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Role of Na^+/Ca^{2+} Exchange in the Control of Contractility in Rabbit Basilar Arterial Smooth Muscle

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=ABSTRACT=

The contraction of rabbit basilar artery was examined as a function of changes in the Na^+ electrochemical gradient in order to determine the contribution of Na^+/Ca^{2+} exchange to the modulation of contractility. Ouabain (10^{-5} M) or K^+-free Tyrode solution caused an increase in tonic tension even in the presence of a Ca^{2+} channel blocker (10^{-6} M verapamil) and an α-receptor blocker (10^{-5} M phentolamine). After treatment with ouabain (10^{-5} M), contractions were augmented by reduction of external Na^+ concentration. The longer the treatment with ouabain (10^{-5} M) was, the larger the amplitude of Na^+-free contracture was. Na^+-free contracture was induced by either substitution of equimolar Tris for Na^+ or substitution of equimolar Li^+ for Na^+. The competition between Na^+ and Ca^{2+} for the Na^+/Ca^{2+} exchange carrier would exist, because it was observed that contractility was dependent on the Na^+ electrochemical gradient or the extracellular Ca^{2+} concentration (2 mM, 4 mM). Ryanodine (10^{-7} M), the blocker of intracellular Ca^{2+} release from the sarcoplasmic reticulum, did not suppress the development of Na^+-free contracture. The contractile response to norepinephrine (10^{-6} M) was augmented by reducing the extracellular Na^+ concentration. The relaxation rate from caffeine-induced contraction was dependent on the extracellular Na^+ concentration (0 mM, 140 mM).

From the above results, it could be suggested that Na^+/Ca^{2+} exchange can move Ca^{2+} either into or out of rabbit basilar arterial smooth muscle. Ca^{2+} entry or extrusion is dependent upon the Na^+ electrochemical gradient. Na^+/Ca^{2+} exchange plays a significant role in the regulation of contractility in rabbit basilar arterial smooth muscle.

Key Words: Na^+/Ca^{2+} exchange, Rabbit basilar arterial smooth muscle, Contractility, Na^+ electrochemical gradient

INTRODUCTION

It has been known that the immediate trigger for contraction in vascular smooth muscle is a rise in the cytosolic free calcium concentration, $[Ca^{2+}]_i$ (Kamm et al, 1989). This rise in $[Ca^{2+}]_i$ activates the calmodulin-myosin light chain kinase cascade that initiates smooth muscle contraction (Itoh et al, 1989). Moreover, maintained tension, or tone, in vascular smooth muscle is due to the maintenance of $[Ca^{2+}]_i$ above the contraction threshold (Hai et al, 1989). The precise relationship between $[Ca^{2+}]_i$ and tonic tension is poorly understood (Morgan

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et al, 1984), but withdrawal of extracellular Ca^{2+}, which reduces $[Ca^{2+}]_i$ (Goldman et al, 1990), dissipates tonic tension (Gerthoffer et al, 1983).

The finding of reciprocal transmembrane movements of Na^+ and Ca^{2+} on a bidirectional Na^+/Ca^{2+} exchange mechanism (Reuter & Seitz, 1868) excited much interest about the role of this mechanism in the regulation of intracellular Ca^{2+} and hence the contractile state of muscular tissues. A considerable volume of research has led to the firm establishment of the existence and importance of Na^+/Ca^{2+} exchange in cardiac muscle. Its role in the control of contractility observed on reduction of extracellular Na^+ or elevation of intracellular Na^+ is widely acknowledged (Reeves, 1986) and its electrogenic contribution to the cardiac action potential has been indentified (Noble, 1986). Its current was also recorded in isolated cardiac cells (Kimura et al, 1986). These facts regarding the cardiac muscle can not be extended to vascular smooth muscle at present. Although some reports have demonstrated that Na^+ gradient reduction promotes Ca^{2+} entry and slow Ca^{2+} extrusion via Na^+/Ca^{2+} exchange in vascular smooth muscles, there remains a controversy over whether Na^+/Ca^{2+} exchange plays a physiological role (Bradiry et al, 1985); Mulvany, 1985). The physiological role of the Na^+/Ca^{2+} exchange mechanism has not been fully understood in the cerebrovascular smooth muscle. In the present study, we have investigated the physiological role of Na^+/Ca^{2+} exchange in the regulation of contractility in rabbit basilar arterial smooth muscle.

