

# Sodium and Calcium Interactions in Vascular Smooth Muscle Cells of the Rabbit Ear Artery

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**ABSTRACT** The effects of Na-free and of K-free solutions on the membrane potential, on tension development, and on  $^{45}\text{Ca}$  exchange have been investigated in rabbit ear artery. The contraction induced by Na-free solutions and the tension which develops in K-free solutions after a delay of about 1 h are both submaximal. Exposure for 4 h to K-free solutions does not affect the membrane potential, whereas Na-free solutions depolarize the cells by 10–20 mV, depending on the Na-substitute. Neither the amplitude nor the rate constant of the slowly exchanging  $^{45}\text{Ca}$ -fraction is affected by these experimental procedures. Substituting external Na by choline or TMA induces a transient increase of the  $^{45}\text{Ca}$ -efflux rate which does not occur in a Ca-free efflux medium, and which can be blocked with La. K readmission to Na-enriched tissues hyperpolarizes the cells up to  $-100$  mV and induces a relaxation, without exerting any effect on the  $^{45}\text{Ca}$  efflux rate. The release of Ca from intracellular stores, induced by histamine and FCCP, and its subsequent extrusion through the plasma membrane produce a transient stimulation of the  $^{45}\text{Ca}$  efflux, which is not affected by the reduction of the Na gradient. The transient contraction induced by histamine in Ca-free solutions is affected in a different way by different Na substitutes. The results do not fit the Na-Ca exchange hypothesis but are consistent with an effect of the Na gradient on the passive Ca influx.

## INTRODUCTION

The antagonism between  $[\text{Na}]_o$  and  $[\text{Ca}]_o$  on the tension development of vascular smooth muscle cells, and the contraction induced in these tissues by ouabain or K-free solutions, have been interpreted as evidence in favor of a Na-Ca exchange mechanism being involved in the regulation of  $[\text{Ca}]_i$ . This hypothesis has been proposed by Reuter et al. (1973), and Blaustein (1976, 1977). Such an interpretation is based on the assumptions that the change of tension development is a measure of the change in the intracellular Ca-concentration, and that this change in  $[\text{Ca}]_i$  is mainly caused by a decrease of the net transmembrane Ca extrusion, because of the reduction of the Na gradient. However, the extrapolation from contractile response to transmembrane Ca movements may be rather speculative because other factors, which could affect the contractile state of the smooth muscle cells, do not necessarily remain constant under the different experimental conditions. It is also possible that the intracellular Ca-buffering mechanisms, as well as the Ca permeability of the plasma membrane are affected by these different experimental conditions.

Moreover, it has to be pointed out that Na-free or K-free solutions and solutions containing ouabain cause a release of prostaglandin E in guinea pig taenia coli (Coburn and Soltoff, 1977). Also, a release of noradrenaline has been described in arteries with a rich sympathetic innervation during exposure to K-free solutions (Bonaccorsi et al., 1977). It is the purpose of the present experiments to analyze further these contractile responses and to complement these observations with  $^{45}\text{Ca}$  flux studies in order to get a better insight into the actual role of Na ions in the activation of vascular smooth muscle and their interaction with the transmembrane Ca movements. In addition, the effect of the different experimental treatments on the resting potential has been investigated.

A preliminary communication on some of these data has been presented (Droogmans and Casteels, 1977).

#### MATERIALS AND METHODS

Rabbits (2–3 kg body wt) were stunned and bled. The ear artery was dissected and transferred to warmed and oxygenated physiological solution. It was cleaned of its periarterial connective tissue, and helical strips were cut. For contraction experiments these helical strips were mounted in an organ bath (10 ml), and tension was recorded by means of an isometric force transducer. The membrane potential was measured intracellularly by means of glass microelectrodes with a resistance of 60–100 M $\Omega$ .

For flux experiments the tissues were mounted isometrically on Teflon holders and allowed to equilibrate for 1 h in control physiological solution. Afterwards they were transferred to the radioactive loading solution and washed in the different efflux media. At the end of the efflux period the tissues were blotted, weighed, ashed and dissolved (Casteels, 1969; Casteels and van Breemen, 1975). The radioactivity in the effluent samples and the tissues were counted, and from these data the rate of efflux, the tissue tracer content, and the fractional loss have been calculated.

The control physiological solution was a HEPES-buffered modified Krebs' solution containing (in millimolar): 135.5 Na; 5.9 K; 1.5 Ca; 1.2 Mg; 143.8 Cl; 11.6 HEPES; 11.5 glucose. It was bubbled with O<sub>2</sub> at pH 7.3, and the temperature was kept constant at 37°C.

Na-free solutions were obtained by replacing Na by either choline or tetramethylammonium (TMA). For these solutions 11.6 mM HEPES was titrated with KOH instead of with NaOH, and the amount of added KCl was reduced so as to maintain  $[\text{K}]_o$  at its value of 5.9 mM. Atropine (100 mg/liter) was added to all Na-free solutions. K-free solutions were prepared by replacing KCl by NaCl. All these modified solutions contained phentolamine ( $10^{-5}$  M) to eliminate a possible effect on the release of noradrenaline from the nerve endings. Ca-free solutions always contained 2 mM EGTA.

