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DOPAMINE-INDUCED DEPRESSION OF ADRENERGIC NERVE-MEDIATED CONTRACTION OF SMOOTH MUSCLE

C. BELL & G. MATALANIS

Department of Physiology, University of Melbourne, Parkville, Victoria, 3052, Australia

1 Dopamine (0.25–1.0 μM) applied extraluminally depressed vasoconstrictor responses of the perfused rabbit ear artery to low frequency adrenergic nerve stimulation.

2 The depressant effect of dopamine was prevented in the presence of haloperidol (0.1 μM) but not in the presence of phentolamine (0.03–0.14 μM).

3 Extraluminal dopamine (1 μM) or intraluminal injection of dopamine in amounts up to 0.025 μmol had no dilator effect on arteries partially constricted by infusion of noradrenaline.

4 Dopamine also depressed contractile responses of guinea-pig vas deferens to low frequency adrenergic nerve stimulation. However, in this tissue the effect of dopamine was prevented by phentolamine but not affected by haloperidol.

5 In neither the rabbit ear artery nor the guinea-pig vas deferens did haloperidol increase the magnitude of responses to nerve stimulation or affect muscle sensitivity to noradrenaline.

6 We conclude that the adrenergic axons in the rabbit ear artery, but not the guinea-pig vas deferens, possess specific receptors for dopamine whose activation depresses axonal conduction or transmitter release. However, our results do not favour the view that these receptors are activated during normal transmission at physiological frequencies.

Introduction

It is well documented that α-adrenoceptor agonists depress and α-adrenoceptor antagonists increase the release of noradrenaline from adrenergic axons in response to nerve stimulation (Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Starke, 1972 and others), suggesting the existence of presynaptic α-adrenoceptors. From such data it has been postulated that these receptors may be involved in feedback modulation of adrenergic transmission (Farnebo & Hamberger, 1971; Starke, 1971; Kirpekar & Puig, 1971).

Recently it has been reported that low concentrations of dopamine also depress transmitter overflow from the rabbit ear artery (McCulloch, Rand & Story, 1973). This effect of dopamine is prevented by dopamine-receptor antagonists but not by α-adrenoceptor antagonists, suggesting the existence of presynaptic dopamine receptors (Hope, McCulloch, Rand & Story, 1975).

The present experiments have been performed in order to determine the effect of activation of these receptors on neurogenic vasoconstriction in the rabbit ear artery and to obtain evidence as to whether they are involved in normal transmission. Results obtained have been compared with those obtained with the guinea-pig vas deferens.

Methods

Young adult (2.0–2.5 kg) albino rabbits and adult (400–600 g) male guinea-pigs were killed by cervical dislocation. Rabbit ear arteries were prepared as described by de la Lande & Rand (1965) and perfused at a constant rate of 5 ml/min with McEwen's (1956) solution at 37°C. Vasoconstriction was monitored as changes in perfusion pressure via a pressure transducer coupled to the perfusion system distal to the pump.

Vasa deferentia were dissected free of the hypogastric ganglia but without removal of their mesenteric investment and mounted vertically in Huković's (1961) solution at 37°C with a resting longitudinal tension of 250 mg. Longitudinal contractions were monitored via an isotonic strain gauge.

Stimulation of intramural adrenergic nerves was carried out with Pt ring electrodes 10 mm in diameter and 5 mm apart, which were positioned around the proximal end of the artery and the urethral end of the vas deferens. A Grass S44 stimulator was used to deliver trains of 1 ms square wave pulses at 2 pulses/s and 70 volts. With the arteries, the stimulating trains were for 12 s every 200 seconds. With the vasa deferentia, the trains were for 5 s every 80 seconds. The contractile responses obtained were due solely to

activation of adrenergic axons and not to activation of the smooth muscle cells, as they were abolished by tetrodotoxin 1.6 μM or guanethidine sulphate 4 μM.

Drugs used were: dopamine hydrochloride (Sigma), haloperidol (Serenace, Searle), noradrenaline hydrochloride (Sigma) and phentolamine mesylate (Regitine, Ciba). Dopamine and noradrenaline were stored as 1 mg/ml solutions in 0.001 N HCl and diluted into 0.9% w/v NaCl solution (saline) on the day of use. All saline used contained 0.6 mM ascorbic acid. Haloperidol and phentolamine were diluted into saline on the day of use from the commercially available ampoules.

Differences of means were assessed using a paired Student's *t* test.

Results

Rabbit ear artery

Nerve stimulation produced increases in perfusion pressure which varied from 20 to 250 mmHg in different preparations. The mean ± s.e. mean for all preparations used was 97 ± 16.3 mmHg ($n = 14$) (1 mmHg = 1.333 mbar).

When dopamine was added to the bathing fluid surrounding the artery to produce a final concentration of 0.25–1.0 μM, the amplitude of responses to nerve stimulation was decreased (Table 1, Figure 1). This depression was rapidly reversed by washing the bath free of dopamine and was reproducible. The maximum depressant effect was observed in the presence of 1.0 μM dopamine. Concentrations greater than this caused direct vasoconstriction which prevented assessment of the amplitude of neurogenic responses.

