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EVIDENCE FOR ELECTROGENIC SODIUM PUMPING IN THE DUCTAL EPITHELIUM OF RABBIT SALIVARY GLAND AND ITS RELATIONSHIP WITH $(Na^+ + K^+)$-ATPase.

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SUMMARY

The temperature dependence of the transepithelial potential difference (PD) of the main duct of the submaxillary gland has been measured during in vitro perfusion studies. The magnitude of the PD depends strongly on the anion composition of the perfusing and bathing fluids. The following combinations of perfusion and bathing fluids, respectively, were used: (1) Na_2SO_4/NaCl, (2) Na_2SO_4/Na_2SO_4, (3) NaCl/NaCl, (4) NaCl/Na_2SO_4. The mean transepithelial potential differences at 35 °C with these four sets of conditions were, respectively: 144, 148, 10 and −15 mV, serosal side positive with respect to lumen. From the data obtained it was possible to construct Arrhenius plots of temperature dependence of the PD for the four sets of experimental conditions. They all show a breakpoint between 16 and 19 °C. The apparent activation energies in the four situations above the breakpoint are 4.2, 1.4, 12.0 and 10.6 kcal/mol, respectively. Below the breakpoint they are 29.9, 37.5, 29.0 and 31.3 kcal/mol, respectively. The rapid change in the PD as a function of temperature (which can also be achieved by the addition of ouabain), the effects of the removal of K^+ on the serosal side on the PD, the decrease in the PD after the addition of ouabain or CN^-, and the activation energies and breakpoints all lead to the conclusion that a large part of the PD is caused by an electrogenic sodium pump which is very probably the enzyme $(Na^+ + K^+)$-ATPase. When the duct is perfused with Na_2SO_4 we find, above the breakpoint in the Arrhenius plots, a lower activation energy than is found when perfusing with NaCl.

INTRODUCTION

The submaxillary duct functions as a transport channel for precursor salivary fluid, the composition of which is modified during passage through the duct. One of the ions extensively reabsorbed by the epithelium is sodium. The net sodium flux from mucosa to serosa is about 600 nequiv./cm^2 per min, when the duct is perfused and bathed with solutions containing Na^+, Cl^- and HCO_3^- [1, 2]. Knauf and Frömter [3] have measured the temperature dependence of the net sodium flux under these conditions. The rate of sodium reabsorption at 26 °C is half the rate at

36 °C which gives a Q_{10} for the reabsorption process of about 2. The temperature dependence of the transepithelial potential difference (PD) has also been measured, but using different bathing and perfusion fluids. These authors reported a Q_{10} of 1.09 for the temperature dependence of the PD.

Their conclusion that in the range of 36–26 °C the PD and the transepithelial sodium transport are influenced differently by changes in temperature is not justified since the temperature dependence of PD and net sodium reabsorption have been measured under different conditions. Moreover, they did not correct their potential changes, obtained on lowering the temperature, for the temperature term in the Nernst equation.

For these reasons we reinvestigated the problem of temperature dependence of the PD, using a perfusion chamber in which the bathing fluid could be rapidly cooled or warmed by means of a Peltier element.

METHODS

White rabbits of both sexes were anaesthetized with urethane (about 35 g/kg body weight). The main duct of the submaxillary gland was excised and mounted in a micro perfusion chamber as described previously by Knauf [1] in which the base and the stirring equipment were modified in order to facilitate cooling and warming of the serosal bath.

Microperfusion chamber. The base of the chamber consists of a silver plate (1 mm thick) assuring good thermal contact between the serosal fluid and the Peltier element (Sirigor, Siemens). The Peltier element is used for warming and cooling the serosal bathing fluid. The opposite side of the Peltier element (Fig. 1) is in contact with a metal block cooled with running tap water. The Peltier element is electrically isolated from the silver plate and the metal block by means of a mica sheet. In order to eliminate thermal gradients, the bathing fluid is vigorously stirred. Stirring is accomplished by means of a stirring paddle driven by an electric motor (600 rev./min) in the serosal compartment (Fig. 2).

The temperature of the bathing fluid is measured with a thermocouple (Cu-constantane). The reference probe is placed in melting ice. The voltage generated by the thermocouple is amplified to an output sensitivity of 10 mV/°C and is monitored on a recorder. Bath temperature can be varied between 0 and 50 °C and the chosen

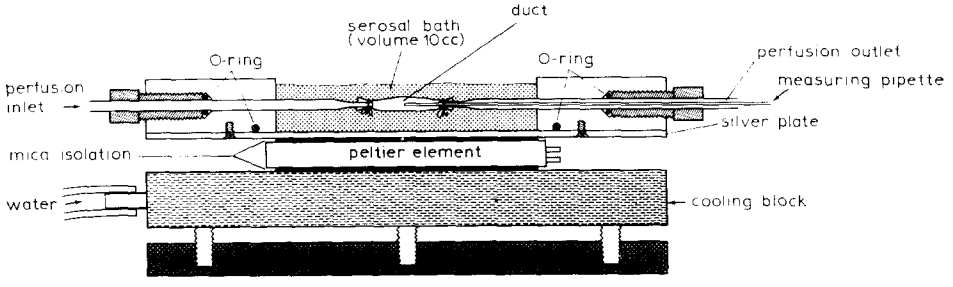

Fig. 1. Perfusion chamber. A schematic longitudinal cross-section at the position of the perfusion pipettes is shown.

