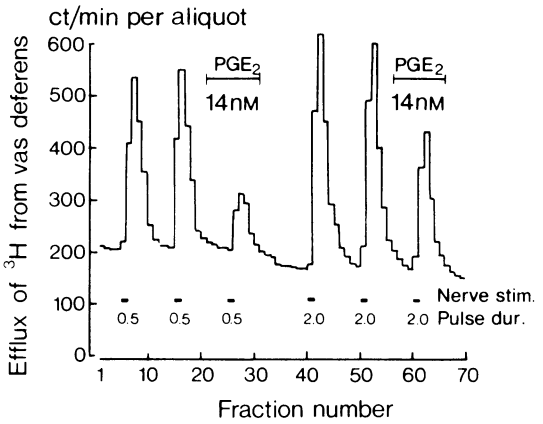

Figure 1 Continuously superfused guinea-pig vas deferens, loaded with [^{3}H]-(–)-noradrenaline (NA). Inhibitory effect of prostaglandin E_2 (PGE_2) on release of tracer by transmural nerve stimulation (5 Hz, 450 pulses) at pulse durations of 0.5 ms or 2.0 ms. Fraction numbers = time in minutes.

and carefully dissected free from adjacent tissue. The isolated preparation was incubated for 1 h in Tyrode solution containing 10 μCi/ml of [^{3}H]-(–)-NA (sp. act. 5.4 Ci/mmol, N.E.N.) and was then thoroughly rinsed and continuously superfused in a 2 ml organ bath with NA-free Tyrode at a rate of 1 ml/minute. The composition of the solution was (mM): NaCl 136.7, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5 and ascorbic acid 0.1. The solution was kept at 37°C and bubbled with 5% CO_2 in O_2. The preparation was electrically stimulated by means of platinum electrodes in the wall of the bath and a Grass S4 stimulator delivering trains of biphasic pulses (5 Hz, 0.5–2.0 ms duration, 450 pulses, supramaximal voltage) at 10 min intervals. The superfusate was divided into 1 min samples and the radioactivity was determined by counting 0.5–1.0 ml aliquots in a Packard liquid scintillation spectrometer using Instagel (Packard Instr. Co.) as counting medium. Quenching was monitored by internal standards.

The following drugs were used: desipramine hydrochloride, indomethacin, (±)-normetanephrine hydrochloride, phenoxybenzamine hydrochloride, prostaglandin E_2, rubidium chloride and tetraethylammonium chloride.

Experimental data were expressed as means ± s.e. Significance was calculated according to Student's *t*-test for paired and unpaired data.

Table 1 Superfused guinea-pig vasa deferentia, loaded with [^{3}H]-(–)-noradrenaline (NA) and subjected to transmural nerve stimulation (5 Hz, 450 pulses). Percentage increase in evoked release of [^{3}H]-(–)-NA by raising the pulse duration from 0.5 to 2.0 ms, and by administration of tetraethylammonium (2 mM) or rubidium (6 mM) (with pulse duration kept at 1 ms).

% increase in evoked [^{3}H]-(–)-Na release		
Pulse duration	*Tetraethylammonium*	*Rubidium*
101 ± 23 (8)	165 ± 12 (5)	77 ± 13 (5)
$P<0.01$	$P<0.001$	$P<0.01$

Mean values ± s.e. of 5 to 8 experiments.

Results

The superfused guinea-pig vas deferens, in which the NA stores were prelabelled with [^{3}H]-(–)-NA, was transmurally stimulated (5 Hz, 0.5–2.0 ms, 450 pulses supramaximal voltage) at 10 min intervals. This type of stimulation, which specifically activates nerve fibres, causes an increased overflow of tracer, the bulk of which consists of intact [^{3}H]-(–)-NA (Hedqvist, 1974b).

With other stimulation parameters kept constant, the overflow of [^{3}H]-(–)-NA was found to vary with the pulse duration. Thus, increasing the pulse duration from 0.5 to 2.0 ms increased the overflow of [^{3}H]-(–)-NA by 101% (Table 1). Prostaglandin E_2 (14 nM) in the superfusing solution, markedly and reversibly inhibited the overflow of [^{3}H]-(–)-NA in response to transmural nerve stimulation at a pulse duration of 0.5 ms (Figure 1). The inhibition by prostaglandin E_2 remained essentially the same when this procedure was repeated several times. On the other hand, increasing the pulse duration to 2 ms significantly reduced the inhibitory action of prostaglandin E_2 on evoked [^{3}H]-(–)-NA overflow (Figure 1 and Table 2).

In agreement with observations on the cat spleen (Thoenen, Haefely & Staehelin, 1967; Kirpekar, Prat, Puig & Wakade, 1972), TEA (2 mM) and rubidium (6 mM) significantly increased the overflow of [^{3}H]-(–)-NA induced by transmural nerve stimulation (5 Hz, 1 ms, 450 pulses) (Table 1), without affecting the spontaneous efflux of tracer. The enhancing effect of TEA and rubidium remained after combined treatment with desipramine (0.5 μM) and normetanephrine (1 μM), given in order to block NA uptake mechanisms. TEA and rubidium were also tested with respect to their capacity to interfere with the effects of prostaglandin E_2 on NA release. Both compounds were found to reduce significantly the inhibitory action of prostaglandin E_2 on [^{3}H]-(–)-NA overflow induced by transmural nerve stimulation (Table 2).

