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MECHANISM OF THE LOCAL VASCULAR ACTIONS OF 1, 1-DIMETHYL-4-PHENYLPIPERAZINIUM (DMPP), A POTENT GANGLIONIC STIMULANT

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Recent reports from this laboratory demonstrated that 1, 1–dimethyl-4-phenylpiperazinium (DMPP), a potent ganglionic stimulant, produced a local vasodilator and a local vasoconstrictor effect when injected intra-arterially into the intact hind limb of the dog (Winbury, Wolf & Tabachnick, 1958; Winbury, 1958). Under normal conditions the dilator action predominated and resisted atropine or acute denervation of the limb; the vasoconstrictor action was observed only after ganglionic block with hexamethonium. Burn & Rand (1958), however, observed only a constrictor response in the isolated perfused hind limb of the dog, and Page & McCubbin (1953) reported that DMPP produced no effect in the same preparation.

Nicotine, another ganglionic stimulant, has a direct vasoconstrictor effect in the isolated rabbit ear and in the hind limb of the dog; these effects were blocked by hexamethonium or by pre-treatment with reserpine (Kottegoda, 1953; Ginzel & Kottegoda, 1953; Burn & Rand, 1958). The response was attributed to the release of norepinephrine from chromaffin tissue in or near the blood vessels. In fact, Burn & Rand (1958) demonstrated that the decline in the vasoconstrictor activity of nicotine following reserpine coincided with a decline in the norepinephrine content of the blood vessels. Hilton (1954), working with the hind limb of the cat, reported that nicotine can elicit both a vasodilator and a vasoconstrictor response in the muscle of the hind limb of the cat via axon reflexes in cholinergic or adrenergic fibres, respectively. Folkow (1955, 1956) questions the conclusions of Hilton (1954) and suggests that if such an axon reflex is present it differs from those previously described.

With this background to nicotine and DMPP we decided to analyse further the mechanisms of the local vascular actions of DMPP by use of various types of blocking agents and by chronic denervation. The experiments to be described suggest that the dilator and constrictor actions of DMPP in the

innervated limb are mediated by distinct nerve pathways, and that after chronic total denervation of the limb DMPP exerts a direct relaxant action on the smooth muscle of the vessel wall.

METHODS

These studies were carried out in 56 dogs, measuring the total blood flow in one or both hind limbs. The majority of animals were anaesthetized with sodium thiopentone, 20–30 mg/kg I.V. followed by α-chloralose, 60–80 mg/kg I.V. Some animals were anaesthetized with sodium pentobarbitone, 30 mg/kg I.V. Additional anaesthetic was used as required to maintain a steady level of blood flow.

The blood-flow rate (inflow) was recorded with a rotameter (Shipley & Wilson, 1951) connected between the central end of a cut femoral or carotid artery and the peripheral end of a cut femoral artery. Heparin, 1500 u./kg, was administered 5–10 min before cannulation. Pontamine Fast Pink BL, 10–25 ml. of a 5% solution, was used to enhance the anticoagulant action (Winbury, Michiels, Hambourger, Stockfisch & Cook, 1950). The pressure in the rotameter circuit was determined with a pressure transducer at the inflow tubing close to the inflow cannula and was recorded simultaneously with the blood-flow rate on a direct-writing electronic recorder. In a few experiments the blood flow to the muscle was measured separately (no skin) according to the procedure of Lanier, Green, Hardaway, Johnson & Donald (1953).

All intra-arterial injections were administered into the outflow tubing of the rotameter circuit (Winbury *et al.* 1950) close to the outflow cannula. Compounds were dissolved in NaCl solution 0·9% (w/v) and injection volumes were kept below 0·2 ml. Intravenous injections were given into a cannulated jugular vein.

In several animals one hind limb was totally denervated 7–12 days before the experiment by cutting the sciatic plexus and femoral nerve and stripping the adventitia from the femoral artery. When the flow studies were performed, the femoral artery in the denervated limb was cannulated distal to the operative site.

RESULTS

Intact innervation

Control responses to DMPP and nicotine. Intra-arterial injection of DMPP (100–300 μg total dose) usually resulted in a prompt increase in blood flow, similar to that observed after nicotine (20–200 μg) or acetylcholine (ACh, 0·1–1 μg) (Figs. 1–5). Similar dilator effects were noted in the few experiments on muscle blood flow. In a few animals a vasoconstrictor response to DMPP was observed at the start of the experiment but this changed to a dilator response within a short period of time. Constrictor responses were not observed with nicotine or ACh.

Effect of ganglionic blockade: reversal of DMPP and blockade of nicotine. After hexamethonium 2 mg/kg I.V. the dilator response to nicotine was blocked while the dilator response to DMPP was changed to a *constrictor* response, (Figs. 1, 4). The response to ACh was not affected by hexamethonium.

