EFFECTS OF ANTICHOLINERGICS ON THE AFFERENT DISCHARGES FROM THE MUSCLE SPINDLE OF THE BULLFROG IN VITRO

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Stretching of the muscle spindle produces a receptor potential (a local depolarization) in the muscle spindle which gives rise to repetitive spikes in the sensory nerve (1). In addition, it has been suggested that the dynamic component of the receptor potential is simply the result of a change in membrane capacity, and that the successive static component may be due to a change in membrane permeability (1).

Henatsch and Schulte (2) found that the afferent discharges from the muscle spindle were increased by acetylcholine or succinylcholine in the muscle of frog. Ottoson (3) reported that acetylcholine \((1 \times 10^{-4} \text{ g/ml})\) had no significant effect on the sensory endings while higher concentrations caused a slight reduction of their activity. Some reports (4-6) indicated that d-tubocurarine or atropine affected the discharges from the muscle spindle. The problem thus remains whether acetylcholine or cholinesters play an essential role in the generation or transmission of spikes.

It was expected that use of various anticholinergics might provide an important clue in elucidating the mechanism of static generation and transmission of spikes. Strychnine was shown to have curare-like action (7) and also depressive action on the afferent discharges from the muscle spindle (8). The present study deals with d-tubocurarine, atropine, hexamethonium, hemicholinium-3, procaine and also strychnine. It is concerned with effects on muscle spindle discharges, receptor potential, nerve conduction and muscle twitch.

METHODS

Muscle spindle discharges: The isolated nerve muscle preparation of the m. extensor dig. long. IV of bullfrog (Rana catesbiana) was used as previously described (8,9). The preparation was perfused with amphibian Ringer’s solution (pH 7.6-7.8) composed of NaCl 115.0 mM, KCl 2.7 mM, CaCl₂ 1.8 mM, NaHCO₃ 3.0 mM and glucose 5.5 mM. The afferent impulses produced by stretch of the muscle were amplified with a Nihonkohden AVB-2 amplifier and displayed on a Nihonkohden VC-7 oscilloscope. The same impulses

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were then transformed into square waves and fed into an integrator, the output of which was recorded by a Toa Electronics DC-recorder (EPR-2TB). Test drugs were applied by perfusing the preparation with drug solution (pH 7.6-7.8). The medium in the bath, the working volume of which was 0.8 ml, was at least 80% replaced within 2 min.

To obtain the isolated spindle preparation, muscles were divided until the intramuscular part of the sensory fibre had been cleared and the spindle with a portion of the intrafusal muscle bundle freed from adjacent tissue (10). Thus the afferent discharges from the isolated spindle were obtained.

Receptor potential: Isolated muscle spindle preparations were used. The receptor potential was obtained from the isolated spindle preparation subjected to a brief stretch (300 msec). The potential elicited by stretching was amplified with a Nihonkohden AVH-2 DC-amplifier and displayed on an oscilloscope. At the end of the experiments, the preparation was crushed at the receptor region in order to ascertain that the records were not artefacts.

Nerve conduction: The sciatic nerve trunk was dissected from the bullfrog, divested of the external sheath, and carefully severed to several branches. One of the branches was mounted across five electrodes. The central part of the nerve was stimulated supramaximally by square wave pulses, 0.5 Hz, 0.01 msec in duration from a Nihonkohden MSE-3 stimulator with an isolator. A reservoir, 7 mm wide, in which Ringer's solution had been deposited, was interposed between the stimulating and recording electrodes. The action potential was detected by the recording electrodes, amplified with an AC-amplifier (Nihonkohden AVB-2) and displayed on a Nihonkohden VC-7 oscilloscope. One electrode was grounded to minimize the recording of the stimulus artefacts. Drugs were applied by replacing Ringer's solution with the drug-containing solution.

Muscle twitch: The m. extensor dig. long. IV of the bullfrog was suspended in a bath perfused with amphibian Ringer's solution bubbled with air. The twitch tension of the muscle in response to nerve stimulation was recorded on a kymograph using an isotonic lever. The nerve was stimulated by supramaximal square wave pulses, 0.1 Hz, 0.1 msec in duration from a Nihonkohden MSE-3 stimulator with an isolator.

In some experiments, direct stimulations were also applied on the muscle. For direct stimulations, another electrode was secured to the belly of the muscle. Single maximal contractions in response to alternate direct and indirect stimulations were recorded.

Drugs were applied by perfusing the preparation with the solutions. The medium in the bath, the working volume of which was 3 ml, was at least 90% replaced within 1 min.

