

## brief communication

**Measurement of Na–K pump current in acinar cells of rat lacrimal glands**

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**ABSTRACT** Isolated cells from rat lacrimal glands were voltage clamped using the tight-seal whole-cell recording technique. The intracellular solution contained ATP and an elevated Na concentration (70 mM). Removing external K ions elicited an inward current shift. Ouabain (0.5 mM) induced an inward current shift of identical amplitude, but with slower kinetics. In the

presence of ouabain, removal of K ions did not alter the cell current. The potassium- and ouabain-sensitive current was outward between  $-120$  and  $+20$  mV, and its amplitude decreased below  $-60$  mV.

This current was highly sensitive to temperature, and was not affected by blockers of the K channels which are

present in these cells. It was attributed to an inhibition of the Na–K pump.

The Na–K pump current was estimated to be 15 pA for an average acinar cell at physiological temperature, with 70 mM internal Na ions and 20 mM external K ions. Implications of this value in terms of electrolyte secretion are discussed.

**INTRODUCTION**

In exocrine glands, the Na–K pump has a double function. First, it helps establish adequate ion gradients, as in other animal cells. But second, it plays a key role in energizing fluid secretion (Petersen, 1980). Pump inhibitors leave the first phase of the fluid secretion response unaffected, but they block entirely the sustained phase of the response (Petersen, 1970). This differential effect most likely results from the fact that the early phase of ion secretion utilizes the ion gradients present in the resting preparation as a result of previous pump activity, while the sustained phase directly uses pump activity in order to transfer ions into the secreted fluid. Earlier work has demonstrated the presence of three types of Ca-dependent channels in exocrine glands (Petersen and Maruyama, 1984; Marty et al., 1984). When the cells are stimulated by a secretion-inducing agent such as acetylcholine, the internal Ca rises and Ca-dependent channels are activated. This results in major changes in ion concentrations, notably in a rise of external K and internal Na concentrations (Burgen, 1956). These changes activate the Na–K pump. It is thought that the Ca-induced currents, together with the Na–K pump, are the major elements underlying the fluid extrusion response to acetylcholine. Present models of secretion imply that the ionic fluxes due to the pump should be comparable in size to the Ca-dependent currents, which reach hundreds of pA in stimulated cells (Petersen and Maruyama, 1984; Marty et al., 1984; Suzuki and Petersen, 1985). It therefore seemed interesting to measure the Na–K pump

current in exocrine acinar cells in order to be able to assess more directly the role of the pump in fluid secretion. The experimental approach was guided by the demonstration by Gadsby et al. (1985) that Na–K pump current may be measured at the cellular level using the patch-clamp technique.

We have chosen the rat lacrimal gland since extensive results on acetylcholine-induced currents are available in this preparation. However the results are also presumably relevant to vertebrate salivary glands, which function in a fashion very similar to the rat lacrimal (Petersen, 1980).

**METHODS**

Cells were isolated from rat lacrimal glands and placed in short-term culture conditions as previously described (Marty et al., 1984). The tight-seal whole-cell recording method (Marty and Neher, 1983) was used to measure membrane currents while controlling the cell internal solution. The standard pipette (cell) solution contained (in millimolars): 70 KCl; 70 NaCl; 10 EGTA-KOH; 3 Mg ATP; 5 Hepes-KOH (pH 7.2). The standard external solution contained 125 NaCl; 20 KCl; 1 CaCl<sub>2</sub>; 1 MgCl<sub>2</sub>; 5 Hepes-NaOH (pH 7.2). The internal Na concentration, Na<sub>i</sub>, and the external concentration, K<sub>o</sub>, were selected to reflect conditions pertaining during maximum electrolyte secretion (see Discussion). In some experiments 10 mM decamethonium was added to the internal solution and/or 4 mM tetraethanolamine (TEA) was included in the external solution in order to block Ca-dependent K channels (see Trautmann and Marty, 1984; Llano et al., 1987).

Most experiments were performed at 33°–38°C. The bathing solution was changed through a small hole (200- $\mu$ m diam) located at the tip of a glass capillary shaped as a U (Krishtal and Pidoplichko, 1980). In order to minimize temperature changes occurring during replacement of the external solution with this method, the U-tube ( $\sim$ 400- $\mu$ m external diam) was bent in such a way that the incoming solution, before reaching the hole, traveled over a distance of 1 cm in a portion of the

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tube that was in contact with the heated bath. This resulted in a very marked reduction of the response of a small thermistor probe placed in the bath upon ejection from the U-tube compared with that obtained with a capillary shaped in the conventional manner. Applying a solution identical to that of the bath through the modified U-tube did not cause any detectable change in the cell currents. In particular, the kinetics of K currents was unchanged, indicating that temperature alterations during application were indeed small.

