THE MECHANISM OF ACTION OF MUREXINE ON NEUROMUSCULAR TRANSMISSION IN THE FROG

BY

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Murexine (urocanoylcholine, [2-β-imidazol-4(5)-ylacryloyloxyethyl]trimethylammonium chloride hydrochloride) produced a contracture like acetylcholine in the frog rectus and a neuromuscular block in the rat diaphragm which was not relieved by neostigmine but was antagonized by hexamethonium. Using the foot muscle of the frog, electrical recordings showed that murexine produced a neuromuscular block and depolarized the end-plate region. These effects were similar to those seen with suxamethonium, decamethonium and acetylcholine. While murexine had the same depolarizing potency as decamethonium, it was only one-tenth as active as suxamethonium and acetylcholine. It was concluded that murexine could be classified as a " depolarizing type " of neuromuscular blocking agent but was less potent than suxamethonium.

Murexine (urocanoylcholine, [2-β-imidazol-4(5)-ylacryloyloxyethyl]trimethylammonium chloride hydrochloride) is a naturally occurring choline ester present in high concentration in the hypobranchial body of *Murex trunculus* and other prosobranchiate molluscs. It was first isolated by Erspamer (1948). In a study of the pharmacological actions of murexine, Erspamer and Glässer (1957) reported that it appeared to produce a " depolarizing type " of block in vertebrate neuromuscular transmission. The present paper gives an account of some experiments in which the neuromuscular blocking action of murexine on skeletal muscle has been studied both by pharmacological and by electrical methods.

METHODS

Isolated Frog Rectus Abdominis Muscle.—The muscle was excised from *Rana temporaria* and set up in a 2 ml. bath of normal frog Ringer solution bubbled with air. The contractures were recorded isotonically by a weighted gimbal-lever.

Isolated Rat Phenic Nerve-Diaphragm Preparation.—The method of Bülbring (1946) was modified by using Krebs solution bubbled with 5% CO_2+95% O_2. Single maximal shocks were delivered alternately to the nerve and muscle (Bell, 1957).

Isolated Extensor Longus Digiti IV Muscle *of the Frog.*—The foot muscle was carefully dissected with its nerve and soaked for 30 min. before beginning each experiment in frog Ringer solution containing 1×10^{-6} neostigmine, which was used for all foot muscle experiments. Muscle action potentials, end-plate potentials, and the depolarizing action of drugs

(utilizing the moving fluid electrode technique) were recorded using a D.C. amplifier in the manner described by Nicholls and Quilliam (1956).

RESULTS

Frog Rectus.—When added to the fluid bathing the rectus muscle, murexine produced a contracture which developed more slowly than that following a dose of acetylcholine producing a comparable response (Fig. 1). Murexine was about eight times less potent than acetylcholine. The dose/response relations for acetylcholine (solid circles) and for murexine (open triangles) are illustrated in Fig. 2. The slopes of the two curves are approximately parallel, but the curve for murexine is displaced to the right of that for acetylcholine.

Rat Diaphragm.—Murexine produced a neuromuscular block when added to the fluid bathing the diaphragm. This block was not antagonized by neostigmine but was partly reversed by hexamethonium (Fig. 3). The addition of murexine was followed in the experiment illustrated in Fig. 3 by a transient augmentation of the muscle twitch in response to nerve stimulation before neuromuscular block developed. This increased response was seen only once in 12 experiments, whereas Erspamer and Glässer (1957) reported that they often observed a short-lived increase in twitch tension after murexine which preceded neuromuscular block in their cat sciatic-nerve gastrocnemius preparations. Murexine was about
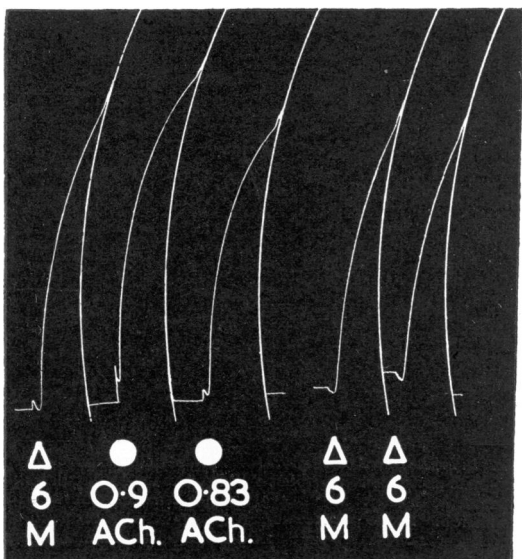
FIG. 1.—Isolated frog rectus abdominis muscle preparation. Comparison of the contractures elicited by a constant dose of murexine (M) with those following two different doses of acetylcholine (ACh). Bath volume, 2 ml. Contact time, 90 sec. The numerals indicate the dose of the drugs used in μg.
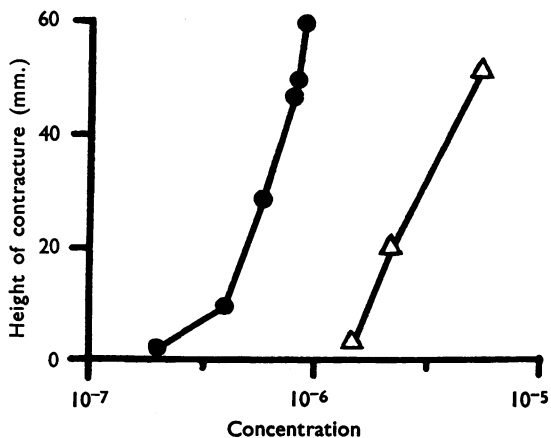

