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ACTIONS OF DIMETHYLPHENYLPIPERAZINIUM

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The actions of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) have been studied to discover under what conditions a blocking action could be seen. Dimethylphenylpiperazinium has a stimulant action on autonomic ganglia, stimulating the superior cervical ganglion and causing contraction of the nictitating membrane. It caused slowing followed by acceleration of the rate of beat of isolated rabbit atria. The denervated gastrocnemius muscle contracted if dimethylphenylpiperazinium was injected intra-arterially. Under other circumstances a blocking action was seen. It blocked peristalsis in the isolated guinea-pig ileum stimulated by raising intraluminal pressure and inhibited the response of the rat diaphragm and of the cat gastrocnemius stimulated through the motor nerve. It is suggested that dimethylphenylpiperazinium acts by depolarization, causing stimulation of resting muscle but inhibition by prolonging depolarization.

1,1 - Dimethyl - 4 - phenylpiperazinium iodide (DMPP) was first investigated by Chen, Portman, and Wickel (1951). It is primarily a hypertensive drug, the rise in blood pressure being due to stimulation of sympathetic ganglia and to the release of catechol amines from the suprarenal gland; it also has a stimulant effect upon plain muscle, like guinea-pig ileum, which is abolished by hexamethonium. It is therefore a "nicotine-like" drug, but unlike nicotine it appears to have little blocking action on ganglia, even in large doses. For this reason it has been used by Chen *et al.* (1951) and by Fakstorp and Pederson (1954) in preference to nicotine as an agonist in estimating the efficiency of ganglion-blocking agents on plain muscle preparations, such as guinea-pig ileum. Most ganglionic stimulators, like nicotine and tetramethylammonium, block ganglionic transmission in large doses or after repeated administration of small doses, so that dimethylphenylpiperazinium seemed to be an exceptional drug and worthy of further investigation. In particular my object was to discover under what conditions, if any, dimethylphenylpiperazinium would block ganglion cells.

Chen and Portman (1954) showed that dimethylphenylpiperazinium iodide caused a block of the peristaltic reflex of the guinea-pig ileum set up as described by Trendelenburg (1917). Leach (1957) confirmed this finding, and he also showed that, when a maintained contraction of the nictitating membrane of the cat was produced by stimulation of the preganglionic cervical sympathetic, dimethylphenylpiperazinium injected intra-arterially caused a lessening of the contraction, which then slowly returned.

The experiments reported here concern the effects of dimethylphenylpiperazinium on the spinal cat, on the isolated atria of the rabbit, on the peristaltic movement of guinea-pig intestine, on the skeletal neuromuscular junction (rat and cat), and upon the vessels of the hindleg of the dog.

METHODS

Spinal cats were prepared by the method described by Burn (1952). The contractions of the nictitating membrane were recorded by using a fine silk thread, attached to the tip of the membrane, which passed round a pulley and to an isotonic frontal writing lever having a magnification of about six times. The isolated atria of the rabbit were dissected clean and suspended in an isolated organ bath in Locke solution containing twice the ordinary amount of dextrose. Aeration was carried out with oxygen, and the beat was recorded by attaching a thread from the tip of one atria to a Starling heart lever. The guinea-pig ileum preparation was that described by Bülbring and Lin (1958) and Bülbring, Crema, and Saxby (1958). The rat diaphragm preparation was that of Bülbring (1946). The response of the gastrocnemius muscle of the spinal cat was recorded by detaching a portion of the os calcis into which the tendon of Achilles is inserted, and connecting it by a wire to an isometric tension lever. A rod passed through a hole drilled near the lower end of the femur was held rigidly between clamps. Electrodes were placed on the sciatic nerve which was stimulated by maximal single rectangular

wave shocks, duration of 0.7 msec. at a frequency of about 13/min. The hindleg of the dog was perfused with blood from a Dale-Schuster pump. A second pump perfused the lungs of another dog to provide oxygenation. Outflow was measured with the recorder of Stephenson (1949).

