Plateau Formation in Action Potential of Guinea-Pig Ureter

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Many authors had been reported the effect of calcium ion on the electrical activity of smooth muscle and the essential role of calcium ion for the spontaneous discharges has been emphasized. Bülbring and Kuriyama (1963) showed that the amplitude and the frequency of action potential and the membrane potential in taenia coli were increased by excess calcium solution. Burnstock et al (1963) also described that the increase in calcium concentration produced the increase in the amplitude of plateau type action potential of guinea pig ureter.

In general, a reduction of calcium ion from the external solution causes a fall in the resting potential on smooth muscle (Bülbring and Kuriyama ; 1963, Burustock and Straub ; 1958, Holman ; 1958). In ureteral smooth muscle, Bennett et al (1962) observed that the amplitude of oscillatory action potential became small until it disappeared in the solution of low calcium concentration. In addition it is well known that calcium ion has the essential role in the excitation-contraction coupling (Bülbring, 1962).

The influence of external calcium ion on the electrical and mechanical activity of guinea-pig ureter had been investigated in this experiment. The interesting results were obtained in the experiment on effect of calcium-free solution. As described in results, the long plateau type action potential was generated in calcium-free solution. Effects of sodium and potassium ion on the generation of such long plateau type action potential and the formation of plateau had been also studied.

METHODS

Isolated ureters of guinea-pig were used as described in the previous paper (Ohkawa, 1967).

The normal Ringer-Locke's solution used in all experiments contained (mM) : NaCl 154.0 ; KCl 5.6 ; $CaCl_2$ 2.2 ; $NaHCO_3$ 2.4 ; glucose 5.0 and was aerated with O_2. Calcium excess solutions were prepared by adding reguired amount of calcium chloride. The calcium-free solution was prepared by omitting the calcium chloride from the normal solution. The excess potassium solutions were prepared by adding potassium chloride and potassium-free by omitting potassium chloride from

the normal solution. The sodium-free solutions were prepared with sucrose, choline and lithium.

Solutions, at 37°C, flowed through the active side of the apparatus, continuously at the rate of 8–10 ml/min.

A sucrose-gap apparatus was used to record the spontaneous electrical activity of strips of ureter and the mechanical activity was recorded by the mechano-electronic transducer RCA 5734 simultaneously.

RESULTS

A. Effect of excess calcium solution

The solution with high calcium concentration produced the changes on the action and membrane potentials and the mechanical activity.

When the calcium chloride concentrtion was two-fold (4.4 mM) of normal, the amplitude of action potential was reduced but its reduction was not distinguished. The frequency of spontaneous action potential of ureter was increased. The frequency, measured 20 min after exposure, changed from the mean of 1 pulse/38sec in the normal calcium to the mean of 1 pulse/23sec in 4.4 mM calcium solution. In a few cases it reduced slightly.

The membrane potential increased in most experiments. This observation consisted with the previous study of Bülbring et al (1963) in taenia coli. The tension of phasic contraction of ureter was increased by this calcium solution. These changes were shown in Fig. 1.

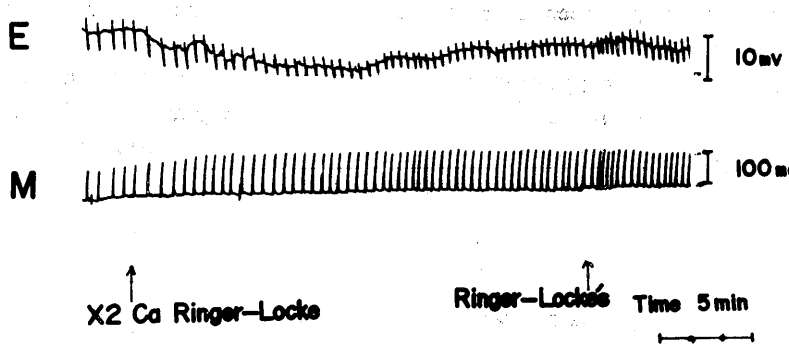

Fig. 1. The effect of increasing the external calcium concentration (4.4 mM) on the electrical activity (E) of ureter. M; mechanical activity.