The numerical values are expressed as mean  $\pm$  SEM.  $n$  represents the number of observations.

#### RESULTS

##### *Membrane Potential and Contraction in Na-Free Solutions*

Exposure of the rabbit ear artery to Na-free solutions induces a contraction, as has been observed in many other vascular smooth muscle cells (Hinke and Wilson, 1962; Sitrin and Bohr, 1971; Reuter et al., 1973). This contraction is partially inhibited by the addition of phentolamine, probably because an exposure to Na-free solutions also induces a release of noradrenaline from the

nerve terminals. The amplitude of this contraction remains constant during prolonged exposure to Na-free solution and depends on the nature of the Na substitute: it is higher in solutions containing TMA than in those containing choline (Fig. 1*a*). Moreover, when a contraction has been induced in a Na-free solution containing TMA, a substitution of choline for TMA induces a partial

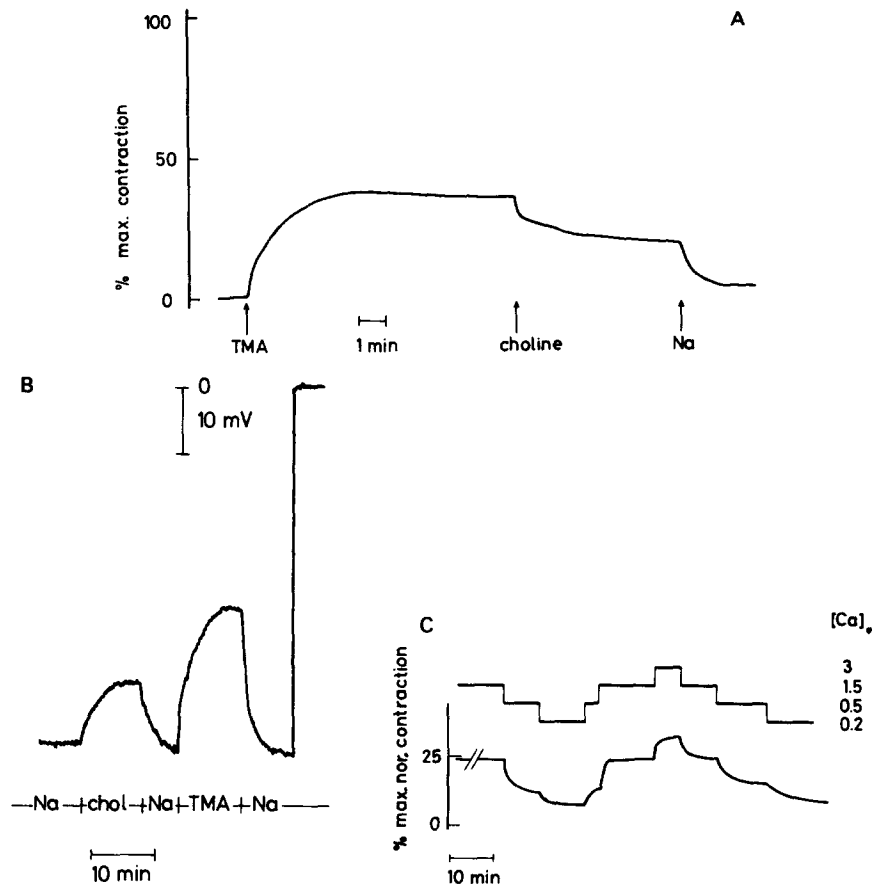


FIGURE 1. Effect of the Na substitutes choline and TMA on (A) the mechanical tension and (B) the resting potential. (C) The effect of changing  $[Ca]_o$  on the tension induced in Na-free choline-containing solutions. The tissues were preincubated for 3 h in a Na-free solution containing 1.5 mM Ca. The tension is expressed as a percentage of the contraction induced by  $10^{-5}$  M noradrenaline (nor.).

relaxation. The study of the membrane potential revealed that in a Na-free solution prepared with choline the cells depolarize from  $-61$  to about  $-50$  mV and that this membrane potential is maintained for at least 2 h. However, the contraction occurring in this Na-free solution cannot be explained by this limited depolarization, because a similar depolarization induced by increasing  $[K]_o$  does not cause an appreciable contraction (Droogmans et al., 1977). Na-

free solutions prepared with TMA depolarize the smooth muscle cells to about  $-40$  mV or about 10 mV more than in Na-free solutions prepared with choline (Fig. 1 *b*). This larger depolarization occurring in TMA solution could play a role in increasing the tension development above its value in choline solutions because a supplementary depolarization by increased  $[K]_o$  in such solution induces a further tension increase. The tension development during exposure to Na-free solution occurs only in the presence of external Ca. Its amplitude can be modified by changing  $[Ca]_o$ : reducing  $[Ca]_o$  causes a partial relaxation, and increasing  $[Ca]_o$  causes an increased tension development (Fig. 1 *c*). These phenomena have been observed after an incubation of more than 3 h in Na-free solution. After such treatment the cellular Na content of the tissues has dropped to unmeasurably low values (less than 0.3 mM/kg wet wt). It seems therefore likely that the contractions induced in Na-free solutions depend on an increased entry of Ca ions, which is not mediated by a carrier mechanism exchanging internal Na for external Ca. Moreover, the relaxation which occurs in Na-free medium on reducing  $[Ca]_o$  cannot be explained by an extrusion mechanism powered by the Na gradient.

