Effects of Various Anions on the Electrical and Mechanical Activity of the Smooth Muscle of Guinea-pig Ureter

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The intracellular chloride ion content in smooth muscle cells is considerably higher than that of skeletal muscle (Barr, 1959; Durbin et al, 1961; Casteels et al, 1956). It is known that chloride equilibrium potential which is calculated on the assumption of passive distribution of chloride ions is different from the resting membrane potential measured intracellularyl (Kao et al, 1964; Casteels, 1965). This result indicates that chloride permeability in smooth muscle membrane is relatively large. In fact, when chloride ions in normal solution were replaced by various monovalent anions or sulfate ion, changes in membrane potential and frequency of action potential were observed in taenia coli of guinea-pig (Kuriyama, 1964).

On the other hand, potentiation in contraction and changes in after-potential of skeletal muscle were observed in anion replaced solution (Kahn et al, 1950; Hill et al, 1954; Lubin, 1957; Frank, 1961; Mashima et al, 1962). The effect of anions on the electrical and mechanical activity of guinea-pig taenia coli was studied by Kuriyama (1963). In this experiment, the influences of anion replacement on the electrical and mechanical activity of ureteral smooth muscle were examined.

METHODS

Guinea-pigs of both sexes, weighing between 200 and 300g, were used. The ureter, 3-4cm in length, was dissected and the electrical and mechanical activity was recorded by means of the sucrose-gap method and RCA 5734. The normal Ringer-Locke solution contained (mM) NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 2.4 and glucose 5, and was aerated with 97% O_2 and 3% CO_2. Chloride-free solutions were prepared by replacing NaCl by an equivalent amount of NaBr, $NaNO_3$, NaI, NaSCN and Na_2SO_4. In SO_4 solution, the calcium concentration was increased to 10mM (c. f., Burnstock et al, 1958). High potassium anion solutions were prepared by replacing NaCl by an equivalent amount of KBr, KNO_3, KI, KSCN and K_2SO_4. The membrane potential changes obtained in

various anion solutions were corrected for the liguid junction potentials between the sucrose (10% w/v) solution, the normal and the test solutions.

RESULTS

I. NO_3 solution

Fig. 1 shows the changes of electrical and mechanical activity in ureteral smooth muscle. When the normal solution was replaced by NO_3 solution, the membrane potential decreased. After 10min in NO_3 solution, the depolarization in the membrane potential was 9.1 mV (mean value in five preparations). The frequency of spontaneous action potentials increased immediately (7/min in NO_3 solution and 1.1/min in normal) and decreased gradually. The amplitude of the action potential gradually decreased in NO_3 solution. In some preparations, large amplitude action potentials were observed in the initial stage. However, after 10min in NO_3 solution the amplitude of action potential was reduced to 50–70 % of normal. The amplitude of action potential decreased with prolonged exposure period, e. g., the amplitude of action potential was 14.2 mV in normal solution and 10.0 mV after 5min, 7.3mV after 10min and 5.8mV after 15min.

The duration of the action potential was increased in NO_3 solution. In normal solution the mean duration of the action potential was 470msec. After 7min in NO_3 solution, it increased to 640msec (135%). The half duration of the action potential was also increased, from 300 msec in normal to 330–360 msec (110–118%) after 6–7min in NO_3 solution. The rate of rise and fall of the action potential was considerably decreased. The decreases in the rate of rise and fall were 30–50% and 40–50% respectively.

When recording the action potential of guinea-pig ureter by the sucrose-gap method, an after polarization potential was often observed; the amplitude of this positive after-potential was 2–3 mV. An after-negative potential was also observed in some preparations. After replacement with NO_3 solution, the after-positive potential was depressed and the after-negative potential which was ovserved in normal solution was incressed in amplitude.

The tension of the phasic contraction was increased in NO_3 solution. It depended on the exposure period, e. g., 168 % increase after 5min and 197 % after 10 min.

