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Mechanisms of Contraction Induced by Sodium Depletion in the Rabbit Renal Artery

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=ABSTRACT=

In the rabbit renal artery, mechanisms of contraction by sodium depletion were investigated. The helical strips of isolated renal artery were immersed in the Tris-buffered salt solution. The contractions were recorded isometrically using a strain-gauge transducer. Na-free solution (Na was substituted by Li, choline or sucrose) produced contractions which were dependent on the nature of the Na substitutes. Na-free solution (choline) produced the contraction in ouabain-pretreated artery (Na loaded artery) even in the presence of verapamil. The amplitude of the contraction was dependent on the duration of the pretreatment with ouabain (10^{-4} M). Monensin potentiated the effect of ouabain on the contraction. Removal of Ca from bathing solution abolished the contraction and the substitution of Sr for Ca produced the contraction. Divalent cations such as Mg, Mn blocked the depolarization-induced contraction, while they had little effect on the Na-free contraction in Na loaded artery. These results suggest that the contraction induced by Na removal is dependent on the cellular Na content and may be caused by Ca influx via the Na-Ca exchange carrier.

Key Words: Na-Ca exchange, Sodium depletion, Ouabain, Verapamil, Rabbit renal artery.

INTRODUCTION

The clinical observation that hypertension in some patients is affected by Na intake has been supported over the last 30 years by series of experimental data. These works have suggested several hypotheses to explain the mechanisms by which Na can affect the tone of blood vessel. In smooth muscle, evidence that changes in intra- and extracellular Na concentrations affect smooth mucsle function by changing the level of myoplasmic free Ca has been presented. However, the mechanism and significance of its role in regulation of myoplasmic Ca is questioned.

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The change of intra- or extracellular Na concentration produces the change in basal tone of vascular smooth muscles. In the guinea-pig and rabbit aorta, extracellular Na depletion produced a contraction, and an increase in cellular Na by ouabain or K-free solution also produced a contraction (Ozaki et al, 1978; Ozaki & Urakawa, 1981; Reuter et al, 1973). In some vascular smooth muscles, contraction induced by low extracellular Na is inhibited by alpha-receptor blockade or denervation (Broeckaert and Godfraind, 1973; Karaki & Urakawa, 1977). However, in other vascular smooth muscles, contractions induced by Na-free medium were not blocked by phentolamine (Droogmans & Casteels, 1979; Ozaki & Urakawa, 1981). Such regional and species differences in the mechanical response in various vascular smooth muscles are commonly observed. Ozaki & Urakawa

(1981) suggested that the contraction induced by low extracellular Na was caused by a Ca influx which is dependent on internal Na (Na-Ca exchange), whereas Droogmans & Casteels (1979) concluded that Na-free contraction was not attributable to a Na-Ca exchange mechanism.

Whether Na-Ca exchange plays a physiological role in smooth muscles, especially in arterial smooth muscle, is still a matter of controversy (Brading & Lategen, 1985; Casteels et al, 1985; Droogman & Casteels, 1979; Mulvany, 1985; van Breemen et al, 1979). The presence of Na-Ca exchange in vascular smooth muscles was suggested by many early studies in which tonic contractions were induced when the external Na was reduced and /or the Na pump was inhibited by cardiotonic steroids (Reuter et al, 1973; Ozaki et al, 1978; Ozaki & Urakawa, 1981). However, in some cases, Ca entry through voltage-gated Ca channel activated by depolarization, and Ca entry activated by the release of endogenous catecholamines or other agonists could not be ruled out as alternative sources of activator Ca (van Breemen et al, 1979).

Recent sarcolemmal vesicle studies demonstrated directely the presence of a Na-Ca exchange mechanism in various types of smooth muscle, including vascular smooth muscle (Morel & Godfraind, 1984; Matlib et al, 1985; Kahn et al, 1988). However, it is difficult to determine whether Na-Ca exchange plays a physiological role in smooth muscles by sarcolemmal vesicle studies.

The present study was designed to investigate the mechanisms of vasoconstriction during incubation in Na-free solution and to determine whether there exists a functional Na-Ca exchanger in intact vessel.

METHOD

Rabbits (1.5~2 kg) were killed by an occipital blow. The renal artery was isolated and transferred to oxygenated physiological salt solution. It was cleaned of its periarterial connective tissue and recovered for 2 hours in oxygenated physiological salt solution at room temperature. For measurement of tension, helical strips about 10 mm long and 2 mm wide were cut.

