RESISTANCE OF NEUROMUSCULAR SYNAPSES TO DECAMETHONIUM. NATURE AND PROPERTIES OF THE POST-RESISTANCE BLOCK

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In 1951, for the first time, Paton [8] reported that in certain conditions decamethonium may act in two phases: initially it may cause a lasting depolarization of the end plates and relaxation of the muscles, but after repeated administration it may lose its depolarizing properties although neuromuscular conduction remains impaired. The block to neuromuscular conduction in these cases is abolished by acetylcholinesterases and intensified by tubocurarine, which led Paton to conclude that the second phase of the decamethonium block is competitive in nature.

A similar type of "double" ("mixed," "dual") block is also caused by succinyl choline (ditilin) when injected repeatedly [15] or by intravenous drip for a long period [5]. A "double" block arises especially easily if depolarizing curare-like substances are administered after competitively acting substances [9] or if they are given concurrently [1]. The development of a "double" block is usually preceded by some degree of resistance of the end plates to the action of decamethonium or succinylcholine [14].

In the present research a systematic study was made of the conditions for the development of resistance of the neuromuscular apparatuses of skeletal muscles to decamethonium, and also of the block to neuromuscular conduction developing during the action of decamethonium against the background of impending resistance to the drug. Since this type of neuromuscular block is always preceded by a period of resistance, we may call it a "post-resistance" block.

EXPERIMENTAL METHOD

Experiments were conducted on cats weighing 1.8-3.6 kg under urethane anesthesia (1.0-1.3 g/kg intraperitoneally). The counteractions of the gastrocnemius muscle were recorded on a kymograph during rhythmic stimulation of the sciatic nerve with rectangular impulses of a frequency of 0.5 cps and duration 0.5 millisecond, and the respiration was also recorded. The test substances were injected into the femoral vein of the opposite limb. Decamethonium was given as a 0.002% solution. As a substance with a competitive action, diplacin (tubocurarine) was used, in a 0.1% solution. The indicator of the "double" or post-resistance block was prostigmine (0.05% solution), which in doses of 0.05-0.1 mg/kg considerably reduces this block.

EXPERIMENTAL RESULTS

The first injection of decamethonium into an intact animal caused a complete block of neuromuscular conduction in a dose of 0.03 mg/kg. In doses of 0.02-0.025 mg/kg, decamethonium diminished the amplitude of the contractions of the gastrocnemius muscle by 50-80%, and in a dose of 0.01 mg/kg it was ineffective. The isoeffective doses of diplacin were 2, 1.5, and 0.5 mg/kg respectively.

The post-resistance block was most easily reproduced when the experiments were performed as follows.

As a result of the injection of decamethonium in a dose of 0.03 mg/kg a complete block of neuromuscular conduction was produced, lasting for 6-14 minutes, after which weak contractions of the gastrocnemius appeared. Injection of diplacin at this moment completely abolished the effect of decamethonium.

Against this background decamethonium was injected repeatedly in doses of 0.02-0.03 mg/kg, which caused no significant change in neuromuscular conduction. This concluded the period of preparation for reproduction of the post-resistance block. From 15 to 20 minutes after this preparation, but not earlier, injection of decamethonium in an

