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AN INVESTIGATION OF CHLORIDE–BICARBONATE EXCHANGE IN THE SHEEP CARDIAC PURKINJE FIBRE

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SUMMARY

1. Intracellular Cl activity (a^i_{Cl}), and intracellular pH (pH_i) were measured in isolated sheep cardiac Purkinje fibres using a liquid ion exchanger Cl-selective micro-electrode and a glass recessed-tip, pH-selective micro-electrode.

2. Removal of external Cl (glucuronate substituted) produced a fall in a^i_{Cl} from about 20 to about 4 mmol/l: the residual level is probably caused by intracellular interference on the Cl-sensitive electrode. Re-exposure of the fibre to increased levels of external Cl produced, in the steady state, increased levels of a^i_{Cl}. The dependence of steady-state a^i_{Cl} upon external Cl activity, a^o_{Cl}, was roughly hyperbolic with 50% recovery occurring at an a^o_{Cl} of about 9·5 mmol/l. At all levels of external Cl tested, Cl was accumulated to a level much higher than that predicted for passive electrochemical equilibrium.

3. Exposure of a Cl-depleted fibre to various levels of external Cl produced an exponential rise with time in a^i_{Cl}. The initial rate-of-rise in a^i_{Cl} was estimated to be a saturating function of a^o_{Cl}, with a half-maximal effect occurring at an a^o_{Cl} of about 33 mmol/l. The rate-of-rise was about 10-fold greater than that predicted from constant-field theory using published values for P_{Cl}, the Cl permeability coefficient.

4. Steady-state a^i_{Cl} was essentially insensitive to changes in external HCO_3 concentration, $[HCO_3]_o$, if these changes were made at a constant external pH, pH_o, i.e. when a reduction in $[HCO_3]_o$ was accompanied by a simultaneous reduction in the partial pressure of CO_2, P_{CO_2}.

5. In contrast, if P_{CO_2} was maintained constant, then a change in $[HCO_3]_o$ (thus producing a change in pH_o) resulted in an inverse change in a^i_{Cl}. This change in a^i_{Cl} was also accompanied by a change in pH_i: when a^i_{Cl} increased, pH_i decreased and vice versa.

6. The anion-exchange inhibitor, DIDS (4,4-diisothiocyanato-stilbene disulphonic acid) abolished the effect on a^i_{Cl} of changes in $[HCO_3]_o$ and pH_o (at constant P_{CO_2}). Furthermore DIDS reduced the influence of pH_o upon pH_i.

7. Both the fall of a^i_{Cl} in Cl-free solution and the subsequent reuptake of Cl following re-exposure to Cl-containing solution were slowed by a reduction in $[HCO_3]_o$ (constant pH_o, reduced P_{CO_2}). Both reuptake and wash-out of Cl were saturating functions of $[HCO_3]_o$ with half-maximal effect occurring at an $[HCO_3]_o$ of 1–1·3 mmol/l.

8. The reuptake of Cl was little affected by removal of external Na (bis,2-hydroxy ethyl, dimethyl ammonium substituted).

9. The reuptake of Cl was unaffected by amiloride (1 mmol/l) but slowed by piretanide (1 mmol/l).

10. The results are discussed in terms of a saturable and reversible Cl–HCO_3 exchange carrier in the sarcolemma with competition between Cl and HCO_3 ions for binding and transport. It is proposed that such a mechanism can account quantitatively for the high a^i_{Cl} measured in the steady state.

INTRODUCTION

The distribution of Cl ions across the sarcolemma of cardiac muscle does not conform to a simple passive distribution of Cl in accordance with the membrane potential (Ladle & Walker, 1975; Vaughan-Jones, 1979*a*; Spitzer & Walker, 1980). The intracellular Cl activity, a^i_{Cl}, is 20–30 mmol/l which, in quiescent tissue, is about five times higher than expected for electrochemical equilibrium. In a previous paper (Vaughan-Jones, 1979*b*) evidence was presented that the high level of intracellular Cl in the sheep-heart Purkinje fibre is achieved via a carrier-mediated uptake of Cl in exchange for the efflux of HCO_3. This anion exchange appears to be reversible so that, in Cl-free media, Cl is lost from the cell in exchange for the uptake of HCO_3. Therefore many Cl movements across the sarcolemma occur, not through membrane channels, but via the anion exchange mechanism.

There has hitherto been no quantitative study in heart of the dependence of the anion exchange system upon Cl and HCO_3. Furthermore, while Cl–HCO_3 exchange appears to operate in other cells, in certain cases it also requires and transports Na ions (Thomas, 1984). In addition, in another excitable tissue, the squid axon, a separate Cl uptake system involving co-transport with Na and K appears to operate (Russell, 1983). The possibility that Na is required for Cl uptake in the Purkinje fibre has not so far been tested.

In the present work I have therefore examined the ability of the sheep cardiac Purkinje fibre to accumulate Cl under conditions where the external and internal levels of Cl and HCO_3 are varied. In addition, I have investigated whether or not the Cl–HCO_3 exchange system requires Na. The experiments are based upon micro-electrode measurements of the intracellular activity of Cl, a^i_{Cl}. The results demonstrate that Cl uptake can proceed in the absence of Na. The activation of Cl transport by Cl and HCO_3 ions is consistent with a saturable carrier which mediates a reversible Cl–HCO_3 exchange across the membrane. The results are discussed in terms of a simple model for Cl–HCO_3 exchange. Some of this work has been presented previously in preliminary form (Vaughan-Jones, 1982*a*, *b*).

METHODS

The method of measurement of a^i_{Cl} and intracellular pH, pH_i, in a Purkinje fibre is identical to that described previously (Vaughan-Jones, 1979*a*, *b*). Briefly, free-running Purkinje fibres were dissected from sheep hearts obtained from a local slaughter house. The fibres were pinned in the experimental bath and perfused with Tyrode solution at 37 °C. Membrane potential was measured with a convential micro-electrode filled with 0·5 M-K_2SO_4 and KCl, 10 mmol/l (resistance, 30 MΩ). This records virtually the same membrane potential as an electrode filled with 3 M-KCl (Vaughan-Jones, 1979*a*), while minimizing Cl leakage into the fibre. a^i_{Cl} was measured with a liquid ion

exchanger, Cl-selective micro-electrode (Walker, 1971; Corning sensor: 477315). pH_i was measured with a glass, recessed-tip pH-sensitive micro-electrode (see Thomas, 1978). Such an electrode was acceptable if it responded to a change in pH with a half-time of 15–20 s. For further details of ion-selective electrode fabrication and calibration, see Vaughan-Jones (1979*a*, *b*) and Thomas (1978).

Measurement of a^i_{Cl}

The present measurements of a^i_{Cl} have not been corrected for possible intracellular interference on the Cl electrode from other anions. The interference from intracellular HCO_3 is known to be low, contributing about 0·5–1·0 mmol/l to the measurement of a^i_{Cl} (Vaughan-Jones, 1979*a*). Other unidentified intracellular interference is unlikely to be more than about 3 mmol/l since a^i_{Cl} falls to this level in Cl-free, HCO_3-free media. Such a level of interference if constant would alter very little the conclusions drawn in the present work. For example, although the dependence of a^i_{Cl} upon external Cl activity, a^o_{Cl}, illustrated in Fig. 1*B* or upon external HCO_3 concentration, $[HCO_3]_o$, in Fig. 4, would be shifted down the ordinate by 3–4 mmol/l, the over-all shape of the relationships would remain unaltered. In addition, estimates of Cl reuptake rate would be unaffected (e.g. Figs. 3, 7 and 8) since these are estimated from eqn. (2) (p. 384 in the text) and do not need to make the assumption of zero intracellular interference. However, it is recognized that the present measurements of a^i_{Cl} may be over-estimated by a few mmol/l.

Estimation of intracellular HCO_3 *activity,* $a^i_{HCO_3}$

This has been estimated from measurements of pH_i, assuming an intracellular HCO_3 activity coefficient, γ_{HCO_3}, of 0·76. This is the same as the Cl activity coefficient estimated for Tyrode solution. The same value is also assumed for extracellular HCO_3 (and, in other calculations, for intracellular Cl). Further assumptions are that P_{CO_2} (the partial pressure of CO_2) and the CO_2 solubility coefficient are the same on either side of the Purkinje fibre membrane. Under these conditions, the H ion equilibrium potential (E_H) and the HCO_3 ion equilibrium potential (E_{HCO_3}) will be equal. Therefore

$$[H^+]_i/[H^+]_o = [HCO_3^-]_o/[HCO_3^-]_i,$$

where $[H^+]_i$ and $[H^+]_o$ are the intracellular and external H ion concentrations respectively and $[HCO_3^-]_i$ and $[HCO_3^-]_o$ are the intracellular and external HCO_3 ion concentrations respectively. Hence

$$pH_o - pH_i = \log([HCO_3^-]_o/[HCO_3^-]_i).$$

Rearranging and converting to activity gives

$$a^i_{HCO_3} = \gamma_{HCO_3}[HCO_3^-]_o/10^{(pH_o - pH_i)}. \quad (1)$$

Hence, knowing the external pH, pH_o, and $[HCO_3^-]_o$, $a^i_{HCO_3}$ can be estimated from experimental measurements of pH_i.

Estimation of transmembrane Cl fluxes

These were estimated from the rate of change of a^i_{Cl}, da^i_{Cl}/dt, measured during removal and re-exposure to extracellular Cl:

$$M_{Cl} = S\, da^i_{Cl}/dt,$$

where M_{Cl} is the net Cl flux per unit area and S the volume:surface-area ratio of the sheep Purkinje fibre (Mobley & Page, 1972). It should be noted that this method for estimating M_{Cl} takes no account of possible changes in S caused by, for example, changes in fibre volume. However, in the present work, a volume change caused by Cl–HCO_3 exchange is likely to be small since equimolar amounts of Cl and HCO_3 ions are counter-exchanged. Nevertheless, since pH_i is strongly buffered, the net change in a^i_{Cl} and $a^i_{HCO_3}$ caused by the exchanger will be different (see Appendix) so that a change in volume may still occur. Indeed, Page & Solomon (1960) report a small volume decrease ($< 10\%$) in cat ventricular muscle exposed to Cl-free solution. It is possible, therefore, that the present estimates of Cl flux attributed to anion exchange are somewhat underestimated.

Solutions

Modified Tyrode solution consisted of NaCl, 118 mmol/l; KCl, 4·5 mmol/l; $CaCl_2$, 2·5 mmol/l; $MgCl_2$, 1·0 mmol/l; glucose, 11·0 mmol/l; equilibrated with nominally 5 % CO_2–95 % O_2 (British Oxygen), pH adjusted to 7·4 at 37 °C with $NaHCO_3$ (20–24 mmol/l). Cl-free solution was made by equimolar substitution of Na glucuronate for NaCl, K glucuronate for KCl, $MgSO_4$ for $MgCl_2$ and by replacement of $CaCl_2$ with Ca gluconate, 12 mmol/l. The level of 12 mmol/l was chosen in order to compensate for the Ca ion binding properties of glucuronate and gluconate (Kenyon & Gibbons, 1977; Vaughan-Jones, 1979*a*). The extent of this Ca binding can be calculated theoretically from the known metal-binding constants of these anions (Sillen & Martell, 1964). It has also been estimated empirically from measurements of Ca activity using Ca-selective electrodes (Kenyon & Gibbons, 1977) although this latter method may over-estimate such Ca binding because of an apparent anion sensitivity of Ca-selective electrodes (Dani, Jorge & Hille, 1983; E. E. Carmeliet, personal communication). Nevertheless, for gluconate and glucuronate anions, the theoretical approach also predicts significant Ca binding (Vaughan-Jones, 1979*a*).

In experiments where external HCO_3 was varied between 0 and 24 mmol/l at constant pH (7·4), $NaHCO_3$ was substituted with equimolar amounts of HEPES (Sigma chemicals) to produce HCO_3 concentrations of 10, 2, 0·7 and nominally 0 mmol/l. These solutions were saturated with 3, 0·6, 0·13 and 0 % CO_2 (and O_2) at 37 °C, and the pH adjusted to 7·4 with 4 N-NaOH. This produces solutions with final concentrations of HCO_3 very close to those added initially. The same procedure was used to produce Cl-free solutions with varying HCO_3 concentrations but constant pH.

In experiments where the HCO_3 concentration was varied (2, 22 and 62 mmol/l) at a constant P_{CO_2} (5 % CO_2–95 % O_2), the NaCl in all solutions was reduced by 30 mmol/l. The three solutions then contained (mmol/l): $NaHCO_3$, 2 and Na glucuronate, 50; $NaHCO_3$, 22 and Na glucuronate, 30; $NaHCO_3$, 62. This ensured a relatively constant osmolarity. Ca gluconate, 5 mmol/l, was also added (in addition to $CaCl_2$). The reduction of Cl in these solutions (a^o_{Cl} reduced by 20 mmol/l) has only a slight effect *per se* on a^i_{Cl} (see Fig. 1*B*).

