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THE MECHANISM OF ACTION OF PARALDEHYDE AND METHYLPENTYNOL ON NEUROMUSCULAR TRANSMISSION IN THE FROG

BY

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Paraldehyde and methylpentynol block neuromuscular transmission (Quilliam, 1955), and the object of the present study was to analyse the mode of action of the two drugs using electrical methods. The experimental evidence obtained suggests that the neuromuscular blocking activity of paraldehyde and of methylpentynol can be accounted for by their action in decreasing ACh release. Some of the results were communicated to the British Pharmacological Society in July, 1954, and in January, 1955.

METHODS

The *extensor longus digiti IV* muscle of the frog was used in all experiments. The preparation was carefully dissected with its nerve and soaked in frog-Ringer solution containing 1×10^{-6} neostigmine for 30 min. before beginning each experiment. Only uninjured preparations were used, since even one injured fibre gives rise to large injury potentials. The muscle was mounted vertically in a bath with the proximal nerve-free end uppermost (Fatt, 1950). Recordings were made through silver-silver chloride electrodes which fed through balanced cathode followers and a D.C. amplifier (Copeland, 1952) to an oscilloscope equipped with a camera.

End-plate potentials were recorded in the manner described by Del Castillo and Stark (1952), square wave stimuli being applied to the nerve through platinum electrodes. A concentration of between 1.6×10^{-6} and 3.2×10^{-6} tubocurarine ensured the production of a pure end-plate potential (e.p.p.) by nerve stimulation.

The depolarizing action of a drug was studied by measuring the potential produced in the region of maximum end-plate density. To avoid errors in localization of this region, a moving fluid electrode was used to record the potential distribution along the whole muscle surface (see Fatt, 1950; Del Castillo and Stark, 1952). Increasing concentrations of a depolarizing drug, such as ACh, produce progressively larger depolarizations of the end-plate region, and a dose-response curve can be drawn of the end-plate depolarization against ACh concentrations (Fatt, 1950).

RESULTS

The Effect of Paraldehyde on the End-plate Potential

Paraldehyde greatly reduced the size of the e.p.p. in every experiment. The e.p.p. (produced by nerve stimulation in the presence of the blocking concentration of tubocurarine) remains constant for many minutes, and the action of various concentrations of paraldehyde can thus be accurately measured. The relationship between the magnitude of the e.p.p. and paraldehyde concentration is plotted in Fig. 1. The effect of paraldehyde was rapidly and usually completely reversed by washing. After treatment with tubocurarine as described, the e.p.p. was completely abolished by the addition of 2×10^{-3} paraldehyde, which was the minimum

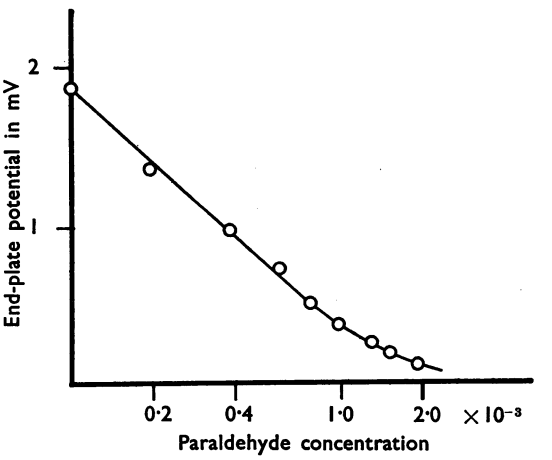

FIG. 1.—To show the relation between the magnitude of the tubocurarine end-plate potential in mV and the log of the paraldehyde concentration in the Ringer solution bathing the *M. extensor longus digiti IV* of the frog.

