Evidence for Sodium-Coupled Acid–Base Transport Across the Basolateral Membrane of the Reabsorptive Duct of the Human Eccrine Sweat Gland

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Intracellular pH was measured in isolated nonperfused ducts of human eccrine sweat glands *in vitro* to investigate basolateral acid–base transport mechanisms. Bath sodium removal led to a bicarbonate-independent, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid insensitive acidification. The recovery of this acidification was ethylisopropyl amiloride sensitive, suggestive of basolateral sodium:hydrogen exchange. Whereas bath chloride removal led to a small acidification this was not 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid sensitive and its causes remain unclear. Elevation of bath potassium to depolarize the basolateral membrane led to a small alkalinization but this was not mimicked by addition of barium or chloride removal. As chloride removal and barium addition would be expected to cause larger depolarizations than potassium elevation these observations do not support a major role for electrogenic acid–base transport. In conclusion, although this study does not support a major role for electrogenic acid–base transport, it has demonstrated the basolateral presence of sodium-coupled acid–base transport in the reabsorptive duct of the human eccrine sweat gland, which most likely represents a sodium:hydrogen exchanger involved in regulation of intracellular pH. *Key words: Na:H exchange/pH. J Invest Dermatol 117:877–879, 2001*

The reabsorptive sweat duct of the human eccrine sweat gland receives a primary secretion of sweat from the initial tubular segment of the sweat gland, the secretory coil, with a pH of approximately 7.2 (Sato *et al*, 1989; Sato and Sato, 1990). By the time this sweat reaches the skin surface, however, it can be greatly acidified by the more distal tubular segment, the reabsorptive duct, such that at low sweat rates the pH can be lower than 5 (Kaiser *et al*, 1974; Bijman and Quinton, 1987). The acidity of this secretion probably helps maintain the ''acid mantle of the skin'', which is thought to limit colonization of pathogenic microflora (Schmid and Korting, 1995). As the length of the reabsorptive duct is as short as 5 mm (Sato *et al*, 1989), large and variable transcellular fluxes of acids or bases must be produced that, while producing an acid sweat, may also have the potential to disturb the intracellular environment. In the absence of studies examining the functionality of acid–base transporters, however, very little is known about the nature of the membrane transporters involved in either vectorial acid secretion or the regulation of intracellular pH. Accordingly, the effects of ion substitution on intracellular pH were observed to identify what acid–base transporters might be present on the basolateral membrane of the reabsorptive duct of the human eccrine sweat gland.

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Abbreviations: BCECF, 2′-7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; EIPA, ethylisopropyl amiloride; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid.

MATERIALS AND METHODS

Tissue preparation Human sweat glands were obtained from slivers of apparently normal skin obtained from adults undergoing surgery who gave informed consent. Ethical committee approval was obtained for this procedure. To prepare single glands from this skin, the shearing technique was used (Lee *et al*, 1984); small slivers of skin were sheared with steel surgical scissors continuously for about 10 min in a small quantity (about 2 ml) of Dulbecco's phosphate-buffered saline to yield a slurry. This was then diluted further and examined under a dissecting microscope, and individual eccrine glands were identified. From these glands single reabsorptive ducts were dissected using fine steel forceps.

Duct set-up Experiments were performed in a thermally controlled Perspex chamber (White Instrument, MD), on the stage of an inverted microscope (Olympus OM-2), and ducts were mounted on a conventional microperfusion apparatus (White Instrument). Access of the bathing medium was denied to the apical membrane by crimping the ends of the ducts in glass micropipettes using the technique first described by Dellasega and Grantham (1973) for renal proximal tubules. Ducts were superfused with saline at a rate of 8 ml per min with a bath volume of 200 µl. The control saline contained (in mM) NaCl 114; K_2HPO_4 2.5; $MgCl_2$ 1; $NaHCO_3$ 25; glucose 5; Na lactate 4; $CaCl_2$ 1. This solution was bubbled with 5% CO_2 in oxygen and adjusted to pH 7.4. Changing solutions was rapidly performed with a pneumatically driven minislider valve (Omnifit, Cambridge, U.K.) All experiments were performed at 37°C.