The effect of three-fold (6.6 mM) was similar to that of two-fold calcium. The amplitude of action potential reduced gradually. It decreased from 25 mV in normal solution to 22 mV after 10 min exposure, 20 mV in 15 min, 18 mV in 21 min, 17 mV in 25 min and 14 mV in 30 min.

The frequency of spontaneous action potential was reduced from the mean of 1 pulse/28sec in normal solution to the mean of 1 pulse/120sec throughout exposure period of 30 min. The membrane potential was increased slightly. In some experiments it was decreased slightly.

The rate of rise in one experiment was changed as follows; 8.6 mV/sec in normal solution, 6.3 mV/sec after 17min and 4.6 mV/sec after 2.4 min, i.e., according to the exposure period the rate of rise was reduced.

The effect of five-fold (11 mM) calcium was investigated. The action potential was diminished immediately but the membrane potential was not changed in this case. The phasic contraction ceased with diminishing the action potential.

In the case of ten-fold (22 mM), similarly the action potential was abolished immediately and the membrane potential was increased. After long exposure period the preparation was again placed in normal solution, then the membrane potential decreased rapidly.

After replacing to the solution contained twenty-fold (44 mM) calcium, the changes were similar to that in the ten-fold calcium. The spontaneous action potential was abolished instantly and the membrane potential was increased.

B. Effect of calcium-free solution

When the exrernal calcium concentration was decreased from normal (2.2 mM) to zero, the membrane potential was not changed or decreased slightly in a few cases. In general, a reduction of calcium ion causes a fall in the membrane potential on striated muscle (Holman et al, 1959 ; Curtis, 1963 ; Ishiko et al, 1957) and smooth muscle (Burnstock et al, 1963). Bülbring and Kuriyama (1963) had found that, in the absence of calcium, the membrane potential on taenia coli of guinea-pig was always decreased, furthermore, the depolarization was very distinguished.

Spontaneous action potential was suddenly inhibited after a few minutes of exposure, while the phasic contraction decreased gradually and finally ceased with the complete inhibition of action potential.

However, 15–20 min after abolition of the spontaneous action potential in calcium-free, spike type action potential reappeared spontaneously with higher frequency than that of normal. The phasic contraction, however, could not be recorded. The shape of reappeared action potential changed from the spike type to the long plateau type according to the exposure time.

Moreover, the oscillatory potentials developed on the later phase of plateau. The duration of action potential was prolonged gradually with exposure period. These changes were shown in Fig. 2.

These results in calcium-free solution were very different from other smooth muscle and cardiac muscle. In dificiency of external calcium (0.27 and 0.027mM), the duration of action potential of papillary muscle was prolonged (Hoffman et al,

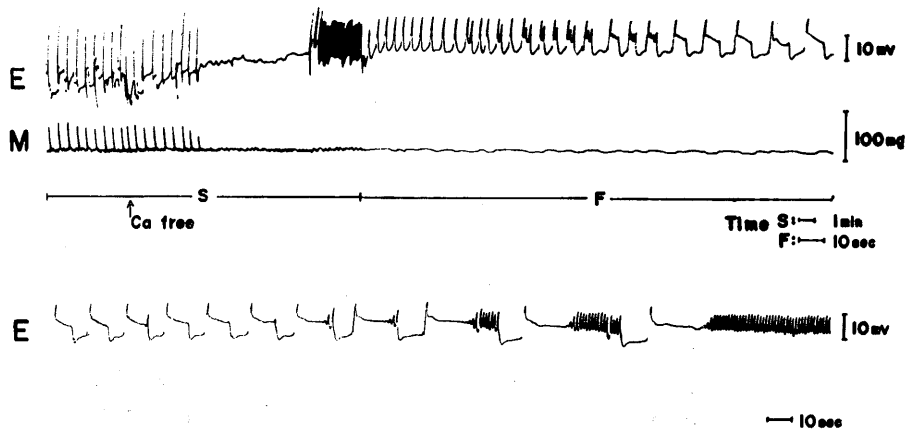

Fig. 2. The effect of calcium-free solution on the electrical and mechanical activity of ureter.
E; electrical activity, M; mechanical activity. Lower record continued directly.