Na-free solution was made by equimolar substitution of NaCl with bis, 2-hydroxy ethyl, dimethyl ammonium chloride (BDAC) and equimolar replacement of $NaHCO_3$ with Tris HCO_3, adjusting the pH of the final solution, saturated with 5 % CO_2–95 % O_2, to 7·4 at 37 °C using 4 N-HCl.

Amiloride was obtained from Sigma, U.K. Piretanide was a gift to Dr J. C. Ellory from Hoechst Pharmaceuticals, Hounslow, Middlesex, U.K.

RESULTS

The dependence of Cl uptake upon external Cl

Fig. 1*A* shows an experiment designed to investigate the dependence of Cl uptake upon external Cl. The fibre was first depleted of its intracellular Cl by exposing it to a Cl-free solution (Cl is substituted by glucuronate). This produced a slow fall of a^i_{Cl} from 18 to 4·7 mmol/l (cf. Vaughan-Jones, 1979*a*). a^o_{Cl} was then raised from 0 to 11·5 mmol/l and this caused a rise of a^i_{Cl}. Further increments of external Cl resulted in additional increases of a^i_{Cl} although it is notable that the response of a^i_{Cl} to changes of a^o_{Cl} displays saturation at the higher levels of a^o_{Cl}. This is seen more clearly in Fig. 1*B* (circles) where the steady-state levels of a^i_{Cl} obtained in Fig. 1*A* have been plotted against a^o_{Cl}. The relationship between a^i_{Cl} and a^o_{Cl} is roughly hyperbolic. In four experiments, the level of external Cl required to produce a half-maximal recovery of a^i_{Cl} was 9·23 mmol/l ± 0·53 (S.E. of mean). Also shown in Fig. 1*B* (triangles) is the relationship that would be predicted if Cl distributed itself passively across the membrane in accordance with a^o_{Cl} and the membrane potential. It is clear that, for all the activities of external Cl tested, a^i_{Cl} is greatly in excess of that expected from passive electrochemical equilibrium.

The reuptake of Cl occurs with an exponential time course (Vaughan-Jones,

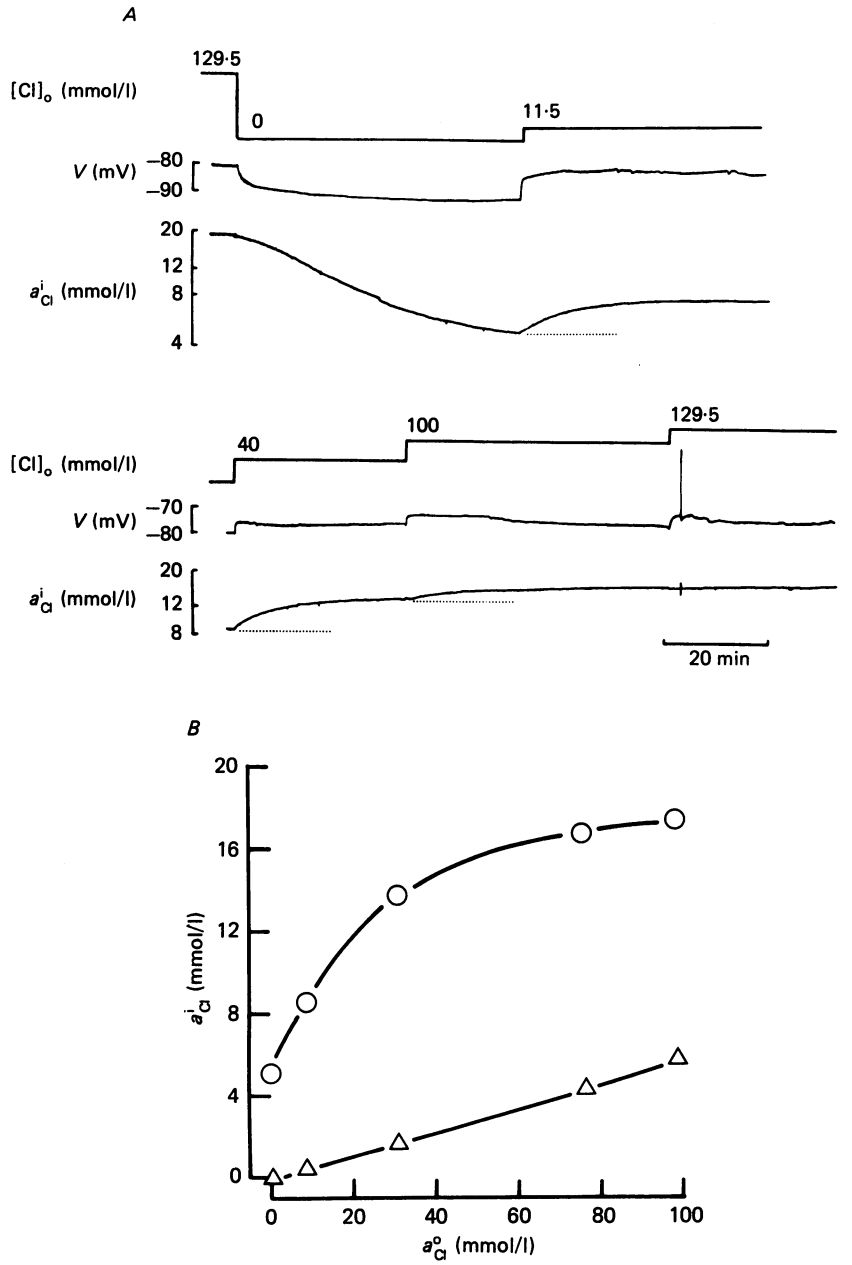

Fig. 1. The dependence of a^i_{Cl} upon a^o_{Cl}. A, time course of changes in a^i_{Cl} (lower trace) and membrane potential, V (upper trace), following removal and readmission of external Cl. Concentrations of external Cl, $[Cl]_o$, shown at top of Figure; glucuronate used as Cl substitute. The lower part of the Figure is continuous with the upper part. *B*, plot of dependence of steady-state a^i_{Cl} upon a^o_{Cl} (open circles); data from *A*. Open triangles show relationship predicted for electrochemical equilibrium using a^o_{Cl} and the measured value of V in each solution where: $a^i_{Cl} = a^o_{Cl}$ e (VF/RT).

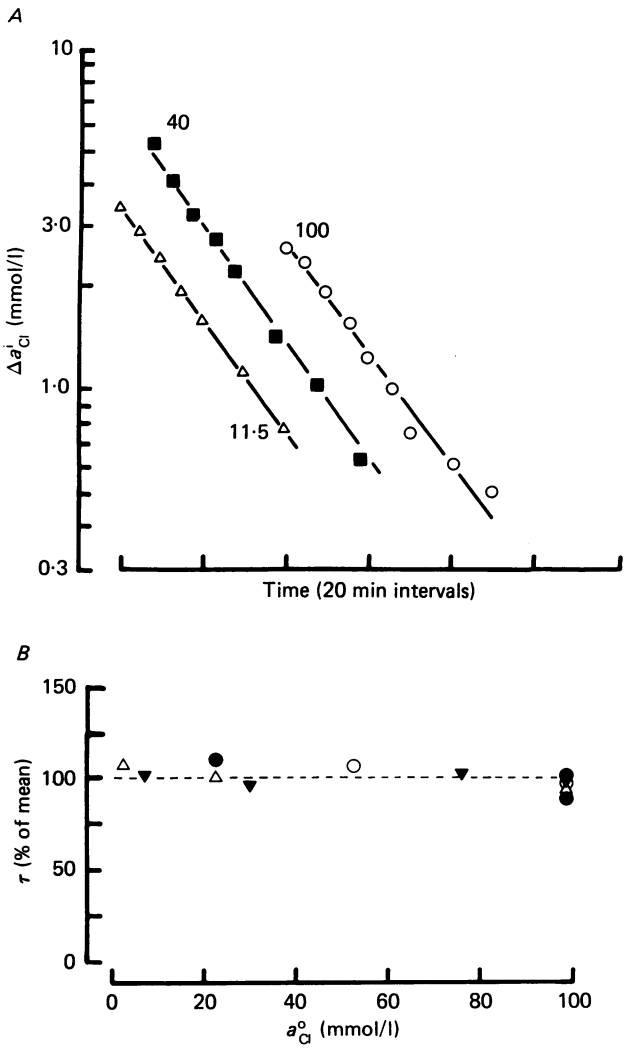

Fig. 2. Time course of rise in a^i_{Cl} following readmission of a^o_{Cl} to a Cl-depleted fibre. *A*, the rise in a^i_{Cl} (Δa^i_{Cl}) is plotted *versus* time (semi-logarithmic plot) following elevation of a^o_{Cl} from zero to, sequentially, 11·5 mmol/l (triangles), 40 mmol/l (squares) and 100 mmol/l (circles). Δa^i_{Cl} was estimated as: (final steady-state $a^i_{Cl} - a^i_{Cl}$). Lines fitted by linear regression. For ease of comparison, respective plots are displaced along the abscissa. Data from Fig. 1*A*. *B*, dependence upon a^o_{Cl} of the time course of rise in a^i_{Cl}. The time constant of rise in a^i_{Cl} (τ), determined from plots similar to that in *A* is plotted *versus* a^o_{Cl}. Different symbols denote experiments on different fibres. τ is expressed as a percentage of the mean determined for each experiment (denoted by dashed line).

1979*b*). In Fig. 2*A*, the recoveries of a^i_{Cl} observed in Fig. 1 have been replotted on semilogarithmic coordinates. It is clear that the time constant for each recovery is virtually identical. Fig. 2*B* shows data pooled from four experiments like that shown in Fig. 1. The time constant for the recovery of a^i_{Cl} has been plotted *versus* a^o_{Cl}. In order to compare different experiments the time constant has been expressed as a

percentage of the mean determined in that particular experiment. It is evident that the time constant of rise in a^i_{Cl} is essentially independent of a^o_{Cl}.

It is of interest to examine the dependence of Cl reuptake rate upon external Cl. One way of doing this is to compare the rates of recovery of a^i_{Cl} at different levels

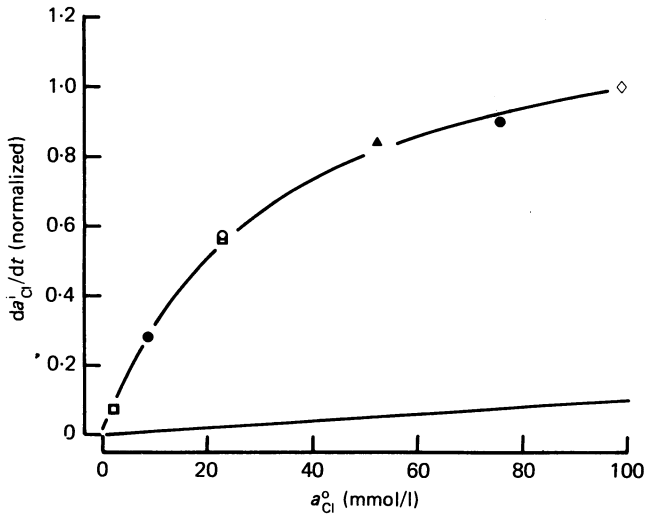

Fig. 3. Dependence of Cl uptake rate upon a^o_{Cl}. Fibres were initially depleted of Cl in a Cl-free solution (glucuronate substituted). External Cl was then elevated to different levels resulting in a rise in a^i_{Cl} (cf. Fig. 1*A*). The initial rate-of-rise in a^i_{Cl} (da^i_{Cl}/dt; mmol/l. min) was calculated for the case where a^i_{Cl} = 4 mmol/l using eqn. (2) in the text. Different symbols denote experiments on different fibres: for each experiment da^i_{Cl}/dt was normalized to the rate (diamond) determined in an a^o_{Cl} of 100 mmol/l. The curve through the points has been fitted using the equation:

$$da^i_{Cl}/dt = \frac{V_{max}}{1 + K^{Cl}_{a0\cdot5}/a^o_{Cl}},$$

where V_{max} = 1·33 and $K^{Cl}_{a0\cdot5}$ = 33·3 mmol/l (curve fitted by linear regression to a double reciprocal plot of data; correlation coefficient, 0·996). Mean da^i_{Cl}/dt in an a^o_{Cl} of 100 mmol/l was 1·5 mmol/l. min. Hence mean V_{max} = 2·0 mmol/l. min. Lower line shows relationship predicted from constant-field theory:

$$da^i_{Cl}/dt = \frac{P_{Cl}a^o_{Cl}\,VF/RT}{1 - e\,(-VF/RT)},$$

where V is the membrane potential (assumed to be −75 mV), and P_{Cl}, the Cl permeability coefficient (assumed to be 3×10^{-8} cm/s; see text for further details).

of a^o_{Cl}. However, the comparison must be made under conditions where the efflux of Cl from the cell is negligible. For this reason the initial rate-of-rise of a^i_{Cl} has been estimated following the readmission of external Cl to a Cl-depleted fibre: this should be a reasonable measure of the unidirectional Cl influx.