Materials: Drugs used were d-tubocurarine chloride (Wako Pure Chem, Industries), atropine sulfate (Wako Pure Chem. Industries), hexamethonium bromide (Yamanouchi), hemicholinium-3 (Aldrich Chem.), procaine hydrochloride (Hoei Yakko) and strychnine nitrate (Hoei Yakko). Each drug was dissolved in amphibian Ringer's solution.
Effect on muscle spindle discharges

When the appropriate stretch was applied to the muscle, afferent discharges (about 20 Hz) occurred immediately, being followed by lasting constant discharges (about 10 Hz) for at least 2 hr. Effects of the drugs on the "static" discharges were studied during the period of constant frequency. Depressant effects of drugs on the rate of the discharge are shown in Fig. 1. Dose-response curves for drugs are depicted in Fig. 2. Atropine (2 x 10^{-4} M) gradually reduced the rate of discharge: 30% and 68% decrease in 10 and 20 min respectively; a concentration of 2.5 x 10^{-5} M also reduced the rate. The dose-response curve for procaine coincided approx. with that for atropine.

The depressant effect of atropine on the isolated muscle spindle was much stronger than that on the whole muscle preparation. On the other hand, the depressant effect of procaine on the isolated muscle spindle was not significantly different from that on the whole muscle preparation.

As is evident in Fig. 2, strychnine markedly reduced the rate of discharge; a low concentration of 1.25 x 10^{-5} M also reduced the rate. In the isolated spindle preparation, the depressant effect of strychnine was significantly stronger than that in whole muscle pre-
FIG. 2. Log concentration-response relationships in the depression of the rate of afferent discharge from muscle spindle. Abscissa: Molar concentration of drugs, on a logarithmic scale. Ordinate: Effect as percentage of depression. Each point represents the mean of depression in separate four experiments, with the S.E. indicated. The points marked with asterisk represent the values obtained from the experiment using the isolated spindle preparation.

After the drug solution of atropine, procaine or strychnine was exchanged for drug-free Ringer's solution, discharges were gradually restored.

The effect of hemicholinium-3 was very weak contrary to our expectation. The dose-dependent relationship for hemicholinium-3 was not obtained in a range from $1 \times 10^{-4}$ M to $4 \times 10^{-4}$ M; a concentration of $8 \times 10^{-4}$ M exerted a marked depression. The gradual appearance of depression and its slow restoration after washing were observed.

d-Tubocurarine exerted a weak effect on the rate of discharge; even a concentration of $1 \times 10^{-3}$ M decreased it at most 50%. Hexamethonium showed no significant effect even in a high concentration of $1 \times 10^{-3}$ M in the isolated spindle preparation as well as in the whole muscle preparation.

Effect on receptor potential

By a brief stretch, a receptor potential was obtained, which disappeared when the preparation was crushed at the receptor region (Fig. 3). During the period of depolarization (receptor potential) repetitive firing of spikes was seen. The receptor potential was not depressed by strychnine, atropine and procaine ($1 \times 10^{-3}$ M), which depressed static discharges. As shown in Fig. 3, the dynamic spike (first spike) was more resistant than the static spikes (continuous spikes) to the drugs used.

Effect on nerve conduction

Action potentials elicited by the stimulation became constant within 20 min after
FIG. 3. Effect on the receptor potential obtained from the isolated spindle preparation subjected to a brief stretch (300 msec).

FIG. 4. Effect on nerve action potential following a single nerve stimulus.
mounting a severed branch of the nerve on electrodes. Drugs were applied for 20 min by replacing Ringer's solution with drug-containing solution. The action potentials were photographed several times during the application of drug. Five serial action potentials were superimposed in each record (Fig. 4). The time course for conduction block after the drug application ($1 \times 10^{-3}$ M and $5 \times 10^{-4}$ M) is shown in Fig. 5.

Procaine in concentrations of $1 \times 10^{-3}$ M and $5 \times 10^{-4}$ M decreased the amplitude to 78% and 20%, respectively. Strychnine ($1 \times 10^{-3}$ M) caused the deformation of the spike shape as well as the decrease in spike amplitude (Fig. 4). Atropine ($1 \times 10^{-3}$ M) showed only 9% inhibition. Hemicholinium-3, d-tubocurarine and hexamethonium ($1 \times 10^{-3}$ M) showed no significant effect on the nerve conduction.