The helical strips were suspended in an organ bath (100 ml) under 0.5 g tension. Bathing solution was gassed with 100% O_2 and the temperature was kept constant at 35°C. The contractions were recorded isometrically using a strain-guage transducer (F-60, Narco Biosystem). The muscles were allowed to equilibrate in normal physiological salt solution for at least 2 hours until the response to high K (40 mM) becomes stable. The amplitude of the contraction in response to high K (40 mM) was considered as a reference response (100%).

The physiological salt solution (PSS) was a Tris-buffered modified Tyrode's solution containing (mM): NaCl 158, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2, glucose 6 and Tris 5 (pH 7.4). The Na free solution was obtained by replacing NaCl with isosmolar sucrose, choline chloride or LiCl. All choline solutions contained atropine sulphate (10^{-6} g/ml) to inhibit any cholinergic effect which the choline chloride might exert. The high K (40 mM) solution was prepared by replacing NaCl with isosmolar K (36 mM).

Drugs used were ouabain (Sigma), verapamil (Sigma), atropine sulphate (Sigma), monensin(Sigma) and phentolamine methylate (CIBA). Monensin was dissolved in ethanol (100%) and diluted with PSS to give a final concentration of ethanol less than 0.2%. We confirmed in preliminary experiments that 0.2% ethanol does not affect vascular smooth muscle contraction.

RESULTS

In Na-free solution in which NaCl had been replaced by sucrose, choline chloride or LiCl, arterial strips showed an increase in basal tone (Fig. 1). The characteristics of

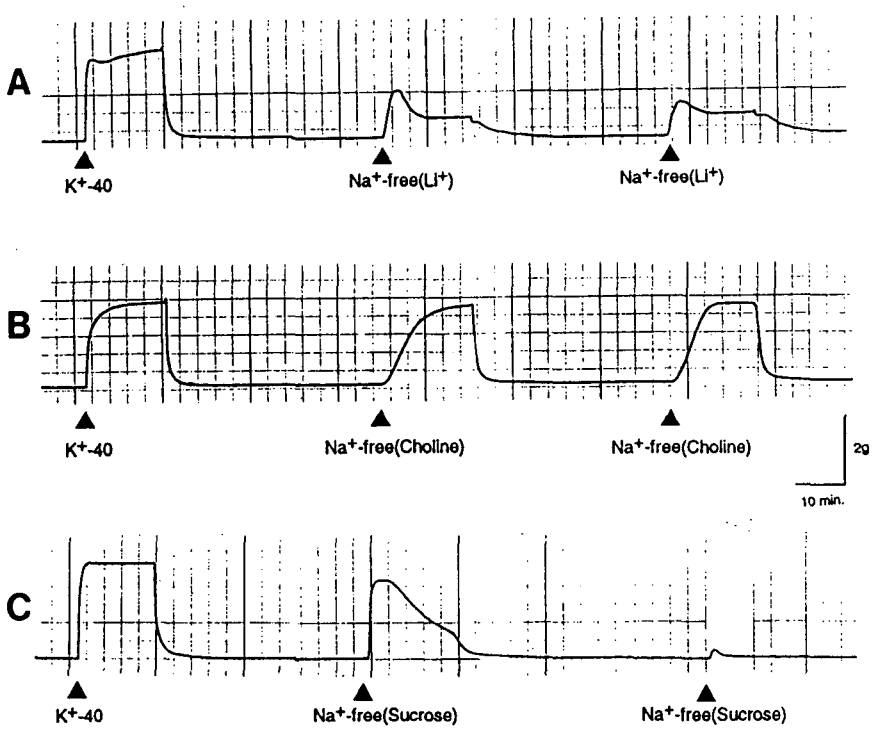

Fig. 1. Tracings of tension development in normal tissue on exposure to Na-free solution. Three different Na substitutes were used; Li(A), choline(B) and sucrose(C).

Characteristics of Na-free contraction were dependent on the nature of Na substitutes. The Na-free contraction (substituted by Li or choline) were reproducible and had phasic and tonic component of contraction. However, the Na-free contraction (substituted by sucrose) did not show the reproducibility and tonic component.

these contraction depended on the nature of the Na substitute: Li solution produced a small phasic contraction (57.8±4.8%) followed by a tonic one with less amplitude. Choline solution also produced a sustained contraction and the amplitude of contraction (96.3±3.0%) was larger than that produced by Li solution. The contraction produced by Li or choline solution was reproducible (Fig. 1A, B). Whereas, in sucrose solution, the contraction was not reproducible and the amplitude of contraction (107.0±5.4%) was larger than those produced by Li or choline solution but tonic contraction did not appear (Fig. 1C). Thus, in all subsequent experiments, Na was replaced by choline chloride.