II. Br solution

The membrane potential was increased in Br solution immediately. After 5min, the mean hyperpolarization value of membrane was 6mV and after 10min, 4mV. The frequency of spontaneous action potential was not changed in Br solution; it was 1.5/min in normal and 1–2/min in Br solution. Fig. 2 shows the spontaneous action potential in Br solution. Changes in the amplitude of the

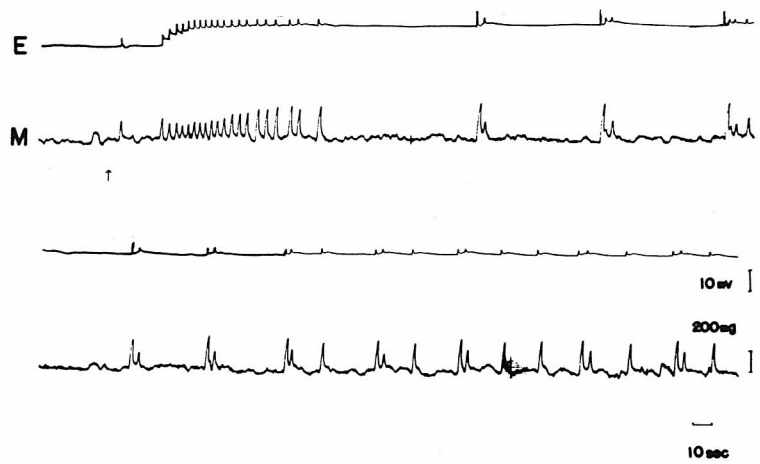

Fig. 1. Effect of NO_3 solution on the electrical and mechanical activity of guinea-pig ureter. E; electrical activity and M; mechanical activity. Arrow indicates the solution change. Lower recording continued directly.

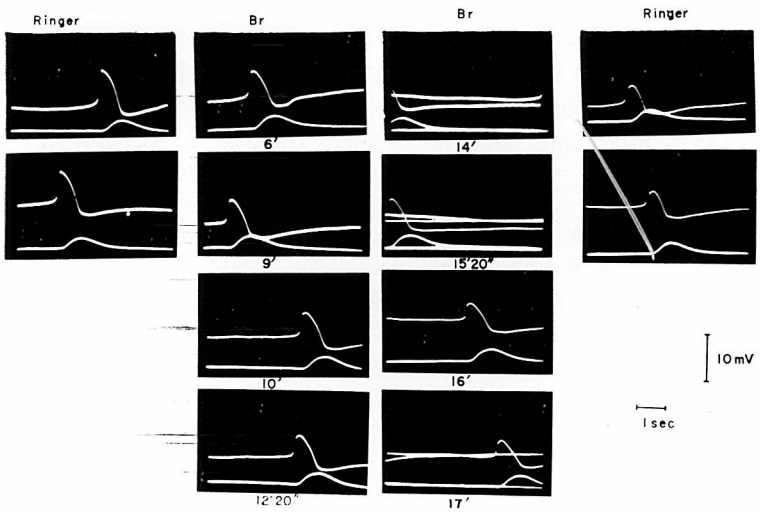

Fig. 2. Effect of Br solution on the electrical and mechanical activity of guinea-pig ureter. Upper; action potentials and lower; contractions. Times indicate the perfused period in Br solution.

action potential were not constant at initial stage, however, it decreased to about 85% of normal in all preparations.

The duration of action potential was slightly prolonged. The half duration of action potential, 470–480 msec in normal, was prolonged to 500–600msec (105–125%) in Br solution.

The rate of fall of the action potential was reduced and the decrease depended

on the exposure time, e. g., 80% after 6min, 70% after 10min and 55% after 15min.

The after-positive potential (1.8–2.9mV) which was observed in normal solution, was decreased or the after-negative potential was observed in Br solution. The tension in phasic contraction was increased immediately and further increased with exposure time, e. g., 116 % after 5min and 119 % after 10 min.

III. I solution

The membrane potential was decreased in I solution immediately, mean depolarization was 4.5 mV after 5min and 7.4 mV after 10 min. The frequency of action potential increased initially and gradually decreased. The frequency in normal solution was 2.5–3.5/min and 2–3/min in I solution.

The amplitude of action potential was decreased below the normal value initially. The tension was increased with the exposure period; 135% after 5min and 137% after 10min.

