Electro- and Pharmacomechanical Coupling in the Smooth Muscle Cells of the Rabbit Ear Artery

G. DROOGMANS, L. RAEMYMAEKERS, and R. CASTEELS
From the Laboratorium voor Fysiologie, Universiteit Leuven, B-3000 Leuven, Belgium

A B S T R A C T A contraction of the rabbit ear artery can be induced by depolarizing the cells with a K-rich solution if Ca is present. $10^{-9}-10^{-8}$ M noradrenaline and $10^{-8}-10^{-7}$ M histamine cause a contraction of this tissue without modifying the membrane potential. If the histamine concentration exceeds $10^{-7}$ M some depolarization of the membrane also occurs. Both noradrenaline and histamine also induce a contraction in Ca-free medium, even if La is present. None of these stimuli produces action potentials or fluctuations of the membrane potential. Besides these tonic contractions, the ear artery can also produce phasic contractions when $10$ mM TEA is added to the medium. Such contractions are caused by the appearance of action potentials which are Ca dependent and which are similar to those appearing in visceral smooth muscle. A study of $^{45}$Ca fluxes has revealed that K depolarization and noradrenaline cause only a small increase in $^{45}$Ca uptake by the cells, while noradrenaline also releases cellular Ca, even in Ca-free medium. A comparison of tension development and $^{45}$Ca release induced by noradrenaline in Ca-free medium suggests that Ca extrusion could be very efficient in the rabbit ear artery and that it could play a direct role in its relaxation.

I N T R O D U C T I O N

The contraction of vascular smooth muscle can be induced by electrical or nonelectrical stimulation. Su et al. (1964) first observed that noradrenaline induces a contraction of the pulmonary artery without depolarizing the cells. Similar findings have been reported by Mekata and Niu (1972) for the common carotid artery of the rabbit and by Mekata (1974) for the aorta. In visceral smooth muscle and in some vascular smooth muscle, such as the portal vein (Ito and Kuriyama, 1971), contractile activity is normally induced by regenerative action potentials. A contractile response in all these tissues can also be evoked by K-rich solutions.

In the present work we have investigated the membrane potential of the smooth muscle cells of the rabbit ear artery and its modification by noradrenaline, histamine, and K-rich solution. Exposure of these cells to solutions containing TEA was found to induce spike-like fluctuations of the membrane potential. We have further investigated the nature of these action potentials and the sources of activator calcium during different stimulating procedures. Preliminary communications on some of these data have been presented (Droogmans and Casteels, 1976; Casteels and Droogmans, 1977).
MATERIALS AND METHODS

Rabbits (2–3 kg body wt) were stunned and bled. The ear artery was dissected and cleaned of its periarterial connective tissue under a dissection microscope while the artery was kept in warmed and oxygenated Krebs solution.

For electrophysiological experiments, segments (2–3 cm long) of the artery were partially mounted on a glass tube of 0.8 mm OD. The free artery segment was internally perfused with physiological solution at a constant driving pressure of about 80 mm Hg. The solution leaving the free artery segment also flowed around the artery through an organ bath. The contraction of the smooth muscle cells was measured from the change in the pressure drop across a glass tube connected in series with the artery. The membrane potential of the smooth muscle cells was measured in the artery segment fixed on the glass tube with microelectrodes filled with 3 M KCI and having a resistance of 60–100 MΩ (Hendrickx and Casteels, 1974).

Constant current pulses lasting from 100 ms to 1 s could be applied through external platinum electrodes mounted longitudinally along the preparation. Changes in membrane voltage were recorded differentially between the intracellular electrode and a second extracellular microelectrode mounted close to the intracellular one in order to reduce the stimulus artefact.

For contraction experiments and flux studies helical strips of the ear artery were cut. These strips were mounted for contraction studies in an organ bath of 10 ml and tension was recorded by means of an isometric force transducer. For efflux studies the tissues were mounted on Teflon rods and allowed to equilibrate for 1 h at 37°C in control physiological solutions. 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 effluent samples and tissues were counted and from these data the rate of efflux, the tissue tracer content, and the rate coefficient have been calculated.

The standard physiological solution was a HEPES-buffered modified Krebs solution containing: 135.5 mM Na; 5.9 mM K; 1.5 mM Ca; 1.2 mM Mg; 143.8 mM Cl; 11.6 mM HEPES; 11.5 mM glucose. The solution was bubbled with O₂ at pH 7.3 and the temperature was kept at 35°C. Solutions with an increased K concentration were obtained by replacing NaCl with KCl, keeping the sum [Na]₀ + [K]₀ constant (Casteels and Kuriyama, 1966). K-rich solutions were obtained by replacing all the NaCl with KCl, giving a K concentration of 138 mM. In order to eliminate possible effects of noradrenaline released by the adrenergic nerve fibres during K depolarization, 10⁻⁵ M phentolamine was added to the solutions. Na-deficient solutions were prepared by replacing NaCl with choline-Cl; the final Na concentration in the solution was about 3 mM. Atropine (100 μg/liter) was added to this Na-deficient solution in order to prevent the cholinomimetic effect of choline.

