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THE MEMBRANE PROPERTIES OF THE SMOOTH MUSCLE CELLS OF THE RABBIT MAIN PULMONARY ARTERY

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SUMMARY

1. The membrane potential of the smooth muscle cells of the rabbit main pulmonary artery amounts to -57 mV, the length constant of the tissue is 1·48 mm and the time constant of the membrane 182 msec. On the basis of the electrical properties of its membrane, this smooth muscle tissue is classified as a single-unit type. During outward current pulses, the membrane shows marked rectification and action potentials can never be generated.

2. Tetraethylammonium (10 mM) and procaine (5 mM) depolarize the membrane and increase the membrane resistance. By studying the effect of both substances on the ^{42}K efflux, it could be concluded that they reduce the K-permeability of the membrane. They also suppress the rectification of the membrane and increase the length constant of the membrane. In the presence of TEA and procaine, a graded response of the membrane can be induced by outward current pulses, but overshoot potentials never occur.

3. Noradrenaline, in concentrations between 2×10^{-8} and 10^{-7} M, evokes contraction without depolarizing the membrane. When the concentration is increased above 2×10^{-7} M, noradrenaline depolarizes the membrane and reduces the membrane resistance. A study of the effect of noradrenaline on the K, Cl and Na fluxes has revealed that it increases the permeability of the membrane for these three ions.

4. The tissue concentrations of Na and K are 80 and 38 m-mole/kg wet wt., respectively. The amount of Cl in the cellular compartment was measured by an extrapolation procedure and found to be 13 m-mole/kg wet wt. The extracellular space measured with [^{14}C]sorbitol is 550 ml./kg wet wt. and the dry wt./wet wt. ratio 19 %. The calculated equilibrium potentials for K, Na and Cl (E_K, E_{Na} and E_{Cl}) are -83, $+59$ and -26 mV,

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respectively. In efflux experiments under steady-state conditions, the following rate constants have been calculated: 0·092 min^{-1} for Na, 0·029 min^{-1} for Cl and 0·0054 min^{-1} for K. The calculated value for the ratio P_{Na}/P_K was 0·22 and for P_{Cl}/P_K 0·63.

5. K-free solution and 2×10^{-6} M ouabain depolarize the cells by about 8 mV. After exposure of the cells to K-free solution, they hyperpolarize on readmission of K, suggesting that part of the membrane potential could be due to electrogenic transport of ions.

6. A decrease of external Ca depolarizes the cells and increases the membrane resistance. Na-deficiency hyperpolarizes these smooth muscle cells but this procedure does not prevent the depolarization induced by Ca deficiency.

INTRODUCTION

In some vascular smooth muscles a contractile response can be evoked by electrical activation. Such activation is exemplified by the smooth muscle cells of the portal vein, in which spontaneous action potentials occur that are similar to the ones described in the different visceral smooth muscles (Bülbring, Brading, Jones & Tomita, 1970). On the other hand, a mechanical response can be induced in the smooth muscle of the pulmonary artery (Su, Bevan & Ursillo, 1964; Somlyo & Somlyo, 1968; Haeusler, 1972), of the common carotid artery (Mekata & Niu, 1972), of the aorta (Mekata, 1974) and of the ear artery of the rabbit (Hendrickx & Casteels, 1974), without occurrence of action potentials.

It is known also that, in the main pulmonary artery, the adrenergic nerves only reach the outer muscle fibres. It is not yet clear whether the muscle fibres at the inner surface of the media are activated by electrotonic current spread or by diffusion of the adrenergic transmitter from the outer layer (Speden, 1970; Burnstock, 1970; Holman, 1969; Bevan & Su, 1973; Burnstock & Costa, 1975). The main pulmonary artery of the rabbit was suggested to be a multi-unit vascular smooth muscle (Su *et al.* 1964), although the morphological arrangements of the cell structure are in favour of a classification into a single-unit vascular smooth muscle (Burnstock, 1970). We have investigated, therefore, the membrane properties of this muscle tissue in order to be able to classify this muscle as either single-unit or multi-unit. Moreover we have investigated the nature of the membrane potential and the passive membrane properties. The effect of TEA, procaine and noradrenaline on these membrane properties has been analysed.

METHODS

Preparation. Albino rabbits of either sex, weighing 2·5–3·0 kg, were stunned and bled. The main pulmonary artery was excised and freed of connective tissue using a dissecting microscope. The tissues were cut circularly at a width of 2·0–2·5 mm and a length of *ca.* 10 mm. Such preparations were used for the electrophysiological studies, for the isometric tension recording and for the analysis of the ion content and ion fluxes. The arteries were transferred immediately after dissection to oxygenated Krebs solution at 35° C and also further cleaning and dissection were performed under these conditions.

Apparatus for recording the membrane potential and for studying the membrane parameters. For recording the membrane potential of the single cells, the conventional micro-electrodes, filled with 3 M-KCl were used (Bülbring & Kuriyama, 1963). These electrodes have been tested at regular intervals for tip potentials in order to avoid systematic errors. They were inserted from the outer surface of the preparation. The tissue chamber had a volume of 2 ml. and was perfused at a rate of 3 ml./min at a temperature of 35–36° C. In order to investigate the passive characteristics of the smooth muscle cells, the partition stimulating method was used as described by Abe & Tomita (1968). During exposure of the cells to depolarizing solutions, a conditioning hyperpolarization could be applied in order to return the cells to their normal resting potential. During such conditioning hyperpolarization, short hyperpolarizing and depolarizing pulses were applied in order to determine the current–voltage relationship.

Ion contents and flux measurements. All tissues, which were used to study the ion distribution in the pulmonary artery, were left for at least 90 min in the normal Krebs solution. The Na and K content were determined by flame photometry, after ashing the tissue at 600° C in platinum crucibles. The extracellular space was determined by exposing the tissue for 1 hr to a Krebs solution containing [^{14}C]sorbitol and by extracting immediately afterwards the tracer by leaving these tissues for 90 min in 3·75 ml. of Krebs solution. The water content of the tissues was determined by subtracting the dry wt., which was measured after drying the tissues for 12 hr at 95° C (Casteels, 1969). From these experimental data, the intracellular K and Na concentration, expressed per l. cell water, could be calculated (Casteels, 1969).

The amount of Cl in the intracellular compartment was determined by exposing tissues to a Krebs solution containing ^{36}Cl for 90 min and washing them immediately afterwards in a non-active Krebs solution at 0° C. The slow part of the efflux was extrapolated to zero time and this intercept can be considered as representing the amount of intracellular Cl per kg wet wt. of tissue. Dividing this value by the volume of intracellular water gives the intracellular Cl concentration (Casteels, 1969). The same procedure has been used also as a control for estimating the amount of intracellular Na per kg wet wt. We have studied also the efflux of ^{22}Na, ^{36}Cl and ^{42}K in normal Krebs solution in order to determine the rate coefficient of these respective effluxes. ^{14}C and ^{36}Cl have been measured by liquid scintillation counting using a scintillation mixture described by Patterson & Greene (1965). ^{42}K and ^{22}Na were measured by a γ-detecting scintillation counter.

