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AN ELECTROGENIC NA^+/K^+ PUMP IN THE CHOROID PLEXUS

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Summary

Intracellular electrical potential and potassium activity was measured by means of microelectrodes in the epithelial cells of choroid plexus from bullfrogs (*Rana catesbeiana*). Ouabain applied from the ventricular side caused an abrupt depolarisation of 10 mV but only a gradual loss of potassium from the cells. Readministration of potassium to the ventricular solution of plexuses which were previously depleted of potassium, caused a hyperpolarisation of about 4 mV. These two experiments are consistent with the notion of an electrogenic Na^+/K^+ pump situated at the ventricular membrane and which pumps potassium into the cell and sodium into the ventricle. The numerical values obtained suggest that 3 sodium ions are pumped for 2 potassium ions. The permeability coefficient for potassium exit from the cell is calculated to be $1.24 \cdot 10^{-5}$ $cm^{-1} \cdot s^{-1}$ expressed per cm^2 of flat epithelium.

Sodium is actively transported across many epithelial cells and the model usually invoked to explain this phenomenon is the first proposed for the frog skin by Koefoed-Johnsen and Ussing [1]. In this model it is envisaged that sodium enters the epithelium across one face of the epithelium down its electrochemical potential gradient, and then it is pumped out across the other face of the cell by a sodium potassium exchange pump. It is proposed that this Na^+/K^+ pump is similar to that found in single cells, and it performs the dual function of transepithelial sodium transport and the regulation of the intracellular sodium and potassium concentrations. Support for this hypothesis comes from observations that: (i) specific inhibitors of $(Na^+ + K^+)$-ATPases, e.g. ouabain, block active sodium transport across epithelia and eliminates the sodium and potassium concentration gradients between the intracellular and extracellular compartments [2—4]; and (ii) $(Na^+ + K^+)$-ATPases are distributed

asymmetrically between the two faces of epithelial cells [5—8]. Nevertheless, some experiments raise uncertainties about the link between net sodium transport across epithelia and the accumulation of potassium within the epithelium [3,9]. The interpretation of these experiments are complicated by the fact that in the epithelia so far studied the Na^+/K^+ pumps are not directly accessible owing to the presence of supporting tissue between the epithelium and the external bathing solutions. In the choroid plexus this problem does not arise as the $(Na^+ + K^+)$-ATPase [10] are located on the apical or brush border surface of the epithelium and this surface of the cell is directly exposed to the external bathing medium [6,11—13]. Thus, we have been able to use direct electrophysiological techniques to study the plexus Na^+/K^+ pump, and correlate these results with tracer flux studies [4,11—13].

Choroid plexuses were removed from mature bullfrogs (*Rana catesbeiana*) and mounted between perspex chambers as described previously [11,12]. Double-barrelled microelectrodes [14,15], one barrel sensitive to the intracellular potential (E_m) and the other to the internal potassium activity (K_i^+), were advanced into the epithelium from the ventricular side of the plexus. E_m was on average −44 mV (Table I) (maximum −70 mV) and was similar to values obtained by high impedance (40−100 MΩ) single barrelled electrodes. K_i^+ was on average 84 mM (Table I) which corresponds to an apparent intracellular K concentration of 110 mM, assuming an activity coefficient of 0.76. This is about 45 mM lower than that obtained by chemical methods [4]. The potassium equilibrium potential across the apical plasma membrane (E_K = 59 mV × $\log K_i^+/K_o^+$) was 101 mV. Thus, potassium was accumulated inside the cell against an electrochemical potential difference of about 57 mV.

Ouabain ($1 \cdot 10^{-4}$ M) in the ventricular solution caused an abrupt (2.4 mV ·

TABLE I

INTRACELLULAR POTENTIALS AND K^+ ACTIVITIES OF THE CHOROID PLEXUS OF BULLFROG

Results are ±S.D., numbers in parenthesis are number of observations; number of potentials.