The effects of phentolamine and verapamil on the choline solution-induced contraction was observed. The alpha adrenoceptor blocking agent, phentolamine (10^{-5} M), added during the contraction by choline solution caused slow and small relaxation (32.3±3.8 %). This contraction was also inhibited completely by verapamil (10^{-5} M), Ca channel blocker(Fig. 2). Ouabain(10^{-5} M) was added to bathing solution in order to load cellular Na. During incubation of the arterial strip with ouabain, slow tonic contraction occurred. This contraction was inhibited by phentolamine (10^{-5} M) or verapamil (10^{-5} M) completely (Fig. 3).

In order to reduce the transsarcolemmal Na gradient, the arterial strip, after loading cellular Na in the PSS containing ouabain for

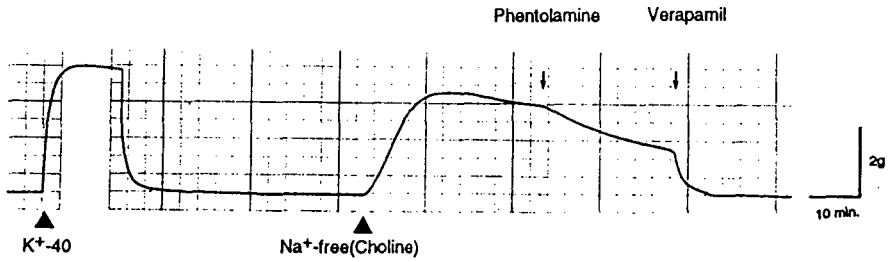

Fig. 2. Effect of phentolamine and verapamil (A) on the Na-free contraction (choline).
Addition of phentolamine (10^{-5} M) to the bath blocked the contraction partially and slowly. Verapamil (10^{-5} M) blocked the contraction completely.

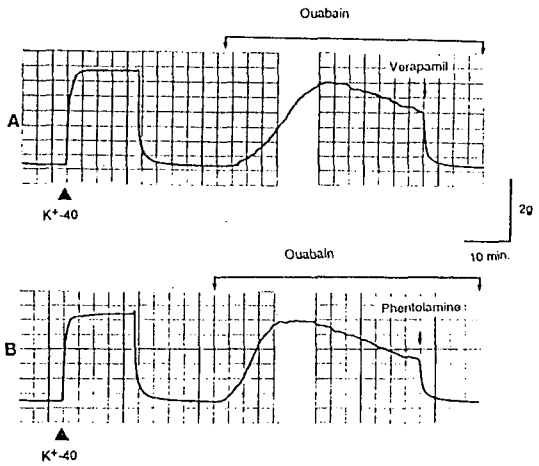

Fig. 3. Effects of verapamil and phentolamine on the contraction induced by ouabain.
Arterial strip contracted by the treatment with ouabain and that contraction was blocked by the addition of verapamil (10^{-5} M) or phentolamine (10^{-5} M).

30 minutes, was exposed to choline solution. Verapamil was added to bathing solution in order to block the Ca transport via the gated Ca channel. The sustained contraction occurred by reduced transsarcolemmal Na gradient (Na gradient-induced contraction) (Fig. 4B). This contraction was not inhibited by verapamil even though the concentration of verapamil was sufficient to inhibit the depolarization-induced contraction, but completely inhibited by removal of extracellular Ca (Fig. 5). Whereas, without incubation with ouabain, arterial strip did not contract when exposed to both ouabain and choline solution in the presence of verapamil (Fig. 4A). The dependence of the response to choline solution on the time of incubation with ouabain was investigated. The longer the incubation period in PSS containing ouabain is, the amplitude of contraction is larger (Fig. 6, 7).

The effect of monensin on the Na gradient-induced contraction was observed. Monensin is monovalent cation-selective ionophore that transport Na through sarcolemma (Pressman, 1976; Ozaki et al, 1982). Therefore, we use the monensin as a tool that increase the cellular Na more rapidly. Monensin was added to the bathing solution during incubation (30 minutes) of the arterial strip in PSS containing ouabain. And then, the arterial strip was exposed to choline solution. The contraction was induced and the amplitude of contraction was larger than that of contraction which is induced by choline solution after incubation of the arterial strips in PSS containing only ouabain (Fig. 6D, 7).

