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Effect of Ionomycin on Cell pH in Isolated Renal Proximal Tubules

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In isolated rabbit proximal tubules the addition of 2.0 μM but not 0.2 μM ionomycin induced a sustained increase in cell pH ($[pH]_i$). This $[pH]_i$ response to 2.0 μM ionomycin was shown to be independent of several transporters such as Na^+/H^+ exchanger, Na^+-HCO_3^- cotransporter, Cl^-/HCO_3^- exchanger, or H^+-ATPase. On the other hand, the removal of extracellular Ca^{2+} abolished the $[pH]_i$ increase or even induced a transient $[pH]_i$ decrease in the presence of ionomycin. These results are consistent with the induction of Ca^{2+}/H^+ exchange by ionomycin. Therefore Ca^{2+} ionophores should be used with caution as probes to estimate renal tubule functions.

Renal proximal tubules reabsorb more than 70 % of the filtered HCO_3, and this process is under control of several hormones such as angiotensin II and parathyroid hormone (1, 2, 3). It is quite reasonable to speculate that, in addition to cAMP-dependent pathway, changes in intracellular calcium concentrations ($[Ca^{2+}]_i$) are also involved in these hormonal regulations. However, the exact influence of $[Ca^{2+}]_i$ changes on proximal functions are not completely clarified, and both stimulatory and inhibitory effects of the increase in $[Ca^{2+}]_i$ on proximal reabsorption have been reported (4, 5). To gain insight into this controversial issue, we examined the effect of ionomycin on steady state cell pH (pH_i) in isolated, lumen-collapsed proximal tubules. In this preparation the major determinant of pH_i would be basolateral Na^+-HCO_3^- cotransporter (6), which is reported to be inhibited by calcium-calmodulin-dependent protein kinase II in a previous study using basolateral membrane vesicles (7).

MATERIALS AND METHODS

The experiments were performed on isolated proximal tubules (superficial S2 segment) from female New Zealand white rabbits (1.5 ~ 2.5 Kg body wt), and only non-perfused, lumen-collapsed tubules were used as previously described (8). Fura 2 was used to measure $[Ca^{2+}]_i$ as previously reported (9), and pH_i was measured with bis(carboxyethyl)carboxyfluorescein (BCECF). Tubules were loaded with either fura 2/AM (20 μM) or BCECF/AM (15 μM), and $[Ca^{2+}]_i$ or pH_i were measured using a microscopic fluorescence photometry system (OSP-10, Olympus, Japan) with appropriate excitation and emission wavelengths. The calibration for $[Ca^{2+}]_i$ was made according to the equation of Grynkiewicz et al. (10), and that for pH_i was made according to the method by Thomas et al. (11). After the incubation period, tubules were perfused peritubularly with prewarmed (38 °C) experimental solutions. The following solutions were used (in mM): standard HCO_3^-- buffer (115 NaCl, 5 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 1 Na_2SO_4, 2 NaH_2PO_4, 25 $NaHCO_3$, 5.5 glucose), Cl^--free HCO_3^-- buffer (Cl^- in standard-HCO_3^- buffer solution was replaced with gluconate, and 5.0 $Ca(gluconate)_2$ was used), standard N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic (HEPES)-buffer (127 NaCl, 5 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 1 Na_2SO_4, 2 NaH_2PO_4, 12 HEPES, 13 Na-HEPES, 5.5 glucose), Na^+-free HEPES-buffer (Na^+ in standard-HEPES buffer solution was replaced with N-methyl-D-glucamine). pH of these solutions were adjusted to 7.4 either by bubbling with 5 % O_2/95 % CO_2 gas (HCO_3^--buffer solutions), or by 1N NaOH (HEPES-buffer solutions). Fura 2/AM and BCECF/AM were obtained from Dojindo Chemical, Japan, and ionomycin, 4, 4′-diisothiocyanatostilbene-2, 2′-disulphonic acid (DIDS), amiloride, and iodoacetic acid (IAA) were from Sigma Chemi-

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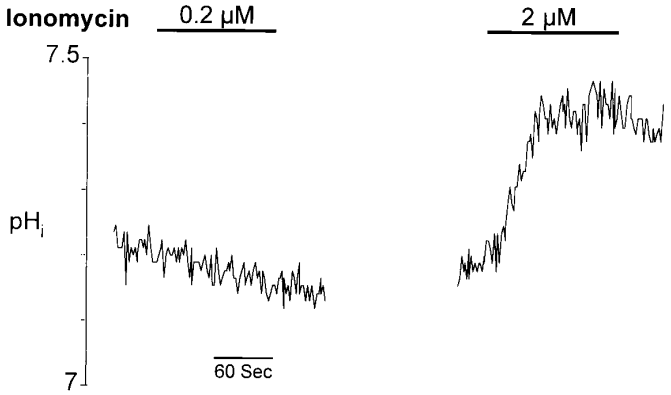

FIG. 1. Effect of ionomycin on pH_i in lumen-collapsed tubules. Tubules were perfused peritubularly with the standard HCO_3^--buffer solution, and different concentrations of ionomycin were added as indicated.

cal Co, USA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) was from Fluka, Germany, and all the other chemicals were from WAKO, Japan.

