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Na–Ca exchange and tension development in arterial smooth muscle

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Replacement of external NaCl by LiCl, choline chloride or sucrose increased tension of rabbit aortic strips and pulmonary arteries despite little change in membrane potential. The Ca content of the aortic strips increased concomitantly with the tension development. A two- to threefold increment in ^{45}Ca influx could be measured when external Na was removed. ^{45}Ca efflux decreased in Na-free, Ca-free solutions where little or no tension developed and increased in Na-free, Ca-containing solution concomitantly with the increase in tension. The possible significance of a Ca transport mechanism in the cell membrane which depends on Na and Ca (Na–Ca exchange) for the regulation of vascular tone is discussed.

Introduction

In cardiac muscle (Reuter & Seitz 1968; Glitsch, Reuter & Scholz 1970) and nerve (Baker, Blaustein, Hodgkin & Steinhardt 1969; Blaustein & Hodgkin 1969) Ca fluxes through the membrane are partially dependent on the Na gradient across the membrane. In these tissues an increase in total intracellular Ca occurs when either $[Na]_o$ is reduced or when $[Na]_i$ is increased. It has been suggested that this Na–Ca exchange mechanism is an important factor in determining the intracellular Ca concentration which can be made available for the activation of the contractile proteins. Isolated arterial smooth muscle preparations (Hinke & Wilson 1962; Sitrin & Bohr 1971) like cardiac muscle (Lüttgau & Niedergerke 1958), develop reversible contractures in the absence of $[Na]_o$, and also when $[Na]_i$ is increased by the removal of $[K]_o$ or by the addition of cardiac glycosides (Leonard 1957). Furthermore, in many types of hypertension the Na content of the vascular wall is increased (Schoffeniels 1969), although it is not known whether this increase is due to a net gain in *intracellular* Na.

In the present paper we report experiments concerning the possible role of Na–Ca exchange across the cell membrane in the regulation of vascular smooth muscle tone.

Tension measurements

First we examined the effect of different replacements of $[Na]_o$ on tension of spirally cut strips of rabbit thoracic aorta or pulmonary artery. After an initial equilibration period in a 'standard' Na medium (composition in mmol/l: NaCl, 137; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.05; tris-HCl buffer, 5, pH 7.4 at 37 °C; glucose, 5), the effect of Na removal was tested by exposing the tissues for 5 to 20 min to media in which part or all NaCl was isosmotically replaced by KCl, LiCl, choline chloride or sucrose. Tension was measured by means of a force displacement transducer (Grass FT 03C) and recorded on a Grass polygraph. There were marked differences between contractures developed in high K solution and the ones which occurred when NaCl was replaced by the other substitutes. In the high K solution maximal force was larger, rise time of tension was shorter and tension started to increase above the resting load (0.5 gf) when only

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about 10 to 15 mmol/l NaCl were replaced by KCl. Maximal tension was attained with 40 to 60 mmol/l $[K]_o$ (figure 1). Simultaneous measurements of the membrane potential of pulmonary arteries with a conventional microelectrode technique showed a depolarization from -42 to -16 mV when $[K]_o$ was increased from 15 to 60 mmol/l. With LiCl, choline chloride or sucrose as substitutes at least 70 % of the NaCl in the solution had to be replaced before the arterial preparations developed any additional tension above the resting load (figure 1).

