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The Action of Ouabain on the Smooth Muscle Cells of the Rat Tail Artery[1] (38199)

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When the rat tail artery is incubated at 2° in a medium containing lithium in place of sodium (LiPSS), extracellular Na is replaced in less than 30 min. At this temperature, however, Li does not readily traverse the cell membrane; as a result, cell Na can be measured as the amount of Na remaining in the tissue after this period of equilibration (1). With this simple method, we have shown that cell Na increases in a 1:1 relation with K loss when the transmembrane gradients are discharged in a K-free medium. In this report, the effects of ouabain on cell Na and K are examined to provide a further test of the usefulness of the method and to complement our recent study of the relation of Na^+–K^+ gradients to vascular resistance (2). Cell K reaches lower, and cell Na higher, levels after prolonged incubation in a medium which lacks K than in one which contains 1 m*M* ouabain.

Methods. The methods have been fully described (1). In brief, the tail artery of an adult male rat is gently excised under pentobarbitone anaesthesia. It is then incubated overnight in a K-free medium in the refrigerator (3°) and, on the next morning, transferred to a continuously aerated normal physiological salt solution (PSS) at 37° for 3 hr to complete the preliminary phase (3). Arteries so prepared are then incubated in one of the experimental media listed in Table I for predetermined intervals. At the end of the prescribed interval, the artery is rapidly transferred to cold LiPSS at 2° for 30 min (or, where required, 60 min) to wash out extracellular Na. It is then quickly but very gently blotted and immediately weighed in a stoppered glass cup. It is then processed by drying to constant weight, defatting, extraction for 7 days in 4 ml of 0.75 *M* nitric acid, and ion analysis by atomic absorption spectrophotometry.

Results are expressed as mean values with the estimated S.E. of the mean. Unless otherwise noted, each individual value in figure or table is the mean of a group of 6 arteries. Exponentials were computed by the method of least squares.

Results. Cell Na and K in K-free or ouabain medium. In this experiment, tissues were incubated either in a K-free medium or in a normal medium containing 1 m*M* of ouabain for 0–3, or 4 hr, followed in each case by a final incubation in LiPSS at 2° for 30 min to wash out extracellular Na. This concentration of ouabain was more than adequate for full blockade since we have already shown that the rate of K loss is not further increased by doubling the dose (2). A maximal rate of K loss is actually attainable in this tissue with 0.75 m*M* ouabain (V. Palatý, personal communication). Each artery was handled individually and groups of 6 were used for measurements at each interval. As shown in Fig. 1, the results can be readily approximated by single exponential functions.

As a preliminary to the quantitative analysis of these observations, the extracellular space of the artery was measured

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TABLE I. The Composition of Physiological Salt Solutions (in m*M*), Aerated with 95% O_2, 5% CO_2. pH 7.4 ± 0.1 at 37°.

Soln.	Na	K	Ca	Mg	Cl	HCO_3	HPO_4	Li	CO_3	glucose
Normal (PSS)	141.2	5	1.7	1.2	123.4	25	1.2	—	—	11
K-free	146.2	—	1.7	1.2	123.4	25	1.2	—	—	11
Li subst. (LiPSS)	—	5	1.7	1.2	123.4	—	1.2	141.2	25	11
K-free Li subst.	1.2[a]	—	1.7	1.2	123.4	—	1.2	141.2	25	11

[a] Required for buffering.

in separate experiments with ^{14}C-inulin and ^{14}C-sorbitol to determine to what extent tissue K values represent cell K. The extracellular fluid volume was 54 ± 2% of tissue water with the first marker and 62 ± 1% with the second. Since only 8–10 mmole of K was thus noncellular, a standard correction seemed justified and 10 mmole/kg dry weight was adopted for converting total K to the cell K. A 10% change