MATERIALS AND METHODS

Preparation of basilar arterial circular muscle

Rabbits of either sex weighing about 2 kg were stunned by a sudden blow to the hind neck and killed by exsanguination via the cutting of both carotid arteries. A basilar arterial segment was then removed and transferred to a dissection bath containing oxygenated Tyrode solution. Circular muscle fibers with a size of 4 mm in length and 0.4 mm in width were obtained from the segment using fine optical scissors. One end of the muscle strip was fixed with a fine stainless steel pin and a loop was made with fine cotton thread around the other end of the muscle to connect it to a force transducer hook for measurement of tension. The endothelium was not destroyed in the present study. The preparation of tissues was done under a zoom stereomicroscope. The muscle strips were allowed to equilibrate for 1 hour without stretching at room temperature, while the bathing Tyrode solution was regularly changed. After an hour of rest, the muscle strip was transferred into the experimental chamber.

Solutions

Preparation solution: Phosphate-buffered Tyrode solution containing (in mM) NaCl 140, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 2, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 12H_2O$ 1.81, glucose 5.5, pH 7.4 (adjusted with NaOH). Working solution: Tris-buffered normal Tyrode solution contains (in mM) NaCl 140, KCl 4, $CaCl_2 \cdot 2H_2O$ 2, $MgCl_2 \cdot 6H_2O$ 1.05, Tris-HCl 5, glucose 5.5, pH7.4 (adjusted with NaOH). 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 with Tris-Cl isosmotically.

Experimental apparatus and protocol

The experimental chamber was made of perspex. It was a horizontal type. The flow was controlled by hydrostatic pressure. Experimental temperature was maintained at about 35°C with a constant temperature circulator (Harvard).

The muscle strips were allowed to relax in the

horizontal chamber for 1 hour in Tris-buffered Tyrode solution at 35°C equilibrated with 100% O_2. Then isometric contractions were recorded using a micro-tension transducer (Harvard) and a recorder (Harvard).

RESULTS

Tonic tension was increased in circular smooth muscle of rabbit basilar artery by ouabain (10^{-5} M) even in the presence of a Ca^{2+} channel blocker (10^{-6} M verapamil) and an α-receptor blocker (10^{-5} M phentolamine). The basal tone of the muscle strip increased gradually and maintained a constant peak level after about 30 minutes exposure to ouabain (10^{-5} M) (Fig. 1).

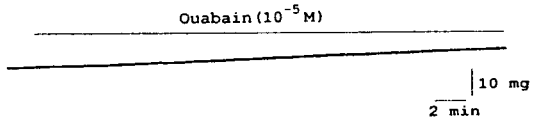

Fig. 1. Effect of ouabain on the resting tension of the rabbit basilar arterial smooth muscle. The superfusion fluid contained verapamil (10^{-6} M) and phentolamine (10^{-5} M). After treatment with verapamil (10^{-6} M) and phentolamine (10^{-5} M) for 10 minutes, ouabain (10^{-5} M) was applied. Ouabain (10^{-5} M) induced an increase in the tonic tension of the basilar artery.

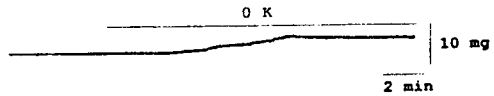

Fig. 2. Effect of K^+-free Tyrode solution on the resting tension of the rabbit basilar arterial smooth muscle. Increased tonic tension was induced in K^+-free Tyrode solution despite pretreatment with verapamil (10^{-6} M) and phentolamine (10^{-5} M) for 10 minutes. The superfusion fluid also contained verapamil (10^{-6} M) and phentolamine (10^{-5} M).

