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THE EFFECTS OF BICARBONATE AND FOREIGN ANIONS ON CHLORIDE TRANSPORT IN SMOOTH MUSCLE OF THE GUINEA-PIG VAS DEFERENS

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SUMMARY

1. The selectivity of the external site of the Cl transporting mechanism in the guinea-pig vas deferens has been investigated by measurement of ^{36}Cl uptake and efflux and by direct measurement of intracellular pH.

2. Replacing 50 % of the Cl in normal Krebs solution inhibited the 15 min uptake of ^{36}Cl in the order $NO_3 > Br > SCN > F > I >$ glucuronate, both in Cl-depleted tissues and tissues pre-incubated in the 50 %-Cl solutions (steady-state uptake). After 3 h incubation in these solutions, the total cellular Cl was reduced by the anions in the order $Br > NO_3 > I > SCN > F >$ glucuronate. Br, NO_3 and I reduced the cellular Cl to less than 50 % of normal, suggesting that they are actively taken up by the cells.

3. The ability of foreign anions to inhibit a 3 min uptake of high specific activity, low concentration Cl (6·5 mM) suggests an apparent affinity series of $NO_3 > Cl = SCN = Br > I > F$ at the external site.

4. Addition of NO_3, Cl, Br, HCO_3, F, SCN or I to a Cl-free, nominally HCO_3-free bathing solution accelerated ^{36}Cl efflux. The first four mentioned were powerful stimulants, the other three less potent. However, the exact position of HCO_3 in the sequence is uncertain. The rapidity with which CO_2 crosses the membrane and forms HCO_3 intracellularly may allow competition between HCO_3 and Cl at the internal site and so distort the result. The action of F is also questionable since this ion drastically reduces the divalent cation activity and is a metabolic inhibitor.

5. Measurement of intracellular pH provided conclusive evidence that Cl, NO_3, Br and I can exchange with HCO_3 across the cell membrane. This exchange is as rapid with NO_3 as with Cl but slower with Br and considerably slower with I. The results also indicate that SCN ions cross the cell membrane.

6. It is concluded that Cl, HCO_3, Br and NO_3 are all translocated by the exchange carrier. I and perhaps SCN also interact with the transport mechanism, but the translocation rate is then greatly reduced. The precise order of the affinity of these anions remains uncertain but the following sequence: $NO_3 > Cl = SCN = Br > I > F$ is considered to be the most likely.

INTRODUCTION

Following the development of a double-barrelled ion-selective micro-electrode which allows direct and continuous recording of intracellular ion activities in mammalian smooth muscle (Aickin, 1981), we were able to demonstrate that a reversible Cl–HCO_3 exchange carrier plays a major role in establishing the high intracellular Cl activity (Aickin & Brading, 1982, 1984). Complementary measurements of ^{36}Cl flux led to the suggestion that the affinity of the internal site of the carrier was greater for HCO_3 than it was for Cl (or alternatively that the outward translocation of the HCO_3-loaded carrier was faster) while that of the external site was unlikely to be so (Aickin & Brading, 1983*a*). These characteristics are reminiscent of the much studied anion exchange carrier, the band III protein of the erythrocyte, and led us to conclude that the HCO_3-dependent fraction of Cl transport in guinea-pig vas deferens may not be significantly different from band III (Aickin & Brading, 1983*a*). The work described here was undertaken with the intention of testing this conclusion and gaining a better understanding of the mechanism mainly responsible for Cl accumulation in smooth muscle. The main aim was to establish the selectivity sequence of the external site.

^{36}Cl flux experiments were primarily used in this study, first, because the Cl-sensitive liquid ion exchanger shows sensitivity to other anions, particularly other halides, and secondly, because valuable information about the properties of the carrier can be obtained in the steady state when its operation is invisible to the ion-selective electrode. However, double-barrelled pH-sensitive micro-electrodes were used to monitor the rate of net transport of HCO_3 ions in the presence of various extracellular anions.

A preliminary report of some of these results has been communicated to the Physiological Society (Aickin & Brading, 1983*b*).

METHODS

Experiments were performed on strips of smooth muscle from the vasa deferentia of albino male guinea-pigs. Preparations were maintained at 35 °C in normal Krebs solution of the following composition (mM): NaCl, 122; KCl, 5·9; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 14; glucose, 11; Na_2HPO_4/NaH_2PO_4 at pH 7·35, 0·1, equilibrated with 3% CO_2, 97% O_2. Cl-free solutions were buffered either with 3% CO_2, 14 mM-HCO_3 as in the normal solution, or with 14 mM-HEPES (2-*N*-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) and NaOH to pH 7·35, equilibrated with 100% O_2 (nominally HCO_3-free solution). They contained (mM): Na glucuronate, 122; K gluconate, 5·9; Ca gluconate, 12; $MgSO_4$, 6. The concentration of divalent cations was raised to compensate for binding by the organic anions (see Aickin & Brading, 1983*a*). Glucuronate and gluconate salts were used as the control Cl substitute because first, they do not interfere with the Cl-sensitive liquid ion exchanger, and secondly, gluconate has been shown not to compete for the transport site of the band III protein in red blood cells (Lambert & Lowe, 1978). Na salts of the anions under investigation replaced Na glucuronate or NaCl isosmotically. In the micro-electrode study, K salts were also replaced isosmotically. Solutions containing NO_3 were prepared immediately prior to use, since, in the presence of glucose, NO_3 is converted progressively to NO_2.

