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PARALDEHYDE AND METHYLPENTYNOL AND GANGLIONIC TRANSMISSION

BY

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Paraldehyde and methylpentynol blocked transmission of nerve impulses through the superior cervical ganglion of the cat when the drugs were administered intra-arterially to the ganglion or intravenously using the nictitating membrane as an indicator. Electrical studies showed that concentrations of methylpentynol and paraldehyde which blocked transmission in the isolated rat superior cervical ganglion were without action on the preganglionic nerve fibre. In amounts which blocked transmission in the isolated rat ganglion, paraldehyde had no depolarizing activity directly on the ganglion cells and did not interfere with the depolarizing activity of added acetylcholine. The results suggest that the block in transmission of the impulse could be accounted for by a decrease in the release of acetylcholine from the preganglionic nerve terminals. In both species the block was reversible.

Paraldehyde and methylpentynol block neuromuscular transmission (Quilliam, 1955). Electrical studies showed that the blocking activity of both drugs could be accounted for if their action decreased acetylcholine release (Nicholls and Quilliam, 1956). In the present work, the effect of paraldehyde and methylpentynol on ganglionic transmission has been studied using, first, the nictitating membrane preparation in the cat and, second, the isolated superior cervical ganglion of the rat with electrical recording of the compound nerve action potentials in the pre- and post-ganglionic nerves. The experimental evidence showed that paraldehyde and methylpentynol could block ganglionic transmission reversibly in the cat and in the rat. The action of methylpentynol at the ganglion has been briefly reported (Quilliam, 1957).

METHODS

Nictitating Membrane Preparation in the Cat

Cats were anaesthetized with pentobarbitone sodium (Nembutal 30 mg./kg., intraperitoneally). The pre- and post-ganglionic nerves to the superior cervical ganglion were cleaned and prepared for stimulation in the usual manner. Rectangular pulses of 0.5 msec. duration at 10/sec. from an electronic stimulator were applied through platinum electrodes to stimulate the nerves. The preganglionic nerve was stimulated continuously and the response of the nictitating membrane on that side was recorded on a smoked paper. When necessary, the response of the preparation to postganglionic stimulation could be tested by electrodes applied to the postganglionic nerve. The voltages of the pulses used in each instance were slightly greater than those producing maximal retraction of the membrane. The lingual artery was prepared for intra-arterial injections of drugs into the ganglion during which the external carotid artery was clamped (Morrison and Paton, 1953).

The Isolated Superior Cervical Ganglion of the Rat

Rats were anaesthetized with urethane (1.2 g./kg. intraperitoneally). The superior cervical ganglion was exposed. The ganglion with its pre- and post-ganglionic nerves was removed from the rat and transferred to Krebs solution at room temperature (20°) and bubbled with 95% O_2 and 5% CO_2. The connective tissue sheaths investing the preparation were then removed.

For the electrical recordings, the ganglion was suspended horizontally between two insulated forceps in a bath filled with liquid paraffin B.P. previously bubbled with 95% O_2 and 5% CO_2. Platinum electrodes were used both for stimulating and recording and were applied to the surface of the preparation in fixed positions at the beginning of each experiment. The two stimulating electrodes (2 mm. apart) were placed as far proximally as was possible on the preganglionic trunk. Single electrical stimuli of sufficient voltage at 0.5 msec. duration were used to produce a maximal action potential in the preganglionic nerve. One pair of recording

electrodes (2 mm. apart) was placed on the preganglionic trunk to record the preganglionic nerve action potential. Between this pair of electrodes and the stimulating electrodes, the preparation was earthed. Another pair of recording electrodes (2 mm. apart) were placed upon the ganglion for recording ganglionic potentials and a further pair of electrodes (2 mm. apart) were applied to the postganglionic trunk as far distally as possible for recording the postganglionic nerve action potential. When recordings were being made, the appropriate pair of electrodes were selected by a switch assembly and the pulses picked up by them were led through a cathode follower, passed to a direct coupled amplifier (Copeland, 1952) and displayed on an oscilloscope equipped with a camera.