Cells dialyzed with our standard, ATP-containing solution displayed a comparatively large leakage conductance ( $\sim 0.5$ – $2$  nS) at  $33^\circ$ – $38^\circ\text{C}$ . This conductance usually rose further after several minutes of whole-cell recording. Furthermore, it was found that the pump-related current decreased with time. For these reasons, measurements were restricted to the first 5–10 min of recording, during which time the cell properties and the pump-related current were stable.

## RESULTS

When the bath K ions (normally at 20 mM) were substituted with Na, the membrane current was found to decrease rapidly and reversibly (Fig. 1). At  $33^\circ$ – $38^\circ\text{C}$ , the amplitude of this effect ranged between 8 and 56 pA in the cells examined (average value 24 pA,  $n = 18$ ). With the largest responses, a clear overshoot of the current was observed after readmission of K ions. This outward readmission current returned to the baseline level with a slow time constant, on the order of 5 s.

It was essential to keep the temperature high in order to obtain sizable  $K_o$  sensitive currents. At room temperature ( $24^\circ$ – $25^\circ\text{C}$ ) removal of external K induced small current changes (4–8 pA,  $n = 3$ ), which were difficult to measure with acceptable accuracy.

To test the hypothesis that the inward current change induced by K removal was due to the inhibition of an

outward current carried by the Na–K pump, effects of a large concentration of ouabain (0.5 mM) were examined under the same experimental conditions. Ouabain was also found to induce a reversible inward current shift. However, the kinetics of the current changes was much slower than with K removal (Fig. 1). The onset of the ouabain-sensitive current had a time constant of  $\sim 4$  s, and recovery required  $\sim 2$  min of washing. In five cells where  $0K_o$  and ouabain were tested sequentially the corresponding current responses were found to be in a ratio of  $0.98 \pm 0.08$  (SD). These results are consistent with the notion that removal of external K and application of ouabain blocked the same Na–K pump current.

A series of control experiments gave further evidence in favor of this view. The inward current response was still observed in the presence of 2–4 mM external TEA (as in Fig. 1, *top*) or 10 mM internal decamethonium. Both compounds are potent inhibitors of the major K conductance system observed in rat lacrimal glands (Trautmann and Marty, 1984; Llano et al., 1987). The inward current shift was not seen if the internal solution lacked Na and Mg ATP, as expected from the known properties of the Na–K pump. Finally, the K removal effect was not observed if the cell was held in a bath solution containing ouabain.

The effects of the K-free solution and of ouabain were only obtained in the first minutes of recording. This indicates that some intracellular compound other than ATP (which is continuously supplied by diffusion from the pipette reservoir) is necessary for pump activity and is lost during prolonged whole-cell recording or else that the cells start to produce a compound which blocks the pump.

The  $0K_o$ -induced current was not studied systematically as a function of the initial  $K_o$  value. However, in the same cell it was found that a  $5K_o$ – $0K_o$  change elicited an inward current of 12 pA while a  $5K_o$ – $20K_o$  change induced an outward current of 6 pA, suggesting that the  $K_o$ -sensitive current is probably close to saturation at 20 mM  $K_o$ . The pump  $I$ – $V$  relation was examined by subtracting the  $I$ – $V$  curve obtained after blockade of the pump from the control  $I$ – $V$  curve. At positive potentials, the control  $I$ – $V$  curve showed an outward rectification probably due to residual K currents (Fig. 2 *A*), which was likely to be altered by manipulations of  $K_o$ . It seemed therefore preferable to block the pump with ouabain than with K removal. Because of the poor stability of the cells at high temperature (see Methods), it was difficult to obtain satisfactory controls after removal of ouabain, and in most cells  $I$ – $V$  curves were simply compared before and during ouabain application. Step pulses of 200 ms duration were given every second from a holding potential of  $-30$  mV, first in the control solution, and then in the presence of ouabain. Difference currents between the two

