J. Physiol. (1984), **349**, *pp.* 619–630
With 6 text-figures
Printed in Great Britain

THE ANIONIC BASIS OF FLUID SECRETION BY THE RABBIT MANDIBULAR SALIVARY GLAND

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(*Received 12 July 1983*)

SUMMARY

1. The role played by anions in salivary secretion has been studied in experiments on the isolated, perfused mandibular gland of the rabbit, in which perfusate Cl^- and/or HCO_3^- were replaced by other anions.

2. Replacement of Cl^- with Br^- had no significant effect on salivary secretion rate, but replacement with the other anions tested caused secretory rate to fall, by 38% (I^-), 50% (NO_3^-), 61% (isethionate, ise^-), and 66% ($CH_3SO_4^-$), respectively.

3. Replacement of perfusate Cl^- with ise^- or $CH_3SO_4^-$ caused the salivary HCO_3^- concentration to rise up to 4-fold. Replacement with Br^- or I^- seemed to have little effect on salivary HCO_3^- concentration but, in contrast to ise^-, Br^- and I^- entered the saliva in concentrations comparable to those of Cl^- during control perfusion.

4. In glands perfused with HCO_3^- and ise^-, the addition of methazolamide, an inhibitor of carbonic anhydrase, caused a further 60% drop in secretory rate, but the saliva remained rich in HCO_3^-.

5. Replacement of perfusate HCO_3^- with Cl^- or ise^- had no effect on salivary secretion or composition.

6. Replacement of both HCO_3^- and Cl^- in the perfusate with ise^- reduced salivary secretion to less than 2% of control levels.

7. In control glands (i.e. perfused with both HCO_3^- and Cl^-), administration of furosemide, an inhibitor of Na^+/Cl^- co-transport, reduced the secretion rate and increased salivary HCO_3^- in a manner indistinguishable from that seen when perfusate Cl^- was replaced with ise^-.

8. In control perfused glands, administration of SITS (4-acetamido-4′-isothiocyano-2,2′-disulphonic acid stilbene), an inhibitor of Cl^-/HCO_3^- antiports, did not cause any change in salivary HCO_3^- concentration. Unexpectedly, it induced a significant increase in salivary secretory rate.

9. The results show that salivary secretion depends on two independent transport systems. One is a Cl^--dependent, furosemide-sensitive system, probably a Na^+/Cl^- symport. The other is an HCO_3^--dependent, methazolamide-sensitive system, and is probably an Na^+/H^+ antiport.

INTRODUCTION

Recently, it has become widely accepted that transepithelial transport of salt and water may involve not only primary active transport, directly dependent on the activity of one or more membrane-bound ATPases (Stekhoven & Bonting, 1981), but also secondary active transport, in which the dissipative flux of one ion (e.g. Na^+) is coupled to the movement of another solute in such a way that the secondarily transported solute is moved against its electrochemical gradient (Young, 1982).

Although most studies on these secondary active transport mechanisms have been performed on absorbing epithelia, interest in the role of co-transport in exocrine secretion has been aroused by the publication of a model for secretion by the shark rectal gland (Silva, Stoff, Field, Fine, Forrest & Epstein, 1977). This model involves secondary active transport of Cl^-, coupled to the influx of Na^+ from interstitium to cytosol across the basolateral plasma membrane of the secretory cells. The Cl^- ions, diffusing from cytosol to lumen via an undefined channel in the apical membrane, thus take a transcellular path and provide a solute drive for secretion; whether the bulk of the transported fluid and other solutes in the secretion also follow a transcellular route or pass paracellularly is disputed (Ussing, Bindslev, Lassen & Sten-Knudsen, 1981).

An alternative transport model has been proposed in order to explain the ability of many epithelia to transport Na^+ and Cl^- independently of one another, and to explain the influence of pH, HCO_3^- and carbonic anhydrase on salt and water transport. This model, originally proposed for the small intestine (Turnberg, Bieberdorf, Morawski & Fordtran, 1970), involves the double exchange of Na^+ for H^+ and Cl^- for HCO_3^- (or OH^-). Since some of our preliminary data (Case, Conigrave, Hunter, Novak, Thompson & Young, 1981; Case & Hunter, 1981; Novak & Young, 1982) were not fully consistent with simple Na^+/Cl^- co-transport, we contemplated this double counter-exchange system as an alternative model for salivary secretion (Case, Conigrave, Favaloro, Novak, Thompson & Young, 1982).

In the present study, using an isolated rabbit mandibular gland preparation, we have manipulated the anionic composition of the gland perfusate and have tested a number of drugs known to interfere with ion transport mechanisms. Our results are compatible with an anion co-transport model for secretion, but a simple Na^+/Cl^- co-transport mechanism alone will not account for all of our findings. We propose instead, that secretion in the rabbit mandibular gland involves two independent systems, a Na^+/Cl^- symport, and a Na^+/H^+ antiport, both located on the basolateral plasma membrane.