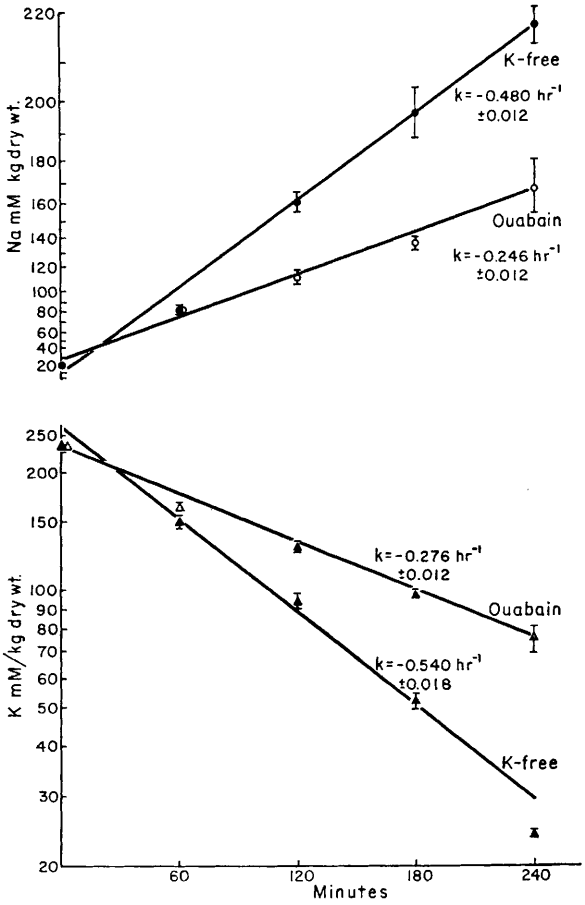

FIG. 1. Changes in Na and K in arteries incubated in K-free or 1 m*M* ouabain PSS followed by 30 min wash in 2° LiPSS to remove extracellular Na. Values are fitted to single exponential functions by method of least squares.

in extracellular water introduces a negligible error of 1 mmole into this corrected cell K value.

The relation of the loss of cell K to the gain in cell Na over the 4 hr duration of the experiments is shown in Table II. Both with ouabain PSS and K-free PSS, a 1:1 Na-K exchange was observed. Since the measurements depend on a slow rate of cell exchange with Li during the final cold wash period, this was checked by exposing an additional group of arteries to 2° LiPSS for 60 min. Similar values were obtained in both cases. The fact that the Li uptake in the 30 min cold wash was the same at the end of the experiment as at the beginning and that the sum of cell Na ± K also remained constant provides additional evidence that only extracellular Na was exchanged during the cold wash.

If it is assumed, as in Fig. 1, that all of the potassium contained within cells is available for replacement by sodium, then alterations in passive permeability may be involved. On the other hand, it is equally possible that the exponential rate constant, as distinct from the rate itself, is approximately the same in both instances, and then a significant difference in asymptotic values would underlie the observations. These possibilities were next explored.

The effect of ouabain and of K-free media on the entrance of Li into cells. To examine the extent to which the previous observations might reflect changes in passive permeability, the effect of ouabain, or of a K-free medium, on the rate with which lithium can enter cells was examined. For this experiment, the arteries were incubated in LiPSS at 37° for specified intervals after which the tissues were transferred to cold PSS at 2° for 30 min to wash out extracellular lithium. Cell lithium, unable to move out of the cell at this temperature, was thus measured directly. Three experiments were carried out. In the first, the simple exchange of Li for K was followed over a 3 hr period; in the second, K was omitted from the LiPSS; in the third, 1 m*M* ouabain was added to the medium. Measurements were made on groups of 6 arteries at 0, 1, 2, and 3 hr. The results, approximated

TABLE II. Cell Monovalent Cation Balances in Rat Tail Artery Following Interruption of Na Transport and Measured after Exchange of Extracellular Na in Cold LiPSS.