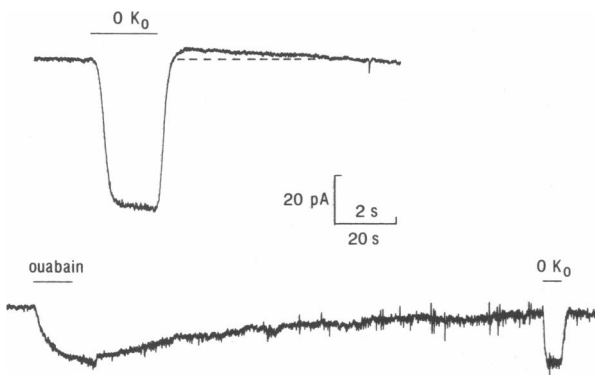


FIGURE 1 Current response to K removal and to ouabain. (*Top*) Large current response to K removal (substituted with Na). Membrane potential  $-30$  mV. Temperature,  $37^\circ\text{C}$ . Cell capacitance, 37 pF. Note the overshoot above basal level (*dotted line*) upon readmission of K. (*Bottom*) Smaller current response (from a different cell) to application of 0.5 mM ouabain (*left*) and K removal (*right*). Temperature,  $34.5^\circ\text{C}$ . Cell capacitance, 45 pF.

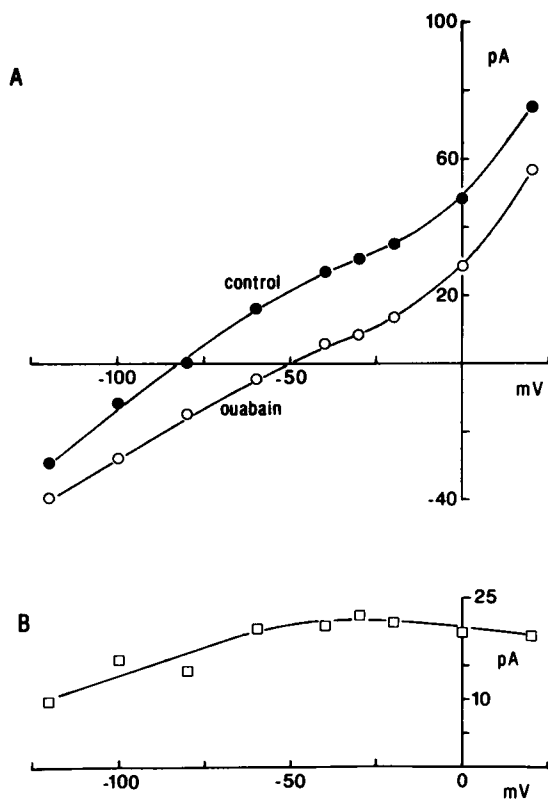


FIGURE 2 Voltage sensitivity of ouabain-sensitive current. Control and test solutions contained 4 mM TEA in order to block voltage-dependent K channels. (A) *I-V* curves in control conditions (solid circles) and in the presence of 0.5 mM ouabain (open circles). (B) *I-V* curves of ouabain-sensitive current obtained from the difference between control and ouabain currents. Temperature, 34.5°C. Cell capacitance, 29 pF.

sets of traces did not show any sign of time dependence. The ouabain-sensitive current was outward from  $-120$  to  $+20$  mV, and the current-voltage relation ran almost parallel to the voltage axis (Fig. 2). There was, however, a current decrease for potentials more negative than  $-60$  mV. In the example shown, there is also a very slight current decrease at positive potentials. Such a trend was present with varying intensity in other cells. This effect may be related to a small  $K_o$  depletion due to the pump activity, since a decrease of  $K_o$  performed late in an experiment (at a stage where ouabain-sensitive currents had been totally eliminated) resulted in a decrease of outward current relaxations obtained at positive test potentials.

## DISCUSSION

The ouabain- and potassium-sensitive current has many of the properties expected for an electrogenic Na-K pump

current. That removal of external K ions or application of a saturating dose of ouabain induced the same maximum current change is a particularly strong argument in favor of a common mechanism in both types of experiments.

The response to ouabain was much slower than that after K removal. Assuming that the pump inhibition results from a simple binding reaction of ouabain, it is possible to estimate association and dissociation rates of  $4 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$  and  $0.02 \text{ s}^{-1}$ , respectively. These are apparent rate constants. Estimating the true rate constants from these results must take into account the fact that ouabain can bind to (and dissociate from) the pump molecule only during certain steps of the Na-K exchange cycle.