Fig. 3 shows data pooled from four such experiments. The initial rates-of-rise of a^i_{Cl} have been normalized and are plotted *versus* the external level of Cl. For each point the initial rate-of-rise of a^i_{Cl} has been estimated for the case when a^i_{Cl} is 4 mmol/l since this is close to the residual level of a^i_{Cl} measured in a Cl-free solution

(see Figs. 1, 7 and 10 and also Vaughan-Jones, 1979*a*). The rate-of-rise of a^{i}_{Cl} was calculated from the equation:

$$\frac{da^{i}_{Cl}}{dt} = K\,(a^{i}_{Cl_\infty} - 4), \tag{2}$$

where K is the rate constant measured for the recovery of a^{i}_{Cl} and $a^{i}_{Cl_\infty}$ is the activity (mmol/l) of intracellular Cl achieved in the steady state. This approach is justified since a^{i}_{Cl} recovers exponentially and, in any given experiment, the rate constant is independent of a^{o}_{Cl} (Fig. 2*B*). The procedure was nevertheless checked in an experiment where the time constant of recovery of a^{i}_{Cl} was measured when a^{o}_{Cl} was raised from 0 to 100 mmol/l and from 30 to 100 mmol/l. The two time constants were found to be in reasonable agreement (13·1 and 11·9 min respectively).

Fig. 3 suggests that the unidirectional influx of Cl is a saturating function of external Cl. It would appear that the Cl reuptake is half-maximal at an a^{o}_{Cl} of about 33 mmol/l. It should be noted that a passive influx of Cl through the membrane conductance cannot account for this result. The lower line in Fig. 3 shows the relationship expected if Cl were moving through membrane channels assuming a Cl permeability coefficient, P_{Cl} (constant-field theory), of 3×10^{-8} cm/s (cf. Fozzard & Lee, 1976; Carmeliet & Verdonck, 1977; Vaughan-Jones, 1979*a*, *b*) and a resting membrane potential of -75 mV. Hence the Cl influx estimated from constant-field theory is, at most, only 10% of the Cl influx actually observed. Furthermore, if Cl reuptake were occurring through membrane channels then, assuming the above value for P_{Cl}, constant-field theory predicts a transient hyperpolarization of up to 15 mV (see eqn. (7) and Hodgkin & Horowicz, 1959). In fact, there is a sustained depolarization of about 10 mV (see e.g. Figs. 1 and 9). This depolarization is related to the Cl substitute used (in this case, glucuronate: assumed to be impermeant through anion channels) rather than to changes in internal or external Cl (Vaughan-Jones, 1979*a*) and there is evidence that it is mediated via an inhibitory effect of certain anions upon the activation of the pace-maker current, i_f, resulting in a decrease of inward current (Van Bogaert & Carmeliet, 1985). There is no evidence, therefore, that Cl reuptake *per se* contributes significantly to the membrane conductance. All of these observations, coupled with the fact that Cl is accumulated to high, non-passive levels within the cell, suggests that Fig. 3 describes the dependence on external Cl of an inwardly directed, electrically silent, Cl transport mechanism.

The dependence of Cl transport upon HCO_3

The steady-state relationship between a^{i}_{Cl} and external HCO_3. In a previous paper evidence was presented that, in the sheep Purkinje fibre, HCO_3 ions are required as counter ions for Cl transport (Vaughan-Jones, 1979*b*). This HCO_3 dependence is now explored quantitatively. Fig. 4 shows results pooled from experiments on twenty-two fibres where Cl was re-added to a Cl-depleted fibre that had been equilibrated in solutions of constant pH (7·4) but at different levels of HCO_3/P_{CO_2} (one such experiment is shown in Fig. 7). In Fig. 4 the level of a^{i}_{Cl} achieved in the steady state has been plotted *versus* $[HCO_3]_o$. It is clear that, over the range investigated, a^{i}_{Cl} is little affected by variations of HCO_3. This is in agreement with the observation made previously that, when using Cl-containing solutions, adding and then removing

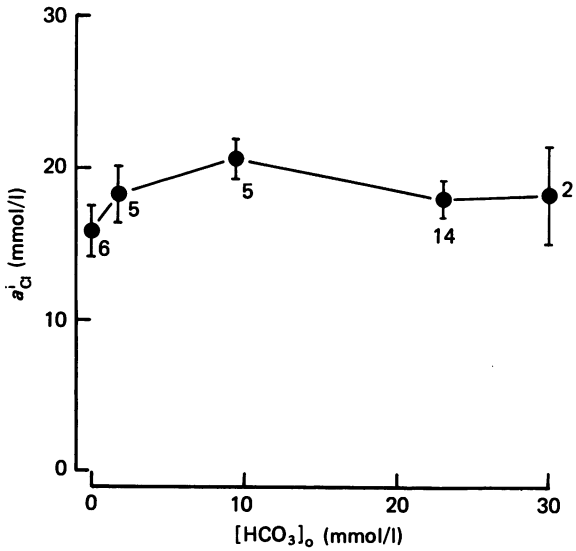

Fig. 4. Dependence of a^i_{Cl} (steady state) upon $[HCO_3]_o$, when pH_o is constant (7·4). Therefore a rise in $[HCO_3]_o$ is also associated with a rise in P_{CO_2} (for $[HCO_3]_o$ of 0·7, 2, 10, 24 and 30 mmol/l, P_{CO_2} was respectively 0·13, 0·6, 3, 5 and 7 % (see Methods for further details)). Data pooled from twenty-two fibres. Bars denote S.E. of mean for the number of observations indicated.

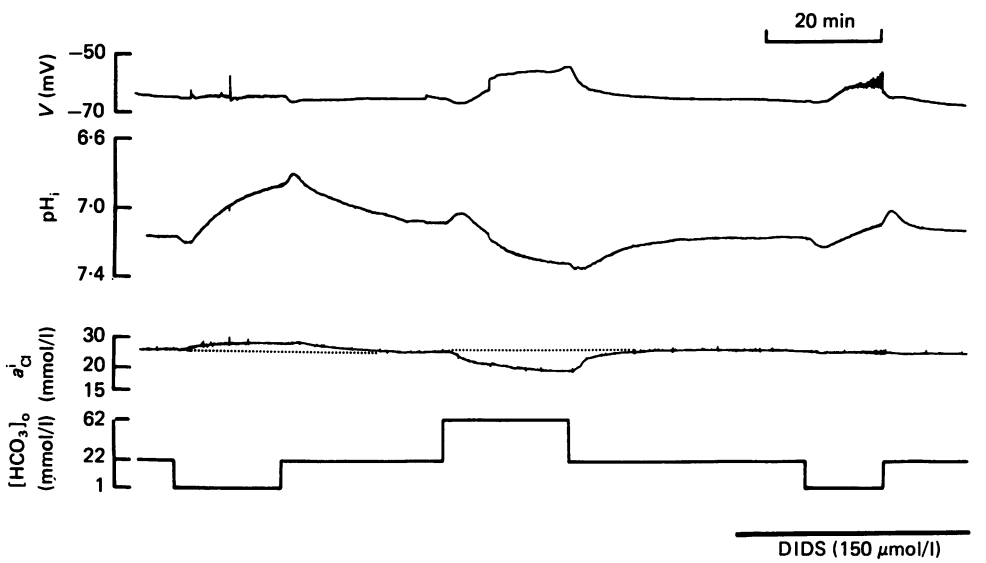

Fig. 5. Effect on a^i_{Cl} (lower trace), pH_i (middle trace) and membrane potential (upper trace) of changing $[HCO_3]_o$ at a constant P_{CO_2} (5 % CO_2–95 % O_2); changes as indicated in lower part of Figure. The drug DIDS was superfused for the period denoted by bar at bottom of Figure. For an $[HCO_3]_o$ of 1 mmol/l, pH_o was 6·4; for an $[HCO_3]_o$ of 62 mmol/l, pH_o was 7·8; for an $[HCO_3]_o$ of 22 mmol/l, pH_o was 7·4.

external HCO_3/CO_2 at a constant pH_o has virtually no effect on a^i_{Cl} (see Vaughan-Jones, 1979*b*, Fig. 5).

In contrast, if external HCO_3 is varied but at a constant P_{CO_2} (hence pH_o also varies), then clear changes of a^i_{Cl} can be observed. This is illustrated in Fig. 5. In this experiment a^i_{Cl} and pH_i were recorded simultaneously. Reducing external HCO_3

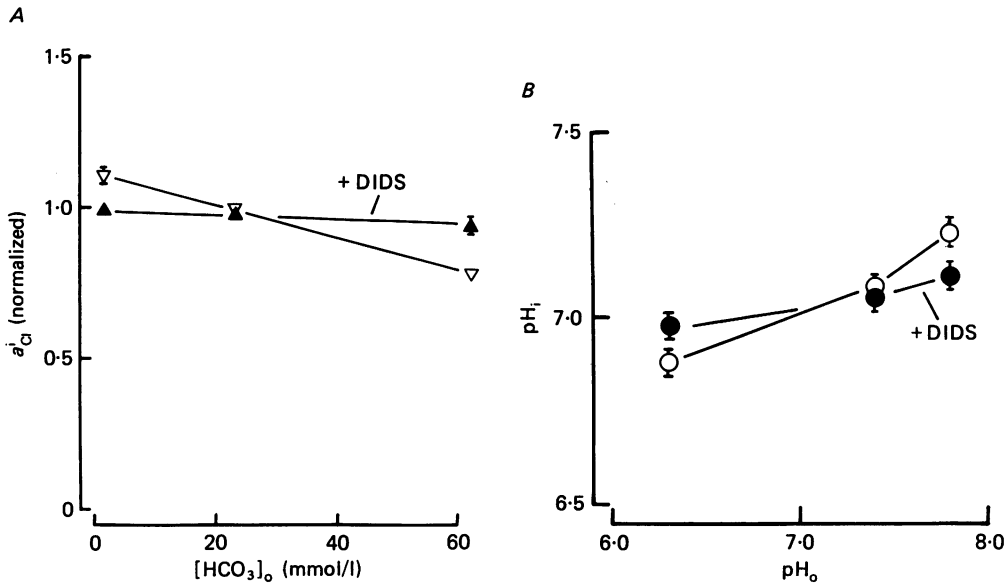

Fig. 6. Effect on a^i_{Cl} and pH_i of changing $[HCO_3]_o$ at constant P_{CO_2}. *A*, dependence of a^i_{Cl} upon $[HCO_3]_o$. The a^i_{Cl} was determined 10–15 min after changing $[HCO_3]_o$ in the absence (open triangles) and in the presence (closed triangles) of the drug DIDS. For each experiment a^i_{Cl} has been normalized to the value obtained in an $[HCO_3]_o$ of 22 mmol/l. *B*, dependence of pH_i upon pH_o at constant P_{CO_2} (5 % CO_2 − 95 % O_2) in the absence (open circles) and presence (filled circles) of DIDS. The three different values of pH_o (abscissa) correspond to the three values of $[HCO_3]_o$ shown in *A*; pH_i determined 10–15 min following a solution change. Bars in *A* and *B* denote S.E. of mean, $n = 7$.

from 22 to 1·0 mmol/l (thus also reducing pH_o from 7·4 to 6·4) produces a rise in a^i_{Cl} accompanied by a slow fall in pH_i. The transient intracellular alkalosis that precedes this fall in pH_i has been observed before (Ellis & Thomas, 1976) and has been attributed to a transient change in P_{CO_2} at the level of the membrane produced because H and HCO_3 ions do not exchange at the same rate in the extracellular spaces following a change in their concentration in the bulk solution (de Hemptinne & Marranes, 1981). Conversely, elevating external HCO_3 from 22 to 62 mmol/l (thus raising pH_o from 7·4 to 7·8) produces a fall in a^i_{Cl} accompanied by a rise in pH_i, this time preceded by a transient acidosis. Fig. 5 also shows that the rise in a^i_{Cl} in response to a reduction in external HCO_3 is inhibited by the drug DIDS (4,4-diisothiocyanato-stilbene disulphonic acid), an inhibitor of anion exchange in the red blood cell (e.g. Cabantchik, Knauf & Rothstein, 1978). In addition, the drug reduces the rate of intracellular acidification that occurs under these conditions. Fig. 6*A* shows results pooled from seven experiments similar to that shown in Fig. 5. The level

of a^i_{Cl} recorded 10–15 min after changing solution has been plotted *versus* the $[HCO_3]_o$. For each experiment a^i_{Cl} has been normalized relative to its level in 22 mmol HCO_3/l. Fig. 6*A* indicates that there is an inverse relationship between a^i_{Cl} and external HCO_3 and that this is no longer apparent following the addition of DIDS. In the absence of the drug, an elevation of external HCO_3 from 1 to 62 mmol/l decreases a^i_{Cl} by 30%.

