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A Study on the Na^+/Ca^{2+} Exchange Mechanism in the Smooth Muscle of Guinea-pig Stomach

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= ABSTRACT =

The effects of changes in extracellular Na^+ and Ca^+ concentration on the membrane potential and contractility were studied in the antral circular muscle of guinea-pig stomach in order to elucidate the existence and the nature of Na^+/Ca^{2+} exchange mechanism.

All experiments were performed in tris-buffered Tyrode solution which was aerated with 100% O_2 and kept at 35°C. The treatment of 10^{-5} M ouabain was performed to induce intracellular Na^+ loading prior to the start of experiment.

The results were as follows:

1. Na^+-free Tyrode or high Ca^{2+}-Tyrode solution hyperpolarized the membrane potential and induced contracture. The time course of contracture was similar to that of change in membrane potential.
2. The degree of hyperpolarization and the amplitude of contracture decreased in accordance with the increase of extracellular Na^+ concentration.
3. Na^+-free contracture was developed even after blocking the influence of intrinsic nerves by the pretreatment with atropine, guanethidine and TTX.
4. Ca^{2+}-channel blockers(D-600 or Mn^{2+}) and the blocker of intracellular Ca^{2+}release from sarcoplasmic reticulum(ryanodine) did not suppress the development of Na^+-free contracture. And also, dinitrophenol had no effect on Na^+-free contracture.
5. Dose-response relationship between extracellular Na^+ concentrations and the magnitude of contractures showed a sigmoid pattern. The slope of straight line from Hill plot was 2.7.
6. In parallel with the increase of extracellular Ca^{2+} concentration, the amplitude of contracture increased dose-dependently and was maximum at 8 mM Ca^{2+}-Tyrode solution.
7. The relationship between extracellular Ca^{2+} concentrations and the magnitude of contractures showed hyperbolic pattern. The slope of straight line from Hill plot was 1.1.

From the above results, it is suggested that Na^+/Ca^{2+} exchange mechanism exists in the antral circular muscle of guinea-pig stomach and this mechanism affects the membrane potential electrogenically.

Key Words: Antral circular muscle, Na^+-free contracture, Ca^{2+}-contracture, membrane potential, Na^+/Ca^{2+} exchange mechanism, Na^+/Ca^{2+} exchange ratio

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INTRODUCTION

Intracellular free calcium serve various biological functions such as excitation-contraction coupling, excitation-secretion coupling and cell-to-cell communication and so on. Intracellular free calcium which is a biological secondary messenger transfer a electrochemical information to intracellular biochemical machinery (Anghileri, 1982). The cytoplasmic Ca^{2+} concentration is generally several orders of magnitude lower than that of extracelular fluids and most of the Ca-regulated physiological process appear to be controlled by intracellular Ca^{2+} concentrations. So the control in the intracellular free calcium concentration is important to have an effective physiological process to be carried out.

The control of the intracellular free calcium concentration is generally carried out as followings. First, it is calcium influx across the cell membrane, Secondly it comes from the calcium release and uptake by intracellular calcium stores such as endoplasmic reticulum (sarcoplasmic reticulum in case of muscle) or mitochondria. In addition to this, cytoplasmic protein such as calmodulin or parvalbumin contributes to the control of the intracellular free calcium concentration by binding with the free calcium. Thirdly, it is Ca efflux across the cell membrane.

Ca influx is done through voltage-sensitive Ca-channel (Reater,1967) and receptor-operated Ca-channel (Bolton, 1979, 1986), Ca efflux is done through ATP-driven Ca pump (Schatzmann & Vincenti, 1969 ; Schatzmann, 1973). Next there is a Na^+/Ca^{2+} exchange mechanism which is known to concern with both the Ca influx and efflux across the cell membrane (Carafoli, 1982).

It is well known that replacement of part of the NaCl in Ringer's fluid with an osmotically equivalent quantity of sucrose has an effect on the frog's cardiac contractility which is similar to that of an excess of calcium (Ringer, 1883; Daly & Clark, 1921). This observation that the contractile tension is determined by the ratio of the Ca concentration to the square of the Na concentration in Ringer's fluid had been confirmed (Lüttgau & Niedergerke, 1958). By the finding of reciprocal transmembrane movements of Na and Caions on a reversible Na^+/Ca^{2+} exchange mechanism (Reuter & Seitz,1968 ; Baker et al, 1969), Reuter & Seitz (1968) found an evidence for a membrane carrier system in the heart involving Na^+ and Ca^+, and demonstrated that Ca efflux rate was sensitive to the concentration gra-dient for Na^+ across the sarcolemma. Next Baker et al (1969) demonstrated, in the internally perfused squid axon, that in addition to inward Na^+ movement coupled to outward Ca^{2+} movement, exchange also occurred in the opposite direction i.e. elevation of internal Na^+ concentration increased Ca influx. Besides cardiac muscle and squid giant axon, in the smooth muscle such as ureter, artery, tonic tension is regulated by a Na^+/Ca^{2+} ex-change mechanism (Aickin et al, 1987 ; Ashida & Blaustein MP, 1987).

Furthermore, in cardiac muscle, it had been suggested that this exchange could be electrogenic and thus voltage dependent (Horackova & vassort, 1979, Kimura et al, Sheu & Blaustein, 1987). However, in the case of the gastric smooth muscle, the Na^+/Ca^{2+} exchange mechanism is not well known. So this present study was carried out to find out a kind of Na^+/Ca^{2+} exchange mechanism in the antral circular muscle of the stomach.

METHOD

Preparation of antral circular muscle.