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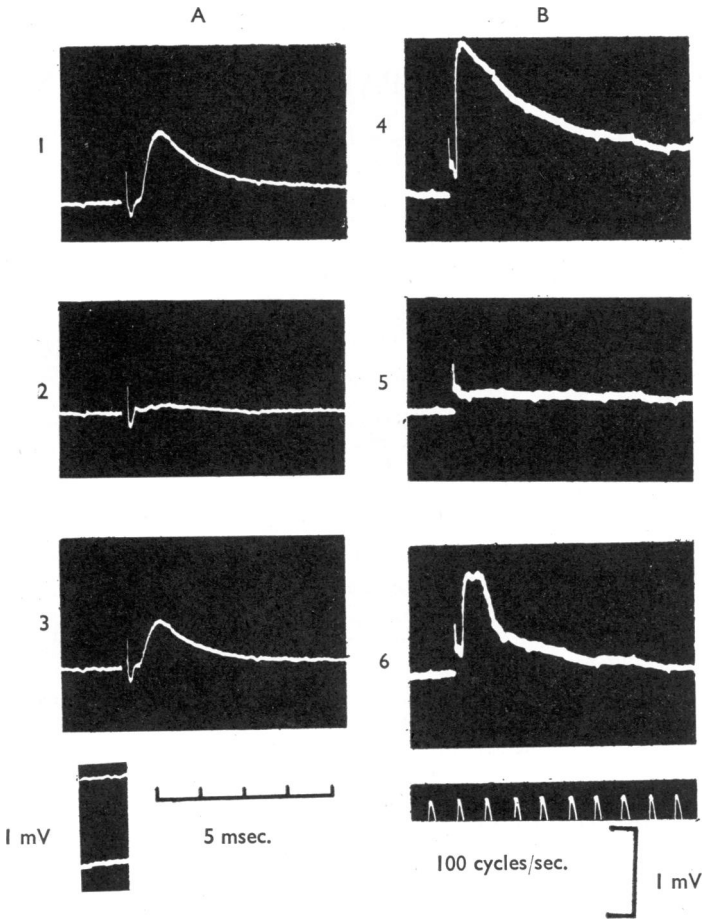

FIG. 2.—The abolition of the end-plate potential recorded from the *M. extensor longus digiti IV* of the frog by that concentration of paraldehyde which had to be attained before neuromuscular block was complete in a fresh muscle. A, Records made in the presence of 3.1×10^{-6} tubocurarine. 1, Before, and 2, during exposure to 2×10^{-3} paraldehyde; 3, recovery of e.p.p. after washing. B, Records made in the presence of 4×10^{-7} ACh. 4, Before, and 5, after soaking in 2.4×10^{-3} paraldehyde; 6, recovery of e.p.p. after washing. In A, note the small downward deflection preceding the e.p.p., due to the nerve action potential which was unaltered by paraldehyde.

concentration of paraldehyde required to block neuromuscular transmission when previously applied to the fresh preparation (Fig. 2A).

Paraldehyde had the same effect when a drug other than tubocurarine was used to demonstrate the e.p.p. Thus in two experiments ACh in concentrations between 2 and 4×10^{-7} was used to produce neuromuscular block (Del Castillo and Stark, 1952); the e.p.p. after nerve stimulation was reversibly abolished by the blocking concentration of paraldehyde (Fig. 2B).

The abolition of the e.p.p. by paraldehyde may be due to one or more of the following mechanisms: (1) block of transmission in the nerve, (2) prevention of the release of ACh from the motor nerve endings, (3) decrease in the sensitivity of the end-plate region to ACh, (4) persistent depolarization of the end-plate region, or (5) decrease in the excitability of the muscle fibres. It was not possible to study directly the release of ACh by the motor nerve endings in this preparation, but the effect of paraldehyde on the nerve, on the end-plate region, and on the muscle membrane could be measured.

Effect of Paraldehyde on Nerve

In all experiments, the nerve action potential was measured before and after adding the blocking concentration of paraldehyde. No change was observed in the amplitude or form of

