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# Evidence for an Anion Exchanger in the Mouse Lacrimal Gland Acinar Cell Membrane

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**Summary.** Anion exchange transport in the mouse lacrimal gland acinar cell membrane was studied by measuring the intracellular $H^+$ ($pH_i$) and $Cl^-$ ($aCl_i$) activities with double-barreled ion-selective microelectrodes. In a $HCO_3^-$-free solution of pH 7.4 (HEPES/Tris buffered), $pH_i$ was 7.25 and $aCl_i$ was 33 mM. By an exposure to a $HCO_3^-$ (25 mM $HCO_3^-$/5% $CO_2$, pH 7.4) solution for 15 min, $aCl_i$ was decreased to 25 mM, and $pH_i$ was transiently decreased to about 7.05 within 1 min, then slowly relaxed to 7.18 in 15 min. Intracellular $HCO_3^-$ concentration $[HCO_3^-]_i$, calculated by the Henderson-Hasselbalch's equation, was 11 mM at 1 min after the exposure and then slowly increased to 15 mM. Readmission of the $HCO_3^-$-free solution reversed the changes in $aCl_i$ and $pH_i$. The intracellular buffering power was about 40 mM/pH. An addition of DIDS (0.2 mM) significantly inhibited the rates of change in $aCl_i$, $pH_i$, and $[HCO_3^-]_i$ caused by admission/withdrawal of the $HCO_3^-$ solution and decreased the buffer value. Replacement of all $Cl^-$ with gluconate in the $HCO_3^-$ solution increased $pH_i$, and readmission of $Cl^-$ decreased $pH_i$. The rates of these changes in $pH_i$ were reduced by DIDS by 32–45% but not by amiloride (0.3 mM). In the $HCO_3^-$ solution, a stimulation of intracellular $HCO_3^-$ production by exposing the tissue to 25 mM $NH_4^+$ increased $aCl_i$ significantly. While in the $HCO_3^-$-free solution or in the $HCO_3^-$ solution containing DIDS, exposure to $NH_4^+$ had little effect on $aCl_i$. All of these findings were consistent with the presence of a reversible, disulfonic stilbene-sensitive $Cl^-/HCO_3^-$ exchanger in the basolateral membrane of the acinar cells. The possibility of anion antiport different from one-for-one $Cl^-/HCO_3^-$ exchange is discussed.

**Key Words** lacrimal gland · intracellular pH · intracellular $Cl^-$ · $Cl^-/HCO_3^-$ exchange · DIDS · membrane transport

## Introduction

Oxygen consumption by the exocrine glands increases significantly during secretion (*see* Herzog, Sies & Miller, 1976) and part of the generated carbon dioxide hydrates to form $H^+$ and $HCO_3^-$ in the intracellular fluid. The carbonic anhydrase (Henniger, Schulte & Spicer, 1983) may augment hydration. However, the regulation mechanisms of $H^+$ and $HCO_3^-$ ion concentrations has not been clearly elucidated. Recently, we showed in the mouse lacrimal gland a $Na^+/H^+$ antiporter existing in the basolateral membrane that was stimulated by the addition of acetylcholine (ACh). We also found that disulfonic stilbene (DIDS) increases the $pH_i$ of ACh-stimulated cells and increases acid extrusion from the acid-loaded cells (Saito et al., 1987*b*, 1988*a*). Furthermore, even in the presence of a high concentration (1 mM) of furosemide, which inhibits putative $NaKCl_2$ cotransport in this tissue (Suzuki & Petersen, 1985; Saito, Ozawa & Nishiyama, 1986), a significant uphill $Cl^-$ uptake was observed after the cessation of the ACh stimulation (Saito et al., 1987*a*). This suggested the presence of a $Cl^-$ uptake mechanism such as $Cl^-/HCO_3^-$ exchange in addition to the $NaKCl_2$ cotransport in this tissue (Ozawa, Saito & Nishiyama, 1988). In the salivary glands (Novak & Young, 1986) and exocrine pancreas (Seow, Lingard & Young, 1986), the effects of $HCO_3^-$ and stilbenes on the rates of fluid secretion suggested the presence of a $Cl^-/HCO_3^-$ antiporter. A recent study of the $pH_i$ in the salivary gland by a weak base DMO distribution method offered further evidence of the $Cl^-/HCO_3^-$ antiporter (Pirani et al., 1987). In the present study we measured the intracellular $H^+$ and $Cl^-$ activities ($aCl_i$) in the acinar cells of the mouse lacrimal gland and studied the effects of the changes in the extra- or intracellular $Cl^-$ and $HCO_3^-$ concentrations in the presence and absence of DIDS. The results were qualitatively consistent with presence of a reversible $Cl^-/HCO_3^-$ antiporter in the basolateral membrane. A preliminary account of the results has been presented (Ozawa, Saito & Nishiyama, 1987; Saito, Ozawa & Nishiyama, 1988*b*).