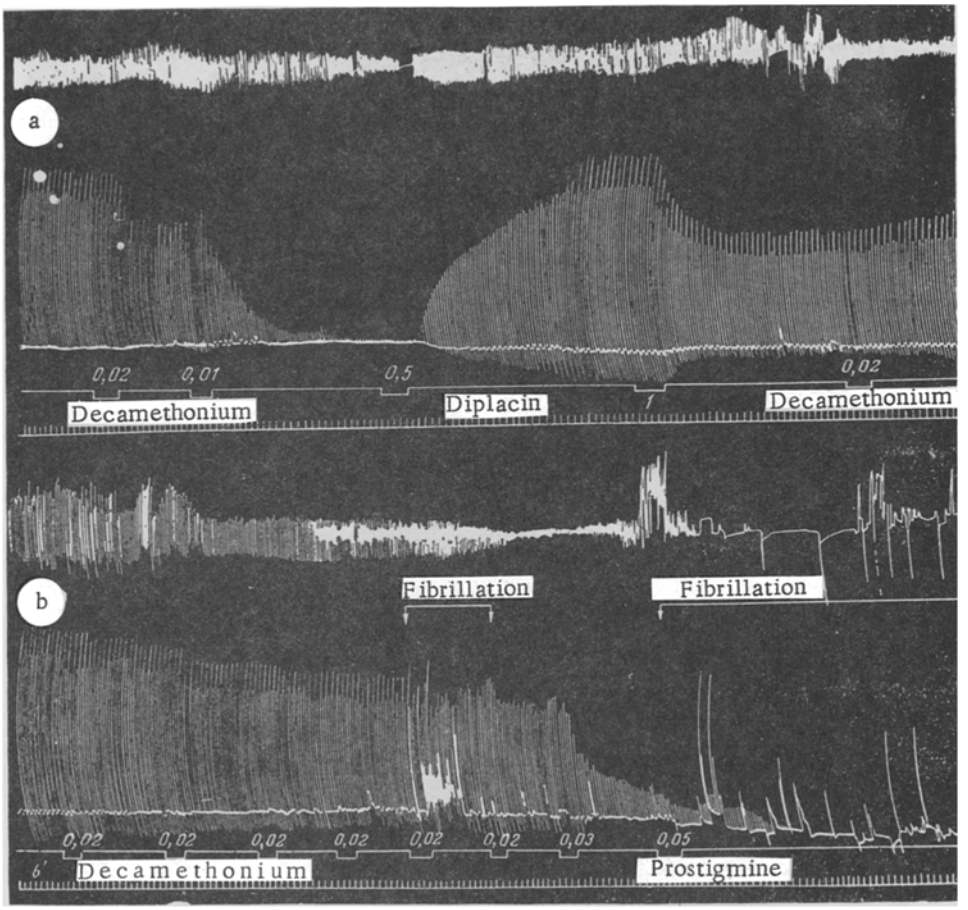

Fig. 1. Kymogram of an experiment in which one injection of diplacin was given. Significance of the curves (from above, down): respiration; contractions of the gastrocnemius muscle; time marker (5 seconds); marker of injection of diplacin; the figures above the time marker denote the time during which the drum was stopped (in minutes); the figures below the time marker denote the dose (in mg/kg).

initially effective dose (0.03 mg/kg) caused only an insignificant effect, and only a further injection of 0.02-0.05 mg/kg decamethonium led to a complete block of the neuromuscular conduction which, however, in contrast to the typical decamethonium block, was weakened or abolished by prostigmine, i.e., it acquired the features of a competitive block (Fig. 2).

In the variant of the experiments described, resistance to decamethonium was comparatively weak, for the total post-resistance block developed after injection of 0.05-0.08 mg/kg decamethonium, i.e., after injection of no less than three times the originally effective dose.

The resistance to decamethonium was stronger if, during the period of preparation for reproduction of the post-resistance block, after the injection of diplacin in a dose of 0.5 mg/kg an additional injection of 0.5-1.0 mg/kg diplacin was given, the original experimental scheme being otherwise unchanged. In these cases the post-resistance block developed after injection of decamethonium in doses exceeding the initial effective dose not less than 5 or 6 times. The extraordinary protection of the specific cholinoreceptors of the skeletal muscles by diplacin, interfering with the development of depolarization of the end plates under the influence of decamethonium, adversely affected the conditions of development of the post-resistance block. This was confirmed by the following variants of the experiments, in which the typical post-resistance block did not generally develop. The results of one of these experiments are shown in Fig. 1.

As a rule the injection of decamethonium in a dose of 0.03 mg/kg produced a complete block of neuromuscular conduction, which was at once abolished by diplacin in a dose of 0.5 mg/kg, after which a further injection of 1

