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THE EFFECTS OF EXTERNAL SODIUM SUBSTITUTION ON CELL SODIUM AND POTASSIUM IN VASCULAR SMOOTH MUSCLE

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SUMMARY

1. The entry of Li into the vascular smooth muscle cells of the rat tail artery follows first-order kinetics with a rate constant of approximately 1·3 hr^{-1} at 10 mM-$[Li]_o$. The rate constant decreases gradually to *ca.* 0·5 hr^{-1} when the $[Li]_o/[Na]_o$ ratio is increased.

2. Replacement of Na with Li over the range of $[Li]_o$ from 1 to 115 mM, accomplished at constant ionic strength and osmolarity of the bathing solution, produces changes in cell Na and K without apparent change in cell water. At equilibrium, cell Li increases in linear proportion to $[Li]_o$, at a ratio of 2:1 throughout the range. The increase in cell Li is associated with inverse falls in both cell K and Na such that the ratio of cell K to cell Na remains constant at *ca.* 10:1 throughout.

3. The changes in the ionic contents, induced by equilibration of the tissue with a Na-free, Li-substituted solution, are reversible.

4. Replacement of Na with sucrose over the range of 40–115 mM results, at equilibrium, in a linear fall in cell Na without conspicuous change in cell K. A constant portion of the cell Na, *ca.* 10 m-mole/kg dry wt., does not participate in this exchange.

5. At equilibrium, reductions in $[Na]_o$ are reflected in corresponding reductions in apparent $[Na]_i$ such that the $[Na]_o/[Na]_i$ ratio remains constant.

INTRODUCTION

It is common practice to explore the role of ions in regulating vascular smooth muscle tension by changing the ionic environment of the tissue (Reuter, Blaustein & Haeusler, 1973). In general, the observations are then interpreted either as a direct effect of the imposed change itself (Holloway & Bohr, 1973) or as the indirect effect of an inferred redistribution of ions (Brace & Anderson, 1973). Because little is known about the steady state ionic redistribution which eventually results from such

alterations of the ionic environment, we decided to examine this directly. The present report concerns the effects of changing $[Na]_o$, with Li or sucrose as contrasting substitutes, on the time course of monovalent cation redistribution as well as on the resulting steady state.

METHODS

Adult male albino rats of an inbred Wistar strain were used throughout. They were anaesthetized with pentobarbitone; the ventral tail artery was rapidly and gently excised, halved, and at once placed into aerated normal physiological salt solution (PSS) for 3 hr. Following this period of recovery, termed pre-incubation, the arteries were transferred to test media for further incubations as will be described for each experiment.

At the end of the prescribed experimental incubation, the arteries were quickly blotted by standard compression at 15 g/cm^2 between aluminium blocks, and then transferred to weighing cups. Wet weights were obtained immediately after the artery was placed in the cup; the tissues were then processed by drying to constant weight, defatting, extraction for 7 days in 4 ml. 0·75 M nitric acid, and atomic absorption analysis (Palatý, Gustafson & Friedman, 1971).

The composition (mM) of the basic physiological salt solution (PSS) was: NaCl, 115·0; $NaHCO_3$, 25·0; NaH_2PO_4, 1·2; KCl, 5·0; $CaCl_2$, 1·7; $MgSO_4$, 1·2 and D-glucose, 11·0. This was modified for low Na solutions by replacement of NaCl up to the maximum value of 115 mM, either with LiCl without changing ionic strength and osmolarity, or with sucrose isosmotically but changing ionic strength. For complete replacement of Na with Li (LiPSS), appropriate amounts of LiCl and Li_2CO_3 and KH_2PO_4 were substituted for the respective Na salts. All media were maintained at pH 7·4 with continuous aeration with 95 % O_2, 5 % CO_2. The detailed composition and method of preparation of these solutions have been presented elsewhere (Friedman, Mar & Nakashima, 1974).

Results are expressed as mean values with the estimated standard error of the mean. Exponentials and linear regressions were computed by the method of least-squares using minicomputer programmes.

RESULTS

The measurement of cell Li

In previous studies we have shown that the movement of Li or of Na across the vascular smooth muscle cell membrane is sufficiently slowed by low temperature to permit simple differentiation of cellular from extracellular locations of these ions (Friedman, 1974, 1975; Friedman *et al.* 1974). In essence, incubation of a tissue sample at 3° C in a complete physiological medium containing Li in place of Na (LiPSS) allows ready replacement of Na in the paracellular matrix, as well as free Na in the extracellular fluid, while leaving cell Na almost unaffected. Conversely, in a tissue enriched with Li, extracellular Li can readily be replaced with Na and cell Li left substantially unaffected.