Guinea-pigs of either sex weighing about 300g were used in the pre-sent experiment. The animals were killed by a blow on the hind neck and exsanguinated by cutting both the carotid arteries. The stomach was extracted quickly and transferred into a chamber containg oxygenated Tyrode solution. The antral part was isolated by removing the other parts with a scissors. The antral part was cut in the longi-

tudinal direction along the lesser curvature.

The content within stomach and the mucosal layer were removed from the muscle layers in phosphate-buffered Tyrode solution aerated with oxygen in the room temperature. The strips of the antral circular muscle were prepared with the size, 5 mm in length and 1 mm in width. The loop was made with the fine cotton thread for connecting with the hook of the force transducer in the one end of the muscle strip.

Above the preparation was done under the zoom stereo microscope. After the muscle strips were rested for 1 hour in the preparation chamber, then the muscle strip was transferred into the experimental chamber.

Solutions

Preparation solution : phosphate-buffered Tyrode solution contains NaCl 147 mM, KCl 4 mM, $MgCl_2 \cdot 6H_2O$ 1.05 mM, $CaCl_2 \cdot 2H_2O$ 2mM, $NaH_2PO_4 \cdot 2H_2O$ 0.42mM, $Na_2HPO_4 \cdot 12H_2O$ 1.81 mM, glucose 5.5 mM, pH 7.4. Working solution: tris-buffered normal tyrode solution contains NaCl 147 mM, KCl 4 mM, $CaCl_2 \cdot 2H_2O$ 2 mM, $MgCl_2 \cdot 6H_2O$ 1.05 mM, tris-HCl 5 mM, glucose 5.5 mM, pH 7.4.

Above solutions were equilibrated with 100% O_2. Na-free solutions were made by replacing NaCl isosmotically with tris-Cl, LiCl. The change in the Na^+ concentration was made by replacing NaCl isosmotically with tris-Cl.

Experimental apparatus and protocol

Experimental chamber was made of perspex (derivative of leucite) and horizontal type. The input and output of the flow was done with hydrostatic pressure. Experimental temperature was maintained at about 35°C with constant temperature circulator(Haake).

The muscle strips were allowed to relax at the horizontal chamber for 1 hour in tris-buffered Tyrode solution at 35°C equilibrated with 100% O_2. Then isometric contractions were recorded by using a force transducer(Harvard & Grass FT-O3) and a recorder (Harvard & Device).

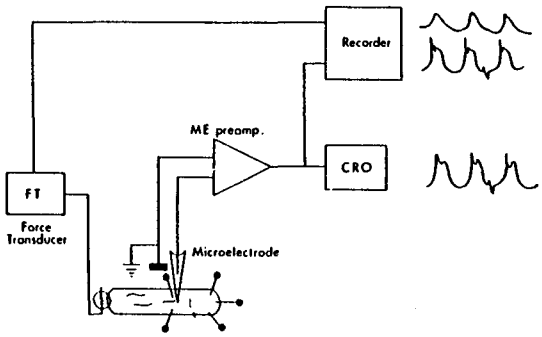

Fig. 1. A schematic representation of the simultaneous recording system for isometric contraction and the electrical activity. The isometric contractions were recorded through a tension transducer from the smooth muscle preparations. Electrical activities were measured intracellularly by use of conventional microelectrode technique.

When spontaneous activity reached the steady-state, length-tension curves were obtained all experiments were performed at optimal length. The membrane potentials were occasionally recorded with intracellular glass microelectrode at the same time of the muscle contraction (Fig. 1). The membrane potential was preamplified, connected to a DC amplifier and recorded by the recorder.

Drugs or chemicals used in this experiment: ouabain, verapamil, guanethidine sulfate, atropine sulfate, tetrodotoxin, D-600, $MnCl_2$, ryanodine.

RESULTS

Na^+/Ca^{2+} exchange mechanism

The antral circulur muscle of the guinea-pig stomach was pretreated with 10^{-5}M ouabain to increase intracellular Na^+ concentration via Na^+-pump inhibition (Fig. 2). The spontaneous contractions disappeared while the basal tone increased gradually and kept constant level (Fig. 2A). After the muscle strip was pretreated with 10^{-6}M verapamil 10^{-6}M atropine, $5x10^{-6}$M guanethidine, and $3x10^{-7}$M Tetrodotoxin(TTX)

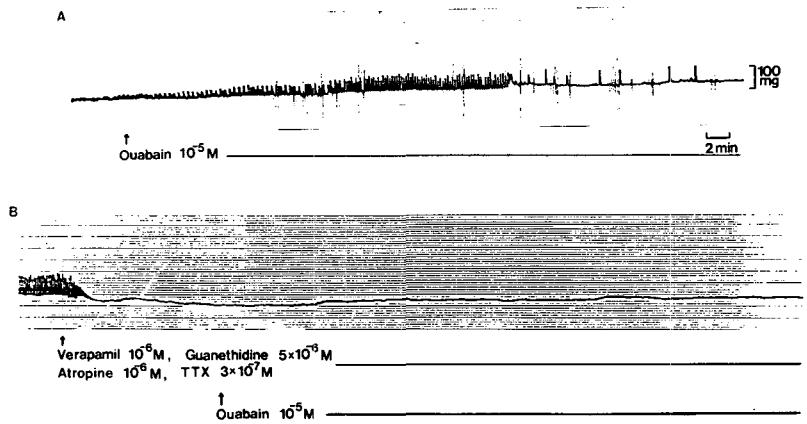

Fig. 2. Effect of ouabain on the spontaneous contractions in the antral circular muscle of guinea-pig stomach. The spontaneous contractions disappeared while the basal tone increased gradually and kept constant level (panel A). In panel B after pretreatment of 10^{-6}M verapamil, 10^{-6}M atropine, $5x10^{-6}$M guanethidine, and $3x10^{-7}$M TTX for 10 minutes, the muscle was exposed to 10^{-5}M ouabain. The basal tone also increased gradually and kept constant level like as in panel A.

