Effects of Sodium Depletion on Contractions Evoked in Intact and Skinned Muscles of the Guinea-pig Mesenteric Artery

Takeo ITOH, Hikaru SUZUKI, and Hiroshi KURIYAMA

*Department of Pharmacology, Faculty of Medicine,
Kyushu University, Fukuoka, 812 Japan*

Abstract In the guinea-pig mesenteric artery, reduction in $[\text{Na}]_o$ by 30 mM (substituted by choline or sucrose; 137 mM $[\text{Na}]_o$ in Krebs solution) generated contraction with no change in membrane potential. In NaCl-free solution (15 mM $[\text{Na}]_o$), the amplitude of phasic contraction reached 0.8 times the contraction evoked by 118 mM $[\text{K}]_o$ with only a slight depolarization. In NaCl-free solution, the amplitude of phasic contraction evoked by noradrenaline (NA) 5×10^{-5} M or caffeine 5 mM increased to roughly twice the amplitude of the contraction evoked in the control solution. In Ca-free solution, the K-, NaCl-free- or Na-free-induced contractions rapidly ceased, but NA-induced contraction ceased within 5 min and the caffeine-induced contraction persisted for more than 15 min. In a skinned fiber, increase of $[\text{Na}]_o$ from 10 to 60 mM suppressed the pCa-tension relationship in the ranges of 10^{-7} and 10^{-5} M free Ca but not with a dose of 30 mM $[\text{Na}]_o$. NA (10^{-5} M) had no effect on skinned fibers. Increase in Na concentration (60 mM) had no effect on Ca accumulation in the store site or on Ca release by caffeine. Possible Na-related mechanisms on the development of mechanical response are discussed in relation to Ca on the surface and in the internal membrane structure. The NaCl-free-induced contraction in smooth muscles of the guinea-pig mesenteric artery is postulated to be due to influx of Ca through the Na channel, rather than the Ca channel.

The removal of Na from the bathing solution produces changes in the tone of vascular smooth muscles. In the rabbit pulmonary artery, Na-free solution produces a contraction, while a relaxation occurs in the porcine coronary artery (CASTEELS *et al.*, 1977; HIRATA *et al.*, 1980). Such regional and species differences in the mechanical response in various visceral muscles are commonly observed. The effects of Na removal are mainly considered to be related to changes in the intracellular free Ca following changes in the Ca flux at the surface membrane or release of the stored Ca (KATASE and TOMITA, 1972; BLAUSTEIN, 1977; FRIEDMAN, 1977; VAN BREEMEN *et al.*, 1978; DROOGMANS and CASTEELS, 1979; BOLTON, 1979; BRADING *et al.*, 1980).

Received for publication March 18, 1981

伊藤猛雄, 鈴木 光, 栗山 熙

BRADING *et al.* (1980) observed the effects of Na removal on the carbachol-induced contraction in the guinea-pig taenia coli and discussed the important role of Ca stores in the production of contraction under conditions of low $[Na]_i$. They stated that Na removal inhibits utilization of these sources of Ca: removal of $[Na]_o$ interferes with transmembrane Ca movement, and loss of $[Na]_i$ prevents refilling of the internal Ca store. On the contrary, in the mesenteric artery, removal or reduction of $[Na]_o$ generates the contraction and enhances the contraction produced by caffeine or noradrenaline (NA). These findings suggest that the properties of the Ca store site differ with the tissue.

We examined the effects of Na removal on the mechanical response evoked by excess $[K]_o$, caffeine, or NA in intact and chemically skinned fibers of the guinea-pig mesenteric artery. This tissue was selected because: i) we have already assessed the passive membrane property (KURIYAMA and SUZUKI, 1981), ii) this muscle cell does not generate spontaneous spikes and the spike is evoked only by nerve-mediated or direct muscle stimulation, *i.e.*, the factor modifying the generation of contraction by Ca-current is deleted in the resting membrane potential, and iii) the chemical skinning method using saponin is applicable to this preparation (ITOH *et al.*, 1981). The possible role of the Ca store site and the Ca flux in modifying the contraction-relaxation mechanism in relation to $[Na]_i$ and $[Na]_o$ is discussed.

METHOD

Materials. Guinea-pigs of either sex were stunned and bled. The dissected mesenteric artery from the jejunum region was about 10 mm in length and about 0.1–0.12 mm in diameter. For tension recording from the chemically skinned muscle cells, the vessel was carefully teased apart using jewellers' forceps, cut along the longitudinal direction, and circularly cut strips of 0.1 mm in width and 0.3 mm in length were prepared. The preparation was set up in a small chamber with a capacity of 0.9 ml through which the test solution was superfused rapidly by jetting from one end and by sucking off simultaneously with a water pump from the other end. Both ends of the preparation were fixed between pieces of Scotch double-stick tape (3M Co., St. Paul, Minn.), and isometric tension was recorded using a strain gauge transducer (U-gauge, Shinko Co.).

