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Effects of Dopamine on Intracellular pH in Opossum Kidney Cells

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Na^+/H^+ exchanger (NHE) has a critical role in regulation of intracellular pH (pHi) in the renal proximal tubular cells. It has recently been shown that dopamine inhibits NHE in the renal proximal tubules. Nevertheless, there is a dearth of information on the effects of long-term (chronic) dopamine treatment on NHE activities. This study was performed to elucidate the pHi regulatory mechanisms during the chronic dopamine treatments in renal proximal tubular OK cells. The resting pHi was greatly decreased by chronic dopamine treatments. The initial rate and the amplitude of intracellular acidification by isosmotical Na^+ removal from the bath medium in chronically dopamine-treated cells were much smaller than those in control. Although it seemed to be attenuated in Na^+-dependent pH regulation system, Na^+-dependent pHi recovery by NHE after intracelluar acid loading in the dopamine-treated groups was not significantly different from the control. The result is interpreted to be due to the balance between the stimulation effects of lower pHi on the NHE activity and counterbalance by dopamine. Our data strongly suggested that chronic dopamine treatment increased intrinsic intracellular buffer capacity, since higher buffer capacity was induced by lower resting pHi and this effect could attenuate pHi changes under extracellular Na^+-free conditions in chronically dopamine-treated cells. Our study also demonstrated that intracellular acidification induced by chronic dopamine treatments was not mediated by changes in NHE activity.

Key Words: Dopamine, Intracellular pH, Renal proximal tubule, OK cell

INTRODUCTION

The systemic concentration of hydrogen ion is closely regulated by kidneys through excretion of acid and reabsorption of bicarbonate (Leaf & Cotran, 1985). A major fraction of bicarbonate reabsorption in the renal proximal tubules is mediated via apical membrane Na^+/H^+ exchanger (NHE) and H^+-ATPase transporter (Aronson, 1983; Alpern, 1990; Krapf & Alpern, 1993; Alpern & Rector Jr, 1996). The NHE activity is influenced by the systemic and/or cellular acid-base status (Ambuhl et al, 1996). NHE is a ubiquitous transporter present in all mammalian cell types, and functions to regulate intracellular pH (pHi) by removing one intracellular H^+ for one inwarded-directed Na^+ whose concentration gradient is established by the Na^+/K^+-ATPase (Bianchini & Grinstein, 1993; Bianchini & Pouysségur, 1995; Noël & Pouysségur, 1995).

It has been shown that dopamine is formed by the mammalian kidney (Lee, 1993) and has natriuretic and antihypertensive effects through the inhibition of the Na^+/K^+-ATPase and/or Na^+/H^+ exchanger via the activation of D_1-like receptors in the renal proximal tubules (Aperia et al, 1987; Bertorello & Aperia, 1990; Felder et al, 1990; Gesek & Schoolwerth, 1990; Ibarra et al, 1993). The effect of dopamine on transepithelial transport of Na^+ seems to be primarily through inhibition of the Na^+/H^+ exchanger, independently or secondarily its inhibitory effects upon Na^+/K^+-ATPase activity (Debska-Slizien et al, 1994; Gomes et al, 2001). It is quite likely that inhibition of NHE activity by dopamine is associated with activation of cAMP-dependent protein kinase (Gomes et al, 2001). However, there is not enough information available for the effects of the chronic treatment of dopamine on the pHi regulation, including buffering capacity.

In the present study, the effects of dopamine on the pHi regulatory system in renal proximal tubular cells were investigated, using fluorescence microscopy to measure pHi in opossum kidney (OK) cells, a cell line of rat renal proximal tubule origin, within an intact monolayer grown on coverslips. It should be noted that many published data have been obtained by the use of OK cells as a model for the epithelial cells of the renal proximal tubule, even at the level of single-cell analysis (Montrose et al, 1989; Montrose & Murer, 1990). The resting pHi, Na^+-independent and -dependent pHi recovery rates after acid-loading, and intrinsic buffer capacities were examined in both acute and chronic dopamine-administered cells under CO_2/HCO_3^--free conditions. Our results suggest that chronic treatment of renal proximal tubular cells with dopamine enhanced the intrinsic buffer capacity without changes of NHE activity by the intracelluar acidification.