RESULTS

Effects in the Spinal Cat.—Dimethylphenylpiperazinium iodide injected intravenously into a spinal cat caused a rise of blood pressure and a contraction of the nictitating membrane. In many instances, 10 or 11 times as much nicotine as dimethylphenylpiperazinium (in terms of base) was required to produce a similar rise in blood pressure and contraction of the nictitating membrane. When the suprarenal glands were excluded, the dose relationship between dimethylphenylpiperazinium and nicotine was of the order of one to three, a value which has been given by other workers using anaesthetized animals. However, when injections were made into the lingual artery the same amount of both drugs was required to produce similar contractions of the nictitating membrane. Fig. 1 shows the contractions of the nictitating membranes produced when dimethylphenylpiperazinium was injected intravenously into a spinal cat. The right membrane (upper record) was normal, but on the left side the superior cervical ganglion had been removed at the beginning of the experiment (lower record). At **A** 80 μg. of dimethylphenylpiperazinium iodide intravenously caused a contraction of both nictitating membranes. These contractions must have been due to liberation of catechol amines from the suprarenal glands, since after exclusion of the suprarenal glands and after evisceration the same dose of dimethylphenylpiperazinium had no effect (**B**). At **C** 500 μg. of dimethylphenylpiperazinium was injected and a contraction of the right membrane occurred, but there was no effect on the left side. When a total of 9.0 mg. of hexamethonium was given intravenously between **C** and **D**, dimethylphenylpiperazinium then had no effect. When dimethylphenylpiperazinium was injected into the lingual artery of a cat anaesthetized with chloralose, an injection of histamine intra-arterially potentiated the action of subsequent injections of dimethylphenylpiperazinium as shown in Fig. 2.

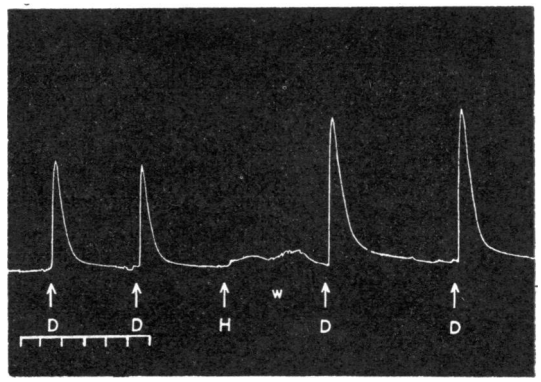


FIG. 2.—Cat, chloralose anaesthesia. Record of the response of the nictitating membrane with the preganglionic fibres cut. Injections given into the lingual artery with the external carotid artery occluded. **D**, 4 μg. of dimethylphenylpiperazinium. **H**, 100 μg. of histamine dihydrochloride. Time, 30 sec.