Fig. 6*B* shows measurements of pH_i obtained in the same experiments as those shown in Fig. 6*A*. It is evident that DIDS also reduces but, notably, does not abolish completely the influence of pH_o upon pH_i. Fig. 6*A* and *B* therefore suggests that, in the Purkinje fibre, the influence of external HCO_3 upon a^i_{Cl} is mediated via Cl–HCO_3 exchange and that this process accounts for part of the observed dependence of pH_i upon pH_o. Hence raising external HCO_3 promotes a net HCO_3 influx in exchange for the efflux of Cl thus producing a rise in intracellular HCO_3 level and a fall in a^i_{Cl}. Assuming that CO_2 is at equilibrium across the sarcolemma, the rise in intracellular HCO_3 will result, as observed, in a rise in pH_i. Conversely a reduction in extracellular HCO_3 will produce a rise in a^i_{Cl} and a fall in pH_i.

It should be noted that the steady-state relationship between pH_i and pH_o in the presence of DIDS may differ from that shown in Fig. 6*B* since, in these particular experiments, pH_i had not always reached a steady state when measurements were made. This is because the changes in pH_i were usually slow following addition of DIDS.

Stoicheiometry of anion exchange. Experiments like that shown in Fig. 5 can be used to estimate the apparent stoicheiometry of Cl–HCO_3 exchange. The DIDS-sensitive changes in a^i_{Cl} and pH_i were measured over a 10 min period and converted respectively into changes in Cl concentration, $\Delta[Cl]_i$ (mequiv/l) and intracellular acid equivalents $\Delta[A]_i$ (mequiv/l), using the equations:

$$\Delta[Cl]_i = \Delta a^i_{Cl}/\gamma_{Cl}, \tag{3}$$

$$\Delta[A]_i = \Delta pH_i \beta + \Delta[HCO_3]_i, \tag{4}$$

where γ_{Cl} is the intracellular activity coefficient for Cl (assumed to be 0·76, see Methods) and β is the 'non-CO_2' intracellular buffering power (assumed to be 30 mmol/l, see Ellis & Thomas, 1976). The change in intracellular HCO_3 concentration, $\Delta[HCO_3]_i$, associated with the DIDS-sensitive change in pH_i, ΔpH_i, was calculated using eqn. (1) in Methods. The ratio $\Delta[A]_i/\Delta[Cl]_i$ was then determined. In ten determinations from seven fibres the mean ratio was $1{\cdot}19 \pm 0{\cdot}21$ (S.E. of mean). Hence the changes in pH_i and a^i_{Cl} are caused by roughly equal changes in intracellular Cl and acid equivalents. Assuming that the DIDS-sensitive changes in a^i_{Cl} and pH_i are caused by transmembrane fluxes of Cl and HCO_3 ions, the above ratio is consistent with a stoicheiometry of 1·19 HCO_3:1 Cl which is reasonably close to the value of 1:1 determined for anion exchange in the red blood cell (see Cabantchik *et al.* 1978). A previous estimate for anion exchange in the Purkinje fibre was 1·45 HCO_3:1 Cl (Vaughan-Jones, 1979*b*).

Effect of HCO_3 upon the rate of Cl uptake. Although varying external HCO_3 at a constant pH_o has little effect upon steady-state a^i_{Cl} (Fig. 4) nevertheless it affects the rate of Cl reuptake by a Cl-depleted fibre. This is illustrated in the experiment shown in Fig. 7*A* and replotted on semilogarithmic axes in Fig. 7*B*. Three reuptakes of Cl

are shown, each occurring at the same pH_0 but at a different $[HCO_3]_0$ and P_{CO_2} (see legend of Fig. 7 for details). Progressively reducing external HCO_3 results in a progressive decline in the rate constant for Cl reuptake.

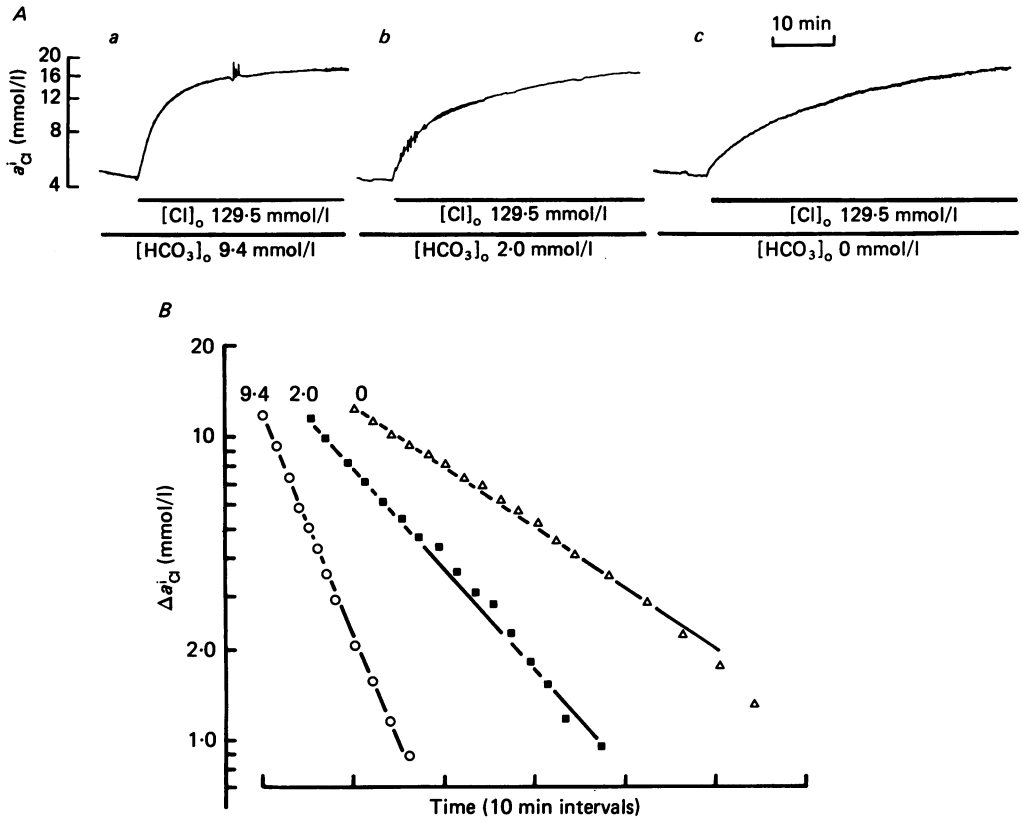

Fig. 7. HCO_3 sensitivity of Cl reuptake. *A*, the Cl-depleted fibre was exposed to Cl-containing solution equilibrated to pH_0 7·4 with HCO_3, 9·4 mmol/l and 3 % CO_2 (*a*), HCO_3, 2·0 mmol/l and 0·6 % CO_2 (*b*), HCO_3, nominally 0 mmol/l and 0 % CO_2 (*c*) (see Methods for further details). *B*, the rise of a^i_{Cl} upon exposure to Cl-containing solution (Δa^i_{Cl}) is plotted semilogarithmically *versus* time for solutions with an $[HCO_3]_0$ of 9·4 mmol/l (open circles), 2·0 mmol/l (filled squares) and nominally 0 mmol/l (open triangles). pH of all solutions, 7·4. Data from *A*. Lines fitted by linear regression (for $[HCO_3]_0$, 0 mmol/l, the line was fitted to all points for $\Delta a^i_{Cl} > 2{\cdot}0$ mmol/l).

The results of experiments like those in Fig. 7 are summarized in Fig. 8. The reciprocal of the half-time for Cl reuptake has been plotted against $[HCO_3]_0$. At low external HCO_3 (< 8 mmol/l) there is a steep dependence of Cl reuptake upon the level of HCO_3 whereas at higher external HCO_3 levels the Cl reuptake displays saturation. The reuptake is half-maximal at about 1·3 mmol HCO_3/l. The inset to Fig. 8 shows that a similar picture emerges when the initial rate of Cl reuptake is plotted *versus* external HCO_3. It is likely that the limit to Cl reuptake under these conditions is provided not by the extracellular but by the intracellular HCO_3 since this will be required for counter-transport with Cl. It is therefore important to

determine $[HCO_3]_i$ under the present conditions. Experimental estimates of intracellular HCO_3 have not been made in the present work. However, it has been shown (Vaughan-Jones, 1979*b*; Vanheel, de Hemptinne & Leusen, 1984) that pH_i is virtually identical to pH_o when Purkinje fibres are bathed in Cl-free, HCO_3-containing solutions. Hence, when external Cl is first readded to a Cl-depleted fibre, intracellular and extracellular HCO_3 levels will be very similar (assuming also that CO_2 is at equilibrium across the surface membrane). The results shown in Fig. 8 may therefore indicate that Cl reuptake is half-maximal when $[HCO_3]_i$ is about 1·3 mmol/l.

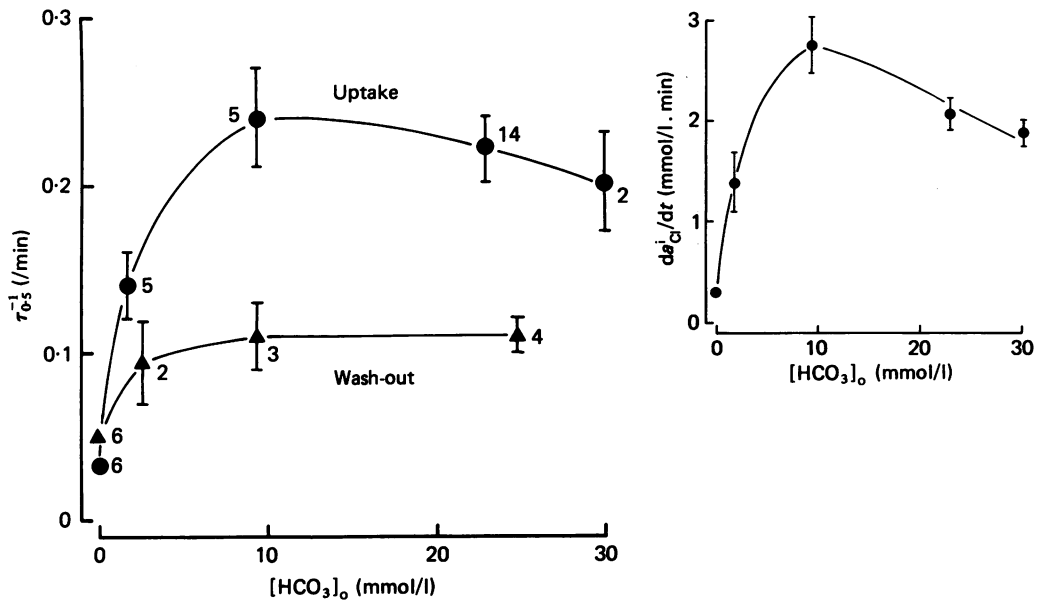

Fig. 8. HCO_3 sensitivity of Cl reuptake and of Cl wash-out. Main Figure, the reciprocal half-time ($\tau_{0.5}^{-1}$) of Cl reuptake following readmission of external Cl to a Cl-depleted fibre has been plotted (circles) *versus* $[HCO_3]_o$ (pH_o, 7·4). Data from experiments similar to Fig. 7. Also plotted (triangles) is the HCO_3 dependence of $\tau_{0.5}^{-1}$ for the fall in a_{Cl}^i measured in Cl-free solution. Data for Fig. 8 pooled from a total of twenty-two fibres. Bars denote S.E. of mean for the number of observations indicated. Curves drawn by eye. Inset, initial rate-of-rise of a_{Cl}^i following exposure to Cl-containing solution is plotted *versus* $[HCO_3]_o$ using data shown in main Figure. The rate-of-rise of a_{Cl}^i was calculated from $\tau_{0.5}^{-1}$ using eqn. (2) in the text. Bars denote S.E. of mean for the number of observations indicated. Curve drawn by eye.

Two other features in Fig. 8 are worthy of note. First, Cl is accumulated, albeit very slowly, in the nominal absence of HCO_3/CO_2 (cf. also Fig. 7 and Vaughan-Jones, 1979*b*) and secondly, the rate of Cl reuptake appears to decrease at high levels (> 10 mmol/l) of HCO_3. The inset to Fig. 8 shows that both phenomena are also apparent when the initial rates of recovery of a_{Cl}^i are examined. These two features will be considered in the Discussion.

Effect of HCO_3 upon the rate of Cl wash-out. In another set of experiments, the effect of changing external HCO_3 levels (at constant pH_o) was tested on the wash-out of Cl into Cl-free solution. The results are also plotted in Fig. 8. In the nominal absence

of HCO_3/CO_2 the half-time of fall of a^i_{Cl} ($\tau_{0.5}$) was approximately doubled, in agreement with previous observations (Vaughan-Jones, 1979*a*). However, variations of external HCO_3 in the range 2–25 mmol/l produced only a small effect on the rate of wash-out of Cl. Therefore a small amount of extracellular HCO_3 is sufficient to sustain a near-maximal efflux of Cl into Cl-free solution. This result is qualitatively similar to the high HCO_3 sensitivity of the Cl influx shown in the same Figure. However, it should be noted that removing HCO_3 only halves the Cl efflux into Cl-free solution whereas removing HCO_3 slows the Cl uptake mechanism about 6-fold. Hence the over-all HCO_3 sensitivity of the Cl uptake process appears to be greater than for the Cl efflux.

