Effect of Aldosterone on Kinetics of Intracellular Sodium in Cortical Portion of Collecting Ducts in Rat Kidney

N. S. Logvinenko, E. I. Solenov, and L. N. Ivanova

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We studied the effect of protein kinase C inhibitor RO-31-8220 (10^{-7} M) on rapid nongenomic effect of aldosterone in cells of isolated segment of distal region of collecting duct in rat kidney. Experiments with fluorescent dye Na-Green showed that the inhibitor abolished the modulating effect of aldosterone (10 nM) on intracellular sodium concentration at external sodium concentration of 14 mM. Aldosterone decreased by half the initial rate of the changes in internal sodium concentration in both 10-day and mature rats ($p<0.05$). Similarly to sodium channel blocker amiloride (10^{-5} M), RO-31-8220 abolished rapid nongenomic effect of aldosterone on the rate of the changes in internal sodium concentration.

Key Words: *aldosterone; kidney; intracellular sodium; protein kinase C*

In principal cells of cortical collecting duct (CCD), the functional effect of aldosterone is mediated via regulation of the expression and activity of epithelial sodium channels, basolateral sodium pump (Na^+,K^+-ATPase) and major membrane proteins involved in the hormone-regulated sodium transport [6]. In addition to genomic (long-term action on the target cells), aldosterone exerts a rapid nongenomic effect. It manifests within seconds or minutes, and in many cases is mediated via kinase cascades of second messengers [5,7,15]. In principal CCD cells, sodium concentration is mostly determined by dynamic equilibrium between sodium influx from the tubular lumen via epithelial sodium channels and its efflux from the cell into interstitium against gradient with utilization of ATP energy of basolateral sodium pump. We previously showed that during the first 10-15 sec, aldosterone (10 nM) increased intracellular sodium concentration ($[Na^+]_i$) in principal CCD cells bathed in 14 mM sodium saline not only in mature rats, but also in 10-day pups [2,3]. At this age, aldosterone cannot exert long-term genomic effect on the control of sodium reabsorption [1]. Moreover, we and other researchers showed that aldosterone induces a rapid elevation of intracellular calcium in cells of the distal segment of the nephron [4,9,14]. The key role of calcium-dependent protein kinase C (PKC) in the realization of rapid nongenomic effects of aldosterone was revealed in many aldosterone-dependent target tissues [5,7].

Our aim was to study the role of calcium-dependent PKC in mediation of rapid nongenomic aldosterone effects in CCD of rat kidney.

MATERIALS AND METHODS

The study was based on microdissection of renal CCD in mature and 10-day rats and continuous recording of fluorescence of Na-Green dye reflecting changes in $[Na^+]_i$ in principal CCD cells (in our modification) [13]. To examine the effect of aldosterone, it was added to the medium in a concentra-

Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia. ***Address for correspondence:*** ninlo@bionet.nsc.ru. N. S. Logvinenko

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tion of 10 nM. Fluorescence was recorded at excitation and emission wavelengths of 480 and 550 nm, respectively; sodium concentration in bathing solution was rapidly changed (137 and 14 mM). Sodium-deficient solution (14 mM NaCl) was prepared on the basis of isoosmolar phosphate-saline buffer (137 mM) where Na^+ ions were partially replaced with 123 mM N-methyl-D-glucamine. The bathing solutions were rapidly replaced within 100 msec. Fluorescence was measured in cells located at the open end of CCD fragment, where the basolateral and apical surfaces of these cells are exposed to the bathing solution. The calibrating solutions were those used to produce sodium gradient, but supplemented with 10 μM nystatin to perforate the cells.

The data were processed by Student's *t* test. Kinetics of changes in intracellular sodium ($V[Na^+]_i$) was based on linear approximation of the initial part of the plot. To this end, a quasilinear part of the curve $V[Na^+]_i(t)$ was selected and approximated by linear regression equation

$$V(t)=A_0+B\times t,$$

where B is coefficient of regression and A_0 is the initial concentration of intracellular sodium (Fig. 1). The initial rate of $V[Na]_i$ change in each test group served as the controls.

RESULTS

Drastic drop in sodium concentration in bathing solution from 137 to 14 mM decreased $[Na]_i$ in the cells at the open end of CCD fragment of rat kidney from 35 to 10 mM ($p<0.05$, Fig. 2). Restoration of external sodium concentration to 137 mM increased $[Na^+]_i$ to its initial level. Aldosterone in a physiological concentration of 10 nM produced no effect on internal sodium under normal conditions with bathing Na^+ concentration of 137 mM, but significantly increased it when external sodium dropped to 14 mM ($p<0.05$). This effect was not observed in the presence of amiloride (10^{-5} M), a