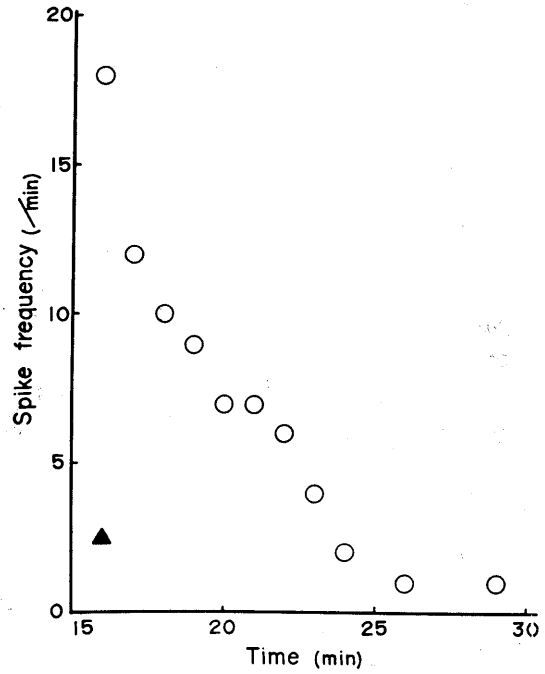

Fig. 3. Relation between the exposure time and the spike frequency in calcium-free solution.
Black triangle indicates the frequency level in normal solution.

1956). It had been found that the shape and amplitude of action potential of Purkinje fiber was relatively insensitive to changes in calcium concentration (Hoffman et al, 1960).

The relation between the exposure time and the spike frequency in calcium-free was shown in Fig. 3. The spike frequency in the initial reappeared stage was higher than that in normal solution and decreased gradually with exposure. After long time of exposure (24 min), the frequency was lower than that in normal. Fig. 4 shows the relation between the exposure time and the duration of reappeared action potential. The duration of action potential gradually prolonged because the spike type changed oneself into the plateau type. The duration of action potential in initial stage of reappearance was very short. However, it was prolonged over 200 sec after exposure of 35 min.

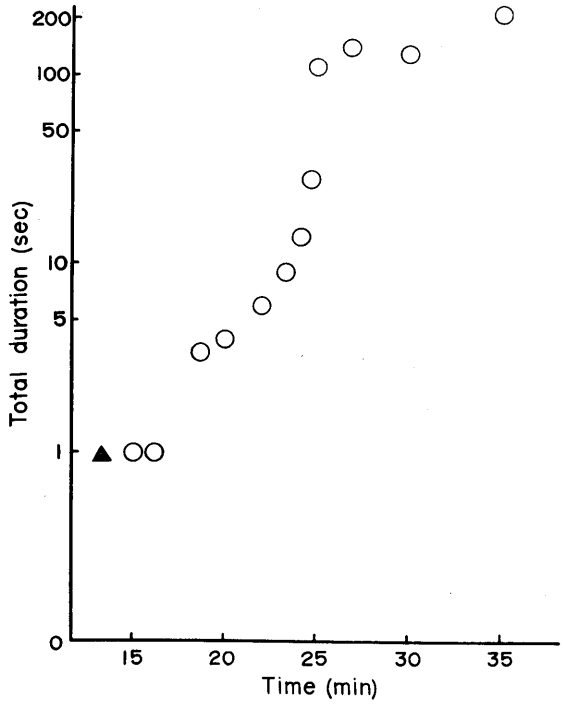

Fig. 4. Relation between the exposure time and the duration of action potential in calcium-free solution.
Black triangle indicates the duration level in normal solution.

C. Effects of sodium and potassium ion on the plateau formation in calcium-free

The following experiments were carried out to investigate what ions affect the plateau formation or plateau duration.

When the plateau type of action potential produced spontaneously in calcium-free prolonged considerably, the ionic environment was changed to excess potassium.

In the excess potassium solution contained 15.3 mM and calcium-free, the plateau of action potential was shortened Finally the membrane potential was arrested on the plateau level or slightly below. These results were shown in Fig. 5. In similar condition (0.25 mM Ca, 18 mMK), the membrane potential was decreased in taenia coli (Bülbring et al, 1963).