When tetraethylammonium-Cl (10–15 mM) was used, the NaCl concentration was reduced in order to maintain isotonicity. Solutions of noradrenaline and histamine (10⁻³ M and 10⁻⁵ M) were made up in isotonic NaCl containing 0.01 mM EDTA and added to the solutions as required.

The La washing solution was prepared by substituting 10 mM LaCl₃ for 40 mM NaCl in a nominal Ca-free Krebs solution. Metabolic inhibition was obtained by adding 10⁻⁵ M iodoacetic acid (IAA) and 10⁻⁴ M dinitrophenol (DNP) to the La washing solution.

The numerical values are expressed as mean ± SEM. n represents the number of observations.

The time course of the ⁴⁰Ca content of the tissues during efflux experiments has been fitted by a sum of three exponentials using an iterative nonlinear least-squares fitting
routine. For fixed values of rate constants it is possible to calculate the corresponding amplitudes of the exponentials by a linear least-squares method. This specific feature of our model makes it possible to limit the nonlinear iteration to the rate constants, which results in an improved convergence of the optimization algorithm (Chambers, 1973). Moreover, the improved convergence compensates largely for the calculations required in order to solve the least-squares problem for each set of intermediary values of rate constants.

RESULTS

Membrane Potential and Mechanical Activity as a Function of [K]0

The resting membrane potential of smooth muscle cells of the ear artery measured with intracellular microelectrodes varies in normal Krebs solution between -58 and -65 mV, with a mean value of -63.3 ± 0.3 mV (mean ± SEM, n = 39).

The values of the membrane potential given in this and in the next section have been obtained on at least five different preparations and represent mean values of 20-40 penetrations lasting at least 1 min. The standard deviation is of the order of 1-2 mV.

Increasing the extracellular K concentration [K]0 results in a graded depolarization of the cell membrane and in a progressive increase of the vascular tone (Fig. 1). The magnitude of the tension is related to the degree of depolarization. No action potentials are observed at any level of K depolarization and it is also impossible to elicit action potentials by applying depolarizing current pulses.

The relation between the membrane potential (Em) and log [K]0 is nonlinear, as has been observed in other smooth muscle cells. The slope of this curve, i.e. dEm/dlog [K]0, increases from 40 mV per 10-fold change of [K]0 at low [K]0 to 53 mV at higher [K]0.

The threshold value of the membrane potential for inducing contraction amounts to -45 to -50 mV. This finding suggests that if a change in membrane potential is an essential link in the excitation contraction coupling for these cells, vasoactive substances should exert a pronounced and dose-dependent effect on this membrane potential.

Effect of Noradrenaline and Histamine on Membrane Potential and on Mechanical Activity

We have studied the effect of noradrenaline in a concentration range from 10^-9 M to 10^-6 M on the membrane potential and on the mechanical activity of the ear artery (Fig. 2 A). The contraction caused by noradrenaline consists of a phasic and a tonic component, as described by Bevan and Waterson (1971) and by Steinsland et al. (1973), but this mechanical response is not accompanied by a transient or sustained change of the membrane potential, even at high concentrations of noradrenaline.

The actions of different concentrations of histamine (between 10^-8 and 10^-5 M) on both the electrical and mechanical properties of the ear artery are represented in Fig. 2 B. Low concentrations (10^-8-5 × 10^-8 M) of histamine cause a contraction without affecting the membrane potential. However, at higher concentrations, exceeding 10^-7 M, histamine causes a dose-dependent depolari-
zation which is accompanied by a contraction. From Fig. 2 B it is obvious that the threshold concentrations of histamine causing contraction or depolarization are significantly different.

**Effect of Noradrenaline on the Ionic Permeabilities of the Cell Membrane**

It has been suggested that the depolarization of vascular smooth muscle cells and the concomitant reduction of membrane resistance during exposure to a solution containing noradrenaline is due to an increase of the Na and Cl conductance of the cell membrane (Mekata and Niu, 1972). Although noradrenaline does not affect the membrane potential in the ear artery of the rabbit it seemed worthwhile to investigate this hypothesis by analyzing the effect of noradrenaline on the passive fluxes of K, Cl, and Na.