pH_i measurements Generally, pH_i was measured as previously described for nonperfused renal proximal tubules (Macri *et al*, 1993). Briefly, ducts were loaded with the AM form of the pH sensitive dye 2′-7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) (Molecular Probes, Oregon) for up to 10 min at room temperature. A PTI dual monochromator spectrofluorometer (PTI, Surbiton, U.K.) was used to excite the dye at 450 and 500 nm. The resulting fluorescence was passed through a 535 nm bandpass filter, detected with a photomultiplier tube,

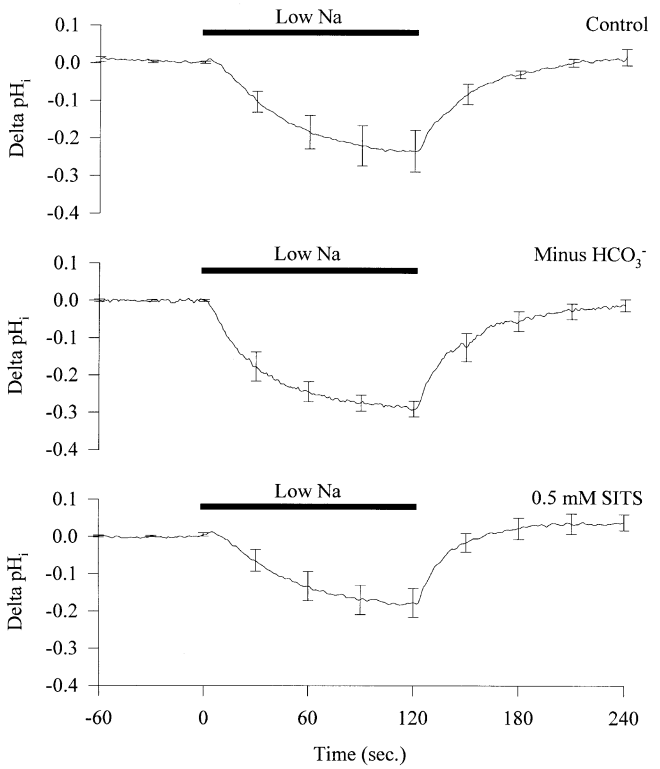

Figure 1. Effect of reducing bath sodium on pH_i in human sweat ducts. Sodium was lowered during periods indicated by horizontal bars under control conditions, absence of bicarbonate, and presence of 0.5 mM SITS. Each trace represents results from five ducts with mean ± SEM.

and digitized for analysis. The ratio of emitted light from the two excitation wavelengths, after correction for background fluorescence, was calculated using PTI software. Calibration of the dye was performed by equilibration of the duct with solutions adjusted to pH 6.75, 7.0, and 7.25 containing (in mM) NaCl 30; $MgCl_2$ 1; glucose 5; Na lactate 4; $CaCl_2$ 2; HEPES 10; KCl 120. The calibration solutions also contained 10 μM of the ionophore nigericin to clamp pH_i to extracellular pH (Thomas *et al*, 1979).

Reagents BCECF-AM and ethylisopropyl amiloride (EIPA) were obtained from Molecular Probes (Oregon). All other reagents were obtained from Sigma (Dorset, U.K.).

Statistics All data are expressed as mean ± SEM. Statistical analysis was performed using Student's *t* test or ANOVA followed by Dunnett's multiple comparison test as appropriate. Statistical significance was assumed for $p < 0.05$.

RESULTS

Sodium-coupled acid–base transport Experiments were performed whereby extracellular sodium was reduced from 143 mM to 4 mM by isosmotic replacement of sodium with N-methyl-D-glucamine for a period of 2 min to investigate the presence of sodium-coupled acid–base transport. These experiments were repeated for ducts that had been exposed to 0.5 mM of the anion transport inhibitor 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) for 5 min and for ducts bathed in a bicarbonate-free medium (bicarbonate replaced with chloride and nominally CO_2 free). In bicarbonate-containing conditions, baseline pH_i was 7.23 ± 0.05 and sodium reduction led to a significant ($p < 0.02$, $n = 5$) acidification of 0.24 ± 0.06. This observation clearly demonstrates the presence of some form of sodium-coupled acid–base transport on the basolateral membrane of this preparation. As shown in **Fig 1** and **Table I**, neither preincubation with 0.5 mM SITS nor removal of external bicarbonate significantly altered the degree of acidification, suggesting that at least a component, if not all, of this response is bicarbonate independent.