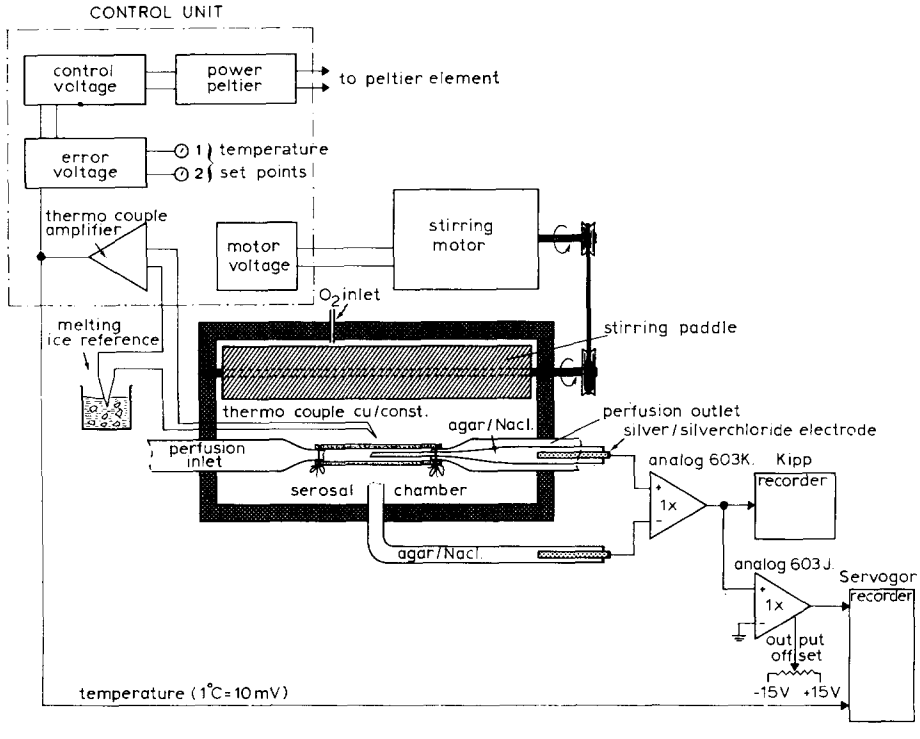

Fig. 2. Perfusion chamber with control unit and recording equipment. The perfusion chamber is seen from above.

temperature can be adjusted by a helipot on an operational unit that regulates the Peltier element voltage. The rate of warming is 0.8 °C/s and the rate of cooling 0.3 °C/s. Oxygenation of the bathing fluid (with 95 % O_2/5 % CO_2) is performed through a small inlet directly into the bathing fluid. The perfusion system is the same as that described by Knauf [1]. Full details of the temperature control unit and perfusion chamber will be published elsewhere (Augustus, J., in preparation).

Perfusion fluids and bathing fluids. Normally two perfusion fluids were used: (1) 150 mM NaCl, (2) 75 mM Na_2SO_4. In some experiments CN^- (5 mM) was added to the perfusion fluid. The composition of the serosal bathing fluid is given in Table I. Mannitol was added to the SO_4^{2-} solutions to maintain the osmolarity at 300$\pm$ 1 mosM. The pH of all solutions was 7.4$\pm$0.2. Because Ca^{2+} and Mg^{2+} in the bathing solution have no influence on PD and none on sodium transport [4], they have been omitted. It is convenient to use abbreviations for the following combinations of perfusion fluid and bathing fluid: System 1 perfusion fluid Na_2SO_4 and bathing fluid A: (SO_4^{2-}/Cl^-), System 2 perfusion fluid Na_2SO_4 and bathing fluid E: (SO_4^{2-}/SO_4^{2-}); System 3 perfusion fluid NaCl and bathing fluid A: (Cl^-/Cl^-); System 4 perfusion fluid NaCl and bathing fluid E: (Cl^-/SO_4^{2-}). The perfusion rate was 12 μl/min.