IV. SCN solution

When replaced by SCN solution, the membrane potential increased initially (by 5.3mV) but gradually recovered (2.0mV increase after 10min). The frequency of action potential increased in many preparations. The amplitude of action potential was decreased in SCN solution. The tention of phasic contraction was immediately increased (185% after 5min and 205% after 10min).

V. SO_4 solution

Fig. 3. shows the effect of SO_4 solution on the electrical and mechanical activity of the ureteral smooth muscle. The membrane potential was greatly decreased, after 10 min in SO_4 solution the membrane potential was reduced by 15mV. The frequency of the spontaneous action potential was immediately increased and the spontaneous action potential abolished after 3-4min.

The amplitude of the action potential was reduced with exposure period. The half duration of action potential was slightly prolonged at the initial stage but decreased to 67–82 % of normal value after 10min incubation.

The rate of rise of the action potential was increase. The after-negative potential was decreased in its amplitude or changed into a small after-positive potential. The tension of phasic contraction was decreased and the degree of decrease in tension was dependent on the exposure period (55 % of normal after 6 min and 45% after 10min).

VI. Phasic contraction in high potassium anion solutions

When NaCl in normal solution was replaced by isotonic potassium anion solutions (NO_3, Br, I and SCN), the phasic contraction was diminished immediately and contractures appeared. The membrane potentials were depolarized in all various high potassium anion solutions. The contractures reached the maximum

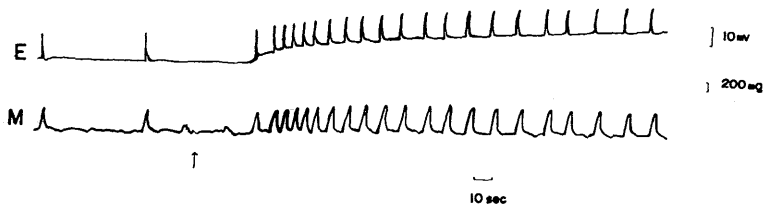

Fig. 3. Effect of SO_4 solution on the electrical and mechanical activity of guinea-pig ureter. E; electrical activity and M; mechanical activity. The solution was changed at the arrow.

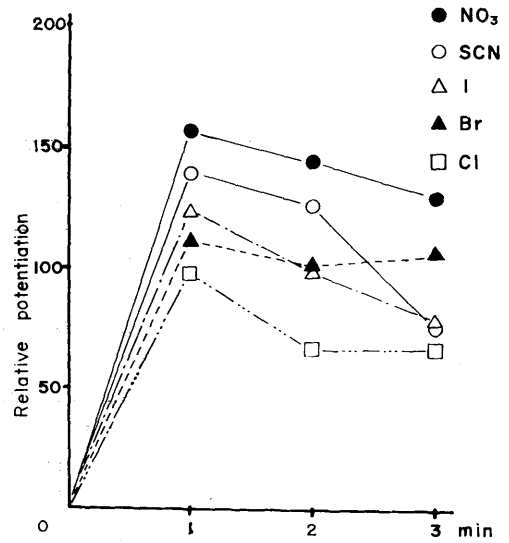

Fig. 4. Comparison of the time course in contracture in high potassium anion solutions. The tension of spontaneous phasic contraction in normal solution was used as 100%.

level after 1 min incubation and decreased gradually. Fig. 4. shows the time course of contractures in various isotonic potassium anion solutions. The maximum contracture levels in various anion solutions were 157 % in NO_3, 139 % in SCN, 123% in I, 112% Br and 99% in Cl solution (the tension of phasic contraction in normal solution was as 100%). The order of magnitude of contractures was consistent with that in phasic contraction in the normal potassium anion solutions.