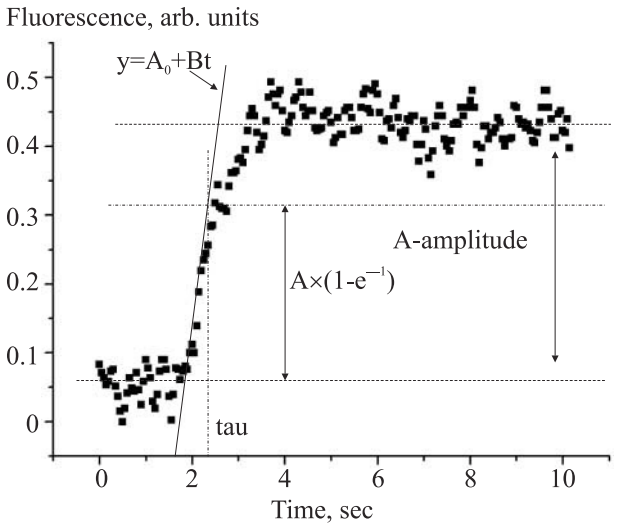

Fig. 1. Kinetics of fluorescence response to drastic elevation of sodium concentration in bathing solution from 14 to 137 mM. Tay is the duration of interval where the plot is quasilinear. $A\times(1-e^{-1})$ is maximum of fluorescence in the linear part of the plot.

blocker of apical epithelial sodium channels (Fig. 2, *b*). Similar effect was produced by RO-31-8220 (PKC inhibitor) at a far lower concentration of 10^{-7} M (Fig. 2, *c*). In this case, the level of intracellular sodium in cells of the open end of CCD fragment did not differ from the control ($p>0.05$). Thus, blockade of Ca^{2+}-dependent pathway of rapid nongenomic effect of aldosterone produced the same effect as amiloride: both agents prevented elevation of $[Na^+]_i$ in sodium-deficient bathing solution. It can be hypothesized that PKC inhibitor moderates activity of preexisting molecules in epithelial sodium channels and thus decrease their open-state probability [11,12].

Table 1 shows the data on the effect of aldosterone on the rate of changes in $V[Na^+]_i$. In both age groups, aldosterone in a physiological concentration of 10 nM significantly decreased the rate of $[Na^+]_i$ drop or rise induced by the corresponding changes in external sodium from 137 to 14 mM and vice versa ($p<0.05$). It decreased this rate virtually 2-fold for influx and efflux of sodium ions induced by opposite changes in sodium gradient across the

TABLE 1. Effect of Aldosterone on Initial Rate of Change of Internal Sodium Concentration $V[Na^+]_i$ in Rat CCD Cells Caused by Changes in External Na^+ Concentration from 14 to 137 mM (Influx) and from 137 to 14 mM (Efflux)

Parameter		10-day pups		Mature rats	
		control	aldosterone, 10 nM	control	aldosterone, 10 nM
$V[Na^+]_i$, mM/sec	influx	1.11±0.19	0.35±0.13*	1.10±0.16	0.48±0.17*
	efflux	0.280±0.088	0.150±0.016*	0.48±0.13	0.23±0.03*

Note. *$p<0.05$ compared to the corresponding control.