Measurement of transepithelial potential difference (PD). The PD was measured by means of two Ag/AgCl electrodes. One was fixed inside an agar-filled glass micropipette. The tip of this pipette was introduced into the lumen of the duct and the

TABLE I

COMPOSITION OF RINGER SOLUTIONS USED ON THE SEROSAL SIDE

Composition of serosal fluids are mM except for haemacel, which is in g/l.

	A	B	C	D	E	F	G	H
NaCl	100	100	100	100	–	–	–	–
Na_2SO_4	–	–	–	–	50	50	50	50
$NaHCO_3$	25	25	25	25	25	25	25	25
KCl	4	4	–	–	–	–	–	–
K_2SO_4	–	–	–	–	2	2	–	–
$MgCl_2$	1	1	1	1	–	–	–	–
$MgSO_4$	–	–	–	–	1	1	1	1
Sodium acetate	10	10	10	10	10	10	10	10
Sodium pyruvate	10	10	10	10	10	10	10	10
Glucose	6	6	6	6	6	6	6	6
Haemacel	30	30	30	30	30	30	30	30
Ouabain	–	1	1	–	–	1	1	–

other electrode inserted into an agar bridge which was placed in the serosal bathing fluid. The composition of the agar bridges was 100 mM NaCl in 3 % agar. When necessary, corrections were made for liquid junction potentials. The PD was amplified once by an instrumental amplifier (Analog 603 K), the output of which was connected to a pen recorder (Kipp). The output of the amplifier was also fed into a second amplifier of the same type provided with an output offset, in order to measure the changes in PD with changes in temperature. The output of the second amplifier was connected to one of the inputs of a servogor dual pen recorder. The other input of the recorder was used to record the temperature of the serosal bath. A schematic drawing of the experimental equipment is given in Fig. 2. The reaction of the measuring electrodes to a temperature change of 35 °C was measured

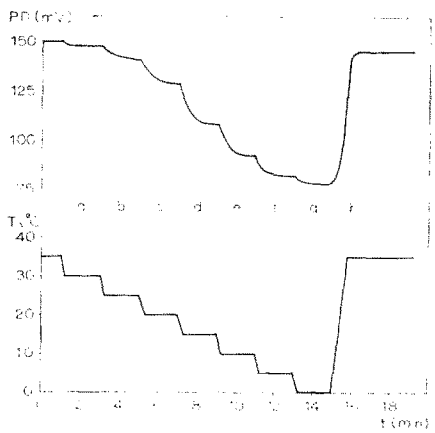

Fig. 3. Typical example of a temperature experiment. The upper curve shows the transepithelial potential difference (PD) as a function of time as the temperature (lower curve) is changed in steps of 5 °C in system 1 (SO_4^{2-}/Cl^-).

before mounting the duct. It never exceeded 50 μV/°C. After the duct was mounted on pipettes in the perfusion chamber the temperature was lowered in successive steps of 5 °C. After each decrease in temperature the PD was allowed to reach a constant value. From 0 °C the temperature was raised to 35 °C in a single step (Fig. 3). The PD at temperature T_i ($i = 1$–7) is given by $\psi(T_i)$, $T_0 = 0$ °C, $T_1 = 5$ °C, etc., so $\psi(T_3)$ is the PD at 15 °C. The change in PD is represented as $\Delta\psi(T_i)$ i.e. $\Delta\psi(T_5)$ is the change in PD when the temperature is changed from T_5 (25 °C) to T_4 (20 °C). All potential differences are referred to the lumen as zero.

RESULTS

In the Methods section four sets of experimental conditions were described in which the temperature dependence of the PD was studied. We shall first consider the results obtained using system 1 (SO_4^{2-}/Cl^-). A typical example of the effect of cooling and warming on the PD is given in Fig. 3. This figure shows that a change in temperature is directly followed by a change in potential, but that the magnitude of the potential change depends on the starting temperature. Upon rewarming to 35 °C the PD returns to the control value (before cooling) within 50 s. In order to interpret these results we have to correct the potential changes for the temperature term in the Nernst equation. Assuming that at 0 °C there is no contribution of a pump mechanism to the PD then $\psi(T_0)$ is only a diffusion potential. By means of the Nernst equation it is possible to calculate the magnitude of the diffusion potential at 35 °C:

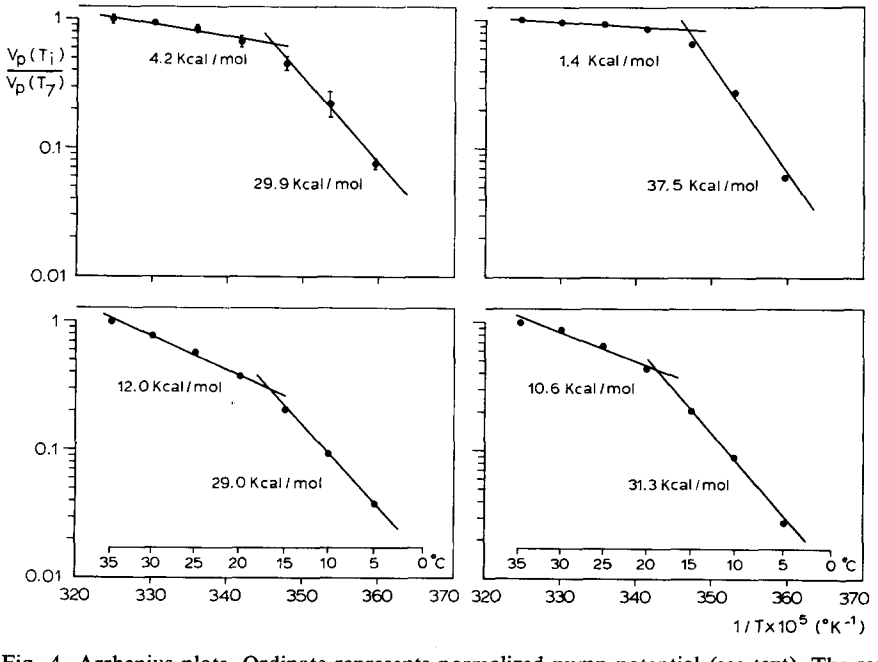

Fig. 4. Arrhenius plots. Ordinate represents normalized pump potential (see text). The results for the systems 1 (SO_4^{2-}/Cl^-), 2 (SO_4^{2-}/SO_4^{2-}), 3 (Cl^-/Cl^-) and 4 (Cl^-/SO_4^{2-}) are shown, respectively, in Fig. 4a, 4b, 4c and 4d. Lines are drawn by hand. Bars in Fig. 4a are twice S.E.

$$\psi_{pas}(T_7) = \psi(T_0)\left(1 - \frac{3}{2}\frac{5}{7}\frac{7}{3}\right) \quad (1)$$

Dividing $\psi_{pas}(T_7) - \psi(T_0)$ by seven we obtain the contribution ΔN, the diffusion potential, which is in this case 1.4 mV/5 °C for every $\Delta\psi(T_i)$. The observed change $\Delta\psi(T_i)$ is much larger than ΔN, which strongly suggests a contribution from an active transport mechanism to the PD. We may now express the change in PD due to the active process as:

$$\Delta V_{pump}(T_i) = \Delta\psi(T_i) - \Delta N. \quad (2)$$

Introducing the pump contribution at temperature T_i, i.e.

$$V_{pump}(T_i) = \sum_{k=1}^{i} \Delta V_{pump}(T_k) \quad (3)$$

($i = 1$–7) and normalizing $V_{pump}(T_i)$ by dividing by $V_{pump}(T_7)$ we can construct an Arrhenius plot in which the logarithm of $V_{pump}(T_i)/V_{pump}(T_7)$ is plotted against $1/T$. The curve obtained is a curvilinear one and is shown in Fig. 4a. It can, however, be very well approximated by two straight lines. The straight lines give the following apparent activation energies: 4.2 kcal/mol above and 29.9 kcal/mol below the break-point, which is at 17 °C. The same procedure is followed for the systems 2 (SO_4^{2-}/SO_4^{2-}), 3 (Cl^-/Cl^-), and 4 (Cl^-/SO_4^{2-}), which are shown in Fig. 4b, 4c and 4d, respectively. Table II gives the results for $\psi(T_7)$, $\psi(T_0)$, $\psi_{pas}(T_7)$ and the contribution

TABLE II

The PD at certain temperatures is given for different experimental conditions: first column $\psi(T_7)$, PD at 35 °C; second column $\psi(T_0)$, PD at 0 °C (diffusional only); third column $\psi_{pas}(T_7)$, diffusional PD at 35 °C calculated from $\psi(T_0)$ using the Nernst equation; fourth column $\psi(T_7) - \psi_{pas}(T_7)$, contribution of the active process to the PD at 35 °C. All PD values are given in mV.