METHODS

Details of surgical procedures for preparing the isolated mandibular gland for perfusion have been published previously (Case, Conigrave, Novak & Young, 1980). Male New Zealand or Half-lop albino rabbits weighing 1·3–3·5 kg were anaesthetized with urethane (1·5 g/kg intraperitoneally, with I.V. supplements as required). Usually, both glands were excised and perfused arterially at 4 ml/min, with the aid of a peristaltic pump, in a humidified chamber (37 °C).

The perfusion fluid was a nutrient, buffered salt solution, containing glucose (5 mmol/l). In experiments conducted according to protocol M (described below), the solution was buffered with HCO_3^- (25 mmol/l) and CO_2 (5% CO_2 in O_2). In experiments conducted according to protocol S,

the perfusate was buffered at pH 7·4 with HEPES (10 mmol/l) and equilibrated with 100 % O_2 or, alternatively, with HEPES and HCO_3^- (25 mmol/l) and equilibrated with 5 % CO_2 in O_2. Details of the composition of all perfusion fluids employed are given in Table 1.

Infusion of acetylcholine into the arterial catheter was begun 15 min after the start of gland perfusion, and maintained for the duration of the experiment (1–4 h). The acetylcholine concentration (0·8 μmol/l) was just sufficient to evoke a maximal secretory response (Case *et al.* 1980). When stimulated in this way, the rabbit mandibular gland responds initially with a high secretory rate (> 160 μl/min), which falls over a period of 30 min to reach a plateau of about 40 μl/min (Case *et al.* 1980, 1982).

TABLE 1. Composition of perfusion solutions in mmol/l. For explanation of the two protocols employed (S and M), see text. Solution A′ (protocol M) was equilibrated with 5 % CO_2 in O_2 (pH 7·4). Solutions A–E (protocol S) were all buffered at pH 7·4 with HEPES: solutions containing HCO_3^- (A and D) were equilibrated with 5 % CO_2 in O_2 and the other solutions (B, C and E) were equilibrated with 100 % O_2

	Protocol M	Protocol S				
	A′	A	B	C	D	E
Na^+	146·0	146·0	146·0	146·0	146·0	146·0
K^+	4·5	4·3	4·3	4·3	4·3	4·3
Ca^{2+}	2·5	1·0	1·0	1·0	1·0	1·0
Mg^{2+}	1·0	1·0	1·0	1·0	1·0	1·0
Cl^-*	126·3	121·3	121·3	—	—	146·3
HCO_3^-	25·0	25·0	—	—	25·0	—
ise$^-$	—	—	25·0	140·0	121·3	—
SO_4^{2-}	1·0	1·0	1·0	3·15†	2·0†	1·0
$H_2PO_4^-/HPO_4^{2-}$	1·0	1·0	1·0	3·0†	1·0	1·0
HEPES‡	—	10·0	10·0	10·0	10·0	10·0

* In some experiments (Fig. 1), Cl^- was replaced with Br^-, I^-, NO_3^-, $CH_3SO_4^-$ or ise$^-$. In Cl^- replacement experiments, acetylcholine (0·8 μmol/l) was used as the perchlorate rather than the chloride salt.

† In these experiments potassium and calcium were added as salts of phosphate, sulphate or bicarbonate, since salts of ise$^-$ were not available to us. Phosphate was omitted from the perfusate altogether in the experiments conducted with solution D depicted in Fig. 4; its omission made no significant difference to the flow response or the salivary electrolyte composition.

‡ At pH 7·4 HEPES has net negative charge of about 50 % of the molar concentration, i.e. 10 mM-HEPES gives about 5 mequiv/l negative charge.

Two alternative experimental protocols were used. In one (protocol M), we did not begin the experiment proper until secretory rates had stabilized at plateau values. Then we changed the perfusion solution at 20 min intervals, alternating between the control perfusate and one or more test perfusates, so that the secretory response during a given test period could be expressed as a percentage of the response during the preceding control period (Fig. 1). In other experiments (protocol S), we used only one perfusion fluid in a given experiment and compared the mean response of a series of glands perfused with one test solution with the response for any other series. In this protocol, saliva was collected initially over periods of 2, 3 and 5 min and, after the first 30 min, over periods of 15 or 30 min. This permitted us to study the secretion in the initial phase of the secretory response, as well as in the plateau phase, but it had the disadvantage that each gland could not be used as its own control. In experiments where we employed inhibitor drugs, we overcame this objection in part by using both glands from each animal, taking one as the control and the other as the test gland (reversing the order in which test and control experiments were done in alternate animals). In such cases, we employed the paired *t* test to assess statistical significance. For quantitative comparison of the initial phase of secretion, we have expressed the

secretory response in terms of the total volume secreted during the first hour of stimulation, and, for the plateau phase, as the mean secretory rate between 60–120 min after the onset of stimulation.