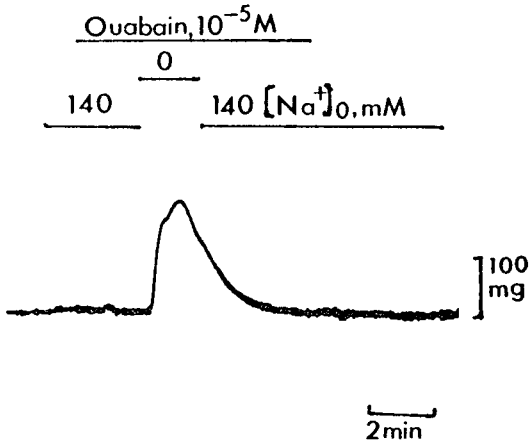

Fig. 3. The effect of sodium-free(tris) solution on contractility in the antral circular muscle of the guinea-pig stomach. The antral circular muscle fibers were pretreated with normal Tyrode solution containing 10^{-5}M ouabain for 1 hour. Then sodium-free Tyrode solution was applied for 2 minutes during the continuous exposure of ouabain, contracture was developed and relaxed spontaneously prior to washout with normal Tyrode solution. Na was replaced by equimolar tris in case of sodium-free Tyrode solution.

to exclude the effect of the neurotransmitter or Ca^{2+}-channel activation, then it was applied to 10^{-5}M ouabain. But the basal tone also increased gradually and kept constant(Fig. 2B).

In the beginnings of all of present experiments, it was pretreated with 10^{-5}M ouabain for 1 hour to maintain the same conditions of them. When Na-free Tyrode solution was applied for 2 minutes, Na^+-free contracture was developed and relaxed spontaneously prior to wash out with normal Tyrode solution(Fig. 3).

From above the result, it was assumed that a possibility about the competition and specificity between Na and Ca ions for a carrier at both sides of the membrane might be exisited, Na-free(LiCl) Tyrode solution was applied instead of Na-free(tris) Tyrode solution (Fig. 4). No difference between Na-free(tris) and Na-free(LiCl) Tyrode solutions for the development of Na^+-free contracture was noted.

In order to observe what kind of an effect on the membrane potential Na-free Tyrode solution has, Na-free contracture and membrane potential were recorded simultaneously under the same conditions(Fig. 5).

When Na^+-free Tyrode solution was perfusated, the membrane potential was hyperpolarized and the concomitant contracture was also developed.

It was assumed the possibility that the change of the extracellular Na concentration would affect on the membrane potential and the contracture(Fig. 6). The amplitude of the hyperpolarization and the contracture was less small in 50 mM Na-Tyrode solution than in Na-

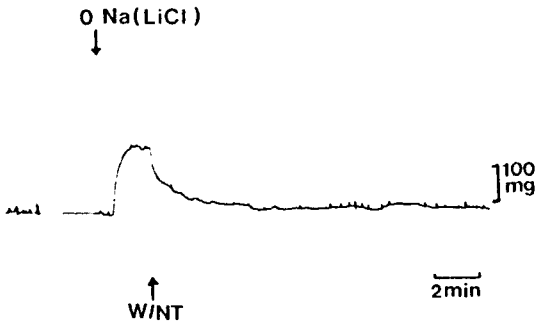

Fig. 4. The effect of sodium-free(LiCl) solution on contractility in the antral circular muscle. No difference between sodium-free (tris) and sodium-free(LiCl) solution for the development of Na^+-free contracture was noted.

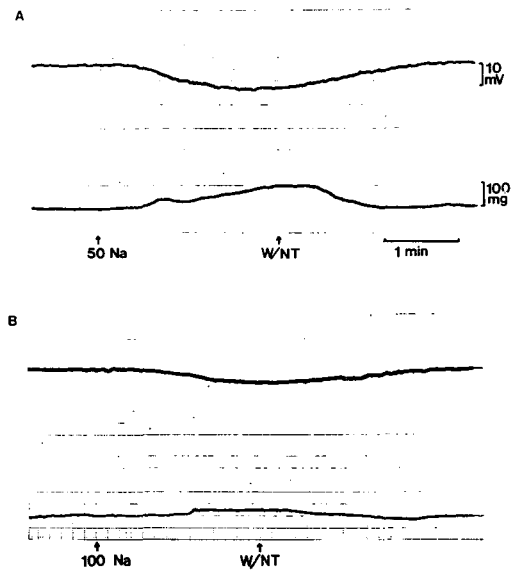

Fig. 6. The effect of extracellular sodium concentration on the magnitude of contracture and the degree of hyperpolarization of the membrane potential. The magnitude of contracture and the degree of hyperpolarization of the membrane potential were larger at 50 mM sodium-Tyrode solution (panel A) than at 100 mM sodium-Tyrode solution (panel B).