Whether bathed in Krebs solution or in liquid paraffin at room temperature, transmission was maintained satisfactorily for 8 to 10 hr.

For recording the depolarizing action of acetylcholine upon the ganglion, the preparation was set up vertically in a 60 ml. bath of Krebs solution at room temperature previously bubbled with 95% O_2 and 5% CO_2 with the postganglionic nerve lowermost and a moving fluid electrode technique was employed similar to that described by Fatt (1950) for the toe muscle of the frog. The present technique differed from that of Pascoe (1956) mainly in that the postganglionic trunk was lowermost. Neostigmine methylsulphate (Roche) 2.5×10^{-6} was added to the Krebs solution bathing the ganglion. The ganglion was exposed to acetylcholine for 2 min. and then washed thoroughly for 45 min.

Drugs

Paraldehyde B.P. and pure methylpentynol (British Schering) were used. They were dissolved in 0.9% sodium chloride solution for intra-arterial injection in the nictitating membrane preparation. As variation in the injection volume led to inconsistent results, the intra-arterial injections were always made in a total volume of 0.25 ml. containing the required quantity of drug.

In the studies of transmission through the isolated rat superior cervical ganglion, hexamethonium bromide (May and Baker) was dissolved in Krebs solution. Paraldehyde and methylpentynol were mixed by vigorous shaking in a stoppered vessel with medicinal liquid paraffin B.P. at room temperature before they were added to the bath containing the preparation. The liquid paraffin was bubbled with 95% O_2 and 5% CO_2 before mixing with the drug. For recording purposes it was an advantage to be able to dispense with aqueous solutions of drugs. Preparations in liquid paraffin produced satisfactory responses for at least as long as those bathed in Krebs solution.

In depolarization experiments, all drugs were dissolved in Krebs solution.