With either protocol, we collected the secreted saliva over timed intervals into tared polycarbonate vessels, which were then re-weighed, stoppered and stored below 4 °C pending analysis. Saliva volume was equated to mass. In our earlier experiments, we did not correct salivary secretory rate for gland mass because of the variable development of oedema, which made determination of the true gland weight uncertain. However, we find that the weight gain due to oedema can be eliminated

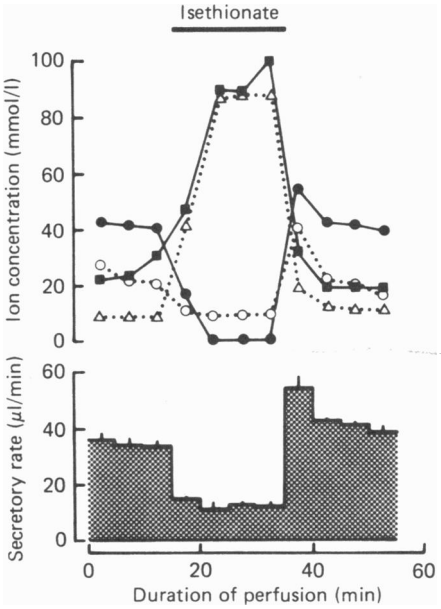

Fig. 1. Salivary secretory rate and electrolyte concentrations for rabbit mandibular glands perfused according to protocol M. The glands were perfused with control perfusate (HCO_3^-/Cl^-), and then switched to a test perfusate containing ise^- instead of Cl^-, but with unchanged HCO_3^-. After 20 min test perfusion, the glands were switched back to control perfusion. Values are means of data from four glands ± S.E. of mean (○ = Na^+, ■ = K^+, ● = Cl^-, △ = HCO_3^-).

at the end of an experiment by pressing the gland firmly between sheets of blotting paper. Consequently, correction of secretory rates for the non-oedematous gland mass has been performed in all experiments conducted according to protocol S. In the present experiments the mean non-oedematous gland mass was 513 mg (S.D. = 77, n = 75), a value close to that reported in our previous studies (Case *et al.* 1980, 1982).

Samples of saliva were analysed for Na^+, K^+, HCO_3^- and Cl^- by standard methods as described previously (Case *et al.* 1980). In order to plot flow rate curves (Figs. 2, 3 and 6), the analytical data were sorted by computer into 'bins' according to flow rate. Bin sizes were chosen to be integral multiples of a standard bin size and, as far as possible, to contain equal numbers of samples. Data are expressed as a mean for each bin ± S.E. of mean.

The following drugs were used: acetylcholine chloride or perchlorate (Sigma), furosemide (gift from Hoechst Roussel Pharmaceuticals), SITS (4-acetamido-4′-isothiocyano-2,2′-disulphonic acid stilbene: Calbiochem-Behring) and methazolamide (American Cyanamid). All drugs were made up in the perfusate just before use. Inhibitors were infused into the gland continuously from the start of perfusion and agonists were administered 15 min after infusion had begun.

RESULTS

Replacement of perfusate Cl^- with other anions, HCO_3^- unchanged

Using protocol M, we examined the effect on salivary secretory rate and composition, of replacing all perfusate Cl^- with either Br^-, I^-, NO_3^-, $CH_3SO_4^-$ or isethionate (ise^-) while maintaining the perfusate concentration of HCO_3^- (as well as phosphate and sulphate) constant. The results are depicted in Fig. 1 and Table 2. Replacement with

TABLE 2. The effect of replacing perfusate Cl^- with Br^-, I^-, NO_3^-, $CH_3SO_4^-$ or ise^-, on the plateau secretory response (protocol M) of the isolated, perfused, rabbit mandibular gland. Values are means for six glands ± S.E. of mean, obtained during 20 min of HCO_3^-/Cl^- perfusion (control) and 20 min of perfusion with a test fluid containing HCO_3^- and another anion

	Secretory rate (μl/min)			
Test anion	Control*	Test	Per cent of control	Significance of difference
Br^-	24·3 ± 3·8	27·2 ± 5·2	109·1 ± 10·7	$P = 0.3319$
I^-	30·4 ± 6·4	19·5 ± 5·3	61·7 ± 4·9	$P = 0.0038$
NO_3^-	29·3 ± 5·1	14·3 ± 2·4	50·1 ± 4·3	$P = 0.0051$
ise^-	22·0 ± 1·5	8·4 ± 0·9	39·0 ± 4·8	$P = 0.0008$
$CH_3SO_4^-$	18·4 ± 0·7	6·1 ± 0·9	24·5 ± 5·2	$P = 0.0003$

* The secretory rates of the five control groups did not differ significantly among themselves ($P = 0.23$) and the grand mean for all control experiments was 24·9 μl/min ± 1·9 S.E. of mean ($n = 30$).