Solutions. As a standard solution, modified Krebs solution of the following composition was used (mM): Na^+, 137·4; K^+, 5·9; Mg^{2+}, 1·2; Ca^{2+}, 2·5; Cl^-, 134·0; $H_2PO_4^-$, 1·2; HCO_4^-, 15·5 and glucose, 11·5. The solution was aerated with 97 % (v/v) O_2 and 3 % (v/v) CO_2, and its pH was maintained at 7·2–7·3. Isotonic K-Krebs solution was prepared by replacing NaCl and $NaHCO_3$ by equivalent amounts of KCl and $KHCO_3$. Na-deficient solution was prepared by replacement of NaCl with isotonic sucrose solution, but it still contained 15·5 mM-$NaHCO_3$. Na-free solution

(sucrose) was prepared by replacement of NaCl, $NaHCO_3$ and KCl by isotonic sucrose and 5·9 mM-$KHCO_3$. The pH was adjusted at 7·2–7·3 with Tris (hydroxymethyl)-aminomethane buffer (Tris buffer). Na-free solution (Tris) was prepared by replacement of Na with isotonic Tris. Ca-free solution was prepared by removing $CaCl_2$ from Krebs solution and by adding 0·1 mM ethylene glycol bis (β-aminoethyl ether)-N,N′-tetra-acetic acid (EGTA). The following drugs were used at concentrations described in the results: noradrenaline-HCl, tetraethylammonium-Cl (TEA-Cl), Procaine-Cl, and G-strophantin (ouabain).

RESULTS

Passive membrane properties of the smooth muscle cells of the main pulmonary artery in Krebs solution

The membrane potential in smooth muscle cells of the rabbit main pulmonary artery amounts to -57 mV (S.D. of observations $= \pm 1{\cdot}8$, $n = 200$). This value is slightly lower than the one measured by Haeusler (1972). The discrepancy may be due to the difference of $[K]_o$ in the physiological medium. The membrane is quiescent, and spikes do not occur spontaneously; neither can they be evoked by application of outward current pulses of strong intensity. In order to measure the electrical properties of the membrane, square pulses of 2 sec duration were applied to the tissue. Fig. 1 shows the current–voltage relationship recorded at five different distances from the stimulating electrode. A roughly linear relationship between the intensity of the applied inward current and the electrotonic potential is observed for a hyperpolarization of up to 15 mV. The marked rectifying properties of the membrane are demonstrated by applying outward current pulses to the tissue.

If the cable equations can be applied to this tissue, we should find a logarithmic relationship between the amplitude of the electrotonic potentials and the distance between the recording and the stimulating electrode. In fact, such a logarithmic relationship was observed in this tissue at any current intensity. The length constant (λ), calculated from the slope of the decay of the electrotonic potential as a function of the distance from the stimulating electrode, has a mean value of 1·48 mm (S.D. of observations $= \pm 0{\cdot}24$, $n = 23$). The time constant of the membrane (τ_m) can be calculated from the relationship between the time after which half of the final steady-state amplitude of the electronic potential has been reached, and the distance between recording and stimulating electrodes. The slope of the line representing this relationship is given by $\tau_m/2\lambda$. The mean time constant measured by this procedure is 182 msec (S.D. of observations $= 43$, $n = 16$). These electrical properties of the membrane confirm that the smooth muscle tissue of the pulmonary artery belongs to the single-unit type.

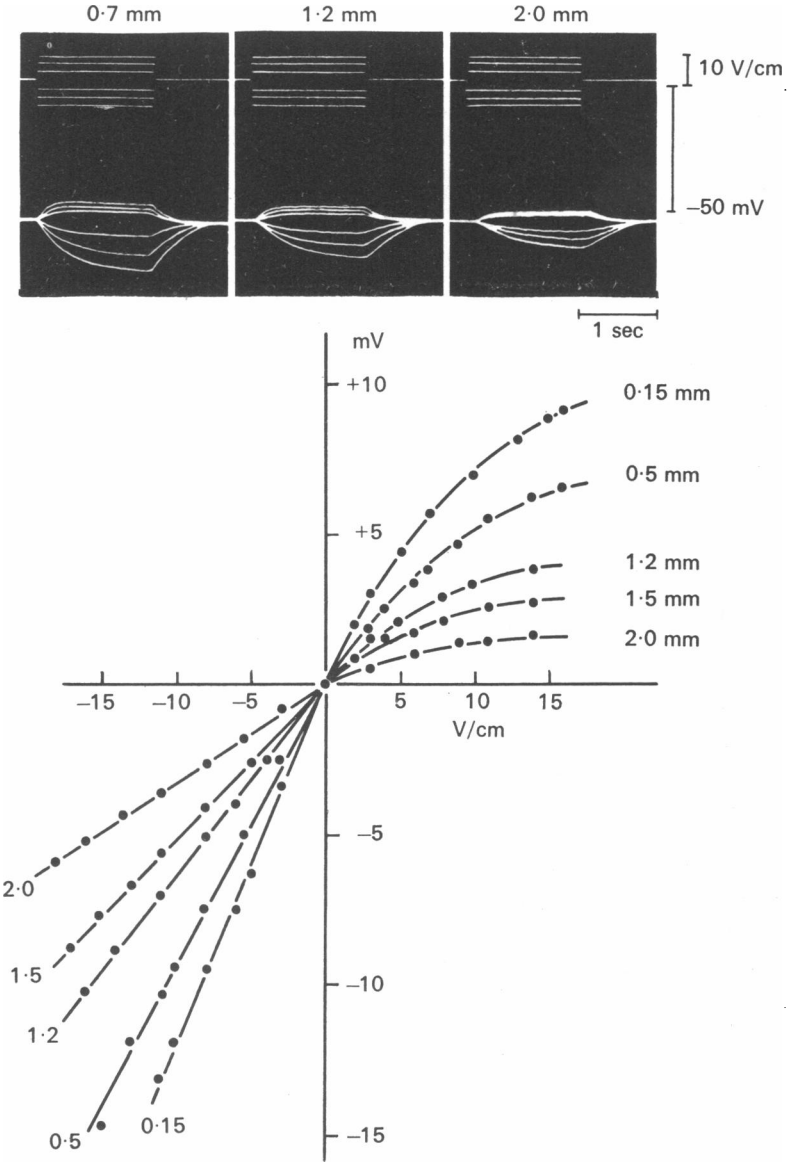

Fig. 1. Current–voltage relationship observed at various distances from the stimulating electrode. The top records show the electrotonic potentials evoked by inward and outward current pulses at three different distances from the stimulating electrode. The lower graph represents the current–voltage relationship observed at various distances from the stimulating electrode. The current intensities are expressed as V/cm on the abscissa and the change of the membrane potential is given on the ordinate in mV.

