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THE EFFECT OF METHYLPENTYNOL AND METHYLPENTYNOL CARBAMATE ON THE PERFUSED SUPERIOR CERVICAL GANGLION OF THE CAT

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The action of methylpentynol and methylpentynol carbamate on the perfused superior cervical ganglion of the cat was analysed. Both drugs, in doses of 1 to 5 mg., depressed the output of acetylcholine. If the acetylcholine output was reduced by more than about 50%, transmission failed. Both drugs also reduced the response of the ganglion to injected acetylcholine. The carbamate, but not methylpentynol itself, had a transient stimulant action on the ganglion, unaccompanied by acetylcholine release.

Quilliam (1957, 1959) has obtained evidence that methylpentynol blocks transmission in the superior cervical ganglion of the cat. This has been confirmed for both methylpentynol and its carbamate (Marley, 1959), although the effect on the blood-bathed ganglion was evanescent. It remained, however, to determine whether these drugs have specific ganglion-blocking properties or depress ganglionic function in a less specific way, as do, for instance, the barbiturates (Exley, 1954).

METHODS

Cats anaesthetized with chloralose (80 mg./kg.) were used. Contractions of the nictitating membrane (after enucleation of the eyeball) were evoked by application of maximal shocks of 0.5 msec. duration at 10 c./s. to the cut peripheral stump of the cervical sympathetic, divided and separated from the vagus in the neck. The contractions were recorded on smoked paper by an isotonic lever with 4.0 g. load and seven-fold magnification. The superior cervical ganglion was perfused by the method of Kibjakow (1933) and Feldberg and Gaddum (1934), the perfusion fluid being warmed to the temperature of the cat by its passage through a polythene tube inserted into the stomach of the animal and which emerged from a small cut in the oesophagus close to the ganglion (Emmelin and Macintosh, 1956). The perfusion fluid was Locke solution, also containing eserine (5×10^{-6}) when acetylcholine output was to be measured. When the perfusion had been established and the last trace of blood washed from the system, samples of the effluent were collected usually for consecutive 6 min. periods, or for longer times if flow was slow. Preganglionic stimulation, when used, was applied only for the first 3 or 4 min. of these periods, the remaining interval being allowed for washing out the acetylcholine released during the stimulation by a sufficient volume of perfusate. The acetylcholine in the perfusate was assayed against acetylcholine chloride on a strip of guinea-pig ileum treated with 5 μg./l. of neostigmine and 10 mg./l. of morphine (Paton, 1957). That the substance in the perfusate released by stimulation of the preganglionic sympathetic was acetylcholine was verified first by showing that the contractor effect on the guinea-pig ileum was lost after the addition of hyoscine (10^{-7}) to the bath, and second by making a sample alkaline and standing it at room temperature for 20 min., neutralizing, then reassaying to demonstrate absence of contractor activity. Activity did not appear unless eserine had been added in adequate proportions to the perfusion fluid. The outputs shown in Figs. 3 and 4 are expressed ng. in unit time, so that the area of each rectangle gives the total output during each period.

For intra-arterial injection, pure methylpentynol was dissolved in Locke solution (20 mg./ml.). A similar concentration of methylpentynol carbamate could be dissolved in Locke solution if the solution was gently warmed and then allowed to cool. Acetylcholine chloride prepared in 0.9% saline was used for assay or injection.

RESULTS

Action on Perfused Superior Cervical Ganglion of the Cat

Both methylpentynol and its carbamate (in intra-arterial doses of 25 to 50 mg.) act at the blood-bathed ganglion to produce a relaxation of the nictitating membrane contracted by continuous preganglionic stimulation of the cervical sympathetic nerve, while the post-