Br^- was without significant effect on secretory rate, whereas replacement with other anions reduced it to varying degrees, ranging from 61·7 % in the case of I^-, to 34·4 % in the case of $CH_3SO_4^-$. The ability of the anions to replace Cl^- was in the sequence:

$$Br^- = Cl^- > I^- > NO_3^- > ise^- = CH_3SO_4^-.$$

Replacement of Cl^- with ise^- was also carried out according to protocol S (curve *D* in Fig. 4). In these experiments, a somewhat more marked reduction in secretory response was observed, so that the total volume of fluid secreted in the first hour of stimulation was only 30 % of the control level. The difference seems likely to reflect a more complete depletion of extra- and intracellular Cl^- when protocol S was employed.

The effects of Cl^- replacement on salivary electrolyte composition are shown in Figs. 1, 2 and 3. Fig. 1 shows four experiments (protocol M) in which a HCO_3^-/Cl^- perfusate was alternated with HCO_3^-/ise^-. Replacement of Cl^- with ise^- caused an immediate rise in salivary HCO_3^- concentration from 8 to 88 mmol/l, and in K^+ concentration from 22 to 90 mmol/l. Similar results were obtained in the one or two experiments with $CH_3SO_4^-$ substitution in which we also measured salivary electrolyte concentrations. In Fig. 2, the pooled salivary electrolyte concentrations from the experiments with HCO_3^-/ise^- perfusate (protocol S) have been plotted as functions of salivary secretory rate, and are compared with the control (HCO_3^-/Cl^-) experiments. The general form of the curves in the two series of experiments was unchanged, but the marked increases in the concentrations of HCO_3^- (60–80 mmol/l), K^+ (15–30 mmol/l) and Na^+ (20–30 mmol/l) are clearly shown. However, by relating concentration to flow rate, this Figure makes clear something that is not apparent

from Fig. 1, namely that the increase in HCO_3^- concentration brought about by Cl^- replacement with ise$^-$ is much greater than the rise in K^+ concentration. For example, at a flow rate of 50 μl/g.min, the increases are 5- and 2-fold, respectively. The residual ion concentration, $[Na^+ + K^+ - Cl^- - HCO_3^-]$, was slightly negative (around -5 mequiv/l) in the control series, probably reflecting the presence of Ca^{2+} and Mg^{2+}, but ise$^-$ substitution caused it to become positive, ranging from 5 mequiv/l at high

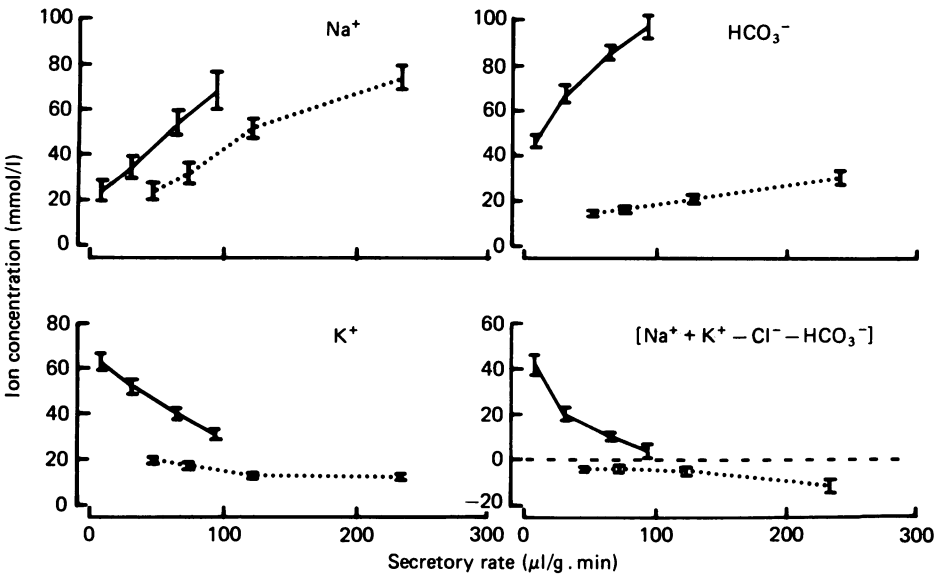

Fig. 2. Salivary electrolyte concentrations, plotted as a function of fluid secretory rate, from experiments on rabbit mandibular glands perfused according to protocol S. Dotted lines show data from control perfusion (HCO_3^-/Cl^-) and continuous lines from ise$^-$-perfused glands (HCO_3^-/ise$^-$). Data are from twelve to thirteen glands in each series; values are means of fifteen to thirty samples ± S.E. of mean. The residual ion concentrations (in mequiv/l) were calculated as $[Na^+ + K^+ - Cl^- - HCO_3^-]$; in the case of ise$^-$-perfused glands, Cl^- was absent from the saliva (see Fig. 1).

flow rates, to 40 mequiv/l at low flow rates. Whether this was due to entry of ise$^-$ into the saliva, or reflects an increased concentration of negatively charged salivary proteins, or both, has not been determined.