In order to determine whether dopamine had any depressant effect on the vascular smooth muscle, partial constriction was maintained in three preparations by intraluminal infusion of noradrenaline at a final concentration of 0.5 μM. Under these conditions resting pressure was maintained at a steady level somewhat above 200 mmHg and intraluminal injections of acetylcholine (0.1–0.25 nmol) produced large falls in perfusion pressure, reflecting vasodilatation. In contrast, intraluminal injections of dopamine in doses from 0.025 nmol to 0.025 μmol had no effect on perfusion pressure. Doses of 0.05 μmol produced vasoconstriction. Addition to the extraluminal bathing fluid of 1 μM dopamine similarly had no dilator effect.

In nine experiments the dopamine-receptor antagonist haloperidol was added to the extraluminal fluid in a final concentration of 0.1 μM after reproducible depressant responses of dopamine on nerve-mediated vasoconstriction had been obtained. In the presence of haloperidol the effect of dopamine was greatly reduced (Table 1). This antagonist effect persisted for up to 30 min after washing the bath free of haloperidol. Haloperidol had no appreciable or consistent effect itself on the amplitude of nerve-mediated vasoconstriction or on constrictor responses to intraluminal injections of 0.1 nmol noradrenaline (Table 2).

In six further experiments the depressant effect of dopamine was tested after addition to the extraluminal bathing fluid of phentolamine in final concentrations of 0.03 μM (three experiments) or 0.14 μM (three experiments). These concentrations of phentolamine produced some (up to 30%) reduction in the amplitude of responses to nerve stimulation and somewhat greater reduction (up to 60%) in the amplitude of similar sized responses to extraluminal addition of 0.05–0.25 μM noradrenaline. In contrast

Table 1 Depression by dopamine of adrenergic nerve-mediated contractions of rabbit ear artery and guinea-pig vas deferens and the effect on this of haloperidol and phentolamine. The magnitude of the effect of dopamine is expressed in terms of percentage reduction of control response

			Effect of dopamine		
Tissue	*Dopamine (μM)*	*Control ($n = 14$)*	*Haloperidol* 0.1 μM ($n = 9$)	*Phentolamine* 0.03 μM ($n = 5$)	0.14 μM ($n = 3$)
Rabbit ear artery	0.25	30 ± 7%	8 ± 3%*	22 ± 3%	15 ± 4%
	1.0	41 ± 6%	12 ± 5%**	41 ± 4%	38 ± 2%
		Control ($n = 14$)	*Phentolamine* 0.3 μM ($n = 5$)	*Haloperidol* 0.1 μM ($n = 2$)	0.19 μM ($n = 3$)
Guinea-pig vas deferens	1.0	21 ± 5%	2 ± 2%*	15, 30%	42 ± 14%
	5.0	40 ± 6%	20 ± 4%	34, 65%	73 ± 8%

* Different from control values for same tissues ($P < 0.05$); ** different from control value for same tissues ($P < 0.02$).

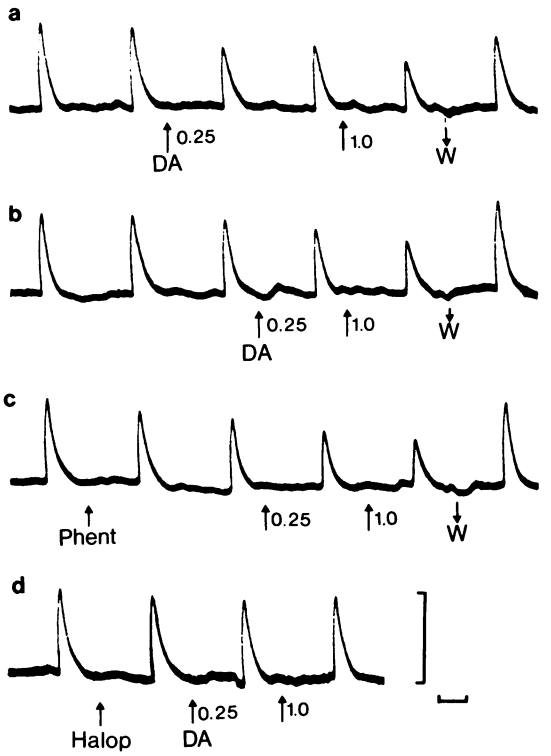

Figure 1 Constrictor responses of a rabbit ear artery perfused with physiological saline at constant rate to stimulation of the periarterial nerve supply (2 Hz, 12 s every 200 seconds). The panels are consecutive and run from above downwards. Addition to the extraluminal bathing fluid of dopamine (DA) at final concentrations of 0.25 μM or 1.0 μM produced dose-dependent depression of the responses which was quickly reversed after the bath was washed free of dopamine (W) and was reproducible. Phentolamine (Phent) 0.14 μM produced slight postsynaptic α-adrenoceptor blockade but did not affect the response to dopamine. By contrast, haloperidol (Halop) 0.1 μM completely prevented the effect of dopamine. Calibrations: 50 mmHg and 1 minute.

phentolamine had no antagonist action towards the depressant effects of either dose of dopamine used (Table 1). Higher concentrations of phentolamine could not be used because they almost completely abolished control responses to nerve stimulation.