The effect of $10^{-6}$ M noradrenaline on the rate coefficient of the $^{42}$K efflux is represented in Fig. 3 A. This change consists of a large initial increase followed by a sustained smaller increase ($0.0108 \pm 0.0004$ min$^{-1}$; $n = 8$), which is about twice the value of the rate constant under control conditions ($0.0050 \pm 0.0002$ min$^{-1}$; $n = 8$). This biphasic effect of noradrenaline resembles only superficially the biphasic tension response because the changes of the efflux proceed at a much slower rate than those of the tension response. The effect of $10^{-6}$ M noradrenaline on the $^{36}$Cl efflux is represented in Fig. 3 B. It causes a sustained increase of the rate constant from $0.054 \pm 0.005$ min$^{-1}$ ($n = 5$) under control conditions to $0.105 \pm 0.06$ min$^{-1}$ ($n = 5$) in the presence of $10^{-6}$ M noradrenaline.
The study of the effect of noradrenaline on Na permeability is more difficult. We should actually investigate the influence on the Na influx, because the Na efflux is largely due to active Na extrusion by the Na-K pump (Casteels, 1969). We have therefore exposed the tissues for 4 h to a K-free solution in order to increase [Na] and performed the efflux in a K-free solution containing 2 x 10^{-5} M ouabain in order to inhibit the remaining active Na efflux and to obtain a Na efflux which is largely passive. This effect of 10^{-6} M noradrenaline is represented in Fig. 3C. It is found that under these conditions noradrenaline increases the rate coefficient of Na efflux from 0.058 ± 0.005 min^{-1} to 0.100 ± 0.008 min^{-1} (n = 6), without causing a change in the membrane potential.
Figure 3. Typical experiment showing the effect of $10^{-6}$ M noradrenaline on the rate constant of the efflux of $^{42}$K (A), $^{36}$Cl (B), and $^{22}$Na (C) from the rabbit ear artery. The loading period in the radioactive solutions for the $^{42}$K experiments was 3 h and for the $^{36}$Cl experiments 1 h. For the study of $^{22}$Na efflux, K-depleted cells were loaded for 1 h. Duration of exposure to noradrenaline is represented by the bar. The initial high rate constant is due to the efflux of radioactivity from the extracellular compartment.
Action Potentials and Contractions Induced by Tetraethylammonium (TEA)

Tetraethylammonium is a substance which is known to induce in some quiescent smooth muscle cells action potentials accompanied by contractions. At a concentration of 10–15 mM it depolarizes the smooth muscle cells of the rabbit ear artery to -40 to -30 mV. This depolarization is accompanied by a tonic contraction. Higher concentrations of TEA do not cause a larger depolarization. If the membrane depolarizes to about -35 mV, the threshold for spontaneous action potentials is reached and spikes, accompanied by phasic contractions, appear (Fig. 4). If the membrane potential remains more negative than -35 mV no spontaneous action potentials occur. However, they can be induced either by injecting current pulses or by a further depolarization of the membrane by increasing [K]o. In the presence of TEA, noradrenaline depolarizes the smooth muscle cells of the ear artery in a dose-dependent way. At a concentration of 5 × 10^{-8} M, noradrenaline depolarizes the cells by 4 ± 0.5 mV (n = 6) while 10^{-7} M noradrenaline depolarizes by 15 ± 2 mV (n = 10) (Fig. 5).

The action of TEA is probably related to its effect on the K permeability of the membrane (Armstrong, 1966). We have therefore investigated the effect of 10 mM TEA on the rate of ⁴²K efflux. In normal Krebs solution TEA causes an
immediate increase in the rate of $^{42}$K efflux because of the concomitant depolarization of the cells and possibly also because of the occurrence of spikes. In order to eliminate these complicating factors we have also studied the effect of TEA in K-rich solution (138 mM) obtained by replacing NaCl by KCl. Under these conditions TEA no longer affects the membrane potential, and we now find a pronounced decrease of the rate of $^{42}$K efflux (Fig. 6).

The nature of the inward current responsible for the upstroke of TEA-induced spikes has been investigated by analyzing the effects of $[\text{Na}]_0$ and $[\text{Ca}]_0$ on the parameters of the action potential. Increasing $[\text{Ca}]_0$ from 1.5 to 4.5 mM augments the amplitude of the action potentials from 43 ± 0.5 mV (20) to 51 ± 0.5 mV (20) and their maximum rate of rise and fall from 0.96 ± 0.02 V/s (20) and 0.24 ± 0.01 V/s (20) to 1.74 ± 0.02 V/s (20) and 0.72 ± 0.02 V/s (20), respectively (Fig. 7). These modifications result in a shortening of the duration of the action potential. The resting potential of TEA-treated tissues is not significantly affected by increasing $[\text{Ca}]_0$ from 1.5 to 4.5 mM. At a $[\text{Ca}]_0$ lower than 0.5 mM no action potentials have been observed. A reduction of $[\text{Na}]_0$ to 3 mM during treatment with TEA, using choline chloride as a substitute for NaCl, transiently hyperpolarizes the cells and inhibits spontaneous electrical activity. After about 1 min the membrane potential returns to its initial value and spontaneous activity reappears. Action potentials can also be evoked by depolarizing current pulses. The amplitude of both spontaneous and evoked action potentials and their maximum rates of rise and fall are, however, reduced. Substances which are considered as Ca antagonists, such as D600 (0.25 mg/l) and Mn$^{2+}$ (1 mM), block the spontaneous action potentials of TEA-treated cells and the accompanying contractions. Because these action potentials present an afterhyperpolarization it is difficult to measure accurately the resting potential during spontaneous electrical activity. There is no evidence that either D600 or Mn$^{2+}$ affects the resting potential (Fig. 8).