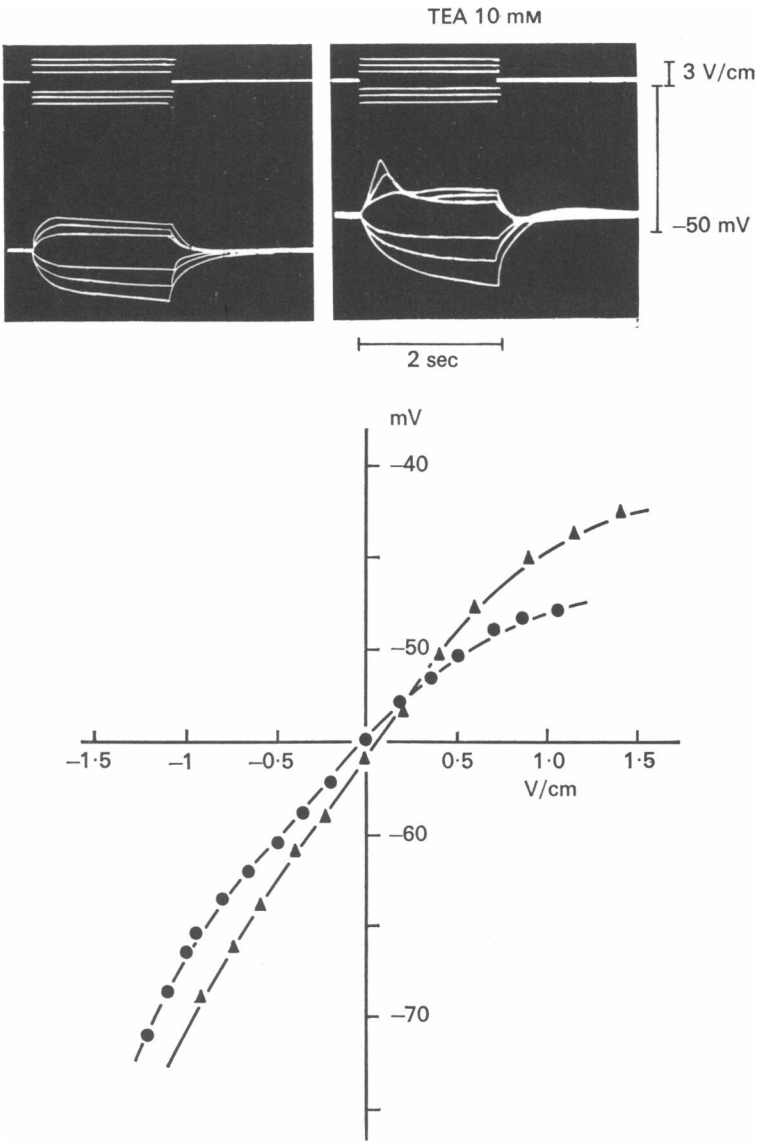

Fig. 2. Effect of 10 mM tetraethylammonium (TEA) on the current–voltage relationships. The recording electrode was inserted into a cell at a distance of 0·5 mm from the stimulating electrode. The top record shows the shapes of the graded responses and of the electrotonic potentials before and during application of TEA. The lower graph shows the effects of TEA on the current–voltage relationships in normal Krebs solution (●) and in Krebs solution containing 10 mM-TEA but during a repolarization of the cells by current injection (▲).

Changes in the membrane properties induced by TEA and procaine

We have investigated the effects of various substances on the membrane properties of the smooth muscle cells of the pulmonary artery. It has been shown that tetraethylammonium (TEA) enhances in many visceral smooth muscle tissues the spike amplitude of spontaneously active cells, and that it can evoke spikes in electrically quiescent smooth muscles (Osa & Kuriyama,

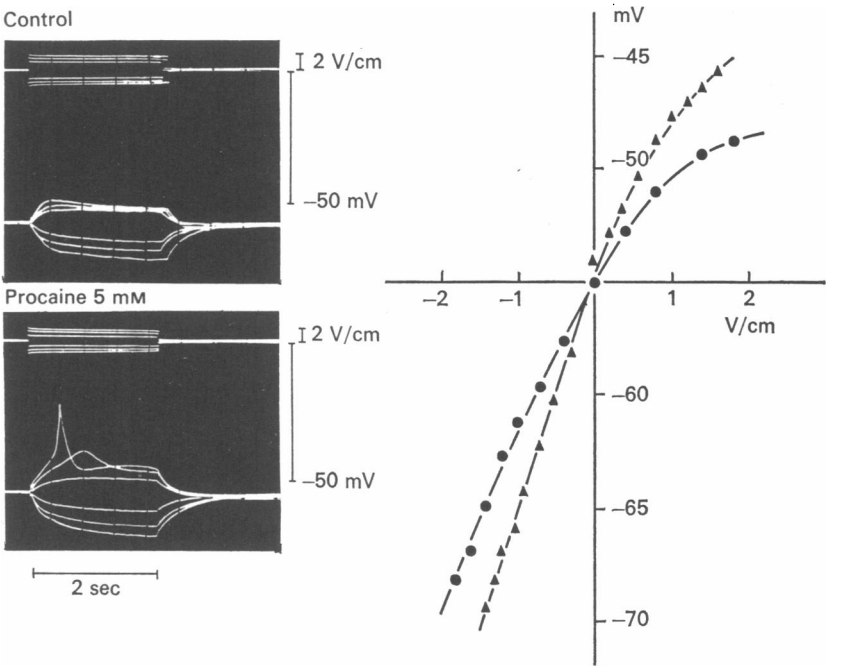

Fig. 3. Effect of procaine (5 mM) on the current–voltage relationship. The distance between recording and stimulating electrode is 0·55 mm. The control is represented by filled circles and the data obtained in the presence of 5 mM procaine by filled triangles. On the left, the electrotonic potentials and graded responses before and during application of procaine are shown.

1970; Ito, Kuriyama & Sakamoto, 1970; Mekata & Niu, 1972; Kirkpatrick, 1975). In the pulmonary artery, 10 mM TEA depolarizes the cells from -57 mV (S.D. of observations = 2·4, n = 20) to -47 mV (S.D. of observations = 2·1, n = 23) and increases the amplitude of the electrotonic potential. Fig. 2 shows the changes of the current–voltage relationship induced by TEA. Throughout the experiment, the recording electrode was placed at 0·5 mm from the stimulating electrode. From these results it can be deduced that 10 mM TEA besides depolarizing the membrane makes the current–voltage relationship steeper than in Krebs solution. The latter

change indicates that the membrane resistance is increased by TEA. This higher membrane resistance explains the finding that, by the presence of TEA, the length constant increases from its control value of 1·47 mm (S.D. of observations = 0·24, n = 6) to 1·90 mm (S.D. of observations = 0·23, n = 6). TEA also reduces the rectifying properties of the membrane and it thereby makes the current–voltage relationship steeper for outward current pulses. Such pulses can, in the presence of TEA, also evoke a graded response.