Procedure	Incubation (min)	2° Li Wash (min)	Na_i	K_i	$Na_i + K_i$	Li_t	H_2O
			(mmole/kg dry wt)				(liter/kg dry wt)
Ouabain PSS	0	30	18.4 ± 0.7	225 ± 8	243 ± 9	356 ± 6	3.44 ± 0.04
	240	30	167 ± 13[a]	66 ± 6[a]	233 ± 13	372 ± 7	3.09 ± 0.17
	240	60	162 ± 7[a]	62 ± 4[a]	224 ± 7	350 ± 8	3.26 ± 0.04
K-free PSS	0	30	20.8 ± 0.6	225 ± 7	246 ± 7	353 ± 6	3.32 ± 0.04
	240	30	217 ± 7[a]	14.2 ± 0.5[a]	232 ± 7	348 ± 7	3.27 ± 0.05
	240	60	217 ± 4[a]	17.3 ± 1.0[a]	235 ± 5	366 ± 4	3.39 ± 0.06

[a] Na_i = residual Na after cold Li wash. K_i = total K less 10 mmole in extracellular water. Li_t = total Li adjusted for gross water change. $P < 0.02$.

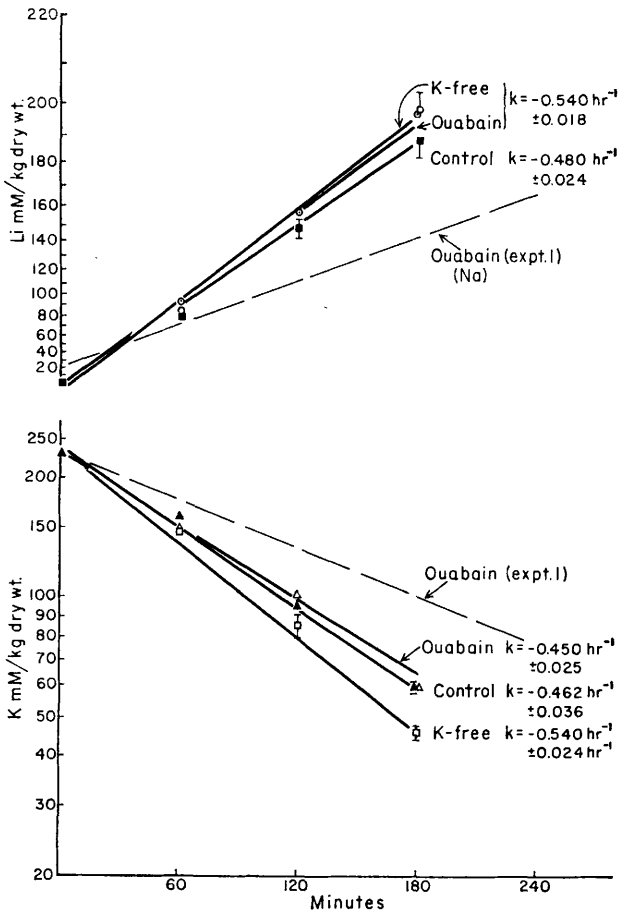

FIG. 2. Changes in Li and K in arteries incubated in LiPSS (control) only, or in LiPSS in the absence of K or presence of 1 m*M* ouabain, followed by 30 min wash in 2° PSS to remove extracellular Li. Values are fitted to single exponential functions by method of least squares.

by single exponential functions, are presented in Fig. 2.

The rate of Li entry into cells was not affected either by the presence of ouabain or by the absence of K in the medium. The rate of loss of K during incubation in LiPSS was not affected by ouabain, but was significantly accelerated in the absence of K.

Cell K at equilibrium in the absence of active Na transport. This experiment was designed to examine the extent to which the observations of the first experiment might reflect differences in asymptotic values. It was evident that prolonged incubations which could involve uncontrollable changes in the structure and viability of the cells might be necessary. To avoid this, the gradients were discharged as usual by overnight refrigeration in K-free medium and were then allowed to recover as usual by equilibration for a further 3 hr at 37°, this time, however, either in the absence of K, or in the presence of K with 1 m*M* of ouabain. The results are shown in Table III.