**Figure 5.** Effect of $10^{-7}$ M noradrenaline on the membrane potential and spike discharge of the ear artery during exposure to a Krebs solution containing 10 mM TEA.

**Effect of External Ca on the Tension Development of the Ear Artery**

In the present investigations we have prevented the action of external Ca on
Figure 6. Effect of 10 mM TEA on 42K efflux from smooth muscle cells of rabbit ear artery during exposure to K-rich solution. The amount of 42K leaving the tissue per minute is plotted on a logarithmic scale against time in minutes. The open circles (○) represent mean values obtained from eight experiments.

Figure 7. Effect of [Ca]₀ on spontaneous action potentials in a solution containing 10 mM TEA and 1.5 mM Ca (A) or 4.5 mM Ca (B). The upper record shows the membrane potential, and the lower, its time derivative. In A the external [Ca] amounts to 1.5 and in B to 4.5 mM.

tension development by using either Ca-free solutions containing 2 mM EGTA or solutions to which La has been added. It is assumed that this latter ion limits to a large extent the passive permeation of Ca across the cell membrane.

The contractions induced by K-rich solution, noradrenaline, or histamine are relaxed by adding 1 mM La to the stimulating solution. However, if the tissues
have been pretreated with La, a K-rich solution will not elicit a contraction, while noradrenaline and histamine will induce a tension development lasting about 1 min. This contraction cannot be induced for a second time in an La-containing solution even if external Ca is present. Pretreatment of the cells in a Ca-free solution containing EGTA exerts an effect similar to that caused by La: noradrenaline and histamine still cause a transient contraction, but K-rich solutions no longer exert any contractile effect. The amplitude of the contraction induced by noradrenaline or by histamine is dose dependent and decreases as a function of the preceding incubation in Ca-free medium. The tension development induced by a single application of $10^{-6}$ M noradrenaline at various times after exposure to a Ca-free medium is represented in Fig. 9. Initially, the tension decreases very rapidly but later on it proceeds exponentially with a half-time of about 35 min.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig8.png}
\caption{Effect of D600 (0.25 mg/liter) on spontaneous activity induced by TEA in rabbit ear artery.}
\end{figure}

\textit{45Ca Fluxes and Their Modification by Noradrenaline and K-Rich Solutions}

It seemed worthwhile to correlate the dependence of the contraction on external Ca or cellular Ca with the \textit{45Ca} flux data. We have therefore investigated the effect of noradrenaline and K-rich solution on \textit{45Ca} uptake and efflux under different experimental conditions. The effect of La and of metabolic inhibition on \textit{45Ca} efflux has also been investigated.

Fig. 10 shows \textit{45Ca} efflux, in a solution containing 1.5 mM Ca, from tissues which have been loaded for 2 h in radioactive solution. This efflux curve is not significantly affected by increasing the loading period to 4 h. This finding suggests that the largest part of the tissue Ca has exchanged during a 2-h exposure to the radioactive solution. We can therefore estimate the exchangeable Ca content from the amount of \textit{45Ca} present in the tissue. The late phase of the decrease of \textit{45Ca} left in the tissue ($\mu$mol \textit{45Ca}·kg$^{-1}$) and of the rate of efflux ($\mu$mol \textit{45Ca}·kg$^{-1}$·min$^{-1}$) both proceed as a single exponential with the same time constant, suggesting that this fraction behaves as a single compartment. However, it is not possible to describe the complete time course of either curve by a two-compartment system and at least three exponentials are required to obtain a good fit of the experimental points. Increasing the number of exponentials to
FIGURE 9. Contraction of the rabbit ear artery induced by $10^{-6}$ M noradrenaline, expressed as percentage of the contraction induced by $10^{-4}$ M noradrenaline in a Ca-containing solution, after different times of exposure to a Ca-free solution containing 2 mM EGTA. The amplitude of this contraction is plotted on a logarithmic scale as a function of the time of exposure to the Ca-free solution. The experimental values have been determined after an equilibration period of 45 min in Ca-containing solution and after exposing the tissues to Ca-free solution as indicated on the abscissa. The data are represented as mean ± SEM. The number of determinations is seven for each point.