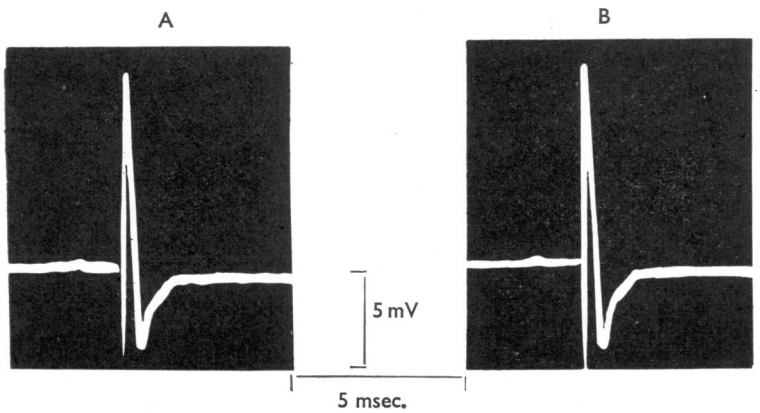

FIG. 3.—The action potential recorded from the motor nerve to the *M. extensor longus digiti IV* of the frog: A, before, and B, after soaking in that concentration of paraldehyde which caused a complete neuromuscular block in the preparation.

the potential (Fig. 3). In some experiments, it was possible to record the nerve action potential as a small downward deflection preceding the e.p.p. Such an experiment is illustrated in Fig. 2A, in which the nerve action potential was uninfluenced by paraldehyde.

Effect of Paraldehyde on the Depolarization Produced by ACh

Increasing concentrations of ACh in the fluid bathing a muscle produced progressively greater depolarizations of the end-plate region. When the e.p.p. reached a critical level, muscle action potentials were initiated. A typical dose-response curve is shown in Fig. 4 (solid circles), which relates the peak depolarization of the end-plate region to ACh concentration. Such a curve is an index of the sensitivity of the end-plate region to added ACh. The effect of paraldehyde on the sensitivity to added ACh has been studied, therefore, in the following way. The ACh dose-response curve was determined in a fresh preparation. Thereafter, the minimum concentration of paraldehyde which produced neuromuscular block—so that nerve stimulation then gave a pure e.p.p.—was determined. The muscle was then soaked in this concentration of paraldehyde and the ACh dose-response curve was measured again. The blocking concentration of paraldehyde had little or no effect upon the position or shape of the curve (open circles in Fig. 4). Furthermore, the threshold concentration of ACh which must be added for the initiation of *action potentials in the muscle* was unaltered by the

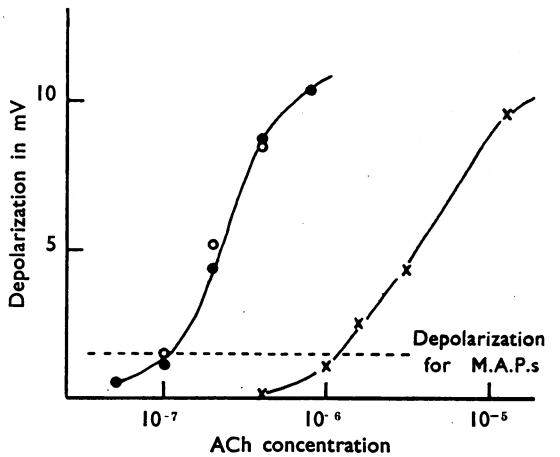

FIG. 4.—*M. extensor longus digiti IV.* Acetylcholine dose-depolarization response curves. Solid circles, normal muscle; open circles, after soaking in paraldehyde (2×10^{-3}); and crosses, after treating with tubocurarine (3.1×10^{-6}). The depolarization required to initiate muscle action potentials is indicated by the dotted line.

blocking concentration of paraldehyde (dotted line in Fig. 4). This is quite different from the action of a competitive blocking agent such as tubocurarine. In the same preparation, the blocking concentration of tubocurarine shifted the ACh dose-response curve to the right by about one $\log_{10}$ unit (crosses in Fig. 4). This shows that, to produce a given depolarization in the presence of tubocurarine, about ten times more ACh must be added to the fluid bathing the muscle. Consequently the pro-