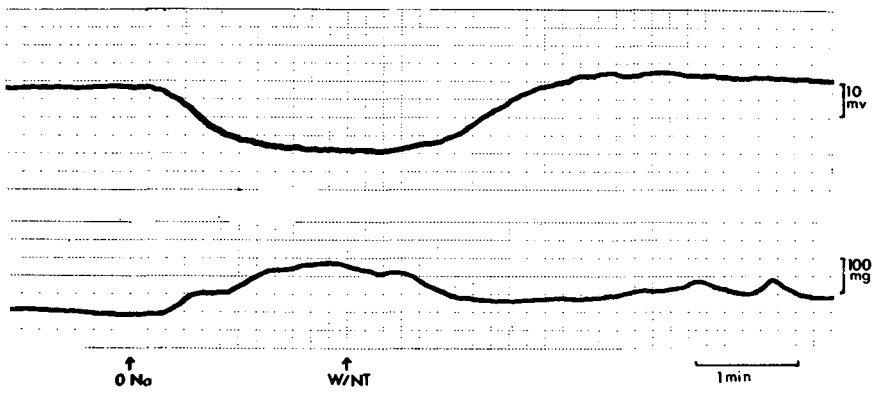

Fig. 5. Relationship between sodium-free contracture and membrane potential. The membrane potential(upper panel) and contracture (lower panel) was recorded simultaneously under the same conditions in a sodium-free Tyrode solution. The membrane potential was hyperpolarized, and the concomitant contracture was observed in sodium-free solution.

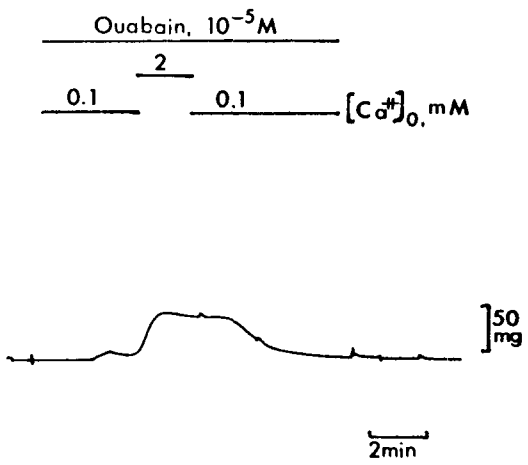

Fig. 7. The effect of calcium-Tyrode solution on contractility in the antral circular muscle. The antral circular muscle fibers were pretreated with Tyrode solution containing 0.1 mM Ca and 10^{-5}M ouabain for 1 hour. When calcium (2 mM)-Tyrode solution was applied for 2 minutes, contracture developed.

free Tyrode solution (Fig. 6A). The amplitude of them was less small in 100 mM Na-Tyrode solution than in 50 mM Na-Tyrode solution(Fig. 6B).

After the muscle strip was pretreated with 0.1 mM Ca- Tyrode solution for 1 hour, 2mM Ca-Tyrode solution was applied. Then the contracture was developed and relaxed spontaneously(Fig. 7).

The 2mM Ca- contracture and the membrane potential were recorded simultaneously under the same condition(Fig. 8). When 2mM Ca-Tyrode solution was perfusated, the membrane potential was hyperpolarized and the contracture was developed with the same time course of the membrane potential.

It was observed whether Na-free contracture and high Ca-contracture were caused by Ca influx via Na/Ca exchange mechanism or by the other mechanisms. The other mechanisms may be voltage-sensitive Ca- channel, receptor-operated Ca-channel,and release from intracellular Ca stores.

The muscle strip was pretreated with organic Ca-blocker, D-600 for 10 minutes, then Na-free

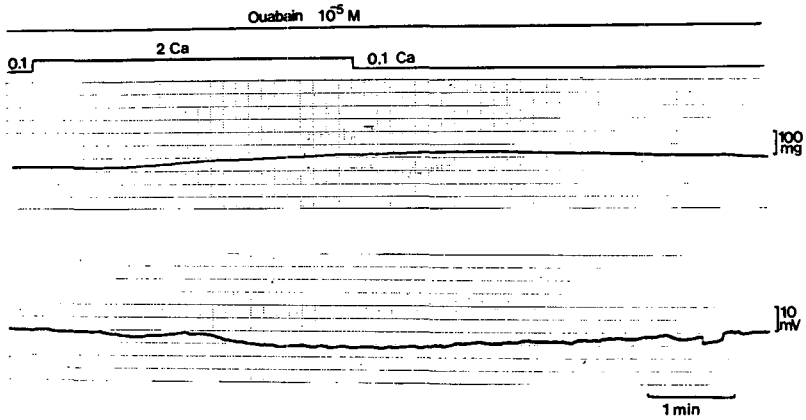

Fig. 8. Relationship between calcium contracture and membrane potential. The contracture (upper panel) and the membrane potential(lower panel) were recorded simultaneously. Increase of extracellular Ca concentration from 0.1 mM to 2 mM under the state of intracellular Na loading induced contracture and hyperpolarization of membrane potential. Note that the time course of contracture is smilar to that of the change of membrane potential.

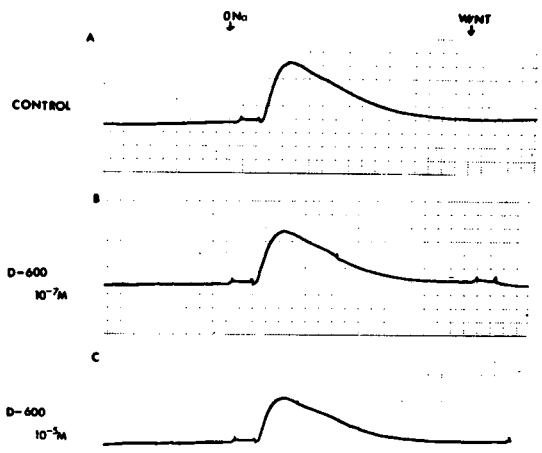

Fig. 9. The effect of D-600 on the sodium-free contracture in the antral circular muscle. (A), Control record of sodium-free contracture. (B,C), Effects of 10^{-7}M and 10^{-5}M D-600 pretreatment on the Na^+-free contracture. D-600 was pretreated for 10 minutes prior to the application of sodium-free solution.