The effect of divalent cations (Mg, Mn) on the Na gradient-induced contraction was investigated. The cumulative addition of Mg (4 ~16 mM) to the bathing solution during the contraction caused a slow and small relaxation (Fig. 8A). On the other hand, the addition

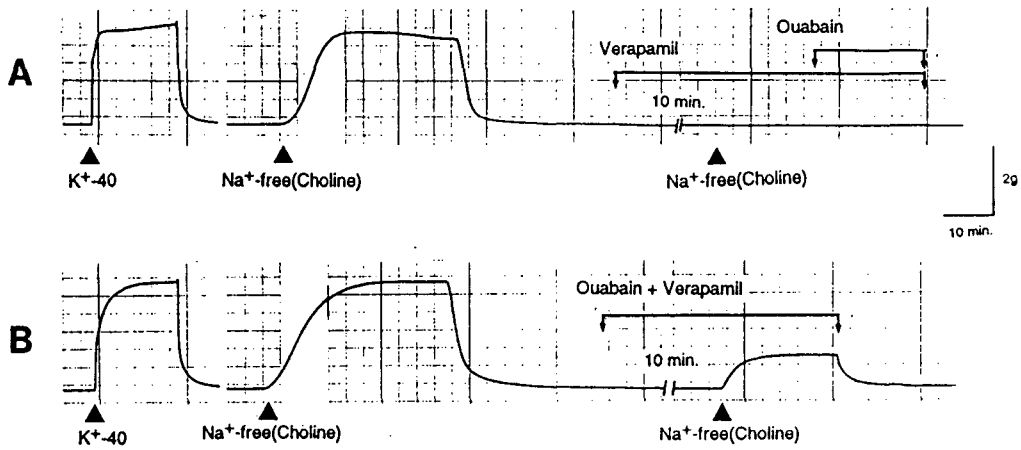

Fig. 4. Comparison between normal arterial strip and Na-loaded arterial strip.

Normal arterial strip did not contract on exposure to the Na-free solution (choline) in the presence of verapamil. Even the addition of ouabain (10^{-5} M) failed to elicit the contraction (A). Na-loaded arterial strip (incubated with 10^{-5} M ouabain for 30 min), however, contracted on exposure to the Na-free solution even in the presence of verapamil (B).

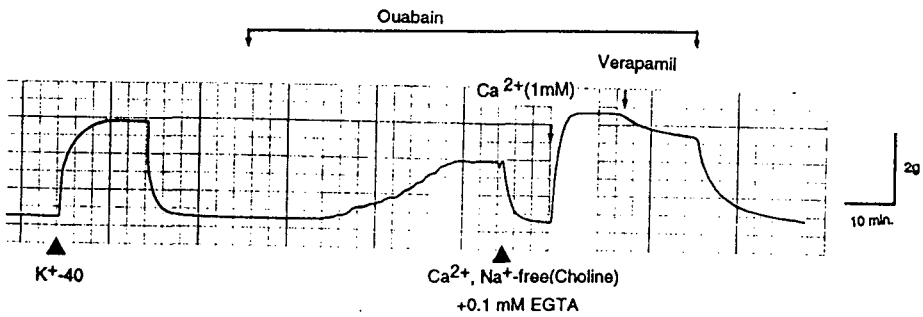

Fig. 5. Effect of verapamil or Ca on the Na-free contraction in Na-loaded arterial strip.

Na-loaded arterial strip did not contract on exposure to Na-free solution (choline) in the absence of external Ca. Verapamil (10^{-5} M) blocked that contraction partially and slowly.

of Mn(2~8 mM) blocked the contraction at early time, but arterial strip showed another contractile response after about 20 minutes (Fig. 8B). The mechanical response of arterial strip to Na depletion in Ca-free solution containing Mg or Mn was examined (Fig. 8C). In the presence of Mg without Ca, arterial strip did not contract even expoed to choline solution after loading cellular Na; however, addition of Ca produced the contraction. On the other hand, in the presence of Mn, arterial strip contracted slowly when exposure to choline solution and addition of Ca inhibited the contractile response. However the contraction produced by high K (40 mM) solution was inhibited completely by adding the low concentration of Mg (1~10 mM) or Mn (0.1~1 mM) (Fig. 8).