For tension recording from the intact tissue, the tissue was prepared the same way as were the skinned preparations. For the membrane potential change, the length and diameter of the preparation were about 10 and 0.1 mm (without dissection), respectively, and superfusion was carried out at a rate of 3 ml/min in a 2-ml organ bath.

Procedures. The experimental procedures were similar to those described by ITOH *et al.* (1981). Tension recording from the skinned muscle preparation: The tissue was superfused with modified Krebs solution of the following composi-

tion (mm: BÜLBRING, 1954); Na^+ 137.4, K^+ 5.9, Mg^{2+} 2.5, Cl^- 134.0, H_2PO_4^- 1.2, HCO_3^- 15.5, and glucose 11.5, and then replaced with 118 mm $[\text{K}]_o$ containing solution in which the NaCl was replaced with KCl isosmotically. After recording the K-induced contraction, the solution was replaced with relaxing solution containing 130 mm KCl, 20 mm Tris-maleate, 5 mm MgCl_2 , 5 mm ATP, and 4 mm EGTA at pH 6.8 (10 mm Na remained as ATP salt). Saponin treatment was then carried out by leaving the preparation for 20 min in the relaxing solution containing 50 $\mu\text{g}/\text{ml}$ saponin (ICN). The preparation was washed again with the same solution and left until the tension level became constant at about zero level. To obtain caffeine-induced contraction, the concentration of EGTA was reduced to 10^{-4} M throughout the experiment. The pH of the caffeine-containing solution was kept at 6.8 by adding KOH instead of KCl to the relaxing solution.

Various Ca concentrations were prepared by adding an appropriate amount of CaCl_2 to EGTA. The apparent binding constant of EGTA for Ca was considered to be 10^6 M^{-1} , at pH 6.8. To determine the binding constant of ATP for Mg at pH 6.8 ($4 \times 10^3 \text{ M}^{-1}$), we used the value calculated by MARTELL and SCHWARZENBACH (1956) as reported by SAIDA and NONOMURA (1978), and the free Mg concentration was kept at 1 mm.

Na-deficient or Na-free solution was prepared by replacement of Na with choline or sucrose and the pH of the solution was adjusted to 7.2 with Tris-buffer. For the choline solution, 10^{-6} g/ml atropine was added to suppress the muscarinic action in most experiments.

Tension recording from the intact muscle preparation: Preparation similar to that used for the skinned muscle tissue was superfused in Krebs solution, and various concentrations of excess K-solution, caffeine, or NA were applied.

The microelectrode method: The glass capillary of the electrode was filled with 3 M KCl, and the resistance was 60–80 M Ω . The measured membrane potential was expressed as mean $\pm$ S.D.

The following drugs were used; *d, l*-noradrenaline-HCl, caffeine, ethylene-glycol-bis(β -aminoethylether)-*N, N'*-tetraacetic acid (EGTA). The stock solution for drugs was freshly prepared just before each experiment.

RESULTS

Effects of Na removal on the membrane potential and mechanical response

Effects of Na-deficient solution on membrane potential and mechanical response were observed. In 15.5 mm $[\text{Na}]_o$, prepared by replacement with choline, the membrane potential was slightly reduced (in the control, the membrane potential was -67.2 ± 2.8 mV, $n=20$, which depolarized to -60.1 ± 3.4 mV, $n=20$ in 15.5 mm Na at about 10 min exposure), and a large contraction which then relaxed to a certain level (phasic and tonic contraction) was evoked.

Figure 1A shows the effects of NaCl-free solution (15.5 mM Na as NaHCO_3) on the membrane potential. NaCl-free solution was prepared by choline-Cl, choline-Cl with atropine (10^{-6} g/ml) or sucrose. To avoid factors introduced by the liquid junction potential, the microelectrode was repetitively inserted into the cell after adjustment of the zero level. Replacement of NaCl with choline produced a transient hyperpolarization of the membrane but the level returned to control level within a few min and then the membrane slightly depolarized. With the simultaneous application of choline and atropine, no such hyperpolarization was observed, and the depolarization appeared. On the other hand, by replacement with sucrose, the membrane was rapidly depolarized and a rather large depolarization level (about -60 mV) was sustained compared to that produced by choline. In this figure, the effects of NaCl-free solution were observed only at 5 min exposure in NaCl-free solution. However, when the tissue was exposed for more than 10 min, the membrane potential measured in choline or sucrose solution showed much the same value, *i.e.*, the membrane depolarized by sucrose solution repolarized to the level of the choline-induced depolarization.