Figure 2 shows the effect of K^+-free media on the resting tension in the presence of verapamil (10^{-6} M) and phentolamine (10^{-5} M). Tonic tension was increased gradually as in the case of ouabain. A constant peak level was reach-

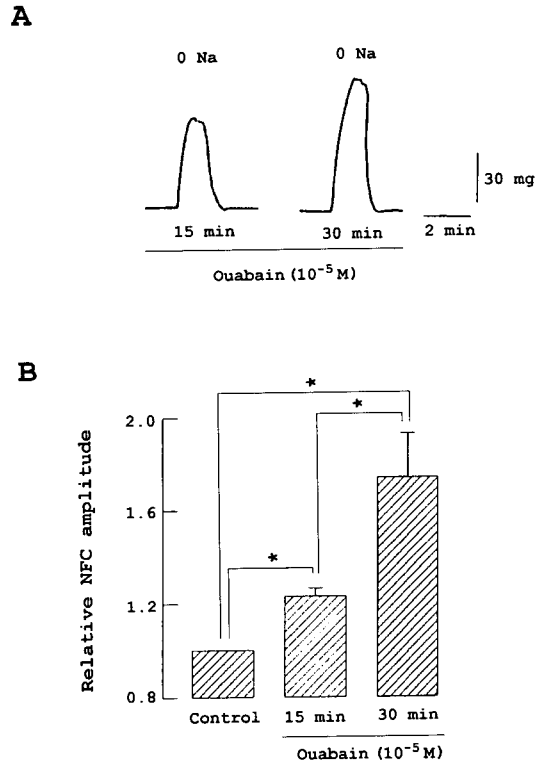

*Fig. 3. Effect of the duration of exposure to ouabain (10^{-5} M) on Na^+-free contracture in the rabbit basilar arterial smooth muscle. A: representative experiment showing effect of ouabain (10^{-5} M) pretreatment for 15 minutes (left panel) and 30 minutes (right panel) on the amplitude of Na^+-free contracture. All solutions contained verapamil (10^{-6} M) and phentolamine (10^{-5} M). B: summarized data from experiment shown in A and 5 other experiments. The amplitudes of Na^+-free contracture are normalized to control Na^+-free contracture amplitude in the absence of ouabain. Error bars indicate ± S.E.M. The amplitude of Na^+-free contracture was larger at 30 minutes than at 15 minutes exposure to ouabain (10^{-5}M); * $P < 0.05$. NFC means Na^+-free contracture.*

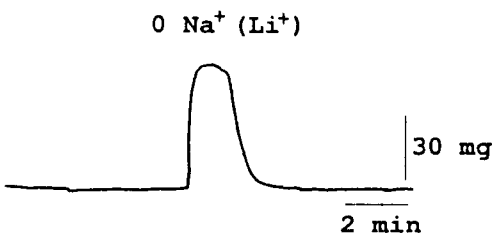

Fig. 4. The induction of Na^+-free (Na^+ was replaced by equimolar LiCl) contracture in the rabbit basilar arterial smooth muscle. The preparation was treated with ouabain (10^{-5} M) for 30 minutes prior to the application of Na^+-free Tyrode solution with equimolar LiCl substituted for NaCl. Na^+-free (LiCl) contracture was similarly induced by Na^+-free (Tris-Cl) solution.

