A STUDY OF ACTION OF DRUGS ON NEURO-MUSCULAR TRANSMISSION WITH THE AID MULTI-BARRELLED INTRACELLULAR MICROELECTRODES

A. I. SHAPOVALOV

Dept. of Pharmacology, Ist. I. P. Pavlov Medical Institute, Leningrad

DEVELOPMENT of electrophysiological technique has provided a means for direct determination of the site of neutropic drug action at cellular level. Intracellular microelectrodes are effective tools for investigation of the mechanism of action of substances affecting synaptic and junctional transmission.

The experimental results presented here are concerned with the mechanism of conduction in the neuro-muscular junction.

METHODS

Experiments were made on the isolated frog's sciatic nerve sartorius muscle preparation soaked in Ringer's solution. In some experiments the ionic composition of bathing medium was modified (calcium-lack and double sodium concentration) so as to induce spontaneous rhythmic activity.

Resting membrane potential, local and propagated potentials of muscle fibre, end-plate potentials and spontaneous miniature end-plate potentials were recorded. Recording was accomplished with the aid of intracellular microelectrode technique. One microelectrode of Ling-Gerard type was used for potential recording, the second—multi-barrelled (2–6 channel) microelectrode was employed for electrical polarization, direct stimulation, injection of drugs and in some cases for potential recording. The drugs were applied iontophoretically to the end-plate; they were discharged by applying outward pulses of current to channels of the microelectrode containing the drug solution.

The method of production of two- and multi-barrelled microelectrodes is described elsewhere (Shapovalov, 1960a, 1961). The tip diameter of the multi-barrelled microelectrode was less than 1 μ. Therefore it could be inserted intracellularly without impairment of the cell. Fig. 1 shows a schematic diagram of the experimental arrangement.

The drugs studied could also be added to the bathing medium. Special precautions were made to reduce mechanical movements of the muscle during stimulation.

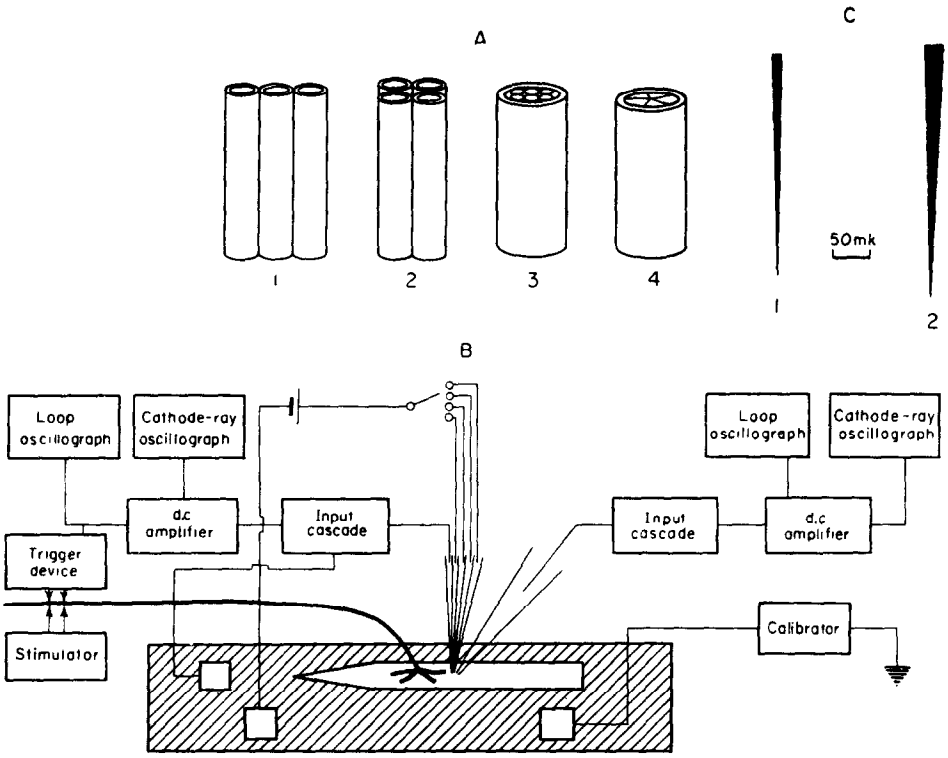

FIG. 1. Multibarrelled microelectrodes (A and C) and schematic diagram of experimental arrangements (B).