After prolonged periods of ganglionic blockade a diminished dilator response returned for both DMPP and nicotine. However, since the constrictor action of DMPP was still present, a two stage response resulted with DMPP, i.e. a slight transient dilatation followed by the more prolonged constriction. Additional doses of hexamethonium did not block this reduced dilator action

of nicotine or DMPP. In a few experiments atropine 1 mg/kg I.V. eliminated the reduced dilator response to nicotine but had no effect on the DMPP dilatation.

Effect of cholinergic blockade: elimination of ACh dilator response. Atropine 1 mg/kg I.V. completely blocked the dilator effect of ACh (Fig. 1). The effect of atropine on DMPP and nicotine was variable. In the majority of experiments atropine had little effect on the DMPP or nicotine dilator action; in a few cases, however, there was a slight reduction of the dilator action. In no case was the dilator response to nicotine or DMPP eliminated by atropine.

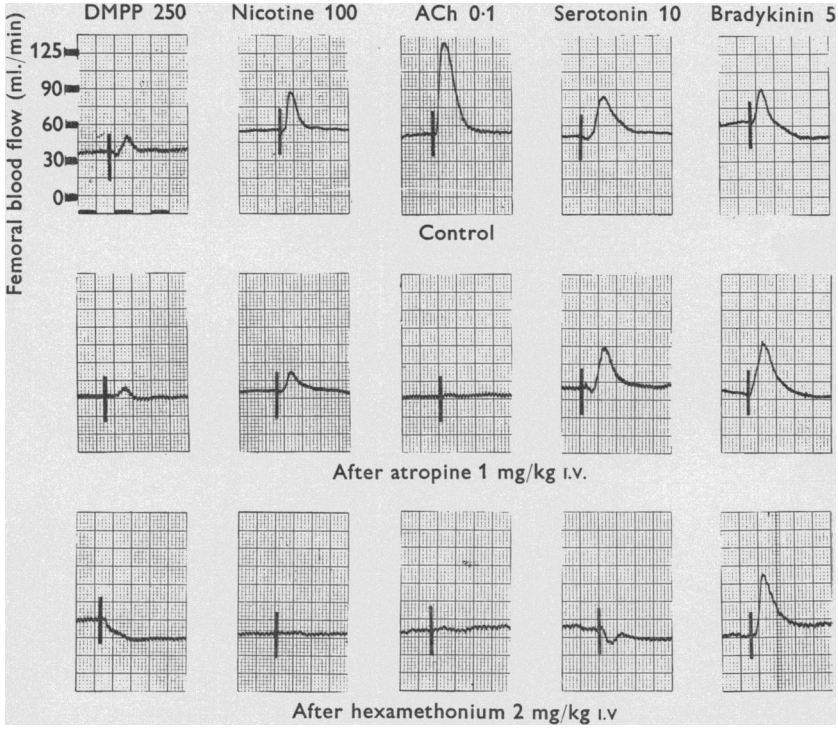

Fig. 1. Effect of atropine and hexamethonium on the vasodilator response to DMPP, nicotine, acetylcholine (ACh), serotonin, and bradykinin; doses in μg. Ordinate, changes in the rate of blood flow (ml./min) in the femoral artery of the dog. The heavy vertical bars indicate the points of injection; time marker 20 sec.

Effect of neostigmine on the dilator response. A dose of neostigmine 10 μg/kg I.V. markedly enhanced the dilator response to ACh but not that to DMPP or nicotine. Higher doses of neostigmine actually decreased the response to DMPP but not that to nicotine.

Effect of cocaine on the response to DMPP and nicotine. The blockade of the dilator response to nicotine and DMPP by hexamethonium suggested that

'ganglionic-like' stimulation (in the periphery) or some related phenomenon may be involved. Cocaine, which blocks nerve transmission (Goodman & Gilman, 1955), was employed further to evaluate the dilator mechanism.

Doses of 5–20 mg were administered by the intra-arterial route in one limb and the flow immediately interrupted for 1–2 min. The other limb served as a control.