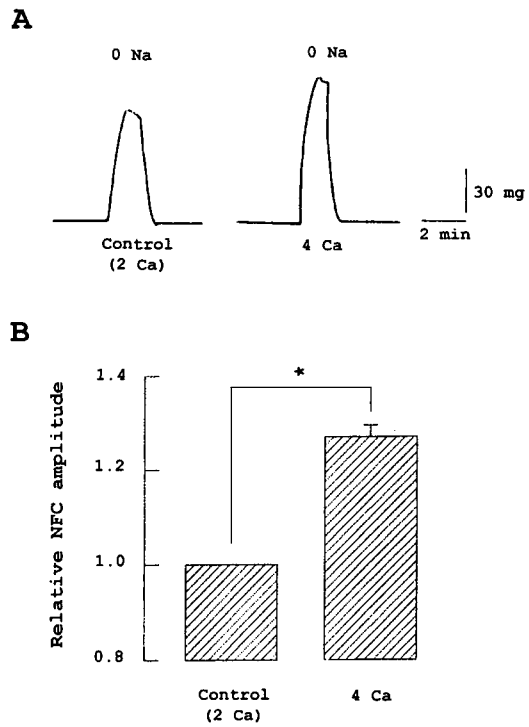

*Fig. 5. Effect of extracellular Ca^{2+} concentration on the Na^+-free contracture in rabbit basilar arterial smooth muscle. A: original records showing response to 2 different concentrations of extracellular Ca^{2+} (2 and 4 mM) during Na^+-free contracture; Na^+ was replaced by equimolar Tris. In both cases, pretreatment with ouabain (10^{-5} M) for 30 minutes took place. B: summarized data from the experiment shown in A and 5 other experiments. The amplitudes of Na^+-free contracture are normalized to control Na^+-free contracture amplitude in the presence of 2 mM Ca^{2+}. The error bar indicates ± S.E.M. The amplitude of Na^+-free contracture was larger in the presence of 4 mM Ca^{2+} than in the presence of 2 mM Ca^{2+}; * $P < 0.05$. NFC means Na^+-free contracture.*

ed at about 10 minutes after exposure to K^+-free Tyrode solution (Fig. 2). These effects may therefore be a direct result of a rise in the intracellular Na^+ concentration, $[Na^+]_i$, due to Na^+ pump inhibition, rather than a consequence of depolarization and activation of voltage-operated Ca^{2+} channels or the release of endogenous α-agonist.

Figure 3 shows the relationship between the duration of Na^+-pump inhibition and the amplitude of Na^+-free contracture. The basilar arterial smooth muscle was treated with ouabain (10^{-5}M) for 15 minutes (Fig. 3A, left panel) and 30 minutes (Fig. 3A, right panel) in the presence of verapamil (10^{-6} M) and phentolamine (10^{-5} M). When Na^+-free Tyrode solution (Na^+ was replaced by equimolar Tris) was applied for 2 minutes, Na^+-free contracture was developed and relaxed spontaneously prior to wash out with normal Tyrode solution. The magnitude of the contracture after treatment with ouabain (10^{-5} M) was larger at 30minutes than at 15 minutes (Fig. 3B, $P<0.05$, $n=6$).

In order to observe whether the specificity between Na^+ and Ca^{2+} for a carrier at both sides of the membrane may exist or not, the following experiment was performed.

After treatment with ouabain (10^{-5} M) for 30 minutes, substitution of equimolar Li^+ for Na^+ also induced contracture as in case of substitution of Tris for Na^+ (Fig. 4). This suggests the possibility that the specificity between Na^+ and Ca^{2+} may exist. All solutions contained verapamil (10^{-6} M) and phentolamine (10^{-5} M).