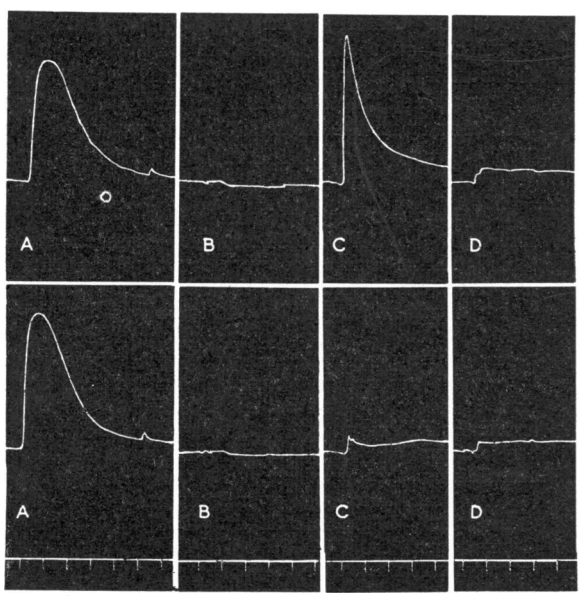
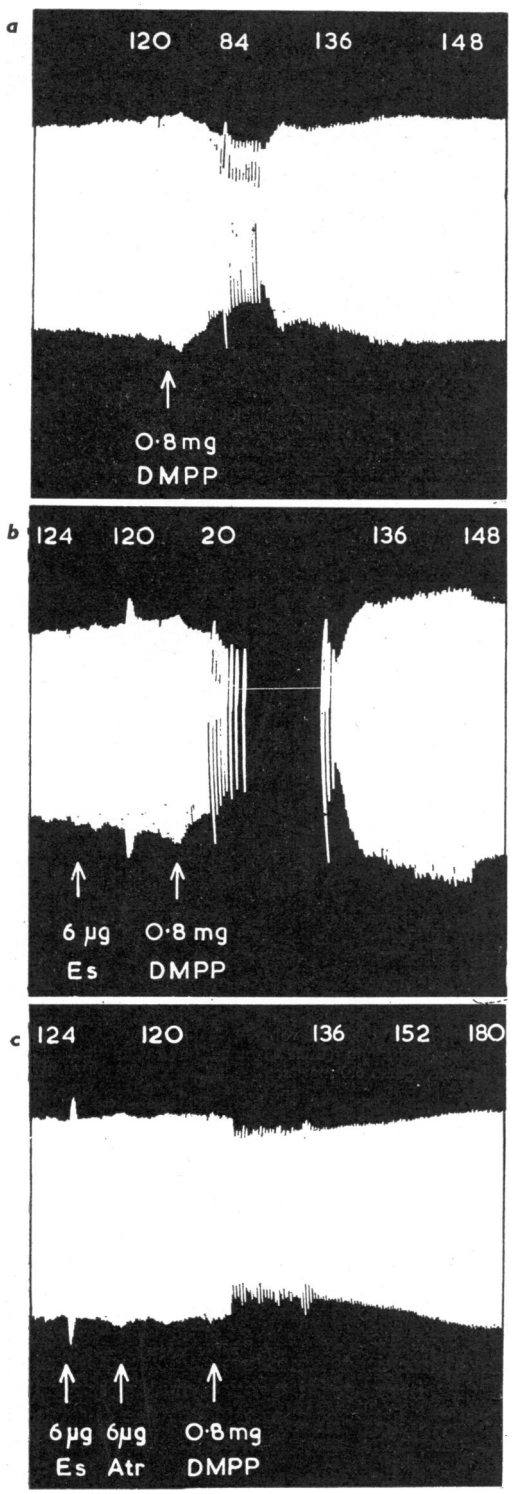


FIG. 1.—Spinal cat. Upper record, right nictitating membrane (preganglionic fibres cut). Lower record, left nictitating membrane (acutely denervated by removal of superior cervical ganglion). **A**, 80 μg. of dimethylphenylpiperazinium. Between **A** and **B**, the suprarenal glands were excluded from the circulation and the cat was eviscerated. **B**, 80 μg. of dimethylphenylpiperazinium. **C**, 500 μg. of dimethylphenylpiperazinium. Between **C** and **D**, 9.0 mg. of hexamethonium was given intravenously. **D**, 1.0 mg. of dimethylphenylpiperazinium. Time, 30 sec.

Effects on the Heart.—When added to a bath containing isolated atria of the rabbit, dimethylphenylpiperazinium iodide caused inhibition followed by stimulation, but after atropine stimulation only was seen. In Fig. 3*a*, 0.8 mg. of the iodide produced a slowing in rate followed by an acceleration, but, as shown in Fig. 3*b*, after 6 μg. of eserine, the slowing was greatly potentiated although the stimulant action which followed was still present. When 6 μg. of



atropine was added, the same dose of dimethylphenylpiperazinium failed to cause slowing, and the acceleration in rate which followed was greater (Fig. 3*c*). The stimulant action of dimethylphenylpiperazinium on isolated atria was abolished by hexamethonium and by ephedrine. Dimethylphenylpiperazinium, injected into the venous inflow of the Starling heart-lung preparation in which the coronary flow was recorded by means of a Morawitz cannula in the coronary sinus, caused increase in both systemic and coronary flow. A similar effect was produced by nicotine, both effects being abolished by hexamethonium.