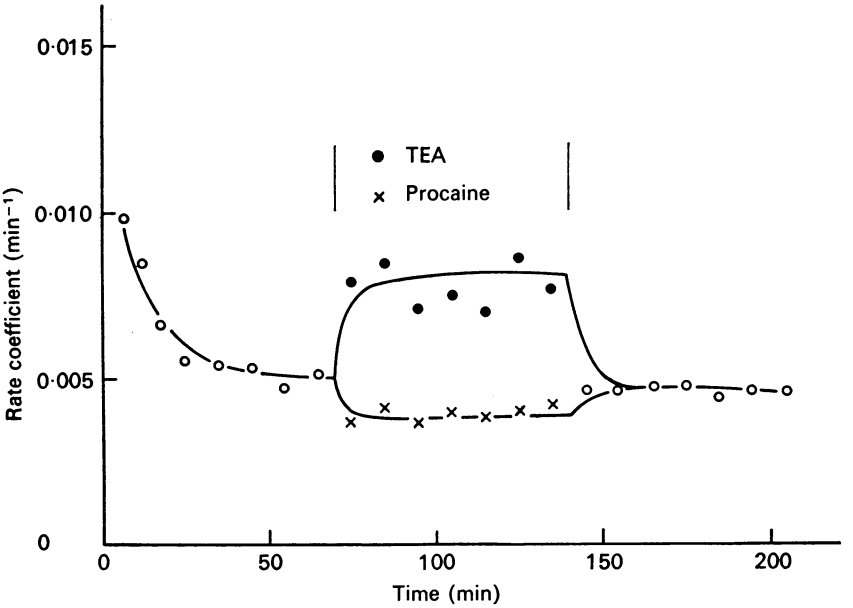

Fig. 4. The rate coefficient of the ^{42}K efflux from the pulmonary artery in normal Krebs solution (○). From min 70 until min 140 of the efflux the normal medium was replaced either by a solution containing 10 mM TEA (●) or 5 mM-procaine (×).

Kurihara (1975) has suggested that procaine also reduces the K-conductance of the membrane in visceral smooth muscles. We have investigated therefore, whether procaine, by analogy with TEA allows the generation of graded responses in the pulmonary artery by reducing the K-conductance. Procaine (5 mM) transiently depolarizes the membrane by 8 mV. The cells gradually repolarize to a membrane potential which after 10 min, is only slightly less than the control value, −53 mV (S.D. of observations = 1·9, n = 30). A comparison of the current–voltage relationship obtained at a distance of 0·55 mm from the stimulating electrode before, and during application of procaine (Fig. 3), indicates that this substance also increases

the membrane resistance and reduces the rectification of the membrane for outward current pulses.

Procaine (5 mM) increases the length constant of the tissue from 1·51 mm (S.D. of observations = 0·24, n = 26) to 2·31 mm (S.D. of observations = 0·21, n = 6). The potentials evoked can attain a larger amplitude (max. 35 mV) than in a solution containing TEA but they never present an overshoot.

The increases of the membrane resistance induced by 10 mM-TEA or 5 mM-procaine could be due to a decrease of the K-permeability of the membrane as suggested by Kurihara (1975). We have investigated, therefore, whether 10 mM-TEA and 5 mM procaine change the K-efflux. These results are represented in Fig. 4. It is obvious that TEA increases appreciably the rate coefficient of the K efflux, while procaine causes a small decrease. In interpreting these findings, we have to take into account that TEA depolarizes the cells by about 10 mV and that procaine, although it causes initially a large depolarization, later on increases the membrane potential by only 2–3 mV. These concomitant changes of the membrane make it difficult to draw any conclusion on the effect of TEA on the passive K permeability of the membrane. We have, therefore, also investigated the effect of both substances on the ^{42}K efflux in K-depolarized cells. The prolonged exposure of the tissues to K-rich solutions modifies appreciably the rate of K-efflux, which no more remains constant. The comparison of the rate of K-efflux in the presence and the absence of TEA or procaine indicates that these two substances reduce the K permeability of these depolarized smooth muscle cells by a factor of 2.

Changes of the membrane properties induced by noradrenaline

The excitatory transmitter in the pulmonary artery is noradrenaline. At concentrations between 2×10^{-8} and 10^{-7} M noradrenaline induces a contraction without depolarizing the cells. At concentrations exceeding 10^{-7} M, the amplitude of the contraction increases and the cells depolarize, without presenting action potentials (Casteels, Kitamura, Kuriyama & Suzuki, 1977). In Fig. 5 we compare the current–voltage relationship in control conditions and in the presence of 10^{-6} M noradrenaline. The cells were depolarized by this concentration, from -56 to -48 mV. Using the partition method of Abe and Tomita (1968), the membrane potential of cells exposed to noradrenaline was returned by current injection from -48 to -58 mV and the current–voltage relationship was determined. A comparison of the current–voltage relationships obtained under these different conditions suggests that noradrenaline decreases the membrane resistance at any given membrane potential.

The change of the membrane resistance by 10^{-6} M noradrenaline and

the concomitant depolarization of the cells suggest that a high concentration of noradrenaline increases the permeability of the membrane for different ions. In order to elucidate this mechanism, we have studied the effect of two concentrations of noradrenaline (5×10^{-8} and 10^{-6} M) on the efflux of ^{42}K and ^{36}Cl. The reason for using two different concentrations of

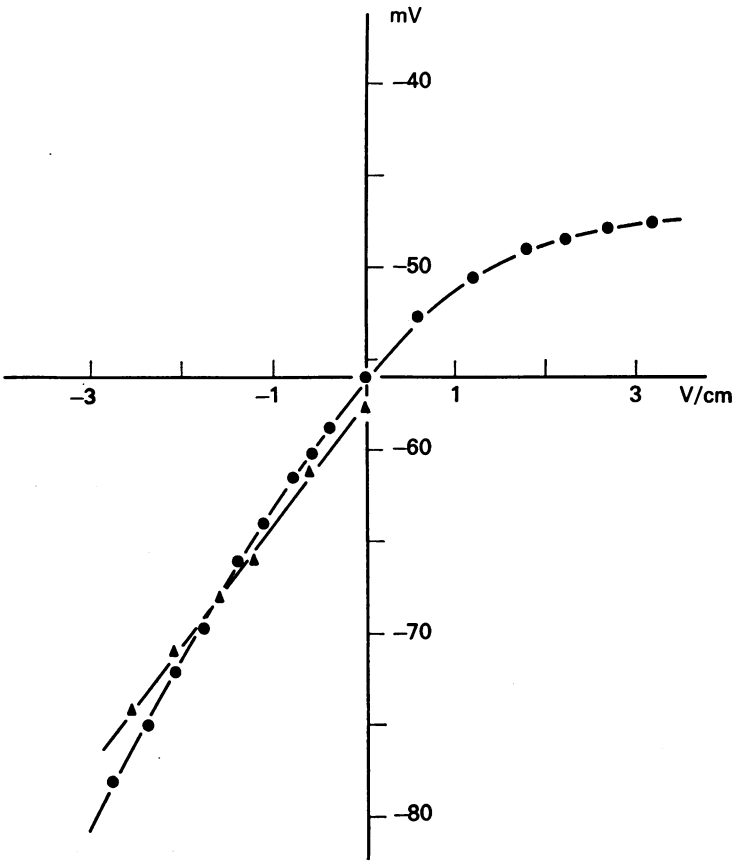

Fig. 5. The current–voltage relationship in normal Krebs solution is represented in filled circles (●) and for tissues exposed to a solution containing 10^{-6} M noradrenaline by filled triangles (▲). These cells were repolarized by current injection to −58 mV.