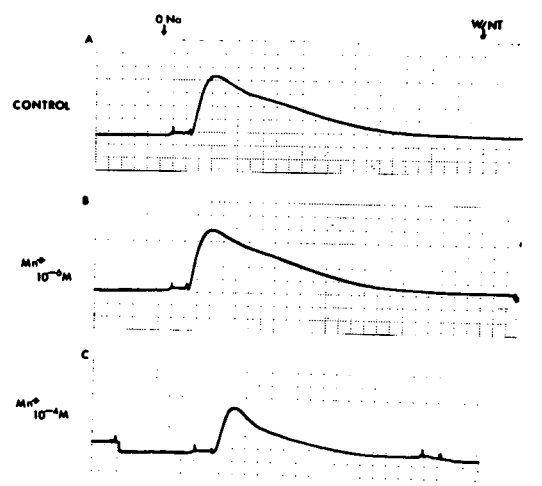

Fig. 10. The effect of manganese ion on the sodium-free contracture in the antral circular muscle. (A), Control record of sodium-free contracture. (B,C), Effects of 10^{-6}M and 10^{-4}M Mn pretreatment. At both the concentration of 10^{-6}M and 10^{-4}M, Mn^{2+} (inorganic blocker of Ca^{2+}-channel) had no effect on the development of sodium-free contracture.

Tyrode solution containing D-600 was applied(Fig. 9). The contracture was developed at low concentration of 10^{-7}M D-600 and at high concentration of 10^{-5}M D-600.

It was assumed a possibility that inorganic Ca-blocker might have a different effect compared with organic Ca-blocker. So muscle strip was pretreated with inorganic Ca blocker, Mn. The contracture was also developed like as D-600 pretreatment (Fig. 10).

In order to observe a nervous effect on the Na-free contracture, after the muscle strip was pretreated with 10^{-6}M atropine, $5x10^{-6}$ M guanethidine, and $3x10^{-7}$ tetrodotoxin (TTX) for 10 minutes, Na-free Tyrode solution was applied. But the contracture was also induced (Fig. 11).

There was a possibility that Na-free contracture might be induced by Ca release from in-

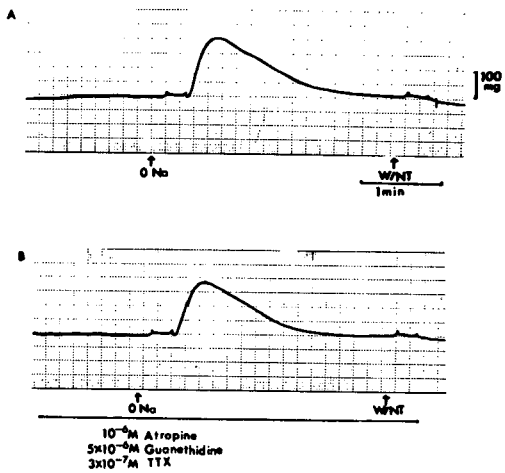

Fig. 11. The effect of 3 blockers on the sodium-free contracture in the antral circular muscle. (A), Control record of sodium-free contracture. (B), Effect of 3 blockers-10^{-6}M atropine, $5x10^{-6}$M guanethidine and $3x10^{-7}$M TTX-pretreatment. 3 blockers were pretreated to investigate nerve effect on sodium-free contracture, but 3 blockers had no effect on the development of sodium-free contracture.

tracellular Ca stores, especially sarcoplasimic reticulum. So the muscle strip was pretreated with ryanodine which was known as a blocker against Ca release from the store to rule out the possibility. But the contracture was also developed (Fig. 12).

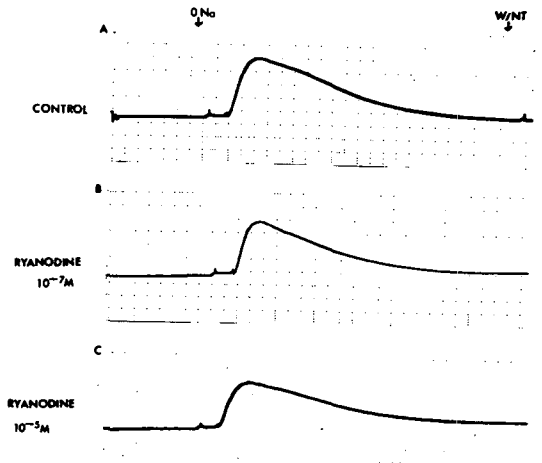

Fig. 12. The effect of ryanodine on the sodium-free contracture in the antral circular muscle. (A), Control record of sodium-free contracture. (B,C), Effect of 10^{-7} M and 10^{-5} M ryanodine pretreatment. Ryanodine was pretreated for 10 minutes prior to the application of sodium-free solution.

The muscle strip was pretreated with dinitrophenol (DNP) which was known as a inhibitor against ATP production, but the contracture was developed (Fig. 13).

From above all the results, it is thought that the Na-free contracture and high Ca-contracture would be induced by Na/Ca exchange mechanism.

Na^+/Ca^{2+} exchange ratio

In order to observe the magnitudes of the contractures to the change of the extracellular Na concentrations normal Tyrode solution was

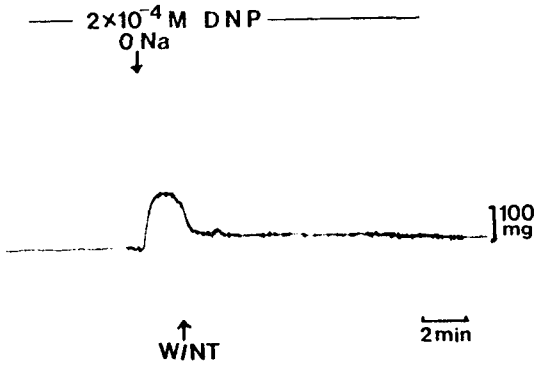

Fig. 13. The effect of DNP (dinitrophenol) on the sodium-free contracture. DNP ($2x10^{-4}$M) was pretreated for 10 minutes.