## Materials and Methods

The methods of tissue preparation, superfusion and electrophysiological instrumentation have been described in detail elsewhere

(Saito et al., 1985, 1987*a,b*). Briefly, the exorbital lacrimal gland of the white mouse was excised, tied on a platform, placed in a chamber and superfused with warmed (37°C), oxygenated saline solutions. The acinar cells were impaled with either $H^+$ or $Cl^-$-selective, double-barreled microelectrode. The outputs of the microelectrode against a reference salt-agar bridge placed in the bath solution were fed to a high input impedance electrometer and recorded on a chart recorder. Membrane potentials stable for at least 30 min and compatible to those reported earlier (Saito et al., 1985, 1987*b*) were accepted.

### Ion-Selective Microelectrodes

Microelectrodes were drawn from double-barreled borosilicate glass tubings and dehydrated by baking on a hot plate at 200°C for 2 hr. The inside surface of one barrel was silanized by exposing it to silicone vapor (trimethylchlorosilane and hexamethyldisilazane were used for $Cl^-$- and pH-electrodes, respectively). The liquid ion exchanger and internal filling solution used for the $Cl^-$-electrode were Corning 477913 (Corning Medical, Medfield, MA) and 0.5 M KCl solution and for the pH-electrode were a neutral carrier-based liquid cocktail (purchased from Fluka, Zurich, Switzerland) and a pH buffer solution of pH 7.0 (Ammann et al., 1981). The electrodes were calibrated for a series of standard solutions before and after the experiments and those of the slope of greater than 50 mV for a decade activity change were employed. The electrodes that showed a sluggish response were bevelled by the method of Lederer, Spindler and Eisner (1979) and those with a response time of less than 10 sec were used for impalements.

### Solutions and Chemicals

The standard $HCO_3^-$ free solution containing (in mM) 143 $Na^+$, 115 $Cl^-$, 25 gluconate, 4.7 $K^+$, 1.1 $Mg^{2+}$, 2.6 $Ca^{2+}$, 4.9 pyruvate, 2.7 fumarate, 4.9 glutamate, 2.8 D-glucose and 5 HEPES/Tris buffer (pH 7.4) was saturated with 100% $O_2$ gas. Bicarbonate-buffered solution ($HCO_3^-$ solution) was prepared by replacing gluconate with $HCO_3^-$ in the standard solution and gassed with a gas mixture of 95% $O_2$ and 5% $CO_2$. Chloride-free $HCO_3^-$-buffer solution was made by replacing all $Cl^-$ with gluconate. 4,4-diisothiocyanatostilbene-2,2-disulfonic acid (DIDS) was purchased from Sigma (St. Louis, MO) and amiloride was a gift from Merck & Co. (Rahway, NJ).

### Statistics

The data values in the text are given as the mean ± SEM. The difference between the two means was tested by Student's *t* test and was considered significant when $P < 0.05$.

## Results

### Effects of External $HCO_3^-$ and DIDS on Intracellular $Cl^-$ Activity

Figure 1 shows the effects of the $HCO_3^-$-solution on basolateral membrane potential ($V_m$) and $aCl_i$. It

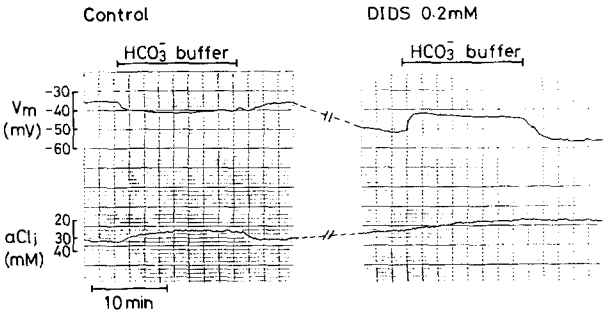


**Fig. 1.** Effects of $HCO_3^-$ solution on membrane potential ($V_m$) and intracellular $Cl^-$ activity ($aCl_i$) in the absence (left panel) and in the presence (right panel) of DIDS; a continuous record from a cell. At the times indicated by the top horizontal bars, $HCO_3^-$-free solution (HEPES/Tris buffer, 100% $O_2$, pH 7.4) was replaced by $HCO_3^-$-solution (25 mM $HCO_3^-$/5% $CO_2$). DIDS (0.2 mM) was added to the solution 16 min before the second exposure to $HCO_3^-$ solution. The $Cl^-$ concentration in the solutions was constant throughout. Exposure to $HCO_3^-$ solution hyperpolarized $V_m$ and decreased $aCl_i$. Addition of DIDS itself significantly hyperpolarized $V_m$, and reduced $aCl_i$ slowly. In the presence of DIDS, the rate of $aCl_i$ decrease induced by exposure to $HCO_3^-$ solution was smaller and the direction of $V_m$ change was reversed (*see also* Table 1)