Guinea-pig vas deferens

Responses to nerve stimulation ranged from 50–400 mg and had a mean value of 234 ± 30.6 mg ($n = 14$). Addition of dopamine in final concentrations between 1 μM and 5 μM to the bathing fluid produced dose-dependent depression of the amplitude of these responses (Table 1) which was readily reversed by washing the bath and was reproducible. Concentrations of dopamine greater than 5 μM caused direct tissue contraction which prevented assessment of the amplitude of neurogenic responses.

In three tissues the depressant effect of dopamine was retested following addition to the bath of haloperidol in a final concentration of 0.1 μM (two experiments) or 0.19 μM (three experiments). Under these conditions the effect of dopamine was enhanced (Table 1). In five further experiments, phentolamine (0.27 μM) greatly reduced the effect of dopamine (Table 1) while having no effect on responses to nerve stimulation itself.

Haloperidol had no effect on the amplitudes of responses to either nerve stimulation or exogenous noradrenaline (10 μM applied for 30 s every 150 s) (Table 2).

Discussion

We have demonstrated that depression of vasoconstrictor responses to adrenergic nerve stimulation of the rabbit ear artery by exogenous dopamine accompanies the depression of tritiated noradrenaline overflow reported by McCulloch *et al.* (1973). As the effect of dopamine was antagonized by the dopamine-receptor antagonist haloperidol at a concentration that lacked α-adrenoceptor blocking activity, but was not antagonized by phentolamine at a concentration sufficient to exert some post-synaptic α-adrenoceptor antagonism, it can be attributed to an action at specific dopamine receptors.

Inhibition of vasoconstrictor responses could occur if dopamine activated inhibitory receptors on the vascular muscle cells. However, this does not seem to be a possible explanation of the present results, as we could not demonstrate any direct relaxant effect of dopamine in the ear artery. It is likely, therefore, that the depressant effect of dopamine on neurogenic vasoconstriction is due to a presynaptic action on axonal conduction or transmitter release.

There is some evidence to suggest that the rabbit ear artery may possess a dilator nervous supply in addition to the adrenergic vasoconstrictor innervation (Holton & Perry, 1951; Kalsner, 1974). The transmitter involved in the mediation of this dilator response is unknown, but from published data seems unlikely to be acetylcholine, histamine, an adenyl nucleotide or a prostaglandin. Our demonstration of the absence of dilator receptors for dopamine on the muscle cells of the artery make dopamine also an unlikely candidate for a role in this response.

We obtained no evidence to support the presence of

Table 2 Effect of haloperidol on excitatory responses of rabbit ear artery and guinea-pig vas deferens to adrenergic nerve stimulation (2 Hz) and to applied noradrenaline (artery, 0.1 nmol intraluminally; vas deferens 10 μM).

Tissue	Haloperidol (μM)	Nerve stimulation Control	Nerve stimulation Haloperidol	Noradrenaline Control	Noradrenaline Haloperidol
Rabbit ear artery	0.1	99 ± 23 mmHg ($n=9$)	88 ± 19 mmHg	67, 73 mmHg ($n=2$)	67, 87 mmHg
Guinea-pig vas deferens	0.1	168, 441 mg ($n=2$)	169, 397 mg	–	–
	0.19	273 ± 64 mg ($n=6$)	290 ± 70 mg	70, 76 mg ($n=2$)	64, 73 mg

presynaptic dopamine-receptors in the guinea-pig vas deferens. In this tissue the depressant action of dopamine on nerve-mediated responses appeared to be solely through activation of α-adrenoceptors, as it was antagonized by phentolamine but unaffected by haloperidol. This difference could be due to species variation. Alternatively it could be related to the differing dimensions of the neuromuscular junctions in the two tissues. In the vas deferens, axons which lie within 200 Å of a muscle cell are common (Merillees, Burnstock & Holman, 1963), while in arteries a minimum axon-muscle separation of at least 800–1000 Å is usual (Bell, 1969; Burnstock, 1970). If the presynaptic amine receptors were to have any physiological function then their distribution might be expected to vary between junctions with different dimensions and therefore different extracellular transmitter kinetics. One piece of evidence against such a postulate is a recent report that inhibition of nerve-mediated contractions of rat vas deferens is antagonized by dopamine-receptor antagonists (Tayo, 1977). As the neuromuscular organization of the rat vas deferens more closely resembles that of the guinea-pig vas deferens than that of arteries (Richardson, 1962; Taxi, 1965), this result would tend to favour species variation as the basis for the difference between tissues seen in our experiments.

Although haloperidol antagonized the depressant effect of dopamine on neurogenic responses in the ear artery, it did not increase the amplitude of the nerve-mediated responses themselves in either this tissue or the guinea-pig vas deferens. This result does not support the theory that presynaptic depression by endogenous dopamine modulates adrenergic transmission at physiological firing frequencies.

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