Before ouabain		
Intracellular potential E_m (mV)	−44.0 ± 2.9	(96;6)
Chemical potential E_K (mV)	+ 101.1 ± 6.3	(81;6)
Electrochemical potential $E_m + E_K$ (mV)	+56.5 ± 7.2	(81;6)
K^+ activity (mM)	83.5	(81;6)
Apparent concentration (mM)	109.9	
Effect of ouabain ($1 \cdot 10^{-4}$ M)		
Initial depolarisation E_m (mV)	−10.2 ± 2.5	(5;5)
Rate of initial depolarisation (mV/s)	2.4 ± 1.1	(5;5)
Onset of change in K_i^+ (s) *	5.5 ± 1.8	(5;5)
Initial rate of change in E_K (mV/s)	0.11 ± 0.038	(5;5)
Repolarisation (mV)	3.3 ± 1.1	(4;4)
Time of repolarisation (s)	55.2 ± 27.0	(4;4)
Effect of readministration of K^+ (10 mM)		
Hyperpolarisation (mV)	4.2 ± 2.9	(24;5)
Rate of hyperpolarisation (mV/s)	1.9 ± 1.9	(24;5)

* Calculated from the time of onset of change in E_m.

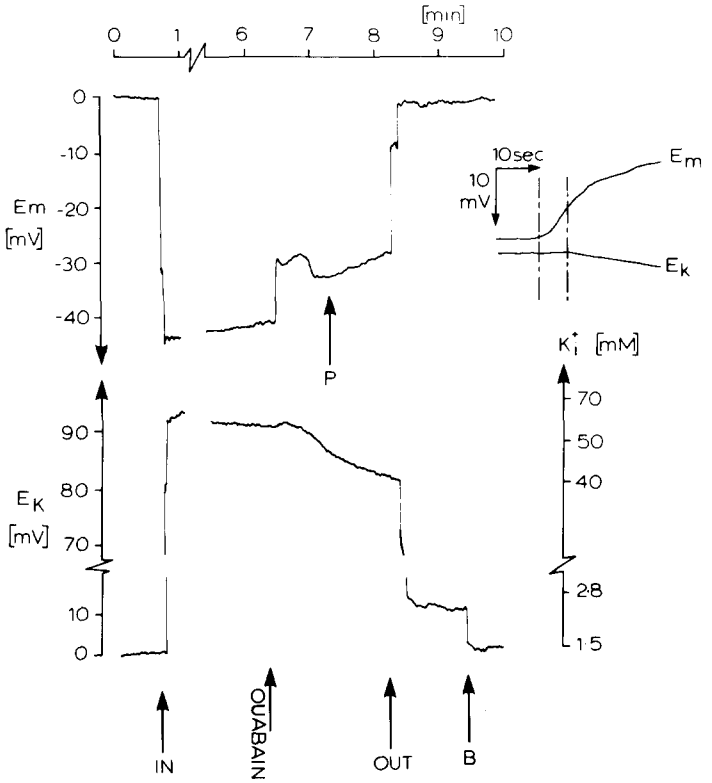

Fig. 1. A recording of the intracellular electrical potential E_m, intracellular K^+-activity (K_i^+) and the chemical potential E_K. Addition of ouabain (10^{-4} M) caused an abrupt depolarisation of −10 mV in E_m complete in about 5 s and a slow decrease in E_K (or K_i^+). There was no significant decrease in K_i^+ until, on average, 5 s after the abrupt depolarisation had started. After an average of 55 s there was (in 4 out of 5 animals) a small repolarisation in E_m of an average 3.3 mV (at P). The change in K_i^+ caused by the ouabain resulted in an elevated K^+ activity adjacent to the cells: when the electrode was retracted from the poisoned cell K_o^+ was about 2.5 mM just outside the cell (B) compared to the 1.5 mM of the perfusion fluid. This layer was up to 600 μm thick. The choroid plexus was mounted in a perspex chamber and bathed in (mM) 85 NaCl/2 KCl/1 $MgSO_4$/1 $CaCl_2$/25 $NaHCO_3$ equilibrium with 95% O_2/5% CO_2, pH 7.3. The electrode was advanced into the cells from the ventricular surface at IN and withdrawn at OUT.

s^{-1}) depolarisation of E_m of 10 mV (Fig. 1), washing away ouabain did not repolarize the cell. This was followed by a slow decline in E_m which was interrupted 55 s after the addition of the glycoside by a small transient repolarisation (P) of 3.3 mV. The reason for this repolarisation is unclear, but it was not observed in experiments with Necturus plexus. Within 15 min E_m decreased to about −12 mV and remained virtually constant for up to 80 min. K_i^+ began to decrease 5.5 s after the onset of the change in E_m, and even 5 s after the fast depolarisation of E_m by 10 mV was complete, K_i^+ had only decreased from 84 to 82 mM. The subsequent decrease in K_i^+ (during which K_i^+ approached electrochemical equilibrium across the cell membrane) could be described by a double-exponential curve with time constants of 15 and 74 min. 80 min after the application of ouabain K_i^+ was only about 5 mM. These experiments suggest that the Na^+/K^+ pump in the apical membrane responsible for Na^+ secretion and K^+ accumulation is electrogenic.