This experiment was designed to re-examine the rate of Li–Na exchange

at 3° C more closely in order to provide appropriate correction factors for the experiments to follow. Twenty-four artery samples were pre-incubated as usual for 3 hr at 37° C in normal PSS to permit recovery from excision. They were then transferred in two groups of twelve to fresh medium in which a nominal 10 or 40 mM-NaCl was replaced by LiCl, and incubated

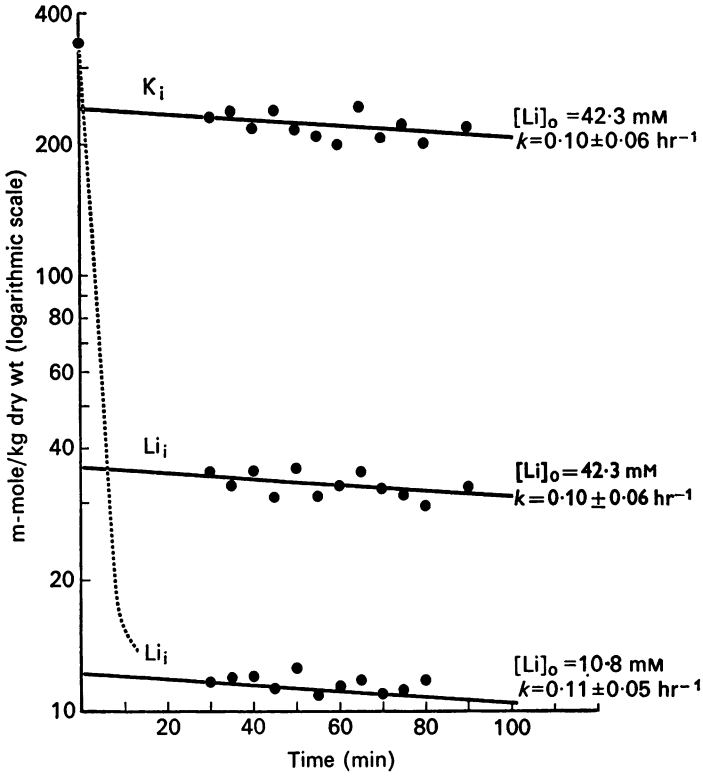

Fig. 1. Arteries were incubated for 1 hr at 37° C in media containing nominal 10 or 40 mM-LiCl as replacement for NaCl. They were then transferred to normal, Li-free medium at 3° C for 30–90 min. A rapid initial loss of extracellular and a continuing slow loss of cellular Li are observed. The rate of cellular loss is independent of the size of the pool and is similar to the rate of loss of cell K at this low temperature. Each point is one sample. Corresponding K values are shown only for the higher Li substitution.

in this Li-enriching medium at 37° C for a further 1 hr. The samples were then rapidly placed in cold (3° C) PSS for intervals from 30 to 90 min to replace extracellular Li with Na before routine tissue processing.

As shown in Fig. 1, a rapid initial washout of extracellular Li was observed as before. This was followed by a slow continuing loss of Li, associated with a parallel loss of K. This slow continuing ion loss was

independent of the degree of cell enrichment with Li and is due, as we have shown (Friedman, 1974), to the replacement of cell Li and K with Na which can still occur even at low temperature. The rate is too slow to be reflected by a measurable increase in total tissue Na. Thus, total tissue Na averaged 344 ± 10 and 352 ± 5 m-mole/kg dry wt. in the two groups, and was not conspicuously different at the beginning or end of the time period.

In view of the slow exchange rates involved, we conclude that 45 min in cold PSS is quite sufficient to wash out extracellular Li. Although a little cell Li may also be removed, this would not exceed 8 % of the total.

The rate of Li influx

Previous experience had shown that Li, under the driving force provided by fully Li-substituted medium, moves into the vascular smooth muscle cell at a rate of about 0·5–0·6 hr^{-1} at 37° C (Friedman, 1975). For the projected series of experiments to be reported here, an estimate of Li influx at lesser initial gradients was required. Accordingly, thirty-six artery samples, pre-incubated as usual in normal PSS for 3 hr at 37° C, were transferred in three groups of twelve to fresh medium in which either a nominal 10, 20 or 40 mM-NaCl were replaced with LiCl. The samples remained in this Li-enriching medium for intervals from 30 to 90 min. They were then washed in cold (3° C) PSS for 45 min to remove extracellular Li before routine processing.

