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Intracellular pH Regulation in the Mouse Lacrimal Gland Acinar Cells

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Summary. Intracellular pH (pH_i) of the acinar cells of the isolated, superfused mouse lacrimal gland has been measured using pH-sensitive microelectrodes. Under nonstimulated condition pH_i was 7.25, which was about 0.5 unit higher than the equilibrium pH. Alterations of the external pH by ±0.4 unit shifted pH_i only by ±0.08 unit. The intracellular buffering value determined by applications of 25 mM NH_4^+ and bicarbonate buffer solution gassed with 5% CO_2/95% O_2 was 26 and 46 mM/pH, respectively. Stimulation with 1 μM acetylcholine (ACh) caused a transient, small decrease and then a sustained increase in pH_i. In the presence of amiloride (0.1 mM) or the absence of Na^+, application of ACh caused a significant decrease in pH_i and removal of amiloride or replacement with Na^+-containing saline, respectively, rapidly increased the pH_i. Pretreatment with DIDS (0.2 mM) did not change the pH_i of the nonstimulated conditions; however, it significantly enhanced the increase in pH_i induced by ACh. The present results showed that (i) there is an active acid extrusion mechanism that is stimulated by ACh; (ii) stimulation with ACh enhances the rate of acid production in the acinar cells; and (iii) the acid extrusion mechanism is inhibited by amiloride addition to and Na^+ removal from the bath solution. We suggest that both Na^+/H^+ and HCO_3^-/Cl^- exchange transport mechanisms are taking roles in the intracellular pH regulation in the lacrimal gland acinar cells.

Key Words intracellular pH · lacrimal gland · amiloride · DIDS · Na^+/H^+ antiport · Cl^-/HCO_3^- · antiport

Introduction

Applications of secretory stimuli to the lacrimal gland significantly enhance the rate of O_2 consumption (Herzog, Sies & Miller, 1976). Enhancement of oxidative metabolism should increase the rate of volatile and nonvolatile acid production in the cytoplasm. Carbon dioxide would be hydrated by the catalytic activity of the cellular carbonic anhydrase (Henniger, Schulte & Spicer, 1983) and increase the cytosolic concentration of H^+ and HCO_3^-. This should cause a significant disturbance of the intracellular pH (pH_i). However, the mechanism of pH_i regulation in the exocrine glands has been poorly understood. In the previous studies it was found that Na^+ influx induced by cholinergic stimulation in the mouse (Saito, Ozawa & Nishiyama, 1987*b*) and rat (Parod & Putney, 1980) lacrimal gland acinar cells was significantly inhibited by amiloride. Also, acid loading the cells by an application of NH_4^+ or CO_2 gas caused an increase of the cytosolic Na^+ activity (Saito et al., 1987*b*). These findings were suggestive of the presence of Na^+/H^+ exchange transport mechanism in this tissue. The presence of Na^+/H^+ exchange has also been shown in a variety of tissues of various origin including the rat exocrine pancreas (Hellmessen et al., 1985) and the mandibular salivary gland (Pirani et al., 1987).

In the present study on the mouse lacrimal gland acinar cells we have measured the pH_i with H^+-selective microelectrodes under various conditions and confirmed the presence of Na^+/H^+ exchange and also obtained evidence suggesting the presence of HCO_3^-/Cl^- exchange transport. A preliminary account of the results has been presented (Saito et al., 1987*c*).

Materials and Methods

The methods of preparation and superfusion of the tissue and electrophysiological instrumentation employed in the present study have been described in detail (Saito et al., 1985, 1987*a,b*). The exorbital lacrimal gland of the white mouse was excised, tied on a platt form, placed in a chamber and superfused with warmed (37°C), oxygenated saline solutions. The acinar cells were impaled with double-barreled pH-sensitive microelectrode and the outputs of the electrode, with reference to the salt-agar bridge placed in the bathing solution, were fed to a high-input impedance electrometer (FD223, W.P. Instruments, New Haven, CT) and recorded on a chart recorder (R-03M, Rikadenki, Tokyo). Recordings stable for at least 30 min and with basolateral membrane potential V_m of greater than −35 mV (Saito et al., 1987*a*) in the standard buffer saline were accepted.

H^+-Selective Microelectrode and Solutions

Double-barreled H^+-selective microelectrodes were constructed. Micropipettes, drawn from inner-fibered borosilicate glass tub-

ings and dehydrated by baking on a hot plate, were silanized (Zeuthen, 1980) by exposing the inside of one barrel of the pipette to hexamethyldisilazane (Sigma Chemical, St. Louis, MO) vapor for about 60 min in a dry chamber filled with N_2 gas. Relative humidity in the chamber was maintained at lower than 30%. Micropipettes were baked again, and after cooling in the atmosphere a small amount of pH-sensitive resin was introduced to the tip portion of the silanized barrel and back filled with a pH-buffer solution (Ammann et al., 1981). The reference barrel was filled with a 0.5 M KCl solution. The electrodes were calibrated for the response with a series of pH standard buffer solutions (pH 6.2–8.0) and those with sluggish response were bevelled as described previously (Saito et al., 1987*b*). Electrodes employed for the impalements had a slope of 58 ± 1-mV/pH and the response time was less than 10 sec. Interference by other ion species in the usual saline solutions was negligible.