The values of $\tau_{0.5}$ measured in the present work are smaller than those reported previously (Vaughan-Jones, 1979*a*). This reflects mainly a difference in the method of measurement of $\tau_{0.5}$. In the present work $\tau_{0.5}$ has been measured as the half-time for the change of a^i_{Cl}, (Δa^i_{Cl}) that occurs when external Cl is removed whereas in previous work $\tau_{0.5}$ was measured as the time taken for a^i_{Cl} to decline to 50 % of its initial value (to be precise, a time constant rather than $\tau_{0.5}$ was quoted previously). Since a^i_{Cl} measured with a Cl-selective micro-electrode falls to about 4 mmol/l rather than to zero (Vaughan-Jones, 1979*a*), the two methods will give different values for $\tau_{0.5}$. It is not known whether the residual level of a^i_{Cl} is caused by a background level of foreign anion interference on the Cl-electrode signal or whether it is really Cl that for some reason cannot leave the cell. However, there is little doubt that the fall of internal Cl, Δa^i_{Cl}, is caused by a loss of Cl from the tissue. Therefore the present method of estimating $\tau_{0.5}$ is a more appropriate measurement.

Does Cl reuptake require Na?

Fig. 9*A* shows an experiment designed to investigate if the presence of Na is necessary for the operation of Cl–HCO_3 exchange. The fibre was first depleted of Cl. External Cl was then readmitted while, at the same time, removing extracellular Na (NaCl replaced by BDAC). The removal of external Na is known to produce a rapid fall of intracellular Na with a half-time of about 30–60 s which is accompanied by a transient hyperpolarization of the membrane potential (Ellis, 1977). Fig. 9*A* shows that Cl can still be re-accumulated to high levels in the absence of extracellular Na and also presumably in the virtual absence of intracellular Na, since the recovery of a^i_{Cl} proceeds for nearly 15 min in Na-free solution. Fig. 9 also shows that re-admitting extracellular Na, which leads to a rapid recovery of the intracellular Na activity (Ellis, 1977), also produces little change in the rate of recovery of a^i_{Cl}.

This can be seen more clearly in Fig. 9*B* which re-plots the recovery of a^i_{Cl} measured in the absence of Na (filled circles). As a comparison, a control recovery of a^i_{Cl} is also plotted (open triangles). This was obtained at the end of the experiment in the presence of Na (see Fig. 9*A*). It is clear that the reuptake of Cl is virtually identical in the two cases. Similar results were seen in three other experiments. Hence it would appear that Cl reuptake in the Purkinje fibre can proceed in the absence of extracellular or intracellular Na. In addition, if other Na-dependent Cl uptake systems exist in this tissue, then their contribution to the net Cl transport in the present experiments must be small.

In a final series of experiments, the diuretic drugs amiloride and piretanide

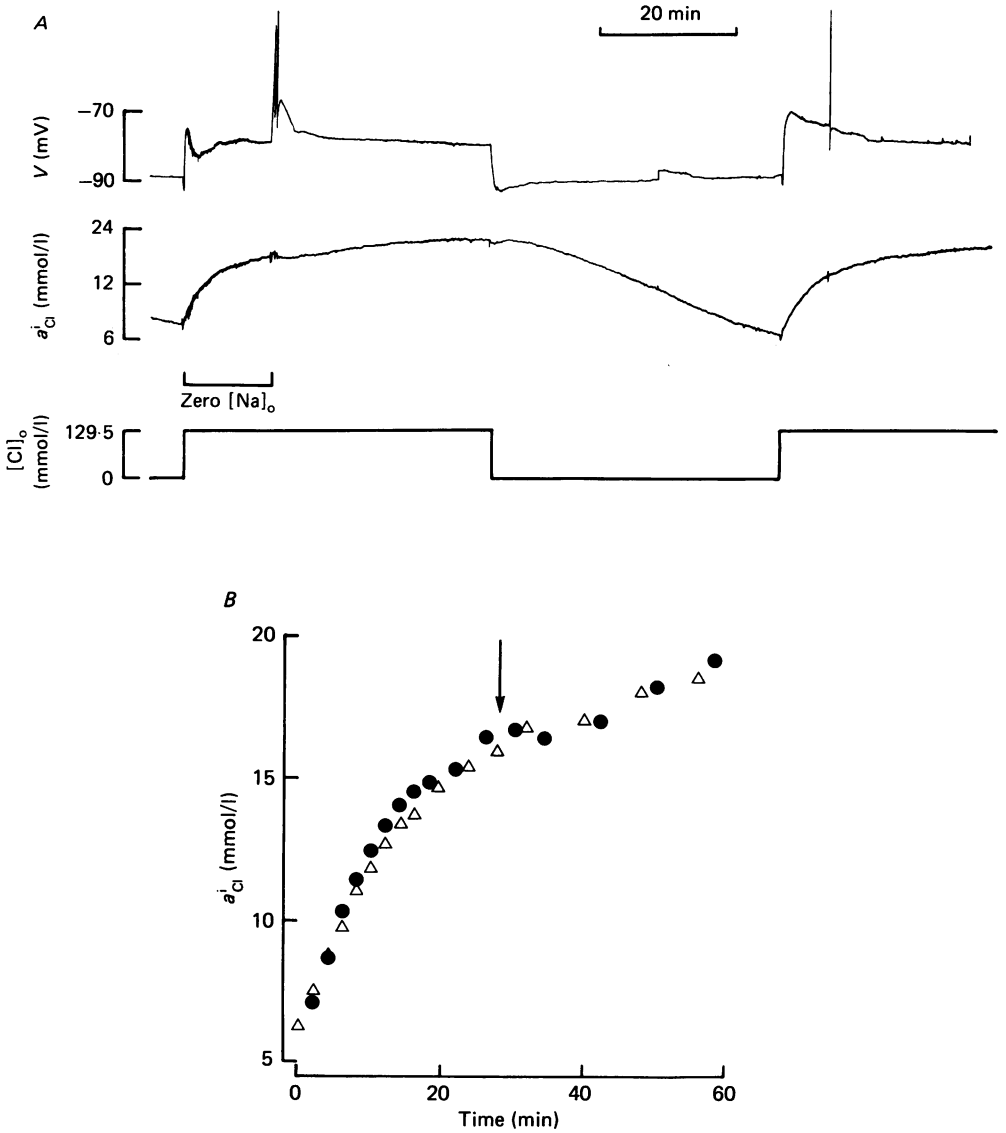

Fig. 9. Cl reuptake is not inhibited by removal of external Na. *A*, the Cl-depleted fibre was exposed to a Cl-containing, Na-free solution (NaCl replaced by bis, 2-hydroxyethyl, dimethyl ammonium Cl; $NaHCO_3$, 23 mmol/l, replaced by Tris HCO_3). The concentration of external Cl, $[Cl]_o$, is indicated at the bottom of the Figure and the period of exposure to Na-free solution is indicated by the bar. Subsequent removal and readdition of external Cl produces respectively a fall in a^i_{Cl} and a rise in a^i_{Cl} which is similar to that observed in Na-free solution. *B*, data from *A*. The reuptake of Cl in Na-free solution is replotted (filled circles). Arrow denotes moment when external Na concentration, $[Na]_o$, was raised from zero to 141 mmol/l. Also plotted (open triangles) is the reuptake observed in the presence of external Na.

were tested upon the reuptake of Cl. Amiloride is believed to inhibit Na–H exchange in a variety of tissues (see e.g. Thomas, 1984), including cardiac muscle (e.g. Ellis & MacLeod, 1985). In the experiment shown in Fig. 10*A*, amiloride (1 mmol/l) had no effect upon the reuptake of Cl. A similar result was obtained in two other fibres. This therefore suggests that the Cl reuptake mechanism in the Purkinje fibre is independent

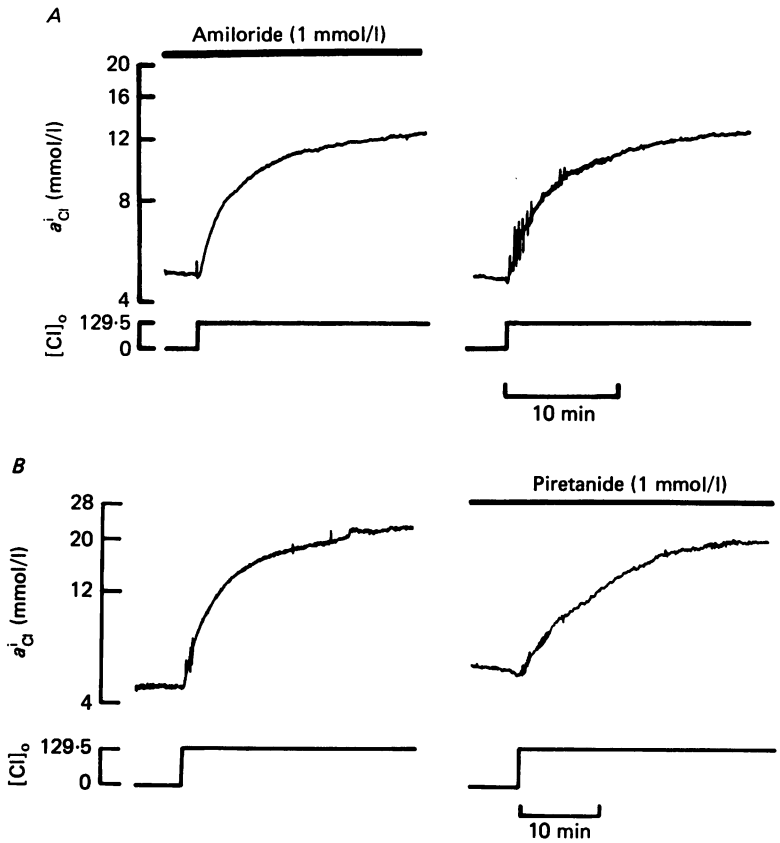

Fig. 10. Amiloride does not slow Cl reuptake but piretanide does. *A*, two Cl reuptakes are illustrated following elevation of external Cl concentration, $[Cl]_o$, from zero to 129·5 mmol/l, the first in the presence and the second in the absence of amiloride, 1 mmol/l. The second reuptake was performed 90 min after washing off amiloride. *B*, two Cl reuptakes are shown. The second, performed in the presence of piretanide, 1 mmol/l, was conducted 90 min after the first Cl reuptake.

of the Na–H exchange mechanism proposed for this tissue (cf. Vaughan-Jones, 1982*a*,*b*). In contrast, piretanide (1 mmol/l) slowed but did not inhibit entirely the reuptake of Cl (Fig. 10*B*). The initial rate of reuptake of Cl was slowed by about 50 %. Piretanide is an analogue of furosemide and has been proposed to be an inhibitor of Na–K–Cl co-transport in the red blood cell (e.g. Ellory & Stewart, 1982). However, the present slowing of Cl movements in the Purkinje fibre is unlikely to be because of inhibition of a Na-dependent co-transport since Na removal does not exert a similar blocking effect.

DISCUSSION

Cl transport in the Purkinje fibre

The present results add considerable weight to the original hypothesis (Vaughan-Jones, 1979*b*) that a Cl–HCO_3 exchange mechanism exists in cardiac muscle and that it is instrumental in determining a^i_{Cl}. An alternative hypothesis is that Cl movements occur exclusively through membrane channels and that, in the steady state, a^i_{Cl} is at electrochemical equilibrium across the membrane, a condition which obtains under most circumstances in frog skeletal muscle (Hodgkin & Horowicz, 1959; Bolton & Vaughan-Jones, 1977). However, many observations in the present work are clearly inconsistent with this alternative hypothesis. First, the steady-state dependence of a^i_{Cl} upon a^o_{Cl} is roughly hyperbolic, displaying saturation at high a^o_{Cl}. This is in contrast to the near-linear dependence expected for a passive distribution of Cl in accordance with the membrane potential (see Fig. 1*B*). Secondly, Fig. 1*B* shows that at all levels of external Cl, steady-state a^i_{Cl} is 3–10 times higher than that expected from passive electrochemical equilibrium. Finally, the kinetics of Cl reuptake by a Cl-depleted fibre are inconsistent with Cl permeation through a simple channel. There are four reasons for this. (i) For all levels of external Cl that have been tested, the initial unidirectional Cl influx is approximately one order of magnitude larger than the influx expected through Cl channels (Fig. 3): such Cl channels in heart have been estimated to have a low constant-field permeability coefficient (Fozzard & Lee, 1976; Carmeliet & Verdonck, 1978). (ii) Rapid movements of Cl across the membrane do not produce the changes of membrane potential expected if they occurred via conductance pathways (see p. 384). This suggests that much Cl transport is electrically silent and associated with the co- or counter-transport of other ions. (iii) The dependence of Cl influx upon external Cl is non-linear, displaying saturation at higher levels of external Cl (Fig. 3): a simple, constant-field model for a Cl channel would again predict a linear dependence. (iv) The wash-out and reuptake of Cl are both influenced by the ambient levels of HCO_3 (Figs. 7 and 8).