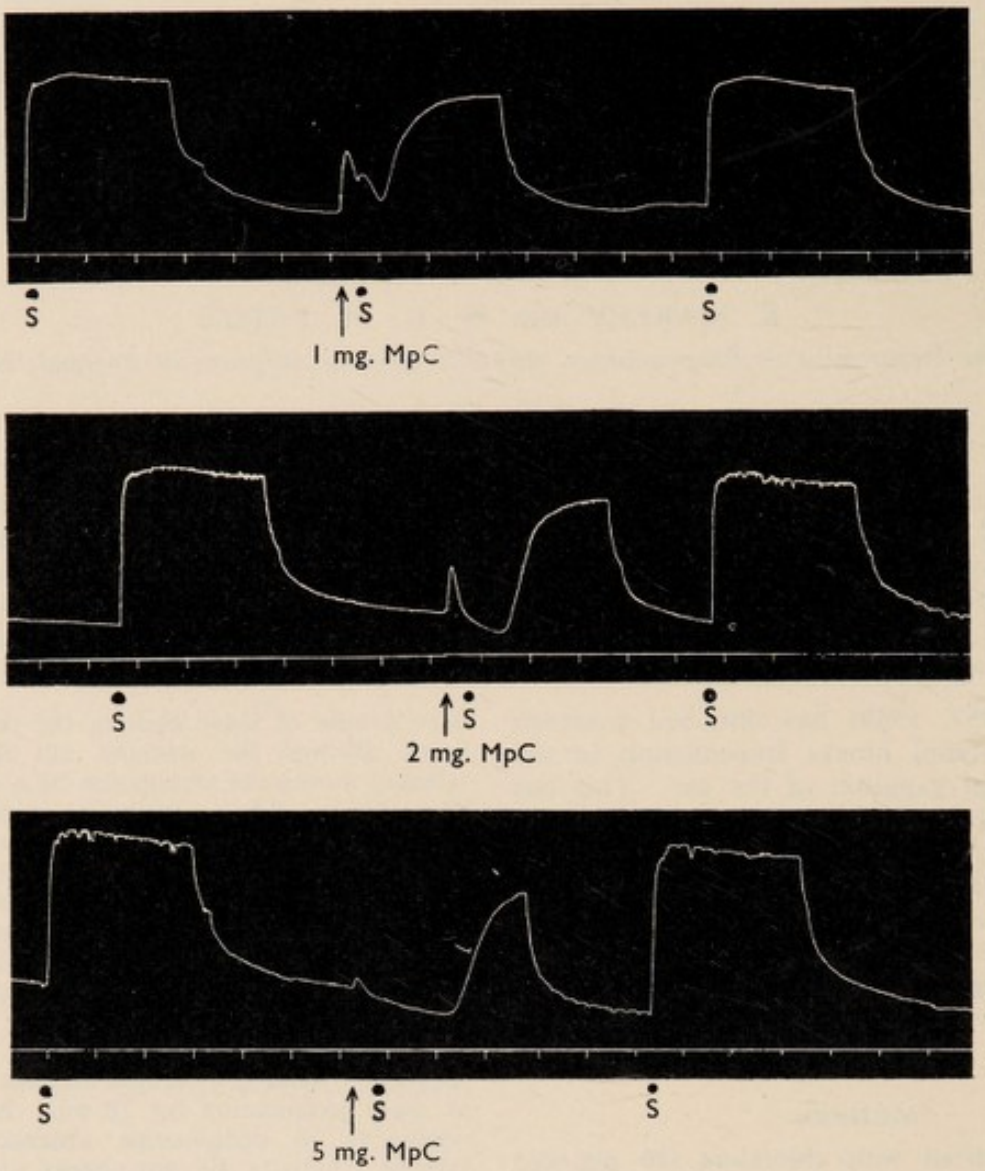

FIG. 1.—Cat, 1.7 kg.; chloralose anaesthesia. Perfused superior cervical ganglion preparation. Effect of various doses of methylpentynol carbamate (**MpC**) given intra-arterially on the contracture of the nictitating membrane excited by stimulation of the cervical sympathetic sustained for 3 min. at 10 sec. (at **S**). Note also small contraction elicited by methylpentynol carbamate. Time, min.

ganglionic response is preserved (Marley, 1959) but the response of the intermittently stimulated nictitating membrane was only reduced by the intravenous infusion of very large doses (1,800 mg.) of either drug. With the perfused ganglion, likewise relatively large doses (1 to 5 mg.) were needed. In this preparation, injections into the perfusion cannula of the carbamate, but not of methylpentynol itself, sometimes produced a brief contraction of the nictitating membrane before the ganglionic block appeared (Fig. 1). This was never seen with injections to the blood-bathed ganglion, nor did the carbamate ever enhance the contraction produced by electrical stimulation of the cervical sympathetic. The contraction produced by the carbamate was not accompanied by release of acetylcholine in the perfusate.