The regular saline contained (in mM): 139, Na^+; 127, Cl^-; 4.7, K^+; 1.1, Mg^{2+}; 2.6, Ca^{2+}; 4.9, glutamate; 4.9, pyruvate; 2.7, fumarate; 2.8, D-glucose; and 5, HEPES/Tris buffer (pH 7.4). In a series of experiments, solutions of pH 7.0 and 7.8 were used. Bicarbonate buffer saline was prepared by replacing 25 mM Cl^- wth isomolar HCO_3^- and gassed with 5% CO_2 and 95% O_2. Sodium-free solution was made by replacing Na^+ with isomolar N-methyl-D-glucamine, and titrated to pH 7.4 with HCl, and the osmolality was adjusted to 285 mOsm/kg H_2O by an addition of the desired amount of D-mannitol. Amiloride was a gift from Merck & Co. (Rahway, NJ), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) was purchased from Sigma Chemical Co. (St. Louis, MO.) and pH-sensitive resin from Science Trading (Frankfurt, FRG).

pH Measurement of the Extracellular Fluid

Output of $H^+/(OH^-)$ from the tissue was observed by monitoring pH of the extracellular fluid. Tissues were minced into small pieces of about 3 mg and packed in a flow cell mixed with small pieces of sintered glass. The flow cell, a cylinder of plexy glass tubing, had a capacity of 0.6 ml, inlet and outlet (i.d. of 1 mm) of the superfusate on the either end, holes for installation of a mini-pH electrode (MEPH-2, WP Instruments, New Haven, CN) in the center of the cylinder and for a reference electrode made of a salt-agar bridge near the outlet of the superfusate. In the lumen of the cylinder a nylon mesh septum was placed in between the two electrodes to hold the minced tissues. Connection of electrodes to the flow cell was tightly sealed with rubber O-rings. For this series of experiments the buffer value of the normal saline was reduced (5 mM HEPES/Tris buffer was replaced by 1 mM Tris buffer of pH 7.2) to magnify the change in the pH. The flow rate of the superfusate was 3 ml/min. After recording the steady-state pH of the extracellular fluid, drugs were added to the superfusate and then the flow was stopped. During this procedure the pH was monitored (Weinman & Reuss, 1982).

Calculation of Intracellular Buffering Power and Net Ion Flux

For quantitative analysis of $H^+/(OH^-)$ ion transport across the plasma membrane, it is necessary to determine the intracellular buffering power β_i. Buffering power is defined as

$$\beta_i = |dB/dpH| \tag{1}$$

where dB represents the amount of added strong base or acid and dpH the induced change in pH. When the solution in the bath is changed from regular HEPES/Tris buffer to HCO_3^-/CO_2 buffer solution, the intracellular buffering power can be estimated by the relation

$$\beta_i = \Delta B/\Delta pH_i = \Delta[HCO_3^-]_i/\Delta pH_i \tag{2}$$

and, assuming free diffusion of CO_2 into the cytoplasm, intracellular HCO_3^- concentration is calculated from the Henderson-Hasselbalch's equation

$$[HCO_3^-]_i = SPCO_2 \cdot 10^{pH_i - pK} \tag{3}$$

where S denoted the solubility coefficient for CO_2 and pK the dissociation constant in the physiological saline at 37°C. A value of 0.0307 mM/mmHg for S and pK value of 6.100 were adopted from Austin et al. (1963).

An alternative method employed to determine buffering power was addition/removal of NH_4^+-containing solutions (Boron & DeWeer, 1976). Assuming that the intracellular NH_3 concentration is equal to that in the external fluid, buffering power is given by

$$\beta_i = \Delta[NH_4^+]_i/\Delta pH_i \tag{4}$$

and

$$[NH_4^+]_i = [NH_3]_i \cdot 10^{pK_a - pH_i} \tag{5}$$

where a pK_a value of 9.37 was used.

Net ion fluxes across the membrane can be calculated by

$$J_i = dC_i/dt \cdot V/A \tag{6}$$

where dC_i/dt denoted the change in the intracellular ion concentration during a given time period and V/A the volume/surface ratio of the cell. In the case of H^+, dC_i represented $\beta_i \cdot dpH_i$. For convenience's sake, we have assumed a constant V/A ratio and dC_i/dt's are given in the following text instead of J_i's.

Statistical Treatments

Values were given as the mean ± SE of the mean. Statistical significance was assessed by Student's t-test and $P < 0.05$ was taken as significant.