FIG. 2.—Dose/response curves obtained from an experiment with an isolated frog rectus muscle. Ordinate, height of contractures in mm. Abscissa, concentration of the drug used. ●=acetylcholine. △=murexine.
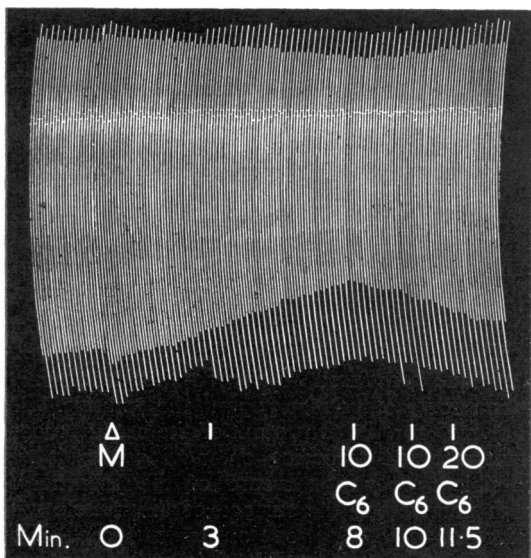

FIG. 3.—Isolated rat phrenic nerve-diaphragm preparation. Alternate stimulation of nerve and muscle. The twitches arising from direct simulation of the muscle are greater in amplitude than the indirect responses. 0.75 mg. murexine (M) was added at time 0. The response to nerve stimulation first suffered slight augmentation for five shocks and then was diminished in amplitude while the response to direct muscle stimulation was unaltered. At 8, 10, 11.5 min., 10, 10, and 20 mg. of hexamethonium (C_6) were added which antagonized the block. Bath volume, 50 ml. Temperature, 36°.
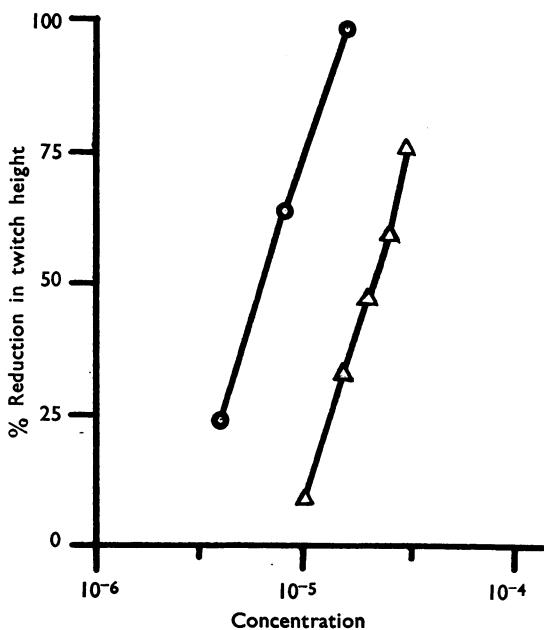

FIG. 4.—Dose/response curves obtained from an experiment with an isolated rat diaphragm. Ordinate, % reduction in twitch height. Abscissa, concentration of the drug used. O=suxamethonium. △=murexine.

