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EFFECTS OF DIPYRIDAMOLE ON NEUROGENIC AND NON-NEUROGENIC DILATOR RESPONSES OF ARTERIAL SMOOTH MUSCLE

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1 In the isolated, perfused parametrial artery of the guinea-pig dipyridamole (10-100 ng/ml) potentiated dilator responses to adenosine 5′-triphosphate (ATP), glyceryl trinitrate and non-cholinergic, non-adrenergic inhibitory nerve stimulation to similar extents. In contrast dilator responses to acetylcholine were generally unaffected or reduced.

2 These results support previous electro-physiological evidence that in this preparation cholinergic vasodilatation is mediated by a cellular mechanism different from that involved in the response to non-cholinergic dilator nervous activation.

3 The results also suggest that potentiation of a neurally-mediated inhibitory response of smooth muscle by dipyridamole does not necessarily implicate ATP as the transmitter involved in this response.

Introduction

Following blockade of the adrenergic vasomotor nerves, the parametrial artery of the guinea-pig responds to perivascular electrical stimulation with two types of neurogenic dilatation. One of these is due to stimulation of cholinergic dilator nerves, and is observed only under conditions of high circulating oestrogen levels, due to the insensitivity of the muscle to acetylcholine at other times (Bell, 1968, 1973). The other response is present in a proportion of tissues examined irrespective of hormonal conditions and is not due to release of acetylcholine or a catecholamine (Bell, 1968).

The existence of non-cholinergic, non-adrenergic inhibitory nerve fibres has been demonstrated in a variety of vascular and non-vascular smooth muscle tissues (see Campbell, 1970; Burnstock, 1972), and recently biochemical and pharmacological evidence has been advanced to suggest that the transmitter released from some or all of these nerves is adenosine 5′-triphosphate (ATP) or a related purine nucleotide. On this basis the nerves have been tentatively called 'purinergic' (Burnstock, 1971). The pharmacological evidence to support this hypothesis consists in the demonstration that relaxations of intestinal muscle in response to 'purinergic' nerve stimulation and to applied ATP are enhanced in the presence of substances which inhibit the cellular uptake of adenosine, while responses to catecholamines are not (Satchell, Lynch, Bourke & Burnstock, 1972). In the present study the effect of the adenosine uptake inhibitor, dipyridamole, on dilator responses of the parametrial artery has been examined.

Methods

Isolated preparations of parametrial arteries from virgin guinea-pigs were cannulated as described previously (Bell, 1968) and perfused at a constant rate of 6 ml/min with McEwan's solution (McEwan, 1956). Changes in vessel resistance were recorded as changes in back pressure with a standard blood pressure transducer and chart recorder.

Perivascular nerve stimulation was elicited with platinum ring electrodes placed around the proximal end of the artery, and 1 ms square wave pulses were delivered at rates of 2-20 pulses/s and supramaximal voltage from a Grass S5 stimulator. Responses to adrenergic nerve stimulation were prevented by inclusion in the perfusion fluid of guanethidine sulphate (Ismelin, Ciba; 1 μg/ml) or phentolamine mesylate (Regitine, Ciba; 2.5 μg/ml) and the vessels were maintained in a partially constricted condition by the intraluminal infusion of noradrenaline (0.5 μg/ml) or, when phentolamine was present, of vasopressin (0.05 u/ml).

Dilator agents used were acetylcholine perchlorate (BDH), adenosine 5′-triphosphate (Merck) and glyceryl trinitrate (Anginine,

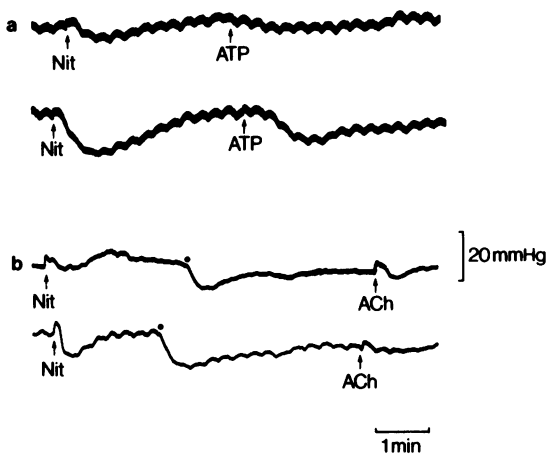

Fig. 1 Perfusion pressure recordings from two preparations (a and b) of isolated, perfused parametrial arteries, showing dilator responses to standard intraluminal doses of glyceryl trinitrate (Nit), ATP and acetylcholine (ACh), and to perivascular nerve stimulation (black dots: 5 pulses/s, 10 s), in the presence of guanethidine (1 μg/ml) and noradrenaline (0.5 μg/ml). Between the upper and lower traces in each panel an intraluminal infusion of dipyridamole (50 ng/ml) was started. Note that dipyridamole increased the amplitude of dilator responses to all stimuli with the exception of acetylcholine. Preparation (b) was taken from an animal pretreated for 3 days with oestradiol (1 mg/kg).