Results

Intracellular pH in the Nonstimulated Conditions and the Effects of pH Alteration in the Bath Solution

On impalement of an acinar cell with the microelectrode, the pH_i was 0.1–0.3 unit more acidic than the external solution. Then pH_i gradually increased to 7.3–7.4 and returned to a steady level of 7.2–7.3 in 30–60 min. During this period, V_m showed a ten-

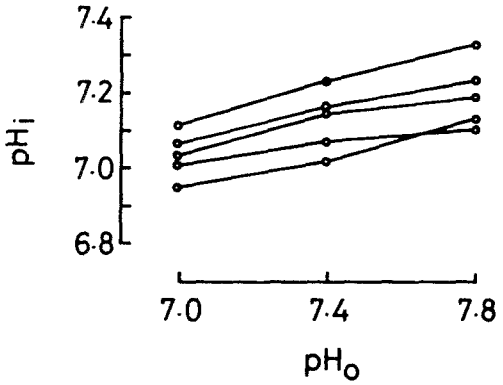

Fig. 1. Effect of changes in pH of bath solution (pH_o) on intracellular pH (pH_i) of the mouse lacrimal gland acinar cell. Data obtained from four samples

dency to hyperpolarize slightly. In the regular buffer solution the steady-state V_m was -39.8 ± 0.6 mV and pH_i was 7.25 ± 0.01 ($n = 55$). The pH_i was about 0.5 unit higher than the equilibrium pH predicted for passive distribution of H^+ ion across the membrane and suggested the presence of active H^+ extrusion. Alterations of the pH in the bath solution (dpH_o) by ± 0.4 unit caused a shift of pH_i (dpH_i) as shown in Fig. 1. The slope dpH_i/dpH_o was 0.2 and constant in the range of pH_o. These results strongly suggested the presence of an effective pH_i regulation mechanism, strong buffering power of the cytosol, and/or very low permeability of the plasma membrane to $H^+(OH^-)$ ions.

Acid-Loading and Intracellular-Buffering Value

Intracellular buffering value was estimated by acid loading procedures (Thomas, 1974; Boron & De-Weer, 1976). When HCO_3^- buffer solution gassed with 5% CO_2/95% O_2 was superfused, pH_i decreased transiently as shown in Fig. 2*a*. From the magnitude of the induced pH_i change and using the relationship described by Eqs. (2) and (3) the intracellular buffering value β_i was calculated. The β_i value determined in 15 cells in 3 tissues was 46.2 ± 4.3 mM/pH. Replacement of Na^+ with NH_4^+ caused an increase in pH_i and removal of NH_4^+ caused an opposite effect on pH_i (Fig. 2*b*). Assuming a homogeneous distribution of NH_3 between extracellular and intracellular compartments and using a pK_a value of 9.37, intracellular concentration of NH_4^+ was calculated by Eq. (5). From the changes in pH_i and $[NH_4^+]_i$ induced by removal of extracellular NH_4^+, β_i value was calculated by Eq. (4). The β_i value of 26.3 ± 1.4 mM/pH ($n = 7$) obtained was comparable to those reported for other tissues studied by the same methods (*see* Roos & Boron, 1981). The presence of amiloride (0.1 mM) or DIDS (0.2 mM) did not significantly affect the buffering power; this was evident from Fig. 2*b* where the size of pH_i change elicited by the removal of NH_4^+ was little affected by these drugs. However, the time course of acid extrusion was retarded by amiloride, and removal of amiloride or the presence of DIDS increased the rate of extrusion.

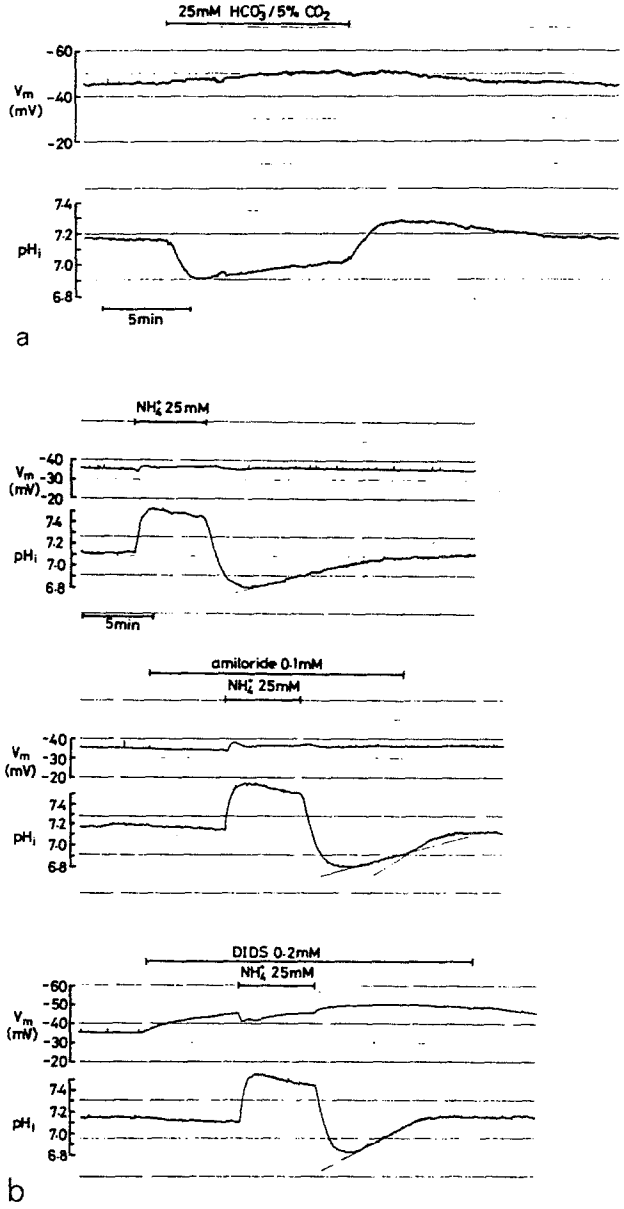

Fig. 2. Effect of acid loading on intracellular pH. The cell was acid loaded by changing the bath solution from nominally CO_2-free HEPES/Tris buffer to bicarbonate buffer solution gassed with 5% CO_2/95% O_2 gas mixture (*a*) or by replacing 25 mM Na^+ with isomolar NH_4^+ (*b*). Note that the removal of amiloride and the presence of DIDS accelerated the slope of acid extrusion