The electrolyte flow relations obtained during HCO_3^-/Br^-, HCO_3^-/I^- and HCO_3^-/NO_3^- perfusion (protocol M), are depicted in Fig. 3. Despite the much narrower flow-rate range obtainable with the M protocol, the expected type of flow dependency for Na^+ and K^+ was observed. The Br^- and I^- ions entered the saliva in high concentrations: for Br^- the average concentration was 69·4 mmol/l (S.E. of mean = 4·5, n = 16) and for I^- it was 85·6 mmol/l (S.E. of mean = 3·5, n = 18). It thus appears that the other halides were secreted as readily as Cl^- and, in contrast to what was seen during ise$^-$ substitution, the HCO_3^- concentration (as judged from the $[Na^+ + K^+ - \text{halide}^-]$ concentration) did not increase.

Replacement of perfusate HCO_3^- (alone or as well as Cl^-) with other anions

In Fig. 4 are depicted the results of five series of experiments conducted according to protocol S. In the control series (*A*), the perfusate contained HCO_3^- and Cl^- in normal extracellular concentrations. In series *D*, the Cl^- was replaced with ise^- while

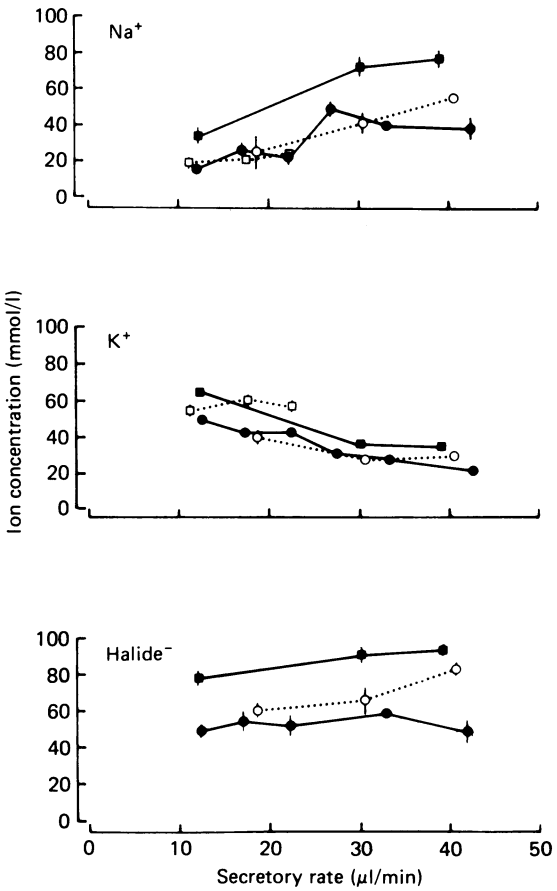

Fig. 3. Salivary electrolyte concentrations, plotted as a function of fluid secretory rate, from experiments on rabbit mandibular glands perfused according to protocol M. All glands were perfused with similar solutions except that the major perfusate anion (normally Cl^-, ●) was changed to I^- (■), Br^- (○) or NO_3^- (□) in the test series. Values are means of four to six samples ± S.E. of mean, from studies in five to six glands for each series. It should be noted that the abscissa has been greatly expanded when compared to that in Fig. 2.

HCO_3^- was held constant. In the three remaining series, perfusate HCO_3^- was removed altogether: in one series (*E*), HCO_3^- was replaced with Cl^-, in another (*B*), HCO_3^- was replaced with ise^-; and in the third series (*C*), both HCO_3^- and Cl^- were replaced with ise^-. (For precise composition of the perfusion fluids employed, see Table 1.)

Removal of HCO_3^- had little effect on secretion, regardless of whether it was

replaced with Cl^- or ise$^-$, provided that the rest of the perfusate Cl^- was unchanged (cf. curves *B* and *E* with *A* in Fig. 4). However, replacement of both HCO_3^- and Cl^- with ise$^-$, virtually abolished secretion (curve *C*); immediately after the onset of stimulation, the secretion rate was 5 μl/g . min, and this fell to less than 1 μl/g . min in 30 min. A similar abolition of the secretory response was observed in four additional experiments, conducted according to protocol M.

