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Action of ANP on the nongenomic dose-dependent biphasic effect of aldosterone on NHE1 in proximal S3 segment

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ABSTRACT

The rapid (2 min) nongenomic effects of aldosterone (ALDO) and/or spironolactone (MR antagonist), RU 486 (GR antagonist), atrial natriuretic peptide (ANP) and dimethyl-BAPTA (BAPTA) on the intracellular pH recovery rate (pHirr) via NHE1 (basolateral Na^+/H^+ exchanger isoform), after the acid load induced by NH_4Cl, and on the cytosolic free calcium concentration ($[Ca^{2+}]_i$) were investigated in the proximal S3 segment isolated from rats, by the probes BCECF-AM and FLUO-4-AM, respectively. The basal pHi was 7.15 ± 0.008 and the basal pHirr was 0.195 ± 0.012 pH units/min (number of tubules/number of tubular areas = 16/96). Our results confirmed the rapid biphasic effect of ALDO on NHE1: ALDO (10^{-12} M) increases the pHirr to approximately 59% of control value, and ALDO (10^{-6} M) decreases it to approximately 49%. Spironolactone did not change these effects, but RU 486 inhibited the stimulatory effect and maintained the inhibitory effect. ANP (10^{-6} M) or BAPTA (5×10^{-5} M) alone had no significant effect on NHE1 but prevented both effects of ALDO on this exchanger. The basal $[Ca^{2+}]_i$ was 104 ± 3 nM (15), and ALDO (10^{-12} or 10^{-6} M) increased the basal $[Ca^{2+}]_i$ to approximately 50% or 124%, respectively. RU 486, ANP and BAPTA decreased the $[Ca^{2+}]_i$ and inhibited the stimulatory effect of both doses of ALDO. The results suggest the involvement of GR on the nongenomic effects of ALDO and indicate a pHirr-regulating role for $[Ca^{2+}]_i$ that is mediated by NHE1, stimulated/impaired by ALDO, and affected by ANP or BAPTA with ALDO. The observed nongenomic hormonal interaction in the S3 segment may represent a rapid and physiologically relevant regulatory mechanism in the intact animal under conditions of volume alterations.

1. Introduction

Recently (over the past seven years), the genomic and nongenomic effects of ALDO on the Na^+/H^+ exchanger of the proximal tubule have been demonstrated [1–4], including a biphasic effect on this transporter in which low doses stimulate and high doses inhibit it [5]. The genomic effects (observed with chronic treatment with ALDO) were sensitive to spironolactone and, therefore, involve the binding of this hormone with its classic receptor (MR) [1,3–6]. However, the receptor and the signal transduction cascades involved in the nongenomic modulation of the Na^+/H^+ exchanger by ALDO need to be clarified. Studies in several cell types and in tubular segments indicate that ERK1/2, PKC and $[Ca^{2+}]_i$ participate in this process [5,7–10].

ANP inhibits the proximal [11–13] and distal reabsorption of fluid [14,15], with cyclic guanosine monophosphate (cGMP) as a second messenger [14]. In the rat proximal tubule, ANP inhibits the sodium [16,17] and bicarbonate [18] reabsorption stimulated by low doses of angiotensin II (ANG II). Studies in MDCK cells demonstrated that ANP abolishes the stimulatory and inhibitory effects of ANG II [19] or arginine vasopressin (AVP) [20] on the Na^+/H^+ exchanger and their stimulatory effects on $[Ca^{2+}]_i$. Taken together, these data suggest that there may be some interaction between these vasoactive peptide hormones in the regulation of extracellular volume.

Given the recently described genomic and nongenomic actions of ALDO in the mechanism of regulation of pHi and $[Ca^{2+}]_i$ in the S3 segment [5] and considering that the physiological doses of ALDO in blood are 10^{-10} to 10^{-9} M and that they can increase or decrease in conditions of extracellular volume modification, the objective of the present study was to examine the mechanism of interaction between the nongenomic effects of ALDO (10^{-12} or 10^{-6} M, 2 min preincubation) and ANP (10^{-6} M) or BAPTA (5×10^{-5} M) on the NHE1 exchanger and $[Ca^{2+}]_i$ in this portion of the proximal tubule of rat kidneys.

Abbreviations: ALDO, Aldosterone; ANP, atrial natriuretic peptide; BAPTA, dimethyl-BAPTA; pHi, intracellular pH; pHirr, intracellular pH recovery rate; NHE1, basolateral Na^+/H^+ exchanger isoform; $[Ca^{2+}]_i$, cytosolic free calcium concentration.

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Table 1
Composition of solutions.