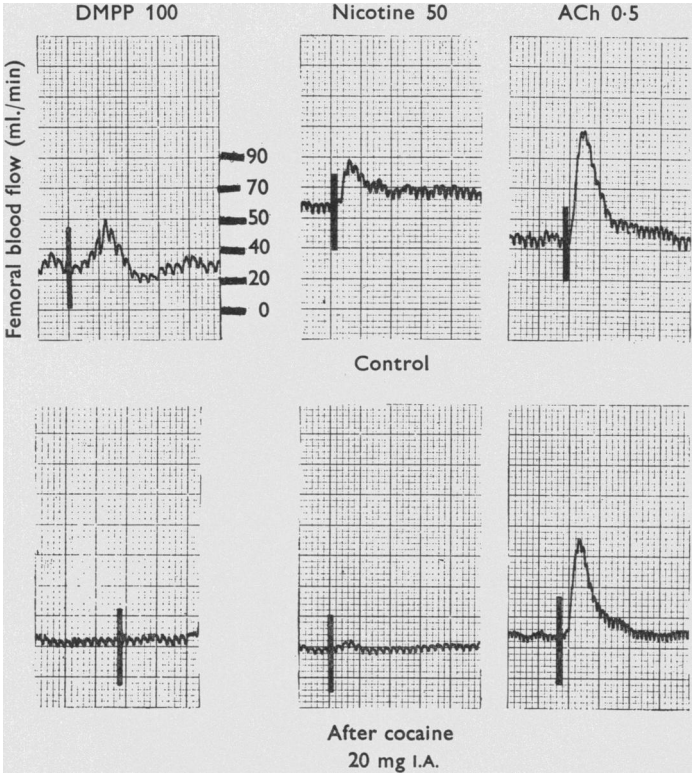

Fig. 2. Effect of cocaine on the vasodilator response to DMPP, nicotine, and ACh. Conventions as for Fig. 1.

The vasodilator action of DMPP was temporarily blocked (15–20 min) by doses of cocaine ranging from 10 to 20 mg (Fig. 2). However, reversal to a constrictor effect was not observed. Smaller doses of cocaine produced only partial blockade. Nicotine was also blocked, but in some cases a small residual response persisted. ACh was not affected by cocaine.

Effect of botulinum toxin on dilator response to DMPP and nicotine. Botulinum toxin prevents transmission in motor nerves as well as autonomic *cholinergic* nerves by blocking the smaller terminal branches close to the myoneural junction (Brooks, 1954, 1956; Wright, 1955; Ambache, 1955). Type A

or type C toxin (2×10^6–10^7 mouse lethal doses) was injected into a femoral artery and the flow interrupted for several minutes.

About 2–3 hr after the injection the dilator response to DMPP and nicotine was blocked in both limbs. In approximately half the animals DMPP now produced a constrictor response (Fig. 3) but no constrictor response was observed with nicotine. The dilator response to ACh was not altered or was reduced slightly. Thus botulinum toxin altered the response to DMPP in the same general way as hexamethonium by specifically blocking the dilator action.

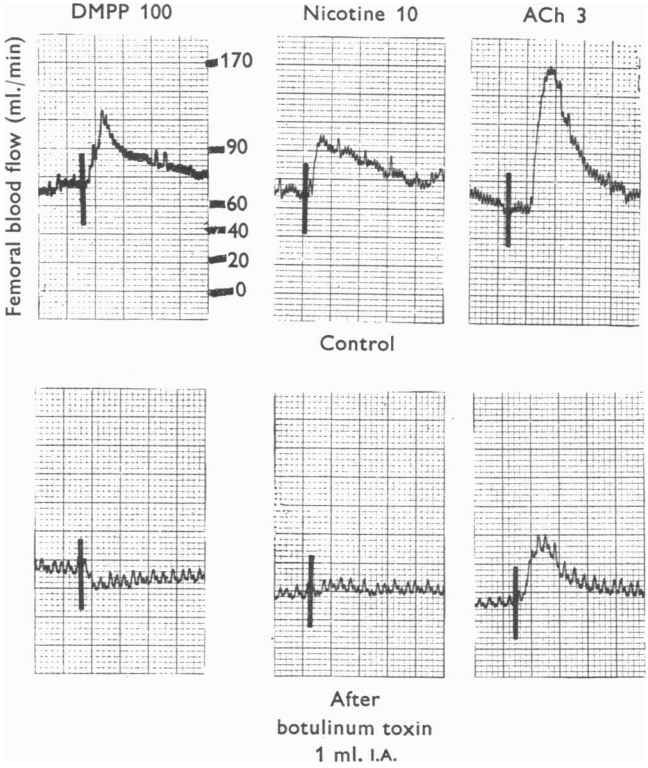

Fig. 3. Effect of botulinum toxin on the vasodilator response to DMPP, nicotine, and ACh. There was an interval of about 2 hr between the control and test records. Conventions as for Fig. 1.

Effect of various agents on the constrictor response to DMPP. Attempts were made to study the mechanism of the constrictor action of DMPP by administration of phentolamine, cocaine, atropine or large doses of hexamethonium. Before administration of these agents it was necessary to block the dilator action of DMPP with hexamethonium 2 mg/kg I.V.

