A previous work (1) has shown that an individual propagated impulse recorded at the sensory nerve terminal of the frog muscle spindle was always followed by a long-lasting positivity of up to 0.1 sec in duration and of 0.1-0.3 mV in the maximal amplitude, during which the appearance of propagated and abortive spikes was suppressed. Time course of the positive after-potential was similar to that of the after-hyperpolarization following spike discharge of amphibian motoneuron, during which excitatory processes were depressed (2). Matthews (3) has suggested that similarity in the electrical responses of the motoneuron and the sensory ending may result from similar properties of polarized surfaces.

Objectives of the present experiments are to determine whether or not the positive after-potential is selectively modulated by certain ions or drugs and to clarify the root of the selective modification from the effects of the drugs on the sensory terminal.

METHODS

Thirty-three experiments were carried out with isolated muscle spindles of the frogs (Rana nigromaculata). The single parent axon of a spindle receptor was isolated along its intramuscular course until the capsule of the spindle receptor was cleared, but the capsule and intrafusal muscle bundle were left attached to the remaining musculature.

The excised preparation was placed in a Ringer’s pool (RA) in a perspex box, and the isolated nerve passed into another Ringer’s pool (RB) through a liquid paraffin pool of 2 mm length. The paraffin pool was situated in a slit of 1 mm at the center of a partition between the two Ringer’s pools. The paraffin gap method has been described in detail elsewhere (1). A pair of calomel electrodes were inserted into subsidiary Ringer’s pools, each of which was connected with RA and RB by means of two Ringer’s agar bridges. The length of paraffin gap was 2 mm.

The muscle was maintained at in situ length (approx. 28 mm) in most experiments, but in some it was stretched by 2, 4 or 6 mm from the in situ length, being referred to as +2, +4 or +6 mm, at constant velocities of 4, 7 and 10 mm/sec. It was maintained there for 0.1 sec or 3 min. Throughout experiments, the muscle tension was monitored by means
of mechano-electric transducer (RCA 5734) to check the effects of some drugs upon muscle fibers where contraction or relaxation should secondarily modify the excitability of the sensory terminal.

Ringer’s solutions containing tetramethylammonium chloride (TMA) at different concentrations were utilized. EDTA and EGTA was diluted at different concentrations in a Ca-free Ringer’s solution. The normal Ringer’s solution perfusing a spindle receptor was replaced by the above test solutions. Successive substitutions were interspersed with normal Ringer’s flow.

RESULTS

After-potential, tension development and steady potential

The amplitude of the positive after-potential following individual propagated spike was selectively increased by 10 mM TMA (Fig. 1A). During application of Ringer’s solution with 10 mM TMA, no discernible change was found in propagated spike and in steady potential. The rising phase of the increased positive after-potential was steeper than that of the control. The increased amplitude of the positive after-potential was reduced to normal size as soon as re-immersing the preparation into the normal Ringer’s solution.

A Ca-free Ringer’s solution with 2 mM EDTA or with 5 mM EGTA gave rise to a selective decrease in the amplitude of the positive after-potential without appreciable change in size of the spike components and in the steady potential (Fig. 1A). The normal amplitude of the positive after-potential could be only incompletely regained by the application of 10 mM TMA.

In Fig. 1B, the response of a single muscle spindle and the tension development during muscle stretch in Ringer’s solution containing 10 mM TMA or 5 mM EGTA were compared with those in normal Ringer’s solution. The tension developed during muscle stretch could be expressed in terms of the two components; the peak tension at the completion of the dynamic phase of stretch, and the static tension at 0.5 sec after the peak during maintenance of the stretch. Table 1 shows the peak and static tensions of a muscle during stretch by 2 mm at three kinds of velocity (4, 7 and 10 mm/sec) from +0, +2, +4, +6 mm initial lengths of the muscle, during application of EGTA or TMA in comparison with normal Ringer’s solution. No significant difference could be detected between the tension development in the applications of drugs and that in the normal Ringer’s solution during the passive stretch of the muscle. These results imply that changes in the positive after-potential or in the discharge pattern following treatment with these drugs may not be attributed to an effect secondary to the change in a viscoelastic property of intrafusal muscle fibers, although the tension development measured in the present experiment depends mainly on the state of the extrafusal muscle fibers.