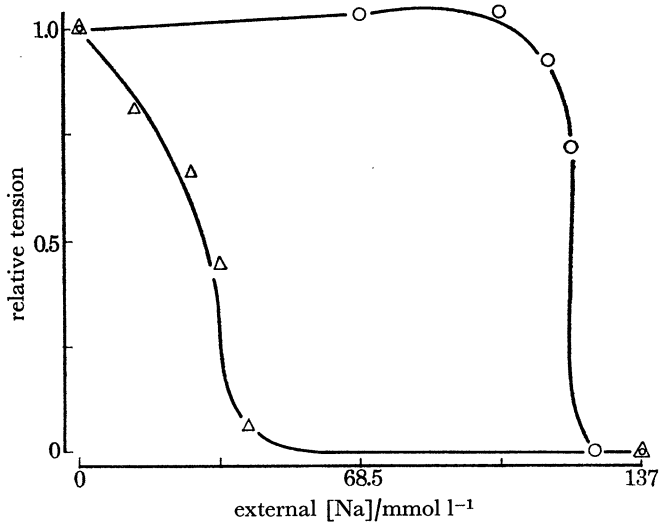

FIGURE 1. The effects of replacing external Na by K (circles) or Li (triangles) on contracture tension of rabbit aortic strips. The K and Li replacement curves were obtained on adjacent segments of tissue from the same aorta, tested simultaneously in different tissue baths. The relative tension of 0 is the baseline tension of 0.5 gf. Maximal tension (1.0) with all of the Na replaced by K was 1.94 gf. For the segment in which Na was replaced by Li, the maximal contracture tension in Na-free solution was 1.29 gf; the tension developed by this segment at the end of the experiment, when 25 % of the Na was replaced by K, was 1.58 gf.

Complete replacement of NaCl by any of these substitutes depolarized the membrane potential of the pulmonary arteries by only 3 to 6 mV (from a resting potential of about -58 mV in the Na medium). These results indicate that in contrast to the K contractures, tension developed in sucrose, choline or Li solutions cannot be explained by depolarization of the membrane potential. The experiments showed further that in the same preparation the absolute force was larger and was maintained longer during incubation in sucrose or choline solutions as compared to Li solution.

In order to establish the competitive nature of $[Na]_o$ and $[Ca]_o$ on tension development, aortic strips were exposed to different Ca concentrations in solutions in which 80, 90 or 100 % of the NaCl had been replaced by sucrose or choline chloride. The data of such an experiment are plotted in figure 2. When only 80 % NaCl was replaced by sucrose the curve relating tension development and $[Ca]_o$ was much less steep than the curve obtained with 100 % replacement of NaCl. When the reciprocal of the tension was plotted as a function of the reciprocal of $[Ca]_o$ the data of figure 2 gave two straight lines which intersected the ordinate at the same point. This indicates competition of $[Na]_o$ and $[Ca]_o$ for binding sites which regulate tension by an unknown mechanism. Quantitatively, 2 Na ions seem to compete with 1 Ca ion, since tension development was approximately the same when the ratio $[Ca]_o:[Na]_o^2$ was kept constant. The ^{45}Ca flux experiments described below indicate that a membrane transport mechanism may be the site of competition between Na and Ca ions.

Moreover, we investigated the effect of increased $[Na]_i$ on tension development of aortic strips. $[Na]_i$ was increased by inhibiting the Na pump of the preparations in K-free or ouabain-containing Na media. When Ca was present in the solutions the preparations developed appreciable contractures within 20 to 30 min. During this time in the presence of 10^{-4} mol/l ouabain the membrane potential of pulmonary arteries fell by only 5 to 10 mV. The addition of 5.4 mmol/l KCl to the K-free solution caused rapid relaxation of the aortic strip. The relaxation of the contractures after removal of ouabain was much slower. When the preparations were loaded with Na by a 20 to 30 min incubation in K-free (or ouabain-containing), Ca-free Na medium no tension developed. However, the tension which developed during the subsequent incubation in Na-free, Ca-containing solution showed a steeper rate of rise and was much larger than after preincubation in the 'standard' Na medium.