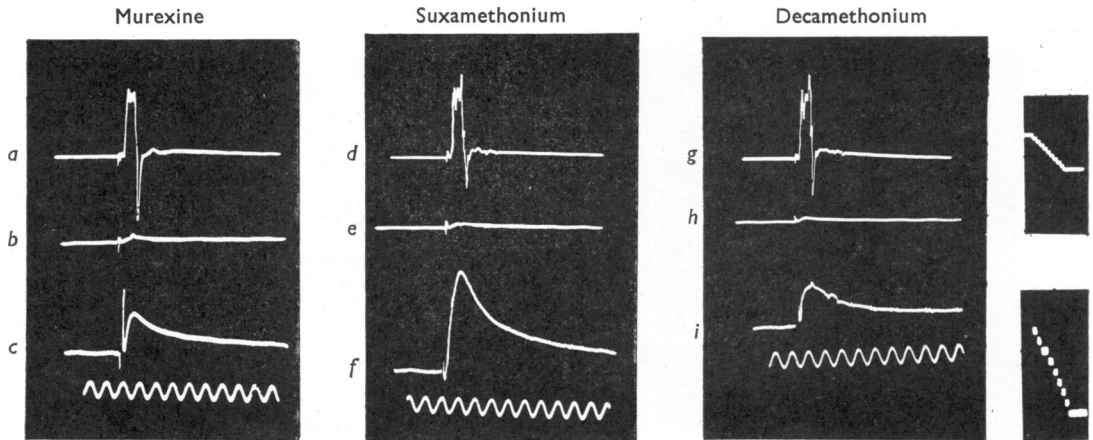Murexine   Suxamethonium   Decamethonium

FIG. 5.—Electrical recordings made from the isolated frog *Extensor Longus Digiti IV Muscle* at the point of entry of its motor nerve. Comparison of the muscle action potential before (*a, d,* and *g*) with the end-plate potential after producing complete neuromuscular block (*b, c, e, f, h,* and *i*). Between (*a*) and (*b*) murexine 2×10^{-5}, (*d*) and (*e*) suxamethonium 1×10^{-6} and (*g*) and (*h*) decamethonium 2×10^{-5} were added to the fluid bathing the muscle to block neuromuscular transmission. The respective end-plate potentials were recorded with the same amplification used for the muscle action potentials (*b, e,* and *h*). Records of the end-plate potentials at greater amplification are given in (*c*), (*f*), and (*i*). The records in (*b*) and (*i*) are modified by a muscle action potential from at least one unblocked neuromuscular junction. At the amplification used for the top and middle rows of records, a calibration (upper right) in 1 mV. steps is given. The calibration for records (*c*) and (*i*) in 100 μV. steps is given in the lower right part of the figure. The amplification used for record (*f*) was three times more than that used in (*c*). Time signal, 100 c./sec.

three times less potent in producing neuro-muscular block than was suxamethonium. The dose/response curves (Fig. 4) for suxamethonium (open circles) and for murexine (open triangles) were parallel, but that for murexine was displaced by about 0.5 log. units to the right of the curve for suxamethonium.

Electrical Studies with Isolated Extensor Longus Digiti IV Muscle *of the Frog*

The foot muscle was mounted vertically in a bath of frog Ringer containing 1×10^{-6} neostigmine with its distal extremity uppermost. For recording purposes one non-polarizable electrode was placed upon its distal end and another in the fluid below the muscle.

Neuromuscular Block.—Murexine in a concentration of 10 to 20 μg./ml. produced a complete neuromuscular block which was recognized by an absence of a twitch and of the muscle action potential when the motor nerve was stimulated and by the presence of an end-plate potential localized to the region around the point of entry of the nerve. In the foot muscle, most of the end-plates are located in this region. The features of the block with murexine are illustrated in Fig. 5a, b, and c and are compared with the block seen with appropriate concentrations of suxamethonium and decamethonium in the same muscle. All the records in Fig. 5 were made

when the bath fluid was lowered to the point of entry of the nerve into the muscle. The upper trace in each section is a record of the normal muscle action potential (Fig. 5a, d, and g). The middle traces are records of the end-plate potential at the same point on the surface of the muscle when completely blocked with 2×10^{-5} murexine (Fig. 5b), 1×10^{-6} suxamethonium (Fig. 5e) and 2×10^{-5} decamethonium (Fig. 5h). The lowermost traces are amplified records of the end-plate potentials seen in the middle traces. When acetylcholine was added to the bath fluid in a concentration which caused complete neuro-muscular block within 2 min., similar electrical results were observed.