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ABBREVIATIONS: pHi, intracellular pH; OK, opossum kidney; βi, intrinsic buffer capacity.

METHODS

Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma (St. Louis, MO, U.S.A.), except the followings: DMEM/F-12 HAM and FBS from GIBCO/BRL (Grand Island, NY, U.S.A.); 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF/AM) from Molecular Probes (Eugene, OR, U.S.A.).

Cell culture

The OK cells were purchased from the American Type Culture Collection (Rockville, MD, USA). For the pHi measurements, the cells ($2\times10^4/cm^2$) were seeded and grown as monolayers on glass coverslips (#1, 25 mm diameter) in 5% CO_2-humidified air at 37° C in a 1 : 1 mixture of Dulbecco's modified Eagle's medium (DMEM) and F-12 HAM supplemented with 5% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. The culture media also contained 24 mM $NaHCO_3$ and 15 mM Hepes. The confluent cells were exposed to 10 μM dopamine for 48 hrs in the FBS-free culture media.

Solutions

All experiments were performed at 37°C with normal Ringer solution, containing (mM): NaCl (120), $CaCl_2$ (1.8), $MgSO_4$ (1), K_2HPO_4 (2.5), glucose (5), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (24), and pH adjusted to 7.4 with Tris/base. Na^+-free solution was prepared by substituting Na^+ with *N*-methyl-D-glucamine ($NMDG^+$). pH calibration solution was prepared by replacing Na^+ with K^+ and adding 10 μM nigericin. All solutions were saturated with humidified pure oxygen gas. The flow rate was 2 ml/min and the bath medium was completely changed within a second.

pHi measurements

pHi measurements are previously described in detail (Kaufman et al, 1993; Wiederkehr et al, 2001). In brief, OK cells were loaded with 5 μM BCECF/AM in Hepes-buffered solution for 20 min at room temperature. The dye-loaded cells were excited alternately with 440 and 490 nm wavelength lights (Omega Optical Inc., Brattleboro, VT, U.S.A.), using a filter wheel (Lambda 10-2, Sutter Instrument Co., Novato, CA, U.S.A.). The resulting emitted fluorescence light was collected with 530 nm filter, using a ×40 oil immersion lens of an inverted microscope (IX70, Olympus, Japan).

The overall image sampling rate by using a cooled CCD camera (SensiCam, PCO Computer Optics GmbH, Germany) was 0.2 Hz. The 490 nm/440 nm emission ratio from the intracellular BCECF was calculated after the background substraction and normalized by means of *in situ* calibration as follows:

$$R=1+\{b\times(10^{(pH-pK)}/(1+10^{(pH-pK)})\}-\{b\times10^{(7-pK)}/(1+10^{(7-pK)})\} \quad \text{(Eq. 1)}$$

pK and b values were obtained from the curve fitting. Standard curve for the relationship between pHi=o, equilibrated pHi and extracellular pH, and the ratio (490/440 nm) of fluorescence intensity in OK cells was constructed (Fig. 1). The values for pKa and b were founded to be 7.15 and 1.36, respectively, in OK cells and these values were used for calibration of pHi.

Na^+-dependent and -independent pHi regulation

The Na^+-dependent and -independent recovery rates of pHi were assayed as the initial rate under normal or Na^+-free conditions after an intracellular acid load imposed by NH_4Cl prepulses. The Na^+-dependent pHi recovery rate, NHE activity, was calculated by subtracting pHi recovery rate under Na^+-free condition from that in the presence of extracelluar Na^+.