was expected that if a $Cl^-/HCO_3^-$ antiporter existed in the acinar cell membrane, an abrupt increase in the external $HCO_3^-$ concentration would cause a decrease in $aCl_i$ and its return to the $HCO_3^-$-free solution would increase $aCl_i$ according to the reversal of the $HCO_3^-$ concentration gradient. With DIDS in the superfusate, smaller changes in $aCl_i$ were expected. The results showed that when the superfusate was changed from the $HCO_3^-$-free to the $HCO_3^-$ solution, $V_m$ hyperpolarized by 4 mV (Fig. 1, Table 1). $aCl_i$ decreased by about 5 mM within 15 min rapidly for the initial 1–2 min period then slowly. Reintroduction of the $HCO_3^-$-free solution restored $V_m$ and $aCl_i$ values to those of the control. However, the much earlier restoration of $aCl_i$ than that of $V_m$ indicated that the increase of $aCl_i$ induced by the removal of the external $HCO_3^-$ was not merely the result of electrodiffusion.

The addition of 0.2 mM DIDS to the $HCO_3^-$-free solution significantly hyperpolarized $V_m$ and slowly but steadily decreased $aCl_i$ by about 2 mM within 20 min. In the presence of DIDS, $V_m$ often depolarized with the exposure to the $HCO_3^-$ solution, but thereafter tended to repolarize. The rate of decrease in $aCl_i$ (0.8 ± 0.2 mM/min) was significantly slower than those in the absence of DIDS (1.5 ± 0.2 mM/min). Readmission of the $HCO_3^-$-free solution caused a significant hyperpolarization of $V_m$. The increase of $aCl_i$ was extremely slow in comparison with those of the controls (Table 1).

**Table 1.** Changes in membrane potential ($V_m$) and intracellular $Cl^-$ activity ($aCl_i$) induced by admission and removal of $HCO_3^-$ solution in the presence and in the absence of DIDS

| | Bath solution | | | | |
|---|---|---|---|---|---|
| | $HCO_3^-$-free solution | | $HCO_3^-$ solution | | $HCO_3^-$-free solution |
| Control ($n$ = 7) | | | | | |
| $V_m$ (mV) | −37.6 ± 1.3 | | [a]−41.6 ± 1.1 | | −37.7 ± 1.0 |
| $\Delta V_m$ (mV) | | −4.0 ± 0.5 | | +3.9 ± 0.3 | |
| $aCl_i$ (mM) | 29.1 ± 1.8 | | 24.0 ± 1.8 | | 28.8 ± 1.5 |
| $\Delta aCl_i$ (mM) | | −5.1 ± 0.8 | | +4.9 ± 0.7 | |
| rate (mM/min) | | −1.5 ± 0.2 | | +1.4 ± 0.2 | |
| Test: DIDS 0.2 mM ($n$ = 5) | | | | | |
| $V_m$ (mV) | [b]−47.2 ± 2.3 | | −46.0 ± 2.4 | | [c]−50.4 ± 2.4 |
| $\Delta V_m$ (mV) | | [b]+1.2 ± 1.5 | | [c]−4.4 ± 1.7 | |
| $aCl_i$ (mM) | 27.6 ± 1.2 | | 24.4 ± 1.2 | | 26.2 ± 1.5 |
| $\Delta aCl_i$ (mM) | | −3.2 ± 0.7 | | [b]+1.8 ± 0.6 | |
| rate (mM/min) | | [a]−0.8 ± 0.2 | | [c]+0.4 ± 0.1 | |

While monitoring the membrane potential ($V_m$) and intracellular $Cl^-$ activity ($aCl_i$), the superfusate was changed in a sequence of $HCO_3^-$-free, $HCO_3^-$ and $HCO_3^-$-free solution. Values are the mean ±SEM. Values for $HCO_3^-$ solution are those 15 min after the admission. During the tests, DIDS was added to the $HCO_3^-$-free solution 15–20 min prior to the experiments and was present throughout. $\Delta V_m$ and $\Delta aCl_i$ denoted the differences in $V_m$ and $aCl_i$, respectively, between two conditions. The rates of change in $aCl_i$ were determined from the slope for the initial 0–2 min period after the admission of new superfusate. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$: significantly different from the control conditions.

The above findings were consistent with those of a $Cl^-/HCO_3^-$ antiport. However, since this acinar cell membrane showed a $Cl^-$ conductance that was inhibited by DIDS (Saito et al., 1987*a*), the changes in $aCl_i$ described above could alternatively be explained by the effects of membrane potential changes and of inhibition of the $Cl^-$ conductance by DIDS. Therefore, to determine whether the observed $Cl^-$ movements were accompanied by $HCO_3^-$ movements, $pH_i$ was monitored under identical experimental conditions.