When the $[\text{Na}]_o$ was reduced to half (69 mM Na) that in the control solution, the membrane potential was similar to that observed in the control solution (-67.2 ± 2.8 mV, $n=20$ in 137 mM *vs.* -66.3 ± 1.9 mV, $n=20$ in 69 mM at about 10 min exposure). However, in the case of the mechanical response, the minimum extent of Na depletion required to produce the contraction was 30 mM, *i.e.*, even in the presence of 107 mM $[\text{Na}]_o$ the contraction was generated. Figure 1B shows the effects of various concentrations of $[\text{Na}]_o$ replaced with choline on the membrane and mechanical responses. The membrane potential was measured during 10–15 min exposure and the maximum amplitude of phasic contraction was measured. Without any marked change in the membrane potential, the amplitude of contraction increased in proportion to the reduction in the $[\text{Na}]_o$ in the ranges of 30 and 137 mM $[\text{Na}]_o$. The amplitude of contraction recorded in the case of 118 mM $[\text{K}]_o$ was registered in the figure as a relative tension of 1.0. In the Na-free solution, the membrane was significantly depolarized (-52.1 ± 4.2 mV, $n=15$ about 10 min exposure), and the contraction reached 0.82 times that evoked by 118 mM $[\text{K}]_o$. The membrane potential observed in 2.5 mM $[\text{Na}]_o$ and Na-free solution significantly differed ($p < 0.01$, *t*-test), while the amplitude of the contraction was similar in both solutions.

Figure 2A shows the relationship between membrane potential and contraction with various concentrations of $[\text{K}]_o$. The contraction was evoked by depolarization of the membrane from -69.2 ± 2.3 mV ($n=15$) to -61.6 ± 2.9 mV ($n=15$), *i.e.*, when the $[\text{K}]_o$ was increased from 5.9 to 11.8 mM, $[\text{Na}]_o$ was reduced conversely from 137 to 131 mM.

Figure 2B summarizes the effects on the mechanical response of Na-deficient solution prepared by replacing Na with choline, sucrose or K. The contraction evoked by 118 mM $[\text{K}]_o$ was registered as a relative tension of 1.0.

Fig. 1. A. Effects of NaCl replacement with choline or sucrose on membrane potential of guinea-pig mesenteric artery. NaCl-free solution was applied for 5 min. To measure the membrane potential, the microelectrode was successively inserted after adjustment of the zero potential level. B. Relationship between the membrane potential and mechanical responses in the various concentration of $[\text{Na}]_o$. The contraction evoked by 118 mM $[\text{K}]_o$ was registered as a relative tension of 1.0. Na was replaced with choline.

Effects of Na-deficient solution on the caffeine-, NA- or K-induced contraction

The contraction evoked by excess $[\text{K}]_o$, NA, or Na-deficient solution was composed of phasic and tonic components, *i.e.*, with application of these agents, the rapidly developing phasic contraction declined to a certain sustained tonic level.

Figure 3A shows the effects of caffeine on the mechanical response before, during and after application of NaCl-free solution (15 mM Na). Caffeine (5 mM) produced a similar amplitude of phasic contraction as that evoked by NaCl-free

Fig. 2. A. Relationship between the membrane potential and mechanical responses in various concentrations of $[K]_o$. The 118 mM K-induced contraction was registered as a relative tension of 1.0. In A and B, vertical bars indicate $2 \times S.D.$ ($n=15-50$ for the membrane potential and $n=4-7$ for the mechanical response). B. Relationship between the mechanical responses and various concentrations of $[Na]_o$. Na was substituted by either K, choline or sucrose. In the solution containing choline, 10^{-6} g/ml atropine was added. Vertical bars indicate S.D. or $2 \times S.D.$

solution. When 5 mM caffeine was applied in the NaCl-free solution, a much larger amplitude of contraction was evoked (1.8 times the control, $n=5$ after 20 min exposure). The caffeine-induced contraction was completely restored after the

tissue had been rinsed with Krebs solution. A similar enhancement by caffeine in NaCl-free solution was also observed by application of 10^{-5} M NA (Fig. 3B). The NA-induced contraction in Krebs solution was much smaller (0.28 times, $n=4$) than that evoked by 5 mM caffeine or NaCl-free solution. NA (10^{-5} M) produced an enlarged contraction in NaCl-free solution (2.0 times the control after 20 min exposure; $n=4$). With over 30 min exposure to the NaCl-free solution, the amplitude of NA-induced contraction was only slightly reduced (1.7 times the control, $n=4$) but it was still much higher than that in the control. Differences between the NA- and caffeine-induced contractions in NaCl-free solution were observed in the recovery from NaCl-free solution, *i.e.*, the caffeine-induced contraction rapidly returned to the control value, but the NA-induced contraction became rapidly much smaller than the control in Krebs solution (within 5 min). Over 30 min was required for recovery to the control level.

When NaCl-free solution was applied following pretreatment with 118 mM $[K]_o$, the tonic contraction produced by excess $[K]_o$ was maintained at a similar amplitude, however, no phasic contraction was evoked (Fig. 3C). By the reversed sequence, excess $[K]_o$ produced a phasic contraction during generation of the tonic contraction evoked by NaCl-free solution, and repetitively applied excess $[K]_o$ in the NaCl-free solution produced a similar amplitude of phasic contractions (Fig. 3C).

