J. exp. Biol. ***177****, 287–292 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

SHORT COMMUNICATION

GRAMICIDIN SWITCHES TRANSPORT IN INSECT EPITHELIA FROM POTASSIUM TO SODIUM

S. H. P. MADDRELL

Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK

and M. J. O'DONNELL

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Accepted 4 December 1992

Recent studies of insect epithelial transport have shown that ion transport in the great majority of cases depends not on the ubiquitous Na^+/K^+-ATPase, which energizes most vertebrate ion-transport systems, but on a vacuolar-type proton pump (V-ATPase; Wieczorek *et al.* 1991; Klein, 1992; Harvey, 1992; Wieczorek, 1992). In the new model for insect epithelia, cation transport is achieved by a combination of a V-type H^+-ATPase and Na^+/H^+ and/or K^+/H^+ antiporters on the apical plasma membrane (Harvey, 1992).

Insect epithelia such as the salivary glands of blowflies and the Malpighian tubules of herbivorous insects secrete fluids containing KCl as the predominant salt (Berridge *et al.* 1976; Ramsay, 1953). In contrast, the Malpighian tubules of most blood feeders, including mosquitoes and tsetse flies, secrete fluids containing predominantly NaCl (Gee, 1976; Williams and Beyenbach, 1983). As a further variant, the upper Malpighian tubules of *Rhodnius prolixus* secrete a fluid containing approximately equimolar NaCl and KCl after the insect has taken a blood meal (Maddrell, 1969).

These apparently diverse patterns of ion transport can be accommodated in a single model in which an apical V-ATPase plus cation/H^+ antiporter(s) can drive transport of Na(Cl) and/or K(Cl) into the lumen. Which of Na^+ or K^+ is actively transported depends on the nature of the passive ion-permeable channels and transporters on the basolateral plasma membranes (Maddrell and O'Donnell, 1992). If this model is correct, it ought to be possible to change substantially the relative concentrations of Na^+ and K^+ in the fluid secreted by insect epithelia by altering the relative permeability of the basolateral membranes.

In the present paper, we describe the dramatic effects of treating two well-studied insect transporting epithelia with gramicidin D, an ionophore known to confer on membranes very high permeabilities to Na^+ and K^+ (see Wills, 1981). Such treatment effectively allows these ions equal ease of access to the intracellular compartment. We chose to investigate the effects of gramicidin D on the salivary glands of *Calliphora*

Key words: potassium ions, sodium ions, excretion, fluid secretion, *Rhodnius prolixus*, salivary glands, *Calliphora vicina*, Malpighian tubules, epithelial transport, gramicidin D.

erythrocephala, which essentially transport iso-osmotic KCl solution (Berridge *et al.* 1976) and the upper Malpighian tubules of *Rhodnius prolixus*, whose secreted fluid contains nearly equal concentrations of Na^+ and K^+ (Maddrell, 1969). In *Calliphora* salivary glands, the basal plasma membrane preferentially allows entry of K^+ and Cl^- (Berridge *et al.* 1976), whereas in *Rhodnius*, basal entry of ions depends instead on the furosemide-sensitive $Na^+/K^+/2Cl^-$ cotransporter (O'Donnell and Maddrell, 1984).

Tubules and salivary glands were isolated as described previously (Maddrell *et al.* 1988) and bathed either in standard saline (Maddrell *et al.* 1993) containing 146mmol l^{-1} Na^+ and 8.6mmol l^{-1} K^+, or in a saline containing 77mmol l^{-1} Na^+ and 77mmol l^{-1} K^+. In all cases stimulation of fluid transport was achieved by including 10^{-6} mol l^{-1} 5-hydroxytryptamine (5-HT) in the saline (Berridge and Patel, 1968; Maddrell *et al.* 1971, 1991).

Levels of sodium and potassium in droplets of fluid secreted by *Rhodnius* tubules and *Calliphora* salivary glands were measured by ion-selective microelectrodes (Wright and O'Donnell, 1992). Although ion-selective microelectrodes measure the *activity* of an ion, they can be calibrated in terms of *concentration* if it is assumed that the activity coefficients of the experimental droplets and calibration solutions are similar. This assumption is valid if the ionic strength and composition of the experimental droplets and calibration solutions are similar. Electrodes were calibrated in mixtures of KCl and NaCl (in mmol l^{-1}) in which the sum of [NaCl] and [KCl] was 180mmol l^{-1} and the concentrations bracketed the experimental values. Concentrations were calculated from the equation:

