EFFECTS OF METABOLIC INHIBITORS AND OUABAIN ON STATIC AFFERENT DISCHARGES FROM THE ISOLATED FROG MUSCLE SPINDLE

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Abstract—Effects of metabolic inhibitors and ouabain on the rate of afferent discharges from isolated muscle spindle of the bullfrog were examined in vitro to elucidate the mechanism for generating afferent action potentials. Dinitrophenol, NaCN, iodoacetic acid, and ouabain gradually reduced the rate of discharges. These drugs were without direct effect on the nerve axon. The rate of discharges was also reduced by Li\(^+\), which is known to be minimally extruded from the interior of cells by an active transport mechanism. The rate of discharges remained approximately 50\% in the presence of NaCN or in the absence of oxygen, suggesting that the energy required for the maintenance of the Na\(^+\)-pump can be supplied to some extent in an anaerobic condition. It appears that the metabolic inhibitors and ouabain reduce the afferent discharges from the frog muscle spindle by affecting the Na\(^+\)-pump in fibers of nerve terminals.

Since the detailed study of the general properties of the muscle spindle was made by Matthews (1), the mechanisms for generating afferent discharges in the muscle spindle have been investigated. The ultrastructural study of frog muscle spindle (2) has demonstrated that the afferent nerve axon is divided into many unmyelinated branches which form long beaded chains and that the cytoplasm of the sensory endings is characterized by an accumulation of small mitochondria. This demonstration led to the assumption that the terminal region may demand a high energy supply to continue generating afferent action potentials. The present experiments were designed to obtain evidence which would elucidate the mechanisms for generating static afferent action potentials. Dinitrophenol, sodium cyanide, iodoacetic acid, and ouabain were used.

MATERIALS AND METHODS

The experiments were carried out on the bullfrog (*Rana catesbeiana*) from April to October. A nerve-muscle preparation of the m. extensor longus digiti IV was prepared. Only one muscle spindle with its afferent nerve fiber was left, and the extrafusal fibers were then cut or crushed under binocular microscopic observation. Two types of chambers were devised (Fig. 1): chambers A and B for testing the effects of drugs on the muscle spindle and the afferent nerve axon, respectively. In chamber A, one end of the muscle spindle was fixed to the bottom of the chamber, and the other was tied to a metal rod which could be moved by means of a stretching device. In chamber B, both ends of the muscle spindle were fixed at a suitable length.
The preparation in chamber A (2 ml) was continuously perfused at a rate of 0.8 ml/min with amphibian Ringer's solution composed of (mM) NaCl 115.0, KCl 2.7, CaCl₂ 1.8, and glucose 5.5 (pH 7.5 ± 0.2, which was adjusted by adding NaHCO₃). Afferent action potentials from the muscle spindle were detected by calomel electrodes, amplified with an amplifier (Nihonkohden AVB-2), and displayed on an oscilloscope (Nihonkohden VC-17). The same potentials were transformed into square waves and fed into an integrator, the output of which was recorded by a DC-recorder (Toa Electronics EPR-2T). When the effect of a drug on the muscle spindle was to be examined, the preparation was perfused with Ringer's solution containing the drug. One preparation was used only once for the drug application.

To examine the effect of a drug on nerve axon, the axon was perfused with drug-containing Ringer's solution through a special groove (chamber B, 0.05 ml); in this case, the muscle spindle was continuously perfused with drug-free Ringer's solution to exclude the possible effect of leaking drug-containing Ringer's solution on the muscle spindle.

For an oxygen-deficient medium, Ringer's solution was bubbled with N₂-gas for more than 30 min immediately before and during the experiment. Lithium-Ringer's solution was composed of (mM) LiCl 115.0, KCl 2.7, CaCl₂ 1.8, and glucose 5.5 (pH 7.5 ± 0.2, which was adjusted by adding KOH). Drugs used included: dinitrophenol (Wako Pure Chem.), sodium cyanide (NaCN, Wako Pure Chem.), iodoacetic acid (Wako Pure Chem.) and ouabain (Merck). All were dissolved in Ringer's solution. The solution was applied for 30 min, with the exception of dinitrophenol for 20 min, and the preparation was then perfused with Ringer's solution to examine the reversibility of the drug effect.

RESULTS

Effects on afferent discharges from the muscle spindle

Long-lasting afferent discharges (about 10 Hz) were obtained for at least 2 hr by the application of appropriate stretch to the muscle spindle. The experiments were performed during this period of constant frequency.
Metabolic inhibitors and ouabain: Depressant effects of dinitrophenol (1 x 10^{-4} M), NaCN (3 x 10^{-3} M), iodoacetic acid (1 x 10^{-4} M, 1 x 10^{-3} M), and ouabain (1 x 10^{-5} M) and their log concentration-response relationships are shown in Figs. 2 and 3, respectively. When dinitrophenol, iodoacetic acid or ouabain was applied, the rate of afferent discharges decreased gradually, and almost disappeared within 20 to 30 min. Effects of these three drugs were all concentration-dependent. The mode of action of NaCN was different from that of the other three drugs in that even high concentrations did not produce more than 50% reduction in the rate of afferent discharges.