Buffer capacity

Intracellular intrinsic buffer capacity (βi, mmol/l · pH) was measured by the stepwise-reducing ammonium pulse technique from 20 to 5 mM NH_3/NH_4^+ under the Na^+-free conditions, as shown in Fig. 5. The assumption was that NH_3 is rapidly equilibrated as $[NH_3]i=[NH_3]o$. The calculation of the intrinsic buffer capacity was performed according to the formula (equation 2).

$$\beta i = [NH_4^+]/\triangle pHi \quad \text{(Eq. 2)}$$

The amount of $[NH_4^+]i$ is calculated from equation 3.

$$[NH_4^+]i = [NH_4^+]o \cdot 10^{(pHo-pHi)} \quad \text{(Eq. 3)}$$

Data for the intrinsic buffer capacities were fitted to a second-order polynomial equation.

Statistics

All data are expressed as means±S.E.M. of *n* preparations. Analysis of variance and the Student's *t*-test for unpaired data were used to determine statistical significance. A p value <0.05 was assumed to denote a signi ficant difference.

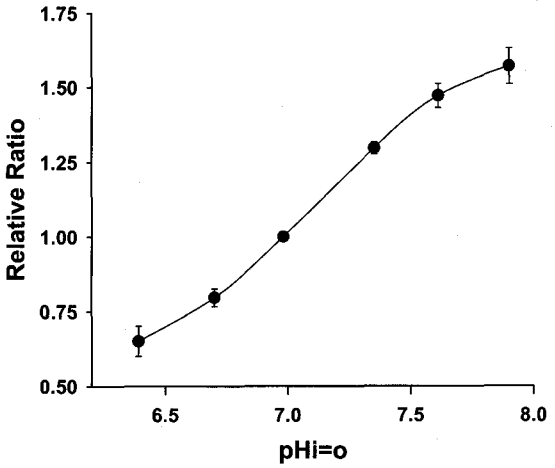

Fig. 1. Relationship between pHi=o and the normalized ratio (490 nm/440 nm) of intracellular 2',7'-bis-(carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF acid). Symbols represent means of four independent determinations; vertical lines show±SE.

RESULTS

Dopamine effects on resting pHi

The pHi at the steady-state in control cells was 6.95±0.03 (n=26). However, intracellular acidification was induced by chronic dopamine treatment (6.69±0.05, n=16) (Fig. 2).

Exracellular Na^+-removal effects on pHi

As shown in Fig. 3, pHi was gradually decreased by isosmotical removal of extracellular Na^+ and approached to the steady-state at 10 min after incubation, strongly implicating that Na^+-dependent or -sensitive pH regulatory mechanisms, possibly NHEs, are normally operating in the cells. As shown in Fig. 4, both the amplitude and the initial rate of pHi changes were markedly diminished in chronically dopamine-treated cells (△pHi, pH units = 0.12±0.02, n=6 vs. 0.23±0.02, n=14; dpHi/dt, pH units/min = 0.034±0.006, n=16 vs. 0.058±0.010, n=14). These results implicate that Na^+-dependent pHi regulation mechanisms could be modified by chronic exposure of OK cells to dopamine. However, we could not exclude the possibility of involvement of the increase of

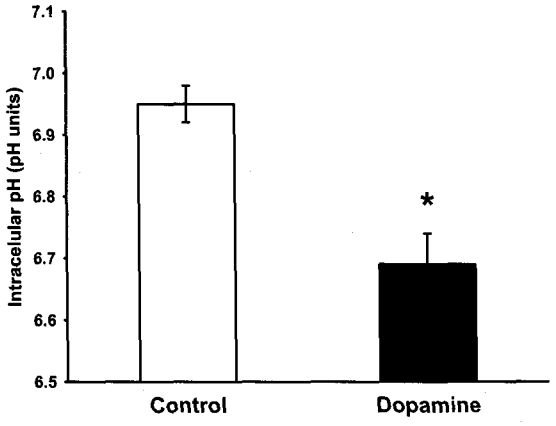

Fig. 2. pHi at resting state. Bars represent means of independent determinations in each group; vertical lines show±SE. n=26 in control group; n=16 in dopamine groups. *$p<0.05$ vs. control.