$$[C^+]_e = [C^+]_c \times 10^{V/s},$$

where $[C^+]_e$ is the concentration of Na^+ or K^+ in the experimental droplet, $[C^+]_c$ is the concentration of the same ion in the calibration solution, V is the voltage change (in mV) when ion-selective and reference electrodes were moved from the calibration solutions to the experimental droplet, and s is the slope of the electrode response (in mV) for a tenfold change in ion concentration. Reference electrodes were constructed from 1mm o.d. glass capillaries with an internal filament. For K^+ measurements, the tip and shank of the reference microelectrode were filled with 1mol l^{-1} sodium acetate and the rest of the barrel was then backfilled with 1mol l^{-1} KCl. Reference microelectrodes were filled with 3 mol l^{-1} KCl for use with Na^+ microelectrodes.

Fig. 1 shows the effects of gramicidin D on the rate of fluid secretion and $[K^+]$ of the secreted fluid of an upper Malpighian tubule of a fifth-instar *Rhodnius*. The tubule was bathed in standard saline containing 146mmol l^{-1} Na^+ and 8.6mmol l^{-1} K^+ (Maddrell *et al.* 1993) to which had been added 3.6 μg ml^{-1} gramicidin D. The transient stimulation of fluid secretion is the result of increased oxygen availability and stirring of the bathing droplet which occurs whenever a solution is added to the bathing droplet (Maddrell *et al.* 1988). The potassium concentration of the secreted fluid rapidly dropped from 96mmol l^{-1} to 5mmol l^{-1} in response to 3.6 μg ml^{-1} gramicidin D (Fig. 1A). The response is even more pronounced with higher concentrations; on exposure to 125 μg ml^{-1} gramicidin D, for example, the potassium concentration of the secreted fluid

dropped from 88mmol l^{-1} to 0.2mmol l^{-1}. For gramicidin concentrations of 1.2 μg ml^{-1} and above, the K^+ concentration dropped on average to 3.3±0.9mmol l^{-1} (mean ± S.E., *N*=5; see Fig. 3). These results show that addition of gramicidin converts *Rhodnius*'

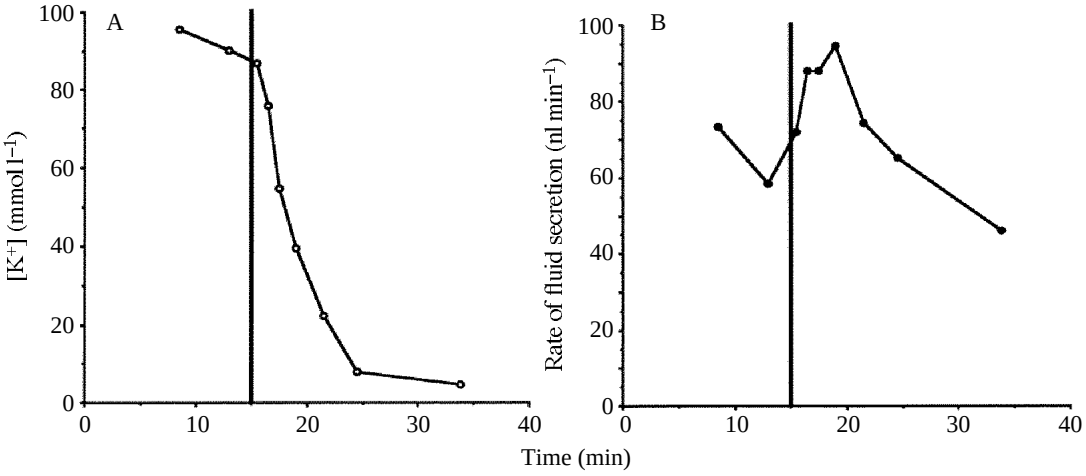

Fig. 1. The effects of 3.6 μg ml^{-1} gramicidin D on K^+ concentration of secreted fluid (A) and the rate of fluid secretion (B) by a Malpighian tubule isolated from a fifth-instar *Rhodnius prolixus*. At 15min (vertical line) after the start of the experiment, 10 μl of saline containing 40 μg ml^{-1} gramicidin D was added to the 100 μl droplet of bathing saline. The decline in $[K^+]$ was complete before the secretion rates dropped below values measured prior to addition of gramicidin, suggesting that the condition of the epithelium, as indicated by its ability to secrete fluid, was not seriously impaired in the short term by exposure to the drug.