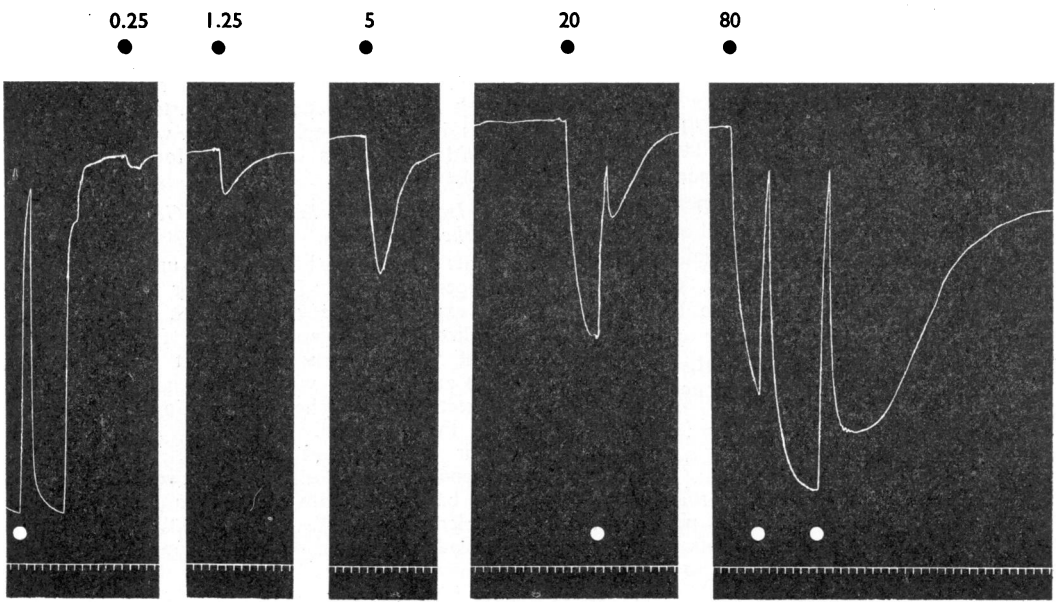

FIG. 1.—Record of response of the nictitating membrane in a cat (4.4 kg., pentobarbitone anaesthesia) to continuous stimulation of the preganglionic nerve to the superior cervical ganglion of that side for the period indicated by the black line below. At the white dots, the postganglionic nerve was stimulated for 30 sec. The numerals above the black dots indicate the quantities of paraldehyde in mg. contained in 0.25 ml. injections intra-arterially to the ganglion. At the time of maximal reduction of the retraction of the nictitating membrane following the last two paraldehyde injections postganglionic stimulation was fully effective. Time, 30 sec.

RESULTS

Nictitating Membrane Preparation in the Cat

Paraldehyde. — If paraldehyde was injected intra-arterially during continuous preganglionic stimulation, the response of the nictitating membrane was reduced and the reduction in the retraction increased with increasing dosage. With total injection volumes of 0.25 ml., 0.25 mg. of paraldehyde usually produced a just perceptible effect. A 50% reduction was caused by about 15 mg. and complete relaxation of the membrane occurred with about 80 mg. of paraldehyde. The block seemed to be ganglionic because post-ganglionic stimulation remained fully effective. Recovery from the blocking action of small and moderate quantities of paraldehyde was full and not unduly delayed. Large doses gave rise to delayed recovery of transmission which was not complete even after 1 hr. These effects are illustrated in Fig. 1.

The time course of the block with paraldehyde was not dissimilar from that seen with a concentration of hexamethonium giving a comparable degree of block.

Much larger doses of paraldehyde had to be given intravenously to produce a block in ganglionic transmission. In one experiment even after 1 ml. of paraldehyde B.P. administered over a period of 6 min. as an intravenous infusion, block was not quite complete. Postganglionic stimulation gave a maximal retraction of the membrane, but the response to preganglionic stimulation showed no sign of recovery 1 hr. later.

Methylpentynol.—With intra-arterial injections in a total volume of 0.25 ml., a reduction in the retraction of the nictitating membrane in response to continuous stimulation usually appeared with 0.25 mg. of methylpentynol. With increasing amounts of methylpentynol, there was an increasing reduction of the retraction. About 3 mg. caused a 50% reduction and 20 mg. usually produced complete or almost complete abolition of the retraction. With all but the largest doses of methylpentynol in this range, the block was much more rapid in onset and the recovery much quicker than with doses of paraldehyde producing comparable reductions in the retraction of the