	$\psi(T_7)$	$\psi(T_0)$	$\psi_{pas}(T_7)$	$V_{pump} = \psi(T_7) - \psi_{pas}(T_7)$
System 1 (SO_4^{2-}/Cl^-)	118	51	58	60
	120	49	55	65
	121	55	62	59
	127	60	68	59
	142	74	84	58
	145	74	84	61
	148	79	91	57
	149	76	85	64
	150	88	100	50
	150	88	99	51
	163	100	113	50
	165	94	106	59
	170	103	116	54
System 2 (SO_4^{2-}/SO_4^{2-})	140	27	30	110
	145	35	40	105
	160	47	53	107
System 3 (Cl^-/Cl^-)	9.2	0	0	9.2
	10.3	0	0	10.3
System 4 (Cl^-/SO_4^{2-})	−28.0	−51.6	−56.9	28.9
	12.8	−39.5	−44.6	31.8

TABLE III

APPARENT ACTIVATION ENERGIES*

(a) Apparent activation energies for the PD temperature dependence**

System	$\psi(T_7)$	E_h	E_l	T_b	n	Ref.
1 (SO_4^{2-}/Cl^-)	144	4.2	29.9	17	13	(this publication)
2 (SO_4^{2-}/SO_4^{2-})	148	1.4	37.5	16	3	(this publication)
3 (Cl^-/Cl^-)	10	12.0	29.0	17	2	(this publication)
4 (Cl^-/SO_4^{2-})	−15	10.6	31.3	18	2	(this publication)

(b) Apparent activation energies for the net Na^+ flux of the duct

System	E_h	Ref.
3 (Cl^-/Cl^-)	11.8	[3]

(c) Apparent activation energies for the PD temperature dependence

System	E_h***	Ref.
2 (SO_4^{2-}/SO_4^{2-})	1.6	[3]

(d) Apparent activation energies for the ($Na^+ + K^+$)ATPase

Source	E_h	E_l	T_b	Ref.
Rabbit kidney	13.5	28.5	18–20	[7]
Rabbit brain	15	34	$\simeq 15$	[9]
Lamb kidney	15.2	32.6	20	[10]

* E_h is the apparent energy of activation above, and E_l the apparent energy of activation below, the breakpoint temperature T_b. E_h and E_l are expressed in kcal/mol and T_b in °C.

** The apparent energies of activation for the PD temperature dependence are corrected with the Nernst equation. The average PD at 35 °C ($\psi(T_7)$) is given in mV. In the last column the number (n) of ducts used is shown.

*** This value of E_h has not been corrected with the Nernst equation.

of the active process to the PD for the various systems employed. Table IIIa gives values for the activation energies and inflection point temperatures.

The Arrhenius plots for the systems 3 (Cl^-/Cl^-) and 4 (Cl^-/SO_4^{2-}) (Fig. 4c and 4d), differ from systems 1 (SO_4^{2-}/Cl^-) and 2 (SO_4^{2-}/SO_4^{2-}) (Fig. 4a and 4b) in their behaviour above the breakpoint.

Since we have found indications of an active transport mechanism with activation energies comparable to those of the ($Na^+ + K^+$)-ATPase enzyme system (Table IIId), we have studied the influence of K^+, ouabain and CN^- on the PD.

Adding ouabain in a concentration of $5 \cdot 10^{-4}$ M to the serosal bathing fluid in condition 1 (SO_4^{2-}/Cl^-), resulted in a rapid decrease in the PD (Fig. 5), within 10–20 s after application. After 3 min the PD reached an almost constant level. Decreasing the temperature in this situation resulted only in a change in potential as predicted on the basis of the temperature term in the Nernst equation. Moreover, the

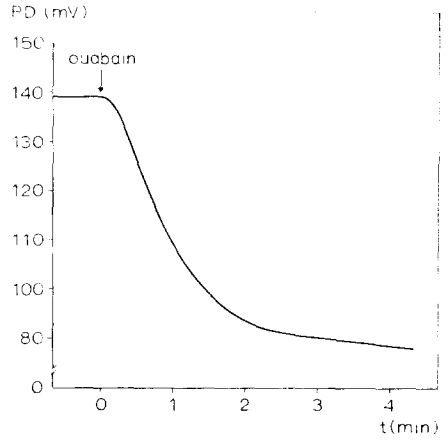

Fig. 5. Application of $5 \cdot 10^{-4}$ M ouabain to the serosal bath results in a rather rapid decrease in transepithelial potential difference (system I (SO_4^{2-}/Cl^-)).

magnitude of the PD change with ouabain is comparable to the temperature-dependent component. This lends strong support to the idea that the active mechanism is related to the ($Na^+ + K^+$)-ATPase system.

