Na/Ca exchangers in collecting cells of rat kidney
A single tubule fura-2 study

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Abstract. Single pieces of fura-2-loaded cortical collecting tube (CCT) isolated either from normal or adrenalectomized (ADX) rats were superfused in vitro, and the cytosolic calcium concentration ([Ca\(^{2+}\)]\text{\textsubscript{i}}) was calculated from fluorescence recordings. The effects of altering the sodium gradient across cell membranes were investigated. Switching external sodium from 164 mM to 27 mM (low [Na\(^{+}\)]\text{\textsubscript{o}}) had little effect on [Ca\(^{2+}\)]\text{\textsubscript{i}}, in normal tubules \((106 \pm 9 \text{ versus } 101 \pm 9 \text{ nM, } n = 13)\); however, it resulted in a large peak of [Ca\(^{2+}\)]\text{\textsubscript{i}} in CCT from ADX-rats \((270 \pm 32 \text{ versus } 135 \pm 11 \text{ nM, } n = 21)\). Since CCT from ADX rats are known to have a reduced Na-pump activity, the effect of ouabain treatment on CCT from normal rats was also tested. When CCT from normal rats were exposed to 1 mM of ouabain, in the presence of 164 mM of [Na\(^{+}\)]\text{\textsubscript{o}}, [Ca\(^{2+}\)]\text{\textsubscript{i}} increased only moderately \((123 \pm 15 \text{ versus } 111 \pm 11 \text{ nM, } n = 13)\); when the low [Na\(^{+}\)]\text{\textsubscript{o}} solution was applied to these ouabain-treated tubules, a large and transient increase in [Ca\(^{2+}\)]\text{\textsubscript{i}} was obtained \((287 \pm 38 \text{ versus } 123 \pm 15 \text{ nM, } n = 13)\). This response was absent with [Ca\(^{2+}\)]\text{\textsubscript{o}} = 0. The data suggest the presence of 3 Na\(^{+}\)/1 Ca\(^{2+}\) exchangers in cell membranes of rat CCT. The calcium flux equation derived by Läuger for the exchanger indicates a non-linear relationship between net calcium flux and driving force which could account for the difference observed here between the poor effect of applying either low [Na\(^{+}\)]\text{\textsubscript{o}} or ouabain alone and the large peak of [Ca\(^{2+}\)]\text{\textsubscript{i}} induced by combining these two conditions.

Key words: Fura-2 - Rat collecting tubule - Na\(^{+}\)/Ca\(^{2+}\) exchanger - Cytosolic calcium

Introduction

The 3 Na\(^{+}\)/1 Ca\(^{2+}\) exchange system is one of the main regulators of the cytosolic calcium concentration ([Ca\(^{2+}\)]\text{\textsubscript{i}}) in many cell types [2, 16]. The presence of this system in renal epithelial cells has been observed in studies using basolateral membrane vesicles from kidney cortex [11, 12, 19, 23] or renal cells in culture [1, 4, 20]. In distal and collecting tubules, the presence of the system has been suggested on the basis of indirect evidence [5, 7], and it has been assumed that cytosolic calcium concentration ([Ca\(^{2+}\)]\text{\textsubscript{i}}) could be involved in the regulation of transepithelial sodium transport via the Na\(^{+}\)/Ca\(^{2+}\) exchange system [8, 22].

By applying the fura-2 fluorescence technique to single pieces of freshly microdissected kidney tubules, we have recently observed in rat cortical collecting tubules (CCT) that [Ca\(^{2+}\)]\text{\textsubscript{i}} is settled mainly by a pump and leak mechanism involving voltage-independent calcium channels and Ca\(^{2+}\)-ATPase [21]. We have also reported that changing external sodium concentrations have only limited effects on cytosolic free calcium concentration in those cells. In addition, cell membrane depolarization with high [K\(^{+}\)] solutions actually resulted in a rapid fall in [Ca\(^{2+}\)]\text{\textsubscript{i}}, instead of an increase, as would be expected from the electrogenic nature of Na\(^{+}\)/Ca\(^{2+}\) exchangers [13]. Thus, no evidence could be obtained from these experiments to demonstrate the presence of a Na\(^{+}\)/Ca\(^{2+}\) exchanger in CCT cell membranes.