The spindle potential could be clearly shown by eliminating the spike component after treatment of the preparation with tetrodotoxin of $5 \times 10^{-8}$ g/ml in concentration, as illustrated in Fig. 1C. When the isolated axon was moved sideways in the center of the paraffin gap during muscle stretch, the spindle potential usually showed a negative deflec-
Fig. 1. A: Effects of 10 mM TMA and 5 mM EGTA upon the amplitude of the positive after-potential following orthodromic propagated spike recorded from a spindle receptor in comparison with those in normal Ringer's solution.
B: Effects of the drugs upon the responses of a spindle receptor (upper traces) and the tension development (lower traces) during stretching the muscle by 2 mm at a velocity of 7 mm/sec from the in situ length (+0 mm) in comparison with those in normal Ringer's solution.
C: Showing no appreciable changes in the amplitudes of the spindle potential in a spindle receptor (upper traces) and tension developments (lower traces) during stretch of the muscle in the same conditions as in B. Tetrodotoxin of $5 \times 10^{-8}$ g/ml was added into Ringer's solutions.

Table 1

| Muscle length (mm) | Stretch velocity (mm/sec) | 5 mM EGTA | Normal Ringer | 10 mM TMA |
|--------------------|---------------------------|-----------|---------------|-----------|
|                    |                           | Peak tension (g) | Static tension (g) | Peak tension (g) | Static tension (g) | Peak tension (g) | Static tension (g) |
| 4                  | 4                         | 0.18 ± 0.02 | 0.17 ± 0.01 | 0.19 ± 0.01 | 0.15 ± 0.01 | 0.18 ± 0.01 | 0.16 ± 0.01 |
| 10                 | 7                         | 0.21 ± 0.01 | 0.16 ± 0.12 | 0.20 ± 0.01 | 0.16 ± 0.01 | 0.19 ± 0.01 | 0.16 ± 0.01 |
| 4                  | 4                         | 0.52 ± 0.04 | 0.42 ± 0.02 | 0.55 ± 0.03 | 0.44 ± 0.02 | 0.47 ± 0.03 | 0.40 ± 0.03 |
| 10                 | 7                         | 0.55 ± 0.03 | 0.46 ± 0.01 | 0.57 ± 0.03 | 0.47 ± 0.04 | 0.53 ± 0.04 | 0.42 ± 0.02 |
| 4                  | 4                         | 0.61 ± 0.04 | 0.47 ± 0.02 | 0.63 ± 0.08 | 0.50 ± 0.02 | 0.59 ± 0.03 | 0.45 ± 0.04 |
| 10                 | 7                         | 0.86 ± 0.08 | 0.78 ± 0.12 | 1.04 ± 0.06 | 0.91 ± 0.04 | 0.92 ± 0.06 | 0.76 ± 0.13 |
| 4                  | 4                         | 0.94 ± 0.11 | 0.81 ± 0.09 | 1.07 ± 0.14 | 0.95 ± 0.12 | 0.87 ± 0.11 | 0.79 ± 0.09 |
| 10                 | 7                         | 1.04 ± 0.08 | 0.81 ± 0.12 | 1.12 ± 0.15 | 0.93 ± 0.08 | 1.02 ± 0.07 | 0.82 ± 0.15 |
| 4                  | 4                         | 1.71 ± 0.18 | 1.48 ± 0.15 | 1.70 ± 0.16 | 1.50 ± 0.16 | 1.91 ± 0.18 | 1.69 ± 0.18 |
| 10                 | 7                         | 1.83 ± 0.18 | 1.51 ± 0.14 | 1.76 ± 0.18 | 1.49 ± 0.16 | 2.02 ± 0.20 | 1.74 ± 0.12 |
| 4                  | 4                         | 1.94 ± 0.10 | 1.56 ± 0.17 | 1.81 ± 0.16 | 1.51 ± 0.17 | 2.06 ± 0.17 | 1.79 ± 0.11 |

