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Cell Na and K in the Rat Tail Artery During the Development of Hypertension Induced by Desoxycorticosterone Acetate[1] (38997)

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Despite much effort, there is as yet no explanation for the close relationship between salt metabolism and many forms of experimental and clinical hypertension. Although there has been rapid progress in the past decade in relating ion movements to acute changes in vascular smooth muscle tension, the case for a similar involvement in sustained hypertensive states remains largely inferential. Much of the difficulty centers about the necessity to distinguish cellular from paracellular ion constituents in chronic conditions. Thus, for example, although the Na content of artery samples is increased in instances of both experimental and clinical hypertension, much of this can be accounted for by an increase in the extensive ion-binding capacity of the polyanionic matrix and need not necessarily involve changes in transmembrane ionic gradients (1–3).

In a continuing effort to quantify cell Na, we recently developed a simple ion exchange method based on the observation that movements of Li across the vascular smooth muscle cell membrane become very small at low temperature (4). Accordingly during incubation of the tissue at about 2° in a large volume of a physiological medium containing Li in place of Na, extracellular Na, whether site-bound in the paracellular matrix or free in the extracellular fluid, is readily washed out, while cell Na remains relatively unaffected. Preliminary observations concerning an elevated vascular smooth muscle cell Na in established hypertension have been presented (5). In this report, we examine in detail the distribution of Na and K in freshly excised and in incubated rat tail arteries during the development of DOCA hypertension. This model was selected for the basic study because of its clear-cut association with salt metabolism.

Methods. Adult male albino rats of an inbred Wistar strain (SPF-Woodlyn Farms), weighing 300 g or more, were used throughout. All animals were anaesthetized with sodium pentobarbital, and in all cases the tail artery was gently excised within less than 30 sec. In experiments with fresh arteries, these were either immediately blotted and transferred to stoppered glass weighing cups for processing or placed in cold LiPSS at 2° as soon as excised to wash out the extracellular Na. In experiments involving incubated vessels, the artery was maintained overnight in a K-free medium in the refrigerator and next morning transferred to a continuously aerated normal physiological salt solution (PSS) at 37° for 3 hr to complete the preliminary phase. With this procedure, which first discharges monovalent cation gradients, the smooth muscle cells consistently reaccumulate high levels of K (6). At the end of this time, the artery was rapidly transferred to cold LiPSS at 2°. Following the period of washing in the lithium substituted medium, the arteries were quickly blotted by compression at 15 g cm^{-2} between aluminum blocks, and then transferred to the 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 of 0.75 *M* nitric acid, and atomic absorption analysis.

The composition of the basic physiological salt solution (PSS), in m*M* 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 dextrose, 11.0. This was modified by equimolar substitution of the Na components by LiCl and Li_2CO_3 where a Li-substituted medium (LiPSS) was required (7).

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Hypertension was induced in uninephrectomized animals by an initial injection of 12.5 mg of DOCA, followed by 6.25 mg at weekly intervals, coupled with saline (1%) as drinking water throughout. Controls were uninephrectomized and drank tap water. On the day preceding artery excision, blood pressure was measured under light ether anaesthesia directly in the femoral artery by means of a Statham transducer.

Results are expressed as mean values with the estimated standard error of the mean. Exponentials were computed by the method of least squares, using a minicomputer program.

Results. Na and K in fresh arteries during the development of DOCA hypertension. In this experiment, arteries were taken after 1, 2, 3, 4, 6, and 8 weeks of continuous treatment with DOCA saline for comparison with matched untreated controls. At each interval, 10 arteries from each group were rapidly excised, two taken whole directly to the weighing cups for processing and the remaining eight were halved and then dropped immediately into cold (2°) LiPSS for extraction. These 16 samples per group were removed to weighing cups over a sequence of timed intervals from 15 to 80 min. Group sizes were limited by the total number of arteries that could be handled effectively in a morning, and by the large number required for the timed sequence in LiPSS.

The two whole arteries that could be spared at each interval for direct analysis were sufficient to provide summed groups of six for the first and second halves of the experiment. The results of this analysis are shown in Table I.

In a recent analysis of fresh arteries taken after 8 weeks of DOCA saline treatment, we observed a significant increase in total Na and a fall in K (5). Here, although the samples were taken at different times and the groups are small, these same changes were quite apparent at even earlier stages of treatment. In this case, the fall in K observed in the pooled groups was marginal, while the increase in Na, at first marginal, was quite marked in the second half of the experiment. Actually, the increase in Na was observed in each artery pair beginning with the 2-week samples.