FIGURE 10. Rate of efflux (×) and $^{44}$Ca content (○) of rabbit ear artery as a function of time. The experimental points are mean values obtained on 10 tissues. The vertical bars represent standard errors of the mean. The left ordinate represents on a logarithmic scale the exchangeable Ca content of the tissues (μmol·kg$^{-1}$ wet wt), the right ordinate the rate of efflux (μmol·kg$^{-1}$ wet wt·min$^{-1}$). On the abscissa time is given in minutes on a linear scale.
four does not significantly improve the fit and we have therefore preferred the simple mathematical model with three exponentials to describe our data. We assume that the initial very rapid loss of $^{45}$Ca consists largely of radioactivity originating from the extracellular fluid and we have therefore not studied this fraction any further. The calculated amplitudes and rate constants of the two remaining fractions, which will be referred to as the fast and the slow component, are given in Table I A. Because of the finite loading time of 2 h the specific activity of Ca in the tissue compartment should theoretically not have reached the value in the loading solution and the amplitudes of the slow compartment given in Table I A may therefore be slightly underestimated. We have also not taken into account that a possible catenary arrangement of the compartments

**TABLE I**

FITTED PARAMETERS OF THE FAST AND SLOW COMPONENT OF THE $^{45}$Ca-EFFLUX FROM RABBIT EAR ARTERY

<table>
<thead>
<tr>
<th>Solution</th>
<th>Fast component</th>
<th>Slow component</th>
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<tbody>
<tr>
<td></td>
<td>Rate constant</td>
<td>Amplitude</td>
</tr>
<tr>
<td></td>
<td>min$^{-1}$</td>
<td>$\mu$mol/kg wet wt</td>
</tr>
<tr>
<td>(A) Efflux in test solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ($n=20$)</td>
<td>0.087$\pm$0.004</td>
<td>195$\pm$11</td>
</tr>
<tr>
<td>Id. + $10^{-6}$ M noradrenaline ($n=6$)</td>
<td>0.091$\pm$0.006</td>
<td>206$\pm$17</td>
</tr>
<tr>
<td>Isotonic K Krebs ($n=6$)</td>
<td>0.092$\pm$0.007</td>
<td>203$\pm$15</td>
</tr>
<tr>
<td>(B) Efflux in Ca-free, 10 mM La-containing Krebs with $10^{-3}$ M IAA, $10^{-4}$ M DNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ($n=10$)</td>
<td>0.085$\pm$0.006</td>
<td>160$\pm$10</td>
</tr>
<tr>
<td>Id. + $10^{-4}$ M noradrenaline ($n=4$)</td>
<td>0.072$\pm$0.008</td>
<td>179$\pm$9</td>
</tr>
<tr>
<td>Isotonic K Krebs ($n=4$)</td>
<td>0.081$\pm$0.006</td>
<td>173$\pm$11</td>
</tr>
</tbody>
</table>

The tissues were loaded for 1 h 45 min in normal physiological solution, followed by an additional loading for 15 min in either Krebs solution containing $10^{-6}$ M noradrenaline or K-rich Krebs solution. The control consisted of a 2-h exposure to normal Krebs solution.

(Casteels and Droogmans, 1976) would require the introduction of corrections. Because this mathematical analysis has been used mainly in order to facilitate a comparison of the different efflux curves, we have not introduced corrections for the above-mentioned systematic errors.

In order to find out whether noradrenaline or K depolarization increases the cellular Ca content, we have compared the efflux curves after 2 h of exposure to normal Krebs solution and the efflux after 1 h 45 min of loading in the same solution and another 15 min of exposure either to a solution containing $10^{-6}$ M noradrenaline or to a K-rich solution. All these loading media have the same specific activity and Ca concentration (1.5 mM). By exposing the tissues to the control radioactive solution for 1 h 45 min most of the exchangeable tissue Ca has been labeled to the same extent as the loading solution and a further increase in the $^{45}$Ca content by the 15-min exposure to noradrenaline or to K-rich solution would indicate that these experimental conditions have induced a net uptake of Ca. The numerical data are given in Table I A. It is obvious that there is no
significant difference between these three effluxes, suggesting that there is no net increase in the cellular Ca by either stimulus. This finding is at variance with the observations in other smooth muscle cells such as the rabbit aorta (van Breemen et al., 1972) and the guinea pig taenia coli (Mayer et al., 1972). A possible explanation is that noradrenaline or K-rich solution only increases the rate of Ca influx without producing a measurable change in the cellular Ca content. Such a mechanism would require a calcium extrusion pump in order to maintain the steady state of cell Ca. This hypothesis has been tested by studying 45Ca uptake during a loading period of 15 min, which is the minimum time of exposure for reliable measurements. This period corresponds to about twice the half-time of exchange of the fast compartment. Such a shortened loading procedure can therefore only be used to measure changes of the uptake rate in the slow component. The tissues have again been exposed either to the control solution, or to a solution containing noradrenaline or to a K-rich solution. The amount of 45Ca taken up in the slow compartment during this loading period increases from a control value of 54 ± 3 (n = 20) μmol/kg wet wt to 65 ± 4 (n = 9) in the presence of noradrenaline and to 61 ± 4 (n = 9) μmol/kg in high-K solution (Table II). These values are lower than the real values because the backflux of labeled Ca has not been taken into account, but they nevertheless suggest that both stimuli increase the rate of influx of Ca into the slow compartment.