As shown in Fig. 2, the rate of Li influx within the range of 10–40 mM replacement of Na in the medium can be readily described by first-order kinetics as with 100 % substitution (Friedman, 1975). A rate constant of approximately 1·3 hr^{-1} with 10 mM replacement ($t_{\frac{1}{2}}$, 0·5 hr) dropping to less than half this value with 40 mM replacement was observed. Values of about 0·5 hr^{-1} ($t_{\frac{1}{2}}$, 1·4 hr) were previously reported with 100 % replacement. This interesting relation of influx rate to the concentration of Li in the medium was not pursued further at this time.

From these results, we conclude that reasonable estimates of the steady state distribution of $[Li]_o/[Li]_i$ at levels below 20 mM-$[Li]_o$ can be derived from 2 hr incubations which conveniently fit a laboratory day. Overnight incubations would certainly be required for the study of higher concentrations of $[Li]_o$.

The equilibrium distribution of Li, Na and K in arteries incubated in media containing less than 20 mM-Li as replacement for Na

Arteries were excised and allowed to recover in PSS at 37° C for 3 hr. They were then transferred in five groups of six to fresh media containing 1·75–16·25 mM-Li as replacement for Na. One group of tissues was trans-

ferred to fresh normal PSS as control. Following incubation at 37° C for 2 hr, the samples were removed, gently blotted free of excess medium, and placed rapidly in cold PSS (3° C) for 45 min to wash out extracellular Li. A duplicate experiment was carried out in which the final wash was in cold LiPSS for 45 min to wash out extracellular Na and permit estimation of cell Na.

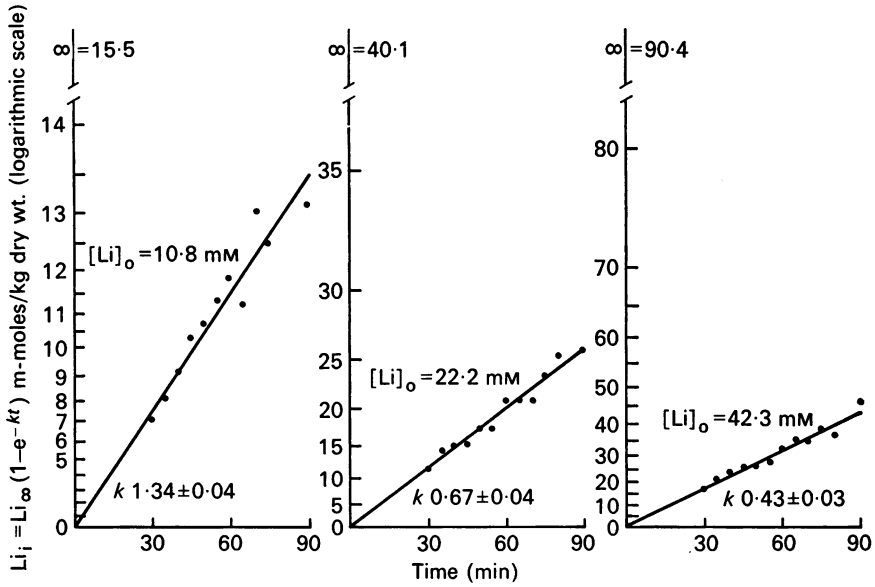

Fig. 2. Arteries were incubated for 30–90 min at 37° C in media containing nominal 10, 20 or 40 mM-LiCl as replacement for NaCl. They were then transferred to normal, Li-free medium at 3° C for 45 min to remove extracellular Li. The values for residual cell Li increase as a simple exponential function of the incubation time. Each point corresponds to one sample and the line of best fit was computed by iterative fitting.

As shown in Fig. 3, cell Li increased in direct proportion to $[Li]_o$ throughout the range of this experiment. According to the raw observations for the 2 hr incubation, the ratio of cell to extracellular increase was 1·5. In the previous experiment the 2 hr values fell short of the asymptote for the exchange by an amount which, at 20 mM-Li substitution, could be as much as 25 %. If the raw data of this experiment are corrected by this maximal amount, the ratio of cellular to extracellular Li is 2·0. Even with this correction, the proportional distribution of Li remains distinctly linear. The gain in cell Li is at the expense of a corresponding loss of cell K. There may be a loss of cell Na as well, but the degree of change is too small in this limited range of Li substitution to be certain.