Electrical recording

The methods for measuring intracellular ion activities have been fully described in Aickin & Brading (1982) and Aickin (1984). Double-barrelled micro-electrodes were used to measure membrane potential and ion activity simultaneously. The unconventional reference liquid ion

exchanger (Thomas & Cohen, 1981) was used in one barrel to measure the membrane potential, and either the Cl-sensitive liquid ion exchanger (Corning 477315) or the H-selective ligand (Ammann, Lanter, Steiner, Schulthess, Shijo & Simon, 1981) was used in the second barrel to measure Cl activity or pH respectively. DIDS (4-4′-diisothiocyanostilbene-2,2′-disulphonic acid: Calbiochem-Behring Corp.), when used, was added to the solutions immediately before use.

^{36}Cl fluxes

Efflux. Two strips were cut from the full length of each vas deferens, and mounted on stainless-steel holders. They were loaded for at least 2 h in ^{36}Cl-containing solution and washed out at 34–36 °C in groups of four using a constant superfusion apparatus and a fraction collector.

Uptake. Four strips were dissected from each vas deferens, mounted on stainless-steel holders, and equilibrated for at least 1 h in normal Krebs solution. Cl-free tissues were prepared by exposure to Cl-free solution for at least 1·25 h with two solution changes. Tissues were exposed to the appropriate ^{36}Cl-containing solutions for the time allocated, and then washed for 5 min in ice-cold, Cl-free, nominally HCO_3-free solution for 5 min before counting. The specific activity of the solutions was also determined. Further details can be found in Aickin & Brading (1983*a*).

RESULTS

Selectivity of Cl over HCO_3

We have previously suggested that the Cl–HCO_3 exchanger can operate in either direction across the cell membrane, implying that both ions can bind to both internal and external sites (Aickin & Brading, 1984). In order to understand the operation of the exchanger and to establish its role in the generation of the non-passive Cl distribution, it is important to establish whether the carrier can distinguish between these two ions. In this paper we are concerned with the selectivity of the external site. Earlier experiments (Aickin & Brading, 1983*a*) showed that if Cl efflux is observed in tissues loaded and washed out in Cl-containing solutions, there is no noticeable effect of removing or readmitting HCO_3^- ions. Neither is the uptake of Cl by normal tissues affected by removing HCO_3 from the bathing solution. These results suggest at least that the affinity of the external site for HCO_3 is unlikely to be much greater than for Cl.

Further information can be obtained by investigating the ability of either ion to support the efflux of Cl^- ions. The results of one such experiment, in which a given amount of either ion was added to a Cl-free, nominally HCO_3-free solution, are illustrated in Fig. 1. It is clear from the Figure that both Cl^- and HCO_3^- ions are capable of activating Cl efflux. However, there is great variability in the degree of stimulation from tissue to tissue (see also Aickin & Brading 1983*a*). In this Figure where each curve shows the means from four tissues, the only significant difference is that 21 mM-Cl stimulates the efflux more effectively than 14 mM-Cl or either concentration of HCO_3.

Because of the variability between tissues, another approach was tried in which equal concentrations of Cl and HCO_3 were exchanged during the course of a wash-out. Fig. 2 shows that if all but 14 mM of the anions in the wash-out solution were replaced by non-permeant species, then changing from Cl to HCO_3 caused a drop in the efflux rate, whereas changing from HCO_3 to Cl increased the efflux rate.

One of the problems in interpreting these results is that removing or adding Cl or HCO_3 leads to a rapid fall or rise in their intracellular level. This consequently alters the substrates available for binding to the internal site. Thus, although the evidence

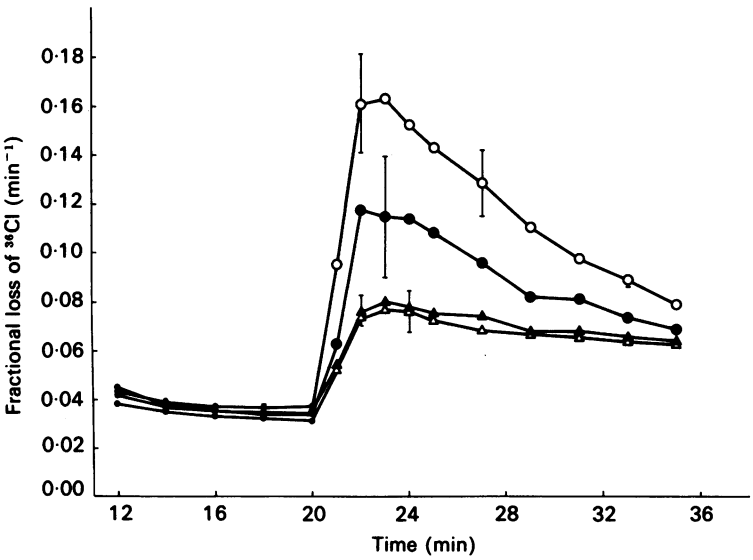

Fig. 1. Stimulation of ^{36}Cl efflux by Cl and HCO_3. Tissues were loaded in normal Krebs solution and washed initially in Cl-free, nominally HCO_3-free solution. Perfusion with media containing Cl (circles) or HCO_3 (triangles) began at the arrow. Filled symbols denote perfusion with solutions containing 14 mM-Cl or HCO_3 and open symbols with solutions containing 21 mM-Cl or HCO_3. Each curve is the mean of four experiments and representative S.E. bars are shown. External pH was 7·35 throughout.