The effects of Ca and Sr on Na gradient-induced contraction were investigated (Fig. 9). The contraction during exposure to choline solution, after loading cellular Na, occurred

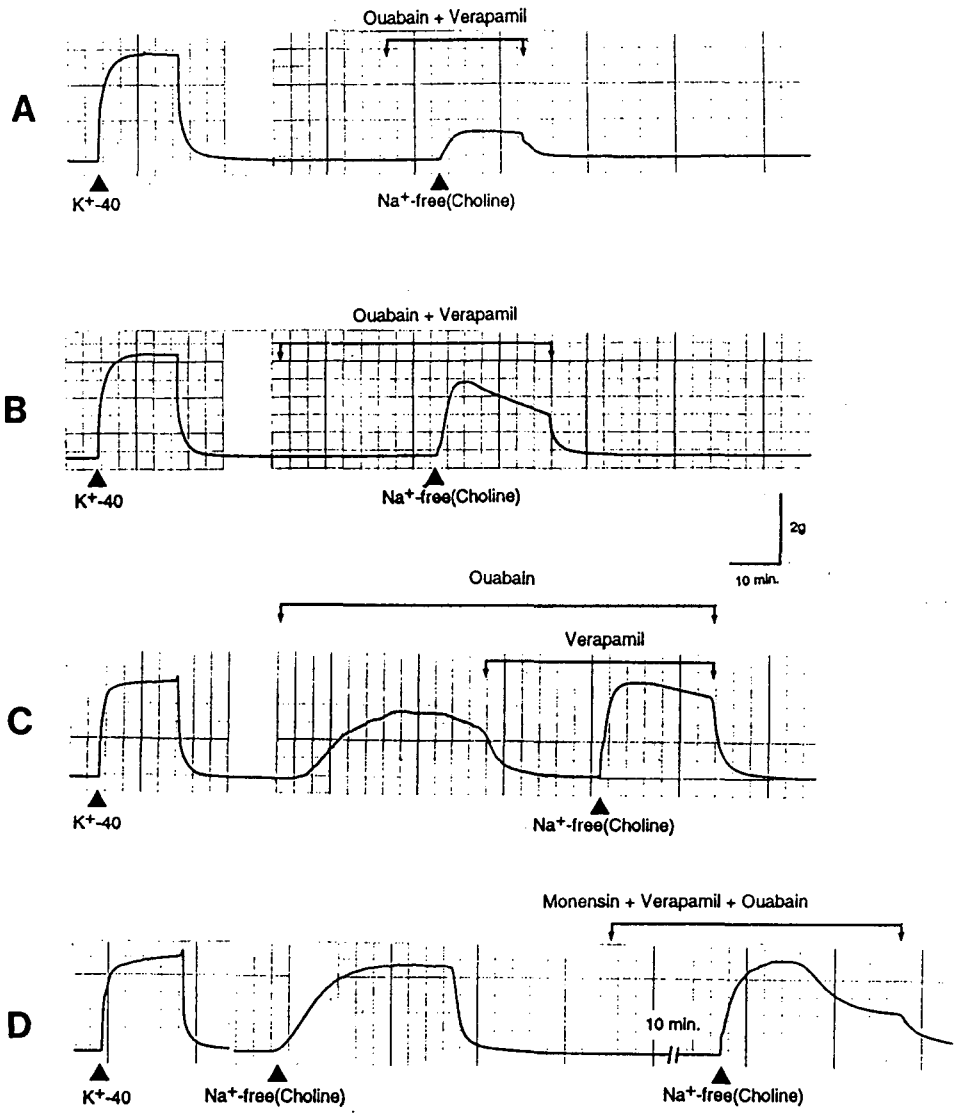

Fig. 6. The relationship between the amplitude of Na-free contraction and duration of incubation with ouabain in Na-loaded arterial strip.

Arterial strips were incubated with ouabain (10^{-5} M) for 10 min (A), 30 min (B), 60 min (C) and with ouabain+monensin (10^{-5} M) for 30 min (D). The amplitudes of Na-free contracture were dependent on the duration of incubation with ouabain. The longer the incubation time is, the amplitude of contraction is larger. Pretreatment of arterial strip with monensin, Na ionophore, in the presence of ouabain increases tension development.

only in the presence of external Ca. Its amplitude could be modified by changing the extracellular Ca concentration. Increasing extracellular Ca concentration caused an increased amplitude of the contraction. Arterial strips began to contract at 0.1 mM $[Ca]_o$ and reached the maximum at 1 mM $[Ca]_o$. It seemed therefore likely that Na gradient-in-

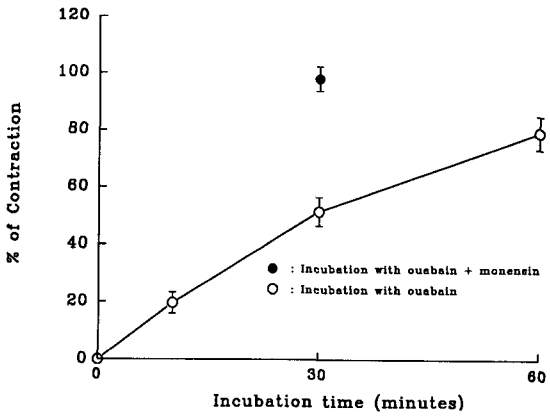

Fig. 7. Relationship between amplitude of Na-free contraction and duration of incubation with ouabain.