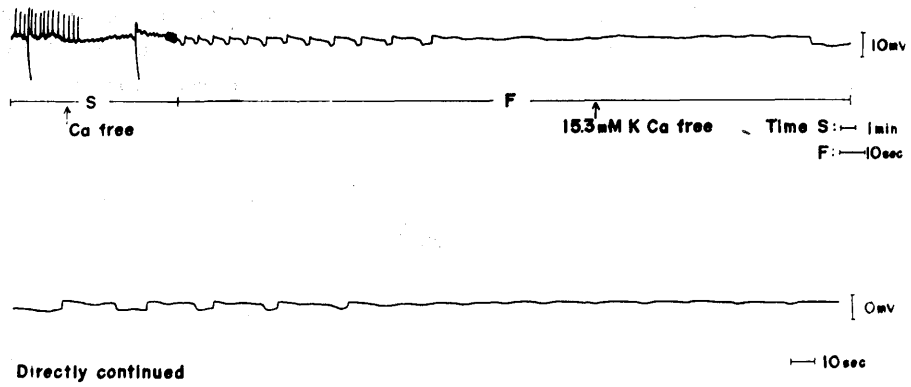

Fig. 5. Effect of excess potassium (15.3 mM) in calcium-free solution on the electrcal activitiy of ureter.

When the external potassium concentration was 44.1 mM, the duration of the plateau was shortened.

In both potassium and calcium-free solution, the membrane potential was not changed and the generation of plateau type action potential was inhibited for a short time. However, after that, the plateau type action potential reappeared and its frequency increased gradually. Finally the spontaneous action potential was abolished.

The duration of action potential was as same as that in before or had a tendency towards decrease except the first action potential in the potassium and calcium-free.

When the preparation was teansferred from calcium-free to the both sodium and calcium-free solution, in which sodium chloride was replaced by sucrose, choline chloride or lithium chloride, the spontaneous plateau type action potential was diminished immediately by these ionic environments, setting the membrane potential on the resting level or slightly below it.

DISCUSSION

A. Excess calcium solution

In excess calcium solution, the membrane potential was increased and the action potential was reduced in amplitude or diminished, rate of rise of action potential was decreased. Hodgkin and Huxley (1949) suggested that calcium and sodium ions have the antagonistic effect on the sodium-carrier in membrane and these ions control the number of free sodium-carrier. If calcium ion is increased in an external solution, the excess calcium may inhibit that sodium ion combines with carrier. Therefore it can be expected to decrease an amplitude of action potential.

On the other hand, the amplitude of action potential in excess calcium was increased in taenia coli (Bülbring and kuriyama, 1963). It was described by Burnstock et al. (1963) that an increase of calcium concentration leads to an increase in the amplitude of the plateau type action potential of guinea-pig's ureter. In this case, the effect of nineteenth-fold of excess calcium decreased the duration of plateau phase since the action potential was converted into the simple spike (Bennett et al, 1962). The later case, it is considered that calcium and sodium ion affect antagonistically on the membrane carrier and calcium ion stabilizes the membrane. Moreover, in taenia coli, after transient cessation of discharge in excess calcium solution (10 mM), spikes returned during prolonged exposure and had an increased amplitude (Holman, 1958).

Our results were not consisted with findings above, but if the antagonistic effect on the carrier exists, the result can be explained without discrepancy.

Holman (1958) suggested that the sodium-carrier mechanism might be poorly developed in taenia coli and found that the rate of rise was increased in the presence of four-fold of normal calcium. Bülbring et al. (1962) obtained similar results on taenia coli that the rate of rise was increased in excess calcium within a range between 5 mM to 15 mM.

It is not clear whether the sodium-carrier mechanism is poorly or not in ureter smooth muscle, but the amplitude of action potential was reduced with hyperpolarization. This result suggests that if the carrier is largely inactivated usually, the influence of antagonistic effect is large.

In Purkinje fibers, the upstroke velocity of the action potential was higher in calcium-rich (Weidmann, 1955). However, in skeletal muscle, the rate of rise was reduced approximately in proportion to the increase in calcium (Ishiko et al, 1957).

Therefore, the effect of excess calcium on the rate of rise is similar to that in skeletal muscle and different from the effect on smooth muscle of taenia coli and cardiac muscle.

