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The effect of 1,1-dimethyl-4-phenyl-piperazinium on the response of mesenteric arteries to sympathetic nerve stimulation

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The effect of 1,1-dimethyl-4-phenylpiperazinium (DMPP) on the response to sympathetic nerve stimulation of rat mesenteric arteries perfused with Tyrode solution at a constant flow has been studied. DMPP (0·3 μg/ml) infused for 3 min enhanced the vasoconstriction caused by stimulation. Infusion of the same concentration for 16–40 min greatly reduced the response to nerve stimulation but did not affect the vasoconstrictor response to injected noradrenaline. The blockade of the response to nerve stimulation produced by DMPP was overcome either by adding (+)-amphetamine to the perfusion fluid or by raising the calcium concentration. Neither effect of DMPP was altered by the infusion of atropine. These effects of DMPP were similar to those seen when acetylcholine was added to the perfusion fluid except that the effects of acetylcholine were diminished or abolished by a concentration of atropine much higher than that of acetylcholine. It is concluded that the receptors at the adrenergic nerve terminals are partly muscarinic and partly nicotinic.

1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP) which acts as a ganglionic stimulant, or as a ganglion blocking drug (Chen, Portman & Wickel, 1951; Page & McCubbin, 1953; Chen & Portman, 1954; Leach, 1957; Ling, 1959; Brownlee & Johnson, 1963) has also been shown to produce an increase in the rate and force of the heart by liberating catecholamines directly from the postganglionic nerve endings (Lindmar & Muscholl, 1961; Bhagat, 1966). Bentley (1962) and Wilson (1962) have shown on the other hand that DMPP inhibits the effect of stimulation of periarterial sympathetic nerves of the rabbit and guinea-pig intestine respectively. Birmingham & Wilson (1965) found that the inhibitory effect of DMPP on the intestine to sympathetic nerve stimulation had features in common with the blocking actions of guanethidine and bretylium. A similar type of blockade was observed by Rand & Wilson (1967) in the rabbit ear vessels.

Recent observations on the rat mesenteric vessels perfused with Tyrode solution have shown (Malik & Ling, 1969) that the vasoconstriction caused by stimulation of postganglionic fibres is increased when acetylcholine is infused in low concentration for a short period (15 s). The same concentration infused for a longer period (15 min) causes marked reduction or blockade of the response to sympathetic nerve stimulation. This blockade can be abolished by a concentration of atropine 20 times greater than that of acetylcholine. The blockade is also reversed by raising the calcium concentration of the perfusion fluid or by simultaneous infusion of (+)-amphetamine. In these last two respects the block by acetylcholine resembles the blockade caused by guanethidine.

The present study describes the effects of DMPP on the response of perfused mesenteric arteries of the rat to sympathetic nerve stimulation.

EXPERIMENTAL

Female albino rats, 250–300 g, were anaesthetized with ether, the abdomen opened and the superior mesenteric artery cannulated and isolated with its small resistance vessels (McGregor, 1965). A Harvard peristaltic pump (Harvard Apparatus Co., Model 1210) was used to perfuse the arteries at a constant flow of 25 ml/min with Tyrode solution of the following composition in mM: NaCl, 136; KCl, 2·7; $CaCl_2$, 1·8; $MgCl_2$, 1:1; $NaHCO_3$, 12; $NaHPO_4$, 0·42 and dextrose, 5·6. The solution was aerated with a mixture of 5% carbon dioxide in oxygen and was maintained at 22°. In some experiments the temperature of the perfusion fluid was 37°. Changes in perfusion pressure were recorded manometrically from the cannulated artery using a frontal writing lever on a kymograph. Before cannulation when the pump was operating and the flow was 25 ml/min the pressure was 60 mm Hg. During perfusion the pressure increased to 85 mm Hg. Thus the average basal pressure during an experiment was 25 mm Hg. Since the mesenteric vessels were cut along the intestine, this pressure was due to the resistance of the arterioles.

Injections of noradrenaline were made directly into the cannula leading to the superior mesenteric artery by means of a Palmer pump (F-30).

The perivascular nerves were stimulated for 20–25 s every 4 min interval with a Grass stimulator (Grass Instrument Co., Model 4C) using biphasic rectangular pulses (20 V; 1 ms; at 7/s).