Arterial strips were incubated with ouabain (○) for 0, 10, 30, and 60 min and with ouabain+monensin (●) for 30 min before they were exposed to Na-free solution. Ordinate: relative contraction. Abscissa: incubation time (min). Given are the mean values±S.E. of 5 experiments. The contractions induced by 40 mM K was used as reference (100%).

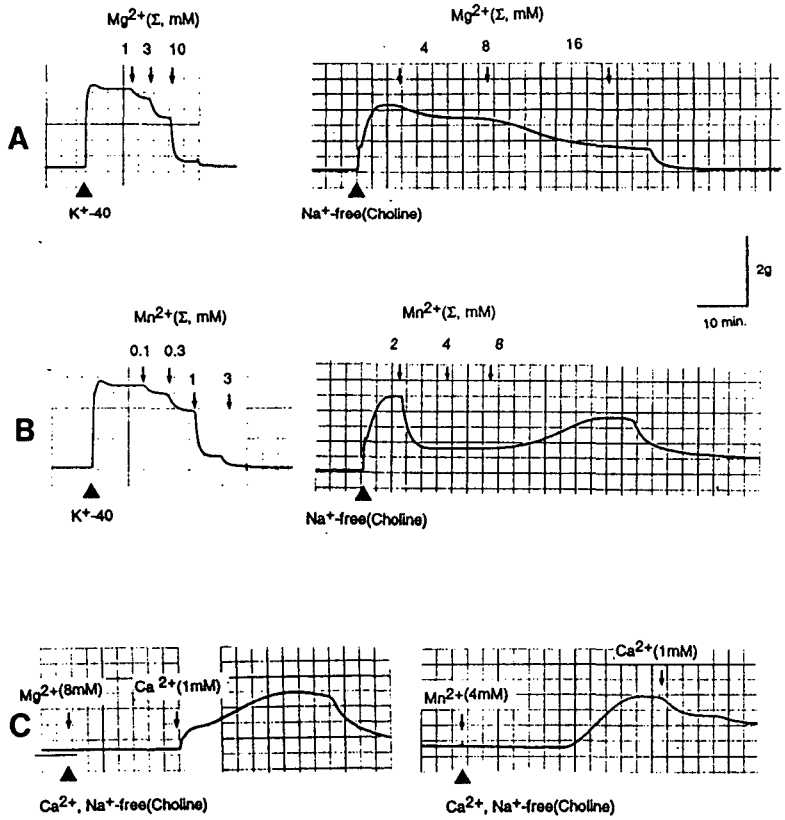

Fig. 8. Effects of Mg and Mn on high K-induced contraction in normal arterial strip or Na-free contraction in Na-loaded arterial strips.

High K-induced contraction was blocked completely by the addition of Mg or Mn. However, Na-free contraction in Na-loaded arterial strips was blocked partially and slowly by the addition of divalent cations. Pretreatment of arterial strip with Mg also failed to block Na-free contraction in the Na-loaded arterial strip. And pretreatment of arterial strip with Mn in the Na-free solution produced the contraction in the absence of external Ca.

**Arterial strips were exposed to choline solution after being pretreated with ouabain in the presence of verapamil for 1 hour.*

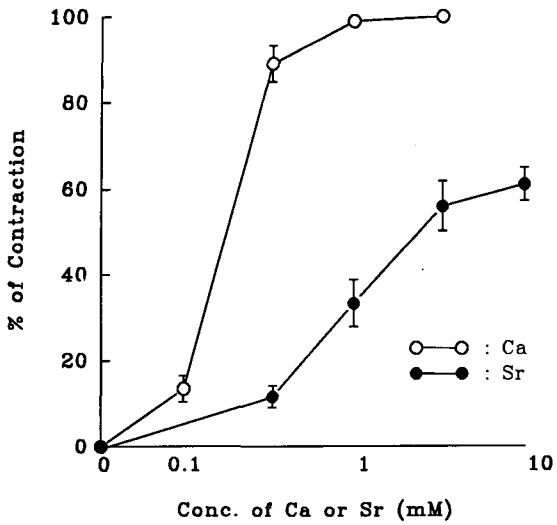

Fig. 9. Effects of Sr or Ca on the Na-free contracture in Na-loaded arterial strips.