*Effect of K-Free Solutions on the Transmembranal Electrochemical Gradient of  $Na^+$  and on the Vascular Tone*

K-free solutions induce a contraction of the ear artery after about 50–60 min of exposure to this solution. This contraction is also partially inhibited by phentolamine and it reaches its maximal amplitude only after about 2 h of exposure. This amplitude of contraction amounts to  $\sim 30\%$  of maximal contraction induced by noradrenaline and does not change significantly during the next 2 h of exposure. Increasing  $[Ca]_o$  or reducing  $[NA]_o$  both enhance the amplitude of this contraction whereas readmission of K to the external solution induces a relaxation within a few minutes (Fig. 2).

Such an exposure of the tissues to a K-free solution with or without ouabain causes a net gain of intracellular Na and a loss of K. During a 4-h treatment with K-free solution the K content of the ear artery decreases from  $34 \pm 2$  mM/kg wet wt ( $n = 5$ ) to  $7.4 \pm 0.8$  mM/kg wet wt ( $n = 5$ ), while the sorbitol space (control  $0.56 \pm 0.04$  [ $n = 7$ ] l/kg wet wt vs. K-free  $0.57 \pm 0.02$  [ $n = 7$ ]) and the dry-wet weight ratio (control  $0.22 \pm 0.01$  [ $n = 7$ ] vs. K-free  $0.21 \pm 0.01$  [ $n = 7$ ]) are not significantly affected by this treatment. From these analytical data a value for the intracellular K concentration of 146 mmol/liter cell water can be calculated. This value is in good agreement with the value obtained in the main pulmonary artery (Casteels et al., 1977). Because of the large value of the extracellular space, it is difficult to obtain reliable estimates of the intracellular Na concentration by the analytical procedure. We have therefore preferred to use an indirect method for estimating the time-course of the change of  $[Na]_i$  during K depletion, which is based on the observation that, in taenia coli (Casteels et al., 1971) under the same experimental conditions, the loss of K is approximately compensated by an equivalent gain of Na. A similar equimolar exchange of  $[K]_i$  for  $[Na]_i$  has also been observed in rat tail artery after treatment with ouabain (Friedman et al., 1973). This assumption makes it possible to estimate the changes in  $[Na]_i$  from the efflux of  $^{42}K$  in K-free

solutions. This efflux proceeds in the rabbit ear artery exponentially with a rate constant of  $0.0053 \pm 0.0005 \text{ min}^{-1}$  ( $n = 10$ ). If we assume that, under control conditions  $[\text{Na}]_i$  is similar to the value calculated for taenia coli (Casteels, 1969) and for the main pulmonary artery (Casteels et al., 1977), the time-course of  $[\text{Na}]_i$  during K depletion can be described by:  $[\text{Na}]_i = 13 + 146 \cdot [1 - \exp(-0.0053 \cdot t)]$ .

