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SECONDARY ACTIVE TRANSPORT OF WATER ACROSS VENTRICULAR CELL MEMBRANE OF CHOROID PLEXUS EPITHELIUM OF *NECTURUS MACULOSUS*

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SUMMARY

1. The interaction between Cl^-, K^+ and H_2O fluxes were studied in the ventricular membrane of the choroid plexus epithelium from *Necturus maculosus* by means of ion-selective microelectrodes. The flux of H_2O was measured by means of K^+ electrodes as the dilution or concentration of intracellular choline ions, Ch_i^+.

2. In one series of experiments Cl^- was readministered to the ventricular solution of tissues incubated in media with low Cl^- concentrations. The resulting influx of Cl^- was associated with an instantaneous influx of K^+ and H_2O.

3. Both the Cl^- and the K^+ influxes were reduced by the diuretic furosemide but were unaffected by inhibitors of Na^+, K^+-ATPase or changes in membrane potentials induced by Ba^{2+}. Since the influx of K^+ proceeds against its electrochemical gradient and is unaffected by changes in membrane potentials, the membrane exhibits secondary active, electroneutral transport of K^+.

4. The influx of water, initiated simultaneously with the influx of K^+ and Cl^-, commenced before these ions had changed the osmolarity of the intracellular solution significantly. The influx of H_2O could proceed against an osmotic gradient. The influx stopped when 100 mmol l^{-1} of mannitol was added to the ventricular solution at the same time as the Cl^- ions. The influx of H_2O was inhibited by K^+ removal, furosemide or high external Ba^{2+} (10 mmol l^{-1}), but not by strophanthidin, ouabain or low concentrations of Ba^{2+} (0·5 mmol l^{-1}). The influx could not continue with other permeable anions, NO_3^-, acetate$^-$ or SCN^-, replacing Cl^-.

5. In another series of experiments Cl^- was removed from the ventricular solution of tissues bathed in saline solutions with normal concentrations of Cl^-. The resulting efflux of Cl^- was associated with an instantaneous efflux of K^+ and H_2O. This efflux of H_2O could proceed against an osmotic gradient of up to 70 mosmol l^{-1}. This effect was inhibited by furosemide, in which case the water fluxes were entirely dependent on the osmotic gradients and the osmotic water permeability L_p of the ventricular membrane.

6. The data suggest that there is a coupling between the flux of KCl and of water in the ventricular membrane, which implies that the reflection coefficient σ for KCl under the given circumstances is less than one. I suggest that the ability of leaky epithelia to transport against osmotic gradients depends on such a coupling, which

derives from the properties of the proteins through which K^+, Cl^- and H_2O leave the cell.

INTRODUCTION

It is well established that movement of ions is a necessary condition for movement of water across plasma membranes as well as epithelial cell layers. The movement of water is coupled to the use of metabolic energy via the ion movements. It is therefore important to define the ion fluxes across the various membranes of the epithelium in order to understand the nature of the solute–solvent coupling.

Leaky epithelia can transport water against an osmotic gradient: 30 mosmol l^{-1} in mammalian kidney (Bomsztyk & Wright 1986), 20 mosmol l^{-1} in *Necturus* gall-bladder (Persson & Spring 1982), 80 mosmol l^{-1} in the rabbit gall-bladder (Diamond 1962, 1964), 150 mosmol l^{-1} for the rat small intestine (Parsons & Wingate, 1961) and more than 20 mosmol l^{-1} in the cerebrospinal fluid system (Heisey, Held & Pappenheimer, 1962; see also House, 1974). In these experiments the absorption takes place from a higher osmolarity to a lower one, and the epithelial cell layers are able to transform the energy contained in the active ion fluxes into osmotic work performed on the water. This finding poses a severe problem for theories which explain the transepithelial water transport by osmotic water flow across the two cell membranes. For a recent review see Tripathi & Boulpaep (1989). According to these theories the cell is hyperosmolar relative to the bath from which the absorption takes place and hypo-osmolar relative to the bath into which the secretion is formed. In the experiments, however, the latter bath is held at a lower osmolarity than the first bath. Thus the efflux of water from the cell takes place against an osmotic gradient.

The same problem is found in leaky epithelia adapted to transport in low external osmolarities. The epithelial cells maintain an intracellular hyperosmolarity relative to the surrounding solutions (Zeuthen, 1981, 1982). The problem could be circumvented, if the lateral intercellular spaces were hyperosmolar relative to the cell and the solutions. Studies with ion-selective microelectrodes, however, show that this is not the case; the spaces are isotonic to the bathing solution (Zeuthen, 1983: Ikonomov, Simon & Frömter, 1985). Thus we have the conceptual problem that water leaves the cell against an osmotic gradient. To explain this, it was suggested (Zeuthen, 1982, 1983) that the serosal membrane of the gall-bladder had a low osmotic reflection coefficient for a K^+ salt, which means that there is an interaction between the flux of water and the flux of salt at the membrane level. The efflux of K^+ could then drive an efflux of H_2O, despite the fact that the cell was hyperosmolar relative to the serosal bath.

The objective of this paper was to determine whether the transport of K^+ and Cl^- interacted with the transport of H_2O in the ventricular membrane of the choroid plexus. This membrane, across which KCl and H_2O leave the cell, is similar to the basolateral membrane of other leaky epithelia, as explained in detail in the preceding paper (Zeuthen, 1991).

A short account of some of the data has previously appeared (Zeuthen, 1990).

METHODS

The methods were the same as in the previous paper (Zeuthen, 1991) and in earlier papers where double-barrelled ion-selective microelectrodes were employed (Zeuthen, 1982, 1987; Zeuthen, Christensen, Bærentsen & la Cour, 1987*a*). The solutions are defined in Table 1. The control solution (solution 1) was also used with no K^+ ions (110 Cl^-, 0 K^+), with 10^{-3} mmol l^{-1} ouabain or

TABLE 1. Composition of solutions (mmol l^{-1})

Solution No.*	Na^+	Cl^-	Mannitol	Measured osmolarity (mosmol l^{-1})
1	106	110	0	210
2	106	110	25	233
3	106	110	50	256
4	106	110	100	307
5	106	4	0	216
6	78	4	0	165
7	53	4	0	108

* The solutions also contained (in mmol l^{-1}) 2 K^+, 1 Mg^{2+}, 1 SO_4^{2-}, 1 Ca^{2+}, 10 glucose. They were buffered with 5 mmol l^{-1} HEPES and bubbled with atmospheric air, pH 7·48. The solutions with 4 mmol l^{-1} of Cl^- contained isethionate or gluconate as Cl^- substitute.

10^{-4} mmol l^{-1} strophanthidin (110 Cl^-, stroph) with 0·5 mmol l^{-1} (110 Cl^-, 0·5 Ba^{2+}) or 10 mmol l^{-1} $BaCl_2$ (110 Cl^-, 10 Ba^{2+}). In the solution containing Ba^{2+}, Na_2SO_4 was omitted. Solutions in which Cl^- was substituted by permeable anions, SCN^-, acetate$^-$ or NO_3^-, were completely Cl^- free, but otherwise as in Table 1. Furosemide was used in concentrations of 10^{-3} or 10^{-4} mmol l^{-1} in the solution (110 Cl^-, fur). There was no difference between the results obtained with ouabain or with strophanthidin, or with high or low concentrations of furosemide; consequently no distinction is made between these solutions. The osmolarities were the same for all solutions employed, about 210 mosmol l^{-1}. Solutions with Cl^- concentrations of 4 mmol l^{-1} had Cl^- substituted by isethionate or gluconate, both of which are known to cause the activity coefficient for Ca^{2+} to decrease to about 0·2. Low Ca^{2+} activity, however, did not change the properties of the tissue (Zeuthen, 1987).