The vasoconstrictor responses to both sympathetic nerve stimulation and injected noradrenaline in all experiments were submaximal.

The drugs were: 1,1-dimethyl-4-phenylpiperazinium iodide, (−)-noradrenaline bitartrate monohydrate and atropine sulphate (K & K Laboratories). Cocaine hydrochloride was generously supplied by British Drug Houses (Toronto), guanethidine sulphate by CIBA (Dorval) and (+)-amphetamine sulphate by Smith, Kline and French (Montreal).

The drugs were dissolved in normal saline just before use and added in the perfusion solution in a volume of not more than 0·5 ml/litre to obtain the final concentration. The final concentration is expressed as that of the salts.

RESULTS

The preparation of the rat mesenteric arteries was stable for periods of more than 4 h. Nerve stimulation and injected noradrenaline produced uniform responses during this time. DMPP in concentrations of less than 2 μg/ml in the perfusion fluid failed to produce any change in the basal perfusion pressure while higher concentrations produced a slight increase.

Effect of DMPP *on the response to sympathetic nerve stimulation*

When DMPP was infused in a concentration of 0·3 μg/ml for 3 min, the vasoconstriction produced by sympathetic postganglionic nerve stimulation was increased. Such an increase is seen in Fig. 1, where it lasted for 56 min. The maximum increase was 60% except in one experiment in which an increase of 170% lasting also 56 min was observed. An increase in response to nerve stimulation after the 3 min infusion of DMPP was observed at 22° and also at 37° in six preparations.

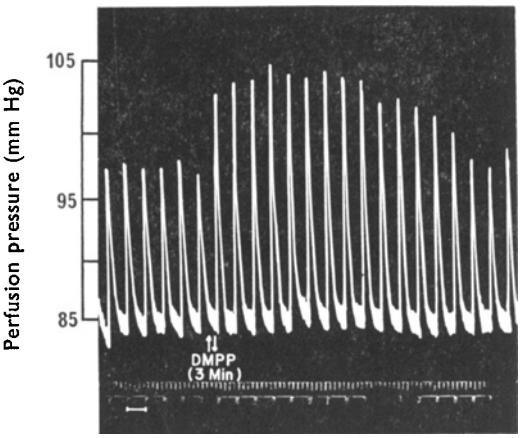

FIG. 1. The potentiating effect of DMPP on the perfusion pressure response of mesenteric arteries of rat to sympathetic nerve stimulation. The mesenteric arteries were perfused with Tyrode solution at a rate of 25 ml/min at 22°. The perivascular nerves were stimulated using biphasic pulses (20 V; 1 ms; at 7/s) every 4 min for 20 s. After 6 control responses were recorded, the infusion of DMPP (0·3 μg/ml) for 3 min markedly increased the response to nerve stimulation. The increase in response was present for about 56 min. Time Marker = 1 min interval.

However, when DMPP in the same concentration (0·3 μg/ml) was infused for 16, 20 or 40 min the initial response was increased but this was followed by inhibition which progressed until the responses to stimulation were abolished. The inhibitory effect of DMPP in 0·3 μg/ml concentration for 32 min is shown in Fig. 2, where the period of infusion was not long enough for complete inhibition. The removal of DMPP from the perfusion fluid only partially restored the responses. Preparations perfused at 37° gave similar results to those at 22° except that DMPP was more active in reducing the response at the lower temperature.

Effect of DMPP *on the response to injected noradrenaline*

To find out whether the inhibitory action of DMPP was due to a diminution in the amount of noradrenaline released or to a failure of the amine to cause vasoconstriction in the presence of DMPP, experiments were made in which the responses to submaximal nerve stimulation and then to submaximal amounts of injected noradrenaline were recorded. Care was taken to use an amount of noradrenaline (3–5 μg) which produced a similar response to that produced by stimulation. The example given in Fig. 2 shows that neither the beginning of the infusion of DMPP, nor the end of the infusion affected the response to injected noradrenaline though the infusion greatly diminished the response to nerve stimulation.