	Solution 1 (control)	Solution 2 (K^+-HEPES)	Solution 3 (NH_4Cl)	Solution 4 ($0Na^+_e$)	Solution 5 (Tyrode's)
NaCl	140	20	120	–	137
KH_2PO_4	2.5	–	2.5	2.5	–
$CaCl_2$	1	1	1	1	1.36
$MgSO_4$	1	1	1	1	–
$MgCl_2$	–	–	–	–	0.49
Glucose	5.5	–	5	5	5.6
L-Alanine	5	–	5	5	–
HEPES	10	5	10	10	5
NH_4Cl	–	–	20	–	–
NMDG	–	–	–	140	–
KCl	–	130	–	–	2.68
$NaHCO_3$	–	–	–	–	12
Na_2HPO_4	–	–	–	–	0.36
Nigericine	–	0.01	–	–	–
pH	7.4	6.18; 7.14; 8.11	7.4	7.4	7.4

Values are expressed in mM. NMDG, *N*-methyl-D-glucamine. HCl or NaOH were used in all Na^+-containing solutions to titrate to the appropriate pH, and KOH was used in Na^+-free solution.

2. Materials and methods

2.1. Preparation of isolated S3 segment of rat

Male Wistar rats (90 g) were anesthetized by tiletamine/zolazepam (30 mg/kg) and xylazine (2 mg/kg). Their kidneys were removed and slices 2 mm in thickness were prepared. Microdissection of the tubules was performed using tweezers under a stereomicroscope in ice-cold normal Ringer solution. The S3 segments were dissected from the outer stripe of the outer medulla [21,22] and were identified as the proximal straight tubule contiguous to the thin descending limb of the loop of Henle. Then, the S3 segments were transferred to glass coverslips prepared with poly-D-lysine for tubule adhesion. The coverslips were mounted on an inverted microscope (Olympus IX70) in a thermostatically regulated perfusion chamber with solutions that were changed by means of valves.

2.2. Viability of the tubules

After the experiments, the integrity of the S3 segments was confirmed by histological analysis and trypan blue exclusion. The tubules were removed to trypan blue (0.4%) prepared in a buffered isotonic salt solution (pH 7.4). This solution (0.1 ml) was added to the bath for 3 min at room temperature, and the color of the cell cytoplasm of the tubules was observed [23].

2.3. Measurement of pHi

For digital imaging of pHi, the S3 segments were incubated in a HEPES-buffered solution with 140 mM Na^+ (control solution, Table 1) containing 12 μM BCECF-AM for 20 min at 37 °C. The pHi was calculated from the fluorescence emission ratio collected every 5 s with an intensified ICCD-350F camera during excitation at 440 and 490 nm and emission at 530 nm. The fluorescence excitation ratio, I_{490}/I_{440}, was displayed in pseudo-color on the monitor, and a maximum of 6 areas per tubule were defined for measurement. The pHi was standardized by the high K^+/nigericin (solution 2, Table 1) technique [24].

2.4. Cell pH recovery rate

After superfusion of the S3 segments with control solution alone to measure the basal pHi, the segment was induced to alkalization by 2 min of exposure to 20 mM NH_4Cl solution (solution 3, Table 1) [25], followed by acidification by the return to control solution. The pHirr was measured in the presence of 140 mM external Na^+ (solution 1), in the absence of external Na^+ (solution 4) or in the presence of the following agents: 500 nM HOE 694 (a specific inhibitor of basolateral NHE1), 4.6×10^{-8} M concanamycin (a H^+-ATPase inhibitor), 10^{-12} or 10^{-6} M ALDO and/or 10 μM spironolactone (a MR inhibitor), 10^{-6} M RU 486 (a GR inhibitor), 10^{-6} M ANP or 5×10^{-5} M BAPTA (a calcium chelator). These drugs were added to the bath at the same time as the acid pulse for a total of 2 min of preincubation. In all experiments, the pHirr (dpHi/dt, pH units/min) was calculated in the first 2 min after the start of the pHi recovery curve, by linear regression analysis. Calculations and graphical representations were performed by an Excel program after importing the results from a data-acquisition program.

2.5. Measurement of $[Ca^{2+}]_i$

The S3 segments were loaded for 15 min with 10 (M of the calcium-sensitive probe FLUO-4-AM [19] at 37 °C and rinsed in Tyrode's solution (solution 5). The FLUO-4 intensity emitted above 505 nm was imaged using laser excitation at 488 nm on a Zeiss LSM 510 confocal microscope. The images were continuously acquired (at time intervals of 2 s) before and after substitution of the experimental solutions. The intracellular calibration was performed using 2.5 mM EGTA in a Ca^{2+}-free bath and then in a 1.36 mM Ca^{2+} bath containing ionomycin (5 μM) to measure the minimum (F_{min}) and the maximum (F_{max}) cell calcium fluorescent signals, respectively. The standard equation $[Ca^{2+}]_i = K_d \times (F - F_{min})/(F_{max} - F)$ was used to calculate the experimental values of $[Ca^{2+}]_i$ [26], using the dissociation constant (K_d) of 345 nM (according to the Molecular Probes catalog).

2.6. Solutions and reagents

The solutions utilized had an osmolality of about 300 mOsmol/kg H_2O and pH 7.4. BCECF-AM and FLUO-4-AM were obtained from Molecular Probes (Eugene, OR, USA). The other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.7. Statistics

The results are presented as means ± SEM. pHirr points are given as *N*/*n*, where *N* is the number of superfused tubules, and *n* is the