However, when a similar decrease in external sodium was applied to CCT cells from adrenalectomized rats, a marked (though transient) rise in [Ca\(^{2+}\)]\text{\textsubscript{i}} was observed, as reported in the present article. We therefore further examine here the respective roles of both external and intracellular sodium concentrations in the regulation of [Ca\(^{2+}\)]\text{\textsubscript{i}} in rat CCT cells. We confirm that cytosolic free calcium concentration is little affected in these cells by either decreasing outer [Na\(^{+}\)] or increasing intracellular [Na\(^{+}\)] separately. However, marked (although transient) increases in [Ca\(^{2+}\)]\text{\textsubscript{i}} were obtained when these two conditions were combined, an observation suggesting the presence of Na\(^{+}\)/Ca\(^{2+}\) exchangers in CCT cell membranes.

Materials and methods

Preparation of fura-2 loaded tubules. Adult (about 200 g) normal and adrenalectomized (ADX) Wistar rats were used. Adrenalectomy was performed 5 to 8 days prior to each experiment as described in [6]. All animals were fed a standard laboratory diet, and were allowed free access to water; in addition, ADX-rats received a 0.9% NaCl solution.

As previously reported in detail [21], the animals were anesthetized before the experiments, their left kidney was perfused with a collagenase containing solution, then the kidney tissue was sliced and incubated again in the presence of collagenase for 15 to 30 min at 30\(^\circ\)C. After rinsing of the tissue, cortical collecting tubules (CCT) were microdissected by hand under stereomicroscopic observation at room temperature in “microdissection solution” prepared by adding 0.1% of bovine serum albumin (BSA) to a “standard solution” of the following composition (in mM): NaCl 137, KCl 5, MgSO\(_4\) 0.8, Na\(_2\)HPO\(_4\) 0.33, KH\(_2\)PO\(_4\) 0.44, MgCl\(_2\) 1, NaHCO\(_3\) 4, CaCl\(_2\) 1, D-glucose 5, Na acetate 10, lactate 5,
pyruvate 1, N-2-hydroxyethyl-piperazine-N-2-ethane-sulfonic acid (HEPES) 20, pH was adjusted to 7.4 with 
NaOH (about 12 mmoles/l), and the osmotic pressure was 
340 mOsmol/kg.

Single pieces of CCT were immersed at room temperature 
in "standard solution" containing 1% agarose (Sigma, 
type IX); each tubule was transferred with about 1 µl of this 
solution and deposited on a microscope glass cover slide; 
then the agarose was gelled by cooling the slide for 1 min at 
temperature. The tubules were loaded with fura-2 by 
incubation for about 30 min at room temperature in "micro-
dissection solution" containing 5 µM of fura-2/AM either 
before of after being embedded into agarose.

Fluorescence measurements and calculation of cytosolic calcium concentration. The coverslide carrying the fura-2-
loaded tubule was glued onto the bottom surface of a meta-