## Effect of External $HCO_3^-$ and DIDS on $pH_i$

Exposure to the $HCO_3^-$ solution was expected to cause a transient decrease in $pH_i$ due to rapid diffusion of $CO_2$ into the tissue and then a slow relaxation towards the initial level due to $H^+$ extrusion (Boron & De Weer, 1976) via the $Na^+/H^+$ exchanger (Saito et al., 1987*b*, 1988*a*,*b*). If a $HCO_3^-/Cl^-$ exchanger existed and transported $HCO_3^-$ out of the cell, the addition of DIDS would inhibit $HCO_3^-$ efflux and stimulate the increase of $pH_i$ in the relaxation phase. Moreover, a decrease in the intracellular buffering power was expected, since the buffering power under this experimental condition involved both a chemical buffering action and an acid/base transport across the membranes.

As shown in Fig. 2, upon exposure to the $HCO_3^-$ solution, $pH_i$ was transiently decreased by about 0.2 unit, probably due to rapid entry of $CO_2$, then relaxed towards the initial level. Intracellular $HCO_3^-$ concentration $[HCO_3^-]_i$ calculated by the Henderson-Hasselbalch's equation was 10.9 mM at the transient peak $pH_i$ (about 1 min after the exposure). Thereafter, $[HCO_3^-]_i$ increased up to 15.3 mM in 15 min (Table 2). The rapid initial increase in $[HCO_3^-]_i$ could be explained by the processes that (i) $CO_2$ diffused into the cell was instantly hydrated to produce $HCO_3^-$ and (ii) extracellular $HCO_3^-$ ions itself entered the cell by simple diffusion or mediated by a specific $HCO_3^-$ transport mechanism. Although the subsequent slow increase in $[HCO_3^-]_i$ cannot be explained in terms of a simple diffusion of $HCO_3^-$ ions with respect to the electrochemical potential gradient across the membrane, it can be expressed as the result of either or both (i) $H^+$ extrusion at a higher rate than that of $HCO_3^-$ extrusion and/or (ii) $HCO_3^-$ uptake against the electrochemical potential gradient mediated by a specific $HCO_3^-$ transport process.

After readmission of the $HCO_3^-$-free solution, $pH_i$ returned to the control level in two phases: an initial peak alkalinization due to a rapid decrease in $CO_2$ concentration and dehydroxylation of $HCO_3^-$ ions ($HCO_3^- \rightarrow OH^- + CO_2$) and a slow acidification due to extrusion of $HCO_3^-$ and/or $OH^-$. The intracellular buffering values were determined from the immediate transient changes in the $pH_i$ (either at the peak ($B_i$), or by extrapolating the slope of $pH_i$ change after the transient peak-to-time zero ($B_i^0$))

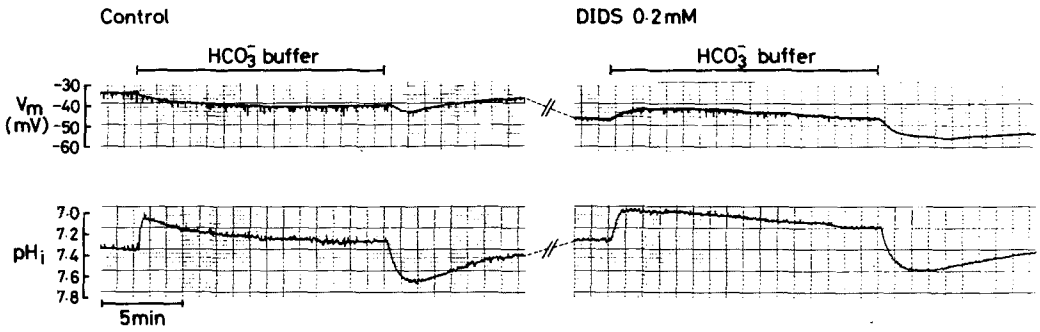


**Fig. 2.** Effects of $HCO_3^-$ solution on intracellular pH in the absence (left panel) and in the presence (right panel) of DIDS; a continuous record from a cell. Experimental condition was the same as in Fig. 1. Immediately after the exposure to the $HCO_3^-$ solution, $pH_i$ decreased transiently reaching the peak in 1 min then relaxed gradually towards the initial level. From the magnitude of the initial peak changes in $pH_i$ or by extrapolating the slope of slow $pH_i$ relaxations to time zero, the intracellular buffering power was determined (*see* text). DIDS decreased both the slope of $pH_i$ relaxation and the buffering power (*see* Table 2)