Table 1. Acetylcholine-induced change in basolateral membrane potential (V_m) and intracellular pH (pH_i) in the absence (controls) and presence (tests) of amiloride, Na^+-free Ringer and disulfonic stilbene DIDS

	Control				Test			
	ACh(−)		ACh(+)		ACh(−)		ACh(+)	
	V_m	pH_i	V_m	pH_i	V_m	pH_i	V_m	pH_i
Amiloride 0.1 mM (n = 5)	−40.0 ±0.7	7.30 ±0.02	−59.3 ±1.3	7.35 ±0.02	−41.3 ±2.1	7.26 ±0.03	−57.7 ±2.2	7.11[a] ±0.03
Na^+-free Ringer (n = 8)	−37.8 ±0.7	7.24 ±0.01	−56.8 ±1.2	7.33 ±0.03	−45.8[a] ±1.2	7.27 ±0.02	−59.9 ±1.3	6.99[a] ±0.05
DIDS 0.2 mM (n = 6)	−41.4 ±1.5	7.25 ±0.02	−60.7 ±1.5	7.32 ±0.03	−47.3[a] ±3.1	7.21 ±0.03	−62.2 ±3.1	7.39[b] ±0.03

Data for control and test conditions were obtained in the same cells. The values in the presence of acetylcholine (ACh 1 μM) are those 5–8 min after the addition when V_m and pH_i reached a quasi-stable level.

[a] Significantly different from the control values ($P < 0.05$).

[b] The magnitude of ACh-induced pH_i change is significantly ($P < 0.02$) larger than the control.

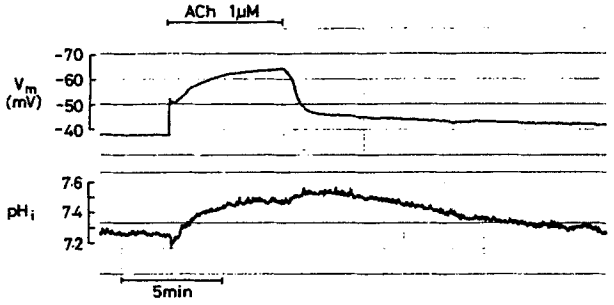

Fig. 3. A representative record of the effect of acetylcholine (ACh, 1 μM) on membrane potential (V_m) and intracellular pH (pH_i) of the mouse lacrimal gland acinar cell. Addition of ACh produced an initial small decrease which was followed by a profound increase in pH_i. Removal of ACh caused a transient further increase in pH_i before the return to the initial level

Effect of Acetylcholine on pH_i

The time-courses of the effect of 1 μM acetylcholine (ACh) on V_m and pH_i were observed (Figs. 3, 4*b,c*, Table 1). Figure 3 is one of the representative records that show a profound alkalinization effect of ACh (*cf.* Figs. 4*b,c*). Immediately after the addition of ACh, membrane hyperpolarization was induced due to activation of the Ca^{2+}-activated K^+ channel (Marty, Tan & Trautmann, 1984). Simultaneously, pH_i decreased slightly and transiently and then increased beyond the nonstimulated level and reached a new stable level in several minutes. Removal of ACh depolarized V_m and increased pH_i further before the slow restoration of the initial level. The rate of increase in pH_i during the 1–2 min period after application of ACh was 0.04 ± 0.01 min^{-1} on the average (n = 26).

Effect of Sodium-Free Ringer Solution, Amiloride and DIDS on Acetylcholine-Induced Change in pH_i

Shortly after the impalement, when the pH_i was in the range of 7.3–7.4, superfusion of amiloride-containing solution or Na^+-free solution caused an immediate decrease in pH_i. The effect of amiloride and Na^+ removal was not additive (Fig. 4*a*), suggesting that the site of interaction of amiloride and Na^+ was common.