After incubation of arterial strips in PSS containing the ouabain (10^{-5} M) and verapamil (10^{-5} M) for 1 hour, arterial strips were exposed to Ca, Na-free solution (choline). And Ca(○) or Sr(●) was cumulatively added to the bath. Ordinate: relative tension. Abscissa: external Ca or Sr concentration (mM). Given are the mean values±S.E. of 5 experiments.

duced contaction depended on increase of Ca ion entry. After loading cellular Na, Sr was added cumulatively to Ca-free choline solution. Addition of Sr also produced a graded contraction. The arterial strips began to contract at 0.3 mM $[Sr]_0$ and reached the maximum at 10 mM $[Sr]_0$. The maximum contraction amplitude reached about 60% as that obtained in the presence of 1 mM $[Ca]_0$.

DISCUSSION

Exposure of the rabbit renal artery to Na-free solution induced a contraction, as has observed in many other vascular smooth muscles (Droogmans & Casteels, 1979; Ozaki & Urakawa, 1981; Reuter et al, 1973; Sitrin & Bohr, 1971). The characteristics of contractile response of renal artery to Na-free solution was dependent on the nature of the Na substitute used.

The contraction induced by choline solution was partially inhibited by the addition of phentolamine, probably because an exposure to Na-free solution induced a release of noradrealine from the nerve terminals. It has been suggested that the contractions induced by Na depletion, K depletion or ouabain in many types of vascular smooth muscle are the result of a release of catecholamines from adrenegic nerve terminals rather than a direct effect on the muscle (Karaki & Urakawa, 1977; Kim & Kim, 1982). Inhibition of the sodium pump in tissue would affect ion transport and ion gradients not only in smooth muscle, but also in nerve fibers present in those tissues, and that part of the contraction was caused by a release of neurotransmitters (Broekaert & Godfraind, 1973; Karaki & Urakawa, 1977). In the present result, arterial strip also contracted when it was incubated with ouabain and this contraction was blocked by addition of phentolamine or verapamil completely. This result suggested that sodium pump inhibition by ouabain could affect ion transport not only in smooth muscle but also in nerve fiber present in that tissue.

Addition of verapamil also completely inhibited choline solution-induced contraction. It has been well known that verapamil blocks the Ca transfer through a Ca channel activated by membrane depolarization and inhibition of electrogenic sodium pump may cause the smooth muscle fibers to depolarize (Casteels et al, 1977; Hendrickx & Casteels, 1974; Webb & Bohr, 1978). Droogmans and Casteels (1979) reported that, after a prolonged incubation in Na-free solution, the vascular smooth muscle of rabbit ear artery was contracted and the membrane was depolarized. On the other hand, Itoh et al. (1981) reported that, in the guinea-pig mesenteric artery, reduction in $[Na]_0$ generated contraction with no change in membrane potential. In the present results, however, it was not possible to conclude that choline solution-induced contraction was due to the change in membrane potential even though the contrac-

tion was inhibited by verapamil. Because such a membrane potential change could not be completely eliminated without electrophysiological study.

The presence and function of a Na-Ca exchange transport system in the sarcolemma of smooth muscle has received a great deal of attention because of its possible role in the regulation of myoplasmic Ca. However, the presence and functional significance of a Na-Ca exchanger in vascular smooth muscle are a controversial issue. It has been suggested that Na plays an important role in regulating the level of myoplasmic Ca, and that a Na-Ca exchange mechanism could be involved in the contraction of vascular smooth muscle (Brading & Widdicombe, 1975; Reuter et al, 1973). Blaustein (1977) and Blaustein et al. (1986) postulated that Na-Ca exchange in vascular smooth muscle plays a key role in the link between Na and hypertension.

The factors that reduce the Na gradient across the cell membrane, e.g., reduction of $[Na]_o$, reduction of $[K]_o$ or cardiac glycosides, are known to induce contraction and an attempt has been made to explain this tension development on the basis of the Na-Ca exchange mechanism (Blaustein, 1974; 1977; Reuter et al, 1973). Several studies demonstrated that vascular smooth muscles of several species and types contracted when transsarcolemmal Na gradient was reduced (Droogmans & Casteels, 1979; Karaki & Urakawa, 1977; Lang & Blaustein, 1980; Ozaki et al. 1978; Ozaki & Urakawa, 1979). On the other hand, Mulvany et al (1982; 1984) have shown that exposure of denervated rings of rat mesenteric arteries to low $[Na]_o$ or to 1 mM ouabain failed to elicit a contractile response and ouabain and K-free solution had depressive effects on the mechanical response of rat mesenteric resistance vessel to norepinephrine. Itoh et al (1981) have shown that, in the guinea pig mesenteric artery, reduction in $[Na]_o$ generated contraction and have postulated that the contraction was due to influx of Ca through the Na channel rather than Ca channel. Yamamoto and Hotta (1985) also suggested that with depletion of both external and internal Na, Ca may enter the cell through channels usually occupied by Na. Thus, the presence of a Na-Ca exchanger in vascular smooth muscle, especially in peripheral resistance arteries, is uncertain. Differences in species, type of artery, and subdivision of artery could give rise to the varying conclusion of these studies. Methodological differences may be of even greater importance.