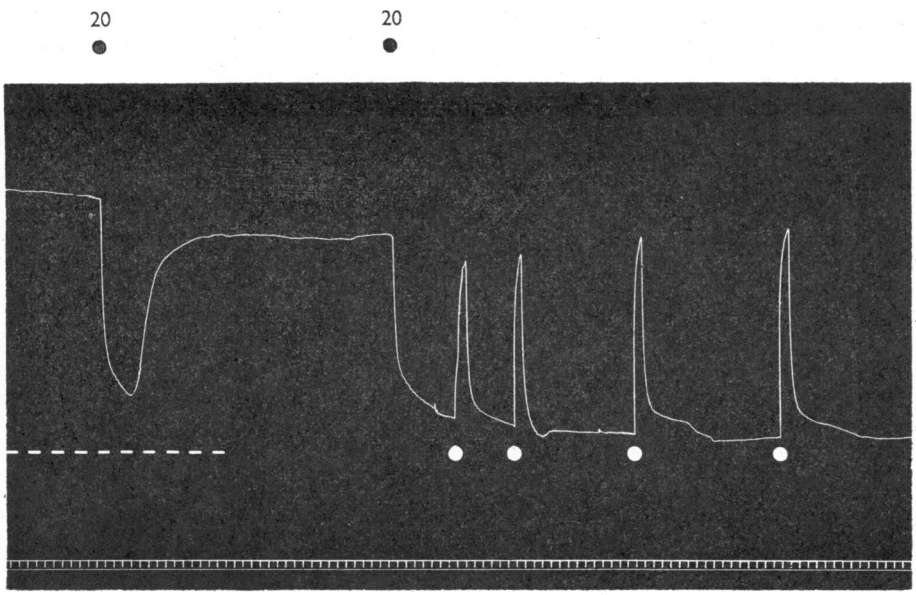

FIG. 2.—Record of response of the nictitating membrane in a cat (3.27 kg., pentobarbitone anaesthesia) to continuous stimulation of the preganglionic nerve to the superior cervical ganglion of that side for the period indicated by the black line below. At the white dots, the postganglionic nerve was stimulated for 30 sec. The numerals above the black dots indicate the quantities of methylpentynol in mg. contained in 0.25 ml. injections intra-arterially to the ganglion. The second injection of 20 mg. of methylpentynol produced almost complete relaxation of the membrane from which no recovery was observed 1 hr. later. The level of complete relaxation of the nictitating membrane is indicated by the horizontal broken white line. Time, 30 sec.