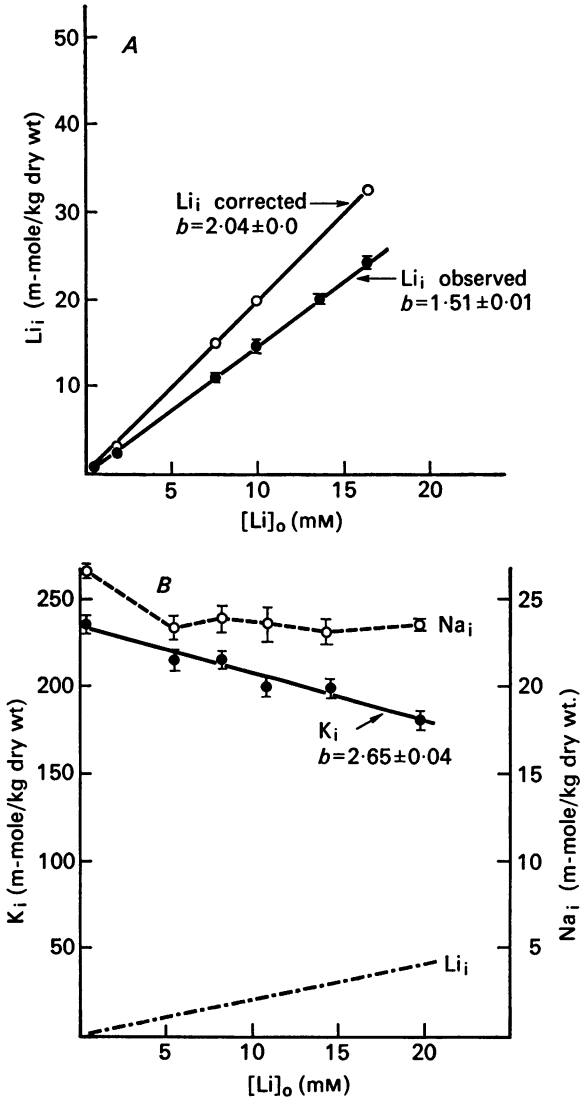

Fig. 3. Arteries were incubated for 2 hr at 37° C in media containing LiCl as replacement for NaCl in the range of 1–20 mM. They were then transferred for 45 min at 3° C either to normal medium for the measurement of cell Li or to Na-free, Li-substituted medium (LiPSS) for the measurement of cell Na. Each point for Li or Na is the average of six arteries, and for K the average of twelve. Vertical lines indicate S.E. of the mean. *A*, cell Li increases linearly with rising $[Li]_o$ even when observed values are maximally corrected for incomplete entrance of Li into cells in 2 hr. *B*, the increase in cell Li is inversely related to cell K and a similar relation with cell Na is suggested.

The equilibrium distribution of Li, Na and K in arteries incubated in media containing more than 40 mM-Li as replacement for Na

Arteries were excised and allowed to recover in PSS at 37° C for 3 hr. They were then transferred in five groups of six to fresh media containing 41–115 mM-Li as replacement for Na, while a sixth group was retained in fresh PSS as control. The upper limit of replacement was chosen as that which could be attained by simple substitution without manipulation of the buffer system of the medium. The samples were incubated overnight at 10° C and on the next morning transferred to fresh, continuously aerated medium at 37° C for a further 3 hr. They were then rapidly removed, gently blotted, and placed in cold PSS for 45 min to wash out extracellular Li. A duplicate experiment was carried out in which the final wash was in cold LiPSS for 45 min to wash out extracellular Na and permit the estimation of cell Na.

Cell Li increased in direct proportion to $[Li]_o$ throughout the range of the experiment at a ratio of 1·9, essentially the same ratio as that observed over the low range of Li substitution (Fig. 4). The increase in cell Li was attained at the expense of both intracellular Na and K in proportion to their normal 1:10 ratio in cells. The constant sum of the three cations imposed by the medium changes was matched by a similar constancy in their intracellular sum.