Burroughs-Wellcome). These were dissolved in McEwan's solution and injected intraluminally in volumes of not more than 0.2 ml.

Where observations on dilator responses to acetylcholine were required the arteries were sensitized to acetylcholine by pretreatment of guinea-pigs for several days before the experiment with oestradiol valerate (Primogyn; 0.5-1 mg/day) intramuscularly (Bell, 1973).

Segments of guinea-pig ileum were mounted in Krebs solution and contractile responses to applied acetylcholine were recorded with isometric tension transducers using a contact time of 15 s and a cycle time of 90 seconds.

Results

Under the experimental conditions employed intraluminal infusion of either noradrenaline (0.5 μg/ml) or vasopressin (0.05 u/ml) produced a stable resting perfusion pressure of about 100 mmHg (1 mmHg = 1.333 mbar), which was maintained for several hours.

Intraluminal injection of glyceryl trinitrate (50-500 ng) and ATP (5-100 μg) produced falls in perfusion pressure which were reproducible at a given dose in any particular preparation and were dose-dependent in magnitude. In arteries from oestrogen-pretreated animals acetylcholine (50-500 ng) also produced dilatation. In about half of the arteries examined perivascular electrical stimulation at frequencies of 2-20 pulses/s produced a dilatation of similar appearance to those obtained using agonist injections (Figure 1). In each experiment the stimulation frequency and the doses of agonists used were adjusted so as to produce dilator responses which were submaximal in amplitude.

Intraluminal infusion of dipyridamole (10-100 ng/ml) usually had no effect of itself on perfusion pressure. However, in a few preparations the highest concentration used produced a sustained fall in perfusion pressure which was only slowly reversed on cessation of infusion. In the presence of dipyridamole, dilator responses to glyceryl trinitrate, ATP and perivascular nerve stimulation were all increased in magnitude. The degree of potentiation obtained varied considerably between different experiments, and to a lesser extent also between the different stimuli in

Table 1 Potentiation of dilator responses of guinea-pig isolated, perfused parametrial arteries to adenosine 5'-triphosphate (ATP), glyceryl trinitrate, perivascular nerve stimulation and acetylcholine (ACh) in the presence of dipyridamole, expressed in terms of percentage of control responses

		Potentiation		
n	*Stimulus*	*Range*	*Mean ± s.e. mean*	*Significance†*
17	ATP	150-500	305 ± 28	$P < 0.001$
23	Glyceryl trinitrate	120-700	270 ± 28	$P < 0.001$
9	Nerve stim.	120-600	276 ± 50	$P < 0.001$
14	ACh*	−100**-200	93 ± 17	NS

* Oestrogen pre-treated animals. ** Response abolished. † Student's *t* test. Difference of means from control values.

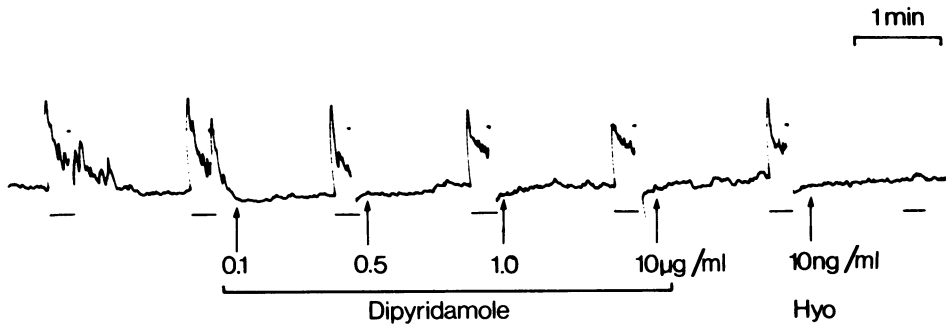

Fig. 2 Contractile responses of a segment of guinea-pig ileum to applied acetylcholine (2.5 ng/ml). The contact time is shown by the black bars. Note that dipyridamole in concentrations up to 10 μg/ml did not appreciably affect responses to acetylcholine, but that hyoscine (Hyo 10 ng/ml) produced complete blockade within one minute.

the same experiment. However, the mean potentiation from a number of experiments was between 270% and 306% for all three stimuli (Fig. 1 Table 1). Dilator responses to acetylcholine were much less affected by dipyridamole, being somewhat (30-100%) potentiated in 4 experiments, not affected in 4 and abolished in 6 experiments (Fig. 1, Table 1).