Figure 2 (typical of 3 experiments) illustrates the inhibitory effect of prostaglandin E_2 on overflow of [^{3}H]-(–)-NA induced by transmural nerve stimulation, before and after administration of the α-adrenoceptor blocking agent, phenoxybenzamine (PB). PB (3 μM)

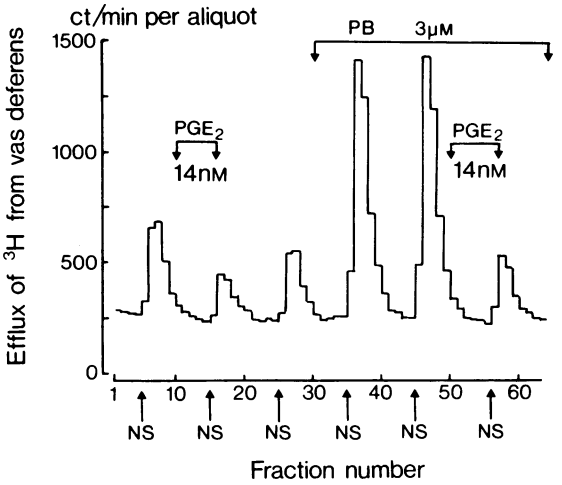

Figure 2 Continuously superfused guinea-pig vas deferens, loaded with [^{3}H]-(–)-noradrenaline (NA). Effect of prostaglandin E_2 (PGE_2) on release of tracer by transmural nerve stimulation (NS) (5 Hz, 1 ms, 450 pulses) before and after phenoxybenzamine (PB) treatment. Fraction numbers = time in minutes.

markedly increased the overflow of [^{3}H]-(–)-NA induced by transmural nerve stimulation (5 Hz, 1 ms, 450 pulses), but it did not reduce the inhibitory effect of prostaglandin E_2. In fact, after PB the preparation proved even more sensitive to prostaglandin E_2, the calculated inhibition being 48% and 73%, respectively before and after PB. After treatment with desipramine (0.5 μM) and normetanephrine (1 μM), PB increased the stimulated overflow of [^{3}H]-(–)-NA by 209 ± 33% (mean ± s.e., $n=4$), indicating that the enhancement was not due to blockade of NA uptake mechanisms. In an additional 4 experiments, in which indomethacin (6 μM) was given in order to block local prostaglandin formation, prostaglandin E_2 remained more effective (15 ± 4%, mean ± s.e., $P<0.05$) in inhibiting evoked [^{3}H]-(–)-NA overflow after PB treatment.

Discussion

The present study showed that prolongation of the pulse duration or administration of TEA or rubidium ions significantly depressed the inhibitory effect of prostaglandin E_2 on [^{3}H]-(–)-NA release induced by transmural nerve stimulation in the guinea-pig vas deferens. In addition, these treatments significantly enhanced the evoked overflow of [^{3}H]-(–)-NA, as previously shown for TEA and rubidium in the cat spleen (Thoenen *et al.*, 1967; Kirpekar *et al.*, 1972). It appears likely that this enhancement actually means increased NA release per nerve impulse rather than altered disposition of released transmitter. Thus, the effect of TEA and rubidium remained after blockade of NA uptake mechanisms. Moreover, it has been shown that the tracer overflowing from the vas deferens in response to transmural nerve stimulation in the absence of drug treatment consists almost wholly of intact [^{3}H]-(–)-NA (Hedqvist, 1974b).

Considerable circumstantial evidence suggests that E prostaglandins inhibit adrenergic neurotransmission by reducing the influx of calcium necessary for the ultimate extrusion of NA into the junctional cleft. Thus, the inhibitory action of prostaglandin E varies inversely with the ambient calcium concentration (Hedqvist, 1970, 1973, 1974a; Stjärne, 1973a, b). On the other hand, NA release by tyramine, which is a calcium-independent process, is unaffected by prostaglandin E (Hedqvist, 1970). It is also worth noticing that the inhibitory action of prostaglandin E on NA release per nerve impulse is diminished by increasing the pulse frequency (Hedqvist, 1973; Stjärne, 1973c; Frame & Hedqvist, 1975), and shortening the pulse interval is considered to leave

Table 2 Superfused guinea-pig vasa deferentia, loaded with [^{3}H]-(–)-noradrenaline (NA) and subjected to transmural nerve stimulation (5 Hz, 1 ms, 450 pulses). Effect of increased pulse duration, administration of tetraethylammonium (TEA) (2 mM), or rubidium (6 mM) on inhibitory action of prostaglandin E_2 (PGE_2, 14 nM) on evoked [^{3}H]-(–)-NA release.