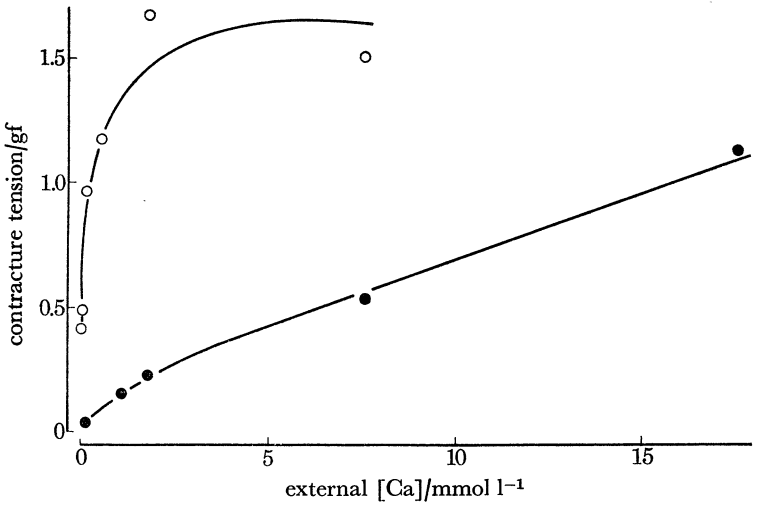

FIGURE 2. Effect of external Ca on tension development of two strips obtained from the same aorta bathed in solutions in which 80% (filled circles; strip 1) or 100% (open circles; strip 2) of the NaCl was replaced by sucrose. The tension developed by the two strips in nominally Ca-free sucrose solutions were 0.05 and 0.33 gf, respectively; these values were subtracted from all tensions recorded in the presence of external Ca.

By analogy to the situation in squid axon (Baker *et al.* 1969) and in cardiac muscle (Glitsch *et al.* 1970) these results suggested to us that a fraction of Ca *influx* might be dependent on $[Na]_i$ and of Ca *efflux* on $[Na]_o$ via a carrier-mediated transport mechanism which has affinity for both Na and Ca ions on either side of the membrane. Such an ion transport system is in principal capable of exchanging $[Na]_o$ for $[Ca]_i$, $[Ca]_o$ for $[Ca]_i$, or $[Na]_o$ for $[Na]_i$. Of these ion exchanges, the physiologically most important is the Na–Ca exchange. The effectiveness of this system to extrude Ca from the cell is dependent upon the concentration ratios $[Na]^\gamma:[Ca]$ on either side of the membrane (where γ is determined by the number of Na ions bound to one carrier site), and on the respective affinities of the carrier to both ions. If no energy source other than the Na gradient across the membrane is utilized to extrude Ca ions from the cells against their electrochemical potential, the steady-state relationship $[Ca]_i:[Ca]_o = [Na]_i^\gamma:[Na]_o^\gamma$ may hold (cf. Reuter & Seitz 1968; Blaustein & Hodgkin 1969). In such a system a reduction in $[Na]_o$ and $[Ca]_o$ should cause a decrease in Ca efflux. We performed Ca influx and efflux experiments in rabbit aortic strips in order to test, whether a Na–Ca exchange mechanism could be detected in these preparations.

Calcium flux experiments

In order to test whether removal of $[Na]_o$ has any effect on calcium content of rabbit aortic strips, spirally cut segments (0.3 to 0.4 cm wide, 5 to 6 cm long; load 0.5 gf) were first incubated for 3 h in 'standard' Na medium. They were then transferred for 20 min either to a fresh standard Na medium (the control group) or to solutions in which all of the NaCl was replaced by LiCl, choline chloride or sucrose. Afterwards all preparations were washed for 30 min in cold (4 °C), Ca-free sucrose solution containing either 0.5 mmol/l EGTA (data in table 1) or 2 mmol/l $LaCl_3$ (cf. van Breemen, Farinas, Gerba & McNaughton 1972) in order to reduce free and bound extracellular Ca as much as possible. Both kinds of washing after the incubation gave similar results. The strips were then blotted on filter paper under constant pressure (250 g), weighed and ashed. The ash was dissolved in 0.1 mol/l HCl + 1 % $LaCl_3$. The Ca concentration in the samples was determined by atomic absorption spectrophotometry. The results of such an experiment are listed in table 1. Net Ca uptake was largest in preparations incubated in solutions where sucrose was the NaCl substitute and least in solutions with LiCl as NaCl substitute. This difference in net uptake of Ca parallels the difference in maximal force developed by the arterial strips under the same experimental conditions.