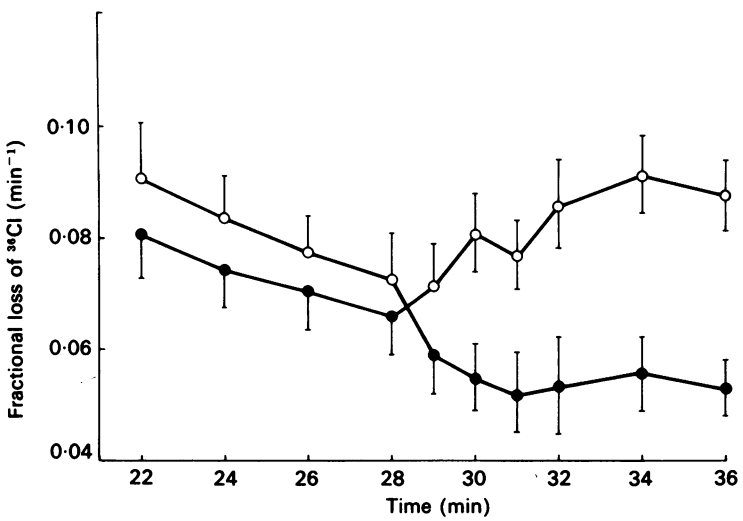

Fig. 2. The effects of exchanging 14 mM-Cl with 14 mM-HCO_3 on ^{36}Cl efflux. ○, during wash-out in nominally HCO_3-free medium containing 14 mM-Cl. ●, during wash-out in Cl-free medium containing 14 mM-HCO_3. Mean ± S.E. of mean, $n = 4$.

suggests that HCO_3 binds less well to the external site than Cl, the decline in efflux rate on changing from Cl- to HCO_3-containing solutions could also reflect competition at the internal site resulting from the rise in intracellular HCO_3. Similarly, the increase in Cl efflux on replacing HCO_3 with Cl could reflect a drop in intracellular HCO_3, particularly in view of the previous suggestion that the internal site has a higher affinity for HCO_3 than for Cl (Aickin & Brading, 1983*a*).

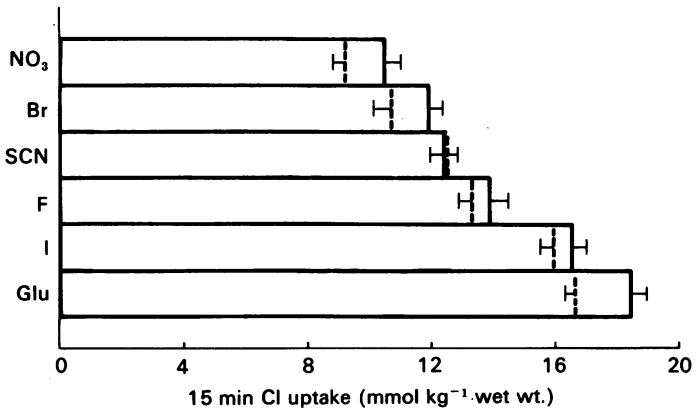

Fig. 3. The inhibitory effect of foreign anions on 15 min uptake of ^{36}Cl. The main histogram shows Cl uptake by Cl-depleted tissues (mean + S.E. of mean, $n = 30$–35) from solutions in which 50 % of the Cl was replaced by the foreign anion. The dotted lines show Cl uptake (mean − S.E. of mean, $n = 8$) by tissues pre-incubated for 1·5 h in label-free, 50 %-Cl solutions. All solutions contained 14 mM-HCO_3 equilibrated with 3 % CO_2, 97 % O_2. Values corrected for Cl remaining in the extracellular space; see Table 1.

The effect of foreign anions on ^{36}Cl uptake

Fig. 3 shows the effects of foreign anions on ^{36}Cl uptake. The tissues were first depleted of Cl by exposure to a Cl-free, HCO_3-containing solution for at least 1·25 h, and then exposed to uptake solutions in which half the normal Cl was replaced by a foreign anion. We chose a 15 min uptake in this experiment, since the uptake of Cl from normal solutions is not quite complete at this time (Aickin & Brading, 1983*a*) and thus any inhibitory effects should be easily noticeable. The order of potency of the anions at inhibiting net Cl uptake under these conditions was: $NO_3 > Br > SCN > F > I >$ glucuronate. Although the initial inhibition of uptake is likely to be due to competition between the foreign anion and Cl for the external site, progressively increasing levels of intracellular Cl, and probably also foreign anion, will compete with HCO_3 at the internal site and so complicate the interpretation of this experiment.