It should be noted that, while more complex models of Cl permeation through channels can encompass some of the observations described above, nevertheless, they cannot account for levels of a^i_{Cl} higher than those predicted from passive electrochemical equilibrium. However, as shown later in the Discussion, such high levels of a^i_{Cl} can be accounted for quantitatively in terms of a Cl–HCO_3 exchange mechanism. Evidence has already been presented for such an exchange in the sarcolemma of the Purkinje fibre (Vaughan-Jones, 1979*b*, 1982*a*, *b*). Therefore, it is reasonable to conclude that the Cl and HCO_3 sensitivity of Cl movements observed in the present work provides a quantitative description of Cl–HCO_3 exchange at the sarcolemma.

The fact that the rise of a^i_{Cl} following readmission of external Cl is little affected by the presence or absence of external Na and intracellular Na or by the drug amiloride suggests that Na ions are not involved in the anion exchange system and, furthermore, suggests that possible contributions to Cl reuptake from other Na-dependent Cl transport systems (e.g. Na–Cl or Na–K–Cl co-transport) are very small. It is notable therefore that piretanide, a putative inhibitor of Na–K–Cl co-transport slows Cl reuptake in the Purkinje fibre (Fig. 10*B*). However, the concentration used

here was rather high (1 mmol/l) and a block of Cl–HCO_3 exchange at such high concentrations of piretanide cannot be excluded (e.g. see inhibitory effects of piretanide on anion exchange in red cells: Brazy & Gunn, 1976).

The present measurements of Cl–HCO_3 exchange will be distorted by any additional parallel movement of Cl through membrane channels. During the Cl reuptake phase at least, this additional contribution is likely to be only 1–10 % of the total net influx of Cl (see Fig. 3) so that it will affect very little any quantitative estimate of Cl–HCO_3 exchange. However, during the somewhat slower Cl wash-out into Cl-free solution, it is less certain that the passive loss of Cl via channels is always such a small fraction of the total Cl efflux (Vaughan-Jones, 1979*b*; Vanheel *et al.* 1984). In addition, a recent report suggests that, in cultured chick heart cells, a K–Cl co-transport mechanism may also contribute a net efflux of Cl (Piwnica-Worms, Jacob, Horres & Lieberman, 1985*b*). Because of these two possible sources of error, quantitative estimates of Cl–HCO_3 exchange based upon measurements of Cl reuptake rather than Cl wash-out will be less prone to error.

The dependence of Cl transport upon HCO_3

The activation by HCO_3 of Cl reuptake is consistent with the existence of a saturable Cl–HCO_3 exchange carrier in the sarcolemma. The rate of Cl uptake is half-maximal at about 1·3 mmol HCO_3/l. As pointed out in the Results, this is likely to reflect a dependence of Cl uptake upon internal rather than external HCO_3 since HCO_3 ions will be transported out of the fibre in exchange for the entry of Cl. Thus it is likely that the initial rate of Cl reuptake is half-maximal at an $[HCO_3]_i$ of 1·3 mmol/l, i.e. an internal activity of 1·0 mmol/l (see Results, p. 389).

It is interesting that the wash-out as well as the reuptake of Cl is sensitive to the extracellular level of HCO_3 (Fig. 8). This is consistent with the proposal that some of the wash-out of Cl is also mediated by Cl–HCO_3 exchange (Vaughan-Jones, 1979*b*). In this case the removal of external Cl permits an efflux of Cl in exchange for the entry of HCO_3 and it is notable that this Cl efflux is associated with a rise in pH_i of about 0·3 units (Vaughan-Jones, 1979*b*, 1982*a*,*b*). Such a wash-out of Cl will require extracellular HCO_3 and it is apparent from Fig. 8 that the rate of wash-out of Cl is also half-maximal at an $[HCO_3]_o$ of about 1·0 mmol/l. It should be noted that a significant Cl efflux via HCO_3-independent pathways (see previous section) will lead to an underestimate of the HCO_3 sensitivity of Cl wash-out. Hence the value for $[HCO_3]_o$ of 1·0 mmol/l should be regarded as an upper limit for the apparent HCO_3 affinity, $K_{0.5}^{HCO_3}$, for Cl efflux.

It should be stressed that although the present figures for half-maximal activation of Cl uptake and wash-out by HCO_3 are considered to be rough estimates rather than precise values (more accurate determinations will require the addition of many more data points to the graphs shown in Fig. 8), nevertheless the main conclusion of this particular series of experiments is clear: Cl reuptake is extremely sensitive to low levels of intracellular HCO_3, i.e. the HCO_3 affinity of the Cl uptake system appears to be high. Similarly, the HCO_3 affinity of the Cl wash-out system also appears to be high. One problem, however, is that the accurate determination of anion affinity upon one (*cis*) side of the membrane requires that the anion exchange carrier is at

saturation upon the other (*trans*) side of the membrane. This is likely to be the case in the present work: the affinity of the exchanger for Cl appears to be similar to that for HCO_3 (i.e. a high affinity, see p. 396) and at no time does the *trans* concentration of anion (Cl and HCO_3) fall below about 20 mmol/l, i.e. at all times the *trans* side of the carrier will be very close to saturation (see Appendix).

Values for $K_{0.5}^{HCO_3}$ of Cl–HCO_3 exchange have been estimated most frequently in the red blood cell. These estimates have been widely discrepant, varying from 0·39 to > 20 mmol/l (Dalmark, 1976; Chow, Crandall & Forster, 1976; Lambert & Lowe, 1978; Wieth, 1979). This is due partly to the fact that the carrier in the erythrocyte is asymmetric with respect to its anion affinity on either side of the membrane (Knauf & Mann, 1984). In the giant barnacle muscle fibre and the squid axon, estimates for $K_{0.5}^{HCO_3}$ have also been made for the extracellular-facing site of the pH_i regulation mechanism (2·3–2·6 mmol/l for squid, Boron & Russell, 1983; Boron, 1985; and 10·3 mmol/l for barnacle, Boron, McCormick & Roos, 1981). A comparison with squid axon and barnacle muscle is not strictly valid however, since, unlike the Purkinje fibre, their anion transport systems are stoicheiometrically linked with the transport of Na ions (see Thomas, 1984) and so may operate via a fundamentally different mechanism. In addition, estimates of $K_{0.5}^{HCO_3}$ in these invertebrate tissues have made no allowance for the fact that the binding of Cl and HCO_3 ions (or, in the case of squid axon, the binding of Cl and $NaCO_3$ ions; Boron, 1985) to transport sites may be predominantly competitive (cf. Boron, Russell, Brodwick, Keifer & Roos, 1978) as is also the case in the red blood cell. Assuming that anion movements conform to a simple Michaelis–Menten mechanism, such competition will lead to erroneously high estimates for $K_{0.5}^{HCO_3}$. In the present work, however, possible problems of anion competition have been minimized through examining the activation of Cl influx by intracellular HCO_3 in the absence of intracellular Cl, i.e. by estimating the initial Cl influx upon readding external Cl to a Cl-depleted fibre. Similarly, the activation of Cl efflux by extracellular HCO_3 has been examined in the absence of extracellular Cl.

Although the possibility of competition betweeen Cl and HCO_3 ions has not been tested directly in the present work, nevertheless certain observations suggest that it does occur in the Purkinje fibre. First, Fig. 8 (inset) shows that while the initial rate of Cl reuptake increases with increasing concentrations of internal HCO_3 in the range 0–10 mmol/l, Cl uptake appears to decline again at high external levels of HCO_3 (> 10 mmol/l). Such a decline at high extracellular levels of HCO_3 is consistent with competition for entry between extracellular Cl and extracellular HCO_3 (cf. Wieth, 1979). Secondly, steady-state a_{Cl}^i varies inversely with $[HCO_3]_o$ displaying no sign of saturation, even at an $[HCO_3]_o$ of 62 mmol/l (Fig. 6*A*). This is despite the first observation that HCO_3-dependent Cl reuptake, determined in the absence of internal Cl, is saturated by about 10 mmol HCO_3/l (Fig. 8). This suggests that, at high Cl levels (as in Fig. 5), the apparent HCO_3 affinity of Cl–HCO_3 exchange is considerably reduced so that the activation of the system by HCO_3 is not at saturation. Such an observation is again consistent with simple competition between Cl and HCO_3 ions for transport sites.

Cl transport in the absence of HCO_3

The HCO_3 sensitivity of Cl reuptake (Fig. 8) is important when considering Cl transport under nominally CO_2/HCO_3-free conditions. It is notable that Cl reuptake is not inhibited entirely in HEPES-buffered solutions (Vaughan-Jones, 1979*b* and see Fig. 7). This may indicate that Cl transport processes other than Cl–HCO_3 exchange can produce a slow reuptake of Cl. An additional explanation however is that the hydration of atmospheric and metabolic CO_2 produces low levels of $[HCO_3]_i$ which can support a slow rate of Cl–HCO_3 exchange. Vanheel *et al.* (1984) have estimated recently that, in HEPES-buffered media, $[HCO_3]_i$ in the quiescent Purkinje fibre is *ca.* 0·5 mmol/l. A similar level may also exist in the localized extracellular space. Reference to Fig. 8 indicates that, in the present work, an intracellular HCO_3 level near the membrane of 0·5 mmol/l would be sufficient to fuel the initial Cl reuptake at rates close to those observed in HEPES-buffered solutions (see also Fig. 13). Such arguments apply not only to Cl reuptake but also to Cl wash-out in Cl-free solution. Vanheel *et al.* (1984) have argued that, in HEPES-buffered media, Cl wash-out from the Purkinje fibre cannot be via Cl–HCO_3 exchange and they suggest that Cl–OH exchange may be operating. In contrast, the present work suggests that Cl–HCO_3 exchange is likely to continue to operate, albeit rather slowly, under nominally CO_2/HCO_3-free conditions.

The dependence of Cl transport upon Cl

The rate of Cl reuptake appears to be a saturating function of external Cl such that an a^o_{Cl} of 33 mmol/l is required to produce a half-maximal rate of Cl reuptake, i.e. the apparent Cl affinity in the presence of HCO_3, $K^{Cl}_{a0·5} = 33$ mmol/l. However, this uptake of Cl is measured in the presence of an $[HCO_3]_o$ of 23 mmol/l and, as discussed above, any competition between external Cl and external HCO_3 for binding will elevate the apparent Cl affinity, $K^{Cl}_{0·5}$. For a system obeying Michaelis–Menten kinetics, this elevation will be given by:

$$K^{Cl}_{a0·5} = K^{Cl}_{0·5}\left(1+\frac{a^o_{HCO_3}}{K^{HCO_3}_{0·5}}\right),$$

where $K^{Cl}_{a0·5}$ is the apparent Cl affinity in the presence of HCO_3 and $K^{Cl}_{0·5}$ is the apparent Cl affinity in the absence of HCO_3. Substituting the values for $K^{Cl}_{a0·5}$, 33 mmol/l; $K^{HCO_3}_{0·5}$, 1·0 mmol/l (expressed as an activity rather than a concentration) and $a^o_{HCO_3}$, 17·25 mmol/l, delivers a value for $K^{Cl}_{0·5}$ of 1·8 mmol/l. Interestingly the ratio $K^{Cl}_{0·5}/K^{HCO_3}_{0·5} = 1·8$ is similar to the ratio for the human erythrocyte estimated by Wieth (1979; $K^{Cl}_{0·5}/K^{HCO_3}_{0·5} = 1·5$). Hence the present data would be consistent with an anion exchange carrier with fairly similar and relatively high affinity for Cl and HCO_3 ions and with competition by anions for binding and subsequent transport.