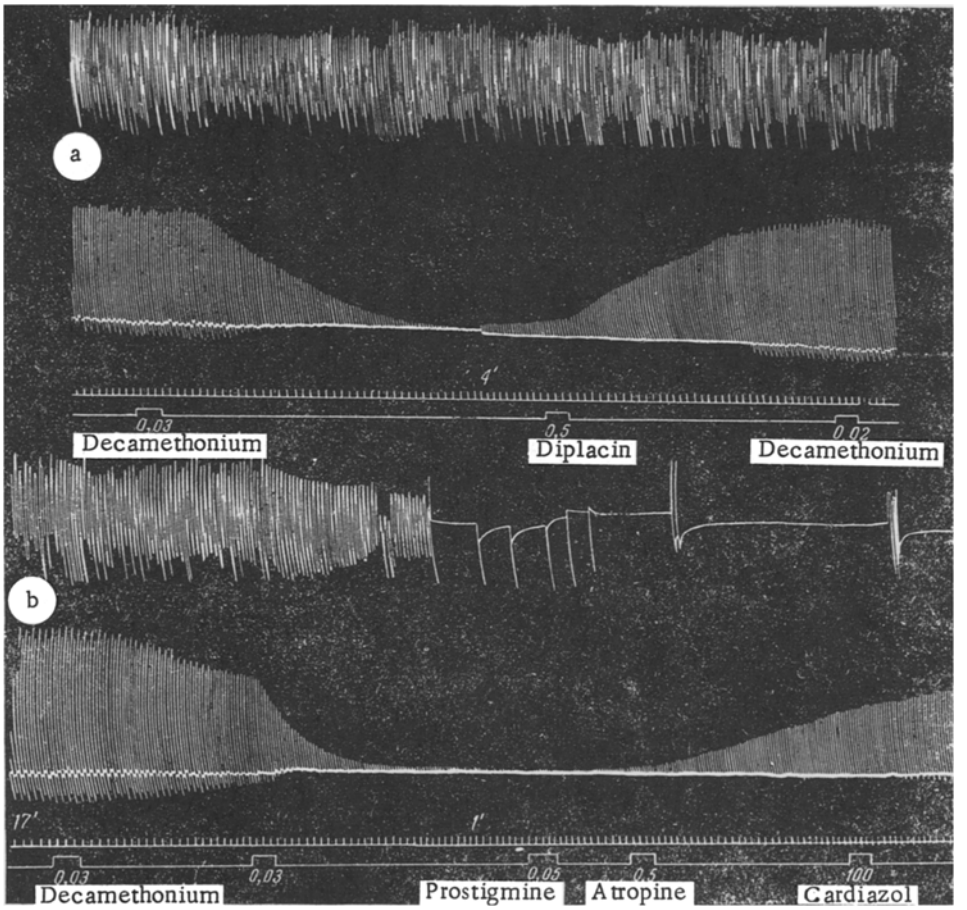

Fig. 2. Kymogram of an experiment in which two injections of diplacin were given. Significance of the curves (from above down): respiration; contraction of the gastrocnemius muscle, injection marker; time marker (5 seconds); the figures above the time marker denote the time during which the drum was stopped (in minutes); the figures below the time marker denote the doses (in mg/kg).

mg/kg diplacin was given and the decamethonium repeated in doses of 0.02-0.03 mg/kg. Immediately after the amplitude of the contractions of the gastrocnemius was restored to its original value, i.e., 4-6 minutes after the preparation we have described, resistance of the neuromuscular synapses to decamethonium could be demonstrated. It was necessary to inject from 5 to 7 initially effective doses of decamethonium in order to obtain a block of neuromuscular conduction. In these experimental conditions, however, in spite of the development of resistance, decamethonium produced its characteristic depolarization block, accompanied by muscular fibrillation and intensified by prostigmine.

The results can be understood if it is assumed that an essential condition for the development of resistance to decamethonium and of a post-resistance block is the penetration of this substance into the depth of the neuromuscular plates or the muscle fibers, and that decamethonium may react in the depth of these structures with certain cholinoreceptors (of the 2nd order). These cholinoreceptors may be the "extrasynaptic receptors of acetylcholine" described by Miledi [7], but in any case they are not the specific cholinoreceptors of the skeletal muscles which, according to Castillo and Katz [4], are situated on the outer surface of the membrane of the end plate.