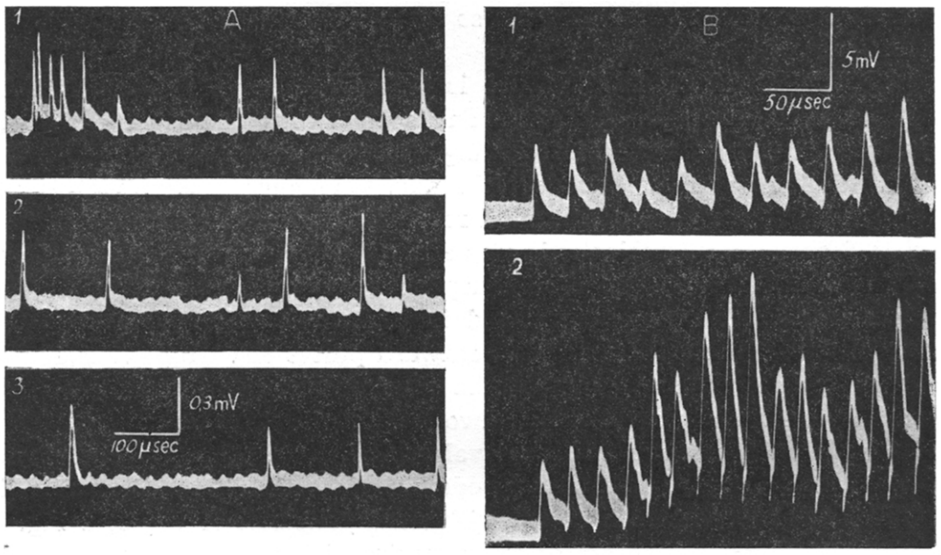

FIG. 2. A. Miniature end-plate potentials before (1) and after administration of blocking neuro-muscular transmission dose of $CoCl_2$ [2,3] B. End-plate potentials of $CoCl_2$ treated muscle fibre under different rates of stimulation.

Recording from the micropipette was accomplished with a cathode follower. This output was fed to a direct current amplifier. The output of the amplifier was fed in parallel into a cathode ray tube and electromagnetic oscillograph (Shapovalov, 1960b).

RESULTS

Some substances (such as $CoCl_2$, $CdCl_2$) depressed neuro-muscular transmission in doses which did not alter significantly amplitude of spontaneous miniature end-plate potentials and slightly diminished their frequency (Fig. 2). The sensitivity of the end-plate membrane to appli-

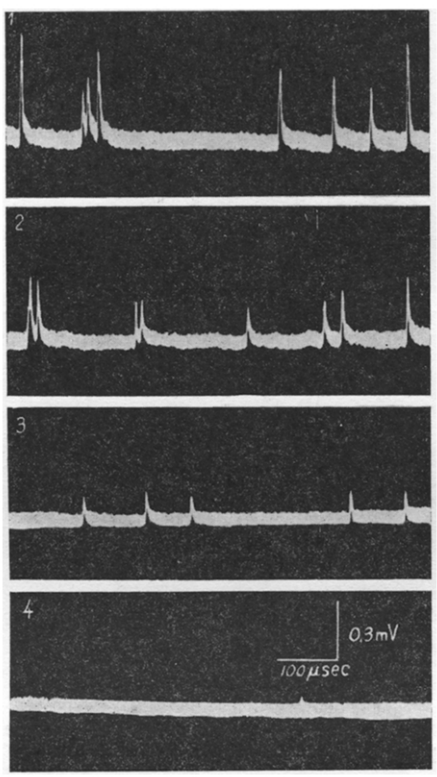

FIG. 3. Effect of diplacine on miniature end-plate potentials. M.e.p.p.'s before (1) and after the application of diplacine (2–4).

cations of acetylcholine and other depolarizing agents (succinylcholine, carbocholine, decamethonium, TMA) remained unchanged. Neuromuscular block produced by $CdCl_2$ and $CoCl_2$ could be relieved at least partly, by high frequency stimulation of the sciatic nerve (Fig. 2), and also by addition of calcium ions.

It was concluded, therefore, that block produced by Co and Cd ions is pre-synaptic in nature.

These substances probably depress the process of mediator release in the motor nerve terminals. Ca-excess also induced pre-synaptic block. But in this case the probable cause of block is the failure of propagation along nerve terminals, for the end-plate potentials, though enhanced in general failed intermittently in an all-or-none manner.