**Table 2.** Effect of DIDS on intracellular pH, $HCO_3^-$ concentration and buffer value and on the rate of acid/base transport

| | Bath solution | | | | |
|---|---|---|---|---|---|
| | *a*) $HCO_3^-$-free solution | $HCO_3^-$ solution | | $HCO_3^-$-free solution | |
| | | *b*) Peak | *c*) 15 min | *b*) Peak | *c*) 15 min |
| Control ($n$ = 9) | | | | | |
| $pH_i$ | 7.26 ± 0.05 | 7.03 ± 0.05 | 7.18 ± 0.04 | 7.46 ± 0.05 | 7.27 ± 0.04 |
| $[HCO_3^-]_i$ (mM) | | 10.5 ± 1.1 | 14.8 ± 1.3 | | |
| $\Delta[HCO_3^-]_i$ (mM) | | +4.3 ± 0.4 | | | |
| $B_i$ (mM/pH) | | 48.5 ± 6.5 | | 55.4 ± 5.8 | |
| ($B_i^0$) (mM/pH) | | (37.2 ± 5.7) | | (43.8 ± 4.1) | |
| $\Delta pH_i \cdot B_i/\Delta t$ (mM/min) | | −1.55 ± 0.16 | | +2.02 ± 0.21 | |
| Test: DIDS 0.2 mM ($n$ = 6) | | | | | |
| $pH_i$ | 7.24 ± 0.06 | 6.96 ± 0.06 | 7.08 ± 0.06 | 7.44 ± 0.07 | 7.28 ± 0.07 |
| $[HCO_3^-]_i$ (mM) | | 8.7 ± 1.0 | 11.5 ± 1.3 | | |
| $\Delta[HCO_3^-]_i$ (mM) | | [a]+2.8 ± 0.4 | | | |
| $B_i$ (mM/pH) | | [a]30.5 ± 3.6 | | [b]31.8 ± 3.0 | |
| ($B_i^0$) (mM/pH) | | (24.3 ± 2.1) | | [b](26.5 ± 2.1) | |
| $\Delta pH_i \cdot B_i/\Delta t$ (mM/min) | | [b]−0.53 ± 0.11 | | [b]+0.76 ± 0.14 | |

The protocol of the experiment is the same as in Table 1. Mean ±SEM. The intracellular $HCO_3^-$ concentration $[HCO_3^-]_i$ was calculated by the Henderson-Hasselbalch's equation. Values for $pH_i$ and $[HCO_3^-]_i$ are *a*) prior to, *b*) at the transient peak change in $pH_i$ (~1 min) and *c*) 15 min after the replacement of the bath solution. $\Delta[HCO_3^-]_i$ shows the difference in $[HCO_3^-]_i$ between *b*) and *c*). Intracellular buffer value $B_i$ was calculated by the peak change in $pH_i$ and $B_i^0$ was by extrapolation of the time course of $pH_i$ change to time zero. $\Delta pH_i \cdot B_i/\Delta t$ denoted the rate of acid/base transport calculated from the steepest slope of $pH_i$ change ($\Delta pH_i/\Delta t$) 1–5 min after the replacement of the bath solution. [a] $P < 0.05$, [b] $P < 0.01$: significantly different from these in the control conditions.

induced by the addition and the withdrawal of the $HCO_3^-$ solution (Roos & Boron, 1981; Saito et al., 1988*a*). As expected, DIDS significantly ($P < 0.05$) reduced the buffer values and the decrease of $pH_i$ after the readmission of the $HCO_3^-$-free solution (right panel in Fig. 2, Table 2). However, in contradiction to our expectation, DIDS reduced the increase of $pH_i$ (i.e., reduced the increase of $[HCO_3^-]_i$) during its exposure to the $HCO_3^-$ solution (right panel in Fig. 2). This could be explained if the slow relaxation of $pH_i$ after the initial peak acidification was caused by both $H^+$ efflux via the $Na^+/H^+$ antiporter (Saito et al., 1987*b*, 1988*a*) and by $HCO_3^-$ *influx* mediated by a specific mechanism across the membrane, and if the latter was inhibited by DIDS. However, under this condition, a one-for-one $Cl^-/$

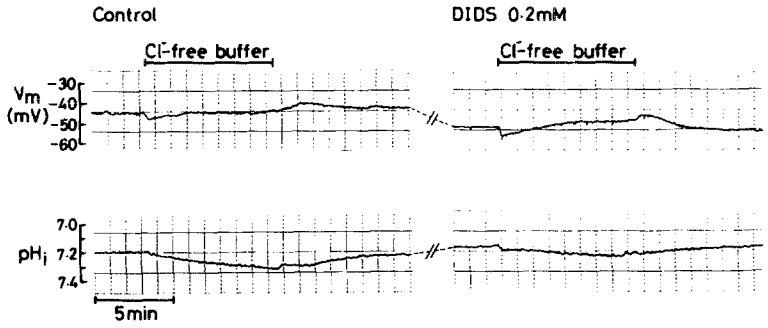


**Fig. 3.** Effect of $Cl^-$ removal from the superfusing solution on $pH_i$ in the absence (left panel) and in the presence (right panel) of DIDS (0.2 mM); a continuous record from a cell. During the time periods marked by the top bars, all $Cl^-$ in the $HCO_3^-$ solution was replaced by equimolar gluconate (*see* Table 3). Note that the rate of increase in $pH_i$ produced by $Cl^-$ removal was slowed by DIDS