Models of Cl–HCO_3 exchange

Since many of the properties of Cl–HCO_3 transport in the Purkinje fibre have now been described, it is important to determine whether they can be reconciled with a simple model of anion exchange. The simplest scheme (Fig. 11*A*) consists of a

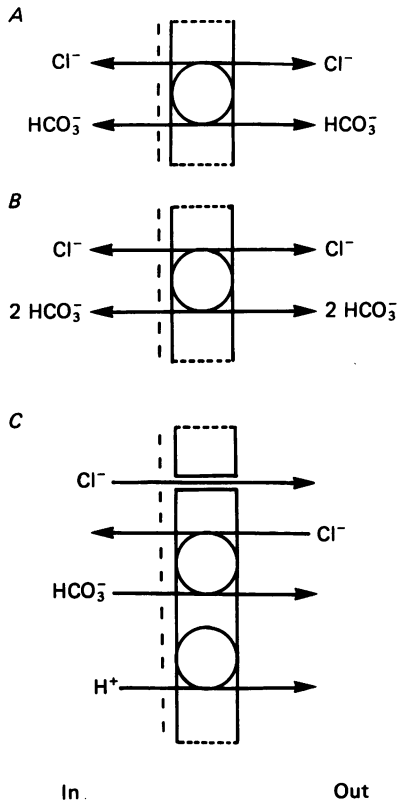

Fig. 11. Three possible models of intracellular Cl regulation by sarcolemmal Cl–HCO_3 exchange in the Purkinje fibre. *A*, the exchange is electroneutral. Cl and HCO_3 ions compete for binding and are exchanged in a one-for-one fashion. The exchanger is at equilibrium. Hence it indulges in both Cl–Cl and HCO_3–HCO_3 exchange depending on the relative affinity of the carrier for Cl and HCO_3 ions. *B*, the exchange is electrogenic with a coupling of 2 HCO_3 for 1 Cl. It is at equilibrium and this equilibrium will be influenced by membrane potential. *C*, the exchange is electroneutral but cannot reach equilibrium because of Cl efflux via another leakage pathway represented here by a Cl channel although other Cl pathways are not ruled out. This scheme recognizes that the net HCO_3 efflux is equivalent to an acid influx which must, in the steady state, be balanced by an equivalent acid efflux via another mechanism, e.g. Na–H exchange. This acid efflux therefore helps indirectly to set the transmembrane HCO_3 gradient and hence a^i_{Cl} via Cl–HCO_3 exchange.

symmetrical carrier in the membrane with fixed affinities for competing Cl and HCO_3 ions and which translocates ions at a rate described by Michaelis–Menten kinetics, and with a stoicheiometry of 1 Cl:1 HCO_3. Thermodynamic considerations predict that, at equilibrium:

$$\frac{a^o_{HCO_3}}{a^i_{HCO_3}} = \frac{a^o_{Cl}}{a^i_{Cl}}.$$

However, in the Purkinje fibre, the Cl activity gradient is more than twice the HCO_3 gradient (see Table 1). Hence such a simple mechanism will not be at equilibrium.

If the carrier operates kinetically, it will mediate a net Cl influx and a net HCO_3 efflux. Therefore the simplest model does not provide an adequate description of the results. There are at least three possible reasons for this.

(i) In the steady state, the carrier may be inactivated by some unidentified allosteric mechanism, cf. the proposed allosteric inactivation of Na–H exchange at

TABLE 1. The various parameters used for the quantitative model presented in eqns. (5)–(9) in text and illustrated in Fig. 11*C*

Parameter	Value	Source
P_{Cl}	3×10^{-8} cm/s	Carmeliet & Verdonck, 1978; Vaughan-Jones, 1979*a*
S	$2{\cdot}56 \times 10^{-4}$ cm	Mobley & Page, 1972
V_{max}^{Cl}	2·0 mmol/l. min	Fig. 3 this paper
$V_{max}^{HCO_3}$	Assumed to equal V_{max}^{Cl}	
$K_{0.5}^{HCO_3}$	1·0 mmol/l*	This paper
$K_{0.5}^{Cl}$	1·8 mmol/l*	This paper
a_{Cl}^{o} (normal)	100 mmol/l	See Methods
$a_{HCO_3}^{o}$ (normal)	17·25 mmol/l	See Methods
$a_{HCO_3}^{i}$ (normal)	8·0 mmol/l	Vaughan-Jones, 1979*b*
V	−75 mV	

P_{Cl} is the Cl permeability coefficient (constant field); S, the volume:surface-area ratio of the Purkinje fibre; V_{max}^{Cl} and $V_{max}^{HCO_3}$, the maximum Cl and HCO_3 transport rates via the exchanger, assumed to be equal; $K_{0.5}^{HCO_3}$ and $K_{0.5}^{Cl}$, the affinities of the exchanger for HCO_3 and Cl*; V, the membrane potential (assumed to be constant for all computations).

* For the purposes of the present model, it is assumed that $K_{0.5}^{HCO_3}$ and $K_{0.5}^{Cl}$ do not change as the carrier moves from the outward to the inward-facing configuration. The value for $K_{0.5}^{HCO_3}$ is taken from the HCO_3 dependence of Cl reuptake (Fig. 8); $K_{0.5}^{Cl}$ is derived as shown on p. 396.

high pH_i (Aronson, 1985). An alternative explanation is that the apparent HCO_3 affinity of the anion exchanger is so low compared with that for Cl that the carrier mediates only Cl–Cl self exchange even though it is not thermodynamically at equilibrium. Although this latter explanation can account for some of the properties of anion exchange in heart (Vaughan-Jones, 1982*b*), it now seems to be unlikely. The results shown in Fig. 5 show that changes in external HCO_3 can still affect the exchange mechanism when Cl is present on both sides of the membrane. In addition, examination of the Cl dependence and HCO_3 dependence of Cl reuptake suggests that $K_{0.5}^{HCO_3} \ll K_{0.5}^{Cl}$.

(ii) The stoicheiometry of anion exchange may not be 1:1. If it were 2 HCO_3:1 Cl, then, in the steady state, it is possible to show that the system would be close to equilibrium (Fig. 11*B*). However, anion exchange would then become electrogenic and its equilibrium would be sensitive to membrane potential. There is no evidence that anion exchange in the Purkinje fibre is influenced by membrane potential (Vaughan-Jones, 1979*b*). Furthermore, anion exchange in the red blood cell is clearly electroneutral. Estimates of stoicheiometry in the present work also suggest that it is 1 HCO_3:1 Cl (p. 387). Hence it seems unlikely that anion exchange in heart is electrogenic.

(iii) The Cl–HCO_3 exchange may not reach equilibrium because of a net outward movement of Cl via some other membrane pathway. The most obvious pathway

would be a passive net efflux of Cl via membrane channels (Fig. 11*C*) although Cl efflux via other pathways is not excluded (see later, p. 402). There is indeed evidence for a small constant-field Cl conductance in cardiac tissue: equivalent to a P_{Cl} of about 3×10^{-8} cm/s (Fozzard & Lee, 1976); Carmeliet & Verdonck, 1977).

The scheme shown in Fig. 11*C* recognizes that the HCO_3 gradient will be fixed initially by other mechanisms involved in the regulation of pH_i, e.g. Na–H exchange (Ellis & MacLeod, 1985; Piwnica-Worms, Jacob, Horres & Lieberman, 1985*a*). By controlling pH_i, these mechanisms will therefore help to set $a^i_{HCO_3}$. The HCO_3 gradient will then determine a^i_{Cl} via Cl–HCO_3 exchange and via passive Cl movements through the leak pathway. In this case intracellular Cl will be governed by the sum of the net carrier-mediated Cl influx and the net Cl efflux through the leak pathway. Each component can be divided further into a unidirectional influx and efflux of Cl. Hence a net flux of Cl leading to a change in a^i_{Cl} can be written as:

$$S\, da^i_{Cl}/dt = (J^{in}_{Cl} - J^{out}_{Cl}) + (M^{in}_{Cl} - M^{out}_{Cl}), \tag{5}$$

where J^{in}_{Cl} and M^{in}_{Cl} are the unidirectional Cl influx via a carrier (J) and a channel (M) and J^{out}_{Cl} and M^{out}_{Cl} are the respective effluxes. S is the volume:surface-area ratio (Mobley & Page, 1972), assumed to be constant (see Table 1).

If the carrier fluxes conform to simple Michaelis–Menten kinetics (cf. Fig. 3) with competition for transport between HCO_3 and Cl, then

$$(J^{in}_{Cl} - J^{out}_{Cl}) = \frac{a^o_{Cl}\, V_{max}\, S}{a^o_{Cl} + K^{Cl}_{0.5}\left(1 + \frac{a^o_{HCO_3}}{K^{HCO_3}_{0.5}}\right)} - \frac{a^i_{Cl}\, V_{max}\, S}{a^i_{Cl} + K^{Cl}_{0.5}\left(1 + \frac{a^i_{HCO_3}}{K^{HCO_3}_{0.5}}\right)}, \tag{6}$$

where V_{max} is the maximum transport rate. It should be noted that this equation describes a symmetrical carrier. In addition, the equation is applicable only when the carrier, loaded with anions, is at saturation on both sides of the membrane (see Appendix).

Net Cl flux through the leak pathway can, for convenience, be expressed using the constant-field relationship (Hodgkin & Horowicz, 1959).

$$(M^{in}_{Cl} - M^{out}_{Cl}) = \frac{P_{Cl}\, a^o_{Cl}\, e\,(VF/RT)}{1 - e\,(-VF/RT)} - \frac{P_{Cl}\, a^i_{Cl}\, e\,(-VF/RT)}{1 - e\,(VF/RT)}, \tag{7}$$

where V is the membrane potential and F, R and T have their usual meaning.

Using the parameters presented in Table 1, it is possible to predict from eqns. (5)–(7) that a^i_{Cl} in the steady state (i.e. when $da^i_{Cl}/dt = 0$) will be 21 mmol/l (Fig. 12*A*), a value which is very close to that actually observed (Vaughan-Jones, 1979*a*, and present work). Furthermore, such a combination of carrier plus leak predicts a hyperbolic dependence of a^i_{Cl} upon a^o_{Cl} (Fig. 12*B*) and also predicts that, when a Cl-depleted fibre is re-exposed to external Cl, a^i_{Cl} rises along a near-exponential time course with a half-time which is virtually independent of the external level of Cl (Fig. 12*A*, *C*). Additional details of the model presented in eqns. (5)–(7) and Figs. 12 and 13 are given in the Appendix. In fact the predicted rise of a^i_{Cl} in Fig. 12*C* is not a perfect exponential, the rate constant increases slightly as a^i_{Cl} rises and there is some decrease in the over-all half-time at high external Cl. Nevertheless a comparison

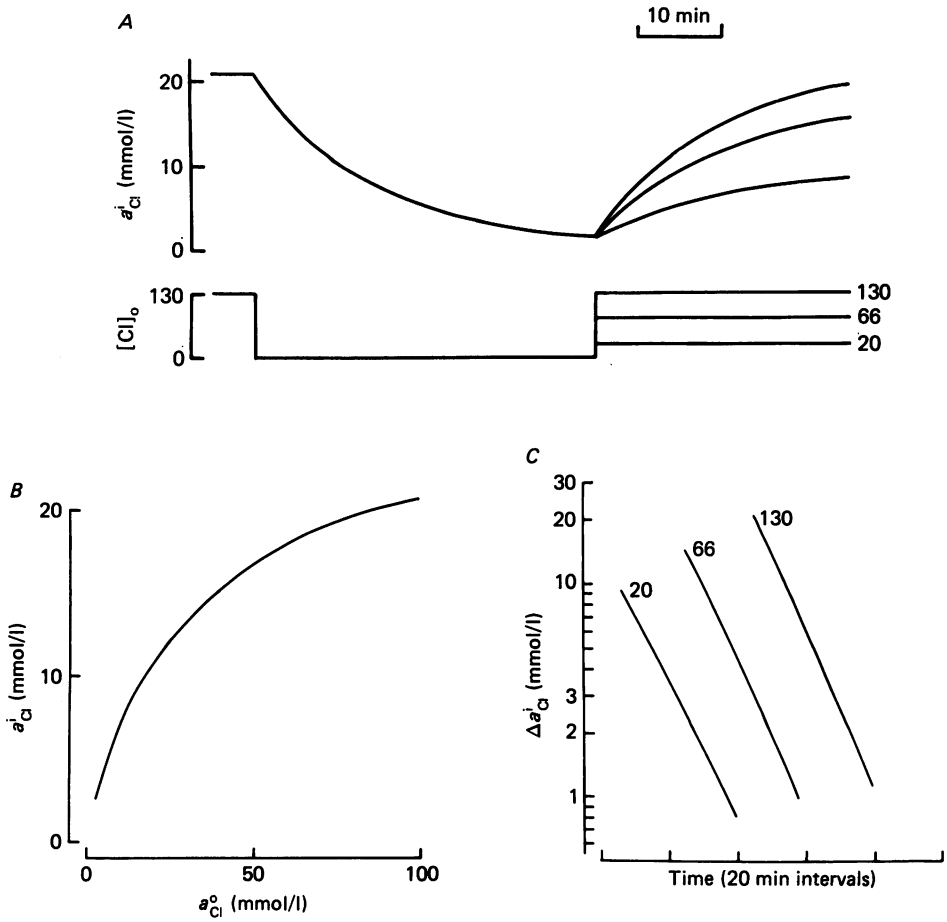

Fig. 12. Predictions of a quantitative model based upon the scheme shown in Fig. 11*C*. Values of the relevant parameters either derived from the present work or taken from previous publications are listed in Table 1. The model is described in eqns. (5)–(7) in the Discussion and eqns. (8) and (9) in the Appendix. *A*, effects on a^i_{Cl} of changing external Cl concentration, $[Cl]_o$, from 130 to 0 mmol/l and then raising it to either 20, 66 or 130 mmol/l. Note that, with Cl, 130 mmol/l, a^i_{Cl} in the steady state is 21 mmol/l (cf. Fig. 1*A*). *B*, dependence of a^i_{Cl} in the steady state upon a^o_{Cl} (cf. Fig. 1*B*). *C*, semi-logarithmic replot of the reuptake of Cl at various external Cl concentrations (as indicated); for convenience the replots have been displaced along the abscissa; data from *A* (the result should be compared with Fig. 2*A*).

of Fig. 12 with the experimental observations of Figs. 1 and 2 indicates a reasonably good agreement.