Phentolamine 2 mg/kg I.V. eliminated the constrictor response (Fig. 4). This dose of phentolamine produced a reversal of the constrictor action of

epinephrine, and markedly reduced the constrictor action of norepinephrine. Intra-arterial injection of cocaine also blocked the constrictor action of DMPP but did not affect the response to epinephrine or norepinephrine. On the other

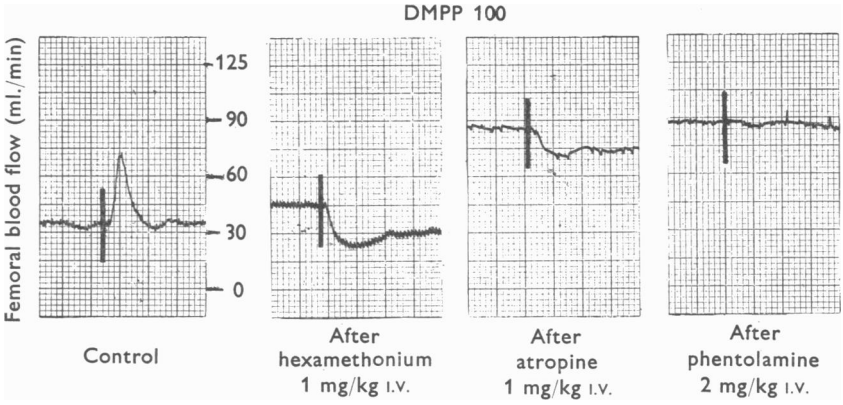

Fig. 4. Effect of atropine and phentolamine on the vasoconstrictor response to DMPP. The constrictor response to DMPP (panel 2) was observed after blockade of the dilator response by hexamethonium. Conventions as for Fig. 1.

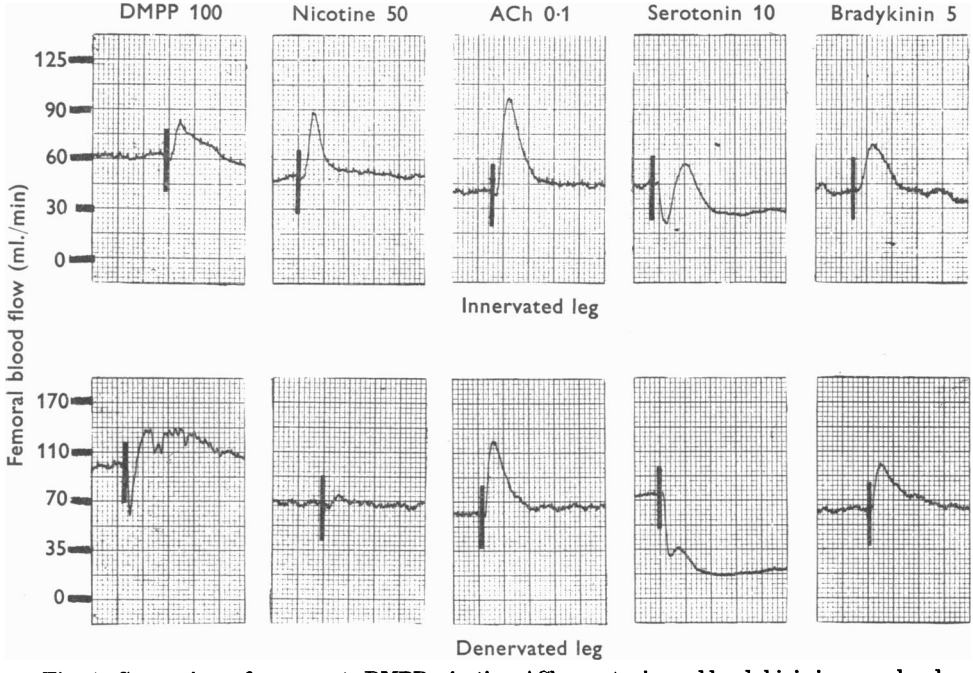

Fig. 5. Comparison of response to DMPP, nicotine, ACh, serotonin, and bradykinin in normal and chronically totally denervated limb; denervation 7 days before experiment. Conventions as for Fig. 1.

hand, the constrictor action of DMPP was not altered by increasing the dosage of hexamethonium to a total of 8 mg/kg or by administration of atropine 1 mg/kg I.V. (Fig. 4).

Effect of reserpine on the response to DMPP and nicotine. Burn & Rand (1958) demonstrated that reserpine pre-treatment depleted the peripheral stores of norepinephrine and prevented the constrictor actions of nicotine and DMPP. In order to ascertain whether or not a local release of catecholamines might be involved in the present results, animals were pre-treated with reserpine 0·5–2 mg/kg intraperitoneal on each of 2 days before the flow study.

Reserpine pre-treatment did not alter the constrictor or the dilator response to DMPP or the dilator response to nicotine. As in the non-reserpinized animal, hexamethonium blocked the DMPP and nicotine dilatation and permitted the DMPP constrictor response to appear; this was blocked by phentolamine. The pressor response to carotid occlusion was absent in the reserpinized animals, suggesting that an adequate dose of reserpine was used.