tion and its size was almost parallel with the tension developed. Dynamic and static components of the spindle potential could be discriminated like those of the tension development, as have been also pointed out by Katz (4) and Ottoson and Shepherd (5). During application of TMA or EGTA, both amplitudes of the dynamic and static components.
in spindle potentials were scarcely distinguishable from those in the normal Ringer's solution, although the rate of discharges superimposed on the spindle potential varied largely. Since the drugs did not produce any appreciable change in the steady potential between the sensory terminal and the proximal portion of the stem axon, the above results were not surprising in view of the close correlation between the amplitude of spindle potential and the amount of steady potential which was reported in a previous paper (6). It was concluded that TMA enhances and EGTA diminishes selectively the amplitude of positive afterpotential without influencing the spindle potential.

Discharge patterns of propagated and abortive spikes during application of TMA or EGTA

Fig. 2 shows trains of propagated and abortive discharges recorded from a spindle receptor during treatments with 5 mM EGTA or 10 mM TMA as compared with those in normal Ringer's solution. The following two facts could be distinguished. 1) Abortive spikes were absent during treatment with EGTA while the population of abortive and propagated spikes during TMA appeared to be less than that under normal condition. 2) Application of EGTA gave rise to a rapid decay in the discharge rate of propagated spikes in the initial period during extension of the muscle, while the decay in the discharge rate during application of TMA was slower than that in the normal Ringer's solution.

The first point of the differences could be substantiated by comparing histograms of the intervals between successive spikes. A set of the interval histograms was obtained from a continuously recording film taken for 3 min, except for 5 sec at the initial period, upon extending the muscle to a desired length. The inter-spike intervals changed drastically during initial 5 sec of muscle extension, but then they were maintained at a rather stable level, which was dependent upon the amount of the extension and independent of the velocity of the extension. Fig. 3 shows histograms obtained from a spindle receptor at three steps of the muscle length in normal Ringer's solution. At a slack state (Fig. 3R), intervals between two propagated spikes (S-S in the figure) distributed in a wide range between 80 and 1600 msec. Similar wide distributions were also obtained for the interval histograms

Fig. 2. Responses of a spindle receptor at 1 sec and 60 sec maintenance of the muscle at +0 mm during application of 5 mM EGTA and 10 mM TMA, in comparison with normal Ringer's solution. Calibration : 20/sec and 200 µV.
Fig. 3. Interval histograms obtained from a spindle receptor at different muscle lengths in normal Ringer's solution. The intervals of the responses were measured from a continuous record for 3 min at individual muscle lengths (at the slack (R), at the in situ length (+0) and at the +2 mm (+2)). The individual histograms of the intervals consist of dividing into following four categories. A-A: Intervals between successive abortive spikes. A-S: Intervals between abortive spike and the subsequent propagated spike. S-S: Intervals between successive propagated spikes. S-A: Intervals between propagated and the subsequent abortive spike. The numbers described under the symbols represent the total number considered in each histogram.

from a propagated spike to subsequent abortive spike (S-A), from an abortive spike to subsequent propagated spike (A-S), and for the interval times between two abortive spikes appearing subsequently (A-A). The number described in each histogram represents the total number of observation in 3 min. The total spike number in 3 min observed during the slack state was 461; hence the mean frequency for all spikes was 2.6 impulses/sec in this case.

Maintaining the muscle at the in situ length (+0 mm) produced a characteristic interval histogram, in which abortive spikes were always followed by an abortive or a propagated spikes appearing with short interval (A-A or A-S in Fig. 3, +0), while propagated spikes were followed by both types of spikes (S-A or S-S) appearing with a delay of 70–
500 msec after a silent period of 50-100 msec. The mean frequency of 9.7 impulses/sec was calculated from the total spike number of 1754 in 3 min. When the muscle was extended to +2 mm, the characteristic pattern of the interval histogram was more accentuated, and the total number and the mean frequency of the spikes were 4488 and 24.9 impulses/sec respectively.