Table 1. The effect of replacement of NaCl in the incubation medium by LiCl, choline chloride or sucrose on Ca content of rabbit aortic strips

All other constituents in the solutions were the same as in the 'standard' Na medium described in the text. The numbers of strips are given in parentheses. Students *t*-test was used to calculate significance of the difference between Ca content of tissues in Na medium and Na-free solutions.

incubation medium	Ca content (μmol per gram wet tissue)	*P*
NaCl	0.65 ± 0.05 (6)	
LiCl	1.00 ± 0.16 (4)	< 0.05
choline chloride	1.14 ± 0.05 (6)	< 0.0005
sucrose	1.57 ± 0.19 (4)	< 0.005

The net Ca gain by preparations exposed to Na-free solutions could either reflect an increase in Ca influx, a decrease in Ca efflux or both. It has been shown by van Breemen *et al.* (1972) that in rabbit aortic strips an almost twofold increase in ^{45}Ca influx occurs when NaCl in the bathing medium is replaced by LiCl. They washed the preparations in a Ca-free solution containing 2 mmol/l $LaCl_3$ after the incubation periods in radioactive solution in order to separate cellular from extracellular ^{45}Ca uptake. We have confirmed their results, and, in addition, have found that ^{45}Ca influx was even larger (about 2.5-fold) in choline chloride than in LiCl solution.

^{45}Ca efflux from aortic strips into inactive rinsing solutions was measured by the method of Reuter & Seitz (1968) after 2 to 3 h loading periods in radioactive 'standard' Na medium. The method allows simultaneous, continuous recording of tension development. The first 40 to 60 min of ^{45}Ca efflux were always into a Ca-free, Na-containing medium; 0.5 mmol/l EGTA was also present in order to allow rapid exchange of extracellular bound and free ^{45}Ca, and thus reduce the contribution of the extracellular ^{45}Ca to the later ^{45}Ca efflux from the strips. Figure 3*a* shows the result obtained with an aortic strip which was first effluxed in a Ca-free Na medium for 40 min and afterwards in Ca-free choline medium for 20 min. The ^{45}Ca efflux decreased in the Na-free medium by approximately 35 % and increased promptly when the

Na-containing solution was re-introduced. When Ca (1.8 mmol/l) was added to the Na medium the efflux increased further by approximately 15 %. In several experiments in which choline chloride was the NaCl substitute, atropine (10^{-5} g/ml) was added in order to exclude a parasympathomimetic effect. However, the presence of atropine had no effect on the results. Moreover, in two experiments a possible contribution of the nerve endings in the aorta to ^{45}Ca efflux was excluded by pretreatment of the animals with 6-hydroxydopamine which destroys the adrenergic nerve endings (Thoenen & Tranzer 1968). No difference could be seen between aortas from pretreated and untreated animals. When a similar experiment as in figure 3*a* was repeated with a strip of adventitia from which all of the muscularis had been removed, replacement of NaCl in the bathing medium by LiCl or choline Cl had no effect on ^{45}Ca efflux (figure 3*b*).

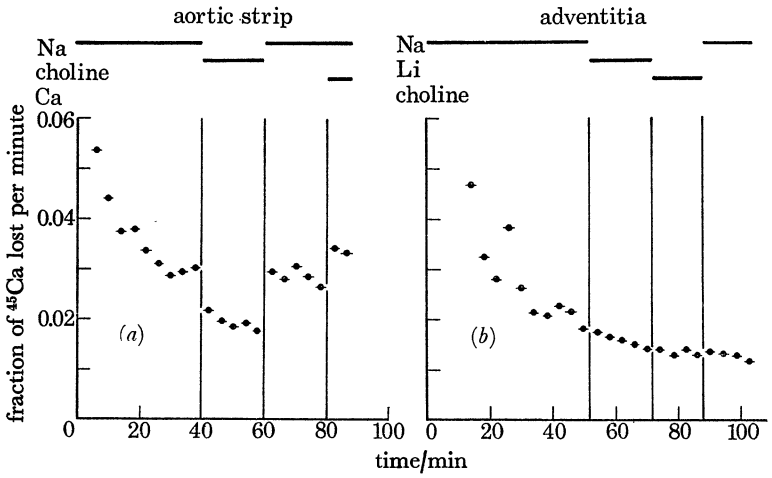

FIGURE 3. The effect of external Na on ^{45}Ca efflux from a rabbit aortic strip (*a*) and from a strip of adventitia isolated from a rabbit aorta (*b*). Ordinate: fraction of ^{45}Ca lost per minute from the tissue into the inactive rinsing solutions (rate coefficient); abscissa: time of ^{45}Ca efflux. The bars above the various efflux periods indicate the nature of the Na substitutes. The Ca-free rinsing solutions contained 0.5 mmol/l EGTA; the solution during the last efflux period of the aortic strip contained 1.8 mmol/l $CaCl_2$. Note the marked decrease of ^{45}Ca efflux from the aortic strip when NaCl in the rinsing solution was replaced by choline chloride and the subsequent increase when Na was readmitted; no such effect occurred with the adventitia.