Effect of other vasoactive agents on blood flow. Several other types of agents were studied for comparative purposes. The following substances consistently produced a dilator response like that of DMPP, nicotine or ACh; isoprenaline 5 μg, histamine 1–5 μg, or bradykinin 5–10 μg. The dilator actions of these substances were not affected by any of the blocking agents previously discussed.

Serotonin 5–10 μg, like DMPP, appeared to have both a dilator action (Fig. 1) and a constrictor. These actions may appear alone or there may be a combined response (Fig. 5). The dilator action of serotonin was blocked by hexamethonium (Fig. 1) and botulinum toxin, but the constrictor response was not altered by any of the blocking agents studied.

Epinephrine (1 μg) and norepinephrine (1 μg) consistently produced a constrictor response, which was reduced or converted to a dilator response by phentolamine.

Chronic total denervation

Control responses. The control responses in the denervated and innervated limbs are shown in Fig. 5. DMPP produced a greater and more prolonged dilator response in the denervated limb than in the innervated limb; in some animals the augmented dilator response was preceded by a transient constrictor action. In contrast, nicotine had a markedly reduced response in the denervated limb, and in several animals, one of which is illustrated in Fig. 7, there was practically no response. The dilator action of serotonin was also blocked by denervation. ACh and bradykinin produced the same effect in both limbs.

Effect of various blocking agents. The dilator effect of DMPP in the denervated limb was *not* blocked by hexamethonium, atropine, phentolamine, botulinum toxin or cocaine (Figs. 6, 7). The results for the first three autonomic blocking agents, given sequentially, are illustrated in Fig. 6. The reduced

dilator responses for DMPP, ACh, and bradykinin after hexamethonium are probably associated with the marked reduction in blood pressure. These responses increased as the pressure increased. However, the blockade of the slight dilator effect of nicotine appears to be real, since the response did not

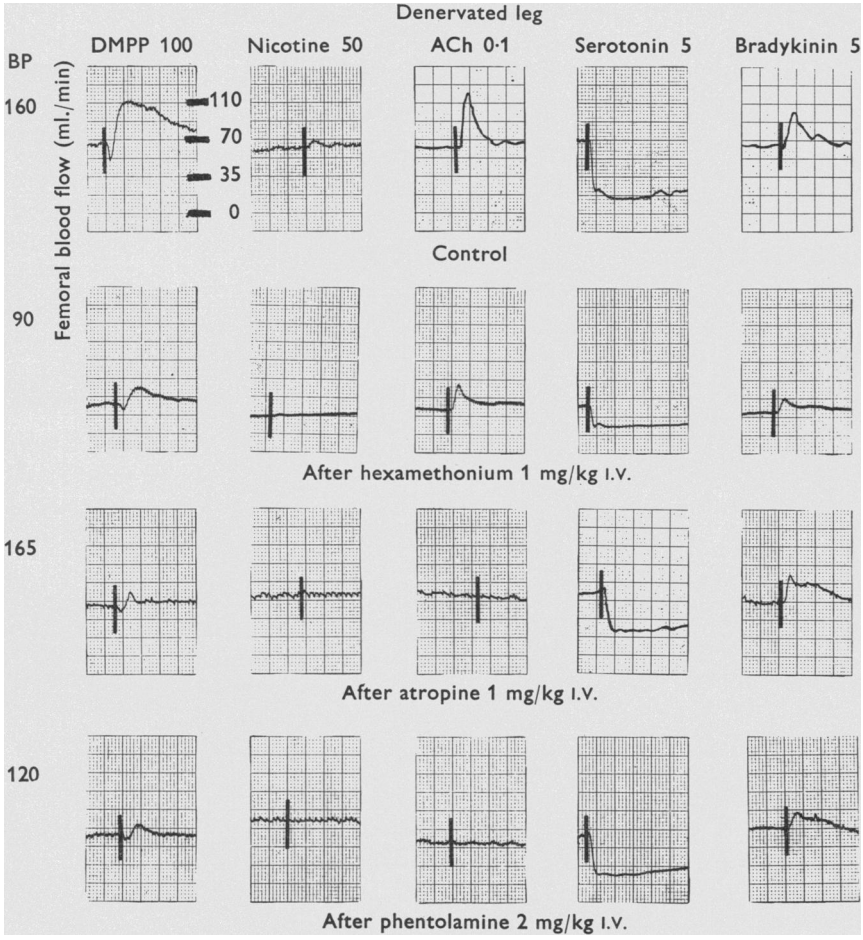

Fig. 6. Effect of hexamethonium, atropine, and phentolamine on the vascular response to DMPP, nicotine, ACh, serotonin, and bradykinin in the chronically (7 days) denervated limb. The blood pressure (mm Hg) during each phase of the experiment is given on the left. Conventions as for Fig. 1.

return when the perfusion pressure increased. Atropine produced a slight diminution of the DMPP dilator response and completely blocked the ACh effect. Phentolamine produced no further change. Bradykinin and serotonin were not affected by any of these agents.