Cl^- was recorded by the electrode as the electrode potential and the initial relative rate of change in internal chloride ions (Cl_i^-) was obtained as the electrode potential E_{Cl} divided by the sensitivity of the electrode, S_{Cl} (see also Methods in Zeuthen, 1991). Thus the initial relative rate of change dCl_i^-/Cl_i^-dt is equal to $S_{Cl}^{-1}\, dE_{Cl}/dt$. If the relative change was multiplied by the apparent intracellular concentration, dCl_i^-/dt was obtained. The apparent intracellular concentration equals the true concentration if the intracellular activity coefficient is the same as that of the extracellular solutions. It is reasonable to assume that Cl^- in the water phase of epithelial cells has the same activity coefficient as in free solution. In the following, apparent concentrations will be referred to as concentrations. Cells adapted to low external chloride have an electrode potential which is influenced by other anions than Cl^-. Since we are interested in the *change* in Cl^- content, S_{Cl}^{-1} dE_{Cl}/dt multiplied with the Cl_i^- obtained by the direct translation of the electrode potential into Cl^- concentration, will yield the correct value for the rate of *change* in Cl_i^-. This is because the change is generated by an influx of Cl^- ions. Measurements of K^+ were calculated along similar lines: $dK_i^+/K_i^+dt = S_K^{-1}\, dE_K/dt$.

Measurements of the changes in cellular volume and thereby fluxes of H_2O (J_{H_2O}) were performed by recordings of intracellular Ch^+. This was described in detail in the previous paper (Zeuthen, 1991). If the bathing solution contained 0·5 mmol l^{-1} of ChCl, Ch_i^+ attained values of 1–4 mmol l^{-1} within 30 min. Since the K^+ electrodes were more than 50 times more sensitive to Ch^+ than to K^+, the changes in cell volume could be measured by K^+ microelectrodes as the changes in Ch_i^+. Fluxes of Cl^- were calculated as:

$$J_{Cl} = V\left[\frac{dCl_i^-}{dt} + \left(Cl_i^- \frac{1}{V}\frac{dV}{dt}\right)\right], \quad (1)$$

or expressed in measurable quantities:

$$J_{Cl} = hCl_i^- \left[\frac{1}{S_{Cl}} \frac{dE_{Cl}}{dt} - \frac{1}{S_{Ch}} \frac{dE_{Ch}}{dt} \right], \quad (2)$$

where h is the cell height (estimated as 10 μm), and S_{Cl} is the sensitivity of the Cl^- electrode. S_{Ch} is the sensitivity of the K^+ electrode to Ch^+ and E_{Ch} is the electrode potential originating from Ch^+ (see also Zeuthen, 1991, for further details). Cl_i^- and Ch_i^+ were recorded in different experiments.

The data are presented with the standard error of the mean. Each cell acted as its own control, which means that each transient experiment is compared to the transient obtained by adding Cl^-. The number of such experiments (n) is given in parentheses. All comparisons were performed by means of Student's t test. The statistics of the fluxes were calculated from the statistics of the changes in the ion concentrations under the assumption that the changes in volume were a constant correction.

RESULTS

Nature of the ion fluxes

This part of the investigation was based on experiments where Cl^- was returned to tissues, which had been adapted to low-Cl^- solutions (4 mmol l^{-1}). The tissues were initially bathed in physiological saline (solution 1, Table 1). The tissues were then bathed for at least 5 min in low-Cl^- solutions (solution 5) on the ventricular side. The ion fluxes were studied when Cl^- was added abruptly by changing the ventricular solution to solution 1.

Electrochemical state of cell adapted to low external Cl^- concentrations

The intracellular electrical potential was -80 ± 1 mV ($n = 125$) and the intracellular K^+ concentration was 103 ± 3 mmol l^{-1} ($n = 64$) which is equivalent to an equilibrium potential for potassium of -99 ± 1 mV. Thus K^+ was accumulated above electrochemical equilibrium in the cells by 19 mV. The cells were 3 mV more negative when the external medium contained 0·5 mmol l^{-1} ChCl, -83 ± 1 mV ($n = 74$).

The intracellular concentration of Cl^- was measured to about 10 mmol l^{-1} for cells bathed in low Cl^- concentrations. The concentration represents a combination of a true Cl^- concentration and a virtual concentration which stems from other anions. We do not need to know the fraction of each, since only the sum appears in the calculations.

Influxes of K^+, Cl^- and H_2O in response to addition of Cl^- to the ventricular solution

The addition of Cl^- to the external solution, a change from 4 to 110 mmol l^{-1}, caused an immediate influx of Cl^-, K^+ and H_2O. The initial relative rate of change in Cl_i^- ($dCl_i^-/Cl_i^-\,dt$, Table 2) was 0·085 s^{-1}, which is equivalent to a rate of change in concentration, dCl_i^-/dt, of 0·9 mmol l^{-1} s^{-1} (Table 2). Given the rate of cell swelling dV/Vdt the influx of Cl^- can be calculated as $1{\cdot}03 \times 10^{-9}$ mol cm^{-2} s^{-1} (Table 3). An example of a recording is given in Fig. 1.

The initial relative rate of change of K_i^+ was *negative* (Table 2, Fig. 2) and K_i^+ *decreased* when Cl^- was added to the ventricular solution. If, however, the rate of swelling of the cell (Fig. 3) was taken into account, it turns out that the cell swelled so fast that, despite the fact that K^+ actually enters the cell together with Cl^-, its concentration decreases (Table 1). If the correction for swelling is incorporated, the *influx* of K^+ was $1{\cdot}05 \times 10^{-9}$ mol cm^{-2} (Table 3), surprisingly close to the influx of Cl^-.

The initial relative rate of cell swelling dV/Vdt was $13{\cdot}2 \times 10^{-3}$ s^{-1}. Since the height of the cell was estimated as 10 μm, this is equivalent to an influx of H_2O (J_{H_2O}, Table 3) of $13{\cdot}2 \times 10^{-9}$ l cm^{-2} s^{-1}.

The recordings of Cl_i^-, K_i^+ and Ch_i^+ (H_2O) were performed with a time resolution of 0·5 s (Figs 1, 2 and 3) and $t = 0$ was chosen as the time of the initiation of the change

TABLE 2. Initial rates of change in Cl_i^-, K_i^+, V and E_i during Cl^- influx under different conditions

Solution	dCl_i^-/Cl_i^-dt (10^{-3} s^{-1})	dK_i^+/K_i^+dt (10^{-3} s^{-1})	V/Vdt (10^{-3} s^{-1})
110 Cl^-*	85·3 ± 8·0 (52)	−4·16 ± 0·7 (27)	13·2 ± 0·9 (55)
110 Cl^-, fur	13·6 ± 3·0 (19)	−1·8 ± 0·7 (9)	3·3 ± 0·8 (11)
110 Cl^-, 0 K^+	41·7 ± 6·0 (25)	−12·6 ± 1·8 (10)	7·5 ± 0·9 (8)
110 Cl^-, stroph	84·0 ± 9·0 (14)	−1·5 ± 0·9 (7)	14·7 ± 2·2 (9)
110 Cl^-, 0·5 Ba^{2+}, stroph	—	−1·7 ± 0·61 (7)	13·7 ± 0·9 (11)
110 Cl^-, 10 Ba^{2+}	92·0 ± 8·0 (18)	9·0 ± 1·3 (5)	3·0 ± 0·4 (3)
110 Cl^-, 10 Ba^{2+}, stroph	—	7·7 ± 2·1 (7)	3·3 ± 1·1 (6)
Solution	dE_i/dt (mV s^{-1})	dCl_i^-/dt (mmol l^{-1} s^{-1})	dK_i^+/dt (mmol l^{-1} s^{-1})
110 Cl^-*	−0·97 ± 0·07 (122)	0·89 ± 0·07 (51)	−0·48 ± 0·07 (23)
110 Cl^-, fur	−0·08 ± 0·2 (10)	0·14 ± 0·04 (18)	−0·15 ± 0·08 (9)
110 Cl^-, 0 K^+	−0·86 ± 0·2 (7)	0·39 ± 0·06 (22)	−1·20 ± 0·18 (9)
110 Cl^-, stroph	1·8 ± 0·4 (7)	0·75 ± 0·12 (14)	−0·07 ± 0·06 (12)
110 Cl^-, 0·5 Ba^{2+}, stroph	3·8 ± 0·9 (23)	—	−0·15 ± 0·05 (7)
110 Cl^-, 10 Ba^{2+}	2·7 ± 0·7 (11)	1·1 ± 0·11 (20)	0·65 ± 0·19 (5)
110 Cl^-, 10 Ba^{2+}, stroph	3·6 ± 1·0 (7)	—	0·86 ± 0·22 (7)

Values are means ± S.E.M. with number of experiments (n) in parentheses.
* Concentrations in mmol l^{-1}.

of electrical intracellular potential E_i. Cl_i^- began to change after 2·9 ± 0·6 (17 tissues), Ch_i^+ after 2·3 ± 0·43 s (19 tissues) and K_i^+ after 4·3 ± 1·1 s (11 tissues). Thus the change in Cl_i^- and Ch_i^+ began at the same time. The finding that K_i^+ changes a few seconds later does not mean that K_i^+ does not to begin to change simultaneously with Cl_i^- and Ch_i^+. The rate of change in K_i^+ is relatively lower and consequently the point at which the signal can be distinguished from the noise (~ 0·5 mV) appears later. The time at which E_i begins to change (taken as $t = 0$ for the ionic changes) is a result of the response of the whole tissue. Thus the choice of $t = 0$ is irrelevant in the present context.