Effects on Peristalsis.—The isolated guinea-pig ileum preparation of Bülbring and Lin (1958) was used, and the longitudinal movements, the intraluminal pressure and the flow were recorded. When dimethylphenylpiperazinium iodide (200 µg.) was injected into the fluid passing through the intestine, a lowering of the threshold of the intraluminal pressure occurred (white dots in Fig. 4*b*). There was a brief stimulation of the longitudinal movements, which was followed later by inhibition of the peristalsis. This lowering of the threshold of the intraluminal pressure was larger than when a similar dose of nicotine was given (Fig. 4*a*). When the drugs were added to the fluid in which the intestine was immersed, that is applied to the outside of the intestine, dimethylphenylpiperazinium (50 µg.) stopped peristalsis and flow as shown in the middle and lower record of Fig. 5*a*. Excitation, however, of the circular muscle occurred which can be seen in the uppermost record in Fig. 5*a*. Nicotine acid tartrate (50 µg.) caused complete paralysis without any excitation of the circular muscle (Fig. 5*b*).

Effects on the Skeletal Neuromuscular Junction.—Dimethylphenylpiperazinium added to the fluid bathing the isolated rat phrenic nerve diaphragm preparation caused a reduction in the height of the muscle twitch in response to single stimuli applied to the phrenic nerve. The effect produced by 0.5 mg. was greater than that produced by 6.0 mg. nicotine acid tartrate (Fig. 6). In the gastrocnemius muscle preparation of the cat, dimethyl-

FIG. 3.—Isolated rabbit atria suspended in Locke solution containing twice the normal dextrose concentration. Bath vol., 35 ml. (*a*) 0.8 mg. of dimethylphenylpiperazinium (DMPP) caused slowing of the rate followed by acceleration. (*b*) In the presence of 6 µg. of eserine, 0.8 mg. of dimethylphenylpiperazinium caused arrest, followed by acceleration. (*c*) When atropine (6 µg.) was given after eserine, 0.8 mg. of dimethylphenylpiperazinium caused only acceleration. The numerals above each record give beats/min.

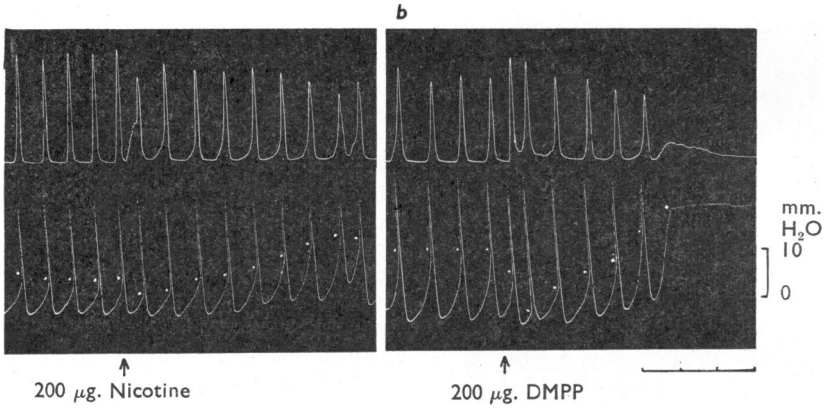


FIG. 4.—Isolated guinea-pig ileum suspended in Tyrode solution to record peristalsis by the method of Bülbring and Lin (1958). Upper record, contractions of the longitudinal muscle. Lower record, intraluminal pressure. (*a*) 200 μg. of nicotine acid tartrate injected into the lumen caused a transitory fall of the threshold of pressure (white dots) required to elicit the peristaltic reflex. (*b*) 200 μg. of dimethylphenylpiperazinium (DMPP) injected into the lumen caused a greater fall of threshold. This stimulation was followed by inhibition of peristalsis. Time, 1 min.