The effect of foreign anions on Cl uptake in the steady state was also investigated, as shown in Fig. 3 (dashed lines). Tissues were pre-incubated for 1·5 h in label-free versions of the various uptake solutions. The order is very similar to that obtained in the Cl-free tissues, but under these conditions the ^{36}Cl uptake will not be a net Cl uptake, but will consist largely of Cl–Cl exchange. In this case a better estimate of the inhibitory effects of the foreign anions can be obtained by expressing the ^{36}Cl

uptake as a percentage of the total Cl present. Table 1 shows the Cl content of tissues exposed for 3·5 h to each of the experimental solutions. The 15 min uptakes expressed as a percentage of the total Cl (mmol kg^{-1} wet weight) are SCN, 66%; NO_3, 70%; glucuronate, 75%; Br, 79%; F, 87% and I, 110%. This order is different from the inhibition of the 15 min uptake by Cl-depleted tissues. It is, however, difficult to interpret, since under steady-state conditions the deficit in Cl in the 50%-substituted solutions could be made up by intracellular foreign anions, differences in intracellular HCO_3 or changes in the fixed intracellular anions, and any of these changes could affect the ion exchange mechanism.

TABLE 1. Cl content of tissues exposed for 3·5 h to Krebs solutions in which half the Cl is replaced by another anion

	Cl (mmol kg^{-1} wet wt.)			Cl (mmol kg^{-1} dry wt.)		
	Total	Corrected	Dry wt./ wet wt.	Total	Corrected (x)	% of x
Cl (100%)	33·9±2·2	30·1	18·7±0·4	181·6±9·3	160·8	100
Glucuronate	24·1±0·7	22·2	19·2±0·4	126·0±3·4	116·0	72·1
F	18·3±0·4	16·4	16·9±0·3	109·1±2·1	97·1	60·4
SCN	20·7±1·1	18·8	20·4±0·3	101·1±4·8	91·9	57·1
I	16·5±0·6	14·6	19·1±0·3	86·8±3·4	76·9	47·8
NO_3	15·1±0·8	13·2	18·5±0·2	93·6±4·4	71·1	44·2
Br	15·4±0·4	13·5	19·4±0·3	79·9±0·8	69·8	43·4

All values are given as mean±S.E. of mean (n = 8). 14 mM-HCO_3 was present in all solutions. The tissues were equilibrated in ^{36}Cl-containing solutions, washed for 5 min in ice-cold, Cl-free Krebs solution, blotted and weighed (wet wt.), dried overnight at 50 °C and reweighed (dry wt.), dissolved in protosol, neutralized and counted. Corrections were made for Cl remaining in the extracellular space by subtracting 3·8 mmol Cl kg^{-1} wet wt. from the tissues in normal Cl, and 1·9 from the tissues in 50%-Cl solution (Aickin & Brading, 1983).

Table 1 also shows total Cl expressed as mmol kg^{-1} dry weight, since this value will allow for any changes in tissue volume. Cellular Cl was considerably reduced by the presence of all of the foreign anions, even glucuronate. We had assumed that glucuronate was a non-permeant anion that was not carried by the Cl transport mechanism (Lambert & Lowe, 1978). However, it is clear that with long exposures to 50%-Cl, 50%-glucuronate solution, the intracellular Cl is depleted more than we had found previously with shorter equilibrations (Aickin & Brading, 1982). It is possible that some penetration of this anion does occur with time, but it is also possible that there is an increase in intracellular HCO_3, or an increase in the intracellular fixed negative charge. Replacing 50% of the Cl with the other foreign anions does however cause considerably greater loss of Cl than with the 50%-glucuronate solution.

To avoid the type of ambiguity implicit in the interpretation of the 15 min uptake experiments due to competition of Cl and other anions at the internal site, we investigated the effect of foreign anions (50 mM) on a 3 min uptake of labelled Cl from a ^{36}Cl solution of high specific activity, containing only 6·5 mM-Cl. A short uptake into Cl-containing tissues was chosen to minimize both backflux of label and changes in intracellular substrate (none of the solutions contained HCO_3). An additional

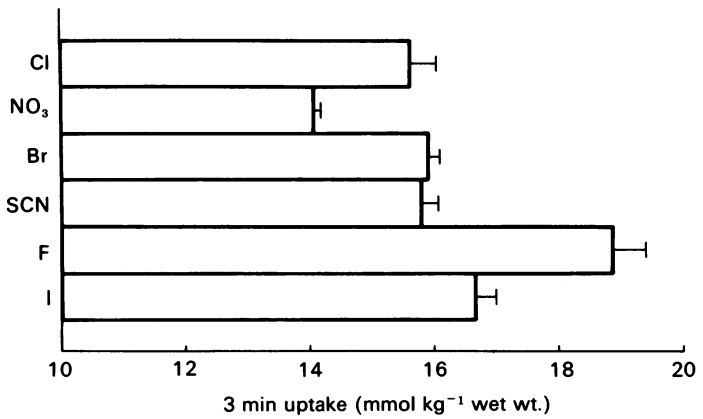

Fig. 4. The inhibitory effect of 50 mM-anion on 3 min ^{36}Cl uptake by Cl-containing tissues. The uptake media were made from Cl-free, nominally HCO_3-free solution with 50 mM-Na(anion) and 6·5 mM-NaCl (incorporating ^{36}Cl) replacing an equivalent amount of Na glucuronate. Means+S.E. of mean, $n = 8$.