Finally, the model predicts (Fig. 13) that when $[HCO_3]_o$ is varied at a constant pH_o, then a^i_{Cl} in the steady state remains virtually unchanged providing that $[HCO_3]_o$ and $[HCO_3]_i$ close to the membrane never fall below about 0·25 mmol/l. This prediction is also very similar to that observed experimentally (Fig. 4). The model therefore confirms that a low level of CO_2 production by the cell is sufficient to maintain a high a^i_{Cl} ($\sim$ 20 mmol/l; see Discussion, p. 396).

The relative insensitivity of a^i_{Cl} to reductions in $[HCO_3]_o$ occurs because P_{CO_2} is also reduced to maintain a constant pH_o. Consequently, because CO_2 is permeant, intracellular levels of HCO_3 are also reduced. Under these conditions, pH_i in the steady state has been shown to be changed very little (see Ellis & Thomas, 1976, Fig. 3). Thus the transmembrane HCO_3 gradient will be little affected and this, via

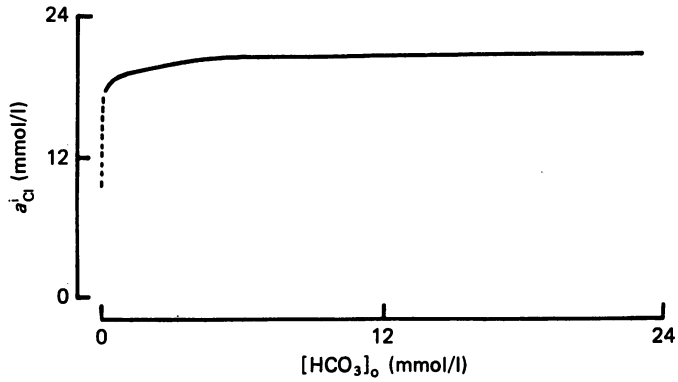

Fig. 13. Predictions of model illustrated in Fig. 11*C*. a^i_{Cl} in the steady state is affected little by reduction in $[HCO_3]_o$ when pH_o is maintained constant, i.e. P_{CO_2} is reduced as $[HCO_3]_o$ is reduced. For simplicity it is assumed that, in the steady state, the transmembrane HCO_3 ratio remains constant as $[HCO_3]_o$ is reduced (see text for a discussion of this). The continuous line represents the prediction of the model for $[HCO_3]_o$ 23–0·25 mmol/l. Below an $[HCO_3]_o$ of 0·25 mmol/l, a^i_{Cl} falls steeply (dashed line). It should be noted, however, that $[HCO_3]_o$ and $[HCO_3]_i$ may be as high as 0·5 mmol/l in nominally HCO_3/CO_2-free solution because of the metabolic production by the fibre of CO_2 (see text) (the prediction shown in Fig. 13 should be compared with Fig. 4).

Cl–HCO_3 exchange, results in a relatively constant a^i_{Cl}. However, if $[HCO_3]_o$ is altered but at a constant P_{CO_2} (hence pH_o varies), then the transmembrane HCO_3 gradient will be changed. Under these conditions a^i_{Cl} is expected to vary as indeed observed in Fig. 5.

Of the schemes (i), (ii) and (iii) discussed above, scheme (iii) (Fig. 11*C*) would therefore appear to be the most compatible with the experimental observations. Scheme (iii) can reproduce reasonably well the changes in a^i_{Cl} following removal and readmission of external Cl and following changes in intracellular and extracellular HCO_3. However, the model makes certain predictions that appear to differ from the experimental observations. For example, inhibition of the anion exchanger with DIDS (see Fig. 5) does not produce the predicted fall in a^i_{Cl}, but this is perhaps not surprising since, in other cells, DIDS also blocks Cl channels (White & Miller, 1979; Inque, 1985). However, DIDS has no discernible effect upon steady-state pH_i (see Fig. 5). This is more difficult to reconcile with the model which predicts that the steady-state efflux of HCO_3 via the exchanger would be about 0·4 mmol/l. min. This is equivalent to a small resting acid influx which would acidify pH_i at a rate of about 0·007 u./min (buffering power is about 54 mequiv/l: non-CO_2 and CO_2-dependent components, Ellis & Thomas, 1976). Hence inhibition of anion exchange should produce a slow increase in pH_i dictated by the rate of acid extrusion via other

mechanisms. It is possible that these other acid extrusion mechanisms are steeply dependent upon pH_i and effectively cease to operate following very small increases in resting pH_i (cf. Frelin, Vigne & Lazdunski, 1985) and this could account for the lack of effect of DIDS on pH_i. Alternatively the resting carrier-mediated HCO_3 efflux predicted from the present work may be over-estimated. This would occur, for example, if the assumed value of P_{Cl} (Table 1) was also an over-estimate since, in the steady state, the passive Cl leak determines HCO_3 efflux via the exchange. In this case, a very low HCO_3 efflux would be difficult to detect experimentally.

Despite the above reservations concerning scheme (iii), it has been presented here in some detail because it at least offers a useful starting point for the consideration of more complex models of anion exchange in heart. In this respect it should be noted that no attempt has been made in the present model to incorporate possible asymmetries of carrier affinity for anions on opposing sides of the membrane (cf. anion exchange in the erythrocyte; Knauf & Mann, 1984). In addition, allosteric effects on the anion carrier cannot yet be ruled out (cf. the modifier site described for anion exchange in the erythrocyte; Cabantchik *et al.* 1978).

The present scheme bears a superficial resemblance to that described in barnacle muscle (Boron *et al.* 1978). In both cases, the anion exchanger mediates Cl–Cl as well as Cl–HCO_3 exchange: an increase in one mode being associated with a decrease in the other and vice versa. In the steady state, the carrier in the present scheme engages in both forms of exchange. Although the present work can only identify net ion movements (i.e. Cl–HCO_3 exchange), nevertheless there is evidence from radioactive tracer studies for Cl–Cl exchange in heart (Polimeni & Page, 1980).

Comparison with other vertebrate muscles

A Cl–HCO_3 exchange mechanism has been identified in smooth muscle, a tissue which also possesses a high a^i_{Cl} (Aickin & Brading, 1984) and in skeletal muscle (eg. Abercrombie, Putnam & Roos, 1983). In smooth muscle the exchanger would appear to be fairly close to equilibrium (a^i_{Cl} is about 50 mmol/l, considerably higher than in the Purkinje fibre). However, there is some evidence for a small additional contribution to Cl accumulation from Na–Cl co-transport (Widdicombe & Brading, 1980; Aickin & Brading, 1984). A carrier-mediated Na–Cl uptake also appears to exist in skeletal muscle (C. Aickin, W. Betz & G. Harris, personal communication) but this has an additional requirement for K (cf. the Na–K–Cl co-transport found in squid axon, Russell, 1983). The present lack of evidence for an Na-dependent Cl uptake in the Purkinje fibre may therefore seem surprising. However, it is possible that Na-dependent Cl transport in this tissue, if it exists, is swamped by the larger Cl movements on anion exchange.

Finally, recent work in cultured chick heart cells (Piwnica-Worms *et al.* 1985*b*) suggests that yet another Cl transport exists in these cells: a K–Cl co-transport. In the steady state this mediates a Cl efflux which, as pointed out by the authors, is in many respects identical to the Cl leak classically assumed to operate via channels. If a similar K–Cl leak exists in the Purkinje fibre its magnitude is also likely to be equivalent to that presently attributed to Cl channels (see Vaughan-Jones 1979*a*, Figs. 10–12). Hence the existence of such a leak would in no way alter the conclusions of the present work except that the Cl channel in Fig. 11*C* would be supplemented or even replaced by a K–Cl co-transporter.

Conclusions

Taken together with previous data (Vaughan-Jones, 1979*a*, *b*) the present results are compatible with a saturable carrier with similar and relatively high affinities for competing Cl and HCO_3 ions which mediates a reversible, electroneutral Cl–HCO_3 exchange across the Purkinje fibre membrane. The exchange is not at equilibrium. This may mean that under normal conditions it is inactivated by some unidentified mechanism or alternatively that it is in a steady state with net Cl influx via the carrier being balanced by a net passive efflux of Cl through some other membrane pathway such as a membrane channel of low constant-field permeability, although other Cl efflux pathways are not ruled out. The anion exchange operates in the absence of Na and is therefore independent of the Na–H exchange that operates in this tissue. However, intracellular Cl regulation may be influenced indirectly by Na–H exchange. This is because Na–H exchange controls pH_i and, in doing so, will help to set the transmembrane HCO_3 gradient which, in turn, will influence a^i_{Cl} via anion exchange. One way, therefore, of examining further the properties of anion exchange in the Purkinje fibre will be to analyse its role in the regulation of pH_i (cf. Vaughan-Jones, 1982*a*, *b*; Vanheel *et al.* 1984).

APPENDIX

The present model of anion exchange is based upon a saturable membrane carrier with competition between Cl and HCO_3 ions for binding and transport. Because the data do not permit a detailed kinetic analysis of the carrier, questions concerning asymmetry and whether the carrier is ping-pong or sequential have been ignored in favour of a simple description (eqn. (6)) which specifies only that the carrier is symmetrical. This is felt to be a justified simplification because the purpose of the model is merely to establish whether or not an anion exchanger can reproduce the basic features of Cl regulation in the Purkinje fibre. Net Cl flux via the carrier (da^i_{Cl}/dt) can be determined using eqns. (5)–(7) in the Discussion. Two additional pieces of information are, however, required.

(i) Following a change in a^i_{Cl}, the new $a^i_{HCO_3}$ must be determined. Because of Cl–HCO_3 exchange, $a^i_{HCO_3}$ will vary inversely with a^i_{Cl} and it will therefore competitively affect unidirectional Cl efflux via the carrier. Since intracellular H ions are buffered whereas intracellular Cl ions are not (see Vaughan-Jones, 1979*a*), the carrier-mediated changes in $a^i_{HCO_3}$ and a^i_{Cl} will not be equimolar even though one Cl ion is exchanged for one HCO_3 ion. From eqn. (4), the change in $a^i_{HCO_3}$ will be less than that for a^i_{Cl} by an amount equal to $\Delta pH_i \beta \gamma_{HCO_3}$ (β is the non-CO_2 buffering power). Furthermore, other transmembrane mechanisms may be contributing to the regulation of pH_i and they will also affect indirectly $a^i_{HCO_3}$. In the present model, the change in $a^i_{HCO_3}$ for a given change in a^i_{Cl} is assumed to be

$$\Delta a^i_{HCO_3} = -0{\cdot}8\ \Delta a^i_{Cl}. \tag{8}$$

This equation is determined empirically from previous experimental observations (Vaughan-Jones, 1979*b*, see Fig. 11 and text: Δa^i_{Cl} upon removing and readmitting

external Cl is 1·3 times the $\Delta a^{i}_{HCO_3}$ as estimated from the change in pH_i). It should be noted that eqn. (8) makes no assumptions concerning the mechanism relating $a^{i}_{HCO_3}$ and a^{i}_{Cl}.

(ii) In the modelling presented in Figs. 12 and 13, the carrier is calculated to be 95–98% saturated on both sides of the membrane. However, even under these conditions, the predicted inward and outward flux of carrier will not be exactly equal. Consequently, in order to produce a working model with conservation of carrier the greater predicted carrier flux has been scaled to equal the smaller. Such a scaling procedure avoids making assumptions concerning the exact mechanism of ion carriage.

The instantaneous carrier-mediated influx and efflux of Cl can be estimated from eqn. (6). The instantaneous influx and efflux of HCO_3 can also be estimated using a similar equation:

$$(J^{in}_{HCO_3} - J^{out}_{HCO_3}) = \frac{a^{o}_{HCO_3} V_{max} S}{a^{o}_{HCO_3} + K^{HCO_3}_{0.5}\left(1 + \frac{a^{o}_{Cl}}{K^{Cl}_{0.5}}\right)} - \frac{a^{i}_{HCO_3} V_{max} S}{a^{i}_{HCO_3} + K^{HCO_3}_{0.5}\left(1 + \frac{a^{i}_{Cl}}{K^{Cl}_{0.5}}\right)}. \quad (9)$$

For simplicity, V_{max} for HCO_3 is assumed to equal that for Cl. Unidirectional carrier-mediated Cl and HCO_3 fluxes determined from eqns. (6) and (9) can be combined to estimate the instantaneous fluxes of the carrier loaded with Cl and HCO_3. Since total carrier influx and efflux must be equal, the rate of carrier flux will be limited by the slower of the two predicted unidirectional carrier fluxes. The counter fluxes of Cl and HCO_3 are then scaled accordingly. The net carrier-mediated flux of Cl can thus be determined at any a^{i}_{Cl} and a^{o}_{Cl}.

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