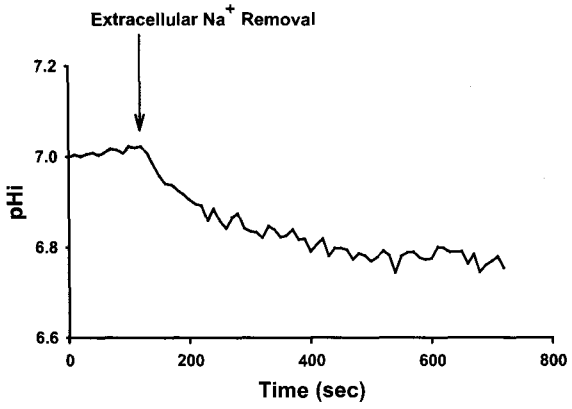

Fig. 3. One representative experiment showing the effects of isosmotical Na^+ remvoal from the bath medium on the pHi in an OK cell. Intracellular acidification occurred by extracellular Na^+ remvoal.

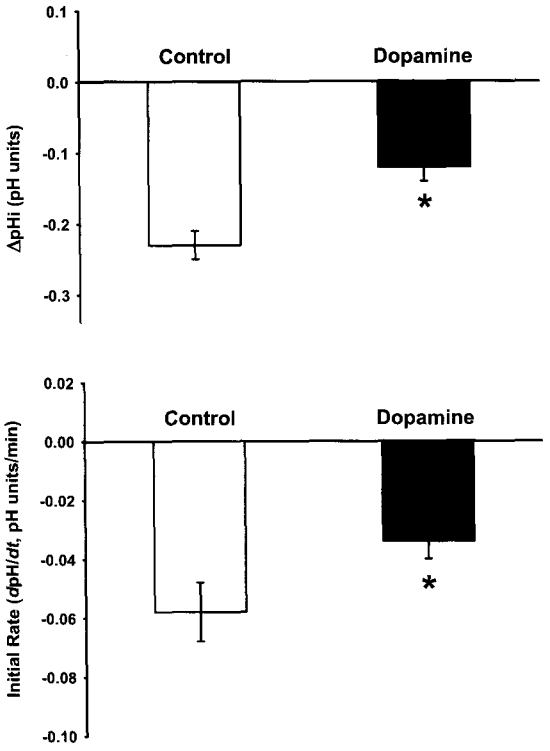

Fig. 4. Effects of Na^+ removal from the bath medium on the initial rates (upper panel) and maximum changes (lower panel) of intracellular acidification measured in monolayers of OK cells. Bars represent means of independent determinations in each group; vertical lines show SE. n=14 in control; n=6 in dopamine groups. *$p<0.05$ vs. control.

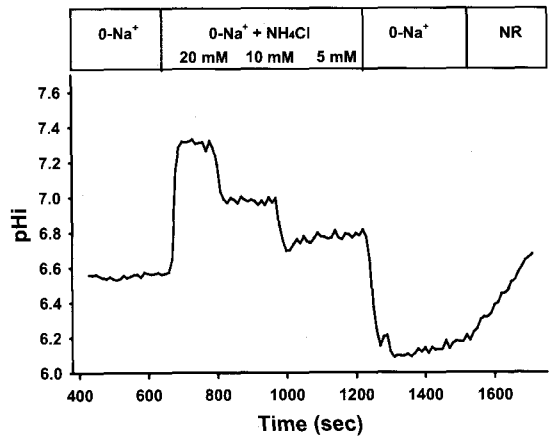

Fig. 5. One representative trace of changes in intracelluar pH induced by ammonium chloride prepulse technique in the absence and presence of extracellular Na^+ in monolayers of OK cells. pHi was decreased by removal of ammonium chloride form the bath medium. There was rapid pHi recovery, when the medium returned to the normal.