Additional support for the involvement of the ($Na^+ + K^+$)-ATPase system in generating part of the PD is provided by the results of the following experiment. The drop in the PD on addition of ouabain was studied at different potassium concentrations in the bathing fluid. It can be clearly seen in Fig. 6 that at high K^+ concentrations (25 mM) the decrease in the PD is slower and less pronounced compared with that seen at lower K^+ concentrations. This might be expected since potassium ions

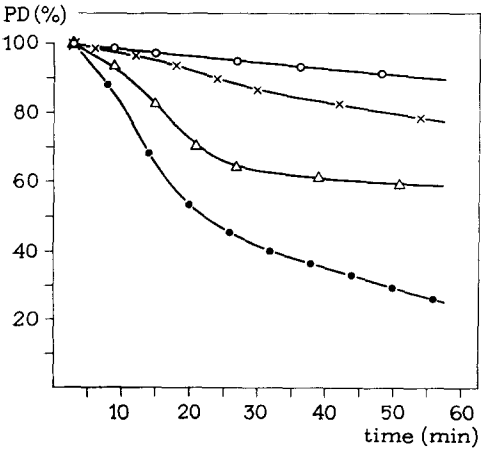

Fig. 6. Decrease in transepithelial potential difference due to ouabain application ($5 \cdot 10^{-5}$ M as percent of the starting value at 35 °C at different potassium concentrations. ○, 40 mM; ×, 25 mM; △, 10 mM; ●, 2 mM. The reason for the slower decrease in the transepithelial PD as compared with Fig. 5 is that (a) the ouabain concentration was $5 \cdot 10^{-5}$ M instead of the normally used concentration of $5 \cdot 10^{-4}$ M and (b) the experiment was carried out in another perfusion chamber in which stirring was not so efficient, i.e. a larger unstrirred layer at the serosal side.

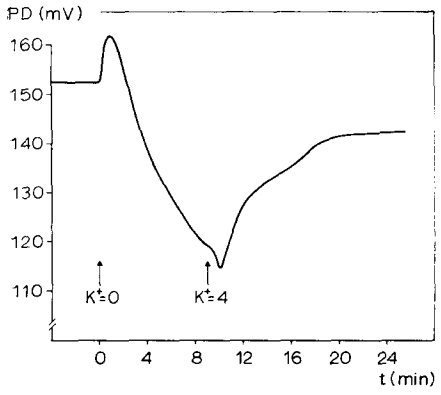

Fig. 7. Effect of K^+ in the serosal bath on transepithelial PD. The K^+ electrode character of the serosal cell membrane became visible in the initial hyperpolarization when K^+ was removed, and in the initial depolarization when 4 mM K^+ was again introduced into the serosal bath (system 1 (SO_4^{2-}/Cl^-)).

antagonise the ouabain effect on $(Na^+ + K^+)$-ATPase.

The normal Ringer used for the serosal bathing fluid contains 4 mM potassium. Removal of K^+ first gives a hyperpolarization and then a rapid decrease in the PD. A depolarization and then a rise in the PD is seen when K^+ is added again (Fig. 7). The hyper- and depolarization may be explained by the K^+ electrode character of the serosal cell membrane [3].

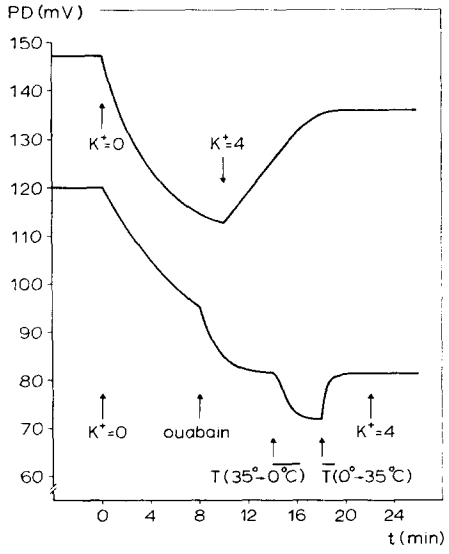

Fig. 8. The upper part shows the effects on transepithelial PD when K^+ was removed from the serosal bath and then re-introduced. The lower curve shows the effect of ouabain ($5 \cdot 10^{-4}$ M) on the transepithelial PD after removing K^+ from the serosal bath. A temperature change of 35–0 °C and than 0–35 °C gives a potential change which corresponds, within experimental error, to the temperature dependence described by the Nernst equation. Upon introduction of K^+ the PD remains stable.

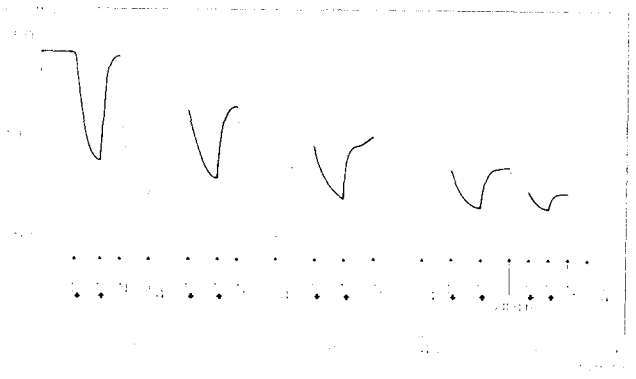

Fig. 9. Results of intermittent application of CN^- ($5 \cdot 10^{-3}$ M) at the mucosal side and subsequent return to the previous situation (Na_2SO_4 Ringer) on the temperature response of the PD (heavy lines). The temperature change was 35 °C down ($T\downarrow$) and then 35 °C up ($T\uparrow$) (System 1 (SO_4^{2-}/Cl^-)).