When furosemide was added together with the Cl^-, it reduced the influx of Cl^-, K^+ and H_2O by 70–80% (Table 3 and Fig. 3). From the rapidity of its action one must conclude that its effect is on the membrane itself. When furosemide was added alone, there was no significant change in K_i^+ or in the intracellular volume.

The requirements for K^+ and Cl^-

In these experiments Cl^- was increased from 4 to 110 mmol l^{-1}, while K^+ was removed simultaneously. This reduced the influx of Cl^- (J_{Cl}) and of water (J_{H_2O}) by 50% (Table 3) compared to the case where K^+ remained in the ventricular solution. This way of testing the effect of K^+ removal might not be optimal, since it is probably

impossible to reduce K^+ at the very surface from 2 to 0 mmol l^{-1} while Cl^- is increased. In three cells in one tissue, K^+ was removed 30 s prior to Cl^- addition. Here the influx of Cl^- was reduced by 75%, which is 25% more than the case where K^+ was removed simultaneously with Cl^-.

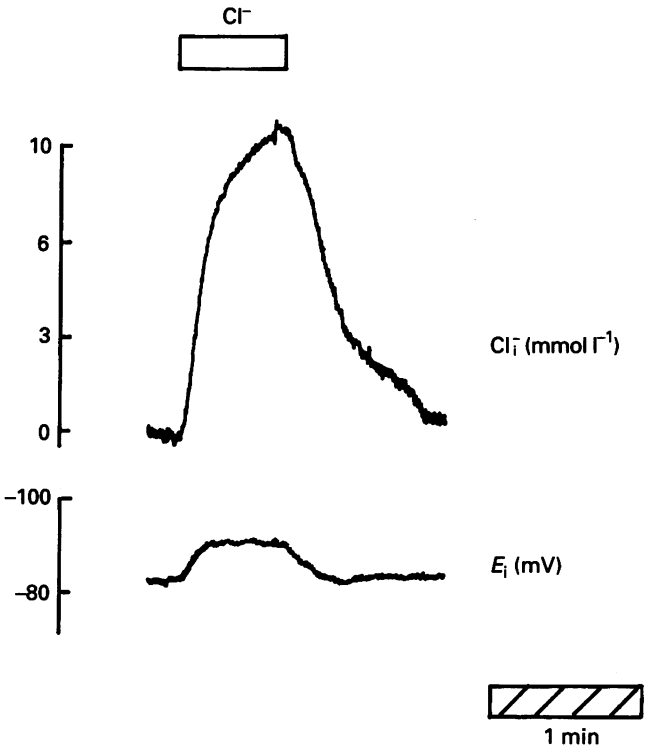

Fig. 1. Changes in intracellular Cl^- concentration (Cl_i^-) and electrical potential (E_i) recorded by a double-barrelled ion-selective microelectrode. The cell was initially bathed in a saline solution with a low Cl^- concentration, 4 mmol l^{-1}. The effect of an abrupt increase in ventricular Cl^- concentration to 110 mmol l^{-1}, as indicated by the open bar marked Cl^-, was tested. The electrical potential was measured relative to the abluminal bath (the blood side). The change of the electrical potential of the luminal (ventricular) bath was +2 mV during the addition of Cl^-; this is not shown.

It was tested whether other permeable anions could replace Cl^-. In four cells the volume changes were studied when NaCl was returned to the ventricular solution, these changes were compared to the effects of using $NaNO_3$, NaSCN or sodium acetate (Fig. 4). These anions were permeable, since they caused a hyperpolarization of the cell. Unlike Cl^-, they did not, however, effect any immediate increase in volume; their initial effect was a cell shrinkage.

The effects of inhibition of the Na^+, K^+-ATPase

When Cl^- was added to the external solution, there was an influx of K^+ into the cell against the electrochemical gradient. If this accumulation was mediated by the pump either directly *via* an increase in pump rate or indirectly via a closure of K^+

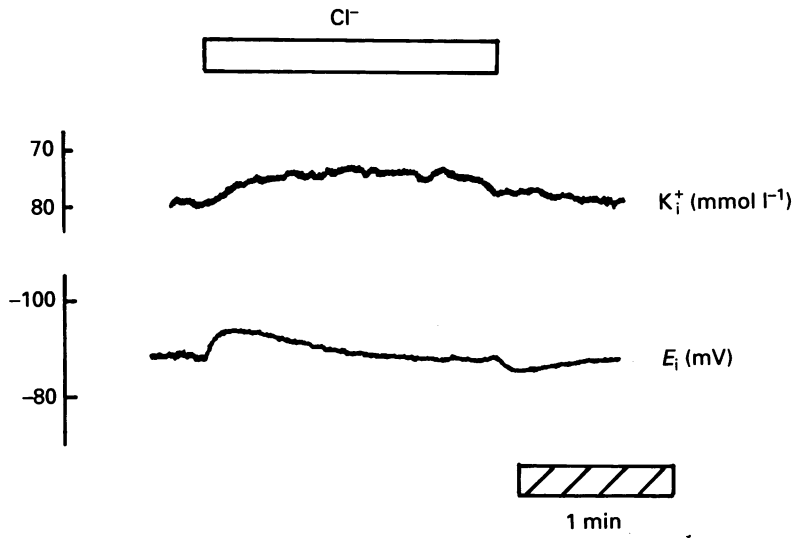

Fig. 2. Changes in intracellular K^+ concentration (K_i^+) and electrical potential (E_i) during influx of Cl^- across the ventricular cell membrane of a cell depleted of Cl^-. Otherwise as in Fig. 1.

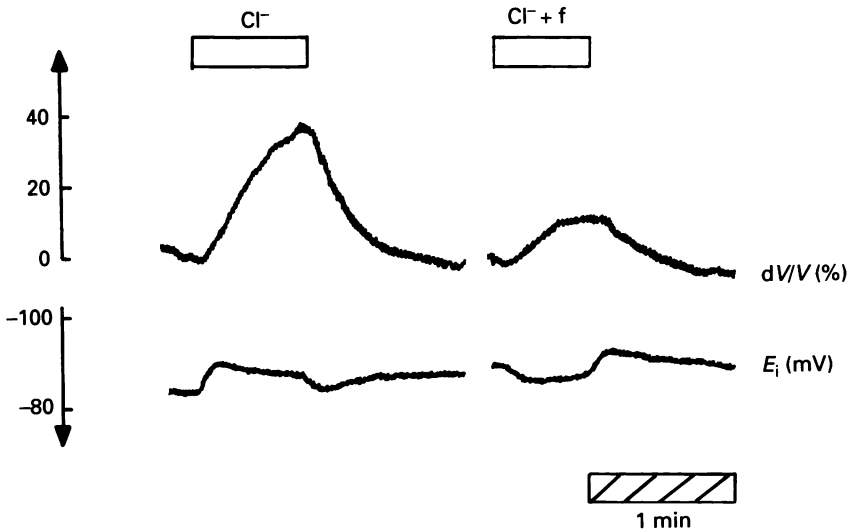

Fig. 3. At the bar marked $Cl^- + f$, the effects of furosemide were tested on the volume changes induced by the addition of Cl^- ions to the ventricular solution. The recordings are successive and from the same cell. Other details as in Figs 1 and 2.