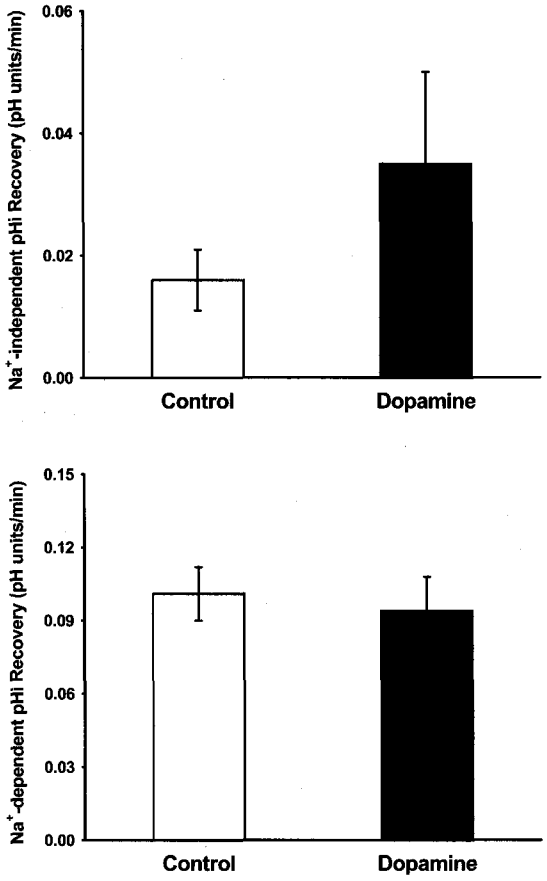

Fig. 6. Na^+-independent and dependent recoveries from acute intracellular acid loading. Although the Na^+-independent recoveries were much greater than control, there was no significant differences between dopamine-treated cells and control. Bars represent means of independent determinations in each group; vertical lines show SE. n = 10 in control; n = 8 in chronic dopamine treatments.

buffering capacity in this process.

Na^+-independent and -dependent pHi recoveries

The slight recovery under the Na^+-free conditions was due to the Na^+-independent pHi recovery, and the rapid recovery occurred in the presence of Na^+ after intracellular acid loading by ammonium prepulse (Fig. 5).

The Na^+-independent recovery rate was increased by 2-fold in chronic dopamine exposures, even though there was no significant difference (0.035 ± 0.015, n=8 vs. 0.016 ± 0.005, n=10)(Fig. 6). These results implicate that chronic treatment with dopamine stimulated the Na^+-independent pH regulatory system. Nevertheless, as shown in Fig. 6, NHE activity in OK cells was not significantly altered by chronic dopamine administration.

Intracellular intrinsic buffer capacities

As shown in Fig. 7, the intracellular intrinsic buffer capacity was increased by reducing pHi. This pattern was

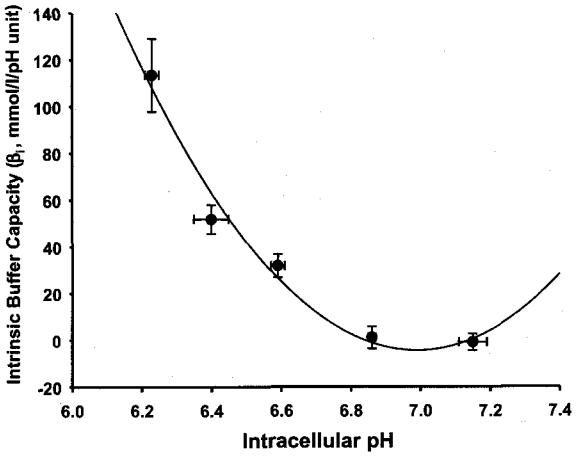

Fig. 7. Relationship between intrinsic buffer capacity (βi) and pHi (pHi). The data from experiments performed on the individual cell monolayers were summarized by averaging βi values, and the regression lines were fit to second order with mean values. Symbols represent means of 6 independent determinations in each group; horizontal and vertical lines show SE.

similar to those obtained by others (Vachon et al, 1995; Feifel et al, 1997; Lagana et al, 2000) after fitting the data with the second order. From these results, we can extrapolate that the chroinically dopamine-exposed cells might have higher buffer capacity, since their pHi was significantly lower than the control.