It is known that calcium ion controls the sodium permeability (Frankenhauer et al, 1957). In Purkinje fibers, Weidmann (1955) assumed that the main effect of

calcium ion was to decrease the sodium permeability of the surface membrane.

As described in results, the rate of rise in excess calcium was decreased. This result suggests that the sodium current across the membrane is decreased. The amplitude in excess calcium decreased, this also suggests the decrease of sodium ion entering to the inside of cells.

Moreover, Weidmann (1955) had described the stabilizing effect of calcium ion. This statement is based on the finding that spontaneous rhythm is slowed or suppressed. This stabilizing effect of calcium ion may due to decrasing the membrane current by sodium ion. It must be care that the meaning of "rate of rise" used in a sucrose-gap method is different from that in an intracellular recording.

B. Calcium-free solution

Obviously the result showed the development of the plateau type action potentials and the prolongation of the duration of plateau phase with exposure period in calcium-free solution. Holman (1958) reported on taenia coli that the spike configuration in calcium-free solution had changed with long exposure, i. e., 45 min after, the amplitude was reduced and the duration was prolonged in company with falling the tension. Hoffman et al. (1956) observed that the duration was increased and the result of the prolongation of the plateau phase in papillary muscle in calcium deficient solution. Also the great reduction in external calcium concentration increased the duration about 15% in ventricular muscle was observed (Hoffman et al, 1956).

In taenia coli the low calcium solution (0.2 mM) caused the decrease of the rate of rise (Bülbring and Kuriyama, 1962). If the rate of rise is larger in calcium-free solution than that in normal solution, the sodium permeability on the membrane may be also larger relatively. In this experiment, the rate of rise was not measured, but after the initial inhibition of the action potential, the spike frequency was higher than that in normal solution. This result suggests that the sodium permeability is increased in calcium-free solution.

From the stand of view on the antagonism between calcium and sodium ion on the sodium carrier, the carrier may carry more sodium in calcium-free solution, therefore, the sodium current may increase.

The action potential was inhibited initially after replacing to the calcium-free solution and the small depolarization was seen in many preparations. After the transient inhibition the reappeared action potential increased its amplitude gradually. The configuration of the action potential was a spike type initially. Furthermore, it was converted into the plateau type action potential with the exposure period; the duration of plateau phase was increased. This may due to the gradual increasing of the sodium permeability in calcium-free solution.

When the calcium-free solution was replaced with the high potassium and calcium-free solution, the plateau type action potential was abolished finally. The

membrane potential was decreased. The abolishment of the plateau type action potential may due to decrease in the membrane potential which leads the inactivation of sodium carrier.

When sodium ion was replaced by other cations, e.g., lithium or choline, the plateau of the action potential could not mainteined. The action potential was abolished immediately by replacing other cations. Namely the generation of the action potential and the maintenance of the plateau level in the action potential requried sodium ion in calcium-free solution. Above result consisted with the previous findings by Bülbring et al. (1963).

The results suggest that the interaction between sodium and calcium ion which exists in normal solution and calcium-free solution is different from the interaction between calcium and other replaced cations in calcium-free solution.

SUMMARY

1. Effects of changes in the external calcium concentration on the electrical and mechanical activity in smooth muscle of ureter of guinea-pig have been studied by means of the sucrose-gap method and the mechano-electronic transducer.
2. Within a range of excess calcium between 4.4mM to 6.6mM, the amplitude and the frequency of action potential were reduced. The membrane potential was increased slightly.
3. Within a range of excess calcium between 11mM to 44mM, the action potential was diminished and the membrane potential was increased slightly.
4. Total absence of calcium in the external solution inhibited the generation of action potential initially and then the plateau type action potential reappeared. The oscillatory potentials developed on the later phase of plateau with the exposure time.
5. In excess potassium and calcium-free solution, the duration of plateau of action potential was shortened and the spike diminished finally.
6. In both potassium and calcium-free solution, the generation of action potential was inhibited transiently and then the action potentials reappeared. The duration of reappeared action potentials was not changed.
7. In both sodium and calcium-free solution, replaced by sucrose, lithium or choline, the plateau type action potential was abolished immediately.

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