TABLE I. TOTAL Na AND K IN FRESH ARTERIES DURING THE DEVELOPMENT OF DOCA-INDUCED HYPERTENSION. GROUPS OF SIX WERE OBTAINED BY EXCISING WHOLE ARTERIES TWO AT A TIME ON THREE OCCASIONS OVER THE INDICATED INTERVAL. THESE WERE ANALYZED DIRECTLY.

	Na (mmole/kg dry wt)		K (mmole/kg dry wt)	
	control	DOCA	control	DOCA
1–3 weeks	308±10	335±14	247±16	232±7
Δ	+28		−15	
4–8 weeks	274±10	341±10*	238±11	228±9
Δ	+67		−10	

* $P < 0.02$.

Measurements in the cold LiPSS extracted arteries are presented in Table II. Total water content of these vessels was unchanged throughout. Cell Na (Na_i), determined by extrapolation back to zero of the timed series incubated in LiPSS for 15–80 min as previously described (5), was higher at all intervals of treatment except the first. However, this increase did not appear to be progressive over the duration of the experiment. The change was not of significant degree at any one time because of the variability inherent in the timed series. However, it was quite significant ($P < .02$) calculated either in terms of χ^2 or as an average for the period. The rate constant for the exponential regression describing the slow rate of exchange of Na_i with Li over the timed interval and used to calculate Na_i was also significantly increased over the whole of the 2- to 8-week period.

Cell K (K_i), i.e., total tissue K minus a constant value of 7 mmole to allow for extracellular K, was diminished over the 2- to 8-week interval, although this was not seen in the 3-week samples.

In sum then, in this experiment the same changes as previously reported for animals treated with DOCA-saline for 8 weeks are here seen as early as after 2 weeks of treatment. This is about the time when the rise in blood pressure becomes evident.

Na and K in incubated arteries during the development of DOCA hypertension. In the

TABLE II. CELL Na AND K IN FRESH ARTERIES DURING THE DEVELOPMENT OF DOCA-INDUCED HYPERTENSION. ARTERIES WERE ANALYZED IN GROUPS OF 16 AFTER IMMERSION IN COLD LiPSS FOR A TIMED SEQUENCE BETWEEN 15 AND 80 MIN.

Duration (weeks)	1	2	3	4	6	8	Average (2–8 weeks)
H_2O, liters kg^{-1} dry wt							
Control	2.84±0.03	2.92±0.04	2.81±0.05	2.87±0.04	2.80±0.03	2.70±0.03	
DOCA	2.91±0.04	3.04±0.04	2.72±0.03	2.93±0.04	2.90±0.04	2.76±0.03	
Na_i, mmoles kg^{-1} dry wt							
Control	49.1±2.9	48.2±2.3	43.3±3.3	45.2±2.6	41.1±3.6	35.8±2.3	42.7±2.1
DOCA	45.4±4.8	56.3±4.7	55.0±5.3	49.6±3.0	53.0±5.0	45.9±4.5	51.9±1.9*
Δ	−3.7	+8.1	+11.7	+4.4	+11.9	+10.1	+9.2*
K_i, mmoles kg^{-1} dry wt							
Control	131±6	139±10	132±10	137±10	146±10	151±10	141±3
DOCA	139±10	118±8	134±10	115±10	105±9*	122±8**	119±5*
Δ	+8	−21	+2	−22	−41*	−29**	−22*
2° Na loss, kh^{-1}[a]							
Control	0.30±0.78	0.21±0.06	0.15±0.11	0.17±0.08	0.20±0.13	0.11±0.09	0.17±0.02
DOCA	0.22±0.14	0.30±0.11	0.36±0.15	0.20±0.09	0.42±0.15	0.31±0.15	0.32±0.04*
Δ	−0.08	+0.09	+0.21	+0.03	+0.22	+0.20	+0.15*
Blood pressure, mmHg systolic							
Control	137±3	141±3	141±4	134±2	134±2	134±2	
DOCA	149±3*	158±4*	163±7*	181±8*	222±3*	219±4*	
diastolic							
Control	80±2	88±2	86±3	83±2	83±2	83±2	
DOCA	87±3	94±4	102±8	112±8*	153±4*	145±4*	

* $P < .02$ control versus DOCA.
** $P < .05$.
[a] $k = [\ln (Na_o - Na_t)]/t$.

preceding experiment, the raised intracellular Na diminished rapidly during incubation in cold LiPSS. This raised the possibility that the increase in cell Na was due to the high intravascular presure and was not an intrinsic characteristic of the vascular smooth muscle cell. If so, it would not be evident in arteries incubated *in vitro*.