If the ganglion was stimulated by acetylcholine injected into the perfusion cannula (eserine being absent from the perfusing fluid), the response of the nictitating membrane was transiently diminished by previous doses of 2.0 mg. methylpentynol or the carbamate into the perfusate, although the response to preganglionic stimulation was unaffected (Fig. 2). The response to injected acetylcholine was thus more readily antagonized than that in normal transmission. The acetylcholine response usually returned to normal within 3 to 6 min. after giving either drug. If the preganglionic response was reduced by higher

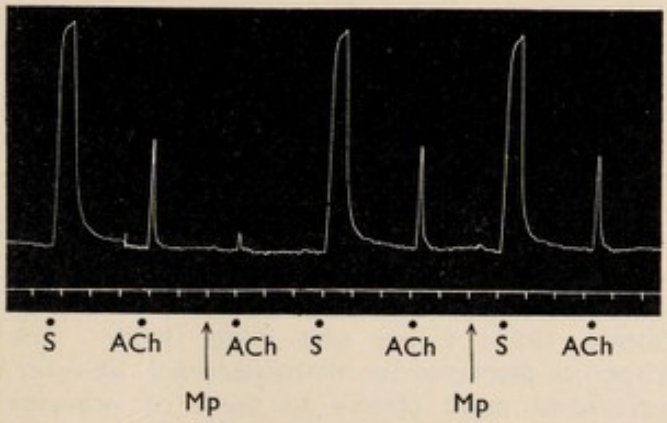

FIG. 2.—Cat, 5.3 kg.; chloralose anaesthesia. Perfused superior cervical ganglion preparation. Effect of 2.0 mg. of methylpentynol (**Mp**) on the response of the nictitating membrane to injections of 50 μg. of acetylcholine (**ACh**) or stimulation (**S**) of the cervical sympathetic 10/sec. for 1 min. Intra-arterial injections.

doses of methylpentynol or the carbamate, the effect was generally of greater duration than with the blood-bathed ganglion.

Effect on Release of Acetylcholine

Five experiments were made. In three, 1 mg. of methylpentynol or the carbamate reduced acetylcholine output during stimulation, although the mechanical response by the nictitating membrane was not necessarily affected. Thus in one experiment, after 1.0 mg. of methylpentynol, the response of the nictitating membrane declined, and the acetylcholine content of the effluent fell from 25 ng./ml. (total, 100 ng.; stimulation period, 4 min.) to 12 ng./ml. (total, 28 ng.). In another preparation after 1.0 mg. of the carbamate (the second injection seen in Fig. 3), the nictitating membrane response was unaltered, although the acetylcholine output fell from 16.2 ng./ml. (total, 27.5 ng.; stimulating period, 3 min.) to 8.3 ng./ml. (total, 13.4 ng.). The action of these doses was sometimes transient; thus, in the experiment in Fig. 3, the first injection of 1 mg. of the carbamate, given 6 min. before stimulation, failed to have any effect. On the other hand, in the same experiment, a larger dose (5 mg.) had a prolonged action; the output fell immediately from 17.5 ng./ml. (total, 24.2 ng.; stimulation period, 3 min.) to 3.7 ng./ml. (total, 3.9 ng.), followed by a partial recovery (10.6 ng./ml.; total 12.8 ng.) during which the nictitating response returned to normal. Sometimes even 1 mg. of either drug had a prolonged action; in one such experiment injections of 3 μg. and 2.5 mg. of choline in successive periods failed to relieve the reduction of acetylcholine output.

In one experiment, 10 mg. of methylpentynol was injected. The response of the nictitating membrane disappeared, but the total quantity of acetylcholine in the immediate stimulated specimen rose from 8 ng./ml. (total, 38.4 ng. in 14 min.) to 37 ng./ml. (total 66.6 ng. in 14 min.) but declined thereafterwards (Fig. 4). That this augmented acetylcholine output may have been merely the result of a damaging action of the drug on the ganglion, releasing substances usually bound, is suggested from another experiment in which a gut-relaxing substance appeared in the perfusate (in the absence of eserine) after the injection of the same amount of methylpentynol. The relaxation of the guinea-pig ileum observed was too great to have been due to methylpentynol itself, and the relaxant substance did not diminish the acetylcholine contractions of the rat uterus. The observation was not pursued, since block could be produced by smaller doses without these additional effects.

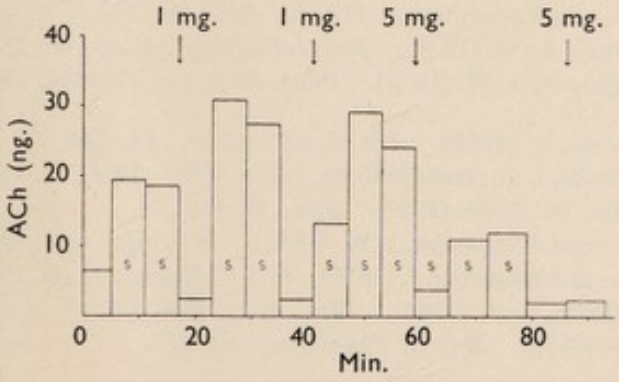

FIG. 3.—Histogram of acetylcholine output from a perfused superior cervical ganglion of the cat. Effect of various doses of methylpentynol carbamate (doses in mg. intra-arterially at arrows) on output of acetylcholine (**ACh**) in 6 min. periods; stimulation of cervical sympathetic for 3 min. during periods marked **S**.