In those experiments in which oestrogen pre-treatment was not performed, the responses to perivascular nerve stimulation could be considered as purely non-cholinergic. It has been shown previously that such responses are affected neither by hyoscine nor physostigmine, although they are abolished in the presence of local anaesthetics (Bell, 1968). In the tissues from oestrogen-sensitized animals, it is possible that the stimulation-induced dilatations were due in part to acetylcholine released from nerve endings but there was no obvious difference between the two groups in the degree of potentiation produced by dipyridamole.

The degree of potentiation of responses did not appear to be much increased if a low concentration of dipyridamole was raised over the concentration range tested. On cessation of dipyridamole infusion all responses returned partially or completely to their control values over the ensuing 30-60 minutes.

In view of the lack of potentiation of responses to acetylcholine in comparison to the other dilator stimuli by dipyridamole, this compound was tested on the guinea-pig ileum for anticholinergic potency. It was found in two preparations of the ileum that contractions in response to acetylcholine (2.5 ng/ml) were little affected by dipyridamole in concentrations up to 10 μg/ml. In contrast, hyoscine in a concentration of 10 ng/ml caused complete abolition of responses (Figure 2).

Discussion

The non-cholinergic, non-adrenergic dilatation which can be elicited by perivascular nerve stimulation in the guinea-pig parametrial artery is associated with hyperpolarization of the arterial smooth muscle cells (Bell, 1969). In contrast, the cholinergic dilatation which can be elicited under conditions of oestrogen–induced sensitization is unaccompanied by changes in muscle membrane potential and has been suggested to be mediated through inhibition of initiation or of propagation of muscle action potentials (Bell, 1969). The present investigation has demonstrated that the non-cholinergic dilator response to nerve stimulation is potentiated by dipyridamole, as are responses to glyceryl trinitrate and ATP, while that to acetylcholine is unaffected or antagonized. The lack of potentiation of acetylcholine cannot be attributed to an anticholinergic activity of dipyridamole, as this appears to be negligible, and so supports the concept that acetylcholine acts to produce dilatation in this system by a cellular mechanism which is different from that involved in the dilator responses to non-cholinergic stimuli.

Satchell *et al.* (1972) observed that in the guinea-pig taenia coli relaxation in response both to applied ATP and to non-cholinergic, non-adrenergic nerve stimulation was potentiated by low concentrations of dipyridamole, while relaxation to catecholamines and sympathetic nerve stimulation was unaffected or reduced. This specificity of action of dipyridamole was used as supportive evidence for ATP being the transmitter released by the non-adrenergic inhibitory nerves. The mechanism underlying the dipyridamole-induced potentiation was suggested to be by blockade of neural uptake of the adenosine produced by degradation of neurally released ATP.

Inhibition of tissue uptake mechanisms for adenosine by dipyridamole has previously been shown in a variety of situations (Koss, Beisenherz & Maerkisch, 1962; Pfleger, Volkmer & Kolassa, 1969; Kolassa, Pfleger & Rummel, 1970; Vigdahl, Mongin & Marquis, 1971).

The present findings, however, have demonstrated that in the guinea-pig parametrial artery dipyridamole causes potentiation to a similar degree of inhibitory responses not only to ATP and to non-cholinergic, non-adrenergic nerve stimulation but also to glyceryl trinitrate. Under these circumstances it is difficult to invoke a pre-synaptic site of action for dipyridamole. Rather it appears that dipyridamole is potentiating muscular relaxation at a post-synaptic level, probably by increasing intracellular cyclic AMP levels (Triner, Vulliemoz, Schwartz & Nahas, 1970; Robison, Butcher & Sutherland, 1971). This could occur through inhibition of phosphodiesterase (Senft, 1968; Triner, *et al.*, 1970; Vigdahl *et al.*, 1971; Triner, Vulliemoz, Verosky, Habif & Nahas, 1972), although the ability of various drugs to potentiate inhibitory responses of smooth muscle to adenine compounds does not appear to correlate well with their potency as phosphodiesterase inhibitors (Satchell *et al.*, 1972; Hamilton, 1972). An alternative mechanism of action would be by alteration of the metabolism or sequestration of muscle cell adenosine.

The specificity of action of dipyridamole noted by Satchell and his co-workers in the taenia coli may reflect some difference between the postsynaptic mechanism of action of catecholamines and that of the other inhibitory stimuli used. It is here reported that vasodilatation associated with muscle membrane hyperpolarization is potentiated by dipyridamole while vasodilatation independent of membrane potential change is not. In this connection, it may be noted that relaxation of the taenia coli due to stimulation of non-cholinergic, non-adrenergic inhibitory nerves is associated with considerable hyperpolarization of the muscle cells (Bennett, Burnstock & Holman, 1966a). The similar degree of mechanical relaxation which can be produced by sympathetic nerve stimulation or by applied catecholamines, however, (Burnstock, Campbell & Rand, 1966; Satchell *et al.*, 1972) is accompanied by cessation of action potential firing but little change in muscle membrane potential (Bülbring & Kuriyama, 1963; Bennett, Burnstock & Holman, 1966b).

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