In order to test the possibility that the competi-

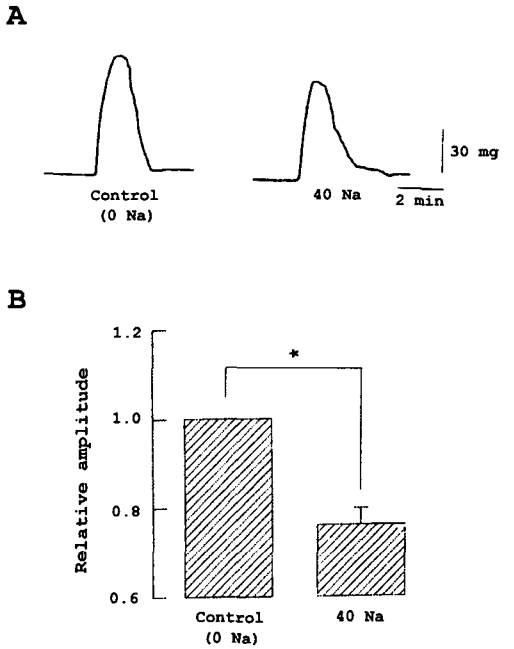

Fig. 6. Effect of extracellular Na^+ concentration on Na^+-free contracture in the rabbit basilar arterial smooth muscle. A: original records showing response to 2 different concentrations of extracellular Na^+ (0 and 40 mM); Na^+ was replaced by equimolar Tris. Pretreatment for 30 minutes with ouabain (10^{-5} M) took place in both cases. B: summarized data from the experiment shown in A and 5 other experiments. The contractile responses of basilar arterial smooth muscle are normalized to control Na^+-free contracture amplitude in the absence of ouabain. The error bar indicates ± S.E.M. Contractility was larger in the Na^+-free solution than in the 40 mM Na^+-containing solution; $^*P < 0.05$.

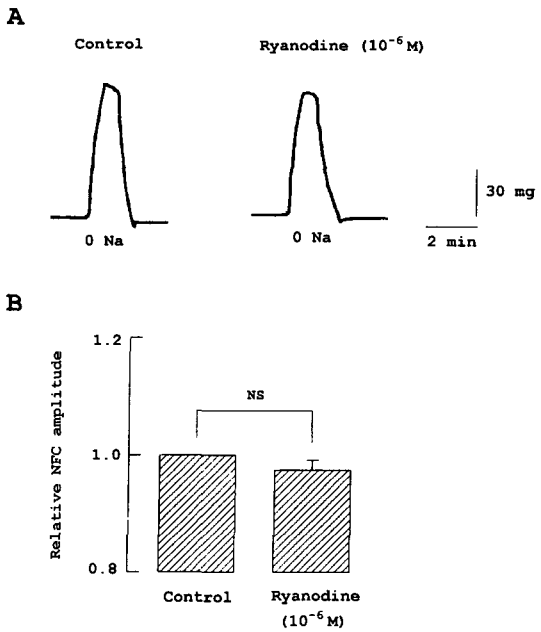

Fig. 7. The effect of ryanodine on Na^+-free contracture in the rabbit basilar arterial smooth muscle. A: representative experiment showing effect of ryanodine on the Na^+-free contracture. Pretrearment with ryanodine (10^{-7} M) took place for 10 minutes prior to the application of Na^+-free solution. All solutions contained ouabain (10^{-5} M), verapamil (10^{-6} M) and phentolamine (10^{-5} M). The Na^+-free contracture was induced even in the presence of ryanodine (10^{-7} M). B: ryanodine did not have a significant effect on the Na^+-free contracture. The error bar denotes ± S.E.M. ($n=4$); NS, $P > 0.05$.

tion between Na^+ and Ca^{2+} for a carrier may be present at both sides of the membrane, we investigated the effect of extracellular Na^+ or Ca^{2+} concentration on the amplitude of the Na^+-free contracture (Fig. 5, 6). The muscle strip was treated with ouabain 10^{-5} M for 30minutes in the presence of verapamil (10^{-6} M) and phentolamine (10^{-5} M). Figure 5 shows that the amplitude of the Na^+-free contracture was dependent upon extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). The Na^+-free contraction was augmented when $[Ca^{2+}]_o$ was increased to a higher concentration (from 2 to 4 mM) (Fig. 5B, $P<0.05$, $n=6$).