**Table 3.** Effect of $Cl^-$ removal from the bath solution on membrane potential, intracellular pH and $HCO_3^-$ concentration

| | Bath solution | | |
|---|---|---|---|
| | $Cl^-$ solution | $Cl^-$-free solution | $Cl^-$ solution |
| Control | | | |
| $V_m$ (mV) | −45.0 ± 2.5 | −42.0 ± 1.9 | −44.4 ± 2.0 |
| $pH_i$ | 7.19 ± 0.03 | [a]7.33 ± 0.03 | 7.17 ± 0.03 |
| $[HCO_3^-]_i$ (mM) | 14.4 ± 1.0 | 20.1 ± 1.3 | 13.7 ± 0.9 |
| $\Delta[HCO_3^-]_i$ (mM) | +5.7 ± 0.7 | −6.4 ± 0.6 | |
| Test: DIDS 0.2 mM | | | |
| $V_m$ (mV) | [b]−53.7 ± 1.9 | [b]−52.0 ± 1.4 | [b]−55.5 ± 1.9 |
| $pH_i$ | 7.14 ± 0.03 | [a]7.25 ± 0.03 | 7.18 ± 0.04 |
| $[HCO_3^-]_i$ (mM) | 12.9 ± 0.9 | 16.8 ± 1.0 | 14.3 ± 1.1 |
| $\Delta[HCO_3^-]_i$ (mM) | 3.8 ± 0.4 | [c]−2.5 ± 0.4 | |

[a] The membrane potential ($V_m$) and intracellular pH ($pH_i$) were determined in the control and the test (0.2 mM DIDS added to the solutions) conditions in the same cells ($n = 5$). Mean ±SEM. $HCO_3^-$-buffered solutions (25 mM $HCO_3^-$/5% $CO_2$) were used throughout. Values for the $Cl^-$-free conditions ($Cl^-$ replaced with gluconate) were those 6 min after the replacement. $\Delta[HCO_3^-]_i$ shows the net change in $[HCO_3^-]_i$. [a] $P < 0.05$ significantly different from these in the $Cl^-$ solution. [b] $P < 0.05$; [c] $P < 0.01$: significantly different from these in the absence of DIDS.

$HCO_3^-$ exchanger could mediate $HCO_3^-$ *efflux* but not *influx*, since the inward chemical potential gradient for $Cl^-$ (−30 mV) was greater than that for $HCO_3^-$ (smaller than −24 mV). Accordingly, an inhibition of the one-for-one $Cl^-/HCO_3^-$ exchanger by DIDS, if present, should enhance the increase of $pH_i$.

## Effect of Removal of External $Cl^-$ on $pH_i$

The effect of removing $Cl^-$ from the extracellular fluid was observed on $pH_i$. In the tissues superfused with the $HCO_3^-$ solution, a sudden replacement of the extracellular $Cl^-$ with gluconate was expected to cause efflux of the intracellular $Cl^-$ in exchange for the extracellular $HCO_3^-$. The results showed (Fig. 3, Table 3) that on exposure to the $Cl^-$-free solution, $V_m$ was slightly depolarized, and the $pH_i$ was increased at an initial rate of 0.026 pH/min. Intracellular $HCO_3^-$ concentration was increased ($P < 0.05$) from 14.4 ± 1.0 mM to 20.1 ± 1.3 mM in 6 min. Readmission of $Cl^-$ decreased $pH_i$ at a rate of 0.035 pH/min. Complete recovery of $pH_i$ and $[HCO_3^-]_i$ was obtained in about 15 min. These changes in $pH_i$, induced by the withdrawal and the readmission of $Cl^-$, were significantly decreased by 0.2 mM DIDS (Table 3); $[HCO_3^-]_i$ increased from 12.9 ± 0.9 mM to 16.8 ± 1.0 mM during the $Cl^-$ free condition, and decreased to 14.3 ± 1.1 mM after the readmission of $Cl^-$ to the solution. These changes of $pH_i$ were more significantly inhibited by 1 mM DIDS. On the other hand, the presence of 0.3 mM amiloride, a dose sufficient to inhibit the $Na^+/H^+$ antiport by more than 50% (Saito et al., 1987*b*), had little effect on the increase in $pH_i$ induced by the $Cl^-$-free solution.

**Table 4.** Effect of alkalinizing the intracellular fluid by exposing to $NH_4^+$ containing solution on intracellular $Cl^-$ activity

| | Prior to $NH_4^+$ | | 6 min after $NH_4^+$ |
|---|---|---|---|
| $HCO_3^-$ solution | | | |
| Control ($n$ = 13) | | | |
| $V_m$ (mV) | −40.8 ± 0.6 | | −36.8 ± 0.6 |
| $aCl_i$ (mM) | 32.6 ± 0.6 | | [a]36.6 ± 0.6 |
| $\Delta aCl_i$ (mM) | | +4.0 ± 0.4 | |
| DIDS 0.2 mM ($n$ = 4) | | | |
| $V_m$ (mV) | [a]−53.0 ± 3.9 | | [b]−47.9 ± 2.3 |
| $aCl_i$ (mM) | 33.0 ± 1.6 | | [a]31.5 ± 1.5 |
| $\Delta aCl_i$ (mM) | | [c]−1.5 ± 0.3 | |
| $HCO_3^-$-free solution ($n$ = 7) | | | |
| $V_m$ (mV) | −38.9 ± 0.4 | | −38.4 ± 0.6 |
| $aCl_i$ (mM) | [a]37.5 ± 1.3 | | 38.4 ± 1.5 |
| $\Delta aCl_i$ (mM) | | [c]+0.9 ± 0.6 | |