On the basis of the present results, the contraction produced by choline solution (following loading cellular Na) in the rabbit renal artery exhibited the following properties: 1. The contraction appear even in the presence of Ca channel blocker, verapamil. 2. The amplitude of contraction increased with an increase in incubation time with ouabain. Organic Ca channel blockers do not affect Ca flux through Na-Ca exchange carrier (Ashida & Blaustein, 1987; Maseki et al, 1990; Mulvany et al, 1984). The increase in $[Na]_i$ results in the augmentation of the activity of a Na-Ca exchange carrier system with stimulation of outward Na and inward Ca movement. These data suggest that intracellular Na is important for the efficient influx of Ca and that increase in tension is accounted for by an influx of Ca via the Na-Ca exchange carrier. However, it seems that Na-Ca exchange activity is latent or plays a minor role in Ca flux through the plasma membrane when $[Na]_i$ is at its basal level. In the present results, the exposure of arterial strip to choline solution without loading cellular Na did not induce any contraction in the presence of verapamil. Mulvany et al. (1984) observed that the exposure of denervated rings of rat mesenteric arteries to low $[Na]_o$ or to 1 mM ouabain fails to elicit a contractile response. A contractile response did occur, however, if the rings were incubated with ouabain to load cellular Na and exposed to low $[Na]_o$. Smith et al (1987, 1989) also observed that, in contrast to the Na-loaded cells, removing $[Na]_o$

caused no detectable change in $[Ca]_i$ in cells with normal Na in cultures of rat aortic muscle cells.

It is known that Mg is a general antagonist of Ca. Altura and Altura (1974) have suggested that certain divalent cation binding sites for vascular muscle may be non-specific to Mg and Ca, and that Mg ions may be important in regulating the permeability, translocation and binding of Ca. The present results have shown that raising $[Mg]_o$ inhibit the high K-induced contraction. However, Mg had little effect on the Na gradient-induced contraction. These data suggest that inhibition of the contraction by high Mg is probably due to a decreased inward movement of Ca, and that this may be the result of Mg-Ca competition at the site of a Ca pathway activated by K-depolarization. The less sensitivity of Na-gradient induced-contraction to Mg may be due to a low affinity for the Ca binding site on the Na-Ca exchange carrier. Ozaki & Urakawa (1979) have suggested that the Ca binding site on the Na-Ca exchange carrier was not occupied by Mg ions. However, according to Smith et al. (1987), in cultured arterial smooth muscle cells, divalent cations such as Mg, Mn, Co could competitively inhibited Ca influx via the Na-Ca exchange carrier.

It is likely that Mn itself seems to enter the cell and posses the property of activating the contractile proteins in the smooth muscle. Itoh et al. (1982) reported that Mn induced the contraction in the absence of Ca in the skinned fiber of the guinea-pig stomach. According to Yamamoto & Hotta (1985), rat portal vein contracted on exposure to Na-free solution in the presence of Mn without Ca. In our present result, the tonic contraction with a slow time course was induced by choline solution containing Mn without external Ca, but high K-induced contraction was blocked by raising the $[Mn]_o$. The tonic contraction may be due to that Mn entered the cell and directly activate the contractile protein or release the internally stored Ca. The differential effects of Mg and Mn on those contraction provide additional evidence that Ca transport systems are different between the contractions by Na gradient and by depolarization.

There have been several reports that the action of Sr ion resembles that of Ca ion on the smooth muscle. This similarity includes direct stimulation of muscle contractile element to elicit the contraction (Daniel et al, 1962; Ebashi et al, 1969). Sr can also be transported via not only the gated Ca channel but also Na-Ca exchange carrier (Ozaki & Urakawa, 1979; Smith et al, 1987). The present results showed that Sr replaced Ca in the Na gradient-induced contraction. These data suggest that Sr also seems to enter the cell through Na-Ca exchange carrier.

It can be concluded that the contraction induced by choline solution (following loading cellular Na) may be due to a Ca influx through Na-Ca exchange carrier, but Na-Ca exchange mechanism only play a role under extreme conditions in rabbit renal artery.

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