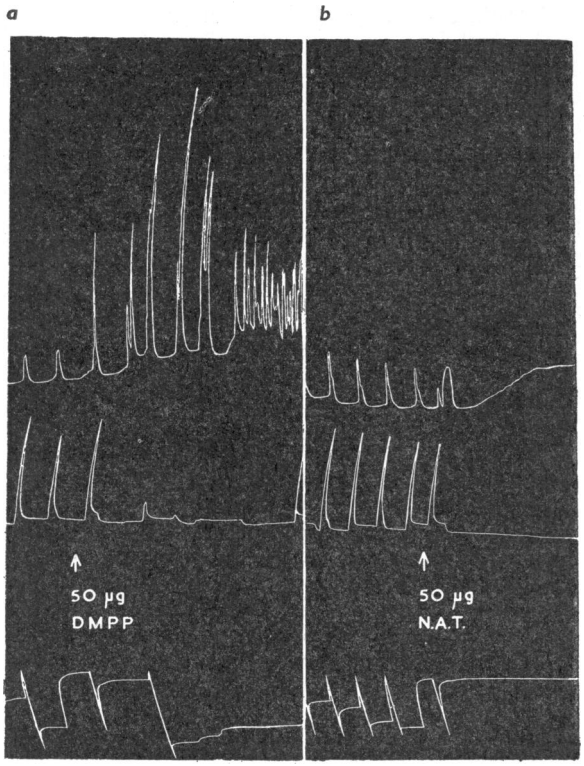


FIG. 5.—Isolated guinea-pig ileum suspended in Tyrode solution. Bath vol., 70 ml. Upper record, contractions of the longitudinal muscle. Middle record, intraluminal pressure. Lower record, outflow. (*a*) 50 μg. of dimethylphenylpiperazinium (DMPP) added to the fluid bathing the outside of the intestine caused an increase in the activity of the intestinal longitudinal muscle (upper record), but caused inhibition of peristalsis and of flow (middle and lower records). (*b*) 50 μg. of nicotine acid tartrate (N.A.T.) caused inhibition of movement and cessation of peristalsis and of flow.

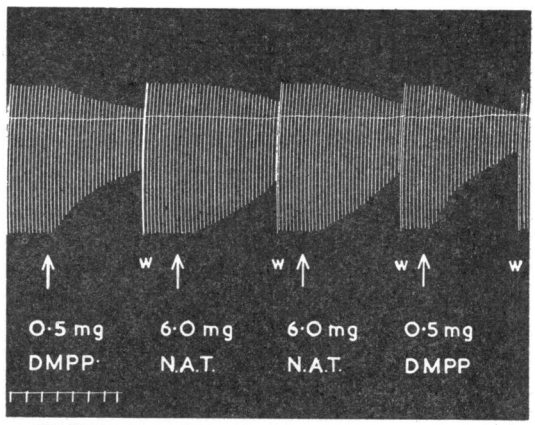


FIG. 6.—Rat phrenic nerve-diaphragm preparation suspended in Tyrode solution. Bath vol., 100 ml. 0.5 mg. of dimethylphenylpiperazinium (DMPP) caused a greater inhibition of stimulation than 6.0 mg. nicotine acid tartrate (N.A.T.). Time, 30 sec.

phenylpiperazinium injected retrogradely into the opposite external iliac artery caused a reduction in the height of the muscle twitch in response to single stimuli applied to the sciatic nerve. The effect produced was similar to that produced by decamethonium (Fig. 7). Much larger doses of tetramethylammonium bromide and of nicotine were needed to produce similar effects. Kaller (1956) suggested that dimethylphenylpiperazinium behaved like decamethonium and suxamethonium, and that it exerted its action by depolarization. This suggestion was supported by the observation that, after the response of the gastrocnemius muscle to stimuli applied to the sciatic nerve had