channels (whereby K^+ ions which had been pumped in could not escape), then the increase would disappear if the pump was inhibited by ouabain or by strophanthidin. These inhibitors, however, when added simultaneously with the Cl^-, had little effect on the influx of Cl^-, K^+ or H_2O. Both $dCl_i^-/Cl_i^- dt$ and $dV/V dt$ (Table 2) as well as J_{Cl}

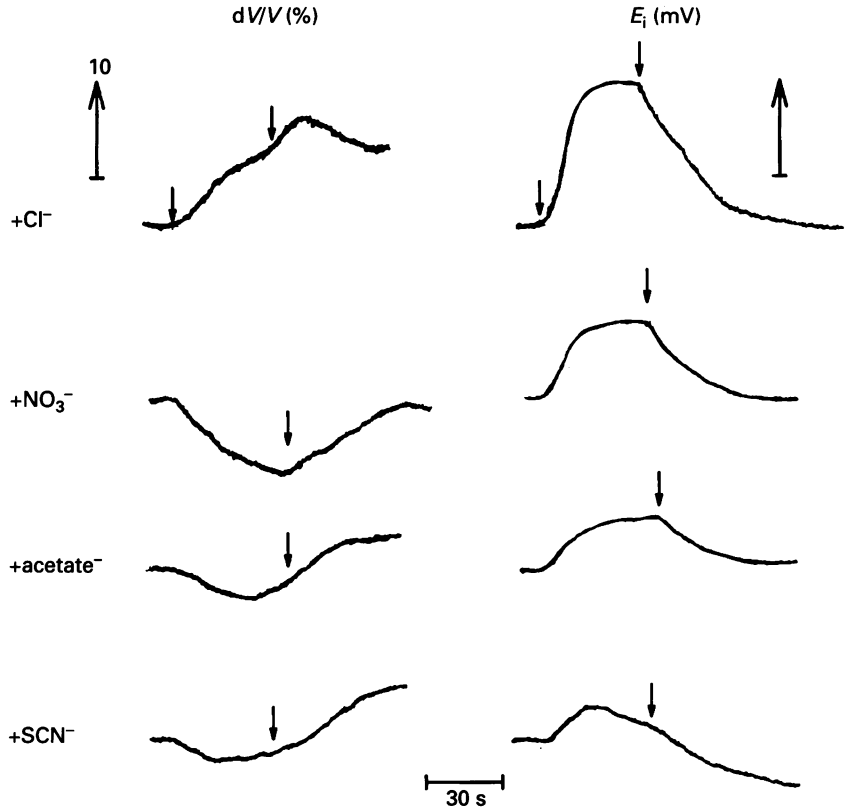

Fig. 4. Relative changes in cellular volume V and electrical potential E_i during readministration of Cl^-, NO_3^-, acetate$^-$ or SCN^- to the ventricular side of tissues previously bathed in solutions of low Cl^- content (4 mmol l^{-1}). The recordings are successive and from the same cell. The shrinkage observed in the three latter traces could reflect a wash-out of some Cl^- which had remained in the cell. The periods of exposure are indicated by the arrows. Other details as in the previous figures.

TABLE 3. Fluxes of Cl^-, K^+, and H_2O into cells when external Cl^- was increased abruptly to 110 mmol l^{-1} under different conditions

Solution	J_{Cl} (10^{-9} mol cm^{-2} s^{-1})	J_K (10^{-9} mol cm^{-2} s^{-1})	J_{H_2O} (10^{-6} cm^3 cm^{-2} s^{-1})
110 Cl^-*	1·03±0·08 (51)	1·05±0·15 (23)	13·2±0·9 (55)
110 Cl^-, fur	0·18±0·05 (18)	0·28±0·15 (9)	3·3±0·8 (11)
110 Cl^-, 0 K^+	0·46±0·07 (22)	−0·48±0·07 (9)	7·5±0·9 (8)
110 Cl^-, stroph	0·99±0·16 (14)	1·41±1·21 (12)	14·7±2·2 (9)
110 Cl^-, 0·5 Ba^{2+}, stroph	—	1·09±0·40 (7)	13·7±0·9 (11)
110 Cl^-, 10 Ba^{2+}	1·12±0·11 (20)	0·87±0·29 (5)	3·0±0·4 (3)
110 Cl^-, 10 Ba^{2+}, stroph	—	1·28±0·33 (7)	3·2±1·1 (6)

Values are means±S.E.M. with numbers of experiments (n) in parentheses.
* Concentrations are in mmol l^{-1}.

and J_{H_2O} (Table 3) were unchanged. dK_i^+/K_i^+dt was reduced by 65%, but since dK_i^+/K_i^+dt is small, this decrease does not count heavily in the calculation of J_K.

Changes in E_i in response to addition of Cl^- to the ventricular solution

When Cl^- was increased from 4 to 110 mmol l^{-1}, there was a hyperpolarization of E_i at a rate of $-0{\cdot}97$ mV s^{-1}. This proceeded to a maximum hyperpolarization of $-8{\cdot}3 \pm 0{\cdot}8$ mV (36 tissues) after about 15 s, whereafter the membrane potential depolarized again and attained a new steady state at -86 mV (see Figs 1–4). The ventricular solution became 2–4 mV more positive during the change to high chloride. Thus the total maximum change of electrical potential across the ventricular membrane amounted to some -14 mV. Although the equilibrium potential E_K remained larger than E_i (it changes from -98 to -102 mV), one might argue (1) that a more negative cell could retain more K^+ which would lead to an influx of K^+, and (2) that the difference between the E_i and E_K is small (as low as 5 mV); consequently any systematic error that would estimate E_i 5 mV too positive would lead to the erroneous conclusion that the K^+ influx proceeded against its electrochemical gradient.

To counter these arguments, it is sufficient to consider that the intracellular electrical potential E_i depolarized at a rate of $1{\cdot}8$ mV s^{-1} when ouabain or strophanthidin was added (Table 1), while the influx of K^+ was unaffected. To further substantiate this point, Ba^{2+} (0·5 or 10 mmol l^{-1}) was added simultaneously with the increase in Cl^-. This caused marked depolarizations, 3–4 mV s^{-1} without affecting the influx of K^+. Thus K^+ moves into the cell at a constant rate, independent of whether the cell hyperpolarized or depolarized. The conclusion is again that the mechanism of K^+ entry is electroneutral and secondarily active.

The electrical potential of the ventricular solution became 2–4 mV more positive relative to the blood side, when Cl^- was returned. These changes are too small to effect any significant ionic currents across the cell membranes, given the electrodiffusive permeabilities of the membranes (Zeuthen, 1987; Zeuthen *et al.* 1987*a*).

High Ba^{2+}-concentrations uncouple KCl transport from H_2O transport

When 10 mM-Ba^{2+} was added simultaneously with the increase in Cl^- from 4 to 110 mmol l^{-1}, J_{H_2O} was reduced by 80%. The Ba^{2+}, however, did not affect the transport of Cl^- and K^+ into the cell (Table 3). It did so without affecting the osmotic water permeability L_p. In four cells L_p was measured by adding 100 mmol l^{-1} of mannitol simultaneously with 10 mmol l^{-1} of Ba^{2+}. L_p was $1{\cdot}9 \pm 0{\cdot}6$ 10^{-4} cm s^{-1} (osmol l^{-1})$^{-1}$, which is not significantly different from values obtained in tissues bathed in Cl^--free saline solutions (see below), and in solutions of normal Cl^- content (Zeuthen, 1991).

Nature of the H_2O fluxes

The nature of the water fluxes was tested with both influxes and effluxes across the ventricular membrane. An influx of H_2O was obtained by returning Cl^- to the ventricular solution of tissues adapted to low external Cl^- concentrations, as described in the previous section. In the present experiments, it was tested whether

the influx of H_2O could be stopped by a simultaneous increase in the osmolarity of the ventricular solution.