Table I. Summary of data for sodium removal experiments

	Baseline pH_i	Intracellular acidification (delta pH_i)
Control	7.23 ± 0.05	0.24 ± 0.06 ($n = 5$)
0 Bicarbonate	7.26 ± 0.04 (NS[a])	0.29 ± 0.02 (NS) ($n = 5$)
SITS	7.29 ± 0.04 (NS)	0.18 ± 0.04 (NS) ($n = 5$)

[a]NS, not significantly different from the corresponding control value.

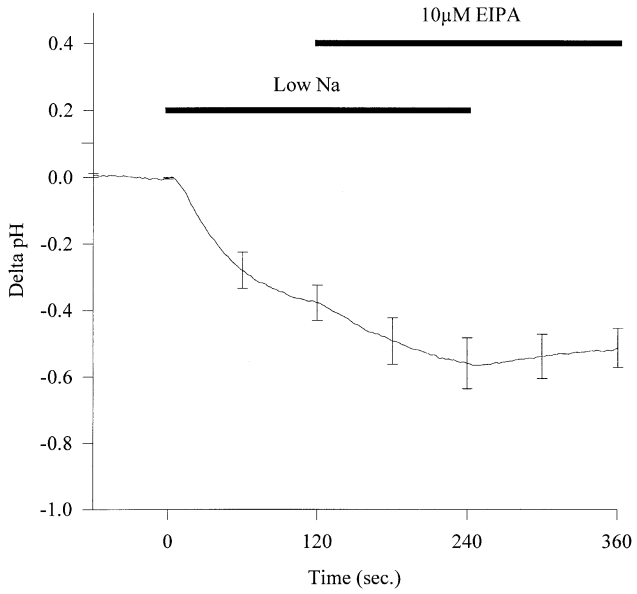

Figure 2. Effect of EIPA on recovery of pH_i from sodium readdition in human sweat ducts. Sodium removal and EIPA addition indicated by horizontal bars. Note that EIPA was not present as sodium was removed. Data shown are the mean ± SEM from six experiments.

Evidence for basolateral sodium:hydrogen exchange A sodium-coupled acid–base transporter is present on the basolateral membrane of a wide variety of epithelial cells as a sodium:hydrogen exchanger sensitive to amiloride and its analogs (Clark and Limbird, 1991; Tse *et al*, 1993). To test for this possibility, sodium removal experiments were performed in bicarbonate-free media but, prior to the readdition of sodium to the bathing medium, ducts were exposed to 10 μmol of the amiloride analog EIPA ($n = 6$; baseline pH_i 7.42 ± 0.06). As shown in **Fig 2** addition of EIPA caused a further acidification in addition to that evoked by sodium removal. When sodium was added back to the bathing medium in the presence of EIPA the recovery of pH_i normally observed was almost abolished (**Fig 2**). These observations support the presence of a sodium:hydrogen exchanger on the basolateral membrane of the human eccrine reabsorptive duct.

Chloride-coupled acid–base transport When chloride was removed from the external medium and replaced with the impermeant anion cyclamate (gluconate in the case of $MgCl_2$) there was a statistically significant ($p < 0.02$, $n = 5$) but very small fall in pH_i of 0.04 ± 0.01 from a baseline pH_i of 7.38 ± 0.02. In some cases this was mildly transient with pH_i being 7.36 ± 0.03 after 2 min of chloride removal. pH_i returned to 7.42 ± 0.02 when

chloride was replaced in the bathing medium. This acidification was not SITS sensitive as, in four of the five experiments, when a second chloride-free pulse was applied after a 5 min preincubation with 0.5 mM SITS in the bathing medium, the second pulse caused an acidification of 0.06 ± 0.01.