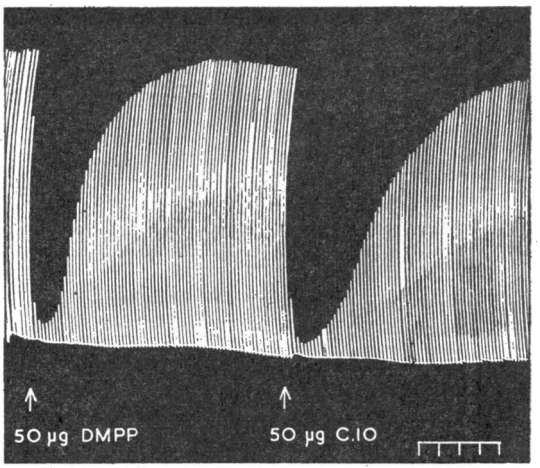


FIG. 7.—Spinal cat. Sciatic nerve gastrocnemius muscle preparation. Injections were made into the opposite iliac artery retrogradely. 50 µg. of dimethylphenylpiperazinium (DMPP) caused a reduction of the response of the muscle to single stimuli applied to the sciatic nerve. The effect was similar to that produced by 50 µg. decamethonium (C.10). Time, 30 sec.

been greatly reduced by dimethylphenylpiperazinium, then during the recovery phase injections of neostigmine produced transient interruption of recovery whereas tubocurarine caused an acceleration of recovery (Fig. 8). In the denervated gastrocnemius muscle preparation, the sciatic nerve having been cut eight days previously, 5 µg. of dimethylphenylpiperazinium given intra-arterially caused a twitch similar in size to, but more sustained than, that produced by 5 µg. of acetylcholine (Fig. 9). 100 µg. of nicotine acid tartrate on the other hand produced a twitch of only half the size.

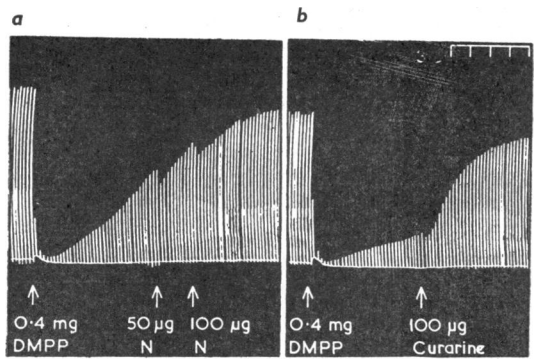


FIG. 8.—Spinal cat. Sciatic nerve gastrocnemius muscle preparation. (a) When the response of the gastrocnemius muscle to stimulation of the sciatic nerve was inhibited by 0.4 mg. of dimethylphenylpiperazinium (DMPP), 50 µg. and 100 µg. of neostigmine (N) caused a further transitory inhibition. (b) After inhibition caused by 0.4 mg. of dimethylphenylpiperazinium, 100 µg. of tubocurarine chloride (Curarine) caused a return of the response to stimulation. Time, 30 sec.

Effect on Blood Vessels.—Dimethylphenylpiperazinium caused constriction when injected into the vessels of the perfused rabbit ear. Moreover, it usually caused constriction when injected into the hindleg of a dog which was being perfused with blood by means of a Dale-Schuster pump as shown in Fig. 10. There was a rise in systemic pressure and a decrease in outflow. The effect was variable, and in some preparations scarcely any change was seen. In the denervated leg in which the sciatic nerve had been cut 11 days previously, dimethylphenylpiperazinium caused a fall of pressure and an increase in outflow, as it also did in a dog which had received daily injections of reserpine for two days. Under both these conditions nicotine also caused vasodilatation.