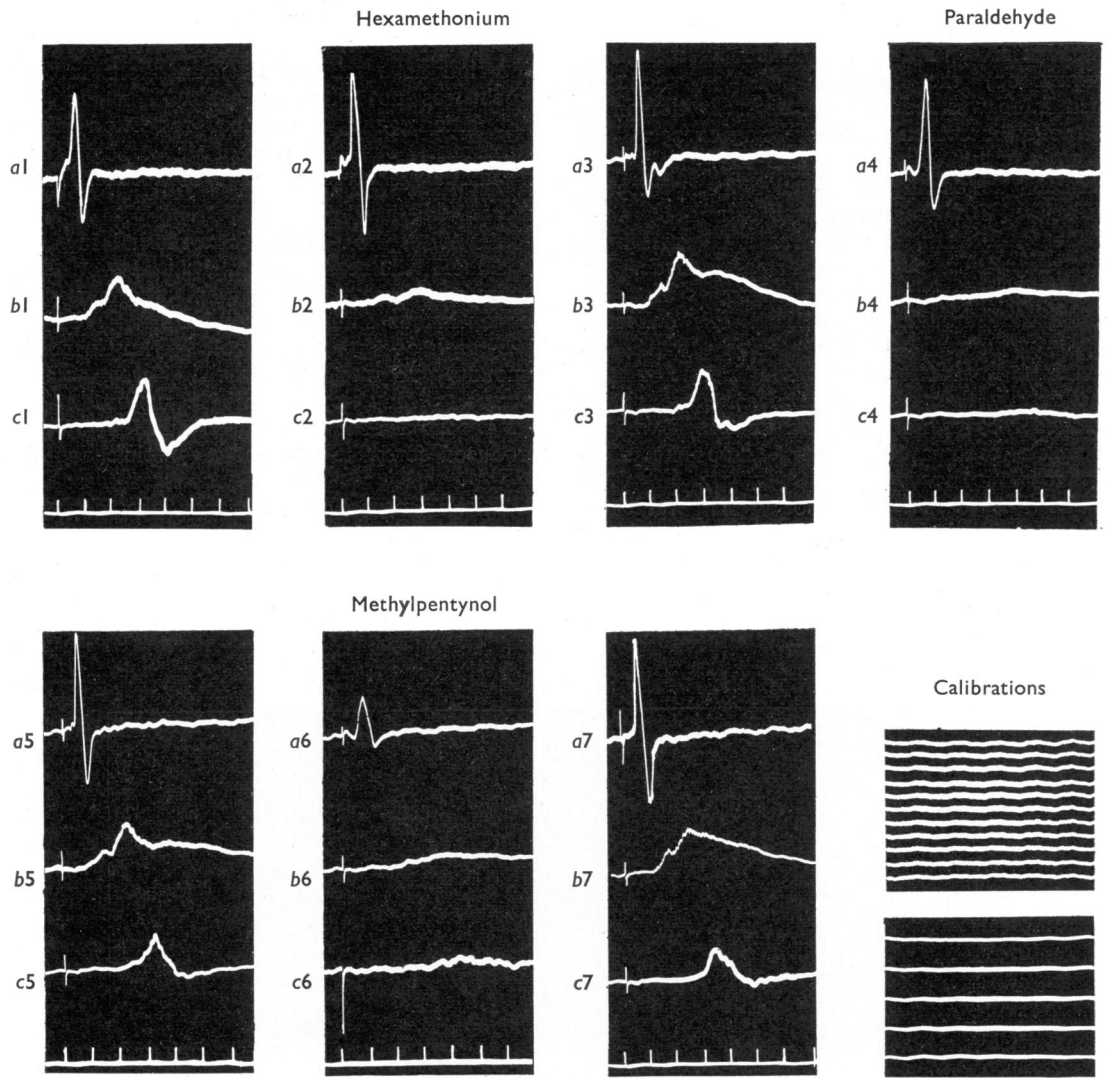

FIG. 3.—The isolated superior cervical ganglion of the rat bathed in liquid paraffin. Recordings of the electrical changes in the preganglionic nerve (series ***a1***, ***a2***, ***a3***, etc.), in the ganglion (series ***b1***, ***b2***, ***b3***, etc.) and in the postganglionic nerve (series ***c1***, ***c2***, ***c3***, etc.) after a single stimulus applied to the preganglionic nerve. See text for fuller explanation. When the ganglion was treated with 500 μg./ml. of hexamethonium in Krebs solution, the ganglionic potential was reduced (***b2***) and the postganglionic nerve action potential was abolished (***c2***) indicating a complete block of ganglionic transmission. After washing in Krebs solution, transmission through the ganglion was restored as is shown by the return of the ganglionic potential (***b3***) and the postganglionic nerve action potential (***c3***). Similar reversible blocks of ganglionic transmission are shown with paraldehyde (12 mg./ml.) and methylpentynol (4 mg./ml.) each of which was applied to the preparation mixed with liquid paraffin. With methylpentynol, the preganglionic action potential was reduced. Only a small increase in the amount of methylpentynol above that producing ganglionic block caused an effect on the preganglionic nerve. The blocking actions of both paraldehyde and methylpentynol were reversed by washing in liquid paraffin. Time, 20 msec. The upper calibration is a series of 100 μV. steps and applies to all records in the series indicated by the letter ***a*** and ***b*** and also to ***c6***. The lower calibration is in 1 mV. steps and refers to all records in the series labelled ***c*** except ***c6***.

membrane. With 20 mg. of methylpentynol, the recovery from the block was slower than with smaller quantities. After repeated intra-arterial injections of 20 mg. of methylpentynol, the block was sufficiently prolonged to test the response to postganglionic stimulation which was full or only slightly and temporarily impaired. There was little if any recovery of ganglionic transmission after methylpentynol block when two or more intra-arterial injections of 20 mg. had been given (Fig. 2).

When 0.5 to 1 ml. of pure methylpentynol was slowly administered intravenously, a prolonged block of ganglionic transmission occurred.

Electrical Responses Recorded from the Isolated Superior Cervical Ganglion of the Rat

Effects on Transmission Through the Ganglion.—Fig. 3 illustrates the electrical responses recorded from an isolated ganglion preparation bathed in liquid paraffin at room temperature when the preganglionic nerve was stimulated electrically. The nerve action potential in the preganglionic nerve is shown in Fig. 3*a1*. Potentials recorded from the preganglionic nerve are all denoted as Fig. 3*a1*, *a2*, *a3*, etc. The potential from the ganglion is depicted in Fig. 3*b1*; all ganglion potentials are labelled Fig. 3*b1*, *b2*, *b3*, etc. The action potential recorded from the postganglionic nerve is shown in Fig. 3*c1*, all postganglionic potentials being designated Fig. 3*c1*, *c2*, *c3*, etc.

