MECHANISM OF EFFECT OF THE DIETHYLAMINOETHYL ESTER OF DIPHENYLPROPYLACETIC ACID (SKF-525A) ON NEUROMUSCULAR SYNAPSES

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On the basis of experiments on frog nerve-muscle preparations a hypothesis is put forward to the effect that SKF-525A does not act on cholinergic receptors but on the subsequent links of the mechanism of changes in ionic permeability.

The diethylaminoethyl ester of diphenylpropylacetic acid (SKF-525A) possesses a unique pharmacologic action. Its intrinsic effects are very few, but at the same time it potentiates or prolongs the effects of a wide range of different therapeutic substances [6]. Even in large doses (50 mg/kg, intravenously) in dogs SKF-525A does not disturb conduction through myoneural synapses, but potentiates the action of muscle relaxants, especially those of the depolarizing type [7-9, 17].

Since the neuromuscular block produced by muscle relaxants of depolarizing type is based on desensitization of the postsynaptic membrane to the action of mediator, it was decided to use microelectrophysiological methods to investigate the effect of SKF-525A on neuromuscular synapses.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated nerve-muscle preparation (sciatic nerve and sartorius muscle) of the frog Rana temporaria. A solution of the following composition was used: 111 mmoles Na^+, 2.5 mmoles K^+, 1.8 mmoles Ca^{++}, 120.6 mmoles Cl^-, 2.4 mmoles HCO_3; pH 7.4. Muscle contractions were prevented by adding 8-12 mmoles Mg^{++} to the normal solution. The nerve was stimulated with short square pulses every 2 sec. The end-plate potential (EPP) was recorded intracellularly by means of ordinary glass microelectrodes filled with KCl. After suitable amplification, the EPP's were photographed from the oscilloscope screen. The method of microiontophoretic application of acetylcholine (AC) which was used was fully described previously [1-3]. Desensitization was produced by the method of Katz and Thesleff [12].

EXPERIMENTAL RESULTS AND DISCUSSION

The addition of SKF-525A to the solution surrounding the nerve-muscle preparation in concentrations of $1 \cdot 10^{-5} - 1 \cdot 10^{-4}$ M had no effect on the EPP amplitude but had a marked effect on its shape: the time of increase of the EPP from its beginning to the maximum was slightly reduced, and the half-decline period (from the maximum to the level of 50% of the maximum amplitude) was definitely shortened (Fig. 1A, B). During the action of SKF-525A in a concentration of $1 \cdot 10^{-4}$ M on 7 end plates, the half-decline period was shortened on the average by 32±5%. An increase in the concentration of SKF-525A to $2 \cdot 10^{-4}$-$5 \cdot 10^{-4}$ M caused an initial omission of individual EPP's, followed by their complete block, evidently because of its anesthetic action on the motor nerve.

In experiments on nerve-muscle preparations treated with neostigmine ($2 \cdot 10^{-6}$-$3 \cdot 10^{-6}$ g/ml), SKF-525A in a concentration of $2 \cdot 10^{-5}$-$3 \cdot 10^{-5}$ M reduced the EPP amplitude by 50% (Fig. 1C, D). This effect of the SKF-525A was seen much more clearly after microapplication of AC to the end plate. A decrease

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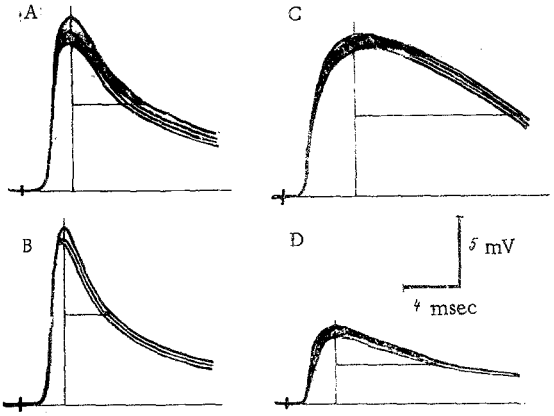

Fig. 1. Effect of SKF-525A on end-plate potential (EPP) of frog sartorius muscle (intracellular recording). A and C) control; B) after exposure for 15 min to $1 \cdot 10^{-4}$ M solution of SKF-525A; D) after exposure for 15 min to $3 \cdot 10^{-5}$ solution of SKF-525A; A and B) on normal muscle; C and D) on muscle treated with neostigmine. Calibration of amplification and time identical for all frames. Each frame represents several EPP's photographed by superposition. Fluctuations of quantum composition can be seen. Times of increase and half-decline for maximal EPP in each group are shown graphically.

in amplitude of the responses was observed after the action of SKF-525A solution in a concentration of $6 \cdot 10^{-7} - 1 \cdot 10^{-6}$ M (Fig. 2A, D). The longer the single response to AC application, or the more frequently AC was applied, the more marked the inhibitory effect of SKF-525A (Fig. 2B, D).

A difference was thus discovered between the action of SKF-525A on the effects of natural mediator liberated from the nerve ending and of AC liberated from the micropipet: SKF-525A inhibited the effects of applied AC (in the presence of intact cholinesterase) much more strongly (by more than 200 times).

SKF-525A speeds up desensitization of the postsynaptic membrane to the depolarizing action of AC very considerably. If infrequent applications of AC (each lasting 10 msec) were made to the sensitive point of the end plate, the response amplitude remained unchanged for a long time. But if, however, against the background of these infrequent applications, a second flow, of low intensity but long duration (10-20 sec) of AC was discharged from the second barrel of the same micropipet, a slowly declining depolarization wave was produced, the responses to the short applications of AC decreasing gradually against its background. In the presence of SKF-525A the rate of this decline was substantially increased (Fig. 2C, F). Threshold concentrations of SKF-525A, causing definite speeding up of desensitization, were $2 \cdot 10^{-7} - 3 \cdot 10^{-7}$ M.