noradrenaline is that the lower one (5×10^{-8} M) does not affect the membrane potential and will thereby allow us to observe, eventually, a direct effect of this mediator on the membrane permeability, while for the higher concentration (10^{-6} M) the concomitant depolarization will make the interpretation more difficult. The effect of both concentrations of noradrenaline

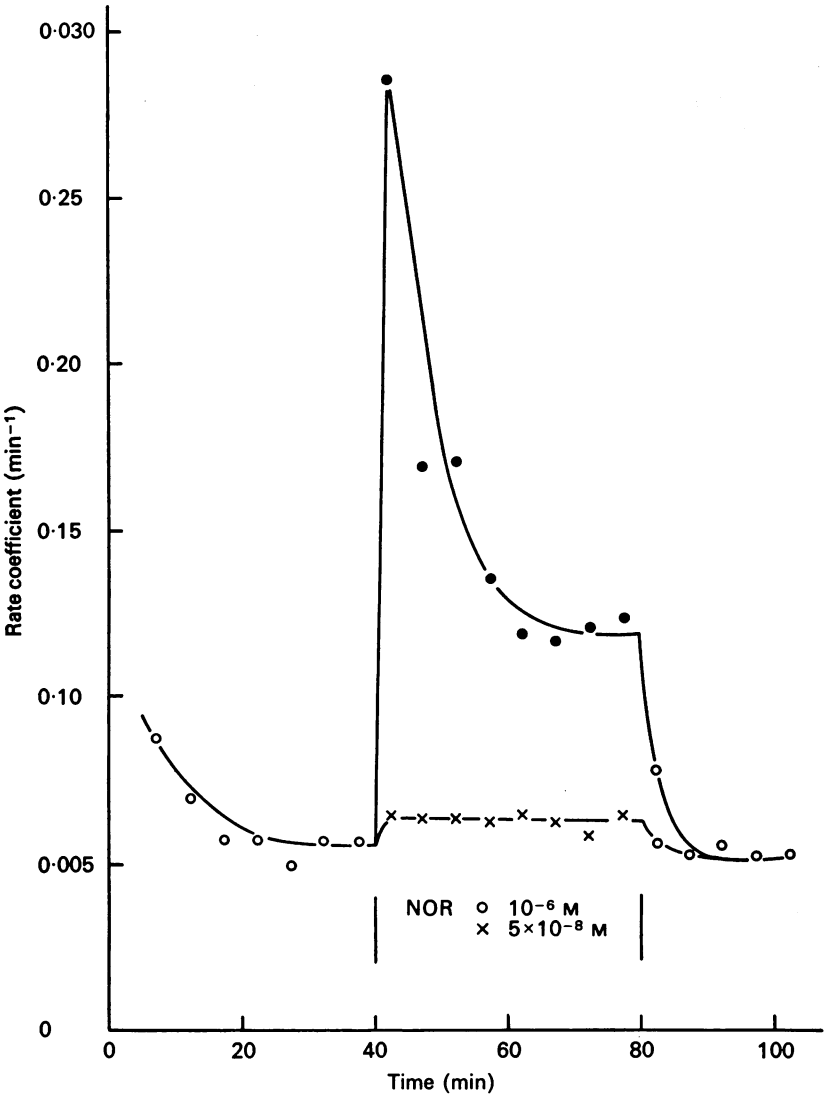

Fig. 6. The effect of 10^{-6} M-noradrenaline (●) and of 5×10^{-8} M noradrenaline (×) on the rate coefficient of the ^{42}K efflux. Noradrenaline was added to the washing medium from min 40 until min 80.

on the ^{42}K efflux is represented in Fig. 6. Noradrenaline (10^{-6} M) induces a large initial increase of the rate of ^{42}K-efflux, which is followed by a lower steady increase. In interpreting this effect, we have to take into account that 10^{-6} M noradrenaline depolarized the cells. Also, 5×10^{-8} M noradrenaline increases the rate of efflux significantly, but here this increase is steady.

The effect of noradrenaline on the ^{36}Cl efflux presents a similar picture (Fig. 7). In order to demonstrate an effect of noradrenaline on the rate of Na-efflux, we have used cells which have been enriched in Na by exposure to a K-free solution containing 10^{-5} M ouabain and performed the efflux in a similar solution. This experimental procedure has the advantage of increasing the ^{22}Na content of the tissue and of making the Na-efflux largely passive. Fig. 8 shows that, in these experiments also, 10^{-6} M noradrenaline increases appreciably the Na-permeability.

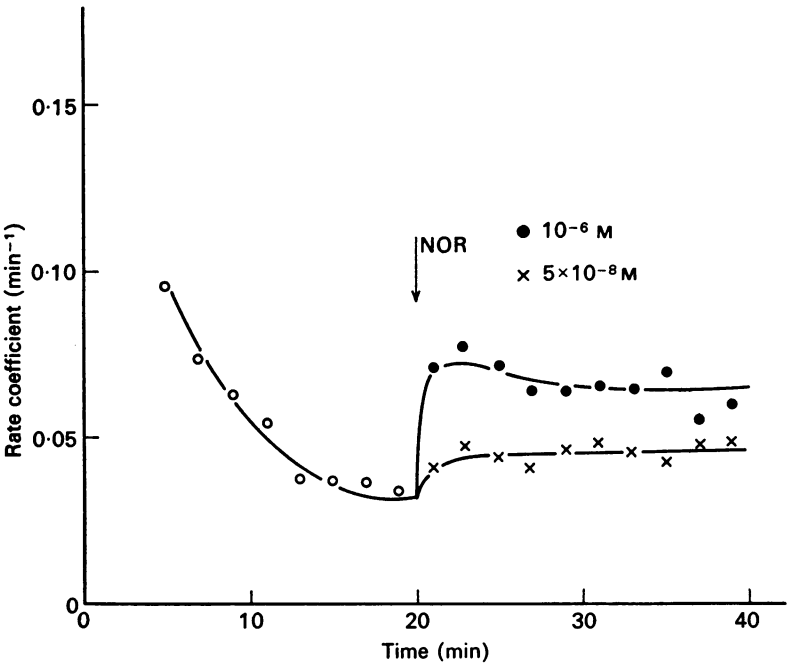

Fig. 7. The modifications of the rate coefficient of ^{36}Cl efflux from the rabbit pulmonary artery by 10^{-6} M (●) and 5×10^{-8} M (×) noradrenaline. This substance was added to the washing solution at min 20 of the efflux, as indicated by the arrow. The rate coefficient during exposure to control solution is represented by the open circles.