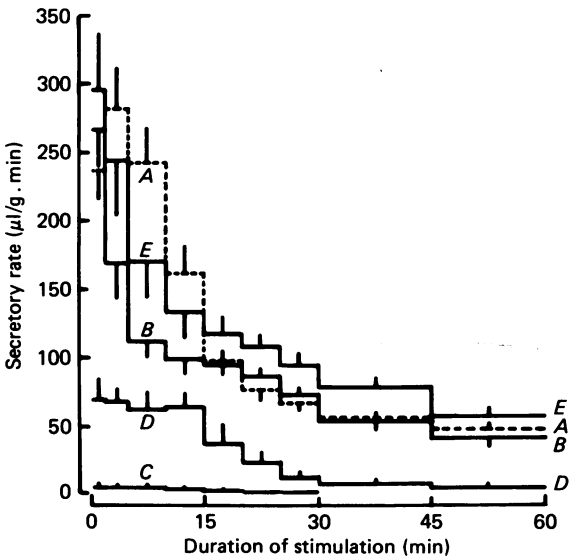

Fig. 4. Secretory response of isolated perfused rabbit mandibular glands stimulated continuously with acetylcholine (protocol S). The control perfusate contained 25 mM-HCO_3^- and 121 mM-Cl^- (curve *A*; dotted line); in the other series, Cl^- was replaced with ise$^-$ (*D*), HCO_3^- was replaced with ise$^-$ (*B*), both Cl^- and HCO_3^- were replaced with ise$^-$ (*C*), and HCO_3^- was replaced with Cl^- (*E*). Each horizontal bar shows the mean ± S.E. of mean of data from four to eighteen glands.

It thus appeared that either Cl^- or HCO_3^- could support secretion. However, since the perfusate HCO_3^- concentration during replacement of Cl^- with ise$^-$ was only 25 mmol/l, it was obviously inappropriate to compare the secretory response under these conditions with that seen when HCO_3^- was replaced with ise$^-$ (when the Cl^- concentration was 121·3 mmol/l). To enable a better comparison, we performed four experiments in which perfusate Cl^- was reduced to 25 mmol/l and the remaining anions (Cl^- and HCO_3^-) were replaced with ise$^-$. In this series, the 1 h fluid output was 0·89 ml/g (S.E. of mean = 0·60, $n = 4$) and the plateau secretion rate after 1 h was 0·68 μl/g . min (S.E. of mean = 0·30, $n = 4$). These secretion rates are of similar size to those obtained during HCO_3^-/ise$^-$ perfusion, suggesting that HCO_3^- and Cl^- are equally able to sustain secretion.

Effects of methazolamide, furosemide and SITS

During control perfusion (HCO_3^-/Cl^-), as we have shown previously (Case *et al.* 1982), methazolamide (0·1 mmol/l) had no effect on the output of saliva or the salivary HCO_3^- concentration. Fluid output during the first hour of secretion was

4·52 ml±0·34 (S.E. of mean; $n = 10$) during control perfusion, and 4·72 ml±0·52 ($n = 7$) in the presence of methazolamide; the difference was not significant ($P = 0·746$). In these experiments, the respective HCO_3^- concentrations in saliva samples collected between 30 and 45 min after the onset of stimulation were 10·6 mmol/l±1·0 ($n = 6$) and 8·8 mmol/l±0·5 ($n = 7$); they did not differ significantly from one another ($P = 0·164$). During perfusion with HCO_3^- and ise^-, however, methazolamide caused more than a 55% reduction in fluid output (1·99 ml±0·26 *versus* 0·88 ml±0·16; $P = 0·009$, $n = 4$) but had no significant effect on HCO_3^- concentration in samples collected at comparable flow rates (57·1 mmol/l±9·5 *versus* 48·4 ml±7·0; $P = 0·137$, $n = 4$).

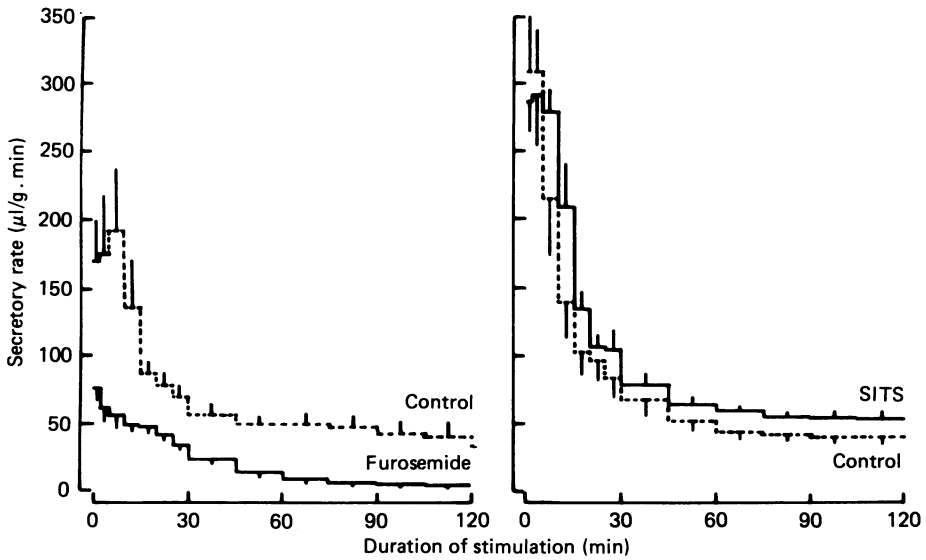

Fig. 5. Secretory responses of HCO_3^-/Cl^- perfused glands. Continuous lines show the response of glands infused with furosemide (1·0 mM) or with SITS (0·1 mM). Dotted lines show the response of corresponding control experiments done on the paired glands. Each horizontal bar represents the mean of four to six experiments±S.E. of mean.