These results suggest that there is a Na-sensitive fraction of Ca efflux from the aortic strip which is not due to cation exchange at extracellular Ca binding sites but more likely reflects a ^{45}Ca efflux from the smooth muscle cells. This conclusion is supported by the experiment shown in figure 4. In this experiment ^{45}Ca efflux from the aortic strip was measured in solutions which were of different ionic composition but which all contained 0.4 mmol/l $LaCl_3$. Under this condition most of the extracellular Ca binding sites should be occupied by La (van Breemen *et al.* 1972). On the other hand, in cardiac muscle La has no effect on the dependence of Ca efflux on $[Na]_o$ and $[Ca]_o$ (Katzung, Reuter & Porzig 1973). In the presence of $LaCl_3$, the average rate coefficients (fractions of ^{45}Ca lost per minute) of Ca efflux from aortic strips were approximately one half of those measured at the same times in the absence of $LaCl_3$ (cf. figures 3*a* and 5). Despite this reduction of Ca efflux by La, removal of $[Na]_o$ caused a further decrease similar to that shown in figure 3*a*. The addition of $[Na]_o$ and $[Ca]_o$ increased ^{45}Ca efflux. The decrease of ^{45}Ca efflux after removal of $[Na]_o$ also occurred in the presence of $[Ca]_o$. Readmittance of Na increased Ca efflux again. No tension was developed by the preparations in La-containing solution when $[Na]_o$ was reduced. However, the addition of adrenaline at the end of the

experiment caused an appreciable increase in tension, indicating that the ability of the preparations to contract was not impaired.

Without La in the rinsing solutions removal of $[Na]_o$ in the presence of $[Ca]_o$ increased ^{45}Ca efflux from the aortic strips. Figure 5 shows ^{45}Ca efflux and the simultaneously recorded tension developed by the strip when NaCl was replaced by LiCl. The first hour of ^{45}Ca efflux in Ca-free, EGTA-containing Na medium is not shown in this figure. When NaCl was substituted by LiCl in the presence of Ca the muscle developed tension which was not well maintained. Coincident with the increase in tension ^{45}Ca efflux increased. Removal of Ca from the LiCl solution caused further relaxation (after a slight increase in tension which was not observed in all experiments) and a further drop in ^{45}Ca efflux. Addition of Ca to the solution increased both tension and ^{45}Ca efflux again. This result suggests that the rise in Ca efflux during substitution of NaCl by LiCl or choline chloride in the presence of $[Ca]_o$ is largely due to an exchange of intracellular for extracellular calcium. The Ca–Ca exchange should be particularly prominent when both the intra- and extracellular Ca ion concentrations are high. In the present experiments the rather high intracellular Ca ion concentration may be reflected by the contracture. In the absence of $[Na]_o$ the transport sites at the outer surface of the membrane should be mainly occupied by Ca. Since under this condition there is no inwardly directed Na gradient, net outward transport of Ca should be inhibited while net inward movement of Ca increases. This explains the increase in the Ca content of the tissue demonstrated in table 1.

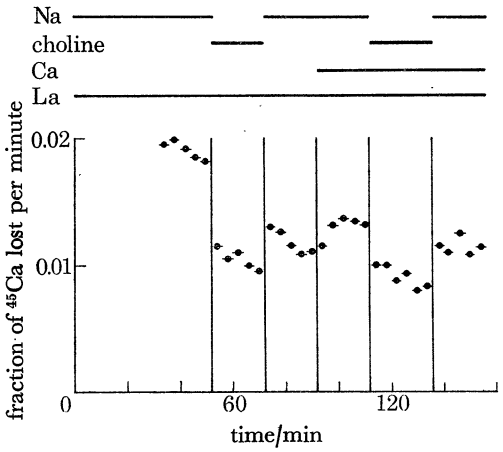

FIGURE 4. The effects of external Na and Ca on ^{45}Ca efflux from a rabbit aortic strip. All inactive rinsing solutions contained 0.4 mmol/l $LaCl_3$ in order to reduce binding of extracellular Ca; the bars above the various efflux periods indicate the changes in ionic composition of the rinsing solutions as compared to the standard Na medium (see text). Ordinate and abscissa as in figure 3.