After establishment of the steady-state pH_i of 7.2–7.3, pH_i was little affected by Na^+-free saline (7.24 ± 0.01 *vs.* 7.27 ± 0.02), while V_m was significantly hyperpolarized (−37.8 ± 0.01 *vs.* −45.8 ± 1.2 mV). In the absence of Na^+, an addition of ACh caused a significant decrease in pH_i (Fig. 4*b*, Table 1). The rate of pH_i decrease (dpH_i/dt) during the initial 1–2 min period was 0.07 ± 0.01 min^{-1}. Readministration of Na^+-containing saline soon after the removal of ACh caused a rapid increase in pH_i (dpH_i/dt = 0.43 ± 0.03 min^{-1}, n = 6), transiently exceeding the normal pH_i level. These findings indicated that stimulation with ACh increases the rate of intracellular acid production; however, in the absence of Na^+ in the superfusate, acid is not effectively extruded out of the cells. Addition of 0.1 mM amiloride in the superfusate caused similar effects on pH_i to those elicited by Na^+ removal from the

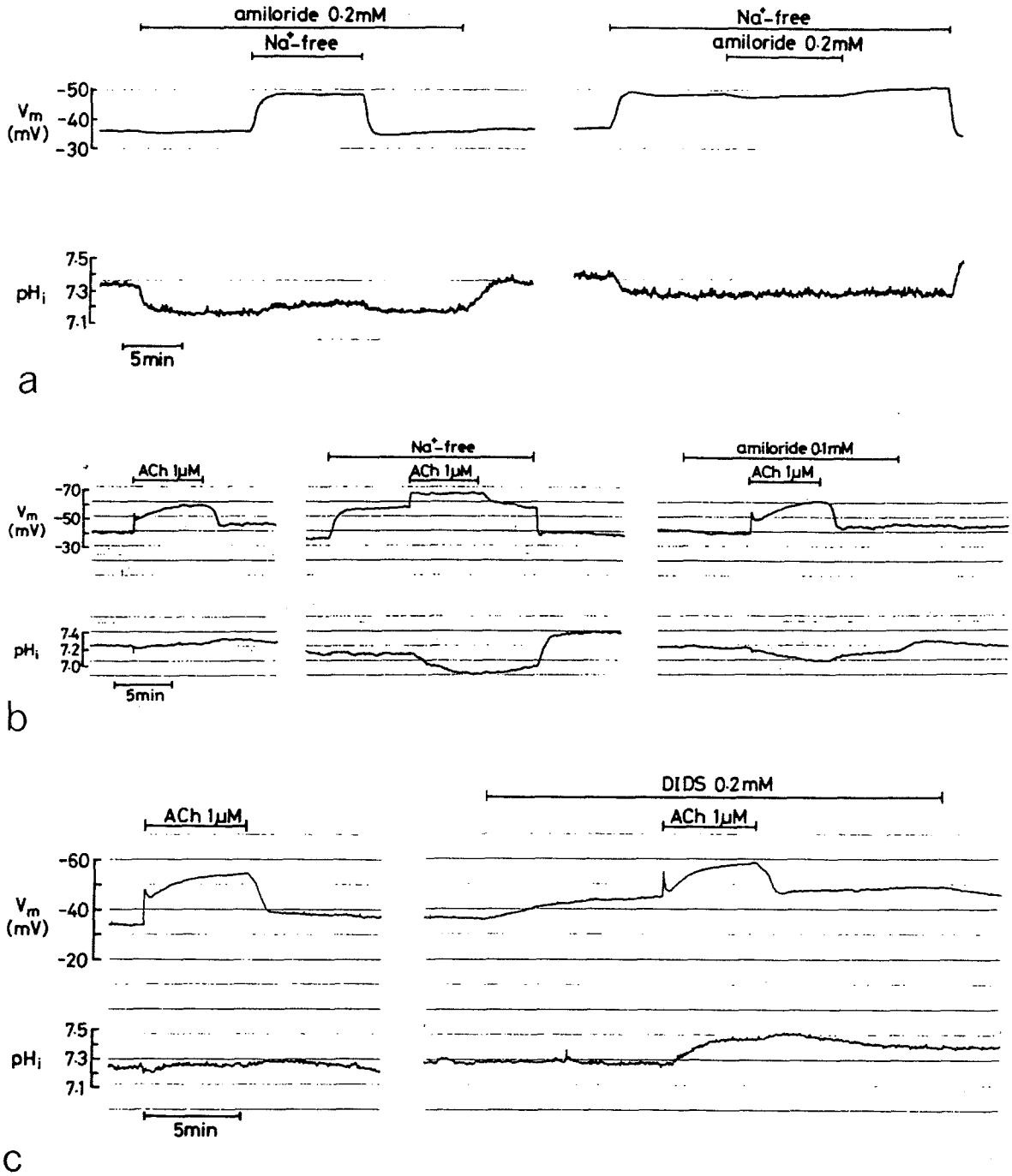

Fig. 4. Effect of addition of amiloride and disulfonic stilbene DIDS and replacement of the normal bath solution with Na^+-free N-methyl-D-glucamine solution on intracellular pH. (*a*) The effects of Na^+-free solution are shown in the presence of 0.2 mM amiloride and vice versa, when intracellular pH was alkalized by the microelectrode impalement (*also see* Results in the text). (*b* and *c*) The effects of ACh are shown after the establishment of the steady-state pH_i in Na^+-free saline (*b*), in the presence of 0.1 mM amiloride (*b*) and 0.2 mM DIDS (*c*)

solution (Fig. 4*b*, Table 1). At this dose of amiloride, the rate of pH_i decrease induced by ACh was 0.02 ± 0.01 min^{-1}. The effect of amiloride on V_m was negligible. The disulfonic stilbene derivative DIDS (0.2 mM) itself caused little effect on pH_i of the nonstimulated tissues; however, it hyperpolarized V_m by 6 ± 1 mV. The cause of DIDS-induced hyperpolarization was suggested to be due to an inhibition of the Cl^- permeability of the basolateral membrane (Saito et al., 1987*a*). In the presence of DIDS, in contrast to the effect of Na^+ removal or amiloride addition, the magnitude of ACh-induced