An efflux of H_2O was obtained by removing Cl^- from the ventricular solution of a tissue adapted to solutions of normal Cl^- concentrations (110 mmol l^{-1}). It was tested

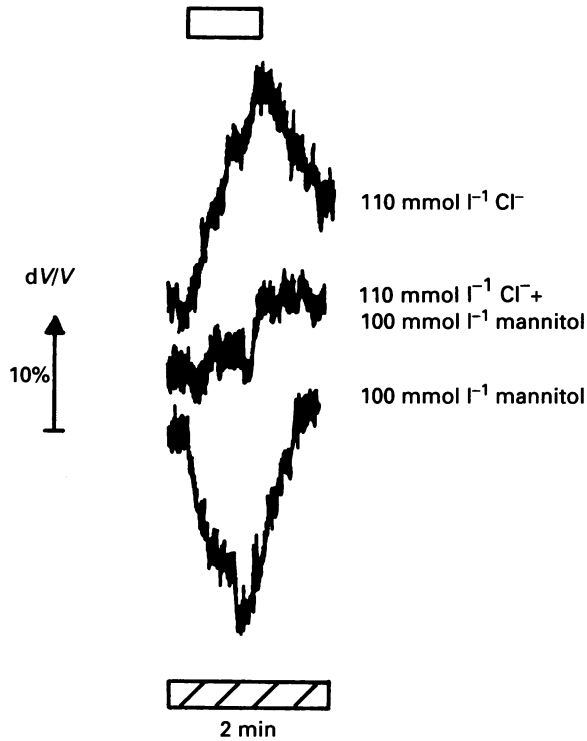

Fig. 5. Relative changes in cell volume V in response to abrupt changes in ventricular solution composition; successive recordings from the same cell which initially was bathed in saline solutions with a low content of Cl^- (4 mmol l^{-1}, solution 5, Table 1). At $t = 0$ the ventricular solution was changed to a saline solution with 110 mmol l^{-1} of Cl^- (solution 1) which caused the cell to swell, upper trace. If 100 mmol l^{-1} of mannitol was added at the same time as the increase in Cl^- (110 mmol l^{-1} $Cl^- + 100$ mmol l^{-1} mannitol, solution 4) then the cell swelled only slightly. If the ventricular solution was changed from the saline with low Cl^- content (solution 5) to one of similar composition but where 100 mmol l^{-1} of mannitol had been added, then the cell shrank.

whether the efflux of H_2O could be stopped by a simultaneous decrease in the osmolarity of the ventricular solution. Numerical values and statistics are given in Figs 7, 8, 9 and Table 4.

The influx of H_2O induced by the influx of Cl^- and K^+ can proceed against an osmotic gradient of more than 100 mosmol l^{-1}

It was shown above that, if a cell had been depleted of Cl^- and if Cl^- was returned abruptly to the ventricular solution (a change from 4 to 110 mmol l^{-1}), then there was an immediate influx of K^+ and of H_2O. In the present series of experiments this

influx of water was inhibited by adding various amounts of mannitol simultaneously with the Cl^- to the external solution. An example is given in Fig. 5, which shows that the swelling of the cell induced by the influx of Cl^- and K^+ cannot be stopped, unless there is an addition of about 100 mosmol l^{-1} of mannitol to the external solution. In all experiments the rate of changes in the intracellular concentrations were too small

TABLE 4. Changes in intracellular amounts of K^+ and Cl^- during simultaneous changes in ventricular Cl^- concentration and osmolarity

Solution change	dK_i^+/K_i^+dt (10^{-3} s^{-1})	dK_i^+/dt (mmol l^{-1} s^{-1})	dCl_i^-/Cl_i^-dt (10^{-3} s^{-1})	dCl_i^-/dt (mmol l^{-1} s^{-1})
		Decreases in external Cl^- and osmolarity		
1–5	4·4±0·8 (5)	0·36±0·07 (5)	−90·5±20·6 (17)	−2·5±0·23 (16)
1–5 (+fur)	—	—	−26·0±5·4 (3)	−1·0±0·3 (4)
1–6	5·1±2·1 (5)	0·39±0·16 (5)	−98·7±10·0 (6)	−2·6±0·4 (6)
1–7	1·6±3·3 (6)	0·13±0·26 (7)	−117·0±6·0 (11)	−3·1±1·1 (11)
1–7 (+fur)	—	—	−62·5±3·6 (4)	−1·3±0·2 (4)
		Increases in external Cl^- and osmolarity		
5–1	−4·2±0·7 (27)	−0·48±0·07 (23)	85·3±8·0 (52)	0·89±0·07 (51)
5–4	−4·1±1·4 (3)	−0·60±0·12 (4)	111·7±13 (6)	1·10±0·14 (6)

Values are means±S.E.M. with numbers of experiments (n) in parentheses.

to affect the magnitude of the osmotic gradient, since the sum of dCl_i^-/dt and dK_i^+/dt is about −2 mmol l^{-1} s^{-1} (Table 4).

The water influxes are summed up and compared with the other findings in Fig. 7. The points form a line which is shifted towards positive (influx) values of J_{H_2O} compared to the line obtained in experiments where only the osmolarity was changed (data from Zeuthen, 1991). It looks as if the joint Cl^- and K^+ influx adds a constant influx of 12×10^{-9} l cm^{-2} s^{-1} to the osmotically induced efflux J_{H_2O}, irrespective of the osmotic concentration of the bulk solutions. The L_p was not affected by the flux of ions; $\Delta J_{H_2O}/\Delta$Osm was the same irrespective of the ion fluxes.

The efflux of H_2O induced by the efflux of Cl^- and K^+ can proceed against an osmotic gradient of up to 70 mosmol l^{-1}

Cells were bathed in control saline (solution 1) and the external solution was changed to solution 5 (Table 1), a change from 110 to 4 mmol l^{-1} Cl^-. This caused an efflux of water, which began within 0·5 s of the Cl^- efflux (Fig. 6). This experiment was then repeated, but with each millimole of NaCl being replaced by only 0·75 mmol of sodium isethionate. Thus Cl^- was removed from the ventricular solution simultaneously with a reduction of osmolarity of about 45 mosmol l^{-1}, this is a change from solution 1 to 6. Despite the fact that the initial osmotic forces indicate an influx of water, an efflux resulted (Fig. 6). In this experiment the osmotic forces which are directed into the cell are not strong enough to match the efflux generated by the efflux of K^+ and Cl^-. If the external osmolarity was reduced by about 100 mosmol l^{-1} (a change from solution 1 to solution 7) at the same time as Cl^- was removed, the direction of water transport was reversed and water moved into the

cell, but at a rate which was not significantly different from zero (Figs 6 and 7). In this case the inwardly directed osmotic gradient matched the effect of the outwardly directed Cl^- and K^+ flux. In all experiments the rates of change in intracellular ion concentrations were too small to affect the magnitude of the osmotic gradient, since the sum of dCl_i^-/dt and dK_i^+/dt was about $-2{\cdot}5$ mmol l^{-1} s^{-1} (Table 4).