Ionic concentrations and membrane potential in the smooth muscle cells of the pulmonary artery

The total ion content of the tissues, measured by flame photometry, gave 80 m-mole/kg wet wt. (S.D. of observations = 1·3, n = 12) for Na and 38 m-mole/kg wet wt. (S.D. of observations = 1·3, n = 12) for K. The [^{14}C]sorbitol space was found to be 550 ml./kg wet wt. (S.D. of observations = 16, n = 13) and the dry wt./wet wt. ratio 19% (S.D. of observations =

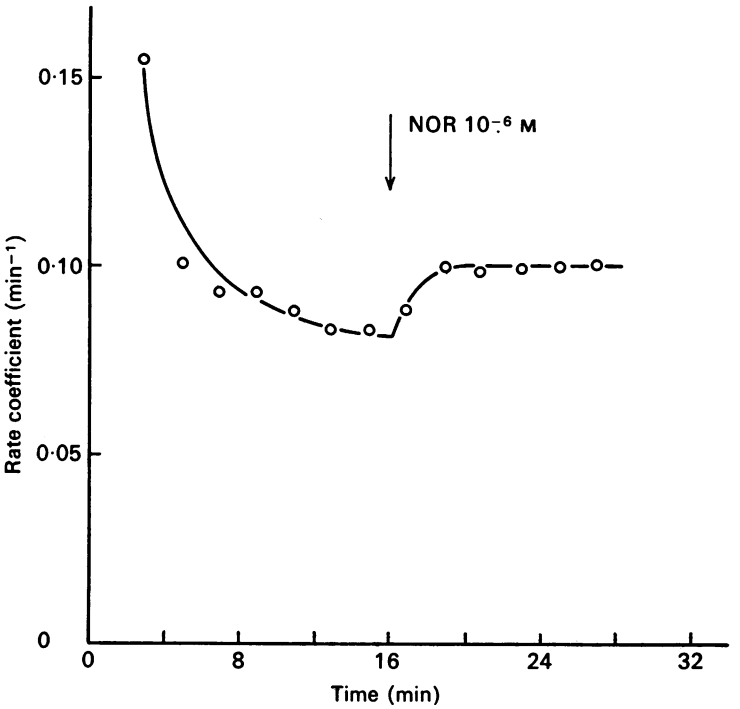

Fig. 8. The effect of 10^{-6} M noradrenaline on the rate coefficient of ^{22}Na efflux from K-depleted cells in a K-free medium. Noradrenaline was added to the washing medium at min 16, as indicated by the arrow.

TABLE 1. Numerical values obtained by ion analysis and flux experiments on strips of the rabbit pulmonary artery. Because the cell dimensions are not known, the flux is expressed by the product of the flux and the ratio of surface/volume (A/V). In order to eliminate (A/V) we have calculated the ratio of the permeability coefficients P_i/P_K

Ion	Extracellular concentration (mM)	Intracellular concentration (mM)	Equilibrium potential (mV)	Rate coefficient of efflux (min^{-1})	Flux A/V mM. (l. cell water) min^{-1}	P_i/P_K
Na	137	15	+59	0·092	1·38	0·22
	134	51	−26	0·029	1·45	0·63
	5·9	134	−83	0·0054	0·72	1

0·1, n = 13). From these data we have calculated a value for $[Na]_i$ expressed per l. cell water of 18 mM and for $[K]_i$ of 134 mM. Because the extracellular space is large, and because Na could bind to extracellular sites, appreciable errors could be introduced in the calculation of the amount of intracellular Na. We have also estimated, therefore, the amount of intracellular Na by

the extrapolation procedure. This value of intracellular Na amounts to 3·9 mM/kg (S.D. of observations = 1·4, n = 6), and using an amount of fibre water of 260 ml./kg wet wt., we find for $[Na]_i$ 15 mM/l. cell water. The amount of intracellular Cl estimated by the same extrapolation procedure, is 13·2 mM/kg wet wt. (S.D. of observations = 1, n = 6) or 51 mM/l. cell water. From the intracellular and extracellular ion concentration, the equilibrium potentials (E) for the three monovalent ions have been calculated. E_K, E_{Na} and E_{Cl} amount to −83, +59 and −26 mV, respectively.

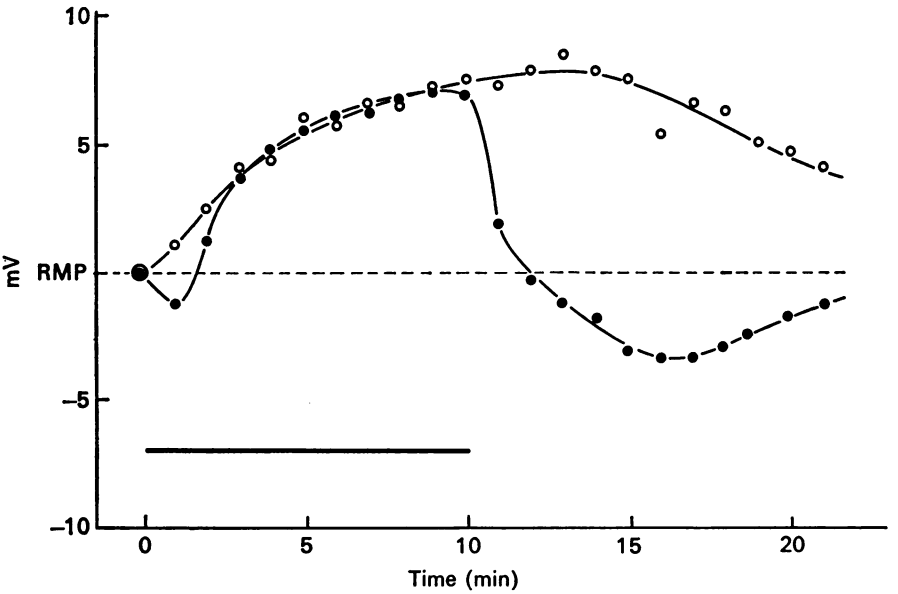

Fig. 9. Effects of K-free solution (●) and of a solution containing 2×10^{-6} M ouabain (○) on the membrane potential. The time of exposure to these solutions is represented by the continuous line. The changes of the resting potential in mV are indicated by + for depolarization and by − for hyperpolarization.

In order to determine the relationship between these ionic gradients and the membrane potential of these smooth muscle cells, we have studied the efflux of ^{22}Na, ^{42}K and ^{36}Cl from the pulmonary artery in steady-state conditions. From these efflux experiments, the following rate constants have been deduced: 0·092 min^{-1} for Na, 0·029 min^{-1} for Cl and 0·0054 min^{-1} for K. From these ionic concentrations the membrane potential and the rate constant we have calculated for the ratio of P_{Na}/P_K a value of 0·22 and for the ratio of P_{Cl}/P_K a value of 0·63 (Table 1). In these calculations we may assume that the Na-influx equals the Na-efflux, because the tissues are in a steady state. The ionic concentrations, expressed per l. cell water,

can be used instead of the values expressed per l. myoplasm, because only relative values of the permeability coefficients are considered. Introducing these different values in the Goldman equation gives a diffusion potential of -31 mV.