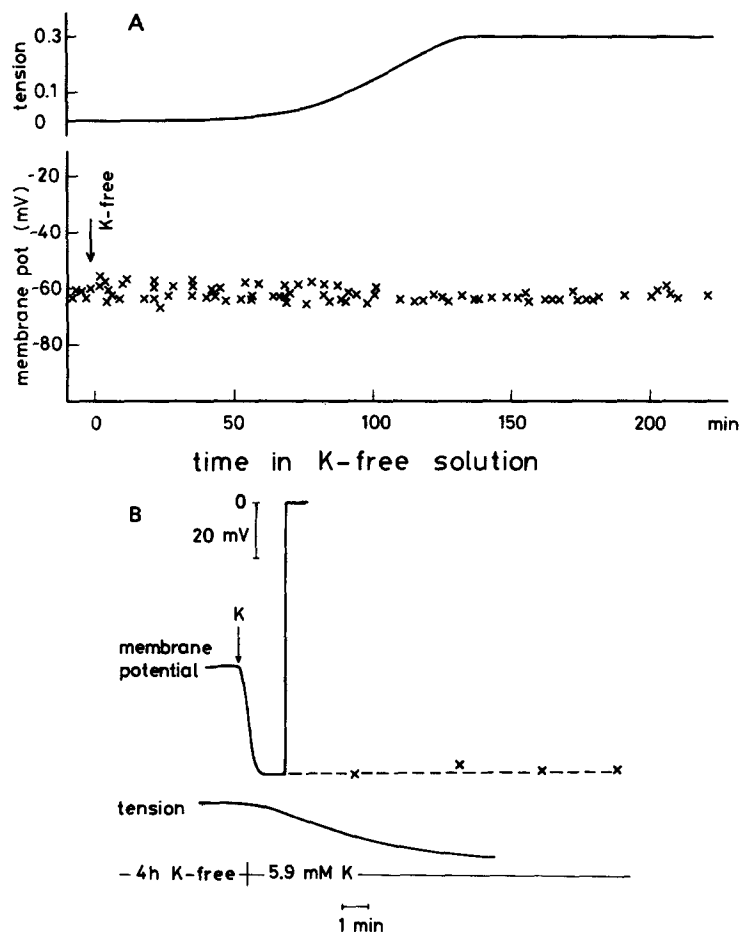


FIGURE 2. Effect of (A) K-free solutions and (B) K readmission on the membrane potential and on the tension development of rabbit ear artery. The tension is expressed as a percentage of a maximal noradrenaline contraction.

In spite of the pronounced changes in the ion gradients occurring in K-free solution, the resting potential of the smooth muscle cells mostly remains remarkably constant during the 4-h treatment in this solution (Fig. 2). In some tissues, a gradual depolarization starts after about 2–3 h of exposure, but after 4 h of K-depletion the resting potential in such tissues still amounts to about –45 to –50 mV.

Readmission of K to K-depleted cells in the absence of ouabain immediately hyperpolarizes them to about  $-100$  mV. The resting potential then slowly returns to its control value. It has to be pointed out that hyperpolarization as observed in single cells appears more rapidly than the accompanying relaxation (Fig. 2).

*Effects of  $[Na]_o$  and of the Membrane Potential on the  $^{45}Ca$  Efflux*

In contrast to the findings in other smooth muscle such as rabbit aorta (Deth and Casteels, 1977) and taenia coli (Casteels and van Breemen, 1975), the slow phase of the  $^{45}Ca$  efflux shows an almost exponential time-course, and evidence has been presented (Droogmans et al., 1977) that this phase might represent transmembrane Ca exchange. It is not possible at the moment to explain this

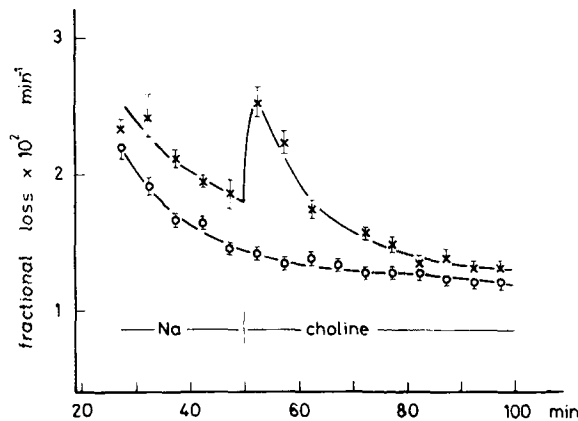


FIGURE 3. Effect of Na-free solutions on the  $^{45}Ca$  efflux from rabbit ear artery in (O) Ca-free and (X) 1.5 mM Ca-containing solution. Ordinate: fractional loss in minutes<sup>-1</sup>. Abscissa: time in minutes. The vertical bars represent SEM. Each curve is the mean of six tissues.

discrepancy between different tissues. We have therefore investigated the effect of the external sodium concentration on the slow phase of the  $^{45}Ca$  efflux from tissues which had been loaded for 3 h in labeled physiological solutions. In these experiments mainly choline was used as Na substitute but similar results have also been obtained with TMA. A reduction of  $[Na]_o$  to zero during an efflux in Ca-free, EGTA-containing solutions does not affect appreciably the rate of efflux. However, if Ca is present in the washing solution, such a reduction of  $[Na]_o$  transiently increases the  $^{45}Ca$  efflux (Fig. 3). This effect of Na-free solutions on the  $^{45}Ca$  efflux is similar to the effect of K-rich solutions, as described by Droogmans et al. (1977). Both conditions induce a transient stimulation of the rate of  $^{45}Ca$  efflux on condition that extracellular Ca is present. In the presence of 1 mM La and 1.5 mM Ca, the efflux rate of  $^{45}Ca$  is not significantly affected by reducing  $[Na]_o$ : the ratio of the efflux rate in Na-free solution to its value in Na-containing solution amounts to  $0.96 \pm 0.004$  ( $n = 5$ ). This finding suggests that Na-free solutions induce tension development by increasing the Ca influx. The Na-Ca exchange model also predicts that for a coupling ratio different from 2:1, the rate of the  $^{45}Ca$  efflux should

depend on the membrane potential (Mullins, 1976). This prediction has been tested by investigating the effect of the hyperpolarization, which occurs on readmission of K to K-depleted tissues, on the  $^{45}\text{Ca}$  efflux of the ear artery. Fig. 4 represents the  $^{45}\text{Ca}$  efflux from tissues, which have been loaded for 3 h in a K-free solution, washed for the first 60 min in a K-free solution, and for the later part of the efflux, in a solution containing 5.9 mM K. The pronounced hyperpolarization occurring during the K reaccumulation does not affect significantly the rate of  $^{45}\text{Ca}$  efflux. The ratio of the rate constant measured just after the readmission of K to the rate constant measured before readmission amounts to  $0.99 \pm 0.03$  ( $n = 12$ ). The same experimental results have been obtained both in Ca-containing and in Ca-free solutions.