These results suggest that NaCl-free solution consistently increases the amplitude of caffeine- or NA-induced contraction and affects to some extent the K-induced contraction.

Figure 4 shows the caffeine- and NA-induced contractions in the Ca-free solution. In the NaCl-free solution, the caffeine- or caffeine with 122 mM $[K]_o$ -induced contraction was enhanced to a greater extent than the contraction evoked by caffeine alone. When effects of these procedures were observed in Ca-free (2 mM EGTA) solution, the amplitude of phasic contraction evoked by caffeine (5 mM), caffeine (5 mM) with NaCl-free or caffeine (5 mM) with 122 mM $[K]_o$ solution was decreased. The contraction evoked by 10^{-5} M NA ceased within 5 min. The amplitudes of the phasic contraction evoked by caffeine, caffeine with NaCl-free solution, and caffeine with 122 mM $[K]_o$ solution differed in the Ca-containing solution, but the relative amplitudes of phasic contraction became similar after 5 min exposure in Ca-free solution. In Ca-free solution, the contraction evoked by 122 mM $[K]_o$ or NaCl-free solution ceased within 1 min. These results indicate that, in the mesenteric artery, 122 mM $[K]_o$ and NaCl-free solution increase the Ca influx, and 10^{-5} M NA, in part, releases the Ca stored in the cells as does caffeine.

Effects of Na on chemically skinned muscles

The chemically skinned muscles were prepared by addition of 50 μ g/ml saponin to the relaxing solution for 20 min. The criteria for the chemically skinned

A

Fig. 3A.

B

Fig. 3B.

Fig. 3C.

Fig. 3. A. Effects of 5 mM caffeine on the mechanical response before, during and after application of NaCl-free solution. As a control, 118 mM [K]_o was applied (a). B. Effects of noradrenaline on the mechanical response evoked before, during and after application of NaCl-free solution. a, before and during application of NaCl-free solution, noradrenaline (NA: 10^{-5} M) was successively applied; b, effects of noradrenaline (NA: 10^{-5} M) on the mechanical response after pretreatment with NaCl-free solution. C. Effects of 118 mM [K]_o solution on the mechanical response before, during and after application of NaCl-free solution. In a, NaCl-free solution was successively applied after application of 118 mM [K]_o solution.

fiber preparation were the same as that described by ITOH *et al.* (1981), *i.e.*, the amplitude of contraction evoked by 10^{-5} M free Ca in skinned fibers was larger than that evoked by 118 mM [K]_o in intact muscle cells.

Figure 5A shows the relationship between pCa and tension recorded from skinned muscles of the mesenteric artery. In the relaxing solution, 10 mM Na was in the form of a salt of Na₂-ATP, and the effects of 30 mM Na- or 60 mM Na-containing relaxing solution on the Ca-induced contraction were compared with the control (10 mM Na) relaxing solution. The amplitude of contraction was measured in response to cumulatively applied various concentrations of free Ca. The minimum concentration of Ca required to produce a contraction was just above the concentration of 10^{-7} M, and 3×10^{-7} M Ca produced a markedly developed contraction. Maximum tension was induced by 10^{-5} M Ca. By addition of 20 mM Na (total 30 mM Na) the pCa-tension relationship was not markedly modified in comparison to that observed in 10 mM Na.

When 50 mM Na was added (total 60 mM), the amplitude of contraction evoked

Fig. 4. Effects of Ca-free (EGTA) solution on the mechanical response evoked by caffeine, caffeine with NaCl-free, caffeine with 122 mM $[K]_0$ or noradrenaline ($n=4-8$). C: control (2.5 mM Ca). The amplitude of contraction evoked by caffeine with 122 mM $[K]_0$ solution was registered as a relative tension of 1.0. In a series of experiments, the individual test solution was applied after 20 min superfusion with Krebs solution.

by less than 10^{-5} M Ca was suppressed, and this suppression was even more dominant in incubation media containing lower concentrations of Ca. However, the suppression was smaller in cases where the contraction was evoked in the presence of 2 mM free Mg solution (control; 1 mM). As shown in Fig. 5A, 2 mM free Mg-containing relaxing solution consistently suppressed the Ca-induced contraction in all ranges of Ca concentration from 10^{-7} to 10^{-4} M. Figure 5B shows a typical example of contractions evoked by various concentrations of Ca cumulatively applied to skinned muscles. As the control, 118 mM $[K]_0$ -induced contraction in intact muscle tissue was observed. After treatment with saponin in the relaxing solution, application of 3×10^{-7} M Ca produced a contraction and during this application, an additional 50 mM Na replacing K suppressed the contraction to some extent. A similar suppression was also observed in the presence of 3×10^{-6} M Ca. Application of 3×10^{-6} M Ca to the relaxing solution produced a larger contraction than that evoked by 118 mM $[K]_0$ in the intact muscle cell. When the application procedure of excess Na was reversed, *i.e.*, when 10 mM Na-containing solution was applied following pretreatment with 60 mM Na-containing solution in the presence of Ca, the amplitude of Ca-induced contraction increased.