RESULTS

In the standard HCO_3^--buffer solution, the addition of 0.2 μM ionomycin increased $[Ca^{2+}]_i$ by 190 ± 70 nM (mean ± S.E.M., n = 6) in a reversible manner. On the other hand, the addition of 2 μM ionomycin increased $[Ca^{2+}]_i$ by more than 2000 nM, and this increase was often irreversible at all or only partially reversible (n = 5). As shown in Fig. 1, while 0.2 μM ionomycin did not affect the steady state pH_i in the standard HCO_3^--buffer solution, 2.0 μM ionomycin irreversibly increased pH_i by 0.26 ± 0.06 pH unit (n = 4). In the tubules incubated in the Cl^--free HCO_3^--buffer solution for more than 15 min, 2.0 μM ionomycin elicited a comparable cell alkalinization (ΔpH_i: +0.18 ± 0.02 pH unit, n = 3). Treatment with 0.5 mM DIDS also did not inhibit the pH_i response to 2.0 μM ionomycin (ΔpH_i: +0.30 ± 0.09 pH unit, n = 4). These results suggest that the effect of this ionophore on pH_i may not come from the changes in activities of Cl^-/HCO_3^- exchanger or Na^+-HCO_3^- cotransporter. To further examine the mechanism of pH_i increase by ionomycin, we incubated the tubules in the standard HEPES-buffer solution containing 20 mM NH_4Cl for 5 min. As shown in Fig. 2, the removal of NH_4Cl induced a profound cell acidification, and pH_i stayed at a very low level (usually below 6.5) for up to 15 min in the Na^+-free HEPES-buffer solution. In these tubules the addition of 0.2 μM ionomycin did not affect the steady state pH_i level. However, 2.0 μM ionomycin induced a marked pH_i increase, and in 4 of 4 tested tubules pH_i reached the pre-NH_4Cl level ($\sim$ 7.2) within 3 min in the absence of extracellular Na^+. The addition of 1 mM amiloride did not affect this pH_i recovery (n = 4), ruling out the involvement of Na^+/H^+ exchanger. Tubule treatment with 2 mM cyanide plus 2 mM IAA (n = 4), which should significantly suppress the metabolism in this segment (12), also did not modify the pH_i response to 2.0 μM ionomycin. Similarly, the pH_i response was unaffected either with 2 mM N-ethylmaleimide (n = 4) or 1 μM bafilomycin A_1 (n = 7), indicating that the involvement of a vacuolar-type H^+-ATPase is unlikely (13, 14). On the other hand, ionomycin did not induce the pH_i increase in the absence of extracellular Ca^{2+} (n = 2). Furthermore, the removal of extracellular Ca^{2+} promptly abolished the pH_i increase in the presence of 2.0 μM ionomycin, or even induced a transient pH_i decrease as shown in Fig. 3.