The values (mean ±SEM) are those prior to and 6 min after the application of solutions containing 25 mM $NH_4^+$ ($Na^+$ replaced by $NH_4^+$). $\Delta aCl_i$ denotes the net change in $aCl_i$. [a]$P < 0.05$; [b]$P < 0.01$, [c]$P < 0.001$: significantly different from the respective controls.

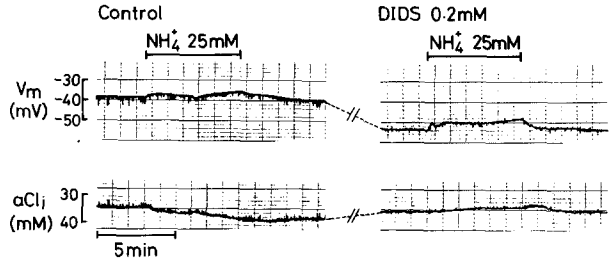


**Fig. 4.** Effect of $NH_4^+$ on $aCl_i$ in the absence (left panel) and in the presence (right panel) of DIDS (0.2 mM). Intracellular alkalinization was induced by replacing 25 mM $Na^+$ with $NH_4^+$ in the $HCO_3^-$ solution. Non-ionic diffusion of $NH_3$ into the cell alkalinizes the intracellular fluid via a reaction $NH_3 + H^+ \rightarrow NH_4^+$, which in turn stimulates hydration of $CO_2$, increases the intracellular $HCO_3^-$ concentration and $Cl^-/HCO_3^-$ exchange transport across the membrane entailing an increase in the $aCl_i$. The increase in $aCl_i$ induced by $NH_4^+$ was completely abolished in the presence of DIDS (*see also* Table 4)

### Effect of $NH_4^+$ on Intracellular $Cl^-$ Activity

Prior to this experiment it was predicted that, in the $HCO_3^-$ solution, the addition of $NH_4^+$ would increase the intracellular $HCO_3^-$ concentration, which in turn would increase $aCl_i$ via $Cl^-/HCO_3^-$ exchange. In a solution of pH 7.4, about 1% of the administered $NH_4^+$ ($pKa$ = 9.37 at 37°C) exists as $NH_3$, which freely diffuses into the cell, combines with intracellular $H^+$ and forms $NH_4^+$ (*see* Boron & De Weer, 1976), thereby accelerating hydration of $CO_2$ and increasing $[HCO_3^-]_i$. As predicted (Fig. 4 and Table 4), the addition of 25 mM $NH_4^+$ increased $aCl_i$ from 32.6 ± 0.6 to 36.6 ± 0.6 mM ($P < 0.05$) in 6 min and this $NH_4^+$-induced increase in $aCl_i$ was almost abolished in the presence of 0.2 mM DIDS. In $HCO_3^-$-free solution, the addition of $NH_4^+$ did not increase $aCl_i$ significantly (37.5 mM *vs.* 38.4 mM).

## Discussion

Chloride uptake by the lacrimal acinar cells across the basolateral membrane has been attributed to either $NaKCl_2$ cotransport (Suzuki & Petersen, 1985; Saito et al., 1986; Ozawa et al., 1988) or $Cl^-$ conductance (Marty, Tan & Trautmann, 1984). The present study offered evidence of an anion exchanger in the basolateral membrane that should contribute for uphill $Cl^-$ uptake. The results showed that under constant $Cl^-$ concentration in the superfusates (i) $aCl_i$ was increased either by a decrease in the extracellular $HCO_3^-$ concentration (Fig. 1, Table 1) or by an increase in $[HCO_3^-]_i$ (Fig. 4, Table 4), (ii) under constant $HCO_3^-$ and $CO_2$ concentrations in the superfusate, $[HCO_3^-]_i$ was increased by a reduction of the extracellular $Cl^-$ concentration (Fig. 3, Table 3), and (iii) all of these changes in $aCl_i$ and $[HCO_3^-]_i$ were significantly inhibited by DIDS. These findings indicated that a $Cl^-$ flux in one direction was coupled to a $HCO_3^-$ flux in the opposite direction across the basolateral membrane in a DIDS-sensitive manner. This mutual transconcentration effect between $Cl^-$ and $HCO_3^-$ can best be explained by a $Cl^-/HCO_3^-$ antiporter in the membrane. The antiporter is most likely located in the

basolateral membrane since it is well known that disulfonic stilbenes do not permeate the biological membranes (Cabantchik, Knauf & Rothstein, 1978). The antiporter would then contribute to the uphill $Cl^-$ uptake into the acinar cells particularly when intracellular $HCO_3^-$ production was enhanced by secretory stimuli (Fig. 4, Table 4). Chloride ions thus accumulated leave the cell across the luminal membrane through a $Ca^{2+}$-dependent $Cl^-$ channel (Marty et al., 1984; Findlay & Petersen, 1985; Saito et al., 1987*a*) providing electrical and osmotic driving forces for $Na^+$ and $H_2O$ transport into the acinar lumen.