Application of 10 mM TMA diminished the population of the propagated spikes in the interval histograms and enhanced relatively that of the abortive ones, as shown in Fig. 4. At a slack state of the muscle, the same spindle receptor as mentioned above discharged only 35 propagated spikes for 3 min which corresponded to one eighth of that in the normal Ringer's solution, but discharged 203 abortive spikes which exceeded that of the normal. Extending the muscle to +0 mm or +2 mm gave rise to an obvious enhancement in the population of abortive spikes with less increase seen in propagated spikes. The total number and the mean frequency for all spikes was 1248 and 6.9 impulses/sec respectively at +0 mm muscle length and 1688 and 9.4 impulses/sec respectively at +2 mm. These values were

Fig. 4. Interval histograms obtained from the same spindle receptor as that in Fig. 3 during treatment with 10 mM TMA. The intervals of the responses were measured from a continuous record for 3 min at individual muscle length (at the slack (R), at the in situ length (+0) and at the +2 mm (+2)). See also caption to Fig. 3.
always less than those in normal Ringer's solution.

Fig. 5 represents histograms obtained from the same spindle receptor as mentioned above at three steps of muscle length during application of 5 mM EGTA. The spindle responded with propagated spikes alone without abortive spike. The interval histogram was also characterized by a short silent period, which varied from 32 to 6 msec by extending the muscle from a slack state to -1-2 mm, in comparison with those (40-80 msec) in normal Ringer's solution or during application of TMA. Since the total number and the mean frequency of the propagated spikes was 686 and 3.8 impulses/sec respectively at a slack state or 1760 and 9.8 impulses/sec respectively at +0 mm, discharge rates were higher than those in the normal Ringer's solution or in TMA. At the muscle length of +2 mm, the total number and the mean frequency were 1562 and 8.7 impulses/sec respectively, though a summit of the histogram occurred at a narrow range between 20 and 30 msec, because the discharge rate of up to 40 impulses/sec for about 10 sec after stretching the muscle to +2 mm decayed rapidly and disappeared 2 min after initiation of the stretching.

Fig. 6 represents time courses of discharge rates of propagated and abortive spikes
recorded from a spindle receptor upon maintaining the muscle at different lengths, during application of 10 mM TMA or 5 mM EGTA, in comparison with those in normal Ringer's solution. Discharge rate during initial 5 sec after completion of muscle stretch was discarded, because the rates depended upon the velocity of the muscle stretch as has been reported by Matthews (3). The rates of propagated and abortive discharges in normal Ringer's solution decayed slowly for 1 min during maintaining the muscle at different lengths (Fig. 6 Normal). During application of 10 mM TMA, the discharge rates of propagated and abortive spikes in the same spindle receptor as mentioned above were lower than those in normal Ringer's solution and scarcely decayed during sustained muscle stretch (Fig. 6 TMA).

Application of 5 mM EGTA resulted in an increase in the initial discharge rate confined to propagated spikes alone of up to 58 impulses/sec at +2 mm muscle length (Fig. 6 EGTA). The high frequency discharge decreased rapidly (approx. 7 impulses/sec 1 min

![Graph showing changes in frequencies of propagated and abortive discharges](image)

**Fig. 6.** Changes in frequencies of propagated (points connected with line) and of abortive discharges (non-connected points) in a spindle receptor terminal for 1 min from 5 sec after the start of the maintained muscle stretch at the slack (○), at the in situ (×) or at the −2 mm (●) length. At the lowest; in normal Ringer's solution. In the middle; during treatment with 10 mM TMA. At the top; during treatment with 5 mM EGTA.
after the beginning of the muscle extension), and disappeared within 2 min. A relatively rapid decrease from 54 to 10.3 impulses/sec was observed during the initial 1 min at +0 mm muscle length, but a slower decrease in the discharge rate was found at a slack state of the muscle. When the muscle was stretched again to +2 mm at a short interval after relaxation, the initial discharge rate of the propagated spikes was regained (approx. 60 impulses/sec) but lasted no longer than 1 min. This suggests that the rapid decrease of discharge rates during application of EGTA may not be caused by precipitating of adaptation but may be due to a mechanism like fatigue in the generating mechanism of impulses.