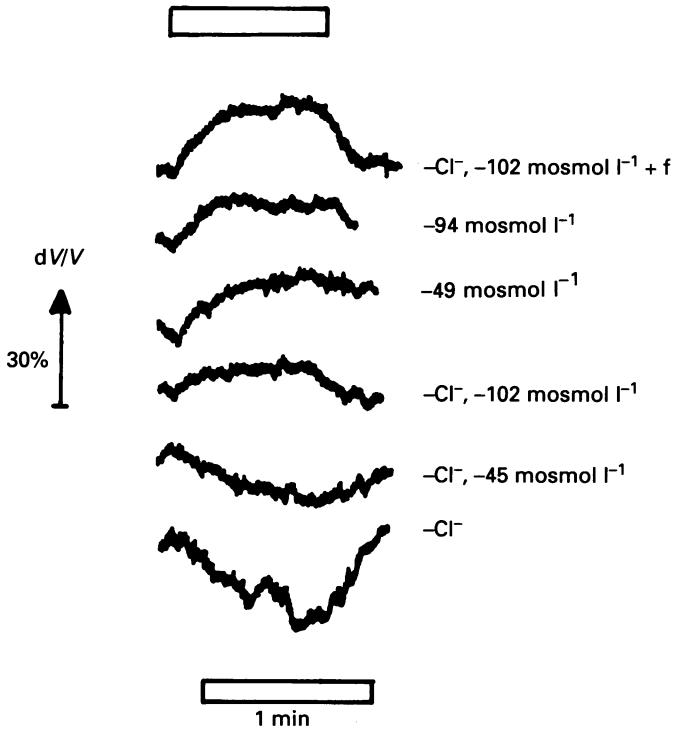

Fig. 6. Relative changes in cell volume (dV/V) in response to abrupt changes of the composition of ventricular solution. The cell was bathed in control saline (solution 1, Table 1) before and after each test, the duration of which is indicated by the open bar. The figure shows successive recordings from the same cell, beginning with the lower one. The composition of the test solution is indicated at the right. Removal of Cl^- caused the cell to shrink ($-Cl^-$; solution 5). Removal of Cl^- together with an intermediate reduction of osmolarity caused a shrinkage ($-Cl^-$, -45 mosmol l^{-1}; solution 6); removal of Cl^- and a large reduction of osmolarity caused swelling ($-Cl^-$, -102 mosmol l^{-1}, solution 7); intermediate reduction of osmolarity caused cell swelling (-49 mosmol l^{-1}; obtained by removal of NaCl); a large reduction of osmolarity caused swelling (-94 mosmol l^{-1}; obtained by removal of NaCl); and finally a removal of Cl^- and a large reduction of osmolarity (solution 7) in the presence of furosemide caused a swelling of the cell.

Membranes treated with furosemide responded to simultaneous changes in external osmolarity and Cl^- as if only the osmolarity had been changed: furosemide abolished the effects of removing external Cl^-, but did nothing to change the water permeability and the H_2O fluxes induced by changing osmolarity (Zeuthen, 1991).

The experiments are summed up in Fig. 7. When Cl^- is removed at the same time as the osmolarity is reduced, values for J_{H_2O} lie around a line which is shifted towards

negative values (efflux) of J_{H_2O}. It appears as if the removal of Cl^- causes a constant efflux of water of some 10×10^{-9} l cm^{-2} s^{-1}, which is superimposed upon the water flux induced by simple osmosis. If furosemide was applied simultaneously with the removal of Cl^- and the reduction in osmolarity, then the values of J_{H_2O} are similar to those obtained in the experiment where only osmolarity was changed.

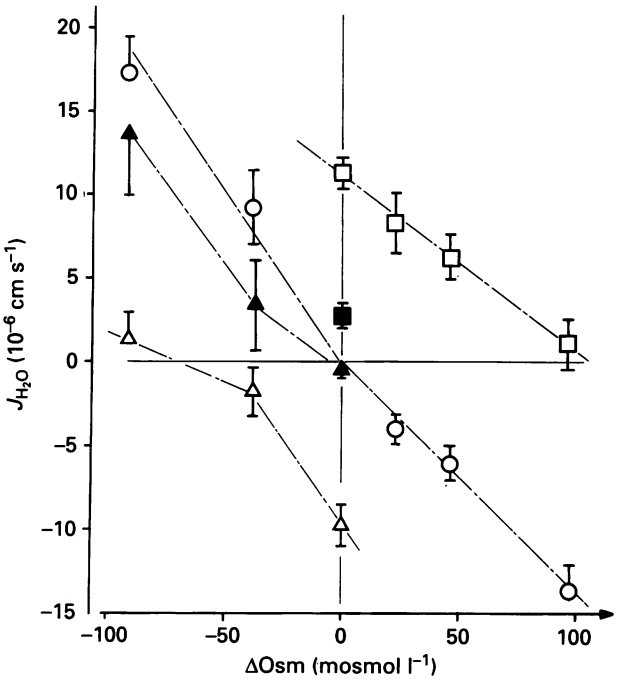

Fig. 7. Influxes (positive) and effluxes (negative) of H_2O across the ventricular membrane. ○, the fluxes obtained when only osmolarity was changed (data from Zeuthen, 1991). □, the fluxes when the osmotic changes were performed simultaneously with a Cl^- influx into cells previously depleted of Cl^- (as in Fig. 5). △, the fluxes when the osmotic changes were performed simultaneously with a Cl^- efflux from cells initially bathed in physiological saline (as in Fig. 6). Filled symbols show the effects of simultaneous addition of furosemide. Positive changes in osmolarity (ΔOsm) were obtained by adding mannitol (shifts from solutions 1 to 2, 3 or 4, Table 1), negative changes by removal of NaCl.

The water permeability in low Cl^- solutions

In order to asses the water permeability of cells adapted to solutions with Cl^- concentrations less than 4 mmol l^{-1} (solution 5, Table 1), Ch_i^+ was recorded while 100 mmol l^{-1} of mannitol was added to the ventricular solution. The cell shrank at a rate indicative of an L_p of $1{\cdot}4 \pm 0{\cdot}4 \times 10^{-4}$ cm s^{-1} (osmol l^{-1})$^{-1}$ ($n = 11$), which is identical to the L_p determined in cells adapted to normal osmolarities in Cl^- solutions (Zeuthen, 1991). In some tissues, which had been in low Cl^{-1} concentrations for more than an hour, the addition of mannitol did not, however, induce any cell shrinkage. One possibility could be that solid material inside the cell prevented further shrinkage.

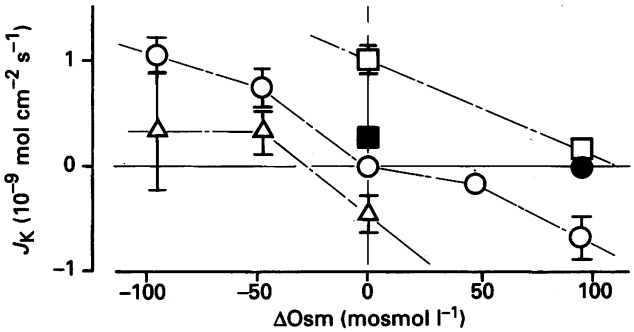

Fig. 8. Influxes (positive) and effluxes (negative) of K^+ during the same osmotic experiments described in Fig. 7. ○, the fluxes obtained when osmolarity alone was changed, data from previous paper. □, the fluxes when the osmotic changes were performed simultaneously with a Cl^- influx into cells previously depleted of Cl^-. △, the fluxes when the osmotic changes were performed simultaneously with the Cl^- efflux from cells initially bathed in physiological saline (solution 1, Table 1). Filled symbols show the effects of simultaneous addition of furosemide. The fluxes were calculated from data in Table 4 and Fig. 7. Error bars are shown when larger than points.

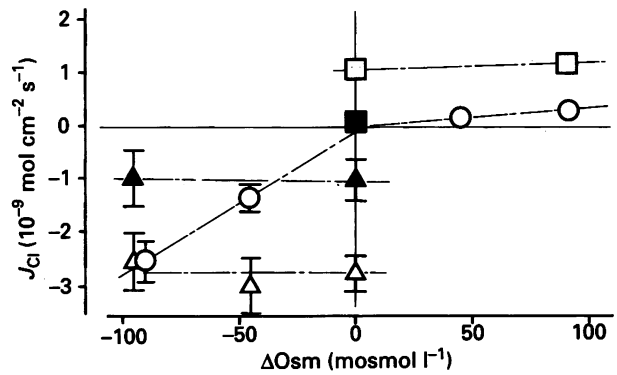

Fig. 9. Influxes (positive) and effluxes (negative) of Cl^- during changes in: external osmolarities (○), changes in external osmolarities and addition of Cl^- (□), changes in external osmolarities and removal of external Cl^- (△). Filled symbols represent the effects of furosemide. Otherwise as described in Fig. 8.

Fluxes of K^+ and Cl^- during simultaneous changes in external Cl^- concentration and osmolarity

K^+ fluxes. Both osmotic water flow (Zeuthen, 1991) and Cl^- fluxes (present paper) induced movements of K^+ across the membrane. In the combined experiment, where the osmolarity and the Cl^- concentration were changed simultaneously, the K^+ flux appeared as a superposition of those K^+ fluxes which would result from each experiment alone (Fig. 8).