The present results, however, do not exclude the role of the $NaKCl_2$ cotransporter in $Cl^-$ uptake into the acinar cells. The presence of the $Cl^-/HCO_3^-$ exchanger rather explains $Cl^-$ movements such as the uphill $Cl^-$ uptake observed after the cessation of ACh stimulation in the presence of 1 mM furosemide (Saito et al., 1987*a*) that was difficult to interpret in terms of $NaKCl_2$ cotransport alone. Our recent studies on the mechanism of $Cl^-$ uptake after ACh-stimulation showed that furosemide or DIDS alone, each in a 1 mM concentration, inhibited $Cl^-$ uptake only by 40–30%, however, the inhibitory effects were additive and a combination of the two drugs inhibited more than 70% of the uptake (Ozawa et al., 1988). Thus we suspect the coexistence of both $NaKCl_2$ cotransport and $Cl^-/HCO_3^-$ antiport in the basolateral membrane. A similar model of $Cl^-$ transport across the basolateral membrane has been proposed for the mandibular salivary gland (Novak & Young, 1986; Pirani et al., 1987).

However, the detailed mechanism of the $Cl^-/HCO_3^-$ antiport in this tissue awaits further elucidation. Among various kinds of tissues, different kinds of anion antiporter have been suggested: namely, electroneutral $Cl^-/HCO_3^-$ (*see* Knauf, 1986; Hoffmann, 1986), $NaCO_3^-/Cl^-$, $Na^+(HCO_3^-)_2/Cl^-$ and $Na^+ \cdot HCO_3^-/H^+ \cdot Cl^-$ (*see* Boron, 1986) antiporters. One interesting finding in respect to the mechanism of the anion exchanger in this tissue was that DIDS inhibited the rate of the increase in $pH_i$ during exposure to the $HCO_3^-$ solution (Fig. 2). The rate of $pH_i$ increase was reduced from 0.034/min to 0.017/min in the presence of DIDS. This was contrary to our prediction that DIDS would facilitate the rate of acid extrusion from the cells by inhibiting the $Cl^-/HCO_3^-$ antiporter, an acid-loading machinery. In the previous study (Saito et al., 1988*a*), we observed that DIDS facilitated acid-extrusion from the cells stimulated by ACh or acid-loaded by a $NH_4^+$-prepulse method (Boron & De Weer, 1976) in the $HCO_3^-$-free solution. This finding ruled out the possibility of inhibition of the $Na^+/H^+$ antiporter by DIDS. Also in the present study amiloride had little effect on the increase of $pH_i$ induced by an exposure to the $Cl^-$-free solution. Therefore, the possibility of electroneutral $Cl^-/HCO_3^-$ antiport in this tissue must be re-examined. In the present study, intracellular $HCO_3^-$ concentration reached to about 11 mM in one min after exposure to the $HCO_3^-$ solution and increased further thereafter. This increase in $[HCO_3^-]_i$ was significantly inhibited by DIDS (Table 2). Simple diffusion or one-for-one $Cl^-/HCO_3^-$ antiport cannot explain the increase in the $HCO_3^-$ concentration beyond 10 mM with respect to the electrochemical potential gradient across the membrane. Therefore, we speculate that the coupling ratio of this DIDS-sensitive $Cl^-/HCO_3^-$ exchanger is smaller than unity, or the anion exchanger is also coupled to potential gradient of other ion species such as $H^+$, $Na^+$ or $K^+$. In our preliminary study of this tissue, the rate of $HCO_3^-$ extrusion from the $HCO_3^-$-loaded cells in the standard solution was enhanced by an increase in the external $Cl^-$ concentration but was extremely slow in a $Na^+$-free N-methyl-D-glucamine solution rich in $Cl^-$ (*unpublished data*). Furthermore, it is difficult to interpret the reason why the exposure to the $HCO_3^-$ solution causes a membrane hyperpolarization in the absence of DIDS and a depolarization in the presence of DIDS (Figs. 1 and 2). These changes in $V_m$ may be relevant to the DIDS-sensitive $HCO_3^-$ transport mechanism or be derived from pH-sensitive membrane conductance such as those demonstrated in other tissues. Further studies on these uncertainties are needed.

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