These results indicate that the Ca receptor in the contractile protein is slightly suppressed by excess Na in the presence of relatively low concentrations of free Ca, *i.e.*, lowering the external Na enhanced the sensitivity of the Ca receptor.

Since K_2 -ATP is a labile compound, experiments on the effect of less than 10 mM Na on the pCa-tension relationship were not done.

Fig. 5. A. The pCa and tension relationship observed in skinned muscle fibers. The tension produced by application of 10^{-5}M free Ca was registered as a relative tension of 1.0. Four different ionic compositions were used in the relaxing solution. B. Actual records of tension produced by various concentrations of free Ca. Concentrations of Na and Ca were shown in the figure. In a, the contraction evoked by 118 mM $[\text{K}]_o$ from the intact muscle tissue served as the control. b shows the Ca-induced contraction of skinned fibers in various concentrations of Ca and Na.

Fig. 6. Effects of noradrenaline (3×10^{-5} M) on release of accumulated Ca from the store site in skinned muscle fibers. a, after procedures 1 and 2, 5 mM caffeine was applied; b, 3×10^{-5} M noradrenaline was applied instead of caffeine; c, after control. The procedures were the same as those described in a.

The effects of excess Na solution on the caffeine-induced contraction in skinned muscles were observed. After the skinned fiber was left for 5 min in 10^{-4} M EGTA-containing relaxing solution, 10^{-6} M Ca with 10^{-4} M EGTA-containing solution was applied to the tissue for 3 min (procedure 1) then the tissue was washed for 3 min with 10^{-4} M EGTA-containing relaxing solution (procedure 2). After accumulation of Ca in the store site by the above procedures, 5 mM caffeine was applied to the tissue (procedure 3) and the amplitude of caffeine-induced contraction was compared in 10 mM Na and 60 mM Na.

In skinned muscles, when the amplitude of caffeine-induced contractions in the presence of 60 mM Na was compared with that in 10 mM Na during procedure 1 (60 mM Na/10 mM Na), the amplitude ratio was $105.4 \pm 7.9\%$ ($n=7$), and when 60 mM Na was applied during procedure 2 or 3, the amplitude ratio was $97.9 \pm 4.9\%$ ($n=4$) or $71.7 \pm 5.0\%$ ($n=4$), respectively. During procedures 1 and 2, when 60 mM Na was added, the amplitude ratio was $98.3 \pm 3.3\%$ ($n=3$). When 60 mM Na was applied to the tissue throughout the experimental procedures (1+2+3), the amplitude ratio was reduced to $70.0 \pm 9.7\%$ ($n=5$). These results indicate that accumulation of Ca in the store site is not affected by the presence of either 60 mM or 10 mM Na, but sensitivities of Ca-receptors to free Ca and Ca-releasing sites to caffeine are suppressed in the presence of 60 mM Na compared to those in 10 mM Na. The effects of 60 mM Na on Ca accumulation and release from skinned fibers are summarized in Table 1.

Figure 6 shows the effects of NA on a skinned fiber. When NA (3×10^{-5} M) instead of caffeine was applied to the tissue during procedure 3, no contraction was evoked. Furthermore, application of NA did not affect the caffeine-induced contraction, even when NA was applied during procedure 2.

DISCUSSION

In Ca-free solution, the K- and NaCl-free-induced contractions in the guinea-pig mesenteric artery rapidly ceased, the NA-induced contraction ceased within

Table 1. Effects of excess Na (60 mM) on the caffeine-induced contraction in skinned muscle fibers. Excess Na was applied during the Ca-accumulating process (procedure 1; 10^{-6} M Ca, 10^{-4} M EGTA, and 60 or 10 mM Na-containing relaxing solution was applied for 3 min), during the removal of free Ca (procedure 2; 10^{-4} M EGTA and 60 or 10 mM Na-containing solution for 3 min) and during application of 5 mM caffeine (procedure 3). The values (%) are calculated from amplitudes of the caffeine-induced contraction with or without treatment of 60 mM Na. The amplitude of caffeine-induced contraction in the control (10 mM Na in the relaxing solution) was registered as 100%.