Curare-like substances (diplacine, paramione, ditiline, decamethonium), as is now widely accepted, depressed neuromuscular transmission chiefly by diminution of sensitivity of chemoreceptor membrane to acetylcholine. They diminished the amplitude of end-plate potentials

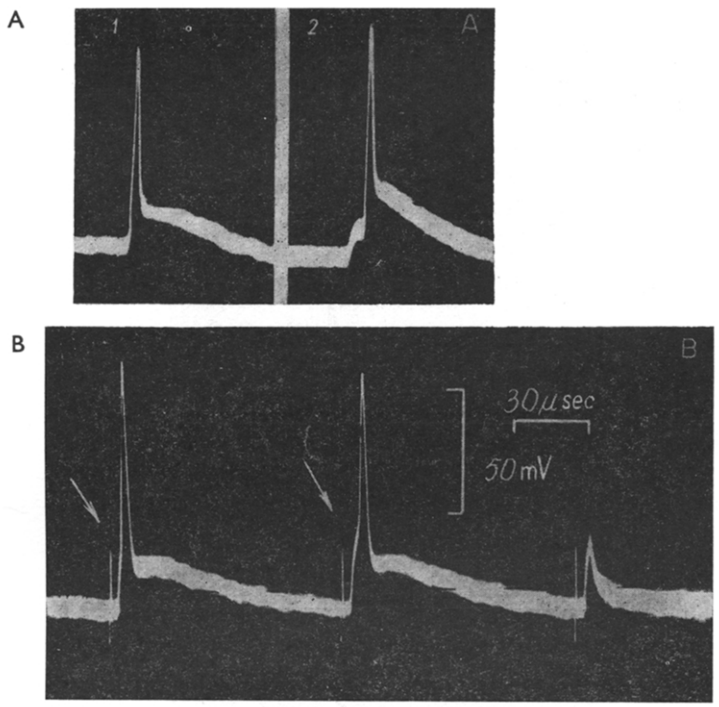

FIG. 4. Augmentation of end-plate potential-muscle spike delay by hyperpolarization (A) and after administration of urethane (B).

and of spontaneous miniature potentials (Fig. 3) and sensitivity of the end-plate to externally applied acetylcholine. In accordance with the results obtained by Castillo and Katz (1957) all effects of curare-like substances of concurrent and depolarizing type were seen only upon extracellular application. Nevertheless the interpretation of this fact may be different from that proposed by Castillo and Katz. As had already been mentioned by Clark (1937) these results may be due not to the extracellular position of chemoreceptors, but to the inactivation of drugs injected inside the cell by combination with cell constituents.

Some drugs may depress neuro-muscular transmission without affecting activity of presynaptic nerve terminals or end-plate membrane. Narcotics (urethane, barbiturates), calcium-excess and also, to a degree, depolarizing curare-like substances depressed the generation of a propagating spike in the zone near the end-plate. This effect is especially pronounced when repetitive stimulation was used, and is accompanied by an increase in the threshold of the muscle fibre membrane to direct stimulation. Hyperpolarization of the muscle fibre membrane caused similar effects (Fig. 4). This was signalled by enhancement of end-plate potential-muscle spike delay.

DISCUSSION

It follows from the results presented here, that different drugs can produce neuro-muscular block affecting different parts of the junction: presynaptic nerve terminals, sub-synaptic receptor membrane and the so-called electrically excitable (Grundfest, 1957) membrane of the muscle fibre. In the latter case depression of propagation in the region of contact of the sub-synaptic membrane (end-plate) with adjacent muscle fibre membrane occurs. Some drugs, for instance local anaesthetic (procaine) can depress conduction in all parts of the junction simultaneously.

It is of interest to mention that similar results are obtained in our experiments on spinal neurons. It may be concluded, therefore, that drugs affecting synaptic transmission affect not only the specific neuroreceptor subsynaptic membrane, but also the membrane of presynaptic nerve terminals and the electrically excitable postsynaptic membrane.

At present we have no direct tests for investigation of events taking place in presynaptic terminations. On the other hand, the electrical reactions of the post-synaptic membrane near the chemoreceptor zone may be effectively investigated. It was found that electric activity of muscle fibre membrane may be considerably altered by calcium lack, calcium chelating substances (such as EDTA), and by some other drugs, such as veratrine or phenyldiguanide. Under such conditions the membrane potential of the cell frequently exhibited fluctuations, the slow waves of depolarization, which on reaching the critical level induced multiple discharges. Such local potentials could appear spontaneously, and also in response to direct or indirect stimulation. Drugs which diminish the excitability of the muscle fibre membrane (narcotics, procaine) abolished these slow waves of depolarization and multiple firing.

A certain similarity was found between the slow local potentials, arising in the muscle fibre membrane, and local reactions of the end-plate region. Slow waves of depolarization could be diminished by electrical depolarization, and enhanced by hyperpolarization, though

in the former case they persisted even if depolarization of the muscle fibre membrane was carried as far as the null line.