No change in total water content was observed in any group in this experiment (Table 1). Since ionic balance and ionic strength, as well as osmolarity of the medium, were the same in all groups, there was evidently no significant change in cell water. Our best estimate for free cell water is *ca.* 1 l./kg dry wt. (Friedman *et al.* 1974), so that cell ion content converts easily into a maximum value for cell ion concentration by assuming all the ion to be free. In the case of Na, we have reason to think that only about 60 % of intracellular Na is normally free (Friedman *et al.* 1974), and on this basis a calculated minimal value for cell $[Na]_i$ is shown in Table 1. The calculation assumes that, broadly speaking, this same proportionality is maintained as Li enters the cell and replaces Na in all its locations. This assumption is supported by the observation that the linear function describing total cell Na in relation to $[Li]_o$ intersects zero when Na in the medium is totally replaced by Li.

These observations indicate that, at equilibrium, the transmembrane concentration gradients for Na and for Li remain remarkably constant over a very broad range of external concentration. To test objectively that a steady state obtained and was reversible, arteries were incubated at room temperature overnight in LiPSS, a medium in which Na is fully replaced by Li. They were then transferred for recovery to normal medium

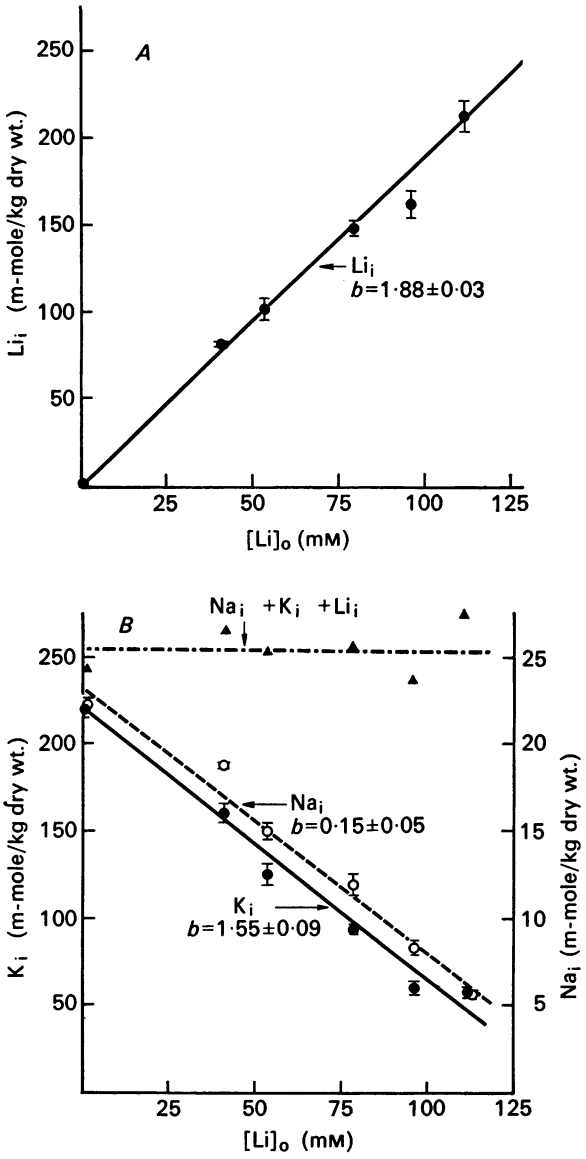

Fig. 4. Arteries were incubated overnight (18 hr) at 10° C and for an additional 3 hr at 37° C in media containing LiCl as replacement for NaCl in the range of 40–115 mM. They were then transferred for 45 min at 3° C either to normal medium for the measurement of cell Li or to Na-free, Li-substituted medium (LiPSS) for the measurement of cell Na. Each point for Li or Na is the average of six arteries, and for K the average of twelve. Vertical lines indicate S.E. of the mean. *A*, cell Li increases linearly with rising $[Li]_o$. *B*, the increase in cell Li is inversely related to both cell Na and cell K. The ratio of cell Na to K, and the sum of cell Na, K and Li remain constant.

for 1, 2 or 3 hr, or to fresh LiPSS for an additional 2 hr period. The tissues were then washed in cold PSS for 45 min to wash out extracellular Li. A progressive extrusion of cell Li and re-uptake of K during recovery from even this extreme degree of Li enrichment is evident (Table 2).