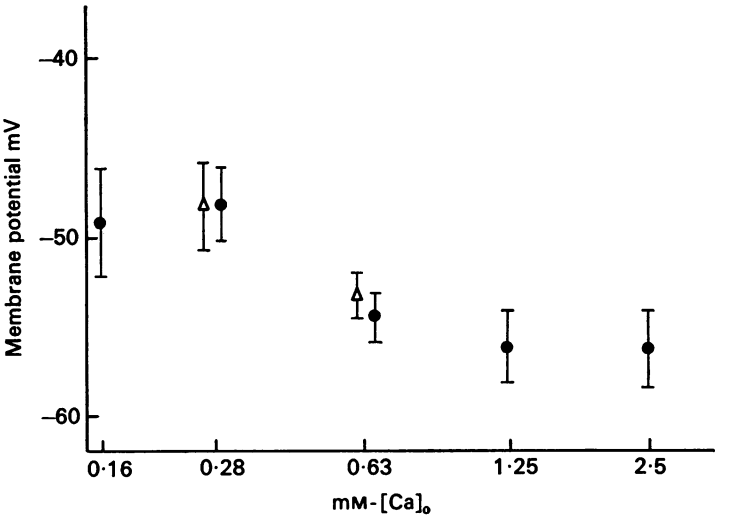

Fig. 10. Effects of various concentrations, $[Ca]_o$, on the membrane potential. The external Ca concentration is represented on the abscissa on a logarithmic scale, the membrane potential on a linear scale on the ordinate. The data obtained in a Krebs solution with different $[Ca]_o$ but with $[Na]_o$ = 137 mM are represented by filled circles. The values obtained in a solution in which $[Na]_o$ was reduced to 15 mM are indicated by the open triangles.

In order to determine a possible contribution of the electrogenic ion pump to the membrane potential of the smooth muscle cells of the main pulmonary artery, we have investigated also the effect of K-free solution and of 2×10^{-6} M ouabain on the membrane potential. These results are represented in Fig. 9. K-free solution depolarizes the cell, after inducing a transient and short-lasting hyperpolarization. A 10 min exposure to K-free solution increases the membrane potential to -50 mV (S.D. of observations = 2·3, n = 30). Readmission of K in the external medium hyperpolarizes the cell to -61 mV. The membrane potential returns to its control value within less than 15 min after readmitting K. Exposure of the cells to a solution containing 2×10^{-6} M ouabain depolarizes the membrane within 10 min from -56 mV (S.D. of observations = 2·1, n = 30) to -49 mV (S.D. of observations = 2·4, n = 30).

Modification of the membrane properties by changing the external ionic composition

Decreasing the external [Ca] from 2·5 to 0·16 mM depolarizes the cells (Fig. 10). A concomitant reduction of $[Na]_o$ to 15 mM, maintaining isotonicity by adding sucrose, does not affect the degree of depolarization caused by lowering $[Ca]_o$. The depolarization of the cells occurring in a solution containing 0·28 mM-Ca^{2+} is accompanied by an increase of the membrane resistance, as can be deduced from the record represented in

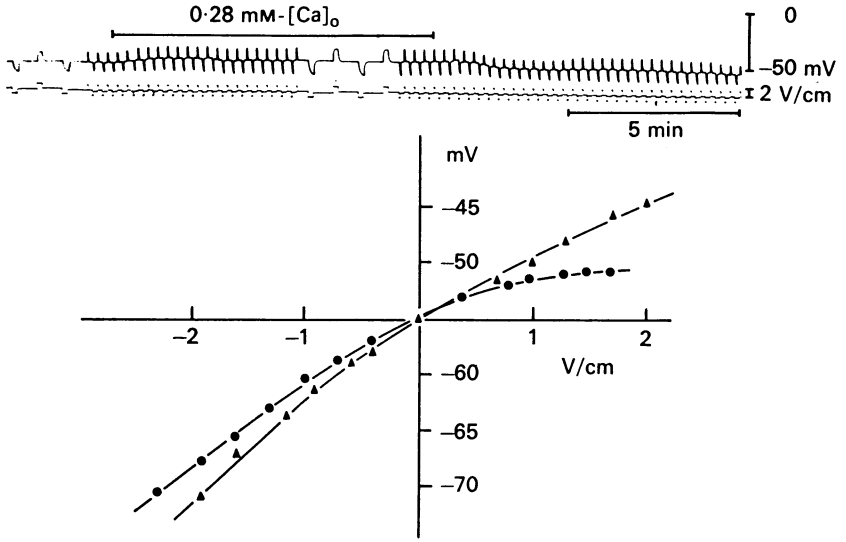

Fig. 11. Effects of Ca-deficient solution (0·28 mM) on the membrane potential, the electrotonic potential and current–voltage relationships. The top record shows the changes of the membrane potential and of the amplitudes of the electrotonic potential evoked by inward and outward current pulses before, during and after application of the Ca-deficient solution. In the lower graph, the current–voltage relationship observed in normal solution (●) and in Ca-deficient solution during repolarization of the cells by current injection (▲) is represented. The micro-electrode was inserted in a cell at a distance of 0·5 mm from the stimulating electrode.

Fig. 11. The amplitude of the electrotonic potentials evoked by inward and outward current pulses increases during exposure to Ca-deficient solutions. In this Fig. we present also the current–voltage relationship obtained in normal Krebs solution and in a Ca-deficient solution (0·28 mM-Ca^{2+}) during repolarization of the cells by current injection.

Comparing these curves indicates that Ca-deficiency makes the current–

voltage relationship steeper than under control conditions. Also the rectification of outward current pulses is suppressed.

We have investigated also the effect of Na deficiency on the membrane potential. Na-free solution prepared by substituting sucrose for NaCl transiently depolarizes the membrane. After 5 min the cells repolarize to a value which is slightly more negative than the control value. If Tris is used