DISCUSSION

Continuous depolarization of the membrane of ureteral smooth muscle cells was observed by replacing Cl by NO_3, I and SO_4. At 10min, the order of the

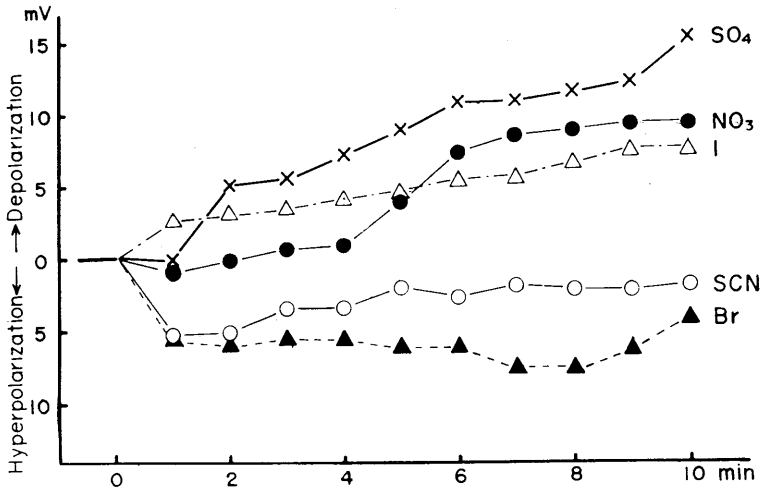

Fig. 5. Comparison of the membrane potential changes of guinea-pig ureter in various anion solutions. Times indicate the exposure period in various anion solutions

depolarizations was $SO_4 > NO_3 > I$. In SCN solution, the change of the membrane potential was slightly increased after 10min exposure. Continued hyperpolarization was observed in Br solution. These changes in the membrane potential were shown in Fig. 5. These results were different from the results obtained from taenia coli (Kuriyama, 1963) in some parts. However, the result in SO_4 solution was consistent with previous papers (Burnstock et al, 1958 ; Holman, 1958). In skeletal muscle, the membrane potential was not affected by replacing Cl by these anion solutions (Lubin, 1957 ; Oseki, 1959). The membrane potential of cardiac muscle is also not changed by replacing anion solutions (Carmeliet, 1961 ; Abe et al, 1964). However, Hutter (1961) had observed hyperpolarization of the cardiac muscle in NO_3 and I solution. In ureteral smooth muscle, considerable changes in membrane potential were observed in various anion solutions from the initial stage. This result indicates that chloride ion contributes to the membrane potential of this smooth muscle. SO_4 ion may be less permeable to the cell membrane (Boyle et al, 1961 ; Araki et al, 1961). It is considered that this anion may also be less permeable or non-permeable to the ureteral smooth muscle cell membrane. The mechanism of depolarization by such an impermeable or less permeable anion is considered as follows :

The current which carried by chloride ion through the cell membrane is given by

$$I_{Cl} = g_{Cl} (E - E_{Cl}) \qquad (1)$$

When chloride ion is replaced by other anion, the current carried by the anion is given by

$$I_A = g_A (E - E_A) \quad (2)$$

At this time, the anion equilibrium potential is given by

$$E_A = \frac{RT}{F} \ln \frac{[Cl]_0 + \beta[A]_0}{[Cl] + \beta[A]} \quad (3)$$

$$\beta = \frac{P_A}{P_{Cl}} \qquad (0 \leqq \beta \leqq 1)$$

The chloride equilibrium potential is determined by the assumption of passive distribution of chloride ions across the membrane (Hutter et al, 1961). However, a difference between the chloride equilibrium potential and the resting membrane potential of the uterine smooth muscle was obtained by Kao et al (1964). This result suggests that chlorde ion is not only distributed according to the Donnan equilibrium and that an active transport system may relate to the chloride ion distribution. In smooth muscle, it is considered that the chloride ion equilibrium potential is less than that of the resting membrane potential. When chloride ion is replaced by an other anion, the anion equilibrium potential decreases below the chloride equilibrium potential. Therefore, the potential difference which is the driving force to move the ions is increased. Even if the membrane condition is unchanged, the depolarizing current is increased under such conditions. The depolarization by NO_3 and I solution might be depend on that these anions are less permeable than chloride ion ($\beta < 1$).

Hutter et al (1959) and Hutter et al (1960) observed a decrease in the membrane conductance due to replacing by SO_4 and Br in skeletal muscle. In Purkinje fiber, the increase in the membrane resistance was also observed in Na-acetylglycinate solution (Carmeliet, 1961).