To examine this question, arteries were taken after 2, 4, and 8 weeks of continuous treatment with DOCA-saline for comparison, in groups of six, with matched untreated controls. Immediately after excision, the arteries were halved and allowed to recover for 90 min in aerated PSS at 37°. They were then transferred to a K-free medium for overnight incubation in a refrigerator (about 6°) and, on the next morning, returned to PSS at 37° for 3 hr to reestablish the ionic gradients as previously described (4, 6). They were then transferred to cold LiPSS for 45 min, removed and blotted, and then taken for weighing and processing. The results are presented in Table III.

As in the case of fresh arteries, no change in total water was observed. Cell Na in the controls was similar to our previously reported values and the averages ranged within narrow limits from 22 to 25 mmoles/kg dry weight. By contrast, average cell Na in the arteries from the DOCA treated animals was never as high as 20 mmoles and was less than the controls at all intervals, a highly significant result. Cell K did not change despite treatment until the last interval, when it was significantly lower than controls. The Li uptake was increased at both 4 and 8 weeks. Such an increase is expected, since the ion binding capacity of the paracellular matrix has been shown to increase under this treatment (3). On the other hand, the possibility that some of this Li was cellular and had exchanged for cell Na cannot be ruled out by this experiment. The second experiment of this series was designed to examine this possibility.

In this experiment, arteries were taken after 4 and 8 weeks of continuous treatment with DOCA-saline for comparison, in groups

TABLE III. CELL Na AND K IN INCUBATED ARTERIES DURING THE DEVELOPMENT OF DOCA-INDUCED HYPERTENSION. ARTERIES WERE ANALYZED AFTER OVERNIGHT INCUBATION IN K-FREE PSS FOLLOWED BY 3 HR RECOVERY IN PSS AND 45 MIN IMMERSION IN COLD LiPSS.

Duration (weeks)	2	4	8
H_2O, liters kg^{-1} dry wt			
Control	3.15 ± 0.05	3.20 ± 0.06	3.06 ± 0.09
DOCA	3.03 ± 0.03	3.19 ± 0.04	3.04 ± 0.05
Na_i, mmoles kg^{-1} dry wt			
Control	22.6 ± 1.6	24.5 ± 2.0	23.2 ± 1.6
DOCA	18.2 ± 0.8**	18.8 ± 0.4*	18.5 ± 1.0**
Δ	−4.4**	−5.7*	−4.7**
K_i, mmoles kg^{-1} dry wt	—		
Control	197 ± 7	216 ± 8	195 ± 9
DOCA	194 ± 5	206 ± 5	151 ± 11*
Δ	−3	−10	−44*
Li, mmoles kg^{-1} dry wt			
Control	328 ± 6	292 ± 3	298 ± 5
DOCA	321 ± 6	313 ± 6*	348 ± 7*
Δ	−7	+21*	+50*

* $P < .02$.
** $P < .05$.

TABLE IV. CELL Na AND K IN INCUBATED ARTERIES DURING DEVELOPMENT OF DOCA-INDUCED HYPERTENSION. ARTERIES WERE ANALYZED AFTER OVERNIGHT INCUBATION IN K-FREE PSS FOLLOWED BY 3 HR RECOVERY IN PSS AND 45, 90, OR 135 MIN IMMERSION IN COLD LiPSS.

Group	Control	DOCA 4 weeks	DOCA 8 weeks
H_2O, liters kg^{-1} dry wt			
45 min	2.97 ± 0.06	2.98 ± 0.02	3.02 ± 0.03
90 min	2.94 ± 0.05	2.99 ± 0.07	2.80 ± 0.03
135 min	3.04 ± 0.08	2.99 ± 0.03	3.03 ± 0.02
Na_i, mmoles kg^{-1} dry wt			
45 min	26.4 ± 0.6	23.9 ± 0.9*	21.8 ± 1.4*
90 min	23.0 ± 0.9	21.2 ± 0.7	19.1 ± 1.2*
135 min	20.1 ± 0.7	20.8 ± 0.7	18.5 ± 1.1
Intercept	30.7 ± 1.8	25.6 ± 1.3**	24.9 ± 2.0*
K_i, mmoles kg^{-1} dry wt			
45 min	220 ± 5	216 ± 6	173 ± 8*
90 min	216 ± 9	209 ± 6	179 ± 10*
135 min	204 ± 5	195 ± 7	179 ± 6*
Intercept	232 ± 11	229 ± 11	174 ± 12*
Li, mmoles kg^{-1} dry wt			
45 min	307 ± 7	318 ± 4	336 ± 10
90 min	313 ± 4	313 ± 6	348 ± 10*
135 min	315 ± 6	324 ± 3	342 ± 7*

* $P < .02$.
** $P < .05$.

of six, with matched controls. They were prepared exactly as in the previous experiment but, to increase precision, whole arteries were used throughout. After equilibration as before, they were transferred to cold LiPSS (2°) for incubation for 45, 90, or 135 min for a high precision analysis of the Li–Na exchange as previously described (7). The results are presented in Table IV.