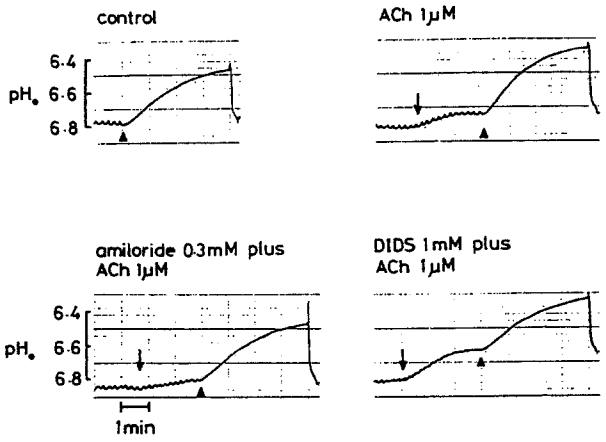

Fig. 5. Acetylcholine-induced change in pH of the external fluid near the surface of lacrimal gland fragments. The lacrimal gland was fragmented, packed in a flow cell, and superfused. The pH of the superfusate was monitored among the tissue fragments. At arrows, either ACh (1 μM), amiloride (0.3 mM) plus ACh or DIDS (1 mM) plus ACh was added to the superfusate. At triangles, the superfusate flow was stopped. At the end of the records, superfusion was resumed

increase in pH_i at the steady state became greater than the control condition ($P < 0.02$). The rate of pH_i increase (0.02 ± 0.01 min^{-1}) induced by ACh in the presence of DIDS was not different from that without DIDS. Wash-out of DIDS slowly restored V_m towards the initial level; however, sometimes pH_i remained at a higher level than the control (Fig. 4*c*). This seemed to indicate that DIDS stimulates the rate of acid extrusion or inhibits the rate of alkali extrusion from the acinar cells.

Extracellular pH Measurement—A Stop Flow Method

In order to confirm that the effects of amiloride and DIDS described above were due to the effect on the $H^+(OH^-)$ transport across the basolateral membrane but not on the intracellular buffering power, extracellular pH (pH_o) was monitored as described in Materials and Methods. If observed changes in the pH_i under various experimental conditions represented the changes in the $H^+(OH^-)$ transport across the basolateral plasma membrane, it was expected that the effects of these drugs on pH_o should be opposite in direction to that on pH_i. As depicted in Fig. 5, under constant flow of the superfusate the pH_o was slightly acidic near the surface of the minced tissues. Addition of ACh slightly decreased pH_o and cessation of the flow resulted in a further steep decrease in pH_o. Addition of amiloride slightly reduced the ACh-induced decrease in pH_o under steady perfusate flow as well as after cessation of the flow. On the other hand, addition of DIDS enhanced the rate of pH_o decrease induced by ACh. These results are consistent with our expectations described above and confirmed that addition of ACh augments the rate of acid output from the acinar cells, amiloride inhibits the acid extrusion and DIDS inhibits alkali extrusion across the plasma membrane.

Discussion

This is the first presentation of the results of direct pH_i measurements in the exocrine glands using a H^+-selective microelectrode. There have been a few reports of pH_i measurement in the exocrine glands where ^{31}P NMR signal (Murakami et al., 1983), weak base distribution in the tissue (Cho, Curry & Jacobsen, 1969; Putney & Borzelleca, 1971*a*,*b*; Pirani et al., 1987) or fluorescence of a pH-sensitive indicator (Hellmessen et al., 1985) was observed. The advantage and disadvantage of the use of pH-sensitive microelectrode and other methods have been discussed (Roos & Boron, 1981). Present study offered the continuous time course of pH_i changes in the same cell under the effect of secretory stimuli, acid-loading, amiloride, Na^+-free solution and DIDS.

The pH_i in the nonstimulated condition was 7.25 ± 0.01 ($n = 55$) on the average. This value is comparable to those reported for other exocrine glands (Putney & Borzelleca, 1971*a*,*b*; Hellmessen et al., 1985; Pirani et al., 1987). After the impalement of the acinar cells, the pH_i underwent a slow, significant increase to about pH 7.4, which gradually returned to around 7.2–7.3 in 30–60 min. The result in Fig. 4*a* was recorded shortly after the impalement when the pH_i was close to 7.4. Under such condition, addition of amiloride or replacement of Na^+ with N-methyl-D-glucamine in the bath solution caused a rapid decrease in pH_i to about 7.2. This finding suggested that the acid extrusion mechanism was in an active condition during this period. On the other hand, the possibility of a leak of the superfusate into the cell can be ruled out since during this period the membrane potential continued to hyperpolarize. We speculate that the acid extrusion mechanism was transiently activated by some factor induced by the mechanical stress to the cell.