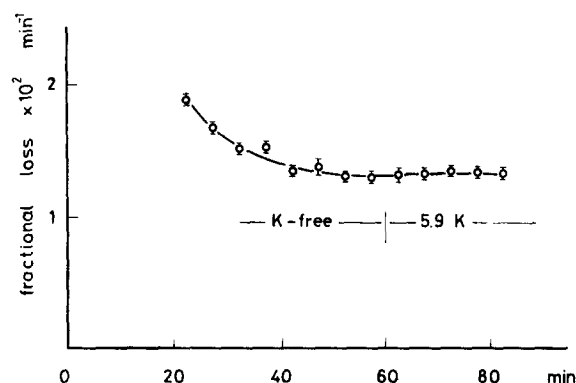


FIGURE 4. Effect of K readmission to K-depleted tissues on the  $^{45}\text{Ca}$  efflux from rabbit ear artery. The efflux has been performed in Ca-free solutions. Ordinate: fractional loss in minutes $^{-1}$ . Abscissa: time in minutes. The vertical bars represent SEM. Mean of 12 tissues.

#### *Effect of $[\text{Na}]_o$ and of the Na Gradient on the Cellular Ca Content and on the Ca Influx*

The Ca which remains in the tissue after washing away the external Ca by e.g., a La-containing solution, is often called the cellular Ca. This Ca fraction is heterogeneous and its value depends on the feasibility of eliminating most of the external Ca without affecting the cellular Ca. In estimating this cellular Ca content by washing the tissues in a La-containing solution, it is assumed that the slowing down of the transmembrane Ca flux will make it possible to separate the extracellular Ca from the bulk of the Ca located inside the cell. Extrapolating this slow phase back to zero time would give an acceptable estimate of this calcium fraction. However, the slow phase of the  $^{45}\text{Ca}$  efflux from rabbit ear artery proceeds exponentially in normal physiological solution, and the amplitude of this fraction can be used as an estimate of the cellular Ca content, as described in a previous paper (Droogmans et al., 1977).

The similarity of the effects of Na-free solution and K-rich solution on the  $^{45}\text{Ca}$  efflux of the ear artery suggests that an increased influx of Ca rather than a decreased efflux might cause the observed changes of the tension development and Ca flux. We have therefore tried to investigate the effect of Na-free

solutions and of K depletion on the so-called cellular Ca content and on the rate of  $^{45}\text{Ca}$  influx.

In order to measure the cellular Ca content, the tissues have been loaded for 3 h in  $^{45}\text{Ca}$  solutions: the control tissues in a normal solution and the K-depleted tissues in K-free solutions. The Na-free tissues have first been loaded for 2 h 30 min in the control solution and then for an additional 30 min in a Na-free solution of the same specific activity. The efflux was performed in a Ca-containing solution of the same composition as the loading solution. The fitted parameters of the slow component of the  $^{45}\text{Ca}$  efflux under the different experimental conditions are summarized in Table I. It can be observed that the total  $^{45}\text{Ca}$  content is not significantly affected by the different experimental conditions. Moreover, the exchange rate of this fraction was not affected either.

TABLE I  
EFFECT OF Na-FREE SOLUTIONS AND K DEPLETION ON  
THE SLOW PHASE OF THE  $^{45}\text{Ca}$  EFFLUX

Experimental conditions	Amplitude $\mu\text{mol/kg wet wt}$	Rate constant $\times 10^3 \text{ min}^{-1}$
n-Hepes (n=13)	149±9	1.69±0.08
Na-free (n=11)	148±8	1.56±0.07
K-free (n=10)	134±10	1.58±0.12

TABLE II  
EFFECT OF Na-FREE SOLUTIONS AND K DEPLETION ON  
THE UPTAKE OF  $^{45}\text{Ca}$  OCCURRING FOR 15 MIN

Experimental conditions	n-Hepes	Na-free	K-free
$^{45}\text{Ca}$ uptake, $\mu\text{mol/kg wet wt}$ (n)	35±3 (11)	40±3 (5)	42±4 (6)

We have also tried to estimate the rate of  $^{45}\text{Ca}$  influx from the amount of  $^{45}\text{Ca}$  taken up in the slowly exchanging Ca fraction during a short loading period of 15 min in either normal Krebs' solutions, Na-free solutions, or K-free solutions. For the study of the effect of K-free solution on the Ca uptake, tissues have been pretreated for 3 h in a K-free medium before exposing them to an identical  $^{45}\text{Ca}$ -labeled solution. After this loading period the tissues were washed in the same solution as the loading one and the amplitude of the slowly exchanging fractions has been determined. The experimental results are summarized in Table II. The difference in loading procedure did not affect significantly the  $^{45}\text{Ca}$  uptake. These findings are not unexpected because it had been observed that even K depolarization does not appreciably increase  $^{45}\text{Ca}$  uptake (Droogmans et al., 1977).