Figure 6 shows the relationship between extracellular Na^+ concentration ($[Na^+]_o$) and contractility. Increase in $[Na^+]_o$ (from 0 to 40 mM) attenuated the contraction of rabbit basilar arterial smooth muscle (Fig. 6B, $P<0.05$, $n=4$).

On the other hand, we investigated the effect of ryanodine (10^{-6} M), which inhibits Ca^{2+} release from the Sarcoplasmic reticulum, on the Na^+-free contracture (Fig. 7). The Na^+-free contracture

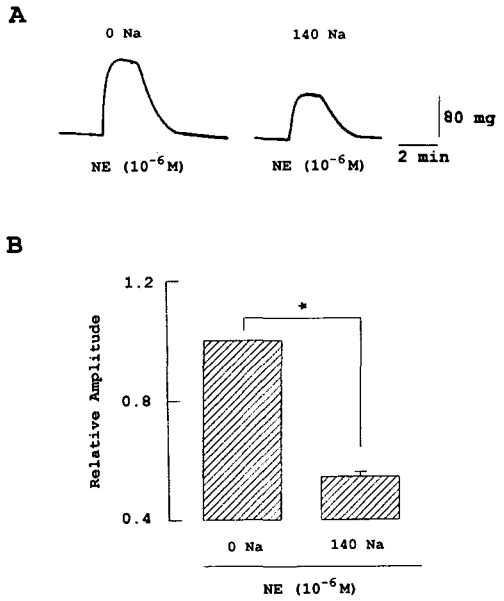

Fig. 8. Effect of $[Na^+]_o$ on contractile response of rabbit basilar arterial smooth muscle to 10^{-6} M norepinephrine. A: original records from one experiment illustrating contractions evoked in solution containing 0 mM Na^+ (left panel) and 140 mM Na^+ (right panel). Norepinephrine-induced contraction was augmented in the Na^+-free Tyrode solution compared with the normal Tyrode solution (140 mM Na^+). B: comparison of effect of $[Na^+]_o$ on the norepinephrine-induced contraction amplitude; summarized data from 4 cells in each case. Error bar indicates ± S.E.M.; $^*P < 0.05$.

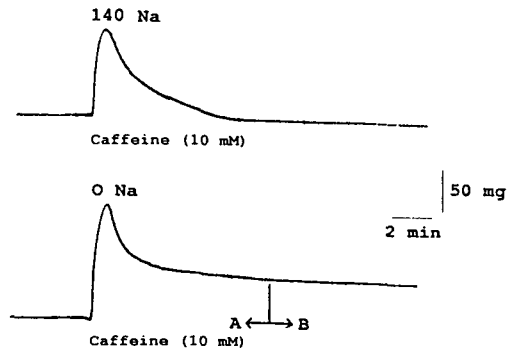

Fig. 9. Effect of extracellular Na^+ concentration on the relaxation rate of caffeine-induced contracture in rabbit basilar arterial smooth muscle. Although the first component of relaxation had almost same slope the relaxation rate was generally slower in Na^+-free Tyrode solution than in normal Tyrode solution. Furthermore, the portion B of caffeine-induced contracture in Na^+-free Tyrode solution did not relax fully to its original resting state.

was also induced even in the presence of ryanodine (Fig.7, $P > 0.05$, $n=4$). From this result, we can exclude the possibility that Na^+-free contracture may be induced by Ca^{2+} release from intracellular Ca^{2+} stores (i.e., the sarcoplasmic reticulum).

Figure 8A shows the relationship between $[Na^+]_o$ and norepinephrine-induced contraction in basilar arterial smooth muscle. The amplitude of norepinephrine-induced contraction was inversely related to $[Na^+]_o$. The contraction amplitude increased significantly when $[Na^+]_o$ was lowered to 0 mM (Na^+ replaced by equimolar Tris)(Fig. 8B, $P < 0.05$, $n=4$).