Our results show that the Na-K pump current is outward between  $-120$  and  $+20$  mV in 20 mM  $K_o$ , and that it is almost voltage-independent over this potential range. In heart muscle cells, a decrease of the pump current was found at very negative potentials (Gadsby et al., 1985). A similar trend was consistently observed in the present experiments. The small, more variable decline seen at positive potentials (Fig. 2) is likely to be due to  $K_o$  depletion in a restricted extracellular space, perhaps comprised between the cell and the dish surface. A similar explanation also accounts for the K readmission effect illustrated in Fig. 1.

The Na-K pump current varied from 10 to 50 pA. Because some of the variability probably originated in differences in the metabolic state of the cells, the upper value may be considered as being the most likely to reflect the conditions pertaining *in vivo*. This figure is quite large if compared with the average size of the lacrimal gland cells. In our experiments, we actually selected large cells in order to obtain large pump currents. The average capacitance of the cells was 40 pF, corresponding to an estimated surface area of  $4 \times 10^{-5} \text{ cm}^2$ . An average lacrimal cell would have a capacitance of 13 pF, a surface area of  $1.3 \times 10^{-5} \text{ cm}^2$ , and a diameter of 20  $\mu\text{m}$ . The corrected pump current value for such a standard cell is 16 pA. The resulting pump current density of  $1.25 \mu\text{A}/\text{cm}^2$  is very similar to that ( $1 \mu\text{A}/\text{cm}^2$ ) found in cardiac muscle cells (Gadsby et al., 1985), a tissue where the pump activity is particularly high.

During electrolyte secretion, acinar cells release K towards the blood, causing a pronounced rise of  $K_o$  on the basolateral aspect of the secreting cells, where the Na-K pump is located (Burgen, 1956, Bundgaard et al., 1977; Speight and Chisholm, 1984). Quantitative figures for  $K_o$  are not available, but a value of 20 mM, as chosen in the present study, may be considered as conservative. Simultaneously with the increase of  $K_o$ , there is a decrease of  $K_i$  and an increase of  $\text{Na}_i$  (Burgen, 1956). In the dog submandibular, Burgen (1956) estimated that  $\text{Na}_i$  could reach 85 mM. More recently, x-ray microprobe analysis

was carried out on the dog submandibular and on the rat parotid, giving maximal  $\text{Na}_i$  values of 55 and 65 mM (Sasaki et al., 1983; Izutsu and Johnson, 1986). Thus the value of 70 mM chosen in the present experiments is likely to reflect conditions pertaining in vivo during stimulation.

For a 3Na/2K transport stoichiometry, a net pump current of 16 pA corresponds to an outflow of 48 pA of Na ions and to an inflow of 32 pA of K ions. This figure is directly relevant to the transcellular transfer of ions during fluid secretion, since in all models developed so far (Petersen and Maruyama, 1984; Marty et al., 1984; Suzuki and Petersen, 1985), the Na-K pump sets the rate of the sustained phase of secretion. The 32 pA value compares favorably with the sustained secretion rate for K (of 1.1  $\mu\text{eq/g}$  per min) observed in the dog submandibular gland (Burgen, 1956), which corresponds to a K current of 10 pA (assuming a cell diameter of 20  $\mu\text{m}$  and a corresponding volume of 4.2 pl).

Finally, the figure of 16 pA per cell can be compared with expectations derived from the number of ouabain-binding sites per cell. The relevant figure is not known in rat lacrimal glands, but the guinea-pig parotid glands have  $3 \times 10^6$  ouabain-binding sites per cell (Hootman and Williams, 1985). Combined with a turnover rate of 150 Na ions/s (estimated from results reviewed in Cerejido et al., 1980) this corresponds to a net current of 24 pA (assuming a 3Na-2K stoichiometry). The agreement between the predicted and measured current values is to some extent fortuitous, since the net pump current, the number of pump molecules and the pump rate, are at best known within a factor of 2. But it does suggest that the measured current is a reasonable estimate of the pump current operating in vivo.

In conclusion, the value found for the pump current is consistent both with expectations derived from previous work on ouabain binding and with predictions based on models where the steady-state secretion is directly linked to pump activity.

We thank Dr. D. Gadsby for helpful comments on the manuscript.

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Received for publication 13 June 1988 and in final form 28 September 1988.

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