DISCUSSION

The stimulant action of dimethylphenylpiperazinium on sympathetic ganglia is well shown in the spinal cat, a preparation which has not been much used by other workers in testing its action. Injection of the drug into the lingual artery (with the external carotid occluded) caused an immediate contraction of the nictitating membrane, the magnitude of the effect being similar to that produced by an equal dose of nicotine. When injected intravenously, however, the drug appeared to be considerably more potent than nicotine: the ratio of the doses of dimethylphenylpiperazinium and nicotine required to produce approximately equal rises of blood

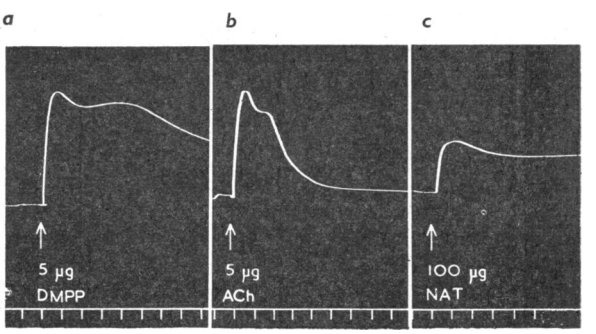


FIG. 9.—Cat, chloralose anaesthesia. Denervated gastrocnemius muscle preparation. Injections were made into the opposite iliac artery retrogradely. (a) 5 µg. of dimethylphenylpiperazinium (DMPP) caused a muscle twitch similar to (b) 5 µg. of acetylcholine (ACh). (c) 100 µg. of nicotine acid tartrate (NAT) produced a smaller effect. Time, 30 sec.

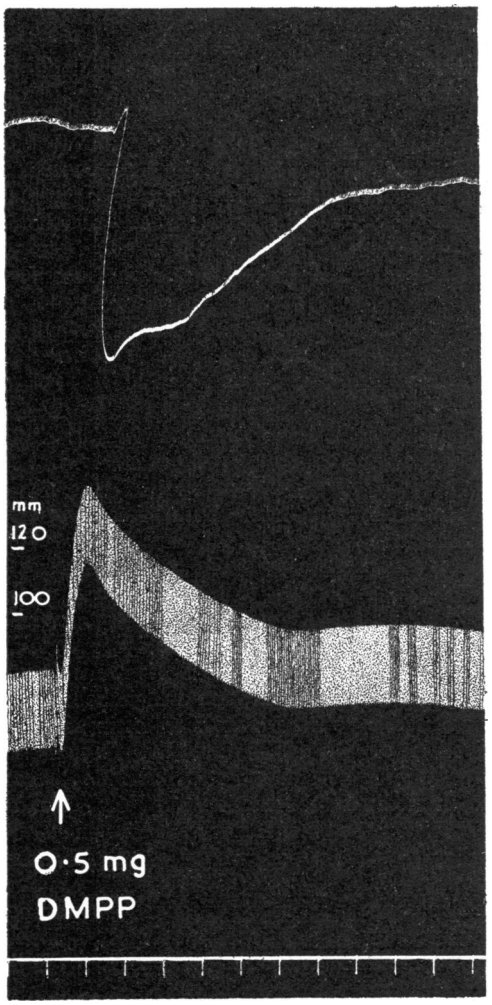


FIG. 10.—Dog hindleg perfused with blood from a Dale-Schuster pump. Upper record, venous outflow. Lower record, arterial pressure. 0.5 mg. dimethylphenylpiperazinium (DMPP) caused a rise in pressure due to vasoconstriction, and fall in outflow. Time, 30 sec.

pressure and contractions of the nictitating membrane was about 1:11 (in terms of base). These two effects were mainly due to the liberation of catecholamines from the adrenal glands, since after exclusion of the glands the pressor effect of previously potent doses was greatly reduced and no contraction of the nictitating membrane was observed. With larger doses of each drug under these conditions, dimethylphenylpiperazinium was about three times as effective as nicotine in causing a rise of blood pressure and contraction of the nictitating membrane.