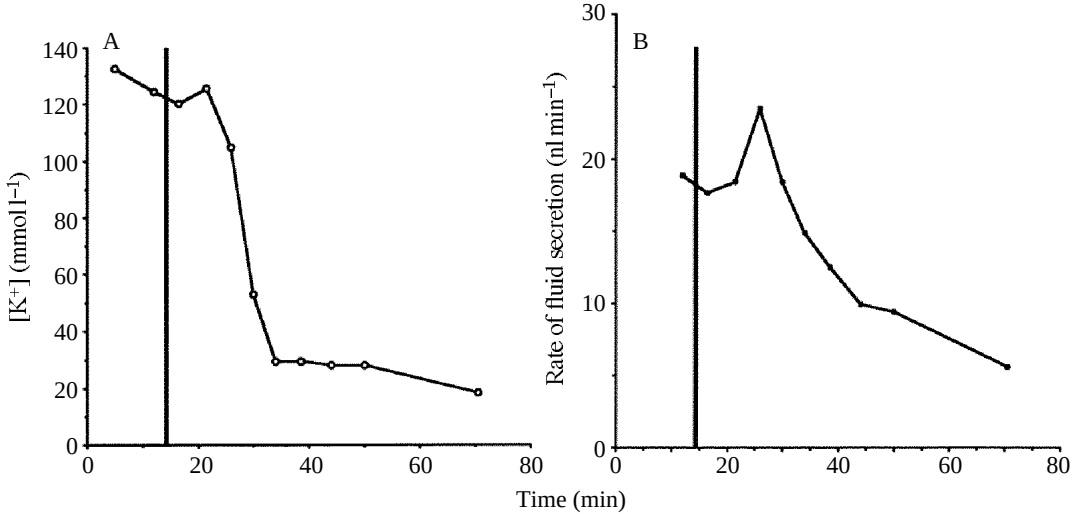

Fig. 2. The effects of 22.5 μg ml^{-1} gramicidin D on K^+ concentration of secreted fluid (A) and the rate of fluid secretion (B) by an isolated salivary gland of *Calliphora erythrocephala*. At the time indicated by the vertical line, 6 μl of saline containing 0.4mgml^{-1} gramicidin D was added to 100 μl of bathing saline.

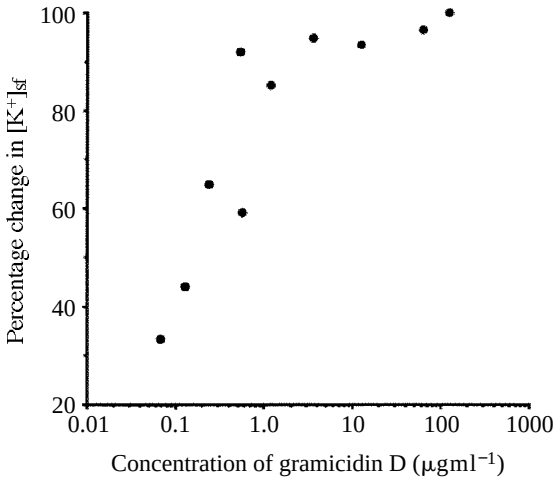

Fig. 3. Dose–response curve for the effects of gramicidin D on the K^+ concentration of fluid secreted by the upper Malpighian tubules of fifth-instar *Rhodnius*. The percentage change in K^+ concentration of the secreted fluid ($[K^+]_{sf}$) was calculated from the concentrations measured before and 20min after the addition of the indicated dose of gramicidin D.

Malpighian tubule, an epithelium that normally secretes equimolar NaCl and KCl, into one which no longer secretes significant amounts of potassium.

Experiments with isolated salivary glands of *Calliphora* indicate that this epithelium, which normally secretes potassium, can be converted into a sodium-secreting epithelium by addition of gramicidin. Fig. 2 shows the effects adding 22.5 $\mu g\,ml^{-1}$ gramicidin D to the saline bathing an isolated salivary gland; the potassium concentration of the secreted fluid dropped from 125 $mmol\,l^{-1}$ to 18 $mmol\,l^{-1}$. On average, the potassium concentration of the secreted fluid changed from 150.1±10.9 $mmol\,l^{-1}$ to 23.3±1.4 $mmol\,l^{-1}$ (means ± S.E., N=7) after addition of 22.5 $\mu g\,ml^{-1}$ gramicidin. The sodium concentration in the secreted fluid after treatment was 152.5±3.1 $mmol\,l^{-1}$ (mean ± S.E., N=4).

Both the Malpighian tubules of *Rhodnius* and the salivary glands of *Calliphora* are extremely sensitive to gramicidin D. Although the dose of the drug required for maximal effect on mammalian epithelia such as the descending colon and the urinary bladder of rabbits is 0.13$mg\,ml^{-1}$ (Wills, 1981), potassium concentrations in the fluid secreted by Malpighian tubules are decreased substantially by concentrations more than 1000-fold lower (Fig. 3).