The effects of furosemide (1 mmol/l), administered during HCO_3^-/Cl^- perfusion, on salivary flow rate and electrolyte content are shown, respectively, in Figs. 5 and 6. Furosemide reduced secretion by more than 60%. Thus, the 1 h fluid output in the control series was 5·24 ml/g (S.E. of mean = 0·82, $n = 4$) and in the furosemide series it was 2·04 ml/g (S.E. of mean = 1·20, $n = 4$); the difference was highly significant ($P = 0·010$). The drug also caused a dramatic increase in salivary HCO_3^- concentration, which rose from a control value of about 13 mmol/l to levels of 55–80 mmol/l (Fig. 6). This change was accompanied by a sharp drop in Cl^- concentration and a rise in K^+ concentration, but there appeared to be no change in Na^+ concentration other than that attributable to the fall in salivary secretory rate. When furosemide was administered to glands perfused with a Cl^--containing fluid from which all HCO_3^- had been removed, secretion was virtually abolished, falling to less than 1% of control levels within 30 min of drug administration.

The effect of SITS (0·1 mmol/l) is also shown in Figs. 5 and 6. This drug had a small

stimulatory effect on salivary secretion (Fig. 5). This was most marked in the plateau region where the control secretory rate was 42·17 μl/g.min (S.E. of mean = 4·40, $n = 6$) and the SITS secretory rate was 55·92 μl/g.min (S.E. of mean = 3·24, $n = 6$) ($P = 0{\cdot}003$). The 1 h fluid outputs, respectively, were 6·57 ml/g (S.E. of mean = 0·91, $n = 6$) and 7·79 ml/g (S.E. of mean = 0·49, $n = 6$) ($P = 0{\cdot}094$). SITS had no effect on salivary HCO_3^- or K^+ concentrations, but Na^+ and Cl^- concentrations were reduced by about 12 mmol/l at all flow rates.

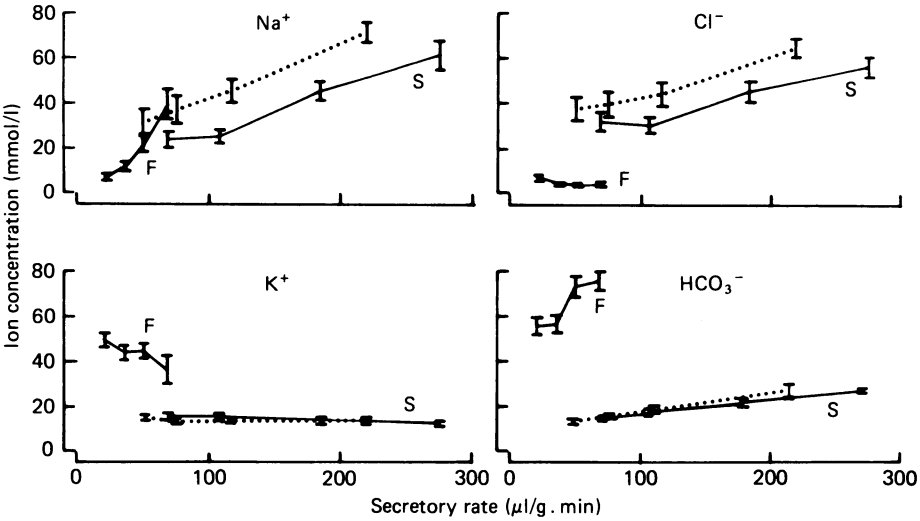

Fig. 6. Salivary electrolyte concentrations plotted as functions of flow rate from the experiments depicted in Fig. 5. The two control series depicted in Fig. 5 have been merged for this Figure, and are shown by the dotted lines. Values are means of eight to twenty samples ± S.E. of mean. Curves labelled F represent the effects of furosemide (1·0 mM) and those labelled S represent the effect of SITS (0·1 mM).