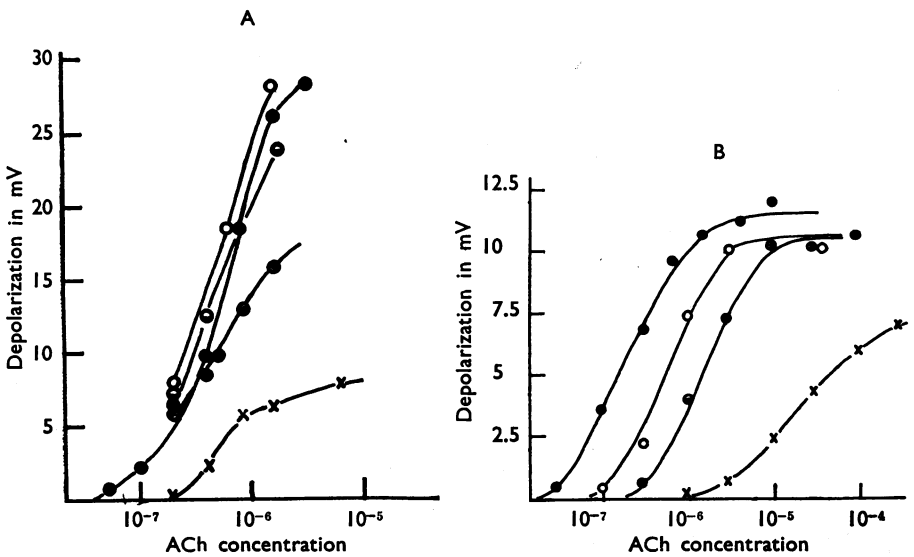

FIG. 5.—Dose-response curves as in Fig. 4. A, in paraldehyde (O, 5×10^{-4}; ◒, 1×10^{-3}; ⊙, 2×10^{-3}; x, 3×10^{-3}; ●, control). B, in tubocurarine (O, 5×10^{-7}; ⊙, 10^{-6}; x, 10^{-5}; ●, control).

duction of neuromuscular block and the abolition of the e.p.p. do not seem to be due to a post-junctional action of paraldehyde.

High concentrations of paraldehyde, on the other hand, did modify the dose-response relation but not in the same way as did tubocurarine. Increasing paraldehyde concentrations reduced the maximum depolarization attained, but produced only a slight shift to the right (Fig. 5A). Some depression of the maximum was seen with the blocking concentration (2×10^{-3}), but this effect was reversible. The gross depression of the maximum with higher paraldehyde concentrations (e.g. 3×10^{-3}) was not reversed even by prolonged washing. Even the highest concentrations of paraldehyde used did not depolarize the muscle membrane at rest.

Increasing concentrations of tubocurarine tested in the same way shifted the ACh dose-response curve progressively to the right (Fig. 5B). As high a concentration as 1×10^{-5} was needed before some depression of the maximum, of the type seen with paraldehyde, occurred. In none of our experiments with tubocurarine alone was any depolarization of the muscle membrane seen under resting conditions.

Methylpentynol

In concentrations which caused neuromuscular block, methylpentynol suppressed the e.p.p. seen after tubocurarine or ACh. After exposing a foot muscle to that concentration of methylpentynol which caused neuromuscular block (1.2×10^{-3}), the depolarizing action of small doses of ACh was unimpaired whereas larger doses were about half as active as in a normal preparation (Fig. 6). The degree of depolarization necessary to set up muscle action potentials in response to added ACh was not modified by methylpentynol.

While in most muscles methylpentynol in a concentration of 1×10^{-3} produced no depolarization, a few preparations did show evidence of a slight depolarization. However, unlike paraldehyde, higher concentrations of methylpentynol, for example 7×10^{-3}, gave a marked and persistent depolarization which was greatest in the region of maximum end-plate density and was not reversed by washing.

Procaine

When a muscle was soaked in a concentration of procaine which caused neuromuscular block, the ACh dose-response curve was shifted to the right but not so far as with a blocking concentration of tubocurarine (Fig. 7). The blocking concentration of procaine had no effect upon the size or shape of the nerve action potential. The competitive effect precluded critical study by our methods of any effect which procaine might have had upon ACh release at the neuromuscular junction.