The rectangular pulses of depolarizing current elicited local reactions with unitary or multiple discharges depending on the strength of the

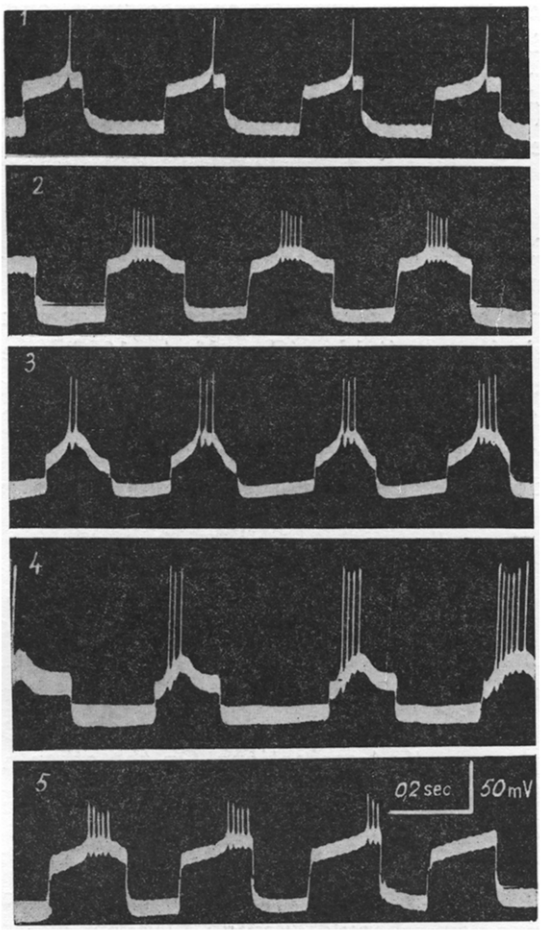

FIG. 5. Responses of muscle fibre membrane to rhythmic cathodic pulses. Facilitation (3, 4) and depression (5) of consecutive responses.

stimulating current. The rhythmic cathodic pulses caused the driving (facilitation) or depression of consecutive responses (Fig. 5). This latter effect, which in our opinion is similar to the desensitization in the end-plate region could be enhanced and speeded under the influence of narcotics and procaine.

End-plate potentials and depolarization of the end-plate induced by microapplication of acetylcholine, succinylcholine, decamethonium, tetramethylammonium and carbamylcholine summate with local potentials

of the muscle fibre membrane. This results in enhancement of frequency of spontaneous firing, or may induce long lasting activity in a silent cell (Fig. 6). Changes of membrane potential of a muscle fibre induced by nerve stimulation or microapplication of drugs through the multi-barrelled microelectrodes affect the local potentials in the same sense as the cathode of the direct current applied to the fibre.

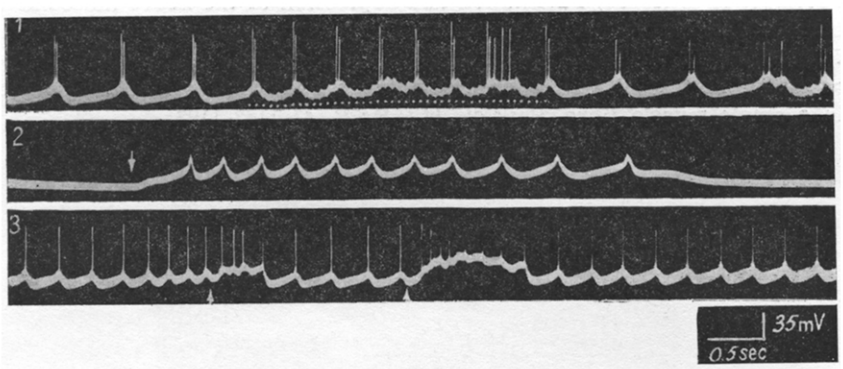

FIG. 6. Effect of end-plate potentials (1) and microapplication of succinylcholine (2) and acetylcholine (3) on spontaneous activity. End-plate potentials are marked by dots, microapplications by arrows.

It may be supposed that local reactions of so-called electrically excitable membrane may be very similar to the reactions of the sub-synaptic cholinoreceptive membrane of the end-plate.

The action of drugs on the membrane, surrounding the sub-synaptic chemoreceptor spots may be of great importance in their modulation of synaptic conduction.

SUMMARY

In experiments carried out on frog's neuro-muscular preparations the action of drugs on junctional transmission was studied with the aid of multi-barrelled intracellular microelectrodes.

It was found that different drugs produce neuro-muscular block affecting different parts of the junction: presynaptic nerve terminals ($CoCl_2$, $CdCl_2$), sub-synaptic membrane (curarelike substances), electrically excitable membrane of muscle fibre (narcotics). Some drugs can depress conduction in all parts of the junction.

Pharmacological and physiological peculiarites of the muscle fibre membrane are discussed. A certain similarity is believed to exist between some reactions (facilitation, desensitization) of the sub-synaptic membrane of the end-plate and of the surrounding electrically excitable membrane of the muscle fibre.

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