The decrease in the PD after removal, and the increase in the PD after application of K^+, is due to the K^+ requirement of the $(Na^+ + K^+)$-ATPase.

Removing K^+ and then, while the PD is still decreasing, applying ouabain, results in a fast change in the PD which is smaller than when K^+ is present. Lowering the temperature to 0 °C and then warming up to 35 °C results in a change in the PD which is fully predicted by the temperature term in the Nernst equation. Reintroducing K^+ into the system does not result in an increase in the PD, since the pump is now inhibited by ouabain (Fig. 8).

The fact that the initial hyperpolarization does not occur when K^+ is removed, and that depolarization occurs when K^+ is introduced, is due to loss of electrical contact with the duct when changing the bathing fluid.

CN^- application indirectly inhibits $(Na^+ + K^+)$-ATPase activity by inhibition of respiration. Fig. 9 shows that after intermittent application of CN^- to the mucosal side (on the serosal side it complexes with the silver bottom of the perfusion chamber).

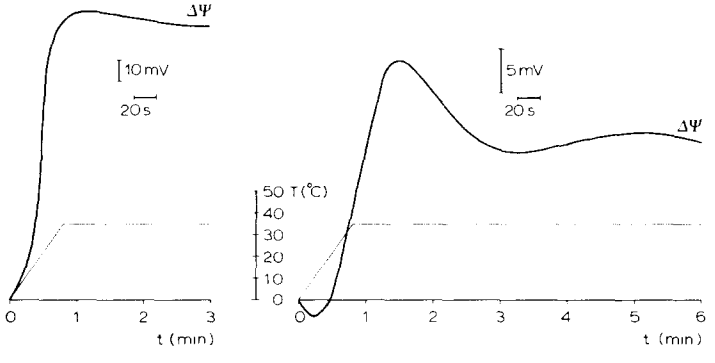

Fig. 10. Overshoot of transepithelial potential difference when warming up from 0 to 35 °C. Potential change $\Delta\psi$ (thick line) and temperature change (thin line) are given as functions of time. Fig. 5a represents the results for system 1 (SO_4^{2-}/Cl^-) and 5b for system 4 (Cl^-/SO_4^{2-}). Note the oscillations in the last figure.

the temperature response becomes smaller. After several applications only the temperature dependence described by the Nernst equation remains.

In every experiment where the duct was cooled to 0 °C it was also rapidly rewarmed to 35 °C, as is shown in Fig. 3.

In all these experiments the potential change was fast and, in general, an overshoot was observed (Fig. 10a and 10b). The magnitude of the overshoot is dependent on the duration of the preceeding cooling period. This can be explained by assuming that sodium enters the cells without being pumped out again. Rewarming reactivates the pump and more Na^+ is transported than in the steady state. If the pump is electrogenic in nature then an overshoot is to be expected [5]. All these observations strongly suggest an electrogenic role of the $(Na^+ + K^+)$-ATPase in the generation of the PD across ductal epithelium of the submaxillary gland.

DISCUSSION

The magnitude of the depolarization observed after cooling to 0 °C is much larger than expected from the temperature term in the Nernst equation. This in itself is not enough to conclude that an active transport mechanism is contributing to the development of the PD because cellular and extracellular resistances (permeabilities) are also temperature dependent. Nevertheless the temperature response after application of ouabain or CN^- corresponds with the temperature term in the Nernst equation (see Fig. 8 and 9). This can be understood from the epithelial model presented by Schultz [6], where only resistance ratios occur in the expression for the transepithelial PD. Together with the observation that the magnitude of the depolarization following application of ouabain is about the same as the magnitude of the depolarization after cooling to 0 °C, provided that this depolarization is corrected with the temperature-dependence factor in the Nernst equation, leads to the conclusion that an active process must be involved in the generation of the PD. There are several indications that the active process is linked to the $(Na^+ + K^+)$-ATPase: (1) the depolarization with ouabain (Fig. 5); (2) the magnitude of depolarization after removal of K^+ (Fig. 7 and 8); (3) the competition effect of K^+ concentration and ouabain on the PD (Fig. 6); (4) the effect of CN^- on the PD (Fig. 9).

We have now established (a) the relation of the active process with the $(Na^+ + K^+)$-ATPase and thus with the sodium pump and (b) the temperature dependence of the PD after inhibition of the sodium pump which corresponds, within experimental error, to the temperature term in the Nernst equation.