Recent work by Burn and Rand (1957) has provided new evidence of the release of noradrenaline from skin and blood vessels. Catecholamines, presumably adrenaline and noradrenaline, are known to be present in various organs (Shaw, 1938; Raab, 1943; von Euler, 1946), and noradrenaline was found in the walls of arteries and veins by Schmiterlöw (1948). These observations suggest a possible explanation for the greater potency of dimethylphenylpiperazinium compared with nicotine, when the adrenal glands were excluded: just as dimethylphenylpiperazinium was more potent than nicotine in liberating catecholamines from the adrenal glands, so it may be more effective in releasing them from other tissues. The experiments on the perfused hindleg of the dog support this suggestion: whereas nicotine had very little effect upon this preparation, dimethylphenylpiperazinium always had some constrictor effect and sometimes it was pronounced (see Fig. 10). On the other hand, after previous denervation of the limb or treatment of the dog with reserpine, procedures which deplete the noradrenaline stores, both dimethylphenylpiperazinium and nicotine caused vasodilatation.

Previous workers have shown that dimethylphenylpiperazinium added to the fluid bathing a piece of isolated guinea-pig ileum produced a contraction similar to that produced by nicotine. When the guinea-pig ileum is set up so that peristalsis is recorded, a nervous mechanism is involved with release of the transmitter, and under these conditions nicotine and dimethylpiperazinium caused first stimulation followed by inhibition.

Dimethylphenylpiperazinium had much more effect than nicotine on striated muscle. When injected intra-arterially to the denervated gastrocnemius muscle preparation of the cat it produced a contraction of longer duration but of similar size to that produced by the same dose of acetylcholine. Nicotine produced a much smaller effect in 20 times the dose. The contractions of the normal gastrocnemius muscle preparation produced by stimulation of the sciatic nerve were depressed by dimethylphenylpiperazinium, the inhibition being increased by neostigmine and relieved by tubocurarine. Thus it acted in a manner similar to decamethonium which is known to produce its effect by depolarization.

When dimethylphenylpiperazinium acts alone it has a stimulant action. It stimulated the superior cervical ganglion to cause contraction of the nictitating membrane and the suprarenal

medulla to cause a rise of blood pressure. The guinea-pig ileum was stimulated to cause contraction, as was also the denervated gastrocnemius muscle of the cat when dimethylphenylpiperazinium was injected intra-arterially. When dimethylphenylpiperazinium was given during nerve stimulation, it caused inhibition. Thus it lessened the maintained contraction of the nictitating membrane and reduced the effect of repeated stimuli as was shown by Leach (1957). It inhibited peristalsis produced by raising the intraluminal pressure in the isolated guinea-pig ileum, and it also inhibited the response of the rat diaphragm and the cat gastrocnemius muscle to stimulation through the motor nerve.

Paton and Perry (1953) suggested that the action of nicotine was first to cause a depolarization and then to cause competitive block. It seems probable that dimethylphenylpiperazinium exerts its action by depolarization, causing stimulation of the resting muscle, but causing inhibition by prolonging the depolarization when the muscle is already depolarized. Page and McCubbin (1953) suggested that blocking action was not seen because of the rapid elimination of dimethylphenylpiperazinium from the site of action. This would explain the transitory blocking action, and absence of any subsequent competitive block such as is produced by nicotine.

Because of its good stimulant action and lack of permanent blocking action dimethylphenylpiperazinium would seem to be a useful agent for use in laboratory investigations when repeated stimulation of ganglia is required.

I wish to thank Professor J. H. Burn for giving me the opportunity to do this work and for allowing me to use his preparations of the dog hindleg perfusion. I am also indebted to Dr. E. Bülbring for performing the operation of cutting the sciatic nerve of the cat. My thanks are also due to Messrs. Parke, Davis and Co. Ltd. for a supply of dimethylphenylpiperazinium iodide.

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