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Epithelia are responsible for the directional transport of selected materials to and from the external environment: absorption involves transport into the epithelial cell across the apical (luminal) cell membrane, followed by exit across the basolateral (contraluminal) membrane; for secretion the sequence is reversed.

Quantitatively, ions and water dominate the transport activity of most epithelia. Although the species of ions transported and the direction of transport may differ, this diversity of function seems to be achieved through the co-ordinated activity of a relatively small number of transport proteins (pumps, carriers and channels), which are combined in different proportions and at different cellular locations (i.e. apical or basolateral membranes) in different epithelia. Those commonly involved are listed in Table 1.

A general description of the function and regulation of these transport proteins has been published elsewhere [1], while a detailed description of some of them follows in this

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### Table 1. Inhibitors of pumps, carriers and channels in epithelia*

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitor</th>
<th>Effective inhibitory concentration</th>
<th>Other inhibitory effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺,K⁺-ATPase</td>
<td>Ouabain</td>
<td>10⁻⁴ M</td>
<td>Na⁺ channel (10⁻⁵ M)</td>
</tr>
<tr>
<td>Na⁺/H⁺ antiport</td>
<td>Amiloride</td>
<td>&gt; 10⁻⁴ M</td>
<td>Na⁺,K⁺-ATPase (10⁻⁵ M)</td>
</tr>
<tr>
<td>Cl⁻/HCO₃⁻ antiport</td>
<td>Disulphonic stilbene (e.g. DIDS, SITS)</td>
<td>&gt; 10⁻⁴ M</td>
<td>Electrogenic HCO₃⁻ transport</td>
</tr>
<tr>
<td>Na⁺/K⁺/2Cl⁻ symport</td>
<td>Bumetanide (also furosemide less selective and piretanide)</td>
<td>10⁻⁶ - 10⁻³ M (10⁻³ M)</td>
<td>Na⁺/Cl⁻ symport (10⁻⁵ M)</td>
</tr>
<tr>
<td>Na⁺ channel</td>
<td>Amiloride</td>
<td>10⁻⁴ M</td>
<td>See: Na⁺/H⁺ antiport</td>
</tr>
<tr>
<td>Ca²⁺-activated K⁺ channel</td>
<td>Ba⁺⁺</td>
<td>5 x 10⁻⁵ M</td>
<td>K⁺/Cl⁻ symport</td>
</tr>
<tr>
<td>Secretory cell Cl⁻ channels</td>
<td>Diphenylamine-2-carboxylate</td>
<td>8 x 10⁻⁵ M (kidney)</td>
<td></td>
</tr>
</tbody>
</table>

*Modified from Case et al. [21] where references to all effects of inhibitors may be found.
†Depends greatly on species.
colloquium. The purpose of this paper is to consider how they may function in a model epithelium (salivary acini) so as to produce a primary secretion of saliva. (The subsequent modification of this primary secretion which occurs in the ducal system will not be considered.)

Models of secretion

Elaboration of primary saliva is usually explained in terms of a neutral symport model of secretion in which the secondary active transport of Cl\(^-\) across the luminal membrane is thought to drive secretion (Fig. 1a). In this model, originally proposed as a result of studies on the shark rectal gland [2], a basolaterally located Na\(^+\)/K\(^+\)/2Cl\(^-\) symporter actively accumulates Cl\(^-\) so that the intracellular Cl\(^-\) activity lies above the electrochemical equilibrium value. The energy for accumulation of Cl\(^-\) via the symporter is provided by the Na\(^+\) gradient which in turn is maintained by the activity of basolaterally located Na\(^+\),K\(^+\)-ATPase. Recycling of K\(^+\) occurs laterally sited Na\(^+\),K\(^+\)-ATPase. Recycling of K\(^+\) allows Cl\(^-\) to flow out of the cell into the duct lumen down its electrochemical gradient. Na\(^+\) is thought to follow the transport of Cl\(^-\) by diffusion via the paracellular pathway. Thus a net movement of NaCl occurs through a K+-selective ion channel also located in the basolateral membrane. A Cl\(^-\) channel in the apical membrane allows Cl\(^-\) to flow out of the cell into the duct lumen and water follows by osmosis.

While considerable evidence has been adduced in support of this model from studies on salivary gland acini, the model is unable to account for a number of features of salivary secretion, notably: (i) primary saliva contains HCO\(_3\)- at concentrations of about 30 mmol/l [3]; (ii) in studies on perfused salivary glands [4-6], the use of transport inhibitors suggests that accumulation of intracellular Cl\(^-\) cannot be achieved solely through Na\(^+\)/K\(^+\)/2Cl\(^-\) symport and that parallel Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)- antiporters may be involved (as illustrated in Fig. 1b); (iii) in perfused glands, provided HCO\(_3\)- is present in the perfusate, fluid secretion continues in the complete absence of Cl\(^-\) or when Na\(^+\)/K\(^+\)/2Cl\(^-\) symport is inhibited by bumetanide [4,7,8].

The neutral symport model of secretion clearly cannot account for these observations which demonstrate that HCO\(_3\)- plays an important role in electrolyte transport in salivary acini. The same is probably true of electrolyte transport in many other epithelia including renal proximal tubule [9], corneal endothelium [10], gallbladder [11], choroid plexus [12] and, of course, pancreatic ductal epithelium [13].