Depolarization.—The moving fluid electrode technique was used to record the depolarizing action of compounds upon the muscle. By allowing the fluid to run out of the bath at a constant rate and recording on moving film, it was possible to obtain a record of the potential at each point along the surface of the muscle with reference to the electrode on its distal end. Such records are shown in Fig. 6 in which the horizontal axis corresponds to distance from the distal end of the muscle. The lowermost records in each group were made when normal frog Ringer bathing the muscle was allowed to run out of the bath. The small deflexions indicate little injury to the muscle cells. When a depolarizing drug was added to the bath fluid, depolarization developed
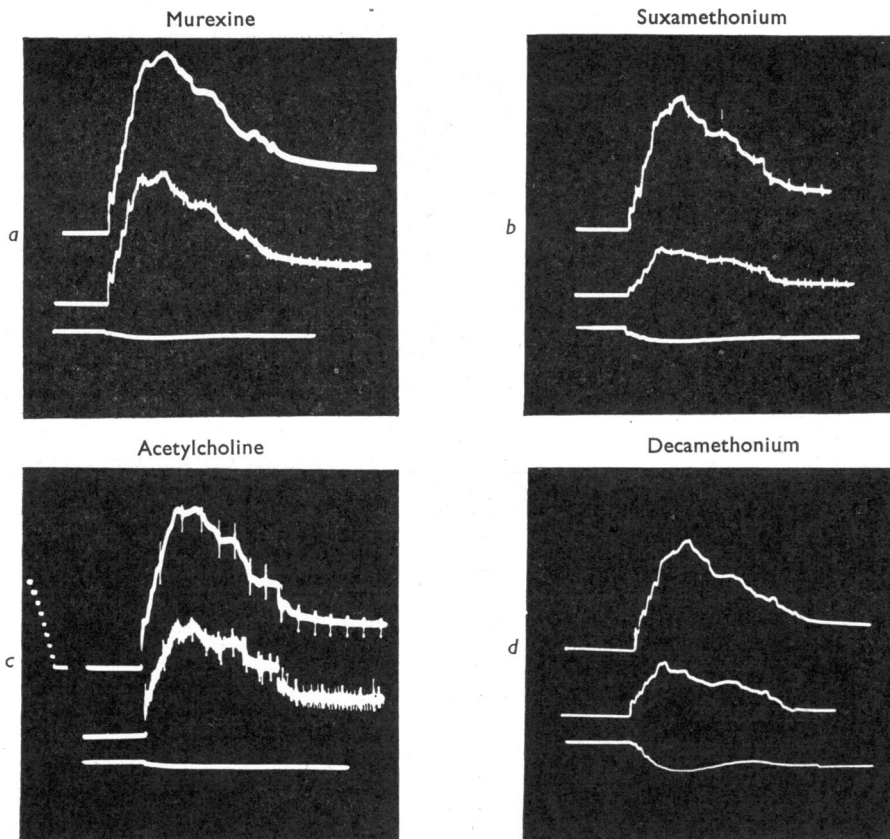
Fig. 6.—Records of depolarization of the *Extensor Longus Digiti IV Muscle* made with the moving fluid electrode technique as the solution ran out of the bath. The ordinate is voltage and a calibration signal in 1 mV. steps is provided on the left of record (c). Depolarization is shown by a vertical deflexion of the trace. The lowest trace in each group was made when frog Ringer solution ran out of the bath and shows that there was little injury to the muscle during dissection. The middle traces give the voltages of depolarization at 0.5 min. and the upper traces the maximum response after 2 min. exposure to depolarizing drugs. The peak of each depolarization record corresponded to the end-plate focus in the muscle. Asynchronous muscle action potentials fired off by the depolarization appear as brief vertical deflexions of the record. These potentials are most profuse in the early stages of depolarization represented by the middle traces in each group. The depolarizing agents used were in (a) murexine 2×10^{-5}, in (b) suxamethonium 1×10^{-6}, in (c) acetylcholine 1×10^{-6}, and in (d) decamethonium 2×10^{-5}. All the drugs produced substantial depolarization and asynchronous muscle action potentials although those with decamethonium were not photographed.

and was recorded as an upward deflexion of the trace. The middle record in each group shows the degree of depolarization which had developed in 0.5 min. and the upper records are of the maximum depolarization which was attained in 2 min. In all the experiments in which the muscles were in good condition, depolarizing drugs also gave rise to asynchronous muscle action potentials which were observed on the oscilloscope and were recorded upon the " depolarization sweeps " as in Fig. 6. The potentials were particularly in evidence during the early phase of the depolarizing action (Fig. 6 middle traces).