Effect of (+)*-amphetamine*

When (+)-amphetamine was added to the fluid perfusing the mesenteric arteries in a concentration of 0·2 μg/ml it caused a large increase in the response to stimulation of the sympathetic fibres; in one experiment the increase was 75%. Since (+)-amphetamine has been shown to reverse the blockade of responses to sympathetic impulses produced by guanethidine and bretylium (Day, 1962), and also blockade of responses to sympathetic impulses produced by DMPP in the rabbit intestine (Birmingham & Wilson, 1965), experiments were made to see if (+)-amphetamine

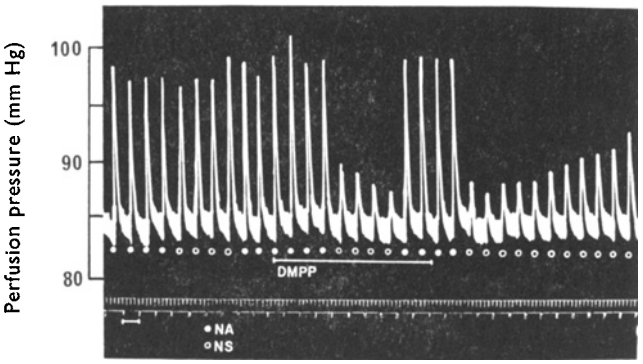

FIG. 2. Comparison of the inhibitory effect of DMPP on the responses to submaximal sympathetic nerve stimulation and to submaximal amounts of injected noradrenaline. Recording as in Fig. 1. The responses to injected noradrenaline (NA—3μg) were obtained by injecting it directly in to the cannula leading to superior mesenteric artery. Responses to sympathetic nerve stimulation (NS) were greatly inhibited, while those to injected NA remained unaffected during the infusion of DMPP (0·3 μg/ml) for 42 min. When the drug-free Tyrode solution was resumed the responses to nerve stimulation were restored partially.

would also reverse the blockade of the vasoconstrictor responses produced by DMPP in the mesenteric arteries. Fig. 3 A shows that simultaneous infusion of (+)-amphetamine (0·2μg/ml) reversed the blockade of the response to nerve stimulation produced by DMPP (2μg/ml); this effect was observed on 6 preparations.

Effect of increased calcium concentration

Since Burn & Welsh (1967) showed that increased calcium (Ca^{++}) concentration reversed the blockade of sympathetic impulses produced by guanethidine, it was of interest to see whether increased Ca^{++} concentration would also reverse the blockade of the response to nerve stimulation produced by DMPP. When Ca^{++} concentration of the perfusion fluid was raised to 4 times the normal value (i.e. from 1·8 to 7·2 mM by adding $CaCl_2$ to the perfusion fluid) the blockade produced by DMPP was partially reversed but reappeared again during the infusion of DMPP as shown in Fig. 3 B. Similar observations were made in 11 experiments.

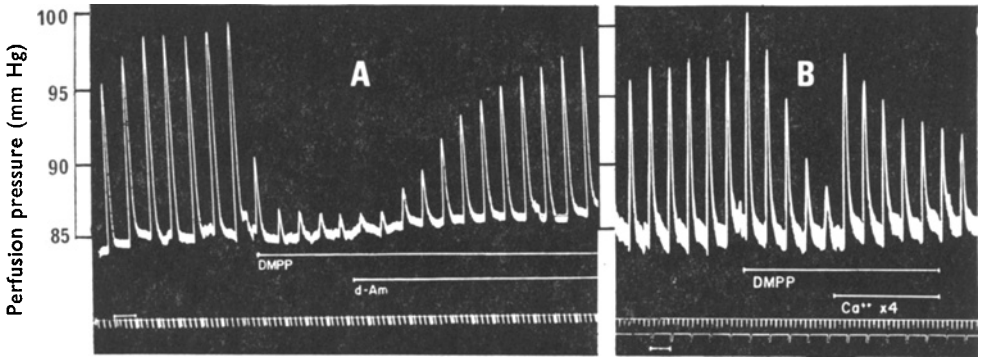

FIG. 3. A. Effect of (+)-amphetamine on the blocking action of DMPP. Recording as in Fig. 1. The responses to nerve stimulation were almost abolished when DMPP was infused in concentrations of 2 μg/ml of the perfusion fluid. (+)-Amphetamine (d-Am 0·2 μg/ml) reversed the blockade produced by DMPP.

B. Effect of increased calcium concentration on DMPP-induced blockade. Recording as in Fig. 1. Responses to nerve stimulation were inhibited by DMPP (0·3 μg/ml). Raising the calcium (Ca^{++}) concentration in the perfusion fluid to 4 times the normal value (i.e. to 7·2 mM) reversed partially the blockade produced by DMPP but it reappeared again during the infusion of DMPP.