These blocking agents produced the usual effects in the contralateral inner-

vated limb. Hexamethonium reversed DMPP, and blocked nicotine, vasodilator responses; atropine blocked ACh dilatation. It is obvious the dilator action of DMPP has a different mechanism after chronic denervation. This is not the case for ACh, bradykinin, and the small residual action of nicotine.

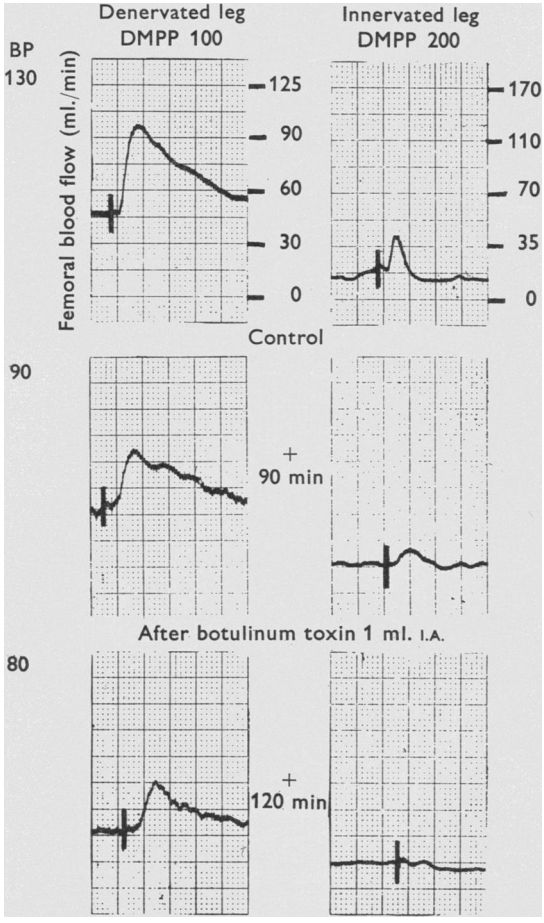

Fig. 7. Effect of botulinum toxin on the vasodilator response to DMPP in the normal and (7 days) denervated limb. Conventions as for Fig. 1. Note change in ordinate scale.

DISCUSSION

These studies showed that DMPP has both a dilator and a constrictor action, on intra-arterial administration, which are mediated by different neural pathways. The dilator action usually predominated under control conditions and the constrictor action was observed only after elimination of the dilator action.

The most significant observations are as follows: (1) The dilator response to DMPP was converted to a constrictor response by hexamethonium or

botulinum toxin. (2) The dilator response to DMPP was blocked by cocaine but no constrictor response appeared. (3) The constrictor response to DMPP was blocked by phentolamine or cocaine. (4) The constrictor response was not blocked by hexamethonium or reserpine. (5) Atropine did not markedly alter the constrictor or dilator actions of DMPP. (6) Neostigmine did not enhance the dilator action of DMPP. (7) Chronic denervation did not eliminate the dilator action of DMPP.

On the basis of these results a mechanism for the actions of DMPP in the innervated limb has been proposed. Both the dilator and constrictor actions appear to involve a peripheral nerve pathway, because cocaine blocked both actions. Since acute denervation produced no change in the responses to DMPP (Winbury *et al.* 1958) central mechanisms (cord or higher reflexes) can be excluded.

Dilator response. The pathway for the dilator response is presumed to be activated in a 'ganglion-like' fashion since the response was effectively blocked by hexamethonium. Whether or not peripheral ganglia in fact exist cannot be answered with the present experiments, but it is suggested that there is some hypothetical site that is excited by DMPP in a way analogous to ganglionic stimulation.

On the basis of the blockade of the dilator response by botulinum toxin this pathway may be considered 'cholinergic-like'. The evidence for a selective action of botulinum toxin on cholinergic fibres is quite convincing (Wright, 1955; Ambache, 1955; Hilton & Lewis, 1955). However, the present studies do not provide evidence for release of the typical cholinergic neurohumoral transmitter (ACh) at the nerve endings, since the dilator action of DMPP was not blocked by atropine nor was it potentiated by neostigmine. Acetylcholine itself was blocked by atropine and potentiated by neostigmine.