TABLE 1. Cell Na, Li and K concentrations (mM) and transmembrane distribution ratios in groups of six arteries incubated for 18 hr at 10° C, followed by 3 hr at 37° C in media containing 40–115 mM-Li as replacement for Na. Calculations use a sorbitol space derived value for cell water as a fraction of total water

Incubation medium						
$[Na]_o$ (mM)	136	101	83	62	40	27
$[Li]_o$ (mM)	0	41	53	79	97	112
$[K]_o$ (mM)	5·5	5·4	5·4	5·4	5·4	5·4
Artery						
H_2O_{total} (l./kg dry wt.)	3·03 ± 0·05	3·18 ± 0·04	3·13 ± 0·05	3·11 ± 0·04	2·99 ± 0·06	3·08 ± 0·05
H_2O_{cell} (l.(est.)/kg dry wt.)	1·0	1·0	1·0	1·0	1·0	1·0
$[Na]_i$ (mM)*						
Maximum	22·1 ± 0·4	18·6 ± 0·2	14·9 ± 0·4	11·9 ± 0·7	8·3 ± 0·4	5·6 ± 0·2
Minimum	13·3	11·2	8·9	7·1	5·0	3·4
$[Li]_i$ (mM)	0	82 ± 2	101 ± 8	149 ± 4	161 ± 9	213 ± 9
$[K]_i$ (mM)	221 ± 4	159 ± 6	125 ± 6	94 ± 2	60 ± 3	54 ± 2
E_{Na}	+58	+55	+56	+55	+52	+52
E_{Li}		−17	−16	−16	−13	−16
E_K	−93	−85	−79	−71	−60	−57

* $[Na]_i$ maximal = cell Na ÷ 1·0 l. cell water, minimal = (cell Na × 0·6) ÷ 1·0 l. cell water (see text).

± = S.E. of mean.

TABLE 2. Total H_2O, cell K and cell Li (mg/kg dry wt.) in arteries incubated overnight at 22° C in medium in which Li replaces Na (LiPSS) followed either by an additional 2 hr in the same medium or a return to normal medium (PSS) for 3 hr at 37° C

	Artery			No. of
Procedure	H_2O (l.)	K_i (m-mole)	Li_i (m-mole)	samples
LiPSS 20 hr	3·35 ± 0·05	20 ± 0·7	252 ± 5	12
LiPSS 21 hr	3·33 ± 0·02	20 ± 0·6	250 ± 6	12
LiPSS 22 hr	3·28 ± 0·04	20 ± 0·6	247 ± 7	12
LiPSS 20 hr				
+PSS 1 hr	3·17 ± 0·06	108 ± 0·5	130 ± 9	12
+PSS 2 hr	3·18 ± 0·05	160 ± 0·6	69 ± 5	12
+PSS 3 hr	3·11 ± 0·06	186 ± 0·7	32 ± 4	12

± = S.E. of mean.

The equilibrium distribution of Na and K in arteries incubated in media containing sucrose as replacement for more than 40 mM-Na

Arteries were excised and allowed to recover in PSS at 37° C for 3 hr. They were transferred in five groups of six to fresh media containing 80–230 mM sucrose as replacement for NaCl, while a sixth group was retained in fresh PSS as control. These limits of replacement were chosen to match the Li replacement of the previous experiment. The samples were incubated overnight at 10° C and on the next morning transferred to fresh, continuously aerated medium at 37° C for a further 3 hr. They were then rapidly removed, gently blotted, and placed carefully in cold LiPSS for 45 min to wash out extracellular Na.