This rather limited effect of K depolarization on 45Ca uptake could also be due either to a rapid extrusion of 45Ca on re-exposure of the cells to normal solution during the efflux or to an increased exchangeability of cellular Ca, as has been observed by Deth and Casteels (1977) in the rabbit aorta after exposure to K-rich solution.

In order to reduce possible interference of these mechanisms in the estimation of cellular 45Ca content we have tried to reduce transmembrane Ca movement by using a solution containing 10 mM La and 10⁻⁴ M DNP and 10⁻³ M iodoacetic acid. The use of this solution is justified by the well-known action of La on transmembrane movement of Ca in smooth muscle (van Breemen et al., 1972) and by the observation that the metabolic inhibition of smooth muscle cells by DNP and iodoacetic acid causes a still larger reduction of the rate constant of the efflux than the use of a solution containing La only. This effect of metabolic inhibition is most probably related to the rapid decrease of ATP content occurring under these conditions (Casteels et al., 1972).

Performing 45Ca effluxes in an La solution added with metabolic inhibitors might give a more accurate estimate of the amplitude of the slow component by reducing its exchange rate. However, the data represented in Table I show that this efflux procedure does not affect the amplitude of the slow component and that the only difference between the efflux in normal Krebs solution and that in the La-containing solution consists in a reduction of the rate constant of the slow component by a factor of 2.6.

We have also applied this La efflux procedure to the tissues loaded over a period of 15 min. These results, which are qualitatively similar to those obtained by performing the efflux in normal Krebs solution, are given in Table II B.
Effect of Noradrenaline and K-Rich Solution on $^{45}$Ca Efflux

The addition of noradrenaline to the washing solution during the efflux procedure results in a transient increase in the rate of $^{45}$Ca efflux (Fig. 11 A). The amplitude of this increase becomes smaller for applications of noradrenaline occurring later during the efflux procedure and is also dose dependent. In a Ca-free medium containing 2 mM EGTA (Fig. 11 B) and in a Ca-containing solution added with 10 mM La (Fig. 11 C), the addition of $10^{-6}$ M noradrenaline causes a similar release of $^{45}$Ca. This release accompanies a transient tension development lasting about 1 min. It can be assumed that this stimulation of $^{45}$Ca efflux by noradrenaline is initiated by a release of Ca from an intracellular store.

We have tried to estimate the size and the exchange properties of this Ca store. The efflux was performed in either normal Krebs solution or in an La-containing solution with added DNP and IAA.

### Table II

<table>
<thead>
<tr>
<th>Solution</th>
<th>Fast component</th>
<th>Slow component</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Efflux in Krebs solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ($n = 20$)</td>
<td>178±12</td>
<td>54±3</td>
</tr>
<tr>
<td>Id. + $10^{-6}$ M noradrenaline ($n = 6$)</td>
<td>210±15</td>
<td>65±4</td>
</tr>
<tr>
<td>Isotonic K Krebs ($n = 6$)</td>
<td>177±21</td>
<td>61±4</td>
</tr>
</tbody>
</table>

| (B) Efflux in La-containing-solution + metabolic inhibition |                |                |
| Control ($n = 5$)                                      | 139±24         | 59±3           |
| Id. + noradrenaline $10^{-6}$ M ($n = 5$)               | 165±26         | 90±7           |
| Isotonic K Krebs ($n = 5$)                             | 164±18         | 94±5           |

*Control, $10^{-6}$ M noradrenaline or K-rich solution.

Figure 11. Effect of noradrenaline ($10^{-6}$ M) on the rate of $^{45}$Ca efflux ($\mu$mol·kg$^{-1}$·min$^{-1}$) from rabbit ear artery after a loading period of 2 h, in the presence of 1.5 mM extracellular Ca (A), in Ca-free solution containing 2 mM EGTA (B), and in a 1.5 mM Ca solution containing 10 mM La (C).
compartment affected by noradrenaline from the $^{45}\text{Ca}$ efflux into Ca-free solution in the presence and the absence of noradrenaline. The tissues were exposed for 2 h to the radioactive solution and we can therefore assume that the specific activity of the exchangeable Ca in the tissue reached the same value as in the loading solution. We can therefore calculate from these effluxes in Ca-free solution the net changes of the Ca content. If noradrenaline ($10^{-6}$ M) is added at the start of the efflux in Ca-free medium, Ca is released from the noradrenaline-sensitive compartment and causes a transient contraction. The later part of the efflux is no longer affected by noradrenaline and we may assume that this rest efflux represents the loss of Ca from all other tissue compartments. We can now estimate the amplitude and rate of depletion in Ca-free medium of the noradrenaline-sensitive compartment by subtracting point by point the $^{45}\text{Ca}$ efflux in the presence of noradrenaline from the efflux in a control Ca-free solution (Fig. 12). This curve should represent the amount of $^{45}\text{Ca}$ which remains available in the noradrenaline-sensitive compartment during prolonged exposure to a Ca-free medium.