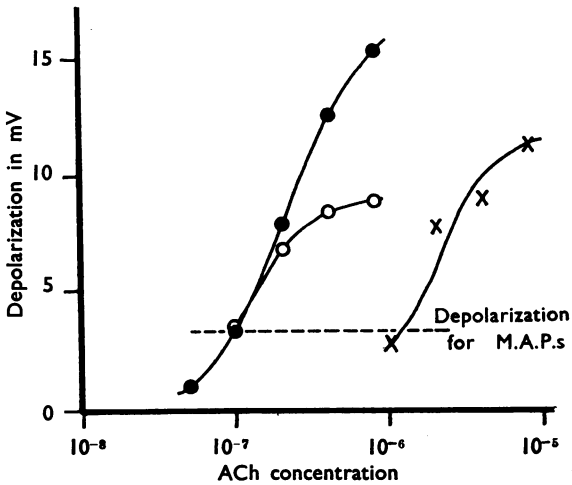

FIG. 6.—Dose-response curves as in Fig. 4. Solid circles, normal muscle; open circles, after soaking in methylpentynol (1.2×10^{-3}); and crosses, in tubocurarine (1.5×10^{-6}).

DISCUSSION

The results reported in this paper show that paraldehyde and methylpentynol, in concentrations

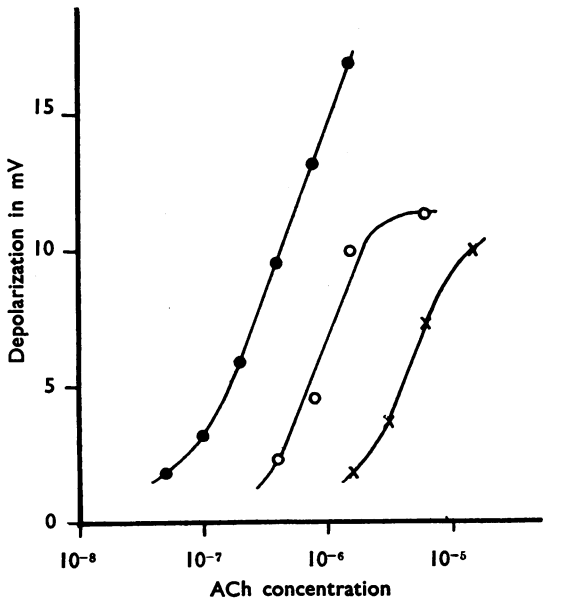

FIG. 7.—Dose-response curves as in Fig. 4. Solid circles, fresh muscle; open circles, after soaking in procaine (5.5×10^{-5}); and crosses, in tubocurarine (2.25×10^{-6}).

which block neuromuscular transmission, suppress the e.p.p. observed after tubocurarine or ACh. The actions of paraldehyde and methylpentynol differ from those of decamethonium and tubocurarine respectively in that they neither depolarize the muscle cell nor compete for ACh receptors. The two hypnotic drugs appear neither to modify the response of the muscle cell to added ACh nor to interfere with conduction of nerve impulses in nerve trunks. Thus, it seems that they must abolish the e.p.p. by reducing the amount of ACh released by nerve impulses at the terminals of the motor nerves, and that they owe their neuromuscular blocking activity to this action. Their effect does not damage the preparation and is readily reversible.

In the experiments with the blocking concentration of procaine, the dose-response curve was shifted to the right, but this shift was not as great as that produced by the blocking concentration of tubocurarine in the same muscle. In part at least, the neuromuscular blocking activity of procaine can be accounted for by a competitive action, and this effect precluded further analysis by our electrical methods of the mode of action of this drug.

Summary

1. Paraldehyde and methylpentynol reversibly block neuromuscular transmission in a frog nerve-muscle preparation.

2. Using electrical recording methods, it was found that both drugs abolished the end-plate potential set up by nerve stimulation in preparations in which transmission was blocked with tubocurarine or ACh.

3. The blocking concentrations of paraldehyde and methylpentynol do not, however, (*a*) block transmission in the nerve, (*b*) depolarize the end-plate region, (*c*) act like tubocurarine or (*d*) decrease the excitability of the muscle membrane.

4. It was concluded that the neuromuscular blocking activity of paraldehyde and methylpentynol could be accounted for only if they reduced the amount of ACh released by nerve impulses. This action does not damage the muscle cell and is reversible.

5. Procaine, in contrast, acted in a similar manner to tubocurarine by reducing the sensitivity of the end-plate region to ACh. It is suggested that the blocking action of procaine at the neuromuscular junction can be accounted for, in part at least, by its competitive action.

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