50 mM-Cl was used as the control. Under these conditions a somewhat different affinity sequence is seen (Fig. 4). The competing anions reduced the uptake of labelled Cl in the order: $NO_3 > Cl = SCN = Br > I > F$.

The effect of foreign anions on ^{36}Cl efflux

The foregoing results suggest that foreign anions compete with Cl at the external site and may be transported by the carrier. If so, foreign anions ought to stimulate Cl efflux. An experimentally simple procedure to test this is through the uptake/efflux approach. The tissues were loaded to equilibrium with ^{36}Cl in nominally HCO_3-free Krebs solution, then exposed to Cl-free (glucuronate) solution for 15 min to wash out most of the extracellular label. Finally, they were washed for a further 10 min in solutions containing 120 mM-anion. All solutions were nominally HCO_3-free. The tissues were then counted to estimate how much of the original intracellular Cl remained. The potency of the anions in activating the loss of intracellular Cl was in the order: $Cl = Br = NO_3 > F > SCN > I =$ glucuronate.

The time course of the activation of ^{36}Cl efflux by the various anions is shown in Fig. 5. This illustrates the results of an experiment in which tissues, loaded in normal solution, were washed out in Cl-free, nominally HCO_3-free solution before 120 mM-anion was applied. I and SCN are clearly less able to stimulate Cl efflux than Cl, Br or NO_3, but the differences are not significant within the two groups.

In an attempt to differentiate between the ability of Cl, Br and NO_3 to activate Cl efflux, tissues were loaded in normal Krebs solution and washed out in nominally HCO_3-free solution containing only 14 mM-Cl. Half-way through the wash-out, the Cl was replaced by 14 mM-NO_3 or 14 mM-Br. The results are shown in Fig. 6. Replacing Cl with Br caused a significant initial drop in the rate of loss of Cl, followed by a slow recovery or increase in the rate, whereas replacing Cl with NO_3 resulted in a small increase in Cl loss. This experiment suggests that the order of effectiveness in activating Cl efflux is: $NO_3 > Cl > Br$.

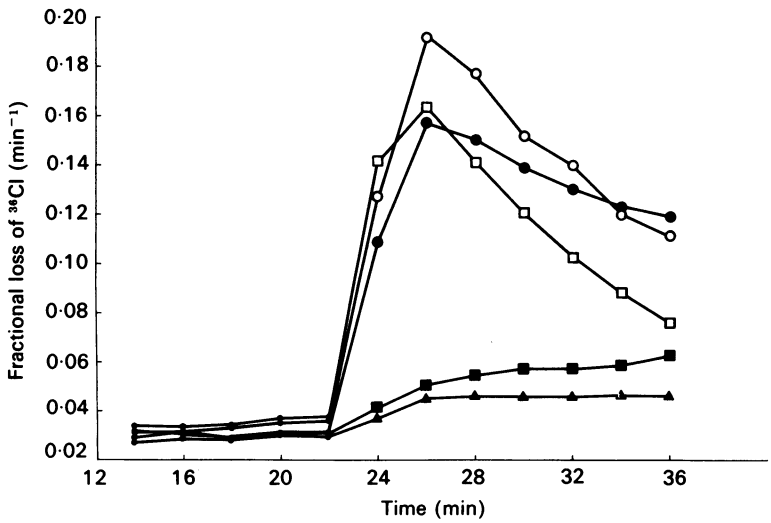

Fig. 5. The effect of 120 mM-anion on ^{36}Cl efflux. Tissues were loaded in normal solution and washed out initially in Cl-free, nominally HCO_3-free solution for 22 min, after which solutions containing 120 mM of the anion under investigation were applied. ○, Br; ●, Cl; □, NO_3; ■, SCN; ▲, I. All solutions were nominally HCO_3-free. Points are means of four experiments.

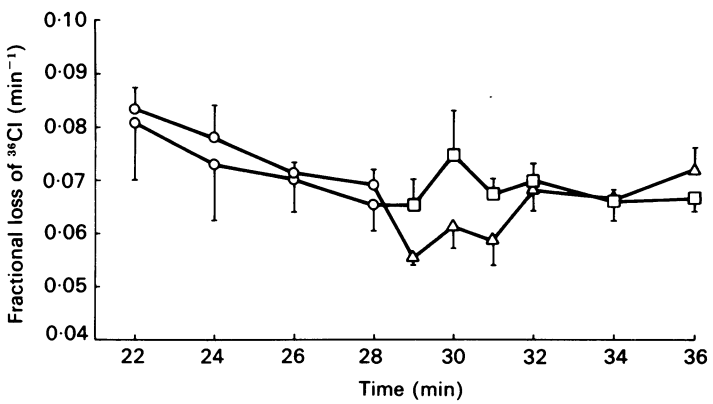

Fig. 6. The effects of exchanging 14 mM-Cl for 14 mM-NO_3 or 14 mM-Br on the ^{36}Cl efflux. ○, wash-out in 14 mM-Cl solution; □, wash-out in 14 mM-NO_3; △, wash-out in 14 mM-Br. All solutions were nominally HCO_3-free. Means ± S.E. of means, $n = 4$.