Electrogenicity of basolateral acid–base transport The existence of channels conductive for bicarbonate has been proposed for a variety of epithelia (Marty *et al*, 1984; Saito and Wright, 1984; Brown *et al*, 1989). In addition, it is conceivable that acid–base transport can be mediated by proton or hydroxyl channels (Lyall and Biber, 1994). To examine whether these possibilities are likely to exist for the basolateral membrane of the sweat duct, maneuvers designed to depolarize the basolateral membrane were performed in the presence of bicarbonate. Although raising external potassium from 5 to 30 mM caused a statistically significant, but very small, alkalosis of 0.04 ± 0.01, which was fully reversible ($n = 4$, $p < 0.05$, baseline pH_i 7.48 ± 0.07), a 2 min exposure of the basolateral membrane to 1 mM of the potassium channel inhibitor barium had no significant effect on pH_i ($n = 4$, $p > 0.1$, baseline pH_i 7.45 ± 0.04). In this analysis of the effect of membrane depolarization, however, the chloride removal experiments should also be considered as, although no alkalosis was observed in these experiments (see above), chloride removal has been shown to cause a far greater depolarization than either barium or potassium elevation (Reddy and Quinton, 1991). Thus, it seems that electrogenic acid–base transport does not play a major role under these experimental conditions.

DISCUSSION

Having the ability to acidify sweat to a pH of almost 4.5, the reabsorptive duct of the human sweat gland can generate a transepithelial bicarbonate gradient of almost 1000:1 (assuming a plasma bicarbonate concentration of 25 mM and a calculated sweat bicarbonate concentration of about 0.03 mM). This requires a powerful transport mechanism to acidify the primary sweat in conjunction with high bicarbonate reabsorption rates. With large variations in transepithelial proton fluxes there could also be significant challenges to the maintenance of an intracellular pH compatible with normal cell function. Histochemical evidence for a vacuolar type proton ATPase in the apical membrane of the reabsorptive duct has recently been reported (Bovell *et al*, 2000), which may well play a key role in the acidification of sweat. To date, however, there has been little work examining functional aspects of acid–base transport in this tissue either for acidification of sweat or for regulation of intracellular pH of cells of the sweat gland.

In this study, we observed the presence of sodium-coupled acid–base transport on the basolateral membrane of the reabsorptive duct. At least a component of this appears to be EIPA sensitive, indicative of a sodium:hydrogen exchanger (Clark and Limbird, 1991). If involved in vectorial acid–base transport across the epithelium, basolateral sodium:hydrogen exchange would act to secrete rather than absorb bicarbonate. Hence the function of this transporter, as in other epithelia, is almost certainly to regulate intracellular pH rather than to contribute directly to bicarbonate reabsorption.

Removal of bath chloride led to a small, SITS-insensitive acidification. The mechanism behind this is unclear but may involve some form of cell-shrinkage-induced inhibition of membrane transport as has been reported for some sodium:hydrogen exchange isoforms (Nath *et al*, 1996).

Maneuvers designed to depolarize the basolateral membrane were generally ineffective at raising pH_i, suggesting the absence of any major electrogenic acid–base transporter such as a bicarbonate channel, proton/hydroxyl channel, or sodium bicarbonate cotransporter. This is consistent with the report of Quinton and Reddy (1993) that neither apical nor basolateral membranes of the reabsorptive duct are electrically conductive to bicarbonate. In this study neither addition of 1 mM barium nor reduction of bath chloride resulted in an elevation of pH_i, even though both would be expected to cause an intracellular alkalosis if any functional electrogenic transporters existed. On the other hand, elevation of bath potassium did cause a small alkalinization even though this maneuver should be far less effective at depolarizing the cell than either barium addition or chloride removal (Reddy and Quinton, 1991). This may be due to the presence of an electroneutral potassium bicarbonate cotransporter as has been described in rat renal thick ascending limb (Leviel *et al*, 1992). It is also conceivable that the effect of potassium elevation was a secondary effect of elevated potassium stimulating the sodium pump.

In summary, we have quantitatively estimated pH_i in the human reabsorptive duct to investigate basolateral membrane acid–base transport. Although ion substitution experiments were not supportive of either electrogenic or chloride-coupled transporters having a major role in basolateral acid–base transport, we have demonstrated the existence of sodium-coupled acid–base transport, which is presumably a form of a sodium:hydrogen exchanger whose role is most likely involved in the regulation of intracellular pH.

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