This hypothesis is confirmed by the finding that the amount of $^{45}\text{Ca}$ which is released by $10^{-6}$ M noradrenaline after 40 and 70 min of efflux in Ca-free solution fits almost perfectly the curve represented in Fig. 12. These values have been estimated from the sudden decrease by noradrenaline of the amount of $^{45}\text{Ca}$ present in the tissue (Fig. 12 inset). After 40 min of exposure to Ca-free solution $10^{-6}$ M noradrenaline induces a Ca release of $30 \pm 3 \mu\text{mol/kg wet wt}$ ($n = 9$) and, after 70 min, of $17 \pm 2 \mu\text{mol/kg wet wt}$ ($n = 8$).

Because tension development is determined by the intracellular $[\text{Ca}^{2+}]$ we should also find that the amplitude of the tension development induced by noradrenaline at various times of exposure to Ca-free solution decreases at the same rate. This is confirmed in Fig. 9. Both the noradrenaline-sensitive Ca fraction, which has an amplitude of about $60 \mu\text{mol/kg wet wt}$, and tension development in Ca-free solution decrease exponentially with a rate constant of about $0.020 \text{ min}^{-1}$.

The induction of contraction by K-rich solution was found to depend on the presence of Ca in the bathing medium. If we depolarize, by a K-rich solution containing Ca, strips of the ear artery which have been loaded with $^{45}\text{Ca}$, we observe that the $^{45}\text{Ca}$ efflux increases transiently. However, this effect almost disappears in a Ca-free solution (Fig. 13).

**Discussion**

The membrane potential of the smooth muscle cells of the rabbit ear artery is more negative than the values observed in most other smooth muscle cells and it remains stable, as has already been described by Speden (1967). These two properties could be related to the relatively high K permeability of these smooth muscle cells. This is suggested by the observation that the slope of the curve relating the membrane potential to the logarithm of $[\text{K}]_o$ is steeper in the ear artery than in visceral smooth muscle cells (Casteels and Kuriyama, 1966). A high K permeability may also be responsible for the impossibility of inducing spikes by depolarizing current pulses.
Our experiments indicate that the contraction of the ear artery of the rabbit induced by noradrenaline (5 × 10^{-9}-10^{-6} M) and by low concentration of histamine occurs without any change in the membrane potential. This effect of noradrenaline in the ear artery can be considered as the perfect example of pharmacomechanical coupling (Somlyo and Somlyo, 1968).

![Figure 12](image1)

**Figure 12.** Time course of the amount of {superscript}45Ca which can be released by noradrenaline (×) after different times of exposure to a Ca-free medium containing 2 mM EGTA. The tissues were loaded in radioactive solution for 2 h and the amount of {superscript}45Ca (μmol/kg wet wt) is plotted on a logarithmic scale as a function of time (min) in Ca-free medium. The open circles correspond to the amount of {superscript}45Ca released by noradrenaline when added after 40 and 70 min of efflux in a Ca-free solution. This amount has been estimated, as shown in the inset, from the decrease of the tissue tracer content (△) which occurs on exposure to noradrenaline.

![Figure 13](image2)

**Figure 13.** Effect of K-rich solutions on the rate of efflux of {superscript}45Ca from rabbit ear artery in the presence (A) and in the absence (B) of extracellular Ca.

High concentrations of histamine (exceeding 10^{-7} M) cause a dose-dependent depolarization and contraction of the cells. However, the threshold concentrations of the drug causing contraction and depolarization differ by a factor of 10. This depolarization is probably a secondary action of histamine because the
contraction caused by histamine reaches almost its maximal value when the resting potential has increased to \(-45\) mV, which is the threshold value for inducing contraction by K depolarization.

Our study of the action of noradrenaline on fluxes of univalent ions reveals that this substance increases membrane permeability for all these ions. High concentrations of noradrenaline induce a biphasic increase of the K efflux, consisting of a very large initial increase and a later, smaller but maintained increase. The mechanism responsible for the initial increase has not been elucidated. The increase of the \(^{38}\)Cl efflux is dose dependent and steady. The effect of noradrenaline on Na efflux has been studied in K-depleted cells in which the Na-K pump has been inhibited by ouabain. The Na efflux of these cells is therefore due largely to a passive movement of these ions. These experiments indicate that the Na efflux also increases by noradrenaline. These changes in the ion fluxes occur at a constant value of the membrane potential and of the ion gradients. We can therefore assume that they represent modifications of membrane permeability. A similar nonselective increase in permeability of the univalent ions Na, K, and Cl has also been observed during exposure of visceral smooth muscle to cholinomimetic drugs (Durbin and Jenkinson, 1961). The absence of an effect on the membrane potential can be due to the increase of all ion permeabilities by about the same factor. It is not known whether the electrogenic potential (Hendrickx and Casteels, 1974) is also affected by noradrenaline. A second procedure for inducing a contraction of the ear artery consists of depolarizing the cells by increasing [K]o.