DISCUSSION

The present experiments showed that TMA produced a relative enhancement of the population of abortive spike in comparison with that of propagated spike while EGTA removed the abortive spike. These results suggest two possibilities. (1) TMA may suppress an encoding mechanism from abortive to propagated spike, while Ca++ chelation by EGTA may agitate it, if the abortive spike are responsible for the triggering of the propagated spike. (2) TMA may inhibit the initiation of both the abortive and propagated spide, while EGTA may accelerate it and hence abortive spike may be more synchronized and easily transferred into propagated spikes. Since the total number of the abortive and propagated discharges is always less during application of TMA but greater during application of EGTA than in the normal Ringer's solution, the latter supposition is more tenable than the former. Increase in the total number of spikes by EGTA may not be due to a change in muscle tension, at least not to an increase in the tension of intrafusal muscle fibers, but may be caused by the direct effect of the drug upon the sensory terminal, since no appreciable change in muscle tension was detected during application of the drug.

Mashima and Matsumura (7) have maintained that the Ca deprivation give rise to a decrease in the resting and twitch tension of the frog muscle. There is also much evidence (see 8) that K contracture is depressed in a Ca-free solution or by Ca-chelation.

Application of EGTA resulted also in a prominent increase of discharge frequency in the initial period of muscle stretch, which was followed by a rapid decay. The high frequency of propagated discharges may be due to removal of the positive after-potential which usually depresses the initiation of subsequent discharges throughout the course of its duration, as shown with a conspicuous shortening of the refractory period in S-S during application of EGTA (see Fig. 5). The rapid decay of the discharge rate may result from fatigue at the encoding membrane for impulses. As it seems unlikely that Ca may play an essential role in the ionic processes underlying the transducer action of the sensory membrane of the muscle spindle (9, 10), it is more conceivable that the ion may stabilize the activity only in the generator mechanism for abortive and propagated spikes upon which a sustained discharge may be guaranteed. The view of Tokizane and Shimazu (11) that after-hyperpolarization in spinal motoneuron is likely to be the most important factor in stabilization of firing rate seems to be significant also in the sensory terminal. It is possibly considered that the threshold of spindle receptor to mechanical stimulation is
decreased by perfusion of the receptor with low Ca solution, as has been demonstrated in Pacinian corpuscle by Nishi (12).

The fact that the amplitude of the positive after-potential following an individual propagated spike is augmented by TMA whereas reduced by EGTA cannot be explained by the generally held opinion that simple hydrophobic cations such as TMA depolarize the post-synaptic membranes in the neuromuscular junction or in the autonomic ganglion, though much less potent than acetylcholine (13). This may be accounted for the results of intracellular record from the sensory nerve terminal in future.

SUMMARY

1. By application of certain drugs upon the isolated terminal of the frog muscle spindle, supervened changes in the amplitude of positive after-potential following individual propagated spikes, the population of abortive and propagated spikes, and their discharge patterns were examined.

2. Application of 10 mM tetramethylammonium chloride (TMA) gave rise to an increase in the amplitude of the positive after-potential without appreciable changes in that of the spike components, to an enhancement in the population of abortive spikes relative to that of propagated spikes, and also to a decrease in the overall discharge rate.

3. Following application of 5 mM EGTA or 2 mM EGTA, the positive after-potential and abortive spike were abolished without discernible change in the propagated spike. However, high frequency discharge of propagated spike in the initial period of muscle stretch decayed rapidly. Significant changes in the amplitude of spindle potential, in the amount of steady potential or in the tension development during muscle stretch were produced by these drugs.

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