DISCUSSION

In our previous studies (Case *et al.* 1981; 1982), we showed that omission of HCO_3^- from the fluid perfusing the rabbit mandibular gland had no effect on the secretion rate evoked by acetylcholine, provided that the pH of the extracellular medium was held constant by the inclusion of a suitable alternative buffer (10 mM-HEPES) in the perfusate. This observation has been confirmed in the present study (Fig. 4, curve *E*) and extended by the observation that HCO_3^- can be replaced, not only by Cl^-, but also by the large, relatively impermeable anion, ise^-. In one of our previous studies (Case *et al.* 1981), we proposed that secretion depended on the activity of a Na^+/Cl^- symport located in the basolateral plasma membrane of the secretory cells, but we felt unable at that time to exclude, as an alternative possibility, that there might instead be two antiports involved, driving Na^+/H^+ and Cl^-/HCO_3^- exchange and leading to neutral NaCl secretion.

In the present study we find that while omission of Cl^- from the perfusate altogether (by replacing it with ise^-) reduces secretion (by about 60%), it does not abolish it, provided that HCO_3^- (25 mM) remains. Indeed, the secretory rate supported by 25 mM-HCO_3^- was not less than that supported by 25 mM-Cl^-. Most

important to note is that the secretion formed in the absence of extracellular Cl^- is HCO_3^- rich. In addition, in control perfused glands, we find that after administration of furosemide, a drug believed to block Na^+/Cl^- co-transport systems (Burg, Stoner, Cardinal & Green, 1973), salivary secretory rate is reduced and its composition altered in a manner indistinguishable from that seen when perfusate Cl^- is replaced with ise^-. There has been a previous report (for the perfused cat mandibular gland), that furosemide induces a partial inhibition of salivary secretion (Poulsen, Laugesen & Nielsen, 1982), but, until now, it had not been appreciated that the remaining secretion might be HCO_3^- rich.

These observations suggest that when Cl^- transport across the secretory epithelium is prevented by either of these two manoeuvres, a component of the secretion supported by HCO_3^- transport is unmasked. Under these experimental circumstances, both inhibition of carbonic anhydrase and complete removal of perfusate HCO_3^- cause a marked further reduction in secretion. Omission of HCO_3^- almost abolished secretion, the residuum (2–3% of control) probably being supported by HCO_3^- derived from endogenous CO_2 production. Methazolamide, administered in a dose large enough to have inhibited all carbonic anhydrase activity (Maren, 1980), only reduced secretion by about 60%, but, of course, the perfusate still contained 25 mM-HCO_3^- and 1·4 mM-H_2CO_3 (as CO_2). It thus seems likely that the residual secretion was the amount capable of taking place when HCO_3^- formation is uncatalysed. A similar inhibitory action on HCO_3^--supported secretion in the cat pancreas has been observed when HCO_3^- is removed or carbonic anhydrase inhibited (Case, Scratcherd & Wynne, 1970).

Taken together, these observations suggest that the end-piece epithelial cells possess two separate carrier systems, both of which support secretion. One of these seems to be Cl^--dependent and furosemide-sensitive, and thus corresponds to the classical Na^+/Cl^- symport described in many other tissues. Since secretion was moderately well maintained without a noticeable increase in salivary HCO_3^- content when Cl^- was replaced with I^- or Br^-, it would appear that the proposed symport can accept halides other than Cl^-, albeit with differing affinities, but not ise^- or $CH_3SO_4^-$.

The other transport system, previously unsuspected in salivary end-piece cells, seems to transport HCO_3^- and is methazolamide-sensitive. An obvious analogue for this system would be the Na^+/H^+ antiport postulated for pancreatic duct cells, but one needs also to consider a double antiport system, Na^+/H^+ associated with Cl^-/HCO_3^-, as postulated for many absorptive epithelia (see Introduction). The failure of SITS, a drug thought to inhibit Cl^-/HCO_3^- exchange in other epithelia (Warnock & Eveloff, 1982), to block secretion or to alter the salivary HCO_3^- concentration, seems at first sight to speak against the double antiport system, but the evidence cannot be considered as conclusive. If SITS had blocked a Cl^-/HCO_3^- antiport in our gland preparation, one would expect intracellular HCO_3^- to rise, but whether this would alter secretion rate or salivary HCO_3^- concentration would depend on how HCO_3^- and Cl^- cross the apical cell membrane and to what extent they can compete for transport when doing so. Even if HCO_3^- did enter the lumen more rapidly than Cl^- (and thereby increase secretion rate as we have observed), one might still not see a change in salivary HCO_3^- concentration, since Cl^- and HCO_3^- can exchange across the walls of the excurrent ducts as saliva flows from the

end-pieces to the exterior (Martin, Frömter, Gebler, Knauf & Young, 1973). More detailed studies will be required to define the nature of the HCO_3^- pump, but even on the available data, the conclusion seems inescapable that salivary secretion involves HCO_3^- as well as Cl^- transport, and the predominance of Cl^- transport seen under normal circumstances, is at least in part due merely to the predominance of this anion in the extracellular medium.

This paper was supported by the U.K. Science and Engineering Research Council, the U.K. Cystic Fibrosis Research Trust, the National Health and Medical Research Council of Australia and the Australian Cystic Fibrosis Research Association.

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