Effect of hexamethonium

To exclude the possibility that the blocking action of DMPP on the response of mesenteric arteries to sympathetic nerve stimulation might be due to its ganglion blocking effects, experiments were made with hexamethonium. Hexamethonium (0·5 μg/ml) neither affected the response, nor reversed the blocking action of DMPP on the response to sympathetic nerve stimulation (4 experiments).

Effect of cocaine

Cocaine has been shown to increase the effect of catecholamines by impairing their uptake in adrenergic nerve terminals (see Trendelenburg, 1966). Cocaine (0·1 μg/ml) exerted a transient antagonism to partial inhibition produced by infusing the DMPP (0·3 μg/ml) for 20 min, but failed to reverse the blockade produced by a more prolonged infusion of this or higher concentrations (2 μg/ml).

Effect of bretylium and guanethidine

The effect of bretylium (0·5 μg/ml) and guanethidine (0·5 μg/ml) was investigated on the response of mesenteric arteries to sympathetic nerve stimulation and injected noradrenaline. These agents blocked the response to nerve stimulation without affecting the response to injected noradrenaline. The blockade of response to nerve stimulation produced by guanethidine and bretylium was also reversed by (+)-amphetamine and by raising the concentration of Ca^{++} to 4 times (i.e. to 7·2 mM) the normal value, but was unaffected by hexamethonium or cocaine.

DISCUSSION

The observations which have been made with DMPP follow our earlier observations on acetylcholine (Malik & Ling, 1969). When the sympathetic fibres to the perfused mesenteric arteries of rat were stimulated, the addition of acetylcholine, 2 ng/ml, to the perfusion fluid had two different effects according to the length of time for which the addition was made. When the infusion was for 15 s only, the response to stimulation increased, in some cases being doubled. However, when the addition was made for a longer time, such as 15 min, the response to stimulation was reduced and even abolished. These observations supported the view that the postganglionic fibre first releases acetylcholine and that this in turn releases noradrenaline. It appears that when acetylcholine was infused for a short period of time its effect and that of the acetylcholine released by the sympathetic fibre were additive causing an increased response. However, when it was infused for a longer time (15 min) it would occupy all the receptors on which the acetylcholine released by nerve stimulation could act, and therefore the response to stimulation would be abolished. In connection with this abolition, the surprising observation was made that the responses returned when atropine was infused in a concentration of 100 ng/ml together with the acetylcholine (5 ng/ml). This suggested that the receptors on which the prolonged infusion of acetylcholine acted to cause block were muscarinic as Lindmar, Löffelholz & Muscholl (1968) have concluded.

To see if these receptors were in fact muscarinic, experiments were made with DMPP, which is known to act on nicotinic receptors. DMPP releases noradrenaline from postganglionic terminations in the heart, and has been shown to block sympathetic terminations (Bentley, 1962; Wilson, 1962) like acetylcholine (Brücke, 1935; Burn & Rand, 1960).

The results that have been described show that DMPP, when infused for short periods, also resembles acetylcholine in causing an increased response to sympathetic stimulation. They also show that DMPP, like acetylcholine, when infused for a longer time, blocks the response to sympathetic stimulation, and that the blockade can be removed either by raising the calcium concentration of the perfusing fluid, or by the simultaneous infusion of (+)-amphetamine. The blockade produced by DMPP, 0·3 μg/ml, was however unaffected by atropine, and in this respect differed from the blockade produced by acetylcholine. The conclusion can be drawn that the receptors concerned are partly muscarinic and partly nicotinic, like receptors on sympathetic ganglia. The work of Ambache, Perry & Robertson (1956) demonstrated that muscarine itself will stimulate sympathetic ganglia, and that this action is modified by atropine.

The blocking effect of prolonged infusion with either acetylcholine or DMPP can be explained not as an effect on inhibitory receptors, but as the inhibition which follows full occupation of receptors for stimulation. It has long been known, for example, that receptors for acetylcholine at the neuromuscular junction in skeletal muscle are blocked when the motor nerve is stimulated at high rates in the presence of an anticholinesterase. In this situation the concentration of acetylcholine rises to such a level that all the receptors are occupied causing stimulation of the nerve to be ineffective.

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