Effect on muscle twitch

Due to the possibility that muscle relaxation was partly or wholly responsible for the decrease of afferent discharges, the effect on muscle twitch to indirect stimulation was studied. The effect of drugs was presented as the percentage of depression 20 min after drug application (Fig. 6).

d-Tubocurarine ($4 \times 10^{-4}$ M) and strychnine ($5 \times 10^{-4}$ M) prevented muscle twitch completely. A concentration of $4 \times 10^{-1}$ M of stropine, procaine or hemicholinium-3 caused a complete inhibition. The effect of hemicholinium-3 was characterized by the gradual appearance and slow fading after washing. As to hexamethonium, the depression of muscle twitches was at most 50% even in a concentration of $1 \times 10^{-3}$ M. Muscle twitches
elicited by direct stimulation were not influenced by the concentration of each drug, which completely prevented the twitch elicited indirectly.

**DISCUSSION**

A summary of results is shown in Fig. 7, thus allowing a comparison to be made of the action of drugs of the muscle spindle discharges, neuromuscular transmission and nerve conduction.

As has already been pointed out in the previous report (8), it is unlikely that the depress-
ion of afferent discharges by drugs is due to the extrafusal neuromuscular blocking effect, since the afferent discharges were not influenced by d-tubocurarine (4 x 10^{-4} M) which completely prevented the muscle twitch. If the effect of drugs on extrafusal fibre influences the afferent discharges, then it would be expected that the depression of afferent discharges by drugs in the isolated spindle preparation would be none or less than that in the whole muscle preparation. In fact, this is not the case (Fig. 2), excluding the possibility that the effect on extrafusal fibre leads to the depression of the spindle discharges.

There may be the possibility that the depressant action on the discharges is caused by conduction block of the afferent trunk fibre (1, 11, 12). It seems remote, however, since (i) the local anesthetic activity of procaine did not coincide with the depressant activity on the muscle spindle discharge and (ii) strychnine (5 x 10^{-5} M) and atropine (1 x 10^{-3} M) which depressed static discharges completely, exerted no significant conduction block.

There could also be the possibility that the depression of the afferent discharges by drugs is the result of the conduction block at some part of the myelinated nerve branches. This is ruled out however because, if the conduction was blocked at the part, the dynamic spike as well as the static ones could not pass through the part, and in fact, the dynamic spike was detected even after the static ones were completely abolished.

It may be postulated that drugs affect the mechanism intermediating between receptor potential and abortive spikes or firing spikes. Ottoson et al found that receptor potential was partly resistant to lack of sodium in solution (13) or tetrodotoxin (14). In the present study, strychnine, atropine and procaine (1 x 10^{-3} M) could reduce the rate of discharge without affecting the receptor potential (Fig. 3). Therefore, it is unlikely that the reduction of the rate of discharge is due to the effects of these drugs on the receptor potential.

The depressant effect of drugs on the spindle discharges could be partly attributed to the effects on intrafusal fibre, an enhancement of the adaptation mechanism or the elevation of the threshold of the sensory endings. The present results throw no light on this, however.

Thus the result of the present study suggests that anticholinergics used here may affect the mechanism transforming receptor potential into afferent discharges. Hemicholinium-3 is known to block chemical transmission at the neuromuscular junction mainly by interrupting acetylcholine formation (15). It was found that the muscle spindle discharges were gradually depressed by hemicholinium-3 and slowly restored after washing (Fig. 1) as was seen at the neuromuscular junction with this drug (in the present study and (15)). Similarity for hemicholinium-3 between the mode of action at muscle spindle and that at neuromuscular junction seems to be suggestive. Although the proposal of the chemical transmission as the transforming mechanism is attractive, clear evidence have not yet been obtained.

SUMMARY

1. The effects of atropine, procaine, strychnine, hemicholinium-3, d-tubocurarine and hexamethonium were examined on muscle spindle discharge, neuromuscular junction and nerve conduction to search for clues as to the mechanism of generation of muscle spindle
discharge.

2. Tested drugs except for hexamethonium caused a reduction of the rate of afferent discharge, whereas none of these drugs affected the receptor potential.

3. The depression of afferent discharges by drugs was due neither to the extrajunctional neuromuscular effect, nor to the conduction block of sensory trunk fibre and myelinated nerve branches.

4. It is concluded that the drugs reduce the afferent discharges by affecting the mechanism whereby the receptor potential was transformed into the spikes. It was observed that the mode of action of hemicholinium-3 on the afferent discharge was somewhat similar to that on the neuromuscular junction. The problem still remains unsolved as to whether or not some chemicals take part in the transforming mechanism.

REFERENCES

1) Katz, B.: J. Physiol. 111, 261 (1950)
13) Ottozon, D.: J. Physiol. 171, 109 (1964)