DISCUSSION

Gomes et al (2001) and Wiederkehr (2001) showed that intracellular acidification occurred in OK cells by acute treatment with dopamine, suggesting the direct inhibition effects of dopamine on the NHE. However, there has been no consensus on the effective concentration of dopamine on the cells. Gomes et al (2001) showed that the pHi was almost maximumly decreased at about 1 μM dopamine and inhibited to 40% of maximum intracellular acidification by 100 nM dopamine. Wiederkehr et al (2001) could not observe any significant inhibitory effects on the NHE activity at the naomolar concentration of dopamine, and showed that NHE activities were inhibited to about 50% of the control by 1 mM dopamine treatment. Although the methodological approach was different, their effective dopamine range on the pHi regulation was far apart from each other. Little information is available for chronic effects of dopamine on pHi regulaton in OK cells.

The resting pHi was significantly lowered by the chronic dopamine administration. The results might have been due to the failure or modification of cellular acid-base regulation, especially the NHE activity which is one of the most important pH regulators. When extracellular Na^+ was isosmotically removed, both the initial rate and the amplitude of the Na^+-sensitive pHi changes were diminished in chronically dopamine-treated OK cells. These data suggested the possibility that the intracellular acidification induced by chronic dopamine treatments resulted from the decrease in the NHE activities as well as other pH-regulatory systems. In addition, we could not eliminate

the possibility that changes in the cellular buffer capacity were also involved.

However, as shown in Fig. 6, Na^+-independent recoveries in the chronic dopamine treatments were almost 2-fold increased without significant changes in Na^+-dependent recoveries through NHE activity. Na^+-independent pH regulatory mechanisms seemed to be activated by dopamine exposure. There appeared to be somewhat discrepancy of the data presented in Fig. 4 and 6 in regards to NHE activity in dopamine-exposed OK cells. As shown in Fig. 7, the intracellular intrinsic buffer capacity was enhanced by reducing pHi. Therefore, the decrease of pHi changes in dopamine-treated OK cells under the Na^+-free conditions might have come from the increase in the intrinsic buffering capaicty induced by intracellular acidification.

Even though detailed mechanisms involved in the increased intrinsic buffering capacity in dopamine-exposed OK cells were not examined, we could not find any changes of NHE activity, although it decreased in acute dopamine-exposed cells. Dopamine metabolites, produced by monoamine oxidase and/or catechol-*O*-methyltransferase in the OK cells (Guimarães et al, 1997), could influence the pHi regulatory machinery. However, there is no evidence that dopamine metabolites including norepinephrine inhibit the NHE or other pH regulatory system.

The data in this study suggest that an increase in buffer capacity in chronic dopamine treatment could result in the diminished initial rate and amplitude of changes in pHi after extracellular Na^+ removal, since the cellular response to the anomalous conditions such as blocking the pH regulation mechanism should buffer it rapidly as the first step and probably for awhile. The intracelluar intrinsic buffer capacity seems to be important in correcting the changes of pHi such as NHE inactivation.

Our data strongly suggested that chronic dopamine treatment increases intrinsic intracellular buffer capacity, since higher buffer capacity was induced by lower pHi and these effects could attenuate the pHi changes under extracellular Na^+-free conditions in chronically dopamine-treated OK cells. Our study also demonstrated that intracellular acidification induced by chronic dopamine treatments was not mediated through the changes of NHE activity.

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