Stimulation of Acid Production and Extrusion by Acetylcholine

The effect of ACh on pH_i seems to be twofold: an increase in the intracellular acid production and

stimulation of acid extrusion out of the cells. Addition of ACh caused a small, transient decrease in the pH_i and then an increase to a steady level more alkaline than the control condition (Fig. 3, Table 1). We did not see a significant, sustained acidification of pH_i reported for salivary glands (Murakami et al., 1983; Pirani et al., 1987) even with ACh concentrations lower than 10^{-6}M. The initial decrease in pH_i may be the reflection of enhanced metabolic production of acids. Since in the presence of amiloride or in Na^+-free solution, addition of ACh caused an immediate, continuous decrease in the pH_i, even in the presence of amiloride or in the Na^+-free solution the removal of ACh immediately increased the pH_i (Fig. 4*b*). On the other hand, it is clear that addition of ACh activated the acid extrusion mechanism at the same time and, despite the increased rate of acid production, made pH_i more alkaline than the control conditions (Fig. 3, Table 1). Therefore, when acid production decreased the rate after the removal of ACh, the pH_i showed a further increase. The above consideration was validated by measurements of pH_o during stop-flow condition (Fig. 5). Addition of ACh markedly increased the rate of acid output from the tissue, and the acid output was inhibited by amiloride.

The Na^+/H^+ Antiport

The results of our previous studies on intracellular Na^+ activity were suggestive of the presence of Na^+/H^+ antiport in this tissue. Namely, about 50% of the ACh-induced Na^+ influx is inhibited by amiloride in a 0.1 mM concentration without an effect on the membrane potential or the input resistance, and acid-loading the tissues with NH_4^+ or CO_2 applications caused an increase in the intracellular Na^+ activity that was susceptible to amiloride (Saito et al., 1987*b*). In the present study, by measuring the H^+ ion movements across the membrane, the presence of Na^+/H^+ exchange was further confirmed. The absence of Na^+ or the presence of amiloride in the superfusate abolished the ACh-induced alkalinization in the cytosol and made the cytosol significantly acidic. Readministration of Na^+ in the superfusate or removal of amiloride caused a rapid increase in pH_i (Fig. 4*a*,*b*). Acid extrusion seen after washout of NH_4^+ was also significantly inhibited by the presence of amiloride (Fig. 2*b*). All of these results obtained by intracellular Na^+ and H^+ activity measurements are consistent with the presence of Na^+/H^+ exchange transport.

Under nonstimulated steady state, the Na^+/H^+ exchange should be in a quiescent state, since addition of amiloride or Na^+ removal from the superfusate had little effect on pH_i (Table 1). Under this condition, there exists an inward driving force of -72 mV for Na^+ (Saito et al., 1987*b*) and an outward driving force of 9 mV for H^+. Thus, a steep outward driving force for H^+ via the Na^+/H^+ antiport (Aronson, 1984) is preserved under nonstimulated condition. This preservation of the driving force seems to be important for effective, rapid extrusion of H^+ upon stimulation of the intracellular acid production. Once the pH_i decreased to a critical level, and triggered by some factor, if any, the driving force for H^+ may be released via the activation of the Na^+/H^+ exchanger. In various tissues, an increase in the cellular H^+ concentration itself activates the Na^+/H^+ antiport mechanism through an interaction with the intracellular modifier site (*see* Aronson, 1983; Grinstein & Rothstein, 1986). However, to account for the significant increase in pH_i induced by ACh beyond the normal level (e.g. Fig. 3), participation of some factors (for review *see* Grinstein & Rothstein, 1986) which activate the Na^+/H^+ antiport has to be considered in addition to the effect of intracellular acidification.

Stoichiometry of Na^+/H^+ Antiport

It is of interest to estimate the H^+ ion flux induced by ACh. If Na^+/H^+ antiport is the only acid extrusion mechanism in the plasma membrane, the difference in the rate of pH_i change induced by ACh in the presence and absence of Na^+ in the extracellular fluid may represent the rate of H^+ extrusion via the Na^+/H^+ exchange mechanism. Using this difference and the intracellular buffer value determined with NH_4^+ application, the H^+ flux was calculated. (The buffer value determined by CO_2 exposure was not used, because the value was significantly larger than that determined by NH_4^+ exposure and might be overestimating the actual internal buffer value as discussed by Thomas (1976)).

Table 2 shows the result of calculations. The calculated rate of acid extrusion was 2.95 mM/min, which was slightly smaller than the rate of Na^+ influx induced by ACh (data from Saito et al., 1987*b*). This is consistent with the presence of one-for-one Na^+/H^+ exchange transport. The small difference between H^+ efflux and Na^+ influx could be explained by the existence of Cl^--dependent and furosemide-sensitive Na^+ influx and metabolic substrate-dependent Na^+ influx (Saito et al., 1987*b*). Addition of 0.1 mM amiloride reduced the rate of H^+ extrusion by 53%. This is also consistent with the result of about 50% inhibition of ACh-induced Na^+ influx by 0.1 mM amiloride (Saito et al., 1987*b*).