In the series Fig. 3*a1*, *b1*, and *c1*, the preganglionic nerve action potential arose after a brief latent period (*a1*). The ganglion potential (*b1*) developed after a longer latent period as might be expected from the time required for the impulse to be conducted down the preganglionic fibre and to be transmitted across the ganglionic synapse. The postganglionic potential (*c1*) arose later still as still more time had to elapse between the moment of stimulation (stimulus artifact) and the time at which the postganglionic impulse passed the recording electrodes on the postganglionic trunk.

If transmission of the nerve impulses across the ganglionic synapse was blocked then a reduction of the ganglionic potential (which contains components of electrical changes in the ganglion cells and in the postganglionic fibres arising therefrom) and a reduction or abolition of the postganglionic nerve action potential with partial or complete ganglionic block respectively would be observed. There should be no change in the nerve action potential recorded from the preganglionic nerve if block is confined to the ganglion.

Hexamethonium.—When the ganglion was soaked in Krebs solution containing 500 μg./ml. of hexamethonium bromide, a complete block of ganglionic transmission occurred, the postganglionic potential being abolished (Fig. 3*c2*) and the ganglion potential reduced (*b2*). The preganglionic action potential (*a2*) was slightly increased, possibly for the reasons given below. After washing the preparation thoroughly with Krebs solution, ganglionic transmission was completely restored (Fig. 3*b3* and *c3*).

For recording, Krebs solution was replaced with liquid paraffin. Changes in the film of Krebs fluid investing the nerve when the preparation was immersed in oil may account for the slight differences in recordings between washes with Krebs solution in spite of attempts to remove all the Krebs solution completely from the preparation with slips of blotting paper. Such differences are those seen when the records in Fig. 3*a1*, *a2*, *a3*, *a4*, *a5*, and *a7* are compared.

Paraldehyde.—The miscibility of paraldehyde with liquid paraffin enabled the ganglion to be exposed to various concentrations of this drug without resorting to aqueous solutions. In the experiment illustrated in Fig. 3, 12 mg./ml. of paraldehyde in liquid paraffin was required to produce a complete block of transmission through the isolated ganglion, the ganglionic potential being reduced (*b4*) and the postganglionic nerve action potential abolished (*c4*). Repeated washing in liquid paraffin restored ganglionic transmission (Fig. 3*b5* and *c5*).

Methylpentynol. — When the ganglion was bathed in liquid paraffin containing 4 mg./ml. of methylpentynol, the ganglion potential (*b6*) was reduced and postganglionic nerve action potential abolished (*c6*). There was some reduction in the preganglionic nerve action potential (*a6*). That this block of ganglionic transmission was reversible can be seen from Fig. 3*b7* and *c7* in which, after repeated washing in liquid paraffin, the ganglion and postganglionic nerve action potentials were restored to near their values at the beginning of the experiment.

It was usually possible to find a concentration of methylpentynol which blocked ganglionic transmission without diminishing the preganglionic nerve action potential. Only a slight increase in this concentration produced impairment of conduction in the preganglionic nerve, and the diminished height of the preganglionic nerve action potential illustrated in Fig. 3*a6* may be accounted for in this way. This effect was reversed by washing (Fig. 3*a7*).