Murexine (2×10^{-5}) produced a marked depolarization of the foot muscle closely similar to that produced by appropriate concentrations of suxamethonium, acetylcholine and decamethonium (Fig. 6). Repeated washing restored the polarization of the muscle membrane after exposure to each of the four substances.

The voltage of depolarization produced by a drug is proportional to its concentration, and the dose/response curves for acetylcholine, suxamethonium, murexine, and decamethonium using the same muscle are given in Fig. 7. The curves for acetylcholine (solid circles) and for suxamethonium (open circles) are similar, being parallel to one another and lying in the same region of the graph. Those for murexine (hollow triangles) and decamethonium (solid triangles) fall in close proximity to one another and are approximately parallel to the curves for acetylcholine and suxamethonium but are displaced by about 1 log. unit to the right.
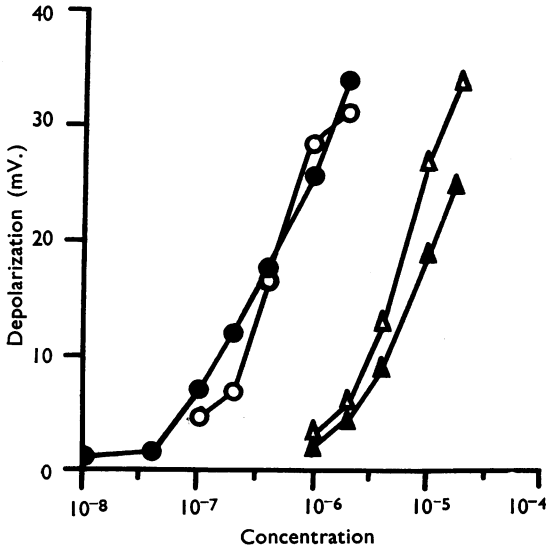
FIG. 7.—Dose/response curves relating voltage of depolarization of the *Extensor Longus Digiti IV Muscle* of the frog with the concentration of the drug used. ● = acetylcholine, O = suxamethonium, △ = murexine, and ▲ = decamethonium.

DISCUSSION

The results of the experiments on isolated tissues are in accord with the findings of Erspamer and Glässer (1957) that murexine can produce a block of vertebrate neuromuscular transmission. The contracture response in the rectus and the relief of the block in the rat diaphragm by hexamethonium suggested that murexine was a "depolarizing type" of blocking agent. As the dose/response curves obtained from the frog rectus and from the rat diaphragm for suxamethonium and murexine were parallel but separated by about 0.5 log. units, it was inferred that murexine blocked by the same type of process as did suxamethonium but was less potent.

With the electrical experiments, it was possible to show that, at the moment of complete neuro-muscular block following murexine, the absence of any muscle twitch in response to nerve stimulation was accompanied by an abolition of the muscle action potential which was replaced by an end-plate potential localized to the end-plate focus in the foot muscle. When the same muscle was exposed to concentrations of suxamethonium, of decamethonium, or of acetylcholine which produced complete neuromuscular block, the events observed with a murexine block were mimicked.

Using the moving fluid technique, it was found by direct measurement that murexine depolarized the foot muscle in a manner closely similar to that seen with acetylcholine, suxamethonium, and decamethonium. Murexine depolarization also gave rise to asynchronous muscle action potentials as did the other three depolarizing drugs. The slope of the curve relating the voltage of depolarization with the concentration in the case of murexine was parallel to those found with the other three agents. The depolarizing potency of murexine on the foot muscle of the frog was about ten times less than that of acetylcholine and of suxamethonium but was equal to that of decamethonium.

It was concluded that murexine could be classified as a "depolarizing type" of neuromuscular blocking agent but that it was three to ten times less potent than suxamethonium.

REFERENCES

Bell, P. M. G. (1957). *J. Physiol.*, **137**, 1P.
Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.
Erspamer, V. (1948). *Experientia*, **4**, 226.
—— and Glässer, A. (1957). *Brit. J. Pharmacol.*, **12**, 176.
Nicholls, J. G., and Quilliam, J. P. (1956). Ibid., **11**, 151.