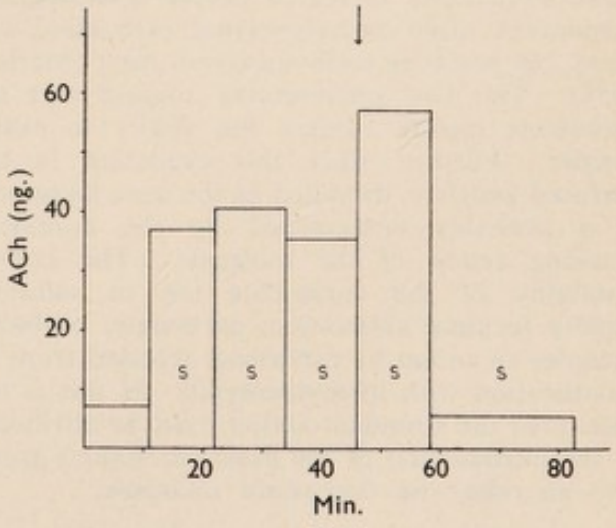

FIG. 4.—Histogram of acetylcholine output from a perfused superior cervical ganglion. Effect of 10 mg. of methylpentynol (intra-arterially at arrow) on output of acetylcholine (**ACh**) in 12 min. periods; stimulation of cervical sympathetic for 3 min. during periods marked **S**.

Methylpentynol or the carbamate in concentrations of 0.3 to 0.5 mg./ml. in the assay bath were found to antagonize acetylcholine about 2.3-fold: methylpentynol or its carbamate was therefore added to the standard acetylcholine solutions during the assays of samples of the perfusate following injections of these drugs.

Discussion

Nicholls and Quilliam (1956) concluded that the neuromuscular blocking activity of methylpentynol could be accounted for only if it reduced the amount of acetylcholine released by nerve impulses. They had shown that neuromuscular blocking concentrations of methylpentynol with a frog nerve-muscle preparation do not block transmission in the nerve, do not depolarize the end plate region, do not act like tubocurarine, and do not decrease the excitability of the muscle membrane. In our experiments, it was shown that these drugs do indeed depress acetylcholine release.

Methylpentynol does not, however, reduce acetylcholine output very powerfully. Transmission failed when the output had been reduced by roughly 50% or more; but it is known that, during continued excitation, outputs considerably lower than this are compatible with almost normal transmission (Brown and Feldberg, 1936). Failure of transmission must also depend, therefore, on the reduction by methylpentynol of the response of the ganglion cell to acetylcholine.

The excitant action of the carbamate is of interest, since it has also been found that, in the intact animal, the pressor response to splanchnic nerve stimulation increased before a subsequent diminution, after methylpentynol carbamate was given, but not after methylpentynol itself (Marley, 1959). The two observations suggest that the carbamate moiety confers the ability to excite ganglia. Further, since this excitation in the perfused ganglion dwindled as the dose increased, it is probably antagonized by the dominant blocking action of the molecule. The known instability of the carbamate ion in solution, rapidly forming ammonium carbonate, probably excludes an action by carbamate released from its esterification with methylpentynol. If this is the case, then the stimulant action must be attributed to the introduction of the basic carbamino group into an otherwise depressant molecule.

The combination of reduced acetylcholine output and antagonism to injected acetylcholine, as a result of injection of these drugs into the ganglion, suggests that they should be regarded as general ganglion depressants. Specific ganglion blocking agents such as curarine, nicotine, the methonium salts, and pempidine produce ganglion block without interfering with release of acetylcholine (Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934; Paton and Zaimis, 1951; Corne and Edge, 1958). The properties displayed by methylpentynol, however, correspond more closely to those of procaine (Harvey, 1939), procaine amide (Paton and Thompson, 1953) and the barbiturates (Exley, 1954). It may well be the case that any drug capable of depressing the activity of nervous tissue in some way will as a general rule reveal this action at the ganglionic synapse too, presynaptically by interfering with normal release of the transmitter, and postsynaptically by desensitizing the recipient neurone to its action.

This work was carried out during the tenure by one of us (E. M.) of a Medical Research Council Clinical Research Fellowship. We are indebted to British Schering Ltd. for a generous supply of methylpentynol and methylpentynol carbamate, and to Mr. D. A. Green and his staff for technical assistance.

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