If the increased Ca entry causes the concentration of ionized Ca in the myoplasm to increase significantly, this would not only account for the tension development in Na-free solution but also for the increase in Ca efflux since more Ca ions are available at the inner surface of the membrane.

CONCLUSION

The results show that in aortic strips, as in cardiac muscle and nerve, Ca influx and efflux are partially dependent on $[Na]_o$. The increase in tension in K-free or ouabain-containing solutions suggests that the fluxes may also be dependent on $[Na]_i$. However, Ca influx and efflux have

not yet been investigated under conditions of increased $[Na]_i$. Such an investigation would be of particular interest in view of the possible relationship between increased $[Na]_i$ and hypertension (Schoffeniels 1969). Increase in peripheral vascular resistance has also been shown to occur in men (Mason & Braunwald 1964) and dogs (Vatner, Higgins, Franklin & Braunwald 1971) after the injection of doses of cardiac glycosides which had little effect on cardiac output.

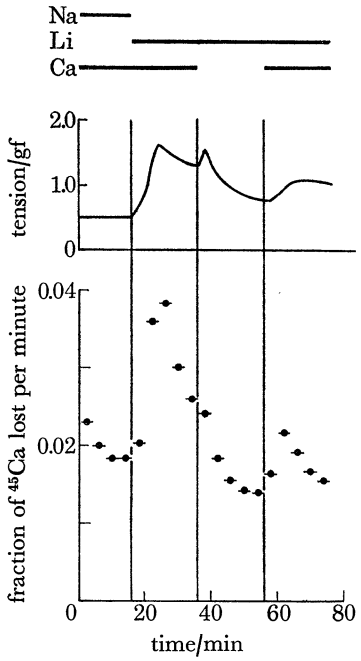

FIGURE 5. The effects of removal external Na and Ca on ^{45}Ca efflux (lower part) and tension development (upper part; graphed from the original record; preload 0.5 gf) measured in a rabbit aortic strip. The Ca-free Li solution contained 0.5 mmol/l EGTA. The initial efflux period in Ca-free Na medium is not shown.

If this increase in vascular tone is due to a rise in $[Ca]_i$ which occurs as a consequence of increased $[Na]_i$, it has to be concluded that the Na–Ca exchange system is an important factor in the regulation of the tone. The fact that the sarcoplasmic reticulum of vascular smooth muscle is less extensive than that of skeletal muscle (Devine, Somlyo & Somlyo 1972), and may have a less active calcium pump (Fitzpatrick, Landon, Debbas & Hurwitz 1972) tends to support this view.

On the other hand, thermodynamic considerations indicate that, on the basis of an electrically neutral 2 Na^+ for 1 Ca^{2+} exchange, the Na gradient across the sarcolemma cannot alone provide the energy to reduce the myoplasmic Ca ion concentration sufficiently to cause complete relaxation of the contractile proteins. An alternative hypothesis is a series system in which the sarcoplasmic reticulum is in close approximation to the sarcolemma; the Na–Ca exchange could take place between the extracellular space and the Ca stores of the sarcoplasmic reticulum, while the main Ca uptake from the myoplasm could occur into the sarcoplasmic reticulum. In this case the Na–Ca exchange system would mainly regulate the total Ca concentration in the sarcoplasmic reticulum and influence the myoplasmic Ca ion concentration only indirectly. Although this seems an attractive hypothesis, supporting evidence is not available at present.

Furthermore, our results show that only about 50 % of total ^{45}Ca efflux from the aortic strips

is dependent on $[Na]_o$ and $[Ca]_o$. It is quite possible that in addition to Na–Ca exchange, another Ca transport system exists in the sarcolemma which helps to keep $[Ca]_i$ at a low level.

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