It was anticipated that tissues incubated in PSS (with ouabain) rather than in a K-free medium would contain more K and correspondingly less Na in proportion to their cell water content. This amounts to about 6 mmole. The observed difference was threefold greater for K and double that again for Na. When these values are intro-

TABLE III. Cell Monovalent Cation Balances in Rat Tail Artery after Overnight Incubation in K-free PSS at 3° Followed by 3 hr at 37° in the Same Medium or in Normal PSS with 1 m*M* Ouabain. Measurements were made after 45 min Exchange of Extracellular Na in Cold LiPSS.

Procedure	Na_i	K_i	$Na_i + K_i$	Li_t	H_2O
	(mmole/kg dry wt)				(liter/kg dry wt)
K-free PSS 37°	261 ± 6	9.6 ± 1.4	271 ± 7	333 ± 4	3.30 ± 0.04
Ouabain PSS 37°	215 ± 7[a]	30.6 ± 1.7[a]	246 ± 8[a]	340 ± 9	3.15 ± 0.07

[a] Δ observed −46 +21. Δ expected −6 +6. $P < 0.02$.

duced as asymptotes into the determination of rate constants, the effect of ouabain in PSS, where Na replaces cell K, is not significantly different from that observed in LiPSS where Li is the replacing ion. This is sufficient to reconcile Fig. 1 with Fig. 2. The faster rates in K-free media reflect the driving force of the steeper K gradient.

Discussion. Despite the large literature concerning ouabain and vascular reactivity, there have been few reports of its effects on ion distribution in vascular smooth muscle. In a recent study, we observed that the rat tail artery lost K about twice as quickly in a K-free medium as when Na transport was interrupted with ouabain (2). The present experiments using cold Li–Na extracellular exchange to define cell Na now provide evidence that the loss of K is coupled with a gain of Na on a 1:1 basis in either a K-free or ouabain containing medium.

Casteels and his associates have been concerned with the effects in taenia coli of these standard methods for interrupting the Na pump (4, 5). Using ethanesulphonate at one time or sorbitol at another to define the extracellular space and so measure cell Na and K by subtraction, no apparent difference was observed in this tissue between the rate of Na–K exchange induced by ouabain or by a K-free medium. No reason was given for the failure to observe an effect proportional to the driving force of the steeper K gradient. One possible explanation may be that these experiments were widely separated in time and methodology. There may indeed be a difference between these tissues since Matthews and Sutter have noted that ouabain induces a faster fall of K in taenia coli than in rabbit anterior mesenteric vein (6).

An important underlying cause of the slower rate of cell K loss induced by ouabain compared with a K-free medium in vascular tissue seems to be the size of the ion pool involved in the 2 cases. If we assume that full equilibration was attained in these tissues, then about 5% of cell K is not affected by ouabain. There is, of course, no absolute evidence that ouabain is 100% effective in blocking the (Na + K)ATPase in this or, indeed, in any tissue but the possibility of residual ATPase activity in the presence of this massive dose is remote (7). For the present, we suggest that this K is held at binding sites with a high degree of selectivity for K^+. Lithium is high concentration can evidently displace K^+ from these sites and they can also be occupied by Na^+ in the absence of K^+.

In addition to the approximately 15 mmole of Na displaced by K when the tissue is returned from the K-free condition to a normal medium with ouabain, an additional complement of about 20 mmole of Na leaves the cell. The interpretation of this must for the present be guarded since [Na] in the 2 solutions was not identical. This amount, however, corresponds closely to the temperature dependent component of Na not associated with K which we have previously noted and which Jones and Karreman have confirmed (8, 9).

Summary. The transmembrane Na and K gradients of the smooth muscle cells of the rat tail artery are discharged about twice

as fast in the absence of external K ions as in the presence of ouabain although a 1:1 exchange is involved in both cases. In large part, this observation reflects the fact that cell Na remains lower and cell K higher after prolonged incubation in a normal medium with maximal ouabain than in a K-free medium. These experiments still do not wholly exclude the possibility that changes in membrane permeability may also be involved.

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