These results show that insect epithelia that normally secrete fluid containing high concentrations of potassium can be converted to epithelia that secrete sodium. This conversion depends on the fact that the treatment with gramicidin allows sodium and potassium more nearly equal access to the intracellular environment (see Wills, 1981). However, in all the experiments described above, the bathing saline was essentially Na^+-rich, reflecting the normal ionic composition of the haemolymph, so that the apical transporters might in these cases have been presented with fluid containing more Na^+ than K^+ (depending on the relative effectiveness of the gramicidin pores and the permeability of the plasma membrane).

It has been argued that insect epithelia such as Malpighian tubules can regulate their intracellular environment to be K^+-rich and Na^+-poor and at the same time secrete fluid containing a predominance either of K^+ or of Na^+ solely through the activity of the apical ion transporter(s) (Maddrell, 1978). This ability was thought to depend on an ability actively to transport both cations from cell to lumen but with a preference for Na^+. *Rhodnius* Malpighian tubules secrete Na^+ more rapidly than K^+ at all intracellular concentrations of Na^+ higher than 20mmol l^{-1}, when $[K^+]_i$ is more than 100mmol l^{-1} (Maddrell, 1978). If this hypothesis is correct and applies to other insect epithelia, then treatment with gramicidin in saline containing equal concentrations of Na^+ and K^+ should lead to the secretion of fluid containing higher concentrations of Na^+ than K^+. In fact, addition of gramicidin D at 20.8 μg ml^{-1} to saline containing equal concentrations of Na^+ and K^+ (77mmol l^{-1}) reduced the K^+ concentration of the fluid secreted by *Rhodnius* tubules from 91.5±2.8mmol l^{-1} to 37.6±1.9mmol l^{-1} (mean ± S.E., N=4). The K^+ concentration of the fluid secreted by *Calliphora* salivary glands dropped from 123.4±5.4mmol l^{-1}, before addition of 45 μg ml^{-1} gramicidin D, to 76.7±7.6mmol l^{-1} (mean ± S.E., N=7) by 25–35min after addition of the drug.

What these results suggest, then, is that indeed the apical ion transporters [V-type H^+-ATPase plus cation/H^+ antiporter(s)] of *Rhodnius* tubules show a preference for Na^+ over K^+ , but that those of *Calliphora* salivary glands show an ability to transport Na^+ and K^+ with equal effectiveness. In both epithelia, nonetheless, transport under all normal conditions will maintain the cell interior as K^+-rich and Na^+-poor while allowing transepithelial transport of either Na^+- or K^+-rich fluids. In *Rhodnius* this result seems to require that the apical cation/H^+ antiporter has an absolute preference for Na^+ over K^+, presumably to match the rapid basal entry of Na^+ *via* the $Na^+/K^+/2Cl^-$ cotransporter. In *Calliphora*, Na^+ enters the cell across the basal side more slowly than K^+ (Berridge *et al.* 1976) so that the equal ability of the apical cation/H^+ antiporters to transfer Na^+ or K^+ into the lumen will still result in intracellular Na^+ levels being kept low. It will be of great interest to discover whether in these epithelia the difference in apical transport of Na^+ and K^+ is achieved by one cation/H^+ antiporter with variable Na^+/K^+ preference or by two distinct Na^+/H^+ and K^+/H^+ antiporters.

These considerations go a long way to explain how it is that ouabain, the potent inhibitor of Na^+/K^+ ATPase, only rarely affects the rate or composition of fluid secretion by insect epithelia (Anstee and Bell, 1975; Maddrell and Overton, 1988). Epithelial fluid transport in insects is often very rapid (in extreme cases each cell can transfer its own volume of fluid in 15s, Maddrell, 1991). It makes good sense that the apical ion transporters driving fluid secretion automatically regulate the intracellular cation levels rather than regulation being provided by the activity of the basal Na^+/K^+ ATPase, as this would need to be present at very high concentrations and, in Na^+-transporting epithelia, would transfer important ions in a direction opposite to that required.

References

ANSTEE, J. H. AND BELL, D. M. (1975) Relationship of Na^+–K^+-activated ATPase to fluid secretion by Malpighian tubules of *Locusta migratoria*. *J. Insect Physiol.* **21**, 1779–1784.

BERRIDGE, M. J., LINDLEY, B. D. AND PRINCE, W. T.(1976). Studies on the mechanism of fluid secretion by isolated salivary glands of *Calliphora*. *J. exp. Biol.* **64**, 311–322.