However, the dopolarization due to replacement by various anion solutions may indicate that the changes in membrane conductance is small and the ratio of the anion current to the total membrane current is large.

Br solution gave hyperpolarization, prolongation of the half duration of action potential, depression of the afterpositive potential and potentiation of phasic contraction. These changes in the electrical and mechanical activity were similar to those in NO_3 solution. These results suggest that Br ion might be less permeable than Cl ion. That is, when Br ion has some interactions with other ions, the membrane potential may increase by less permeable Br ion. From the discussion of the membrane potential changes by anion treatment and the case of Br solution, it is considered that the order of the membrane permeability of these anions is $SO_4^{--} < NO_3^- < I^- < Br^- < Cl^- < SCN.^-$

In general, the frequency of the spontaneous action potential in smooth muscle is increased by anion replacement. After long exposure, it was decreased or spontaneity abolished (Burnstock et al, 1958 ; Holman, 1958 ; Axelsson, 1961 ; Kuriyama, 1963). In this experiment, the frequency of the spontaeous action potential was also increased at the initial stage in SO_4, NO_3 and I solution.

The amplitude of the action potential became smaller than normal except in I solution. In intestinal smooth muscle, the duration of the action potential was prolonged in NO_3 solution (Axelsson, 1961). Similar results were obtained in uterine and skeletal muscle (Harris, 1958; Hutter et al, 1960). In this experiment, it was observed that the half duration of action potential was prolonged in Br and NO_3 solution.

The prolongation of the action potential duration may indicate that chloride ion contributes to the repolarization phase of the action potential. The after-positive potential might be inhibited due to a decrease in the inward anion current which flows during the repolarization phase in Br and NO_3 solution.

The phasic contraction was gradually increased after anion solution. The order of effectiveness in increasing tension was $SCN^- > NO_3^- > I^- > Br^- > Cl^- > SO_4^{--}$

Many authors have reported the potentiation effect due to anion treatment (Kao et al, 1964; Kahn et al, 1950; Hill et al, 1954; Lubin, 1957; Frank, 1961; Mashima et al, 1962; Washio et al, 1963). In skeletal muscle, the potentiation effect was $I^- > NO_3^- > Br^- > Cl^-$ and this order fitted the lyotropic series (Hill et al, 1954). The potentiation effect in the skeletal muscle may relate to (1) excitation-contraction coupling (2) prolongation of the active state (3) specific and direct effect at cell inside and (4) inhibition of calcium bound to relaxing factor. Anions which were used in this experiment might be interact with all above conditions and the degree of the interaction may obey the order of lyotropic series.

In this etxperiment, the potentiation orders in the electrical and the mechanical activity were not the same. The potentiaion effect, therefore, could not be explained by the potentiation of the excitation-contraction coupling only. It is considered that the higher anion in the lyotropic seried has high absorbability and gives rise to the polarity in the membrane; this effect might be similar to depolarize the membrane.

In this prosess, if the following effects that a decrease in a threshold of the electrical activity, a potentiation of the inactivation on the sodium-carrying mechanism and an increase the binding ability of external calcium ion to the membrane are produed, the effect of anions on changes in both frequency and amplitude of spontaneous action potentials and in tension might be explain.

SUMMARY

1. Effects of various anions on the electrical and mechanical activity of guinea-pig ureter was examined by means of the sucrose-gap method and a mechano-electronic transducer simultaneously.

2. The membrane potential was depolarized in SO_4, NO_3 and I solution and

hyperpolarized in Br and SCN solution.

3. The amplitude of the spontaneous action potential was decreased in anion solutions except I solution. The half duration of the action potential was porlonged in NO_3 and Br solution but the change was not constant in SO_4 solution.

4. The frequency of the spontaneous action potential was increased in NO_3, I and SO_4 solution initially but gradually decreased or abolished later.

5. The positive after-potential which was observed in normal solution was depressed in SO_4, NO_3 and Br solution.

6. The phasic contraction was potentiated in anion solutions except SO_4 solution. The order of potentiation at 10 min exposure was $SCN^- > NO_3^- > I^- > Br^- > Cl^- > SO_4^{--}$.

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