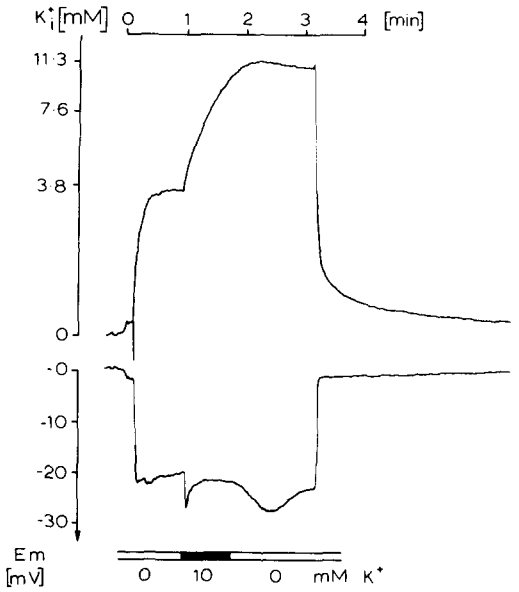

Fig. 2. The effect of readministration of K^+ to the ventricular fluid of a plexus which had been depleted of K^+ by storage for 1—2 h at 2°C in a K^+-free solution. The horizontal bar indicates the period where the ventricular solution from containing 87 mM NaCl and 0 mM KCl was replaced by one containing 77 mM NaCl and 10 mM KCl; it was otherwise composed as in the legend to Fig. 1. The K^+ caused a rapid increase in K_i^+ accompanied by a largely transient hyperpolarisation of E_m which resulted from the rapid onset of the current flow caused by the pump and the slower depolarizing effect of the elevated K_o^+. When K_o^+ was subsequently removed, E_m first hyperpolarized as it approached the new equilibrium potential for K^+ given by the temporarily increased K_i^+.

In a second type of experiment the epithelium was depleted of intracellular potassium by incubation in a potassium-free saline for 1—2 h at 2°C, and E_m and K_i^+ were monitored while potassium was restored to the ventricular fluid. Fig. 2 shows one experiment where K_i^+ was lowered from 84 to 3.8 mM, and potassium (10 mM) was added to the ventricular solution for about 1 min. The addition of potassium caused an abrupt (1.9 mV · s^{-1}) hyperpolarisation of 4.2 mV in E_m and an increase in K_i^+ at an initial rate of 0.3 mM · s^{-1}. This experiment confirms that potassium is actively accumulated within the epithelium by an electrogenic pump in the apical membrane.

The initial rate of fall in K_i^+ in the ouabain-treated epithelium is due to the passive leak of potassium from the intracellular K^+ pool across a membrane or membranes with an effective permeability, P_k, which may be calculated from the constant field equation knowing the electrical and chemical potentials (Table I), i.e.

$$J_K = -dc/dt \cdot H = \frac{-P_K \cdot E_m \cdot F}{RT} \cdot \frac{K_o^+ - K_i^+ \cdot \exp(E_m F/RT)}{1 - \exp(E_m F/RT)} \quad (1)$$

With a cell height of 10 μm, an initial dc/dt of 0.46 · 10^{-6} M · s^{-1} · cm^{-3}, E_m = −33.9 mV, K_o^+ = 1.5 mM and K_i^+ = 84 mM, the equation yields a P_K value of 1.24 · 10^{-5} cm^{-1} · s^{-1} expressed per cm^2 of flat epithelium. Using this estimate of P_k, and values of E_m and K_i^+ for the unpoisoned tissue, the passive potassium

efflux, which in the steady state must equal the active influx, may be calculated. This amounts to $-0.35 \cdot 10^{-9}$ mol $\cdot$ cm$^{-2} \cdot$ s^{-1} which is comparable to that obtained by radioactive tracers for the active potassium flux across the apical membrane [4,12].