Procedure 1 (mM Na)	Procedure 2 (mM Na)	Procedure 3 (mM Na)	%
10	10	10	100 (n=7)
60	10	10	105.9 ± 7.9 (n=7)
10	60	10	97.9 ± 4.9 (n=4)
10	10	60	71.7 ± 5.0 (n=4)
60	60	10	98.3 ± 3.3 (n=3)
60	60	60	70.0 ± 9.7 (n=5)

5 min and the caffeine-induced contraction persisted for more than 15 min. In NaCl-free solution, caffeine and NA markedly enhanced the mechanical response. However, excess $[K]_o$ produced a similar amplitude of phasic contraction with or without pretreatment with NaCl-free solution. In both 118 mM $[K]_o$ and NaCl-free solutions, concentrations of $[Na]_o$ were the same and therefore, the 118 mM K-induced contraction following pretreatment in NaCl-free solution probably produces the same amplitude as that observed without pretreatment. If indeed such is the case, the K-induced contraction may be generated by a voltage-dependent Ca influx and a Na-deficiency-induced Ca influx.

In the guinea-pig taenia coli, the Na content in Na-free solution decreased from 14 mM to nearly zero within 30 min at 37°C (BRADING *et al.*, 1980) or to 90% depletion in the cell within 30 min (VAN BREEMEN *et al.*, 1980). Various vascular smooth muscle cells had almost the same Na content as was detected in the taenia coli (rat aorta, 12 mM; rat portal vein, 13 mM; rabbit main pulmonary artery, 12 mM; rabbit portal vein, 26 mM; review of JONES, 1980). The concentration of $[Na]_i$ used in the case of these vascular muscles was the same as that used in the

relaxing solution to prepare the skinned muscle, in the present experiments.

In skinned muscles of the mesenteric artery, increase in the Na concentration from 10 to 60 mM had no effect on the accumulation of 10^{-6} M Ca, yet excess Na solution inhibited the Ca-releasing site and the Ca receptor of contractile protein by 30%. However, reduction in the Ca sensitivity was less in the presence of free Ca in concentrations over 10^{-5} M. In the ranges of 10 and 30 mM Na, however, no difference was observed in the action on the Ca-receptor, and Ca-releasing mechanism. The property of the saponin-treated chemically skinned muscles of the guinea-pig mesenteric artery was discussed by ITOH *et al.* (1981) in relation to observations reported in the case of the hog coronary artery (FILO *et al.*, 1965), the rabbit pulmonary artery (ENDO *et al.*, 1977) and the guinea-pig taenia coli (SAIDA and NONOMURA, 1978). Under treatment with saponin, the internal structures (mainly sarcoplasmic reticulum) and the Ca receptor of the contractile protein are functionally preserved for measurements of the Ca-releasing ability and superprecipitation of myosin B (SAIDA and NONOMURA, 1978).

If $[\text{Na}]_i$ remains much the same in Krebs and NaCl-free solution (10–30 mM), the Ca receptor of the contractile protein may not be affected, as observed in skinned muscles. However, when $[\text{Na}]_i$ in the NaCl-free solution is reduced to less than 10 mM, it is uncertain whether or not the sensitivity of the Ca receptors remains the same. Reduction of Na below 10 mM by replacement of $\text{Na}_2\text{-ATP}$ with $\text{K}_2\text{-ATP}$ was not done due to instability of $\text{K}_2\text{-ATP}$. In NaCl-free solution (15 mM Na-containing solution), a large contraction was evoked (0.8 times the 118 mM $[\text{K}]_o$ -induced contraction), therefore, the NaCl-free-induced contraction may be due to increase in the free Ca in the cell rather than increase in the sensitivity of the Ca receptor.

REUTER *et al.* (1973) reported that in the case of the rabbit aorta, the Na concentration gradient across the cell membrane was a determinant of $[\text{Ca}]_i$ and measurement of the Ca efflux into the solution containing various concentrations of $[\text{Na}]_o$ revealed a coupling between Na and Ca ions in this vascular muscle cell. BLAUSTEIN (1977) postulated that by equating the electrochemical gradient for Na and Ca, 3 Na would be required to move 1 Ca. Furthermore, the Na-influx and Ca-efflux exchange process is capable of reversal to a Na-efflux and Ca-influx exchange process, under modified ionic environments. BRADING and WIDDICOMBE (1976) found that a fraction of the Ca influx into the taenia coli was dependent on $[\text{Na}]_i$, and a component of Ca efflux was dependent on $[\text{Na}]_o$. This idea was supported by the findings of HIRATA *et al.* (1980). RAEYMAEKER *et al.* (1974) did not find a consistent slowing of Ca efflux when Li or choline was substituted for Na. VAN BREEMEN *et al.* (1973), WEISS (1977), and VAN BREEMEN *et al.* (1980) considered that only a part of the Ca exchange can be explained by this Na-Ca exchange mechanism and that the main mechanism of the generation of contraction is related to the suppression of the Ca-Mg ATPase transport system which reduces $[\text{Ca}]_i$. In the case of this mechanism, the sarcoplasmic reticulum would be functionally

coupled with the sarcolemma directly (VAN BREEMEN *et al.*, 1980; BRADING *et al.*, 1980; JONES, 1980). The Ca-stimulated Mg ATPase is located mainly in the sarcoplasmic reticulum but not or only in part, in the sarcolemma (HESS and FORD, 1974; SLOANE, 1980). WEI *et al.* (1976) and DANIEL *et al.* (1977) reported that the isolated membrane fraction from the rat myometrium (70–80% sarcolemma and 20% sarcoplasmic reticulum) can accumulate Ca. However, they stated that the possible contribution of the sarcoplasmic reticulum contaminated in the fraction had to be considered.