*Effect of  $[\text{Na}]_o$  and of the Na Gradient on the Efflux of  $^{45}\text{Ca}$  Released from Intracellular Stores and on the Accompanying Contraction*

The release of Ca from intracellular stores induced by any substance and its subsequent extrusion through the plasma membrane leads to a transient



stimulation of the  $^{45}\text{Ca}$  efflux. This phenomenon has been described in several vascular smooth muscle cells (rabbit aorta: Deth and van Breemen [1974, 1977], Deth and Casteels [1977]; rabbit ear artery: Droogmans et al. [1977]), and can be induced by agents as noradrenaline, histamine, caffeine, DNP, and FCCP. This increase of  $^{45}\text{Ca}$  efflux occurs both in Ca-free and Ca-containing solutions, and also when La has been added to the washing solution (Droogmans et al., 1977). Because La blocks Ca influx, it is unlikely that the stimulation in Ca-containing solutions represents a Ca-Ca exchange phenomenon.

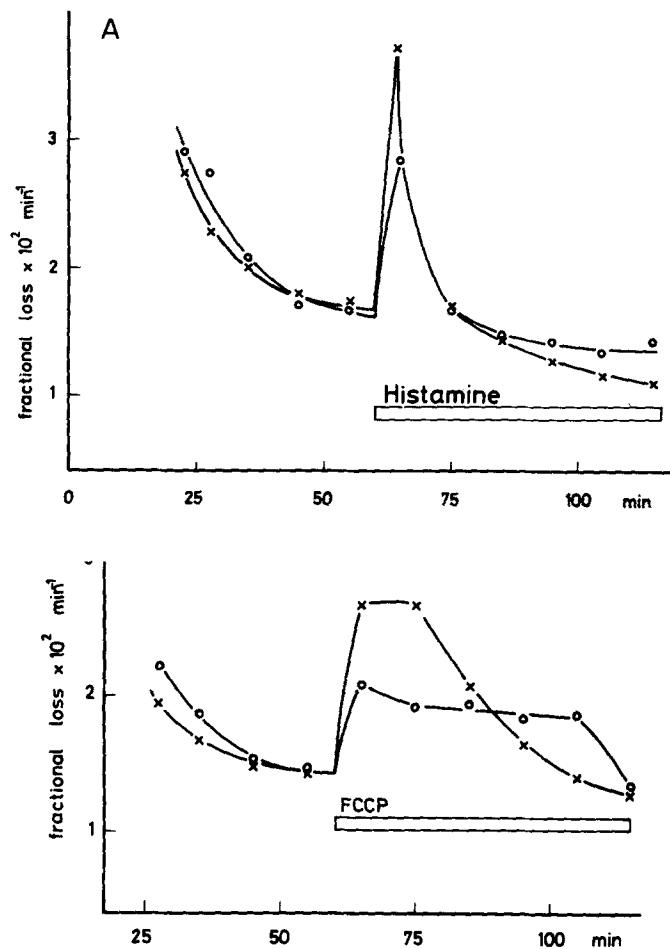


FIGURE 5. Effect of ( $\times$ ) K-free solutions on the stimulation of  $^{45}\text{Ca}$  efflux by (A)  $10^{-5}$  M histamine and (B)  $10^{-6}$  M FCCP. The open circles represent the stimulating effect under normal physiological conditions. The effluxes have been performed in Ca-free solutions. Ordinate: fractional loss in minutes $^{-1}$ . Abscissa: time in minutes.

In Fig. 5 a the augmenting effects of  $10^{-5}$  M histamine on the  $^{45}\text{Ca}$  efflux are compared between tissues exposed to a normal Krebs' solution and tissues which have been depleted of K. Fig., 5 b shows a similar experiment in which  $10^{-6}$  M FCCP, a very potent uncoupler of oxidative phosphorylation, is used. The

effluxes have been performed in Ca-free solutions containing 2 mM EGTA, but similar results have also been obtained in Ca-containing solutions, although here the Ca gradient is inwardly directed.

The experimental results are summarized in Table III. The stimulating effect is expressed by the ratio of the rate constant measured just after stimulation to the rate constant before stimulation. It can be observed that although there might exist some differences between the stimulating effects under the different experimental conditions, the extrusion of  $^{45}\text{Ca}$  after its release is not inhibited in tissues in which the Na gradient is reduced or eliminated by exposure to Na-free solutions, or to K-free solutions.

TABLE III  
EFFECT OF Na-FREE SOLUTIONS AND K DEPLETION ON  
THE STIMULATION OF THE  $^{45}\text{Ca}$  EFFLUX INDUCED BY  
HISTAMINE AND FCCP\*

Stimulating agent	Experimental conditions of tissue		
	Control	Na-free	K-free
Histamine ( $10^{-5}$ M)	$2.27 \pm 0.24$ ( $n=9$ )	$2.37 \pm 0.21$ ( $n=4$ )	$2.17 \pm 0.20$ ( $n=5$ )
FCCP ( $10^{-6}$ M)	$1.57 \pm 0.06$ ( $n=17$ )	$1.71 \pm 0.05$ ( $n=12$ )	$1.92 \pm 0.10$ ( $n=6$ )

\* The stimulating effect is expressed as the ratio of the rate constants after and before stimulation; the effluxes have been performed in a Ca-free solution containing 2 mM EGTA and  $10^{-5}$  M phentolamine.