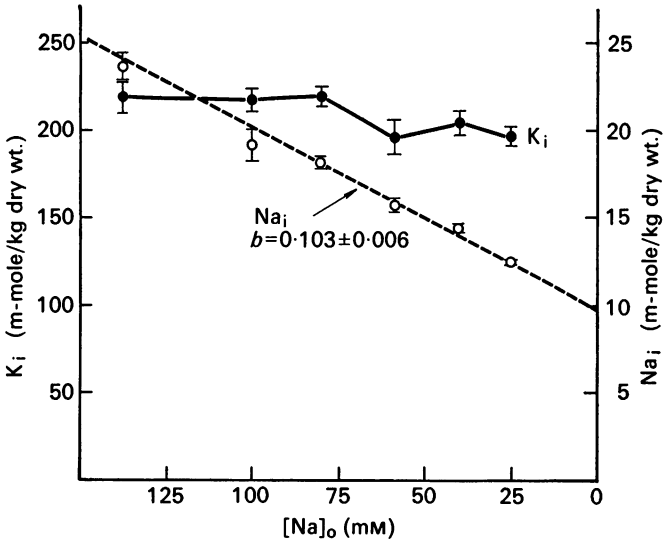

Fig. 5. Arteries were incubated overnight (18 hr) at 10° C and for an additional 3 hr at 37° C in media with sucrose as replacement for 40–115 mM-NaCl. They were then transferred to Na-free, Li-substituted medium (LiPSS) at 3° C for 45 min for the measurement of cell Na. Cell Na falls linearly as $[Na]_o$ is reduced. A constant 9·8 m-mole Na/kg dry wt. does not participate in the exchange.

As seen in Fig. 5, cell Na fell linearly with the fall in $[Na]_o$ as in the preceding experiment. A constant value of 9·8 m-mole of cell Na does not participate in this process, confirming our earlier estimate that only about 60 % of cell Na is normally free. Only a small fall in cell K was observed.

No change in total water content was observed in any group in this experiment (Table 3). Since osmolarity was constant throughout, a value of 1 l./kg dry wt. was assumed as a first approximation for cell water.

From this, it is apparent that the transmembrane Na concentration gradient, either expressed by the ratio $[Na]_o/[Na]_i$ or by the calculated equilibrium potential for Na, E_{Na}, remains constant throughout this broad range of change in $[Na]_o$. The value of E_K remains similarly steady. How the Donnan equilibrium is established is not clear from these data alone.

TABLE 3. Cell Na and K concentrations (mM) and transmembrane distribution ratios in groups of six arteries incubated for 18 hr at 10° C followed by 3 hr at 37° C in media containing sucrose as replacement for 40–115 mM-Na. Calculations use a sorbitol space derived value for cell water as a fraction of total water

Incubation medium						
$[Na]_o$ (mM)	137	100	80	58·5	40·5	25
$[K]_o$ (mM)	5·4	5·5	5·5	5·4	5·2	5·1
m-osmole (sucrose subst.)	288	292	290	282	282	285
Artery						
H_2O_{total} (l./kg dry wt.)	2·95 ± 0·07	2·95 ± 0·05	2·99 ± 0·04	2·93 ± 0·09	2·87 ± 0·09	2·80 ± 0·12
H_2O_{cell} (l.(est.)/kg dry wt.)	0·97	0·97	0·99	0·97	0·95	0·92
$[Na]_i$ (mM)*						
Maximal	23·4 ± 0·9	19·2 ± 0·8	18·1 ± 0·5	15·7 ± 0·5	14·3 ± 0·7	12·4 ± 0·3
Minimal	13·6	9·4	8·3	5·9	4·5	2·6
$[K]_i$ (mM)	218 ± 9	216 ± 6	217 ± 6	194 ± 10	202 ± 7	195 ± 5
E_{Na}	+58	+59	+57	+58	+55	+57
E_K	−93	−92	−93	−91	−92	−92

* $[Na]_i$ maximal = cell Na ÷ 1·0 l. cell water, minimal = (cell Na − 9·8) ÷ 1·0 l. cell water (see text).

± = S.E. of mean.

The equilibrium distribution of Na and K in arteries incubated in media containing an increment of Li or of Na

The previous experiments had indicated that the transmembrane distribution ratio for Na remained constant over a broad range of changes in $[Na]_o$. This experiment was designed to test whether this condition would continue to hold if the cell was faced with an increment of external Li or Na.

Two experiments were carried out. In the first, following the standard 3 hr incubation, arteries were transferred in groups of six to fresh media containing stepped additions of Li as LiCl to a nominal +20 mM level, and allowed to equilibrate overnight at 10° C, and then for an additional 2 hr in fresh medium at 37° C. They were then washed either in cold PSS for the measurement of cell Li or cold LiPSS for the measurement of cell

Na. In the second experiment, the tissues were incubated similarly in media containing stepped additions of Na as NaCl. The increments of Li and Na used in this experiment were modest in order not to impose any extreme osmotic challenge to the cell.

The changes in ion distribution proved to be measurable only at the upper end of the range of ion addition (Table 4). In the case of Li addition, no significant change was observed either in Na or K distribution. As in the previous experiments involving Li substitution, Li was distributed in a 2:1 ratio between cells and environment. Similarly, in the case of Na addition, the increment of Na was distributed in the same ratio between cells and environment as had been observed with decrements of Na in the previous experiments.