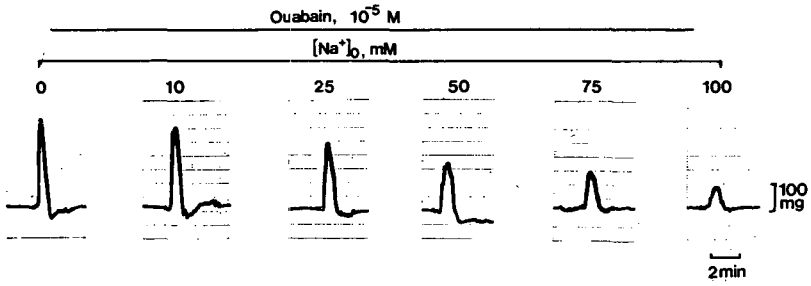

Fig. 14. The effect of extracellular sodium concentration on the magnitude of contracture in the antral circular muscle. The magnitude of the contracture was maximum at 0 mM Na^+ Tyrode solution and decreased in a dose-dependent manner in parallel with the increase of Na concentration.

changed with 0, 10, 25, 50, 100 mM Na-Tyrode solution same as the manner in Fig. 3. The magnitudes of the contractures showed a maximum at 0 mM Na-Tyrode solution, and decreased contrary to the increament of the Na concentration (Fig. 14).

This showed the relationship between the various extracellular sodium concentrations and the magnitudes of the contracture (Fig. 15). The magnitudes of the contractures at various extracellular sodium concentrations were expressed as a percentage of the magnitude of the

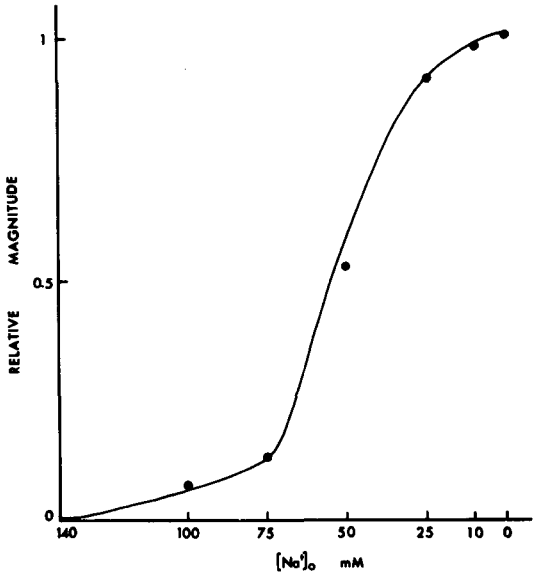

Fig. 15. The relationship between the extracellular sodium concentrations and magnitudes of contractures. The magnitudes of contractures at various extracellular Na^+ concentrations were expressed as a percentage of the magnitude of the contracture in 0 mM Na^+Tyrode solution.

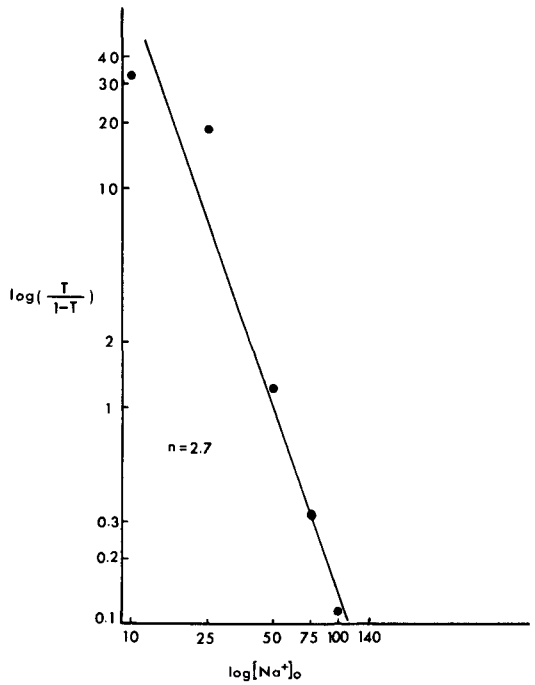

Fig. 16. Hill plot of the effect of the extracellular sodium concentration on the contracture.

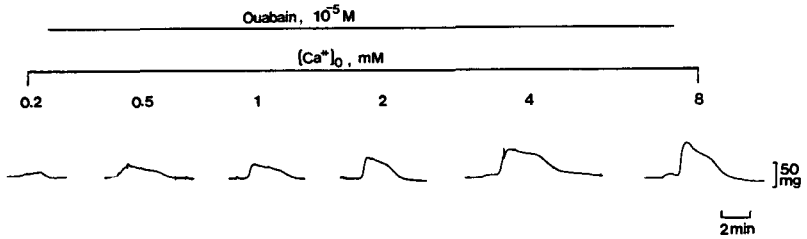

Fig. 17. The effect of extracellular calcium concentration on the magnitude of contracture. The magnitude of the contracture increased in dose-dependent manner as the concentration of extracellular calcium increased.