Because TEA increases the amplitude and duration of the action potentials in some visceral smooth muscle (Ito et al., 1970) and induces fluctuations of the membrane potential in smooth muscle cells which are normally quiescent (Mekata, 1974), we have also studied the effect of this substance on the ear artery. The pronounced depolarization of the cells and the appearance of action potentials induced by TEA can both be explained by assuming that TEA decreases the K permeability of the cells. This is confirmed by our observation that TEA decreases the K efflux from K-depolarized cells. The depolarization induced by noradrenaline in TEA-containing solutions may possibly be due to the fact that noradrenaline under these conditions no longer affects K permeability. The fluctuations in membrane potential which can occur in the presence of TEA resemble the action potentials of visceral smooth muscle. The amplitude and rate of rise of these action potentials depend to a large extent on the external Ca concentration, although the external Na concentration could also play a role, as has been observed by Anderson et al. (1971) for the myometrium. Also, the pattern of tension development during these fluctuations of membrane potential has some of the characteristics of that of visceral smooth muscle. A phasic contraction occurs in association with the spike discharge. This pattern is different from the prolonged tension which is induced by noradrenaline or which accompanies the depolarization of the cells by TEA or K-rich solution.

We have also tried to elucidate to what extent cytoplasmic Ca is increased during stimulation by noradrenaline or K-rich solution. The use of efflux solutions containing La and inhibitors of metabolism does not affect appreciably the amplitude of the fitted components of the \(^{45}\)Ca efflux as compared to the
corresponding quantities under control conditions. Its main effect is to reduce the exchange rate of the slow compartment. It can be proposed that this Ca fraction represents cellular Ca and that the cell membrane is the rate-limiting step in the exchange of cellular Ca with extracellular space, because this slowly exchanging Ca behaves as a homogeneous compartment. The additional reduction of the rate of loss of 45Ca from the cellular compartment by metabolic inhibition suggests that metabolism plays a direct role in Ca extrusion, as has also been observed for guinea pig taenia coli (Casteels and van Breemen, 1975). These findings also suggest that the fast component represents a loss of extracellular Ca.

Depolarization of the cells by high-K solutions can induce a contraction only if Ca is present in the external medium and if this Ca can penetrate the cell membrane. This is a well-known property of all smooth muscle cells (Edman and Schild, 1962; van Breemen et al., 1972). We can therefore assume that depolarization of smooth muscle cells by increased [K]o facilitates the penetration of Ca into the intracellular compartment and augments the amount of cellular Ca. This has been observed for the taenia coli of guinea pig (Mayer et al., 1972) and for the rabbit aorta (van Breemen et al., 1972). However, in the present experiments we could not observe an increase in cellular Ca by supplementary exposure for 15 min to K-rich solution after 1 h 45 min of loading in control solution. By limiting exposure to radioactive solution to 15 min it was found that this K depolarization only increases the rate of Ca influx. These two findings can be reconciled by assuming that the increased rate of influx is compensated by an increased activity of the Ca extrusion pump, thereby limiting the net changes in cellular Ca.

In addition, exposure to noradrenaline causes only an increase in the rate of Ca influx, without changing the net Ca content of the tissue. Here again we have to assume that the cellular Ca content is not significantly changed because of the activity of the Ca extrusion pump. However, in contrast to K-rich solution, noradrenaline still induces a transient tension development and an increased Ca efflux in Ca-free medium or in an La-containing solution. These observations indicate that 10-6 M noradrenaline not only increases the Ca permeability of the cell membrane but also releases cellular Ca. We have to point out that noradrenaline also increases 45Ca efflux in a solution containing Ca and La. La seems therefore to be more effective in preventing external Ca from entering the cells and refilling the Ca stores than in inhibiting the Ca extrusion mechanism. Because of the presence of Ca in the external medium we can conclude that cellular calcium, which is released by noradrenaline, is extruded against its electrochemical gradient. The transient nature of the contraction induced by noradrenaline in Ca-free medium or in an La-containing solution suggests that the transmembrane Ca pump could play a role in relaxing these cells. Because this contraction lasts only about 1 min and cannot be induced for a second time during maintained exposure to Ca-free medium, we propose that Ca released by noradrenaline would be extruded over this short period of time. The calcium compartment on which noradrenaline acts contains about 60 μmol Ca per kg. If this amount of Ca were to be extruded over a period of 1 min, the extrusion rate would have a mean value of 60 μmol kg⁻¹ min⁻¹ which is about 20 times larger.
than the steady-state efflux rate from the slowly exchanging cellular Ca fraction. Such a fast extrusion during the initial minutes of the efflux procedure could explain the small increase in the amplitude of the slow component observed after K depolarization and after exposure to noradrenaline.

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