When the solution containing dinitrophenol, NaCN or ouabain was exchanged for a drug-free Ringer's solution, the rate of afferent discharges was almost restored within 30

Fig. 2. Effect of metabolic inhibitors and ouabain on the rate of afferent discharges from the muscle spindles of the m. extensor longus digiti IV subjected to stretch. Records of impulse frequency using a DC-recorder. Horizontal bar: time for drug perfusion. Vertical bar: frequency of 10 Hz. Photographs of oscillographic display are added. (Spikes retouched.) When spikes decreased in amplitude by application of sodium cyanide, small spikes unable to be discriminated from noises could not be counted, since the trigger level of integration was adjusted a little higher than the noise level.
to 60 min. The inhibitory effect of iodoacetic acid, however, remained unchanged even after perfusion with drug-free solution for more than 1 hr. It should be noted that, in the case of $1 \times 10^{-4}$ M of iodoacetic acid, a marked increase in the rate of discharges was observed in four out of eight experiments (Fig. 2). With $3 \times 10^{-5}$ M, there was no alteration in the rate of discharges.

When NaCN ($3 \times 10^{-3}$ M) was applied to the perfusing medium, the amplitude of the afferent discharges became smaller and finally only small-sized potentials (presumably abortive spikes) remained. No restoration of the amplitude after washing was observed. Drugs other than NaCN had little effect on the amplitude.

Anaerobic condition: The finding that reduction of the rate of afferent discharges was at most 50%, even in high concentrations of NaCN, led to the consideration that the muscle spindle of the frog could generate afferent discharges even under anaerobic conditions. The rate of afferent discharges decreased to about 60% within 30 min after perfusion with the oxygen-deficient Ringer's solution. After the oxygen-deficient solution was exchanged for normal Ringer's solution, the rate of afferent discharges tended to recover (Fig. 4).
Further stretching under the complete inhibition of afferent discharges: After the afferent discharges had been completely inhibited by dinitrophenol ($1 \times 10^{-4}$ M) or ouabain ($1 \times 10^{-5}$ M), a further stretch (1 sec) produced only a few discharges in the early phase of stretching (Fig. 5); the size and shape of the potentials seemed to be substantially the same as those obtained in normal Ringer's solution. This fact indicates that a decrease or disappearance of the discharges is independent of the possible decrease in the muscle tension.

Lithium ion: When the muscle spindle was perfused with a lithium-Ringer's solution including LiCl, the rate of afferent discharges decreased gradually and almost disappeared within 20 min (Fig. 6), their amplitudes being unaffected.

Effects on the afferent nerve axon

In this experiment, we used chamber B, in which only nerve axon was perfused with the drug solution. A concentration of $1 \times 10^{-4}$ M of dinitrophenol or $1 \times 10^{-4}$ M of ouabain, which was ten times higher than required for inhibiting the rate of afferent discharges, had no effects on the rate or amplitude of discharges. Iodoacetic acid, in a concentration of $1 \times 10^{-3}$ M, which completely inhibited the rate of discharges when applied to the muscle...
spindle, had no inhibitory effects on nerve axon; although a higher concentration of \(1 \times 10^{-2}\) M decreased the amplitude and irreversibly abolished the potentials within 20 to 30 min. The rate or amplitude of discharges was not significantly changed by the application of NaCN (\(3 \times 10^{-3}\) M) on the afferent nerve axon. From these results, it is hardly likely that the depressant action on the discharges is caused by conduction block of the afferent nerve fibers. Perfusing with lithium-Ringer's solution did not change the rate or amplitude of discharges.

**DISCUSSION**

Our findings herein indicate that dinitrophenol, NaCN, iodoacetic acid, and ouabain affect the mechanisms of the generation of static afferent discharges without having an inhibitory effect on the nerve axon or tension of the muscle spindle. These findings are in agreement with recent reports by Alekseev and Pavlenko (3, 4), who showed that muscle spindle discharges were decreased by dinitrophenol and ouabain.

It has been shown that the passive permeability mechanism responsible for generating the action potential does not discriminate between Na\(^+\) and Li\(^+\), but that the active transport mechanism is hardly able to extrude Li\(^+\) from the interior of the muscle fibers (5). In the present study, the rate of afferent discharges was seen to be reduced when Li\(^+\) was substituted for Na\(^+\) in the external medium, as was reported by Alekseev et al. (3, 6). Thus it seems likely that the inhibitory effect of the metabolic inhibitors and ouabain as well as Li\(^+\) on the static afferent activities results from the drug-induced insufficiency of the Na\(^+\)-pump; the site of action is thought to be the nerve terminals characterized by the accumulation of mitochondria.

Since the rate of afferent discharges remained approx. 50\% in the presence of NaCN or in the absence of oxygen, it seems feasible that the energy required for the maintenance of the Na\(^+\)-pump can be supplied to some extent even in the anaerobic condition in the frog muscle spindle. In fact, iodoacetic acid, an inhibitor of anaerobic glycolysis, inhibited the afferent discharges irreversibly.

Iodoacetic acid (\(1 \times 10^{-4}\) M) increased the rate of afferent discharges in four out of eight experiments; therefore it would appear that the drug has two effects on the terminal region, one a metabolic-inhibitory effect while the other effect contributes to the initiation of afferent action potentials.

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