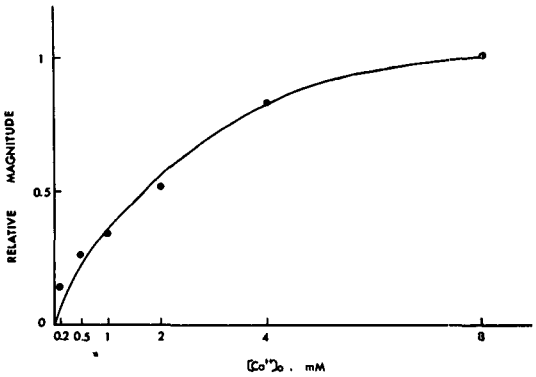

Fig. 18. The relationship between the various extracellular calcium concentrations and magnitudes of contractures. The magnitudes of contractures at various extracellular calcium concentrations were expressed as a percentage of the magnitude of contracture in 8 mM Ca^{2+}-Tyrode solution.

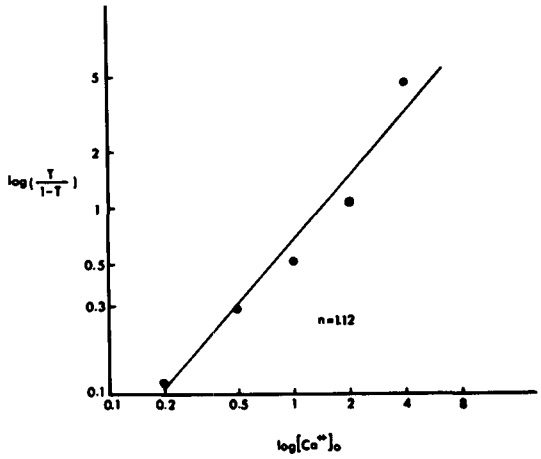

Fig. 19. Hill plot of the effect of the extracellular calcium concentration on the contracture.

contracture in 0 mM Na-Tyrode solution. Relative magnitudes at 10,25,50,75,100 mM Na-Tyrode solutions were 93 ± 1.4, 77 + 3.2, 50 ± 3.0, 27 3.2, 19.2 2.0 (%) (mean S.E.n = 9) respectively. This doseresponse curve showed sigmoid pattern.

The relationhip between the magnitude of contracture (T) and extracellular Na concentration was expressed as Hill plot (Fig. 16). The slope of the Hill plot was 2.7. The number of the slope means the number that extracellular Na ion is bound to the carriers in the extracellular membrane.

In order to observe the magnitudes of the contractures to the change of the extracellular calcium concentrations, the muscle strip was perfusated with 0.2, 0.5, 1, 2, 4, 8 mM Ca-Tyrode solutions (Fig. 17). The perfusion was alternatively changed with high concentration and low concentration to prevent intracellular Ca-depletion. The contracture showed maximum at 8 mM Ca-tyrode solution and increased dose dependently.

This showed the relationship between the various extrcellular calcium concentrations and the magnitudes of the contractures (Fig. 18). The magnitudes of contractures at various extracellular calcium concentrations were expressed as a percentage of the magnitude of the contracture in 8 mM Ca-Tyrode solution. Relative magnitudes at 0,2, 0.5, 1, 2, 4 mM Ca-Tyrode solution were 15 1.6, 22 2.4, 35 2.7, 52 ± 3.4, 83 ± 2.7 (%) (mean S.E.n = 7) respectively. This dose-response curve showed a hyperbolic pattern (Fig. 18).

This dose-response curve was expressed as Hill plot (Fig. 19). The slope of Hill plot was 1.1. The number of the slope means the number that extracellular Ca ion is bound to the carriers in the extracellular membrane.

DISCUSSION

It is generally believed that muscular contraction is produced when the intracellular free calcium concentration is increased beyond a critical level. The source of the intracellular free calcium concentration needed to induce the contraction is thought as extracellular free calcium and intracellular Ca^{2+} stores. In some tissue, these intracellular systems are developed to highly differentiated and prominent degree.

A prime example is the sarcoplasmic reticulum (SR) of fast striate muscle. A similar situation may be assumed for smooth muscle. However, because the sarcoplasmic reticulum is not developed well in smooth muscle (Blbring et al, 1981), the plasma membrane may function similarly to the sarcoplasmic reticulum in regulating the intracellular Ca^{2+} concentration.

It is assumed in the present experiment a hypothesis that Na^+/Ca^{2+} exchange mechanism may be exist in the cell membrane since sarcoplasmic reticulum is poorly developed in the smooth muscle.

The radius of Na^+ is similar to that of Ca^{2+}, that is, the radius of Na^+ is 0.95 0.98 and that of Ca^{2+} is 0.94 0.99 . Na^+ and Ca^{2+} has a competitive relationship on the negatively changed binding site of the surface membrane (Anghileri, 1982). Such a relationship between Na^+ and Ca^{2+} exists not only extracellular membrane but also intracellular membrane. Transmembrane movements of Na^+ and Ca^+ ions on a reversible Na^+/Ca^+ exchange mechanism may be related to the regulation of intracellular Ca^{2+} and hence the contractile state of muscle tissue (Glitsch et al, 1970; Blaustein, 1974; van Breemen et al, 1979; Langer, 1982). It is probably fair to assume that the contractures obtained during the present experiments may be due to extracellular low Na^+ or high Ca^{2+} concentration-dependent Ca^{2+} influx.

The possibilities that the basal tone was increased when the muscle strip was pretreated with ouabain were considered: First, release of the neurotransmitter from endogenous nerve ending due to the inhibition of the Na^+-pump (Katsugar; et al, 1978). Second, the activation of Ca^{2+}-channel caused by the depolarization of the membrane potential due to the inhibition of Na^+-pump (Hirst & van Helden, 1982). Despite of blocking the probable effects of neurotransmitter and the activation of Ca^{2+}-channel, the basal tone was also increased (Fig. 2) This result may be a possibility, intracellular Na^+ increamentdependent Ca^{2+} influx like as positive inotropic effect due to the inhibition of Na^+-pump in the cardiac tissue (Lee et al, 1985).