This justifies our assumptions, stated in the Methods section, that there is no pump contribution of the $(Na^+ + K^+)$-ATPase at 0 °C [7] and that it is valid to treat the potential at 0 °C as a pure diffusional electromotive force with a temperature dependence described by the Nernst equation. The sodium pump can be electrogenic or neutral. Factors in favour of the electrogenic nature are (1) the overshoot that is observed after rewarming when the cooling period was longer than 15 min (see Fig. 10), (2) the fast response of the PD on rewarming (PD restored in about 50 s), and (2) the fast PD change following inhibition with ouabain (steady state in about 4 min).

An electrogenic pump will also have an indirect effect on the PD due to its influence on the cellular concentrations of Na^+ and K^+. So it is not impossible that

cellular leakage of Na^+ into and K^+ out of the cell during the cooling period also contributed to the depolarization. Therefore the pump contribution (V_{pump}) to the PD should give the maximum estimate of electrogenicity.

If cellular leakage of Na^+ and K^+ is the sole source of the observed depolarization during cooling or ouabain application, then of course the pump would be neutral. But in exactly symmetrical solutions (SO_4^{2-}/SO_4^{2-}) a PD of about 20 mV is developed after ouabain application (unpublished observation). This lasts for about 2 h before the PD declines to zero (about 2.5 mV in 15 min). Therefore we also favour an electrogenic rather than a neutral nature for the sodium pump. The concept of the electrogenic pump also provides an explanation for the results of Knauf et al. [3]. When they changed the luminal Na^+ concentration by a factor of ten they found a slope of 54 mV in the plot of PD against the logarithm of the Na^+ concentration. After ouabain they found a slope of only 27 mV for a ten fold change. They concluded that the selectivity of the luminal membrane for Na^+ is highest when the pump mechanism of the serosal membrane is intact. This observation can be more readily explained by the fact that raising luminal Na^+ concentration with an intact pump present not only changes the E_m (mucosal sodium electromotive force) but also activates the pump mechanism because more Na^+ can enter the cell [5].

The same holds true qualitatively for K^+ substitution on the serosal side.

Conclusions about Na^+ or K^+ electrode behaviour based on observed potential change can only be valid if the involvement of possible electrogenic mechanisms, which are also dependent on Na^+ and K^+ concentrations, are excluded.

When the duct is perfused with chloride Ringer we find the same activation energies irrespective of the composition of the serosal Ringer, i.e. Na_2SO_4 or NaCl (Table IIIa).

The apparent activation energies for the potential temperature dependence in the systems 3 (Cl^-/Cl^-) and 4 (Cl^-/SO_4^{2-}) (Table IIIa) correspond well with activation energies reported for ($Na^+ + K^+$)-ATPase (Table IIId). Also the breakpoint temperatures are comparable to those reported for the enzyme system (Table IIIa and IIId).

The breakpoint temperature seems to be dependent on lipid-protein interactions and the phospholipid composition of the membrane [8]. Using a Cl^-/Cl^- system Knauf et al. [3] reported a value of 11.8 kcal/mol for the net Na^+ flux (Table IIIb). These observations strongly suggest, at least for system 3 (Cl^-/Cl^-) and probably also for the system 4 (Cl^-/SO_4^{2-}) that the temperature-sensitive active electrogenic part of the PD and the net Na^+ transport are brought about by ($Na^+ + K^+$)-ATPase. Perfusing the duct with SO_4^{2-}, systems 1 (SO_4^{2-}/Cl^-) and 2 (SO_4^{2-}/SO_4^{2-}), gives different apparent activation energies above the breakpoint temperature as compared to Cl^- perfusion (Table IIIa). An important factor for the functioning of the sodium pump is the entry of Na^+ across the mucosal membrane. Schultz [6] has shown, with a generalized equivalent circuit, that two cell electromotive forces in series (mucosal and serosal) can give rise to a "step" or "well" potential profile depending on the relative magnitudes of the extracellular and cellular resistances. One consequence of the circuit is that increased positivity of the cell interior with respect to the mucosal media can be caused by an increase in extracellular resistance. Because the transepithelial resistance is higher with SO_4^{2-} than with Cl^- perfusion [4] there is probably a potential step profile differing from the Cl^- perfusion condi-

tion; therefore the chemical driving force for Na^+ is opposed by the electrical one. This might be the rate-limiting step for the active Na^+ transport in this condition. The apparent activation energy above the breakpoint (in systems 1 (SO_4^{2-}/Cl^-) and 2 (SO_4^{2-}/SO_4^{2-})) should then be the result of this rate limiting step.

In a later article we intend to take a more detailed look at the contribution of cell leakage and electrogenicity of the pump to the PD change with temperature. Therefore we are now performing experiments in which temperature is reduced from 35 °C to 0 °C within 1 s.

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