Table 2. The rates of H^+ and Na^+ activity change induced by acetylcholine and inhibitors in mouse lacrimal gland acinar cells

	(*n*)	Initial rate (mM/min)
H^+ transport		
a. ACh	(26)	−1.08 ± 0.21
b. amiloride + ACh	(7)	0.47 ± 0.13
c. Na^+-free + ACh	(8)	1.87 ± 0.24
a–*c*		−2.95
b–*c*		−1.40
Na^+ transport		
d. ouabain	(8)	0.92 ± 0.07
e. ACh	(5)	2.21 ± 0.09
f. ouabain + ACh	(5)	3.17 ± 0.82

The rates of H^+ ion transport were calculated by using a buffering power of 26.3 mM/pH determined by NH_4^+ addition/withdrawal method (*see* Materials and Methods). (*a*) The rate induced by ACh in a regular Na^+-containing solution; (*b*) the rate induced by ACh in the presence of amiloride (0.1 mM) in a regular solution; (*c*) the rate induced by ACh (1 μM) in a Na^+-free solution. *a*–*c* may represent the rate of acid production and extrusion and *b*–*c* the rate of acid extrusion in the presence of amiloride. Na^+ transport rates were determined by the change in the intracellular Na^+ activity measured with the Na^+-selective microelectrode method (data from Saito et al., 1987*b*) and represent the rate of Na^+ activity increase induced by ouabain (1 mM) (*d*), ACh (*e*) and ouabain plus ACh (*f*). *f* may represent ACh-induced Na^+ influx. (*n*) Number of observations.

Effect of DIDS on pH_i

The disulfonic stilbene DIDS itself in a concentration of 2×10^{-4} M had little effect on pH_i of the nonstimulated cells. However, as shown in Fig. 4*c* and Table 1, DIDS significantly enhanced the increase in pH_i induced by ACh. A similar result has been obtained in the salivary gland using another disulfonic stilbene SITS (Pirani et al., 1987). Also, the presence of DIDS enhanced the rate of acidification of the extracellular space during the stimulation with ACh (Fig. 5) and acid extrusion after acid loading with NH_4^+ exposure (Fig. 2*b*). We also have observed that Cl^- uptake by the acinar cell was induced by a removal of HCO_3^- from the bath solution and was inhibited by DIDS significantly and that removal of Cl^- from the HCO_3^--buffer solution caused an increase in pH_i (*unpublished observations*). These findings are consistent with the presence of Cl^-/HCO_3^- exchange transport in the basolateral membrane. (Although a HCO_3^--free solution gassed with pure oxygen has been used in the present experiments, endogenous HCO_3^- should reach to a finite concentration). However, the possibility of other stilbene-sensitive HCO_3^- transport mechanism(s) in addition to Cl^-/HCO_3^- antiporter, such as Na^+–HCO_3^- cotransport (Boron & Boulpaep, 1983; Jentsch et al., 1984; Yoshitomi, Burckhardt & Fromter, 1985) still remains. A detailed study of the Cl^-/HCO_3^- exchange will follow.

The Role of Na^+/H^+ Antiport in Secretion

The role of Na^+/H^+ and Cl^-/HCO_3^- antiporters in secretion has been discussed (Case et al., 1982; Novak & Young, 1986; Pirani et al., 1987). The findings collected in the lacrimal gland agree with their view on the roles of these antiporters. Namely, the major role of Na^+/H^+ antiport can be (i) in spite of the enhanced acid production during the secretory states, to maintain pH_i in an optimal range for other cellular functions, e.g. Na^+/K^+ exchange pump (Eaton, Hamilton, & Johnson, 1984), ion permeability of the membranes, etc. (*see* Roos & Boron, 1981), and (ii) to activate the Na^+/K^+ exchange pump (Wright, 1977). Enhancement of Na^+ recycling via the Na^+/H^+ and Na^+/K^+ exchangers across the basolateral membrane entails a steep K^+ gradient across the membrane and maintains the basolateral membrane hyperpolarized during a prolonged exposure to secretory stimuli, thereby providing electrical driving force for Cl^- efflux across the luminal membrane of the acinar cells (Marty et al., 1984; Findlay & Petersen, 1985; Saito et al., 1985). Also, a fraction of Na^+ influx mediated by the basolateral Na^+/H^+ antiporter can be directly transported into the acinar lumen via the Na^+/K^+ ATPase located in the luminal membrane (Wood & Mircheff, 1986). If the Na^+/H^+ antiport is coupled to Cl^-/HCO_3^- exchange transport directly or indirectly, the latter would uptake Cl^- into the acinar cells during the secretory conditions. In this tissue, uphill accumulation of Cl^- in both resting and secretory conditions (Saito et al., 1985) has been attributed to a Na-K-2Cl co-transport mechanism (Suzuki & Petersen, 1984; Saito et al., 1985; Saito, Ozawa & Nishiyama, 1986). However, present results suggest that during the secretory condition Cl^-/HCO_3^- exchange transport is activated by an increase in the cytosolic HCO_3^- concentration and contributes towards supporting sustained secretion by maintaining the intracellular Cl^- concentration above the equilibrium level.

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