calic superfusion chamber (maintained at 37°C), which was 
then fixed onto the stage of a Leitz diavert inverted micro-
scope equipped with a quartz illumination system and a 40-
fold magnification immersion fluorescence objective 
(Nikon). During the experiment, the tubule was continu-
sely superfused with the desired solutions kept at 37°C at 
a rate of 0.4 ml/min ensuring full exchange of the solution 
lighting the cell membranes within a few seconds [21]. The 
tube was excited 10 times per minute throughout the exper-
iment at two wavelengths alternately (336 ± 5 and 
384 ± 5 nm). A 480 nm high-pass filter was used in the 
emission pathway. By means of an adjustable window of a 
field diaphragm, a rectangular area (usually, about 40 µm 
in width and 100 µm in length) was selected from the tubule 
observed under transmitted light. After photomultiplier am-
plification, the fluorescence light emitted from this area of 
the tubule was continuously recorded. If necessary, the cor-
rect positioning and focussing of the tubule could be checked 
at any time during the experiment under transmitted light 
observation. At the end of each experiment, tubule auto-
fluorescence subtraction was performed [22]. An example is 
shown in Fig. 1. As indicated on Table 1, group A, [Ca2+]i; increased 
by an average of 5%, 1 and 5 min after applying the low 
[Na+]o solution (p < 0.05 as compared to control value, 
paired analysis).

However, when CCT from adrenalectomized rats were 
analyzed under similar conditions, reducing [Na+]o from 
164 mM to 27 mM usually resulted in a sharp increase in 
[Ca2+]i; with a peak after about 1-2 min followed by a pro-
gressive decrease to previous values. An example of this kind 
of response is shown in the left hand part of Fig. 2. As 
illustrated by the middle and right hand parts of Fig. 2, 

duction of calcium in the superfusate resulted in a marked fall in [Ca2+]i; moreover, the new steady state value achieved 
with [Ca2+]o = 0 in this tubule was no longer affected by 
creasing [Na+]o, to 27 mM. This observation indicates that 
the increase noted with 1 mM of [Ca2+]o, resulted from a net 
entry of external calcium in response to low [Na+]o. On 

Results

Effect of reducing external sodium concentration

We have previously reported that lowering external sodium 
concentration had little effect on [Ca2+]i in rat CCT cells 
[21]. In the present study, lowering external sodium concen-
tration ([Na+]o) in superfusate from 164 mM to 27 mM by 
substitution with choline or tetrathamylammonium (TMA) 
again produced little effect on intracellular free calcium con-
centration in CCT cells from normal rats. A small and 
transient increase in [Ca2+]i of a duration of about 1 min 
was noted in 4 out of 15 tubules tested. An example is shown 
in Fig. 1. As indicated on Table 1, group A, [Ca2+]i; increased 
by an average of 5%, 1 and 5 min after applying the low 
[Na+]o solution (p < 0.05 as compared to control value, 
paired analysis).

Fig. 1. Effect of low external sodium on [Ca2+]i of normal rat CCT. 
The figure depicts an example of the effect of exchanging for 5 min 
the standard superfusion solution containing 164 mM of sodium 
with a low [Na+]o solution containing 27 mM of Na+ (dashed area). 
The rate of superfusion was maintained constant at 0.4 ml/min 
throughout the experiment. On the ordinate, the free calcium concen-
tration [Ca2+]i (nM) was computed every 6 s from fura-2 fluorescence 
measurements (after autofluorescence subtraction). On the abscissa, 
the origin of the time scale corresponds to the beginning of 
fluorescence recording. Note that the low [Na+]o solution induced 
only a modest and transient increase in [Ca2+]i in this single piece 
of CCT microdissected from a normal rat kidney. Similar small 
peaks of [Ca2+]i were observed in 4 out of 15 tubes treated in this 
way, whereas [Ca2+]i, remained almost constant in the other 11 
tubules.

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Salts used were products of Merck (Darmstadt FRG). 
Fura-2/AM and ionomycin were purchased from Calbio-
chem (San Diego, CA, USA), collagenase (CLS, 194 U/mg) 
from Worthington Biochemical Corp. (Freehold, NJ, USA). 
Agarose (type IX) was purchased from Sigma Chemicals 
(St. Louis, MO, USA).

Statistics and chemicals. Except where otherwise specified,
the data are presented as means ± SEM, n indicating the 
number of different tubules. Statistical analysis was 
performed using either the paired or unpaired Student’s 
t-test, as appropriate. P < 0.05 was considered to represent 
a significant difference.