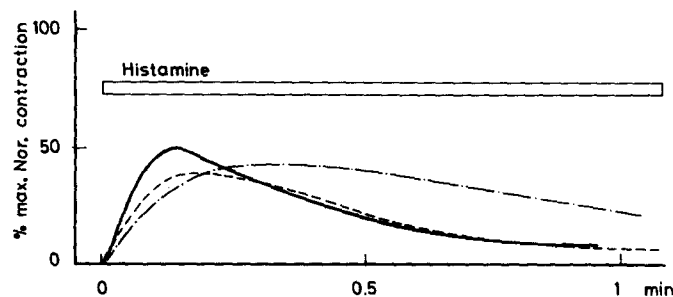


FIGURE 6. Effect of Na-free solutions on the contractions induced by Histamine ( $10^{-5}$  M) in Ca-free solutions, containing either Na (—), or TMA (---), or choline (-·-·-). Histamine has been applied after 3 min exposure to Ca-free solution.

The release of Ca from intracellular stores induces also a transient contraction in a Ca-free solution. Fig. 6 shows a record of such contraction obtained by addition of  $10^{-5}$  M histamine in control solution and in a Na-free solution prepared with choline or with TMA. It is obvious that Na-free solutions not only affect the rate of relaxation, but also the amplitude of the contraction and the rate of tension development. It has to be pointed out that the duration of the preincubation in Na-free solution does not affect this contractile response whereas the nature of the Na substitute does. A substitution of TMA for Na

only results in a decrease of the tension height, but the rate of rise and fall of this contraction are similar to the values observed under control conditions. These tension records also show that the relaxation for none of the experimental conditions follows an exponential time-course, indicating that the reduction of  $[Ca]_i$  does not only depend on transmembrane Ca extrusion.

#### DISCUSSION

The slow phase of the  $^{45}Ca$  efflux from the rabbit ear artery follows an exponential time-course (Droogmans et al., 1977) and therefore this Ca could originate from a single compartment corresponding to the intracellular fraction. This hypothesis is supported by the finding that it can be affected by histamine and FCCP, substances which are acting on different intracellular stores (Deth and Casteels, 1977). The similarity of the exchange rate from either of these stores suggests that a common barrier such as the plasma membrane may be the rate-limiting step for the exchange of the intracellular Ca with the extracellular space. This hypothesis is further supported by the finding that both types of substances only cause a transient stimulation of the  $^{45}Ca$  efflux, and that thereafter the efflux returns to its control value. A fundamental question concerning this calcium efflux is the role of the Na gradient in the extrusion of calcium against its electrochemical gradient.

The hypothesis of a Na-Ca exchange mechanism has been introduced for vascular smooth muscle cells by Reuter et al. (1973) to explain the effects of a modification of the Na gradient on the mechanical activity. Their findings seemed to indicate a coupling ratio of two Na ions for one Ca ion. However as suggested by Blaustein (1977 *a*) and as has been concluded for squid giant axon (Blaustein, 1977 *b*; Mullins, 1977) the coupling ratio should be 3:1 or 4:1 in order to maintain the Ca gradient which is measured under physiological conditions.

A Na-Ca exchange with a 3:1 ratio would be operating close to equilibrium and this would imply that the influx of Ca by passive leak is small compared to its influx through the carrier mechanism. Such an equilibrium which has been implicitly assumed by Blaustein (1976) predicts a tight coupling between  $[Ca]_i$  and the electrochemical gradient for Na. A prolonged exposure to Na-free solution would therefore also largely inhibit the Ca influx as soon as  $[Na]_i$  approaches a value of zero and cause only a limited increase of the cytoplasmic Ca. Our observation that the amplitude of the tension development in Na-free solution remains submaximal and does not increase with time is therefore consistent with this equilibrium model. However, this model does not fit the finding that the rate of  $^{45}Ca$  efflux is not significantly affected when  $[Na]_o$  is reduced to zero in the absence of external Ca. The effect of the increase in tension which occurs in tissues, which have been incubated for more than 3 h in Na-free solution, on increasing  $[Ca]_o$ , as well as the relaxation which occurs on reducing  $[Ca]_o$  under these conditions, cannot be deduced from this hypothesis. The effect of Na-free solution on the  $^{45}Ca$  efflux shows an obvious similarity with the effect of K-rich solution. Both experimental conditions only increase the  $^{45}Ca$  efflux if Ca is present in the washing solution. Moreover, the stimulation of the efflux as well as the accompanying contraction are blocked by La. These findings suggest that the increase of the  $^{45}Ca$  efflux might be due to

an increased Ca influx, and a subsequent stimulation of the Ca extrusion by the increase of  $[Ca]_i$  (van Breemen et al., 1975; Droogmans et al., 1977).