Although we have no data to support the idea that increase in $[Ca]_i$ in the NaCl-free solution is due to suppression of the Ca pump, the Na-Ca exchange diffusion, or to increase in the Ca influx on the plasma membrane, nevertheless, the large phasic contraction and marked accumulation of Ca in the store site in the NaCl-free solution do favour the idea that the Ca influx occurs through the Na channel, under conditions in which $[Na]_o$ levels are low, as has been postulated in the case of the rabbit ear artery (DROOGMANS and CASTEELS, 1979). The supporting evidence is: 1) The NaCl-free-induced contraction was composed of phasic and tonic components, and the velocity of phasic tension development was much the same as that observed during the K-induced contraction. This rapid velocity of tension development is too fast to be accounted for by the retention of Ca due to suppression of the Ca efflux. Furthermore, the minimum reduction in $[Na]_o$ required to produce the contraction was 108 mM $[Na]_o$, and this amount of $[Na]_o$ would probably not reverse the Na-Ca exchange process from Na influx to efflux. Moreover, in K-free solution, smooth muscles of the mesenteric artery generated contraction due to accumulation of $[Ca]_i$ as a consequence of increased $[Na]_i$. However, development of the contraction required a very long latency after replacement with K-free solution (SUZUKI and KURIYAMA, 1980). 2) Diltiazem (a Ca channel blocker or Ca antagonist) suppressed the K-induced and NA-induced contractions in the mesenteric artery, but the contraction evoked in NaCl-free solution was not suppressed (SUZUKI *et al.*, 1981). This means that the Ca influx appearing in the NaCl-free solution may not utilize the Ca channel, at the plasmic membrane.

Finally, the contraction evoked in NaCl-free solution in the guinea-pig mesenteric artery is not a typical example of general features of vascular smooth muscles, *e.g.*, in the porcine coronary artery, the tissue did not contract in NaCl-free or Na-free solution (HIRATA *et al.*, 1980). Regional and species differences in vascular smooth muscle tissues probably account for such differences.

This work was supported by the Ministry of Education and Welfare of Japan (54408, 544020, 577111) and Yamada Science Foundation. We thank M. Ohara for reading the manuscript.

REFERENCES

- BLAUSTEIN, M. P. (1977) Effects of internal and external cations and of ATP on sodium-calcium and calcium-calcium exchange in squid axons. *Biophys. J.*, **20**: 79–111.