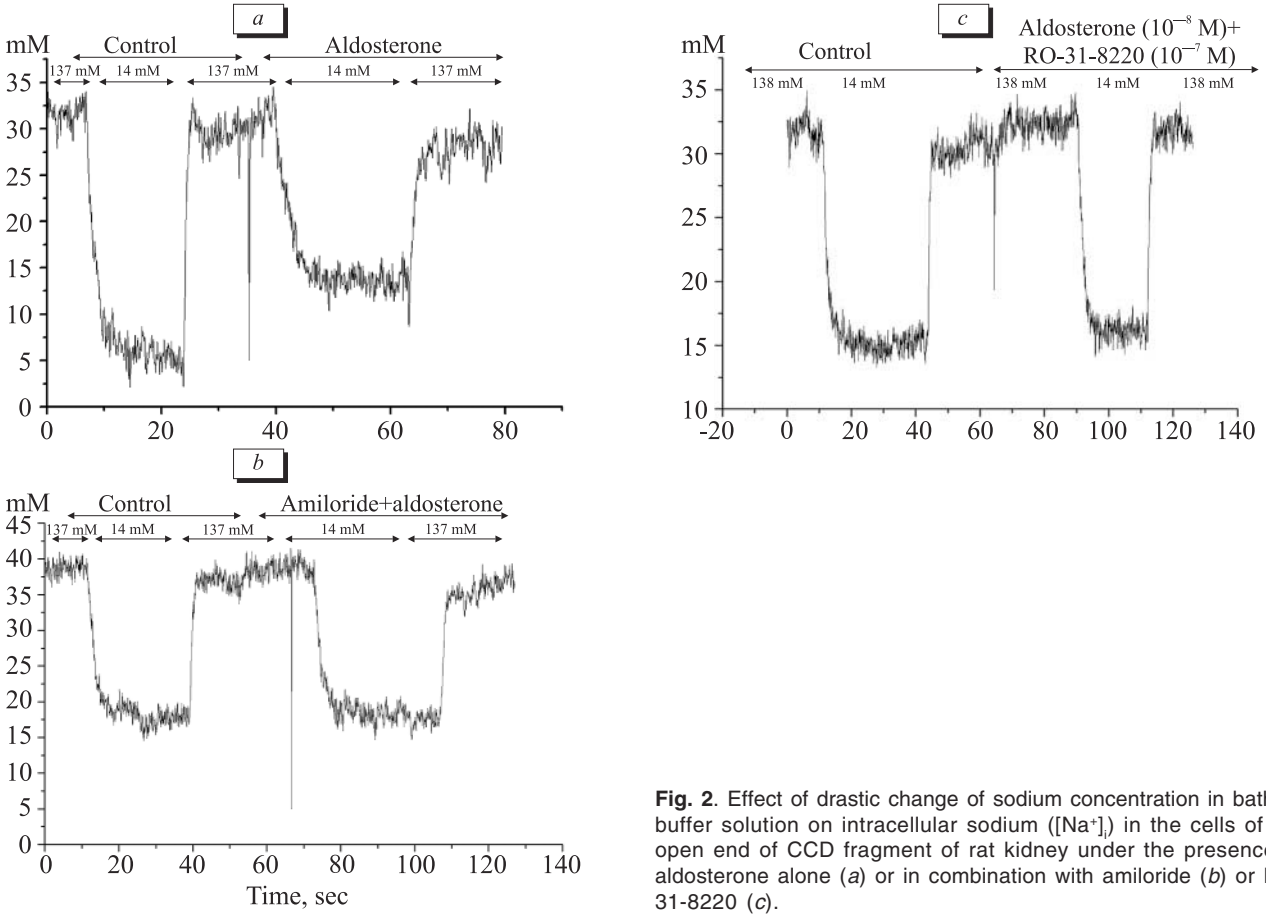

Fig. 2. Effect of drastic change of sodium concentration in bathing buffer solution on intracellular sodium ($[Na^+]_i$) in the cells of the open end of CCD fragment of rat kidney under the presence of aldosterone alone (*a*) or in combination with amiloride (*b*) or RO-31-8220 (*c*).

membrane ($p<0.05$, Table 1, Fig. 3). Both amiloride and RO-31-8220 abolished this effect of aldosterone ($p<0.05$). Specificity of acute effect of aldosterone was corroborated in the experiments with replacement of aldosterone with corticosterone in a 10-fold concentration, which is known to exhibit no aldosterone-like activity [2]. Thus, PKC inhibitor abolished rapid effect of aldosterone on both the steady-state level of intracellular sodium and on the rate of its changes (Figs. 2, 3). The shifts in intracellular sodium concentration result in changes in cell volume. Aldosterone is one of the key steroid hormones involved in the regulation of this process [6,7]. It can be hypothesized that even in the early postnatal ontogeny, aldosterone is involved in the regulation of homeostasis by preventing drastic shifts in cellular volume provoked by changes in luminal sodium concentration. The mechanisms underlying this effect are poorly examined, but this study suggests that Ca^{2+}-dependent PKC plays an important role in its realization. Enhancement of general activity of epithelial sodium channels by aldosterone probably decelerates and diminishes the loss of intracellular sodium and moderates changes in cell volume. Our findings indicate the involvement of fast kinase cascades in the realization of nongenomic effect of aldosterone on activity of epithelial sodium channels, which agrees with published data [5,10,11]. We assume

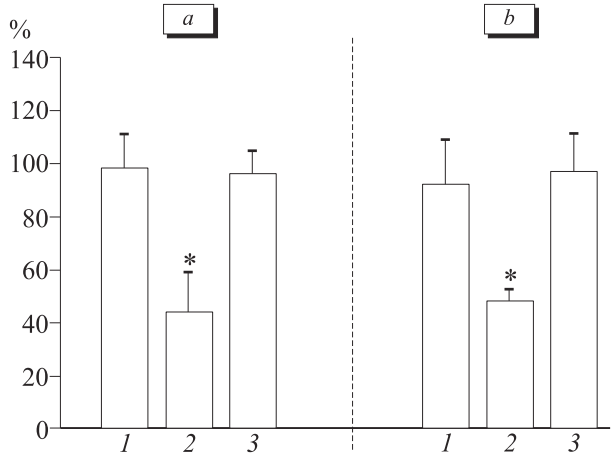

Fig. 3. Initial rate of change of internal sodium concentration $V[Na^+]_i$ in rat CCD cells caused by changes in external Na^+ concentration from 137 to 14 mM (*a*) and from 14 to 137 mM (*b*). *1*) aldosterone (10 nM)+amiloride (10^{-5} M), *2*) aldosterone (10 nM), *3*) aldosterone (10 nM)+RO-31-8220 (10^{-7} M). $p<0.01$ compared to the corresponding control.

PKC to be one of the basic intracellular mediators of aldosterone in the regulation of cell volume in this segment of the nephron.

Thus, we demonstrated involvement of calcium-dependent PKC in implementation of acute aldosterone effects both on the steady-state level of intracellular sodium and on the rate of its changes in the cells of open end of CCD fragment in the kidney of 10-day and mature rats.

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