It is also possible from the present experiments to assess the coupling ratio of the active Na^+/K^+ pump in the apical membrane. Due to the requirement that in the steady state any net active Na^+ and K^+ fluxes must balance the net passive Na^+ and K^+ fluxes, a link exists between (i) the maximum possible contribution of an electrogenic pump to the membrane potential (E_p), (ii) the ratio of the passive sodium and potassium permeabilities ($P_{Na}/P_K = b$) and (iii) the ratio of the number of sodium ions pumped to the number of potassium ions pumped (r), i.e.

$$E_p = 59 \text{ mV} \log 1/r \cdot \frac{rK_o^+ + bNa_o^+}{K_o^+ + bNa_o} \quad (2)$$

where K_o^+ and Na_o^+ are the activities of the external solutions [16,17]. If we assume that the ouabain-induced abrupt electrical depolarization is complete before the ion distributions deviate markedly from their steady-state values (which seems justified for at least K_i^+, Fig. 1): $E_p = -10.2$ mV (Table I). r would then be 1.5 for $b > 0.4$ and 3 for $b < 0.02$. Inspection of the sodium and potassium unidirectional fluxes across the apical membrane [4] and the relative electrochemical driving forces for sodium and potassium (ref. 4 and the present results) show that b needs to be greater than 0.4 to accommodate a passive sodium flux of at least the same order as the potassium flux. Thus, it is likely that $r = 1.5$ which suggests that 3 sodium ions are pumped out across the apical surface for every 2 potassium ions pumped in across this membrane. This is in agreement with the kinetic tracer studies [13] which indicate that the Na^+/K^+ pump has two binding sites on the external surface of the apical membrane. In the present calculations the inherent assumptions are that the constant field equation applies to the ouabain treated tissue, that Cl is passively distributed and that the choroidal epithelial cells have a uniform internal and external environment.

In summary, these electrophysiological experiments on the nature of the Na^+/K^+ pump on the apical surface of the choroid plexus epithelium indicate that the pump is electrogenic and are consistent with a ratio of three sodium ions pumped into the cerebrospinal fluid for every two potassium ions pumped into the epithelium. The result, together with those obtained from tracer studies [4,11—13] lead to the conclusion that the Na^+/K^+ pump in the apical membrane of this tissue is responsible for active sodium secretion across the epithelium and the maintenance of the high potassium and low sodium concentrations within the epithelium.

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References

1 Koefoed-Johnsen, V. and Ussing, H.H. (1958) Acta Physiol. Scand. 42, 298—308
2 MacKnight, A.D.C., Civan, M. and Leaf, A. (1975) J. Membrane Biol. 20, 387—407

3 Schultz, S.G. (1977) in Membrane Transport Processes (Hoffman, J.F., ed.), Vol. 1, pp. 213—227, Raven Press, New York
4 Wright, E.M. (1978) Reviews of Physiology, Biochemistry and Experimental Pharmacology, Springer Verlag, New York, in press
5 Stirling, C.E. (1972) J. Cell. Biol. 53, 704—714
6 Quinton, P.M., Wright, E.M. and Tormey, J.McD. (1973) J. Cell Biol. 58, 724—370
7 Mills, J.W. and Ernst, S.A. (1975) Biochim. Biophys. Acta 375, 268—273
8 Mircheff, A.K. and Wright, E.M. (1976) J. Membrane Biol. 28, 309—333
9 MacKnight, A.D.C. (1977) Kidney Int. 11, 391—414
10 Vates, Jr., T.S., Bonting, S.L. and Oppelt, W.W. (1963) Am. J. Physiol. 206, 1165—1172
11 Wright, E.M. (1972) J. Physiol. 226, 545—571
12 Wright, E.M. (1977) Biochim. Biophys. Acta 468, 486—489
13 Wright, E.M. (1978) in Transport across Biological Membranes (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), Springer-Verlag, New York, in press
14 Zeuthen, T., Hiam, R.C. and Silver, I.A. (1974) in Ion Selective Microelectrodes (Berman, H. and Herbert, N., eds.), pp. 145—156, Plenum Press, London
15 Zeuthen, T. (1978) Membrane Biol. 39, 185—218
16 Thomas, R.C. (1972) Physiol. Rev. 52, 563—594
17 Mullins, L.J. and Noda, K. (1963) J. Gen. Physiol. 47, 117—132