Intracellular pH during secretion

To investigate the role of HCO\(_3\)- in secretion, and hence propose appropriate modifications to the secretory model, we have studied intracellular pH (pH\(_i\)) in the rabbit mandibular salivary gland in two ways: (i) in perfused, whole glands, using \(^{31}\)P n.m.r. spectroscopy [14]; and (ii) in suspensions of dissociated gland acini using the pH-sensitive fluorescent dye, BCECF (2',7'-bis(carboxyethyl)-5(6')-carboxyfluorescein) [15]. Each method has its advantages and disadvantages. The n.m.r. method, which is based on the pH-dependent chemical shift of the intracellular inorganic phosphate (P\(_i\)) resonance, uses intact glands (so that the epithelium is in its normal configuration), but is insensitive, so that time-resolution is poor. By contrast, the fluorescent probe technique has excellent time-resolution, but requires enzymic dissociation of the gland into dispersed acini.

Changes in pH\(_i\) have been studied in response to stimulation with acetylcholine (ACh) and following replacement of extracellular ions and/or exposure to transport inhibitors. Both techniques show that stimulation with ACh (1 \(\mu\)mol/l) causes pH\(_i\) to decrease by about 0.1 pH unit, after which it returns towards control values (BCECF fluorescence) and eventually becomes more alkaline (n.m.r. spectroscopy) (Fig. 2).

The process of intracellular acidification has been most rigorously studied using the BCECF fluorescence technique. It has the following characteristics [15, 16]: (a) The doses of ACh required for maximal and half-maximal acidification (1 \(\mu\)mol/l and 0.1 \(\mu\)mol/l, respectively) are close to those which evoke maximal and half-maximal secretion in the perfused gland [17]; (b) ACh fails to evoke acidification in the absence of extracellular HCO\(_3\)- or presence of acetazolamide. The same is true when pH\(_i\) is measured by n.m.r. spectroscopy [14]. These data suggest that acidification involves efflux of

Fig. 1. Models of Cl\(^-\) secretion in secretory epithelia
(a) Neutral symport; (b) parallel antiport

Fig. 2. The effects of ACh on pH\(_i\) in rabbit mandibular salivary gland acini

The upper record represents data obtained by \(^{31}\)P n.m.r. spectroscopy on whole glands [14]. The lower trace is from BCECF fluorescence studies on dissociated acini, which were continued for only 10 min [15].

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HCO$_3^-$ from acinar cells. (c) Acidification can be elicited by ACh in the absence of extracellular Cl$^-$ and in the presence of the anion antiport inhibitor DIDS (4,4'-diisothiocyanato stilbene-2,2'-disulphonic acid), implying that the mechanism responsible does not involve Cl$^-$/HCO$_3^-$ exchange. It can also be elicited in the absence of Na$^+$, implying that Na$^+$/H$^+$ exchange (or some form of Na$^+$-dependent HCO$_3^-$ symport) can also be eliminated as mechanisms responsible for the acidification. (d) ACh fails to evoke acidification when acini have been preincubated with the Cl$^-$-channel blocker diphenylamine-2-carboxylate.

Taken together, these data are consistent with the idea that the intracellular acidification evoked by ACh is caused by the neutral symport model of secretion. By calculating the initial rates of change in HCO$_3^-$ activity (from the observed change in pH) and in Cl$^-$ activity (from our Cl$^-$-selective microelectrode studies) we estimate a $P_{HCO_3}/P_{Cl}$ selectivity ratio of about 0.6 for this channel [18].

From the pH$_i$ data it can also be calculated that intracellular HCO$_3^-$ activity in mandibular acinar cells is about 9 mmol/l [18]. Assuming the p.d. across the apical membrane is the same as that we have measured across the basolateral membrane (i.e. about ~50 mV), this means there is an outwardly directed gradient for HCO$_3^-$ (as has previously been shown for Cl$^-$ in Cl$^-$-selective electrode studies on gland slices [19, 20]). Thus, the two anions would compete for egress through any apical channels which have been 'opened' through the action of ACh; under normal circumstances this would mean a much greater secretion of Cl$^-$ than HCO$_3^-$, which is what is observed experimentally.

The acidification evoked by sustained stimulation with ACh is transient, and recovery quickly occurs by a process of alkalinization (Fig. 2). This is probably achieved by the Na$^+$/H$^+$ exchanger because it can be entirely prevented by amiloride (1 mmol/l) in both fluorescent probe studies on dissociated acini [15] and $^{31}$P n.m.r. studies on perfused glands [14]. The latter studies also suggest the presence of a DIDS-sensitive HCO$_3^-$ uptake process which helps to maintain intracellular HCO$_3^-$ concentration, and hence pH$_i$, above equilibrium values.

Thus, in conclusion, the pH$_i$ response of the rabbit mandibular salivary gland to ACh appears to be the result of two opposing and independent effects: (i) a rapid acidification, due to HCO$_3^-$ efflux through apical anion channels, and (ii) a more slowly evolving alkalinization, due to the activation of Na$^+$/H$^+$ exchangers (Fig. 3). These two processes may be regulated by the two end-products of phosphoinositol hydrolysis (i.e. Ca$^{2+}$ and protein kinase C, respectively).

This work has been supported by grants from the S.E.R.C., the Wellcome Trust, the Cystic Fibrosis Research Trust, and the Nuffield Foundation.


Received 14 April 1989