Depolarization Experiments.—To find whether paraldehyde in concentrations producing complete ganglionic block depolarized the ganglion cells directly or interfered with the depolarizing action of acetylcholine on the ganglion cells, experiments with the moving fluid electrode technique were carried out. First, a concentration of acetylcholine required to produce about 50% of the maximal depolarization of the ganglion was determined. In one experiment, 25 μg./ml. of acetylcholine in the bath fluid produced 2 mV. depolarization in 2 min. Next, 4 mg./ml. of paraldehyde in Krebs solution was found to abolish the post-ganglionic response to preganglionic stimulation. Under these conditions, no depolarization of the ganglion was recorded and the depolarizing activity of 25 μg./ml. of acetylcholine added to the fluid bathing the ganglion was unimpaired, there being 2 mV. of depolarization after 2 min. Pascoe (1956) reported that the hyperpolarization of the isolated rat superior cervical ganglion after washing off acetylcholine reached a peak in 2 min. This finding was confirmed in the present work and it was observed that paraldehyde in a blocking concentration did not modify the hyperpolarization seen after removal of acetylcholine from the bath fluid.

Discussion

The results show that paraldehyde and methylpentynol can block ganglionic transmission in the cat and in the rat. Previous work has shown that these two drugs can block neuromuscular transmission in the frog and in the rat (Quilliam, 1955). The results of the electrical studies of neuromuscular transmission in the frog by Nicholls and Quilliam (1956) could be accounted for if paraldehyde and methylpentynol reduce the release of acetylcholine. Direct estimates of acetylcholine release from the hind-quarters of the frog when perfused with Ringer solution containing paraldehyde in concentrations which abolished neuromuscular transmission in this preparation appeared to confirm this suggestion (Nicholls and Quilliam, unpublished observations).

The experiments with the nictitating membrane preparation in the cat localized the action of paraldehyde and methylpentynol to the ganglion because postganglionic stimulation was fully effective when the drugs had blocked the response to preganglionic stimulation.

The results from the electrical studies on the isolated rat superior cervical ganglion showed that the two drugs in ganglion blocking concentrations had no action on the preganglionic nerve fibre.

Depolarization studies excluded actions of paraldehyde producing a direct depolarization of the ganglion cell and a competitive block because the depolarizing activity of added acetylcholine was unchanged. In these experiments the amount of paraldehyde used was sufficient to block ganglionic transmission.

Taken together these results suggest that paraldehyde and methylpentynol produce ganglionic block by reducing the release of acetylcholine from the preganglionic nerve terminals. In this connexion it is interesting to note that Marley and Paton (1959) have found that methylpentynol can reduce the output of acetylcholine from the perfused cat superior cervical ganglion and that a reduction of 50% or more of the acetylcholine output was associated with failure of transmission.

Recently much attention has been directed to methylpentynol. Marley and Vane (1958) developed methods by which they were able to study the distribution of the drug and its carbamate in body fluids and in tissues, and suggested that the slow metabolism of both compounds might lead to cumulative effects. Marley (1959) made a wide study of the pharmacology of methylpentynol and its carbamate. This author confirmed the ganglion blocking action of methylpentynol reported by Quilliam (1957) and showed that the drug could also depress mono- and poly-synaptic reflexes.

The experimental evidence already referred to that methylpentynol can block synaptic transmission and the clinical impressions that the drug may allay apprehension (Trotter, 1953; Bourne, 1954) have led to attempts to evaluate the effect of the drug on the central nervous system. Dicker, Steinberg, and Watson (1957) have obtained evidence that methylpentynol appeared to reduce fear in rats. Dicker and Steinberg (1957) attempted to assess the effect of methylpentynol in man. They showed that, although the compound depressed the autonomic reactions of the subject in response to a difficult motor task, it impaired performance and they considered that its mode of action differed from that of hexobarbitone. The ganglion-blocking activity of methylpentynol reported here could account for the depression of autonomic responses found by Dicker and Steinberg (1957).

It is of practical interest that drugs may be applied to the isolated rat superior cervical ganglion mixed with liquid paraffin which is a particularly suitable medium in which to record nerve and ganglion action potentials. However,

with the ganglion immersed in liquid paraffin, about three times the concentration of paraldehyde required in Krebs solution was needed to produce complete block. This difference might be accounted for by the oil/water partition coefficient of the drug.

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