It is unlikely that the dilator action is mediated through the sympathetic cholinergic dilator fibres which supply the muscles of the hind limb, because this pathway is sensitive to atropine (Uvnäs, 1954; Youmans, Green & Denison, 1955; Folkow, 1956). In many ways the dilator of DMPP resembles the vasodilatation produced, by antidromic stimulation, in the skin (not the muscle) of the hind limb of the cat (Folkow, 1955, 1956) and in the isolated sympathectomized rabbit ear (Holton & Perry, 1951; Holton, 1953). The vasodilatation in both these preparations was not blocked by atropine, and in the studies on the rabbit ear the vasodilatation was reduced by neostigmine and other cholinesterase inhibitors.

Constrictor response. The constrictor mechanism is postulated to be mediated via an adrenergic pathway which is not excited by DMPP in a 'ganglion-like' fashion. In contrast to the dilator action, the constriction was not affected by ganglion blockade.

The site of blockade of the constrictor response of DMPP by cocaine is

central to the neuro-effector junction, since epinephrine produced the normal constrictor response. After adrenergic blockade with phentolamine the constrictor action of DMPP was blocked and that of epinephrine was reversed. Doses of reserpine which have been reported (Burn & Rand, 1958) to deplete the norepinephrine stored in chromaffin tissue and arterial walls and to prevent the constrictor response to nicotine and DMPP in the isolated hind limb of the dog, were without effect on the constrictor response to DMPP in our studies.

Chronic denervation. DMPP produced an enhanced dilator response in the chronically denervated limb compared with the contralateral innervated limb. Of greatest interest is the fact that the response to DMPP was not blocked by hexamethonium, cocaine, botulinum toxin, atropine or phentolamine, suggesting an entirely different mode of action in the denervated limb.

It seems most likely that the dilator action of DMPP in the denervated limb results from a direct action on the smooth muscle of the vessel walls similar to that of bradykinin or nitroglycerine. It has been well established that denervation may increase the sensitivity of various organs to sympathomimetic amines; a sensitization to a direct dilator action of DMPP may also result. Presumably this mechanism was of little significance in the innervated limb and the dilatation resulted from the atropine-insensitive neural mechanism.

There is some evidence for a direct dilator action in the innervated limb. For example, in some animals under prolonged ganglionic blockade a slight transient dilator response reappeared, which could not be blocked by larger doses of hexamethonium or by atropine. Further, in studies in which phentolamine blocked the constrictor action of DMPP (after ganglionic blockade), a slight dilator response occasionally reappeared and this could not be blocked by more hexamethonium or atropine.

There are many similarities in the dilator actions of DMPP, serotonin, and nicotine; all three substances were blocked only by hexamethonium and botulinum toxin. However, after chronic denervation differences appeared; the dilator response to serotonin and nicotine was absent but not that to DMPP. There are also differences in the constrictor mechanism for DMPP and serotonin; the action of DMPP, but not that of serotonin, was blocked by phentolamine or cocaine. The reduction in the dilator response to nicotine after chronic denervation is in agreement with the results which Hilton (1954) obtained on the muscles of the hind limb of the cat.

Other compounds classified as ganglionic stimulants, such as tetramethylammonium and the imidazole-acetylcholines, resemble acetylcholine in that the dilator response is blocked by atropine but not by hexamethonium (Winbury, 1957, 1958; Winbury *et al.* 1958).

The following conclusions have thus been drawn about the dilator and constrictor actions of DMPP:

(1) Distinct nerve pathways are involved in the dilator and constrictor actions of DMPP in the innervated limb.

(2) The dilator action indicates the existence of an atropine-insensitive pathway (cholinergic ?) that can be blocked by hexamethonium and botulinum toxin but is not potentiated by neostigmine.

(3) The constrictor action indicates the existence of a pathway (adrenergic) that can be blocked by phentolamine but not by hexamethonium.

(4) Normally the dilator action of DMPP predominated.

(5) There may be a slight direct action of DMPP on the vessel wall, which is masked by 2 and 3 in the innervated limb.

(6) After chronic denervation the limb becomes sensitized to the direct dilator action of DMPP.

SUMMARY

1. The mechanisms of the local vascular actions of 1, 1-dimethyl-4-phenylpiperazinium (DMPP) in the hind limb of the dog were investigated by means of various blocking agents and chronic denervation, and compared with those of nicotine and acetylcholine.

2. DMPP usually produced a local dilator response on intra-arterial injection but vasoconstriction was occasionally observed at the start of the experiment and later changed to vasodilatation.

3. The dilator action of DMPP was converted to a constrictor action by hexamethonium and botulinum toxin. Cocaine blocked the dilator response but no constrictor response appeared; atropine and pre-treatment with reserpine had no significant effect on the dilator response. Neostigmine did not potentiate the dilator response.

4. The constrictor response to DMPP which appears after hexamethonium was blocked by phentolamine or cocaine but not by atropine, pre-treatment with reserpine or large doses of hexamethonium.