When the muscle strip was exposured to Na-free Tyrode solution or 2mM Ca-Tyrode solution , the contractures were developed (Fig. 3-8). These results are thought as Ca^{2+}influx via Na/Ca exchange mechanism, since the contractures were also developed even if in the cases of blocking Ca^{2+}-channnel (Fig. 9, Fig. 10) and the effect of the nerve innervating antral circular muscle (Fig. 11). Ca^{2+} release from the intracellular Ca^{2+} stores may be also another reason to the development of the contracture (Palmer & Posey, 1967, Crompton et al, 1977), but the contracture was developed despite of blocking Ca release from the intracellular Ca^{2+} stores.

The phenomenon of the hyperpolarization of the membrane potential (Fig. 5, 6) may be resulted from the decrease of the membrane conductance for Na^+ due to the removal of extracellular Na^+ since the membrane conductance for Na^+ is generally larger in the tissue with automaticity than in the tissue without automaticity. But this possibility can be denied from the following speculations: First, when extracellular Na^+ concentration was decreased or extracellular Ca^{2+} concentration was increased, Na efflux was induced and membrane potential was hyperpolarized from the studies of both radioisotope flux and intracellular recordings (Rasgado-Flores,1987). Second, when extracellular Na^+ concentration was decreased or extracellular Ca^{2+} concentration was increased, intracellular Na^+ concentration was decreased and the membrane potential was hyperpolarized from both Na^+ -sensitive microelectrode and intracellular recordings (Ellis, 1977, 1978).

The Na-free contracture was developed despite of the pretreatment of dinitrophenol (Fig. 13). From this result Ca extrusion probably appears to depend upon the presence of extracellular Na^+. Because entering Na^+ moves down its steep electrochemical gradient, Na influx may provide thermodynamically some or all of the energy needed to extrude Ca^{2+} against its large electrochemical gradient (Blaustein, 1974: Carafoli, 1982: Dipolo & Beaque, 1984). Unfortunately, the question of whether or not the Na^+ electrochemical gradient serve as the

sole source of energy for maintaining the Ca gradient has not been resolved in this time. But it may be fair to assume two factors. One system is Ca^{2+}-pump, the second system may be the Na/Ca exchange system powered by energy from the Na^+ electrochemical gradient.

From the fact that the magnitudes of the contractures were increased proportional to the decrement of the extracellular Na^+ concentration or the increament of the extracellular Ca^{2+} concentration (Fig. 14, Fig.17), it may be expected that as extracellular Na^+ concentration is decreased or extracellular Ca^{2+} concentration is increased additional Ca^{2+} would link competitively to the carriers in the cell membrane, then this Ca^{2+} participate in the increament of magnitudes of the contractures.

If Na/Ca exchange mechanism plays a role in the transport of Ca^{2+} across the plasma membrane, a question can be arisen :Is there sufficient energy in the electrochemical gradient of Na^+ to maintain, via Na/Ca exchange mechanism, the gradient of calcium outside and inside of cell membrane? In order to answer the question, the information about the stoichiometry of the exchange is needed.

There are various methods which measure Na/Ca exchange ratio : flux analysis utilizing isotope, measurement of the membrane potential, measurement of intracellular Na^+ or Ca^{2+} concentration with ionsensitive microelectrode, intracellular Ca^{2+}-sensitive metallochromatic dye measurement. None of these are complete method in measuring the Na/Ca exchange ratio (Eisner & Lederer, 1985). For example,a complication is caused by the self-exchange fluxes of Na^+ and Ca^{2+} (Na^+/Na^+ exchange, Ca^{2+}/Ca^{2+} exchange) (Blaustein et al, 1977 : Dipolo & Beague, 1986), which the Na/Ca exchange produces. Another problem is produced by membrane channels that are affected by changes of Na^+ or Ca^{2+} concentration (Eisner & Lederer, 1985).

In the present experiments, the measurement of the magnitude of the contracture was mainly used in order to the Na/Ca exchange ratio and partially the membrane potential was recorded to support a indirect method, the present method. The relationship between the extracelluar sodium concentration and the magnitudes of the contractures showed a sigmoid pattern (Fig. 15) and the Hill coefficient was 2.7 (Fig. 16).

The relationship between the extracellular calcium concentrations and the magnitudes of the contractures showed a hyperbolic pattern (Fig.18) and the Hill coefficient was 1.1. So, in the present experiment, though the present method are indirect, it is suggested that Na/Ca exchange ratio is about 3:1 in the antral circular muscle of guinea-pig stomach.

If Na^+/Ca^{2+} exchange ratio is 3:1 or over 3:1,there are several consequences (Mullins, 1979, 1981: Langer, 1982: Brading & Lategan, 1985: Sheu & Blaustein, 1986: Aickin et al, 1987: Ashida & Blaustein, 1987): i) the rate of exchange will be influenced by the membrane potential, ii) the Na^+/Ca^{2+} exchange will be associated with the net flow of ionic current across the plasma membrane iii) the Na/Ca exchanges will participate in the control of contractility.

Already suggested, all of above various methods are not complete and there is the absence of specific inhibitor of Na/Ca exchange and a specific inhibitor for the intracellular Ca^{2+} concentration -modulated Ca channels in this time. So one has no way to measure the Na^+/Ca^{2+} exchange ratio selectively If above problems are overcomed in the later, the physiological role of the Na^+/Ca^{2+} exchange mechanism will be more elucidated in detail.

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