Essentially the same changes were ob-

served here as in the previous experiment. The total water content of the tissue was unchanged by treatment with DOCA, and was uninfluenced by the duration of incubation with LiPSS. Cell Na, measured as residual Na after 45 min immersion in cold LiPSS, was diminished as before in the DOCA-treated groups. There was no evidence from the results with prolonged incubation in cold LiPSS that this fall in Na_i was simply due to an increased rate of loss of cell Na by exchange with Li during incubation.

Cell K, as before, was unchanged in the early stages of treatment, but was significantly lower at 8 weeks. The significant increase in tissue Li measured after 8 weeks of DOCA is evidently not due to any increased rate of Li uptake and thus reflects the known increase in the ion binding capacity of the paracellular matrix (3).

Discussion. These experiments initiate our new program aimed at describing in detail the distribution of monovalent cations in the vascular tree in relation to the hypertensive process. These cations are of particular interest to us because the observed clinical implication of Na metabolism in hypertension still lacks explanation. We have started with DOCA-induced hypertension as a first model because its dependence on salt is unequivocal. In this report, the steady-state transmembrane distribution of Na and K in fresh and incubated rat tail arteries was examined.

We have here observed that the Na content of the artery *in vivo* increases early in the course of DOCA treatment. This was demonstrated in rapidly excised arteries taken "dry" for analysis. It was more precisely shown by measuring the residual Na values after exchanging extracellular Na with Li in a cold Li substituted medium over a timed sequence. In this case, an increase in cell Na was evident as early as 2 weeks after the start of DOCA treatment.

The observed increase of cell Na could well be a consequence of the rise in blood pressure. In favor of this position is the fact that it was only observed when blood pressure was already noticeably elevated and disappeared rapidly when intravascular pressure dropped to zero on excision, and the artery was then processed "wet" through LiPSS. We have shown earlier that an increased intravascular pressure does in fact increase cell Na (8). However, this probable explanation must still be treated cautiously, for we have not yet found a similar increase in cell Na in hypertension induced by renal artery constriction (5). We intend to reexamine this in greater detail.

The idea that the increase in cell Na is a consequence of the rise in intravascular presure is supported by the observations in incubated arteries, which show a quite different steady state. Here, in the absence of any intravascular pressure, cell Na is actually significantly lower than normal after 2 weeks of treatment. The observation does not appear to be fortuitous; it is consistently reproducible, and we have no reason to think it an artifact, even though it is rather unexpected.

We can only speculate at this stage about the steps that relate the vascular ionic pattern to the sustained rise in blood pressure. One simple explanation is that DOCA, like aldosterone, increases the protein synthesizing activity of cells, reflected in part in enhanced Na transport activity (9). Thus, a low cell Na at rest characterizes the transmembrane steady-state distribution of this ion. In the case of vascular smooth muscle, the increased synthesis of new materials by the cell is also reflected in an enhanced production of the protein–polysaccharide paracellular matrix which itself has a high ion binding capacity (3, 10). Thus, even at rest, an increased extracellular uptake of Li is observed. This increased matrix ordinarily holds an increased amount of water (10), but this was presumably squeezed out by the high standard pressure used to blot the arteries in this experiment. The hypertrophy of cells and matrix may be sufficient to account for much of the increase in peripheral vascular resistance and reactivity (11, 12). The larger distributing arteries such as the tail artery are upstream from the major locus of systemic resistance, but receive the full force of the rise in pressure, and this is reflected in the increase in cell Na observed in the freshly excised vessel. However, other

explanations are by no means to be excluded.

Over the years, no consistent picture of the role of K in sustained hypertensive states has emerged. In these experiments, which examined arteries in different ways and found a consistent involvement of Na, an implication of K was less regularly observed. However, a significant loss of K was always observed as a late event in incubated arteries. Since it is not difficult to find morphological evidence of cell loss at this later stage of DOCA-saline treatment, this alone may be sufficient explanation of the K loss.

Summary. An increase in total Na, due in large part to an increase in cell Na, was measured in the freshly excised rat tail artery during the course of DOCA-saline treatment. Since this change was associated with a fall in cell K, was first observed at 2 weeks, coincident with the rise in blood pressure, and was not sustained during subsequent immersion of the artery at zero pressure, it probably reflects the high *in vivo* intravascular pressure. In the incubated artery, cell Na is significantly reduced early n th e course of treatment, while cell K falls late. Thus, Na transport in the artery is under direct attack from the start, but it is suggested that this leads to hypertrophy rather than to vasoconstriction.

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