No. of expts	*Drug treatment*	*Pulse duration (ms)*	*Inhibition by PGE_2 (%)*	*Paired difference (%)*
8	0	0.5	61.2 ± 7.2	23.2 ± 4.7 $P<0.01$
	0	2.0	38.0 ± 6.6	
5	0	1.0	56.9 ± 5.0	15.7 ± 2.8 $P<0.01$
	Rubidium	1.0	41.2 ± 5.7	
5	0	1.0	52.4 ± 4.2	32.8 ± 6.3 $P<0.01$
	TEA	1.0	19.6 ± 2.4	

Mean values ± s.e. of 5 to 8 experiments.

more residual calcium at the active releasing sites in the axon (Katz & Miledi, 1968). In addition, prostaglandin E has been reported to inhibit the uptake of calcium into membranes, and also to facilitate its efflux from membranes (Ramwell & Shaw, 1970; Alm, Efendic & Löw, 1970; Kirtland & Baum, 1972; Carafoli & Crovetti, 1973).

Increased release of transmitter by prolonging the nerve action potential has been considered due to more calcium entering the axon (Katz & Miledi, 1967a, b; Hubbard, 1970). Evidently, TEA and rubidium ions also prolong the nerve action potential (Koketsu, 1958; Baker, Hodgkin & Shaw, 1962), and their enhancing effect on evoked NA release may likewise be explained in terms of an action on calcium influx.

Kirpekar *et al.* (1972) have, as an alternative, explained enhanced NA release by TEA and rubidium as a result of their capacity to inactivate potassium channels (*cf.* Katz & Miledi, 1969), assuming that the outward movement of potassium during the nerve action potential controls the release of NA by modulating the calcium entry into the axon. However, there seems to be little independent evidence to suggest that TEA and rubidium interact with prostaglandin E_2 at the level of the potassium channels. In the experiments of Kirpekar *et al.* (1972), TEA, 1 and 10 mM, were found to cause the same and maximal enhancement of NA release. Supposedly, this means complete inactivation of the potassium channels, as is the case in cholinergic nerves (Hille, 1967; Katz & Miledi, 1969). Yet, in the present experiments prostaglandin E_2 was able to cause a marked inhibition of evoked NA release in the presence of 2 mM TEA. It has also been shown that prostaglandin E_2 markedly inhibits the release of NA evoked by high potassium (Stjärne, 1973d). Therefore, the effect of TEA and rubidium, like that of increased pulse duration, on prostaglandin E_2-induced inhibition of NA release seems to be best explained in terms of an action on the calcium gates allowing more calcium to enter the axon.

Admittedly, increased pulse duration or administration of TEA or rubidium could counteract the inhibitory action of prostaglandin E_2 secondarily to the increased release of NA per nerve impulse. This possibility was ruled out by the finding that PB, which increased the overflow of NA as efficiently as any of the above mentioned treatments, failed to counteract the inhibitory effect of prostaglandin E_2 and rather increased it (cf. Hedqvist, 1974b). It may be noted that the enhancing effect of PB on evoked NA release remains after blockade of NA uptake mechanisms, and that it in all probability represents increased NA release per nerve impulse by blockade of prejunctional α-adrenoceptors (Farnebo & Malmfors, 1971; Enero, Langer, Rothlin & Stefano, 1972; Starke, 1972; Hedqvist, 1973, Stjärne, 1973e).

The increased sensitivity to the inhibitory effect of prostaglandin E_2 after α-adrenoceptor blockade invites a comment. Stjärne (1973f) has proposed that prejunctional α-adrenoceptors control NA release by restriction of availability of external calcium for the secretory mechanism. If this is the case, inhibition of the α-adrenoceptors by PB would increase the availability of calcium, which, being reasonably equivalent to raising the ambient calcium concentration, would be expected to counteract the inhibitory action of prostaglandin E_2 rather than causing the observed opposite effect. Another aspect of this phenomenon is that prostaglandin synthesis inhibitors have been shown to increase the inhibitory effect of prostaglandin E_2 (Hedqvist, 1974b; Frame & Hedqvist, 1975), and PB can in fact inhibit prostaglandin release induced by sympathetic nerve stimulation (Davies, Horton & Withrington, 1967). However, such an action can not explain the increased sensitivity to prostaglandin E_2 in the presence of PB, since, as observed in the present study, it remained after blockade of local prostaglandin formation by indomethacin.

Irrespective of whether the ambient calcium concentration is augmented or the pulse frequency is increased, or, as in the present study, pulse duration is prolonged, or TEA or rubidium ions are administered, there is a decrease in the inhibitory action of prostaglandin E_2 on evoked NA release. One common denominator of all these different treatments seems to be increased availability of calcium at the active NA releasing sites in the axon, and, therefore, it appears highly likely that prostaglandin E_2 interferes with the NA release process actually by inhibiting calcium from reaching these active sites.

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