5. After chronic total denervation DMPP produced an enhanced dilator response which was not blocked by hexamethonium, botulinum toxin, cocaine, atropine or phentolamine.

6. Nicotine produced a local dilator response which was blocked by hexamethonium, botulinum toxin or cocaine but not by atropine. No constrictor response was observed. After chronic denervation nicotine produced no local vascular effect.

7. Acetylcholine produced a local dilator response which was blocked by atropine but not by hexamethonium, botulinum toxin, cocaine or chronic denervation.

8. It was concluded that the vascular actions of DMPP in the innervated limb involved distinct nerve pathways within the limb. The dilator pathway

is sensitive to botulinum toxin but the neurohumoral transmitter does not appear to be cholinergic. The constrictor pathway appears to be adrenergic in nature. The dilator response after chronic denervation would appear to result from a direct action on the vessel.

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REFERENCES

AMBACHE, N. (1955). The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev.* **7**, 467–494.

BROOKS, V. B. (1954). The action of botulinum toxin on motor nerve filaments. *J. Physiol.* **123**, 501–515.

BROOKS, V. B. (1956). An intracellular study of the action of repetitive nerve volleys and of botulinum toxin on miniature end-plate potentials. *J. Physiol.* **134**, 264–277.

BURN, J. H. & RAND, M. J. (1958). Noradrenaline in artery walls and its dispersal by reserpine. *Brit. med. J.* i, 903–908.

FOLKOW, B. (1955). Nervous control of the blood vessels. *Physiol. Rev.* **35**, 629–663.

FOLKOW, B. (1956). Nervous control of the blood vessels. *The Control of the Circulation of the Blood*, Supplemental Volume, pp. 1–86. London: Wm. Dawson and Sons, Ltd.

GINZEL, K. H. & KOTTEGODA, S. R. (1953). Nicotine-like actions in auricles and blood vessels after denervation. *Brit. J. Pharmacol.* **8**, 156–161.

GOODMAN, L. S. & GILMAN, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed. New York: The Macmillan Co.

HILTON, S. M. (1954). The effects of nicotine on the blood vessels of skeletal muscle in the cat. An investigation of vasomotor axon reflexes. *J. Physiol.* **123**, 289–300.

HILTON, S. M. & LEWIS, G. R. (1955). The cause of the vasodilatation accompanying activity in the submandibular salivary gland. *J. Physiol.* **128**, 235–248.

HOLTON, P. (1953). Antidromic vasodilatation and inhibitors of cholinesterase. *J. Physiol.* **120**, 95–104.

HOLTON, P. & PERRY, W. L. M. (1951). On the transmitter responsible for antidromic vasodilatation in the rabbit's ear. *J. Physiol.* **114**, 240–251.

KOTTEGODA, S. R. (1953). The action of nicotine and acetylcholine on the vessels of the rabbit's ear. *Brit. J. Pharmacol.* **8**, 156–161.

LANIER, J. T., GREEN, H. D., HARDAWAY, J., JOHNSON, H. D. & DONALD, W. B. (1953). Fundamental differences in the reactivity of the blood vessels in skin compared with those in muscle. *Circul. Res.* **1**, 40–48.

PAGE, I. H. & MCCUBBIN, J. W. (1953). Cardiovascular action of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). *Amer. J. Med.* **15**, 675–683.

SHIPLEY, R. E. & WILSON, C. (1951). An improved recording rotameter. *Proc. Soc. exp. Biol., N.Y.*, **78**, 724–728.

UVNÄS, B. (1954). Sympathetic vasodilator outflow. *Physiol. Rev.* **34**, 608–614.

WINBURY, M. M. (1957). Muscarinic action of murexine and some related choline esters. *Nature, Lond.*, **180**, 988–989.

WINBURY, M. M. (1958). Comparison of the vascular actions of 1, 1-dimethyl-4-phenylpiperazinium and tetramethyl-ammonium. *J. Pharmacol.* **124**, 25–34.

WINBURY, M. M., MICHIELS, P. M., HAMBOURGER, W. E., STOCKFISCH, W. J. & COOK, D. L. (1950). Coronary dilator action. I. Quantitative assay in the intact dog. *J. Pharmacol.* **99**, 343–349.

WINBURY, M. M., WOLF, J. K. & TABACHNICK, I. I. A. (1958). Cardiovascular actions of three choline esters of carboxyalkylimidazole. *J. Pharmacol.* **122**, 207–214.

WRIGHT, G. P. (1955). The neurotoxins of Clostridium botulinum and Clostridium tetani. *Pharmacol. Rev.* **7**, 413–465.

YOUMANS, P. L., GREEN, H. D. & DENISON, A. B. (1955). Nature of the vasodilator and vasoconstrictor receptors in skeletal muscle in dogs. *Circul. Res.* **3**, 171–180.