Bovet and co-workers [7, 8] consider that muscle relaxants can form complexes not only with true cholinergic receptors, but also with "nonspecific receptors," the role of which is simply to hold in reserve a large number of molecules of muscle relaxant. They further postulate that muscle relaxants of depolarizing type possess a much higher affinity for "nonspecific receptors" than competitive muscle relaxants, and that SKF-525A can break the bond between the muscle relaxant and "nonspecific receptors." The active concentration of muscle relaxant is thereby increased and its effect potentiated. The use of a test based on inclination of a rabbit's head showed that SKF-525A potentiates the action of tubocurarine and flaxedil by 3-4 times and the action of depolarizing muscle relaxants by 8-18 times [8].

However, it is difficult to explain by means of this hypothesis the ability of SKF-525A to potentiate desensitization of the postsynaptic membrane. If it is assumed that AC, like other depolarizing substances, can be bound by "nonspecific receptors," and that SKF-525A interferes with this mechanism of AC inactivation, then the action of SKF-525A would be expected to increase the active AC concentration and, correspondingly, to potentiate its depolarizing effect, as takes place in the presence of acetylcholinesterase substances. If AC was applied sufficiently infrequently, this effect would be manifested as an increase in amplitude of responses to AC. In fact, the effects of AC were depressed (Fig. 2D). With the same frequency

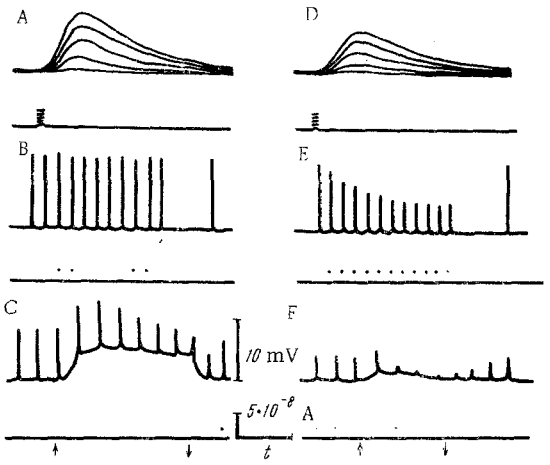

Fig. 2. Effect of SKF-525A on results of electrophoretic microapplication of AC to end plate of frog sartorius muscle. A,B,C) controls; D,E,F) 15-20 min after exposure to SKF-525A ($1 \cdot 10^{-6}$M). In each frame: top beam records responses to microapplication of AC; bottom beam records current through acetylcholine pipet; A and D) responses to microapplication of AC with different pulses liberating AC (photographed by superposition); B and E) series of responses to application of AC every 2 sec; C and F) desensitization to AC during application from 2-channel micropipet, beginning and end of prolonged flow of AC from second channel indicated by arrows (see in text). Calibration of amplification identical for all frames. Time marker (t) for A and D 100 msec; for B, C, E, and F 10 sec.

of application, a gradual decrease in amplitude of the responses was observed (Fig. 2E). After simultaneous application of AC from a two-channel pipet, desensitization could be recorded even when background depolarization was hardly visible (Fig. 2F). Consequently, SKF-525A does not simply increase the active concentration of AC, but also interferes with the desensitization mechanism.

In the discussion of the mechanism of this effect, the marked action of SKF-525A on biological membranes must be taken into consideration [14]. The numerous effects of SKF-525A on the fate of drugs in the body can also be explained by its ability to prevent the penetration of drugs through microsomal membranes and their inactivation by enzyme systems [6]. It may be postulated that the action of SKF-525A on the postysynaptic membrane is no exception and that it is explained by its inherent ability to stabilize biological membranes. This is in good agreement with the hypothesis of the "extra-receptor" mechanism of desensitization [2, 3]. The fact that SKF-525A, even in high concentrations, does not inhibit EPP's, is evidence that it does not interact with cholinergic receptors. The action of SKF-525A on effects of AC application is evidently attributable to its well-developed ability to speed up desensitization (Figs. 1A and 2A). For desensitization to develop, relatively long contact is necessary between the postsynaptic membrane and AC. During the ordinary EPP desensitization does not develop, but during the much longer acetylcholine potential it may occur. This explanation is supported also by the fact that prolongation of the EPP by the action of neostigmine (Fig. 1A, C) leads to a situation in which SKF-525A is able to inhibit the EPP.

The mechanism of the potentiating action of SKF-525A on effects of depolarizing muscle relaxants receives a satisfactory explanation in terms of the suggested hypothesis: SKF-525A does not increase the number of active molecules of muscle relaxant, but potentiates their effect, i.e., desensitization. The problem of the relatively weak effect of SKF-525A on the action of competitive muscle relaxants requires further investigation. The fact that SKF-525A does not potentiate the action of depolarizing muscle relaxants in experiments on cats [9, 17], but has a marked effect in experiments on dogs, rabbits, rats, and frogs [7-9, 11, 17], may be associated with species differences in the ease of onset of densitization of the end plates [14]. It is difficult at the moment to explain the effect of SKF-525A on the shape of the EPP. An ability to shorten the EPP has been described for many substances, but a rational explanation of this phenomenon is still awaited [5, 10, 13, 15, 16].

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