Our experimental methods do not allow us to find a significant increase of the  $^{45}Ca$  uptake during exposure to Na-free solution. Also, the total  $^{45}Ca$  content after a 3-h exposure to Na-free solution has not changed significantly. Similar negative results have also been obtained for K-depolarized tissues (Droogmans et al., 1977), where the concomitant contraction is more pronounced. At present we have no explanation for these rather peculiar observations.

The release of  $^{45}Ca$  from intracellular stores and its subsequent extrusion through the cell membrane do not depend on the presence of an inwardly directed Na gradient. It could be argued that the efflux in Ca-free solutions represents a passive leak of Ca ions down their electrochemical gradient. However, as described in a previous paper (Droogmans et al., 1977), the similarity of the exchange rate in both Ca-free and Ca-containing solutions

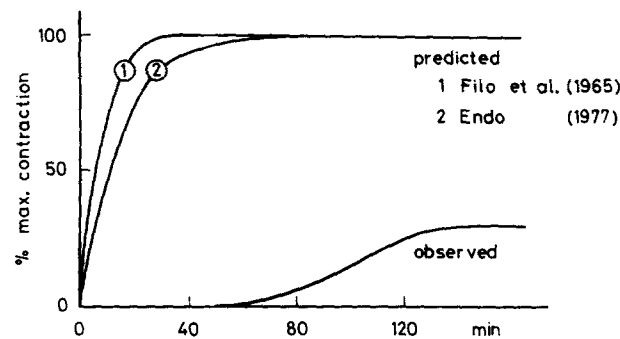


FIGURE 7. Comparison of the tension development observed in K-free solutions to that predicted by an equilibrium Na-Ca exchange model having a coupling ratio of 3:1.

suggests that the same mechanism is responsible for the efflux under both conditions. Also, the effect of Na-free solutions on the pattern of transient contractions induced by histamine in Ca-free medium does not support the Na-Ca exchange hypothesis. Na-free solutions exert a different effect according to the Na substitute, on the amplitude, the rate of contraction, and the rate of relaxation. This rate of relaxation, which is often considered as the relevant parameter reflecting Ca extrusion, can even be normal if TMA is the Na substitute. These findings therefore reflect the complexity of the excitation-contraction coupling in smooth muscle rather than a specific effect on the calcium extrusion mechanism.

K depletion of these vascular smooth muscle cells induces a large increase of  $[Na]_i$ . However, as shown in Fig. 7, the amplitude of the concomitant contraction does not correspond to the values predicted by the equilibrium Na-Ca exchange model given by the equation

$$\frac{[Ca]_o}{[Ca]_i} = \frac{[Na]_o^3}{[Na]_i^3} \exp(-EF/RT),$$

and by the relation between tension development of skinned fibers and pCa as obtained by Filo et al. (1965) and by Endo et al. (1977). It can also be observed that the tension during exposure to K-free solution starts only to increase when  $[Na]_i$  has reached a value of about 50 mM. It can therefore be excluded that  $[Na]_i$  and the Na-K pump would play an important role in regulating the tension development of the rabbit ear artery, as suggested by Blaustein (1977) from his experiments on the rabbit aorta.

The mechanism described in squid giant axon implies in accordance with the theoretical predictions for a 4:1 or 3:1 Na-Ca exchange a strong dependency of the  $^{45}Ca$  efflux on the membrane potential (Mullins and Brinley, 1975). However, in the rabbit ear artery the sudden hyperpolarization of the cells by readmitting K after partial K depletion is not accompanied by a significant modification of the rate of efflux. This negative finding suggests that K reaccumulation and the concomitant hyperpolarization do not modify the Ca efflux, but rather the Ca influx.

An increased rate of Ca influx on reducing the Na gradient can be explained if one assumes the existence of Ca-selective channels, which show also some permeability for Na ions. Because of the much higher concentrations of Na ions compared to Ca ions, both in the extra- and intracellular solutions, a substantial amount of Na ions might flow through these channels even if the permeability for Na is low compared to that for Ca ions. Reducing the number of Na ions or their driving force might therefore increase the number of Ca ions passing through the cell membrane. This might explain why the limited depolarization occurring in Na-free solutions causes a contraction whereas a similar depolarization in Na-containing solutions obtained by changing  $[K]_o$  does not. The higher contraction in TMA also fits this idea, as well as the relaxation which occurs when TMA is replaced by choline.

Evidence for the existence of Ca channels in rabbit ear artery has been presented in a previous paper (Droogmans et al., 1977): the action potentials occurring in TEA containing solutions are inhibited by Ca antagonists, such as D-600 and Mn. Also, the action potential parameters are strongly dependent on  $[Ca]_o$  and  $[Na]_o$ . It is therefore tempting to assume that Na ions can penetrate through the Ca channels, because it might also explain the role of Na ions in the activation of the vascular smooth muscle cells of the rabbit ear artery.

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