If this assumption is granted, it may be supposed that depolarization of the membranes of the end plates, due to the initial effect of decamethonium, creates favorable conditions for its penetration into the depth of the end plates (and perhaps of the muscle fibers?) in view of the disturbance of the ionic permeability of the membranes resulting from depolarization. By reacting in the depth of the neuromuscular plates with the cholinoreceptors of the 2nd order,

decamethonium lowers the affinity of the specific cholinoreceptors for decamethonium reaching the intercellular medium as a result of subsequent injections. The lowering of the affinity of the specific cholinoreceptors for decamethonium must inevitably be accompanied by a decrease in its depolarizing properties. Conditions are thus created in which decamethonium is unable to create depolarization of the post-synaptic membranes and, consequently, a depolarization block. Injection of several times the initially effective dose of decamethonium during this period is not accompanied by a disturbance of neuromuscular conduction, i. e., the neuromuscular apparatus exhibits resistance to decamethonium, based on the phenomenon of noncompetitive auto-antagonism.

The injection of large doses of decamethonium in the period of resistance is accompanied by further penetration of the drug into the depth of the neuromuscular plates, although more slowly than during depolarization of the post-synaptic membranes; it is evidently brought about by diffusion. The penetration by diffusion of large amounts of decamethonium into the depth of the muscle fibers, where it continues to react with the chlorinoreceptors of the 2nd order, leads to a further decrease in the affinity of the specific cholinoreceptors not only for decamethonium, but also for the acetylcholine secreted at the endings of the motor nerves. This leads to the development of a post-resistance decamethonium block, which, consequently, is based on the phenomenon of noncompetitive antagonism with endogenous acetylcholine.

The remarks made above regarding the mechanism of development of resistance to decamethonium and of the post-resistance block presuppose that the following assumptions are correct:

1) the ability of acetylcholine and leptocurare to depolarize the postsynaptic membrane of the neuromuscular synapse is the result of their interaction with the same specific cholinoreceptors of the muscles; this agrees with the results of our previous investigations [2] and with other workers' findings [6, 10];

2) the high sensitivity of the neuromuscular apparatuses to leptocurare is due to their depolarizing properties [9];

3) the nature of the decamethonium block cannot be reduced to a simple depolarization of the postsynaptic membranes [12];

4) the penetration of decamethonium into the depth of the neuromuscular plate aggravates the conditions of depolarization under the influence of subsequent doses of the drug [3];

5) the penetration of decamethonium into the depth of the muscle fibers inevitably modifies the properties of these fibers, and not only the properties of the end plates [11, 13];

Comparison of the Properties of Competitive, Depolarization, and Post-Resistance Blocks

Agent	Type of neuromuscular block		
	competitive	depolarization	post-resistance
Tetanic stimulation of nerve	Temporarily abolishes	Does not change	Temporarily decreases
Prostigmine (0.05-0.1 mg/kg)	Abolishes	Deepens	Decreases
Decamethonium (0.02-0.03 (mg/kg)	Decreases	–	–
Diplacin (0.5-1.0 mg/kg)	–	Abolishes	Deepens
Potassium chloride (10-20)	Decreases or abolishes	Does not change	Decreases
Calcium chloride (10-20)	The same	Decreases	Decreases
Magnesium sulfate (10-20)	Sharply deepens	Deepens	Sharply deepens
Thiopental (10)	Decreases	Temporarily decreases	Decreases
Ether (20-40)	Deepens	Does not change	Does not change
Cadmium sulfate (5)	Abolishes	Decreases	Sharply deepens
Unithiol (75-100)	Does not change	Decreases	Deepens

Note. The meaning of these qualifications is as follows: abolishes – under the influence of the agent the amplitude of the muscle contractions is restored to its initial value; decreases – the amplitude of the contractions is increased, but not to the initial value; deepens – the amplitude of the contractions is decreased by 40-60%; sharply deepens – neuromuscular conduction ceases completely.

6) resistance to decamethonium and the post-resistance block are noncompetitive in nature, i.e., they are due to the interaction of decamethonium not with the specific cholinoreceptors, but with some other receptors of skeletal muscles.

This last assumption may be verified indirectly by studying and comparing the properties of all three types of block: competitive, depolarization, and post-resistance. In this connection we studied the effect of different factors on the course of an incomplete (depression of the contractions of the gastrocnemius muscle by 50-80%) competitive block caused by diplacin and of depolarization and post-resistance blocks caused by decamethonium.

The results given in Table 1 show that the post-resistance block differs essentially from the depolarization block, yet is not identical with the competitive type of neuromuscular block. The effect of the different substances on the course of the post-resistance block is usually weaker than on the course of the competitive block, and qualitative differences are found in the action of ether, cadmium sulfate, and unithiol.

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