The effect of foreign anions on intracellular pH

All of the results described so far may reflect different affinities of the transport mechanism for the various anions or differences in the mobility of the mechanism when associated with different anions. However, interpretation of the data is complicated by the fact that transmembrane Cl movement occurs via at least two transport mechanisms: in the main by Cl–HCO_3 exchange but to about 25% by another, as yet uncharacterized process (Aickin & Brading, 1984). The relative

contributions of the mechanisms may vary under different experimental conditions (e.g. at different levels of intracellular Cl) and their affinities for the foreign anions may not be the same. But perhaps a more serious problem in interpretation of the flux data is that it could be explained equally well on the basis of changes in Cl permeability (P_{Cl}). A foreign anion-induced increase in P_{Cl} could (through passive Cl movements) both decrease the rate of net Cl uptake and increase the rate of Cl efflux. Both these criticisms could theoretically be answered by direct measurement of membrane potential (E_m) and intracellular Cl activity. In practice, the sensitivity of the Cl-selective resin to the foreign anions used in this study would render the results of experiments on inhibition of Cl reaccumulation uninterpretable. Nevertheless, we have previously shown that net transmembrane movement of Cl^- ions is accompanied by an opposite movement of HCO_3^- ions (Aickin & Brading, 1984). Thus reaccumulation of Cl^- ions is accompanied by an intracellular acidification, and loss of Cl^- ions in Cl-free solution is accompanied by an alkalinization, as illustrated in Fig. 7. The H-selective ligand used does not suffer from interference from foreign anions, with the exception of SCN (see below), and has the advantage that, presumably, only movements via the Cl–HCO_3 exchange carrier are monitored.

Fig. 8 shows the effects of various anions on E_m and intracellular pH (pH_i). The preparation had previously been equilibrated in Cl-free, HCO_3-containing solution. Application of a solution in which the major anion was NO_3 (85%) caused an intracellular acidification of the same time course as seen on application of 85%-Cl solution (the mean half-time was $1{\cdot}1 \pm 0{\cdot}1$ min ($n = 3$, S.D. of an observation) in both cases). Similarly, return to glucuronate/gluconate substituted solution caused an alkalinization, i.e. movement of H^+ ions (or their equivalent) *against* the electrochemical gradient, of much the same time course as seen after removal of Cl^- ions (mean half-time of $2{\cdot}0 \pm 0{\cdot}3$ min, $n = 3$ compared with $1{\cdot}9 \pm 0{\cdot}3$ min, $n = 3$; paired observations in three cells). Application of 85%-Br solution also caused an acidification but, like the alkalinization seen on return to the glucuronate/gluconate substituted solution, the rate of change of pH_i was significantly slower than seen on application and removal of Cl^- ions ($2{\cdot}2 \pm 0{\cdot}1$ min, $n = 3$ and $3{\cdot}9 \pm 0{\cdot}2$ min, $n = 3$ on application and removal of Br respectively). I^- ions caused only a small acidification of a mean $0{\cdot}03 \pm 0{\cdot}01$ units ($n = 4$) after a 6 min exposure, which slowly reversed after their removal. It is worth noting that I and Br had similar but smaller effects on E_m than Cl, but NO_3 caused a marked depolarization. Removal of NO_3^- ions caused a further depolarization followed by a slow recovery, usually not complete for about 30 min. Interpretation of the changes in E_m has not been attempted since it requires simultaneous measurement of membrane resistance, and a knowledge of the electrogenicity of ion transport processes which may be affected.

These results, and particularly the alkalinization following removal of the anion, indicate that NO_3, Br, and to a small extent I, are transported by the Cl–HCO_3 exchange carrier. This is confirmed by the observation (not illustrated) that the effects of Br and NO_3 on pH_i are completely inhibited by the anion exchange inhibitor DIDS (130 μM). This concentration was previously shown to abolish totally the effects of alteration of extracellular Cl on pH_i (Aickin & Brading, 1984).

In one experiment, application of 85%-SCN solution caused an apparent alkalinization and hyperpolarization of 10 mV which then gradually declined. Return to the

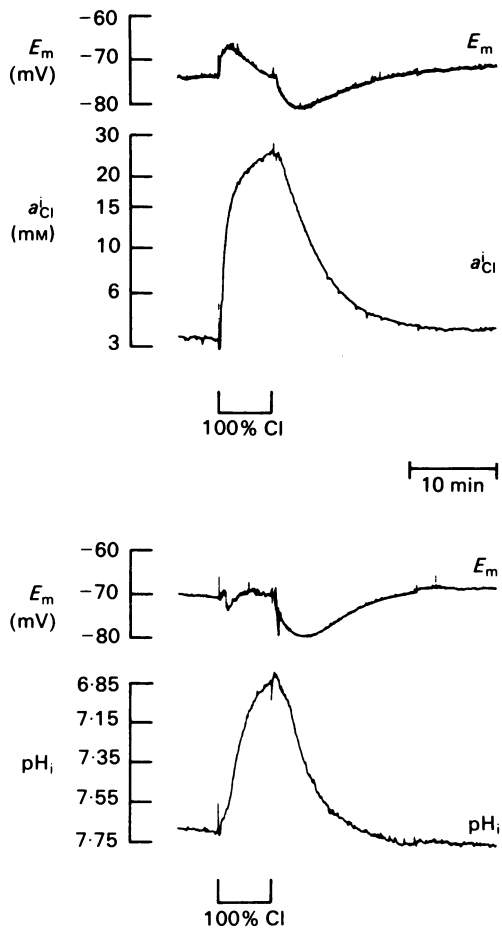

Fig. 7. The effect of a 6 min application of 100 %-Cl to Cl-depleted cells on the intracellular Cl activity (a^i_{Cl}), shown at the top, and the intracellular pH (pH_i), shown at the bottom. The preparations were maintained in Cl-free solution except for the periods indicated. All solutions were buffered with 3 % CO_2, 14 mM-HCO_3 at a pH of 7·36. Each pair of traces show E_m at the top and the ion activity at the bottom, recorded with a double-barrelled micro-electrode.