When Cl^- was reduced from 110 to 4 mmol l^{-1}, there was an efflux of K^+ of about $0{\cdot}5 \times 10^{-9}$ mol cm^{-2} s^{-1}. When the osmolarity of the ventricular solution was reduced simultaneously, the K^+ flux was reduced and changed to an influx for osmotic reductions greater than 50 mosmol l^{-1}.

In the experiments where Cl^- was added to cells which have been adapted to low-Cl^- solutions, a change from 4 to 110 mmol l^{-1}, there was an influx of K^+ of $1{\cdot}1 \times 10^{-9}$ mol cm^{-2} s^{-1}. When the osmolarity of the ventricular solution was increased simultaneously, the influx of K^+ decreased; at osmolarities of 100 mosmol l^{-1} the K^+ flux was arrested. The fluxes are calculated from Zeuthen (1991; Table 2), and the present paper (Tables 2 and 3).

Cl^- fluxes. Those were mainly determined by the Cl^- gradient and were large compared to the fluxes induced by osmosis alone. Since Cl^- also penetrates the membrane by other pathways than the one given by the KCl transport system (Zeuthen, 1987; Zeuthen, Christensen & Cherksey, 1987*b*; Christensen, Simon & Randlev, 1989), it is difficult to specify which fraction of the Cl^- flux originates from water flow and which originates from other transport processes. The fluxes are presented in Fig. 9 and calculated from the same sources as the K^+ fluxes.

DISCUSSION

This paper shows that a flux of Cl^- induces secondary active, electroneutral transport of K^+ across the ventricular membrane. This was accompanied by a transport of H_2O, which could proceed against an osmotic gradient. Conversely, it was shown in the preceding paper (Zeuthen 1991) that an osmotic flux of H_2O could induce a flux of KCl. The data from the two papers suggest a common mechanism for the two effects. In both cases the ratio of salt to water was the same, 30 to 80 mmol l^{-1}, and in both cases the flux of salt was inhibited by furosemide. The findings are consistent with earlier findings in the *Necturus* gall-bladder (Zeuthen, 1982; Fisher & Spring, 1984), where it was shown that osmotic effluxes of H_2O induced a loss of K^+ and Cl^- from the cell. The findings can be expressed by the formal model, Fig. 10.

Secondary active transport of K^+ in relation to cellular ion transport

Since all known mechanisms which could mimic co-transport have been eliminated, it seems fair to suggest that the uphill movement of K^+ is energized by the Cl^- flux and that the coupling takes place in a co-transport protein. This is in agreement with the finding that the transport is inhibited by furosemide in concentrations of 10^{-4} mmol l^{-1}, an effect typical of co-transport. The fluxes of Cl^- and K^+ were of the same magnitude and were independent of the membrane potential. This suggests that the co-transport during influx was electroneutral, although a final discussion on this point ought to include measurements of transport of Na^+ and other ions.

KCl co-transport has been implicated to explain transport of K^+ and Cl^- across the lateral membrane of *Necturus* gall-bladder (Corcia & Armstrong, 1983; Reuss, 1983; Larson & Spring, 1984) and in the kidney (Greger & Schlatter, 1983). In the choroid plexus patch clamp studies of the ventricular membrane (Christensen & Zeuthen, 1987*b*; Brown, Loo & Wright, 1988; Loo, Brown & Wright, 1988; Christensen *et al.* 1989) have shown that transport via channels only can account for 10–50% of the transepithelial transport. Contrary to these views it has been shown that epithelial cells from the choroid plexus of *Necturus maculosus* have electrodiffusive con-

ductances for K^+ and Cl^-, which are sufficient to explain transepithelial transport (Zeuthen, 1987; Zeuthen *et al.* 1987*a*,*b*). If transepithelial transport of salt is transcellular, then two points of view will oppose each other. Either the KCl efflux from the cell is via a neutral co-transport, or it is mediated via electrical pathways

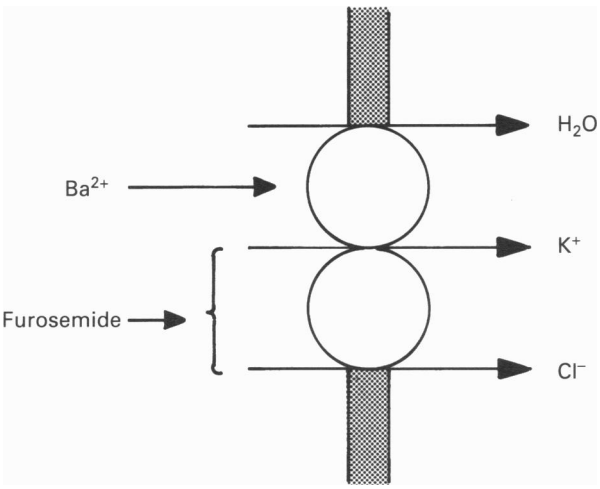

Fig. 10. Formal presentation of KCl–H_2O co-transport which indicates that H_2O fluxes implemented by osmotic gradients can give rise to movements of K^+ and Cl^- which in themselves are inhibited by furosemide (see Zeuthen, 1991). Fluxes of H_2O, K^+ and Cl^- can also be implemented by gradients of Cl^- across the membrane, present paper. Ba^{2+} (10 mmol l^{-1}) inhibits the coupling between the ion fluxes and the H_2O fluxes without affecting the ability of the fluxes to be generated by their appropriate driving forces.

which are characterized by a lack of visible opening and closure events. That such a baseline current can have significant proportions has been suggested by Christensen & Zeuthen (1987*a*). From the present paper it must be concluded that KCl co-transport can be localized to basolateral type of membranes in epithelia, but whether this mode of transport is a consequence of the experimental conditions (cell swelling, removal of Cl^-, etc.) and whether it exists in tissues during normal transport remains to be elucidated.

The mechanism of coupling between KCl and H_2O transport

There are three mechanisms which can be ruled out as explanations for the coupling: (1) increased L_p, (2) unstirred layers and (3) low reflection coefficient for NaCl in the ventricular membrane. Instead I suggest that (4) specific proteins in the ventricular membrane are responsible for the coupling.

(1) When the Cl^- influx was initiated by the increase of Cl^- in the external medium from 4 to 110 mmol l^{-1}, there was an immediate influx of K^+ and of H_2O. It was estimated that the flux of H_2O into the cells had commenced when the intracellular osmolarity given by K^+ and Cl^- had increased by less than one mosmol (Figs 1–3 and Table 3). Such a small driving force is insufficient to explain the flux of H_2O of 13×10^{-9} l cm^{-2} s^{-1} by simple osmosis, since an L_p of $1{\cdot}4 \times 10^{-4}$ cm s^{-1} (osmol l^{-1})$^{-1}$

(Zeuthen, 1991) is two orders of magnitude too low. It is not likely that the L_p is stimulated by a factor of 100 when fluxes of KCl are initiated. L_p was unchanged when the influx of H_2O was reduced by adverse osmotic gradients (see Fig. 7).

(2) The driving forces cannot arise from unstirred layers in the intracellular compartment, if the ion fluxes are evenly distributed across the membrane. When Cl^- was returned to the ventricular solution, it resulted in an influx of KCl, of 10^{-9} mmol cm^{-2} s^{-1}. If this flux was distributed uniformly across the membrane area, then the total concentration gradient inside the cell would be 0·1 mmol l^{-1}. This can be obtained from Fick's first law and assuming a diffusion coefficient of 10^{-5} cm^{-2} s^{-1} for KCl in the cytoplasma and a cell height, h, of 10 μm. This calculation can be performed in different ways (see e.g. p. 112 of House, 1974). Unstirred layers in the ventricular solution were found to be insignificant by means of microelectrodes (Zeuthen, 1991).