Na^+/Ca^{2+} exchange can move Ca^{2+} in either direction across the plasma membrane depending upon Na gradient (Sheu & Blaustein, 1986). Thus, in order to test the possibility that Ca extrusion can be inhibited by reducing $[Na^+]_o$, the effect of $[Na^+]_o$ on the relaxation rate of the caffeine-induced contractions was also investigated. In the experiment of Fig. 9 (lower panel), the amplitude of the caffeine-induced contraction was augmented when Na^+ was replaced by equimolar Tris. Under Na^+-free conditions, the tension did not return to the original base line, whereas, the tension rapidly fell to the original base-line level in the presence of 140 mM Na^+ solution (Fig. 9, upper panel). These results suggest that the Na^+/Ca^{2+} exchange mechanism has a role in determining the relaxation rate.

DISCUSSION

The presence of Na^+/Ca^{2+} exchange in vascular smooth muscle was suggested by many

early studies in which tonic contractions were induced when external Na^+ concentration ($[Na^+]_o$) was reduced, and/or the Na^+ pump was inhibited by cardiotonic steroids (Van Breemen et al, 1979). Moreover, recent sarcolemmal vesicle studies directly demonstrate the presence of a Na^+/Ca^{2+} exchange mechanism in various types of smooth muscle (Casteels et al, 1985). However, it was reported that lowering the electrochemical gradient between extracellular Na^+ concentration and intracellular Na^+ concentration did not always increase tension (Itoh et al, 1982). In some cases, Ca^{2+} entry through voltage-gated Ca^{2+} channels and/or Ca^{2+} entry activated by the release of endogenous catecholamines or other agonists could not be ruled out as alternative sources of Ca^{2+} (Van Breemen et al, 1979). In the present study, the contraction of rabbit basilar arterial smooth muscle was examined as a function of changes in the Na^+ electrochemical gradient in order to determine the physiological role of Na^+/Ca^{2+} exchange in the regulation of contractility.

In the first place, we intend to prove that ouabain- or K^+-free-induced tonic contractions may be developed via the Na^+/Ca^{2+} exchange mechanism. Therefore, it is necessary to rule out the following possibilities that can be caused by Na^+-pump inhibition. One possibility is that Ca^{2+} entry can be activated by the release of an endogenous neurotransmitter due to Na^+-pump inhibition (Katsugari et al, 1978). However, some authors reported that Na^+-pump inhibition-induced contraction still existed in spite of treatment with an α-adrenergic blocker (Ozaki et al, 1978) or nerve destruction by 6-hydroxy dopamine (Mulvany et al, 1984). We also demonstrated that tonic tension was increased by ouabain or K^+-free media even in the presence of the α-receptor blocker phentolamine (Fig. 1, 2). Second, Ca^{2+} entry through a voltage-gated Ca^{2+}-channel can be activated by depolarization due to Na^+-pump inhibition (Hirst & Van Helden, 1982). We can also rule out this possibility because of the ouabain- or K^+-free-induced contractions occurring in the presence of the Ca^{2+}-channel blockers verapamil (Fig. 1, 2). Therefore, ouabain- or K^+-free-induced tonic tension may be a direct result of a rise in the intracellular Na^+ concentration due to Na^+-pump inhibition, rather than a consequence of depolarization and activation of voltage-gated Ca^{2+}-channels or release of an endogenous α-agonist. Some previous reports have supported our interpretations. It has been reported that the ouabain-induced rise in intracellular Ca^{2+} in guinea-pig aorta is blocked by Ni^{2+} (Iwamoto et al, 1992), an inhibitor of Na^+/Ca^{2+} exchange (Kimura et al, 1987), but not by verapamil (Iwamoto et al, 1992).

Intracellular Na^+ increment-dependent Ca^{2+} influx becomes more evident from the relationship between the duration of exposure to ouabain (10^{-5} M) and the amplitude of Na^+-free contracture (Fig. 3). The simplest explanation for the effect of ouabain on the Na^+-free contracture is that ouabain raises intracellular Na^+ concentration and thereby indirectly promotes Ca^{2+} influx and reduces Ca^{2+} efflux via Na^+/Ca^{2+} exchange (Iwamoto et al, 1992).