normal glucuronate/gluconate substituted solution caused a gradual return to the previous pH_i, complete in about 15 min, and a depolarization of about 25 mV. E_m then recovered in about 25 min. These effects were observed twice in the same cell but at the end of the experiment it was found that the 85 %-SCN solution caused a 70 mV shift in the alkaline direction on the potential recorded by the pH barrel. Return to normal Krebs solution slowly reduced this effect. Measurement of the pH of the solutions with a conventional glass electrode confirmed that they were the same. Thus it is concluded that SCN^- ions interfere with the H-selective resin. The apparent intracellular alkalinization seen on application of the SCN solution and acidification on its removal indicate that SCN^- ions both enter and leave the cell.

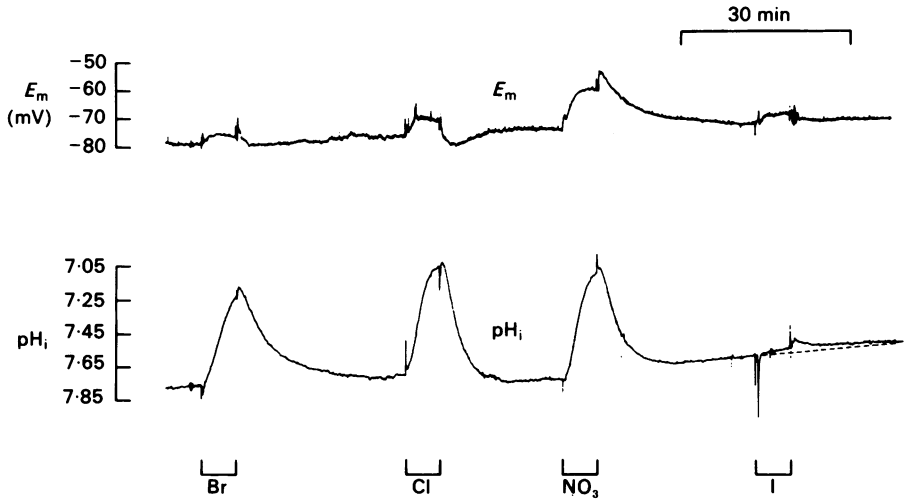

Fig. 8. The effect of 6 min applications of 85 %-Br, 85 %-Cl, 85 %-NO_3 and 85 %-I on E_m and pH_i. The preparation was superfused with Cl-free solution except for the intervals indicated. All solutions were buffered with 3 % CO_2, 14 mM-HCO_3 at pH 7·32. Unfortunately this experiment was marred by drift in the pH barrel and this has been indicated by the dashed line. The effect of I was the greatest seen in four preparations.

DISCUSSION

The observation that NO_3, Br, and to a small extent I reduce pH_i in Cl-depleted cells and, perhaps even more convincingly, increase pH_i against the electrochemical gradient on their removal, leaves no doubt that foreign anions are transported by the Cl–HCO_3 exchange carrier of the guinea-pig vas deferens. This is borne out by the DIDS sensitivity of the pH_i changes. Comparison of the rates of pH_i change indicates that NO_3^- ions are transported as readily as Cl, Br^- ions not as well, and I^- ions only poorly. Unfortunately little other information about the selectivity of the anion exchange carrier can be obtained using this unequivocal technique. Although flux experiments do not suffer from this limitation and offer an alternative and much easier approach, the conclusions that can be drawn from them are not unequivocal due to problems inherent in their interpretation. Nevertheless, we have previously shown that ^{36}Cl flux does reflect transmembrane movements mediated by the anion exchanger (Aickin & Brading, 1983*a*, 1984). Certainly the flux results complement those from measurement of pH_i, for example in the rapid activation of ^{36}Cl efflux elicited by HCO_3, NO_3 and Br, and to a lesser extent by I. Additionally, these experiments show that Cl itself causes a stimulation of ^{36}Cl efflux similar to that effected by NO_3 and Br (information unobtainable with the ion-selective electrodes) and that SCN causes some stimulation (uncertain from the pH_i experiments due to the interference of SCN with the H-selective ligand).