TABLE 4. Cell Na, Li and K concentrations (mM) in groups of six arteries incubated for 18 hr at 10° C followed by 3 hr at 37° C in media containing a nominal 20 mM increment of Li or Na. Calculations use a constant (1 l.) value for cell water

	Li addition		Na addition	
Incubation medium				
$[Na]_o$ (mM)	138	138	139	166
$[Li]_o$ (mM)	0	18·4	—	—
$[K]_o$ (mM)	5·5	5·6	5·5	5·5
Artery				
H_2O_{total} (l./kg dry wt.)	2·89 ± 0·05	3·11 ± 0·06	2·88 ± 0·04	2·93 ± 0·03
$[Na]_i$ (mM maximal)	21·9 ± 1·2	21·9 ± 0·2	19·0 ± 0·4	22·6 ± 0·4
$[Li]_i$ (mM maximal)	0	36·3 ± 1·2	—	—
$[K]_i$ (mM)	197 ± 6	191 ± 5	205 ± 6	210 ± 6

± = S.E. of mean.

DISCUSSION

These experiments provide some basic information concerning ion distribution in vascular smooth muscle. They also have important implications for experiments that involve manipulation of the ionic environment of vascular tissue. To begin with, the entry of Li or of Na into the vascular smooth muscle cell has, as before (Friedman, 1975), been found to be rather slow. A tissue exposed to Li in the medium, either as increment or replacement ion, can take as long as 8 hr to approach a new steady state and even when small amounts are concerned is not likely to do so in much less than 2 hr. In the period of unsteady state, complex readjustments involving water and ions are occurring, and patterns of reactivity or of electrical activity cannot readily be interpreted in terms of precise ionic distribution. This time constraint is no less when Na is added to the medium, or when the adjustment requires the active extrusion of Na from cells, as in response to an abrupt reduction of $[Na]_o$.

In a previous attempt to characterize the Na phases in the vascular smooth muscle cell, we estimated that about 10 m-mole, or about 40 %, of cell Na was in some way restricted and not free in the available cell water (Friedman *et al.* 1974). In the present experiments, objective evidence of this was obtained, thanks to the linearity of a fall of total cell Na which occurs when $[Na]_o$ is reduced and replaced with sucrose. Without in any way indicating the manner of constraint, it may now confidently be asserted that about 10 m-mole/kg dry wt. of cell Na is not readily available to the free cell water. The experiments with Li substitution show that a major part of this constrained Na is capable of being replaced by Li – strong evidence that much of this component is associated with the fixed-charge sites of the cell matrix. This conclusion agrees well with observations in other tissues (Hinke, Caillé & Gayton, 1973; Palatý & Friedman, 1975).

When Li is used to replace Na in the medium over the range of 1–115 mM, or when about 20 mM is added to a normal medium, the ratio $[Li]_i/[Li]_o$ remains constant at *ca.* 2·0. This ratio is about four times lower than would be expected if Li were simply passively distributed. Thus, the estimated equilibrium potential for Li^+ (Table 1) appears to be approximately -16 mV, whereas Hermsmeyer (1976) has measured a membrane potential of *ca.* -55 mV for the smooth muscle cells of this artery. No further conclusions can be drawn, however, from these data concerning the mechanisms which determine the steady state distribution of Li.

In the experiments in which LiCl was used to replace NaCl, cell Na and K were both replaced by Li. Accordingly, within the limits of experimental error, the sum of Na, K and Li remains constant throughout. When Na is replaced by sucrose, however, cell Na falls proportionately while K remains essentially unaffected. This indicates that ionic readjustments become especially complex when the ionic strength of the medium is also varied.

Perhaps the most striking observation concerning steady state ion distribution reported here is the linear relation that is maintained between extracellular and cellular Na over a very broad range of $[Na]_o$ extending from about 20 mM above to 115 mM below the basal physiological level. The ratio $[Na]_o/[Na]_i$, which we have for convenience termed the Na gradient, remains constant over almost a decade span whether $[Na]_o$ is changed by elimination or by ion substitution. The ionic steady state represented by this gradient is metabolically imposed, is a major determinant of the total ionic distribution of the cell, and may well be a characteristic of its membrane.

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