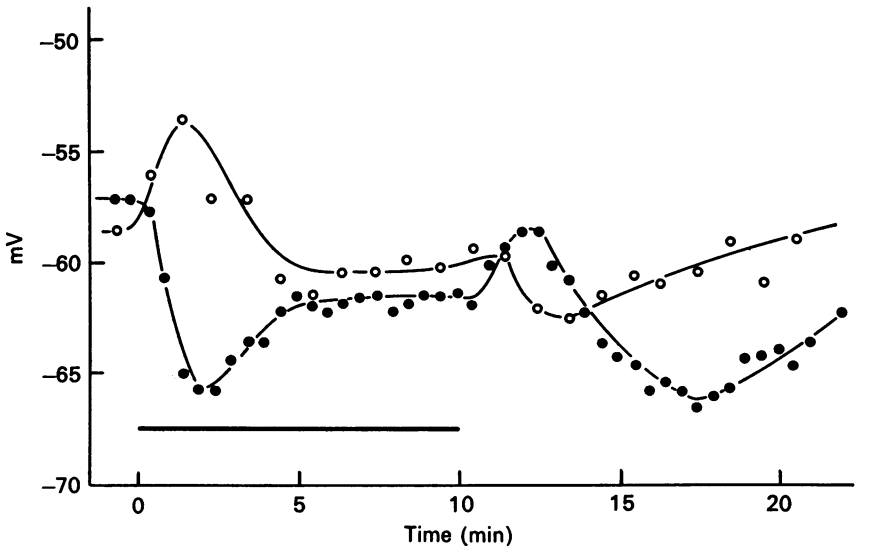

Fig. 12. Effects of Na-free solution on the membrane potential. $[Na]_o$ is replaced either by sucrose (○) or by Tris (●). The membrane potentials obtained in the two types of experiments are superimposed. The duration of the exposure to the Na-deficient solution is indicated by the continuous line. At min 10, the tissues were again superfused with normal Krebs-solution.

as a Na substitute the cells hyperpolarize transiently to −67 mV and immediately afterwards return to a membrane potential which is more negative than in a Na-deficient solution prepared with sucrose. Readmission of normal Krebs solution transiently hyperpolarized the membrane, as shown in Fig. 12. After 10–15 min, the membrane potential has returned to its control value. A study of the current–voltage relationship of the smooth muscle cells in a Na-free solution prepared with Tris indicates that the membrane resistance, for the same value of the membrane potential, decreases to a value of 0·86 times the control value.

DISCUSSION

Our results indicate that the smooth muscle cells of the rabbit main pulmonary artery present cable-like properties. This muscle tissue belongs, therefore, to the single-unit type rather than to the multi-unit type. In this smooth muscle the application of inward and outward current pulses demonstrates the marked rectifying properties of the membrane but these stimuli do not elicit action potentials. These characteristics, the length constant and the time constant of the membrane are roughly the same as those described in other large elastic arteries (Mekata & Niu, 1972).

TEA exerts a similar effect on the main pulmonary artery as on the smooth muscles of the alimentary canal, the carotid artery and trachea (Osa & Kuriyama, 1970; Ito *et al.* 1970; Mekata & Niu, 1972; Kirkpatrick, 1975; Suzuki, Morita & Kuriyama, 1976). It depolarizes the membrane, increases the membrane resistance and suppresses the rectifying properties of the membrane. However, while TEA enhances the spike amplitude in intestinal smooth muscle, or generates action potentials with overshoot in smooth muscle of the stomach fundus, it only generates a graded response of the membrane in the pulmonary artery. Procaine was shown to exert in the pulmonary artery a similar action, but the graded response is larger than the one obtained in a solution containing TEA. TEA (10 mM) causes, in normal Krebs solution, an increase of the K-efflux. The interpretation of this change is difficult because of the accompanying depolarization of the cells. Procaine causes (after the initial large depolarization) a much smaller maintained depolarization (2–3 mV) which is accompanied by a small, but significant decrease of the rate coefficient of the K-efflux. The reduction of the K-permeability by both TEA and procaine is clearly demonstrated by their reducing effect on the rate of K-efflux from K-depolarized cells. We can assume that this modification of the membrane allows the development of a graded response during the application of depolarizing pulses. However, it is not possible to explain at the moment the difference between the effects of TEA and procaine.

According to Bevan & Su (1973) the actual diffusion path between adrenergic nerves and the smooth muscle cells in the rabbit pulmonary artery is about 4 μm. These authors have calculated that the concentration of adrenergic transmitter within the adventitio-medial junction during a 10 Hz stimulation of the sympathetic nerve is 6×10^{-9} M at the nearest postsynaptic membrane and 8×10^{-8} M in the intrasynaptic space. Taking into account the neural uptake and inactivation, an externally applied concentration of noradrenaline (2×10^{-8} to 3×10^{-8} M) might correspond to the above physiological concentration of noradrenaline released by nerve stimulation. However, these concentrations of noradrenaline do not modify

the membrane potential, although they increase slightly the ionic permeabilities of the membrane. If, however, noradrenaline concentrations higher than 10^{-7} M are used, the cells depolarize and the membrane conductance increases as can be deduced from the changes of the current–voltage relationship. Our flux experiments suggest that the mechanism responsible for the simultaneous occurrence of depolarization and decrease of the membrane resistance during application of high concentrations of noradrenaline consists of a dose-dependent increase of the membrane permeability for K^+, Cl^- and Na^+. In order to explain the finding that low concentrations of noradrenaline increase the ionic permeabilities of the membrane, without changing the membrane potential, we have to assume either that the changes of P_K, P_{Cl} and P_{Na} compensate each other, or that a change of the transmembrane diffusion potential is compensated by an adaptation of the electrogenic potential.

The study of the distribution of ions in the pulmonary artery is difficult. One of the main problems is the large extracellular space of the tissue. Such a ratio extracellular space/intracellular space easily results in an underestimation or an overestimation of the intracellular ionic concentration. The values which we have obtained in the pulmonary artery are not very much different from the ones obtained in other smooth muscle cells. $[Na]_i$ is low and $[Cl]_i$ does not fit a passive distribution (Casteels, 1971). A comparison of the $[Na]_i$ values obtained by analytical procedures, and by the extrapolation procedure, does not reveal any difference and consequently there is no reason to assume that an important amount of Na is bound in the extracellular space. The calculation of the membrane potential by the Goldman equation from the ion gradients and the ion fluxes gives a value of -31 mV, a value which is 25 mV less negative than the measured resting potential. The values of the calculated diffusion potential and of the ratio P_{Na}/P_K and of P_{Cl}/P_K are similar to the values obtained for taenia coli cells (Casteels, 1969). The difference between this calculated membrane potential and the measured value could be due to the activity of an electrogenic ion pump. Our present experiments on the effect of K-free solution and ouabain suggest that this mechanism can contribute to the membrane potential. However, these findings do not indicate that the electrogenic potential would amount to 25 mV as suggested by the difference between the measured and calculated membrane potential.

The effect of changes of $[Ca]_o$ and $[Na]_o$ on the membrane potential and membrane properties are still more difficult to interpret. A reduction of $[Ca]_o$ depolarizes the cells and changes the slope of the current–voltage relationship, suggesting a decrease of the membrane conductance. A similar conclusion has been reached by Bülbring & Tomita (1969) for the guinea-pig taenia coli. The finding that a reduction of the external Na does

not prevent the depolarization, caused by decreasing the external Ca concentration, indicates that the effect of the external Ca is more complex than a reduction of P_K. Similar findings have been reported by Casteels, Droogmans & Raeymaekers (1976) for taenia coli.

Also, Na-deficiency could modify the membrane potential by changing the K-permeability. This is indicated by the changes of the current–voltage relationship. The hyperpolarization observed in Na-deficient solution could be due to such an increase of P_K, and to the disappearance of the Na-gradient.

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