- BOLTON, T. B. (1979) Mechanisms of action of transmitters and other substances in smooth muscle. *Physiol. Rev.*, **59**: 606–718.
- BRADING, A. F., BARNETT, M., and SNEDDON, P. (1980) The effect of sodium removal on the contractile responses of the guinea-pig taenia coli to carbachol. *J. Physiol. (Lond.)*, **306**: 411–420.
- BRADING, A. F. and WIDDICOMBE, J. H. (1976) Interaction between sodium and calcium movements in smooth muscle. *In: Colloquium on Smooth Muscle, Pharmacology and Physiology*, ed. by WORCEL, M. and VASSORT, G. INSERM, Paris, pp. 235–245.
- BÜLBRING, E. (1954) Membrane and potentials of smooth muscle fibres of taenia coli of the guinea-pig. *J. Physiol. (Lond.)*, **125**: 302–315.
- CASTEELS, R., KITAMURA, K., KURIYAMA, H., and SUZUKI, H. (1977) The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol. (Lond.)*, **271**: 41–61.
- DANIEL, E. E., KWAN, C. Y., MATLIB, M. A., CRANKSHAW, D., and KIDWAI, A. (1977) Characterization and Ca^{2+} -accumulation by membrane fractions from myometrium and artery. *In: Excitation-Contraction Coupling in Smooth Muscle*, ed. by CASTEELS, R., GODFRAIND, T., and RÜEGG, J. C. Elsevier/North-Holland, Amsterdam, New York, pp. 181–188.
- DROOGMANS, G. and CASTEELS, R. (1979) Sodium and calcium interactions in vascular smooth muscle cells of the rabbit ear artery. *J. Gen. Physiol.*, **74**: 57–70.
- ENDO, M. (1977) Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.*, **57**: 71–108.
- ENDO, M., KITAZAWA, S., YAGI, M., IINO, M., and KAKUTA, Y. (1977) Some properties of chemically skinned smooth muscle fibers. *In: Excitation-Contraction Coupling in Smooth Muscle*, ed. by CASTEELS, R., GODFRAIND, T., and RÜEGG, J. C. Elsevier/North Holland, Amsterdam, New York, pp. 199–209.
- FILO, R. S., BOHR, D. F., and RÜEGG, J. C. (1965) Glycerinated skeletal and smooth muscle: calcium and magnesium dependence. *Science*, **147**: 1581–1583.
- FRIEDMAN, S. M. (1977) The effects of external sodium substitution on cell sodium and potassium in vascular smooth muscle. *J. Physiol. (Lond.)*, **270**: 195–208.
- HESS, M. L. and FORD, G. D. (1974) Calcium accumulation by subcellular fraction from vascular smooth muscle. *J. Mol. Cell. Cardiol.*, **6**: 275–282.
- HIRATA, M., ITOH, T., and KURIYAMA, H. (1980) Effect of external cations of calcium efflux from single cells of the guinea-pig taenia coli and porcine coronary artery. *J. Physiol. (Lond.)*, **310**: 321–336.
- ITOH, T., KURIYAMA, H., and SUZUKI, H. (1981) Excitation-contraction coupling mechanism in the guinea-pig mesenteric artery. *J. Physiol. (Lond.)*, in press.
- JONES, A. W. (1980) Content and fluxes of electrolytes. *In: Handbook of Physiology*, Sec. 2, Circulation, Vol. 2, Vascular Smooth Muscle, ed. by BOHR, D., SOMLYO, A. P., and SPARKS, H., Jr. The Am. Physiol. Soc., Baltimore, pp. 259–299.
- KATASE, T. and TOMITA, T. (1972) Influences of sodium and calcium on the recovery process from potassium contracture in the guinea-pig taenia coli. *J. Physiol. (Lond.)*, **224**: 489–500.
- KURIYAMA, H. and SUZUKI, H. (1981) Adrenergic transmissions in the guinea-pig mesenteric artery and their cholinergic modulations. *J. Physiol. (Lond.)*, **317**: 383–396.
- MARTELL, A. E. and SCHWARZENBACH, G. (1956) Adenosinphosphate und Triphosphate als Komplexbildner für Calcium und Magnesium. *Helv. Chim. Acta*, **39**: 653–666.
- RAEYMAEKER, L., WUYTACK, F., and CASTEELS, R. (1974) Na-Ca exchange in taenia coli of the guinea-pig. *Pflügers Arch.*, **347**: 329–340.
- REUTER, H., BLAUSTEIN, M. P., and HAEUSLER, G. (1973) Na-Ca exchange and tension development in arterial smooth muscle. *Philos. Trans. R. Soc. Lond. (Biol.)*, **265**: 87–94.
- SAIDA, K. and NONOMURA, Y. (1978) Characteristics of Ca^{2+} - and Mg^{2+} -induced tension development in chemically skinned smooth muscle fibers. *J. Gen. Physiol.*, **72**: 1–14.

- SLOANE, B. F. (1980) Isolated membranes and organelles from vascular smooth muscle. *In*: Handbook of Physiology, Sec. 2, Circulation, Vol. 2, Vascular Smooth Muscle, ed. by BOHR, D., SOMLYO, A. P., and SPARKS, H., Jr. The Am. Physiol. Soc., Baltimore, pp. 121-132.
- SUZUKI, H., ITOH, T., and KURIYAMA, H. (1981) Effects of diltiazem on the mesenteric artery of guinea-pig. *Proc. J. Pharmacol. Soc., Annu. Gen. Meeting*, in press.
- SUZUKI, H. and KURIYAMA, H. (1980) Observation of quantal release of noradrenaline from vascular smooth muscles in potassium-free solution. *Jpn. J. Physiol.*, **30**: 665-670.
- VAN BREEMEN, C., AARONSON, P., and LOUTZENHISER, R. (1978) Sodium-calcium interactions in mammalian smooth muscle. *Pharmacol. Rev.*, **30**: 167-208.
- VAN BREEMEN, C., AARONSON, P., LOUTZENHISER, R., and MEISHERI, K. (1980) Ca^{2+} movements in smooth muscle. *Chest (Suppl.)*, **78**: 157-165.
- VAN BREEMEN, C., FARINAS, B. R., CASTEELS, R., GERBA, P., WUYTACK, F., and DETH, R. (1973) Factors controlling cytoplasmic Ca^{2+} concentration. *Philos. Trans. R. Soc. Lond. (Biol.)*, **265**: 57-71.
- WEI, J. W., JANIS, R. A., and DANIEL, E. E. (1976) Studies on subcellular fractions from mesenteric arteries of spontaneously hypertensive rats: Alterations in both calcium uptake and enzyme activities. *Blood Vessels*, **13**: 293-308.
- WEISS, G. B. (1977) Calcium and contractility in vascular smooth muscle. *In*: Advances in General and Cellular Pharmacology, Vol. 2, Chap. 2, ed. by NARAHASHI, T. and BIANCHI, C. P. New York, Plenum, pp. 71-154.