BERRIDGE, M. J. AND PATEL, N. G. (1968). Insect salivary glands: stimulation of fluid secretion by 5-hydroxytryptamine and adenosine 3′,5′-monophosphate. *Science* **162**, 462–463.

GEE, J. D. (1976). Active transport of sodium by the Malpighian tubules of the tsetse fly *Glossina morsitans*. *J. exp. Biol.* **64**, 357–368.

HARVEY, W. R.(1992). Physiology of V-ATPases. *J. exp. Biol.* **172**, 1–17.

KLEIN, U. (1992). The insect V-ATPase, a plasma membrane proton pump energizing secondary active transport: immunological evidence for the occurrence of a V-ATPase in insect ion-transporting epithelia. *J. exp. Biol.* **172**, 345–354.

MADDRELL, S. H. P. (1969). Secretion by the Malpighian tubules of *Rhodnius*. The movements of ions and water. *J. exp. Biol.* **52**, 71–97.

MADDRELL, S. H. P.(1978). Transport in insect excretory epithelia. In *Membrane Transport in Biology*, vol. III, T*ransport Across Multimembrane Systems*. Heidelberg, New York: Springer-Verlag Berlin.

MADDRELL, S. H. P. (1991). The fastest fluid-secreting cell known: the upper Malpighian tubule cell of *Rhodnius*. *BioEssays* **13**, 357–362.

MADDRELL, S. H. P., HERMAN, W. S., FARNDALE, R. W. AND RIEGEL, J. A. (1993). Synergism of hormones controlling epithelial fluid transport in an insect. *J. exp. Biol.* **174,** 65–80.

MADDRELL, S. H. P., HERMAN, W. S., MOONEY, R. L. AND OVERTON, J. A.(1991). 5-Hydroxytryptamine: a second diuretic hormone in *Rhodnius*. *J. exp. Biol.* **156**, 557–566.

MADDRELL, S. H. P., LANE, N. J., HARRISON, J. B., OVERTON, J. A. AND MORETON, R. B. (1988). The initial stages in the action of an insecticidal δ-endotoxin of *Bacillus thuringiensis* var. *israelensis* on the epithelial cells of the Malpighian tubules of the insect, *Rhodnius prolixus*. *J. Cell Sci.* **90**, 131–144.

MADDRELL, S. H. P. AND O'DONNELL, M. J. (1992). Insect Malpighian tubules: V-ATPase action in ion and fluid transport. *J. exp. Biol.* **172**, 417–429.

MADDRELL, S. H. P. AND OVERTON, J. A. (1988). Stimulation of sodium transport and fluid secretion by ouabain in an insect Malpighian tubule. *J. exp. Biol.* **137**, 265–276.

MADDRELL, S. H. P., PILCHER, D. E. M. AND GARDINER, B. O. C. (1971). Pharmacology of the Malpighian tubules of *Rhodnius* and *Carausius*: the structure–activity relationship of tryptamine analogues and the role of cyclic AMP. *J. exp. Biol.* **54**, 779–804.

O'DONNELL, M. J. AND MADDRELL, S. H. P. (1984). Secretion by the Malpighian tubules of *Rhodnius prolixus* Stål: electrical events. *J. exp. Biol.* **110**, 275–290.

RAMSAY, J. A. (1953). Active transport of potassium by the Malpighian tubules of insects. *J. exp. Biol.* **30**, 358–369.

WIECZOREK, H., PUTZENLECHNER, M., ZEISKE, W. AND KLEIN, U. (1991). A vacuolar-type proton pump energizes K^+/H^+ antiport in an animal plasma membrane. *J. biol. Chem.* **266**, 15340–15347.

WIECZOREK, H. (1992). The insect V-ATPase, a plasma membrane proton pump energizing secondary active transport: molecular analysis of electrogenic potassium transport in the tobacco hornworm midgut. *J. exp. Biol.* **172**, 335–343.

WILLIAMS, J. C. AND BEYENBACH, K. W. (1983). Differential effects of secretagogues on Na and K secretion in the Malpighian tubules of *Aedes egypti* (L.). *J. comp. Physiol.* **149**, 511–517.

WILLS, N. K. (1981). Antibiotics as tools for studying the electrical properties of tight epithelia. *Fedn Proc. Fedn Am. Socs exp. Biol.* **40**, 2202–2205.

WRIGHT, J. C. AND O'DONNELL, M. J. (1992). Osmolality and electrolyte composition of pleon fluid in *Porcellio scaber* (Crustacea, Isopoda, Oniscidea): implications for water vapour absorption. *J. exp. Biol.* **164**, 189–203.