If the effects of exchanging 14 mM-Cl with HCO_3, NO_3 or Br on ^{36}Cl efflux are taken at their face value, they suggest that the order of potency for activation of the carrier at the external site is: $NO_3 > Cl > Br > HCO_3$. It is notable that the first three ions are in the same order as found from measurement of pH_i changes. However, the

position of HCO_3 may be erroneously low since the rapid passage of CO_2 across the cell membrane and production of intracellular HCO_3 may result in displacement of Cl from the internal site. This site has previously been suggested to have a higher affinity for HCO_3 than Cl (Aickin & Brading, 1938*a*). The fact that there was no significant difference between the stimulation of ^{36}Cl efflux by 14 or 21 mM-HCO_3 may be taken to suggest that saturation had occurred at these concentrations and thus that HCO_3 had a high affinity. Nevertheless, stimulation by Cl was to a higher level, probably indicating that significant competition of HCO_3 and Cl occurs at the internal site. Although stimulation of Cl efflux, even by Cl itself, shows great and unexplained variability from tissue to tissue, the results consistently show that Cl, NO_3 and Br cause pronounced stimulation while SCN and I have only a relatively weak effect. This is confirmed by the uptake/efflux experiment where Cl, NO_3 and Br equally supported considerable Cl loss while SCN only caused a modest loss and I was insignificantly different from the assumed non-permeant and non-transported glucuronate. Thus, combination of the results from all the efflux experiments suggests an affinity sequence of: $NO_3 > Cl > Br > HCO_3 > SCN > I$.

Bearing in mind the above evidence, results from the 15 min ^{36}Cl uptake by Cl-depleted tissues can be justifiably interpreted as demonstrating that NO_3, Br, SCN and I compete with Cl at the external site and reduce the amount of Cl taken up by the exchange carrier, in the order given. The decreased percentage of Cl found in the tissues after 3 h incubation in 50 %-Cl, 50 %-anion solutions is consistent with these ions being accumulated within the cell. If it can be assumed that the deficit in Cl is made up by accumulation of the foreign anion via the carrier, the percentage Cl present would at first sight suggest an affinity sequence of: $Br \geqslant NO_3 > I > Cl > SCN$. However, this interpretation assumes the same affinities at internal and external sites and we have previously suggested that this is not the case, at least for Cl and HCO_3 (Aickin & Brading, 1983*a*). Because of the problems in interpretation resulting from backflux and alteration of the level of intracellular substrate, we believe that the best estimate the flux technique can provide for the affinity sequence of the external site is from the 3 min uptake into Cl-containing tissues. This yields an apparent affinity sequence of: $NO_3 > Cl = Br = SCN > I > F$. Again, the first three ions are in the same order as derived from the effects on pH_i and ^{36}Cl efflux, although the results suggest a higher affinity for Br. The affinity of SCN appears surprisingly high, considering its limited ability to activate Cl efflux. Similarly, I would appear to have a rather higher affinity than suggested from its very slow effect on pH_i and poor activation of Cl efflux. This could reflect a reduced translocation rate when SCN or I are bound to the carrier.

In interpreting the results, we have assumed that the passive permeability of the cell membrane to the various anions is insignificant. We believe that P_{Cl} is extremely low (Aickin & Brading, 1983*a*). If the permeability is dependent upon the hydrated ionic radius, the order for the anions used would be $Br > I > Cl > NO_3 > SCN > F > HCO_3$ (Ito, Kostyuk & Oshima, 1962), quite different from that found in any of the experimental procedures. This suggests that the passive permeability to Br and I is low, like Cl, while that of the other anions is even lower. Certainly the very small, instantaneous changes in E_m on application of the various anions supports this assumption (see Aickin & Brading, 1983*a*). The fact

that transmembrane Cl and HCO_3 movements activated by NO_3, Br and I can be shown to occur on the exchange carrier suggests that these anions do not increase P_{Cl}, and we have assumed that this is also true of SCN. However, this may not be true for F. The effects of F were investigated with many of the experimental procedures, for completeness sake, and the results suggest that it has a very low affinity for the external site, as judged from the 3 min uptake experiment, yet causes some inhibition of Cl uptake and supports considerable Cl loss. This could be explained by an increase in P_{Cl}. Such an effect would not be surprising since the presence of F dramatically reduces divalent cation activities and inhibits metabolism, both of which have been shown to prevent smooth muscles from maintaining normal membrane properties (Tomita, 1981; Ashoori & Tomita, 1983; Tomita, Takai & Ashoori, 1985).

Although smooth muscle does not lend itself to the elegant kinetic studies possible with erythrocytes, at least with the technique of radioisotope fluxes, the present results do not suggest a mechanism which is fundamentally different from the band III protein. The most likely apparent affinity sequence of: $NO_3 > Cl = Br = SCN > I > F$ (equivalent to Eisenman's series IV, see Diamond & Wright, 1969) is similar to that for band III found by Lambert & Lowe (1978: $HCO_3 > Cl > Br > F$, although they were uncertain of the position of I) and Wieth (1979: $HCO_3 = Cl > NO_3 > F > Br \gg I$). Other work on erythrocytes has suggested that there are modulatory anion binding sites (Dalmark, 1976) and asymmetries in the system with respect either to the internal and external affinities or to the translocation rates in the two directions (e.g. Gunn & Frölich, 1979; Knauf, Law, Tarshis & Furuya, 1984; Knauf & Mann, 1984). Our results may also suggest, although not compellingly, that the mechanism is asymmetric. It is interesting, however, that in the heart, another tissue in which anion exchange has been carefully studied (Vaughan-Jones, 1979*a*, *b*, 1982), the results, at present, are interpretable on a simple model that does not incorporate either asymmetry or modulatory sites (R. D. Vaughan-Jones, personal communication).

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