Figure 4, 5 and 6 indicate the presence of the competition and specificity between Na^+ and Ca^{2+} for a carrier at both sides of the membrane. The radius of Na^+ is similar to that of Ca^{2+}. The radius of Na^+ is 0.95-0.98 Å and that of Ca^{2+} is 0.94-0.99 Å. It has been suggested that Na^+ and Ca^{2+} have a competitive relationship on the negatively charged binding site of the surface membrane (Anghileri, 1982). It is likely that such a relationship between Na^+ and Ca^{2+} exists in either extracellular or intracellular membrane. Furthermore, transmembrane movements of Na^+ and Ca^{2+} ions in a bidirectional Na^+/Ca^{2+} exchange may be related to the regulation of intracellular Ca^{2+} and hence the contractile state of muscle tissue (Langer, 1982).

On the other hand, Daniel (1985) suggested that Ca^{2+} release from the intracellular Ca^{2+} stores may also contribute to the development of

the Na^+-free contracture. However, our results show that the contracture is not induced by intracellular Ca^{2+} release because the Na^+-free contracture developed even in the presence of ryanodine, which facilitates Ca^{2+} release from the intracellular Ca^{2+} stores by holding the sarcoplasmic reticulum Ca^{2+} release channel open (Fig.7).

We demonstrated that norepinephrine-induced contractions were augmented when $[Na^+]_o$ was reduced (Fig. 8). This effect may be expected under conditions in which Ca^{2+} entry is enhanced and/or Ca^{2+} extrusion is reduced. One explanation is that reduction of the Na^+ gradient promotes Ca^{2+} entry into the unstimulated vascular smooth muscle cells via Na^+/Ca^{2+} exchange, and thereby increases the availability of Ca^{2+} for subsequent activation by raising intracellular Ca^{2+} concentration. A rise in intracellular Ca^{2+} concentration should increase the amount of Ca^{2+} stored in the sarcoplasmic reticulum and thus the amount available to be released when the tissue is stimulated by norepinephrine. In addition, reduced Ca^{2+} extrusion via Na^+/Ca^{2+} exchange may prolong the Ca^{2+} transient because the Na^+ electrochemical gradient was reversed by Na^+-free media.

It has been known that the Na^+/Ca^{2+} exchange mechanism can move net Ca^{2+} in either direction across the plasma membrane, depending upon the prevailing electrochemical gradient between extracellular and intracellular Na^+ concentration (Sheu & Blaustein, 1986). When Na^+ entering the cell moves down its steep electrochemical gradient, Na^+ influx may provide thermodynamically some or all of energy needed to extrude Ca^{2+} against its large electrochemical gradient (Carafoli, 1982; Dipolo & Beague, 1984). Our results demonstrated that the amplitude of the caffeine-induced contraction was increased when $[Na^+]_o$ was reduced. In addition, the rate of relaxation following contracture was partially dependent on $[Na^+]_o$ (Fig. 9). It seems likely that reduction of the Na^+ gradient promotes Ca^{2+} influx via Na^+/Ca^{2+} exchange. Moreover, the results also suggest the possibility that the increased sarcoplasmic reticulum store of Ca^{2+} may be reflected by the increase in the amplitude of the caffeine-induced tension transient during exposure to Na^+-free solutions.

From the above results, it could be suggested that Na^+/Ca^{2+} exchange can move Ca^{2+} either into or out of rabbit basilar arterial smooth muscle. Ca^{2+} entry or extrusion is dependent upon the Na^+ electrochemical gradient. Thus Na^+/Ca^{2+} exchange plays a significant role in the regulation of contractility of rabbit basilar arterial smooth muscle by altering the sarcoplasmic reticulum stores of Ca^{2+}.

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