(3) The interactions between the fluxes of ions and water across the ventricular membrane could be explained, if the reflection coefficient σ for some salt was less than one. If, for example, $\sigma_{NaCl} \leqslant \sigma_{Na\text{-isethionate}} = 1$, then the return of Cl to the external solution would cause an immediate influx of water. There is no reason, however, to assume that σ_{NaCl} should be less than one in the membrane under study. NaCl has the same osmotic effects as mannitol (Zeuthen, 1991), which has a reflection coefficient of one across cell membranes. σ_{NaCl} has been recorded to be close to one in membrane vesicles from proximal tubules (Pearce & Verkman, 1989; Van der Goot, Podevin & Corman, 1989) and from small intestine (van Heeswijk & van Os, 1986), but about 0·5 in isolated, non-perfused proximal tubules (Welling, Welling & Ochs, 1987) and in vesicles from rat kidney brush border (Pratz, Ripoche & Corman, 1986). Obviously there is a need to compare the experimental conditions of the various rapports.

(4) The coupling between KCl and H_2O was specific for Cl^- (Fig. 4), required K^+ and was inhibited by low concentrations of furosemide. This strongly suggests, that the coupling depends on co-transport proteins associated with K^+ and Cl^-. Furosemide has been used in affinity gel chromatography to isolate a 260 kDa membrane protein (Cherksey & Zeuthen 1987; Jessen, Cherksey, Zeuthen and Hoffmann, 1989).

Ba^{2+} (10 mmol l^{-1}) uncoupled the KCl co-transport from the transport of H_2O without affecting the magnitude of the KCl fluxes or the water permeability (Fig. 10). Since Ba^{2+} sheds its water coat in order to penetrate into proteins (Latorre & Miller, 1983), this indicates that the site of action must be found in a protein environment, where it is energetically favourable for Ba^{2+} to dehydrate. The Ba^{2+} effect could be explained if the pathways for KCl and water opened up into the same protein environment. The effect is more difficult to explain, however, if the water channel is separate from the KCl co-transporter. In that case Ba^{2+} should interrupt distant communication. The data do not exclude the possibility that there is a population of water channels separate from the KCl–H_2O co-transport system.

In conclusion I suggest that the KCl co-transport system and water channels are parts or subunits of the same protein or that they each reside in proteins which are co-localized in the membrane.

It may be useful at this point to assess some of the consequences of the fact that

a flux of K^+ and Cl^- is located to co-transport proteins. Diffusion towards the proteins would cause a depletion of ions and therefore a decrease in osmolarity around the mouth of the protein, while diffusion away would result in an increase in osmolarity around the mouth of the protein. As a result an osmotic gradient would be established, which had the magnitude and direction to move water along with the ions if the water channel was located close to the pathways for K^+ and Cl^-. The present measurements suggest that the local hyper- and hypo-osmolarities could be of the order of 100 mosmol l^{-1}, since it takes an osmotic gradient of about 100 mosmol l^{-1} between the bulk solutions to stop the transport. Simple mathematical calculations show that radial diffusion could give rise to such osmotic gradients. However, depletion of each type of ion, the resulting concentrations at the active sites of the co-transport proteins and the modifications of these concentrations by fixed charges must be considered in setting up an appropriate model.

The above considerations, while partly realistic, cannot explain all data. When the movements of H_2O were arrested in a balance between an outward directed osmotic gradient generated by mannitol and inward directed force generated by Cl^- movements, the movements of K^+ stopped as well (Fig. 8). If the coupling was purely osmotic, then the flux of K^+ would proceed irrespective of the arrest of the H_2O movements, since there is no *a priori* reason why the K^+ flux driven by the Cl^- flux should be arrested by the arrest of the H_2O flux.

As a working model I therefore suggest that the pathway through the cell membrane can be divided into three components. (1) Permeation through bulk solution and cytoplasm takes place by diffusion in a radial way towards or away from the mouths of the proteins. (2) The central part of the protein has separate pathways for the ions and H_2O and ensures such properties as selectivity, coupling between K^+ and Cl^- and a high osmotic reflection coefficient for the ions, (the pathways for the ions are blocked by furosemide). (3) In between the external solutions and the central part of the protein there is a pathway which is shared by the ions and the H_2O. The properties of this region impose a coupling between the fluxes of H_2O and K^+. It is this coupling which causes the flux of K^+ to be arrested when the flux of water is reduced to zero as a balance between osmotic forces and the Cl^- flux (Fig. 8). Possibly Ba^{2+} interferes with the coupling between the H_2O flux and K^+ flux in this region.

Relations to epithelial transport

Transepithelial, isotonic transport of water occurs by osmotic influx of water into one end of the cell, but since the final secretion is isotonic with the bath, there is the conceptual problem of how water leaves the other end of the cell. In the gall-bladder from the bull-frog, it was shown that, if the tissues were bathed in solution diluted by a factor of 4 relative to physiological values, then the cells were 25 mosmol l^{-1} hyperosmolar relative to both the external bathing solutions and the solutions in the lateral intercellular spaces (Zeuthen, 1983). A similar hyperosmolarity was found in *Necturus* gall-bladder, where it was found to depend on the presence of O_2 (Zeuthen, 1981, 1982). In order to explain the osmotic imbalance and how water could exit from the cell, it was suggested that the reflection coefficient for KCl across the cell membrane was less than one (Zeuthen, 1982, 1983). An epithelial cell, with $\sigma_{KCl} \leqslant 1$ in the membrane across which KCl and H_2O left the cell, would be able to utilize the energy contained in the ionic fluxes to expel H_2O against the osmotic

gradients. It should be emphasized that σ_{KCl} will depend on the transport processes for K^+ and Cl^-. σ_{KCl} will therefore not be constant but will be a function of the concentrations of K^+ and Cl^-.

One of the best established, yet least understood properties of leaky epithelia is their ability to transport fluid against an osmotic gradient. Values between 20 and 150 mosmol l^{-1} have been recorded (for references see the Introduction). This ability of whole tissues has important qualitative and quantitative similarities with the properties which have been described in the present paper for transport across a single epithelial cell membrane: (1) the reduction in the fluxes of water is linearly related to the adverse osmotic gradient which is imposed, (2) the osmolarity at which transport is arrested is similar, around 100 mosmol l^{-1}, and (3) the maintenance of the flux of H_2O against the osmotic gradient requires energy. In the case of the intact tissue this is supplied by the metabolism of glucose; in the case of the single membrane, energy is derived from the imposed gradient of Cl^-. In both cases it is the ion fluxes which couple the energy to the fluxes of water. The present investigation suggests, that the ability of the whole tissue to transport against osmotic gradients derives from the properties of the membrane across which KCl and H_2O leave the cell. It should be emphasized, however, that the present investigation deals with transient states. Thus the suggestion above relies on the assumption that the coupling is also operating in steady states.

The stoichiometry and the energetics of coupling

The stoichiometry of the KCl–H_2O co-transport system is of the magnitude which would be expected from the transepithelial transport of NaCl with a concentration of about 120 mmol l^{-1} in amphibian tissues. The Na^+, K^+-ATPase pumps 3 Na^+ for 2 K^+, which means that the efflux of Na^+ is accompanied by a passive efflux of K^+ (Zeuthen & Wright, 1981) with a concentration two-thirds of the NaCl, 80 mmol l^{-1}. This is close to the ratio found for the KCl–H_2O co-transport system, 40–70 mmol of K^+ per litre of H_2O. The present findings do not aspire to explain the precise mechanism behind isosmotic transport, yet the numbers do indicate that the KCl–H_2O system can function close to isotonicity.

There seems to be energy enough in the gradients of K^+ and Cl^- to move water against the osmotic gradient. The energy which is available from a Cl^- flux of $10{\cdot}3 \times 10^{-9}$ mol cm^{-2} s^{-1}, which is energized by an electrochemical gradient of about 70 mV, is 7×10^{-2} J s^{-1} m^{-2}. This is more than sufficient to move water against its osmotic gradient. A J_{H_2O} of 10^{-9} l cm^{-2} s^{-1}, which moves against an osmotic gradient of 100 mosmol l^{-1}, requires 2×10^{-3} J s^{-1} m^{-2}. This is 1/50 of the energy available from the ion flux. The possibility of co-transport with other ions, Na^+, HCO_3^- etc, will have to be considered in a final evaluation.

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