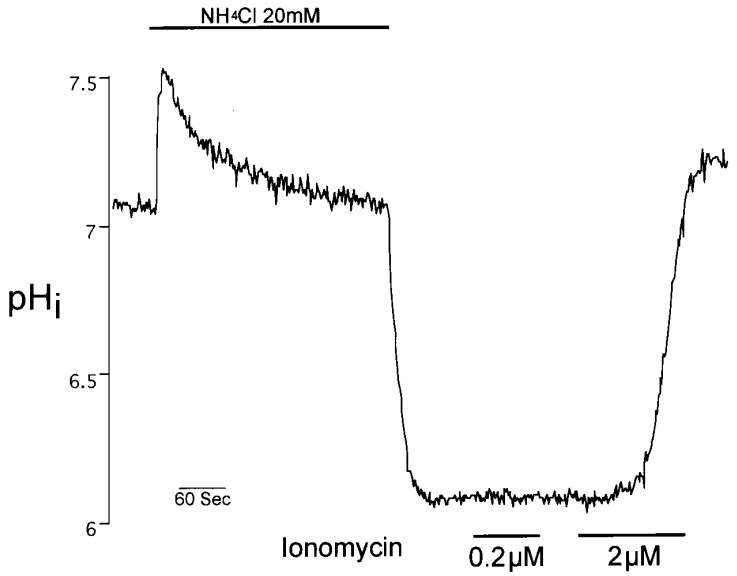

FIG. 2. Effect of ionomycin on pH_i in HCO_3^--free solutions. The tubule was first incubated with the standard HEPES-buffer solution containing 20 mM NH_4Cl (substituted for NaCl) for 5 min. Subsequently, the bath solution was changed to the Na^+-free HEPES-buffer solution, inducing a profound decrease in pH_i. Note 2.0 μM but not 0.2 μM ionomycin induced a marked pH_i recovery.

DISCUSSION

In the present study we observed that the $[Ca^{2+}]_i$ increase within physiological ranges by 0.2 μM ionomycin did not affect steady state pH_i in isolated proximal tubules. On the other

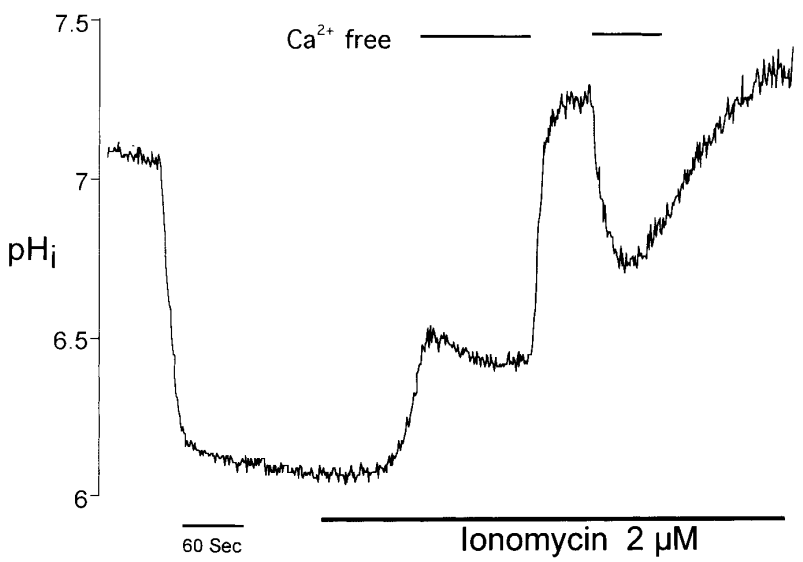

FIG. 3. Effect of extracellular Ca^{2+} removal on the ionomycin-induced pH_i increase. The tubule was acidified as in Fig. 2, then extracellular Ca^{2+} was removed (Ca^{2+}-free plus 0.1 mM EGTA) in the presence of 2.0 μM ionomycin as indicated. A representative trace from three similar experiments is shown.

hand, 2.0 μM ionomycin, while inducing the supra-physiological $[Ca^{2+}]_i$ increase, elicited the sustained increase in pH_i. Additional experiments indicated that the changes in activities of known transporters such as Na^+-HCO_3^- cotransporter, Na^+/H^+ exchanger, Cl^-/HCO_3^- exchangers, or H^+-ATPase (proton pump) cannot explain this pH_i increase. On the other hand, the pH_i response to ionomycin was not only promptly abolished, but its direction was even reversed by the extracellular Ca^{2+} removal. These observations strongly suggest that the pH_i response to 2.0 μM ionomycin is due to the induction of Ca^{2+}/H^+ exchange by the ionophore. It has been known that, in addition to Ca^{2+} releasing effects from the intracellular Ca^{2+} stores, high concentrations of Ca^{2+} ionophores can catalyze electroneutral divalent cation transport, i.e. $Ca^{2+}/2H^+$ exchanger (15, 16). In native tissues pH_i responses to the Ca^{2+} ionophores are quite variable depending on cell types or experimental conditions. However, Asem et al. reported that in chicken granulosa cells the Ca^{2+} ionophores such as ionomycin and 4-Bromo-A23187 induced a similar cytosolic alkalinization, which required extracellular Ca^{2+} but not extracellular Na^+ (17). Our results extend the observations by Asem et al. (17), and suggest that higher concentrations of ionomycin, while elevating $[Ca^{2+}]_i$ to the supra-physiological ranges, could induce the artificial cation exchange in isolated proximal tubules. Therefore, caution must be exercised when the Ca^{2+} ionophores were to be used as probes to investigate the role of Ca^{2+} in acid-base transport in renal tubules.

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