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Maintenance of function in single epithelial cells spatially isolated from similar cells

S. H. P. MADDRELL AND J. A. OVERTON

A.F.R.C Unit of Insect Neurophysiology & Pharmacology, Department of Zoology, Downing Street, Cambridge CB2 3EJ

SUMMARY

We have found in an insect tissue, the Malpighian tubules of *Rhodnius*, instances of single epithelial cells which, as the result of a possible error in development, lie within the epithelium some distance from the main population of similar cells. This spatial separation makes it possible to measure the transport abilities of these cells. Their transport abilities were found to be the same as the cells in the main population. This finding shows that the maintenance of function in individual cells of epithelial tissues may not depend on direct contact with other similar cells.

INTRODUCTION

Many transporting epithelia consist of a single layer of similar cells, all of which are in contact with one another and all of which are thought to carry out the same function. The Malpighian tubules of insects are long blind-ending tubes whose epithelial walls are one cell thick. In the blood-sucking insect *Rhodnius*, the tubules are effectively made up of two separate epithelia: an upper, fluid-secreting region and a lower region that secretes uric acid and reabsorbs KCl (Wigglesworth, 1931*b*; O'Donnell, Maddrell & Gardiner, 1983; Maddrell, 1978). The cells of the two epithelia are easily distinguishable (Wigglesworth, 1931*a*; Wigglesworth & Salpeter, 1962) and, in nearly all cases, the boundary between them is a sharp one. In about 10 % of the tubules, however, there is what appears to be an error in development and single cells typical in appearance of the upper region are found in the lower region (Fig. 1), at a distance of up to about 15 cell diameters (1·25 mm) from the boundary. We have found that in spite of this separation the transport abilities of these single cells are the same as the cells of the upper region that are in contact with one another.

MATERIALS AND METHODS

Insects

The insects used were 5th instar larvae taken from a laboratory culture of *Rhodnius prolixus* maintained at 27°C. For some experiments on transport of p-aminohippuric acid (PAH), the insects were fed 2–5 days previously.

Key words: epithelial transport, single cells, cell–cell contact, sodium transport, organic anion transport, *Rhodnius*.

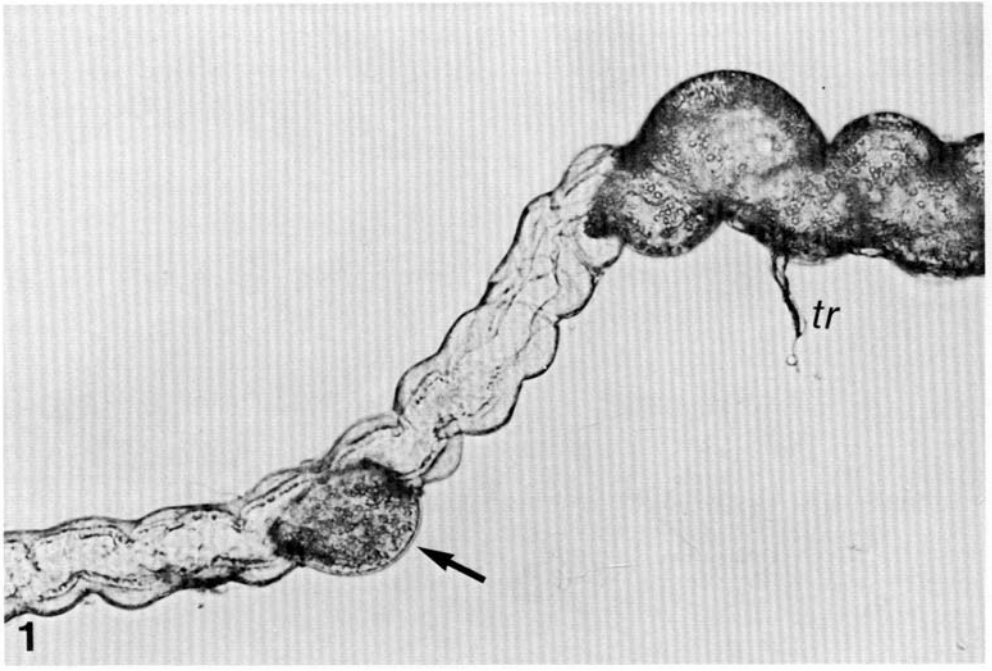

Fig. 1. A part of a Malpighian tubule from a 5th stage *Rhodnius* to show the relatively opaque cells typical of the upper region to the right and translucent cells characteristic of the lower tubule to the left. Among the lower cells is a single dense cell (arrow) very similar in appearance to the cells of the upper tubule. *tr*, a short length of trachea (air supply system) attached to one of the upper cells. ×300.

Isolation of Malpighian tubules

Malpighian tubules were dissected from the insects under saline, whose composition was (mM): NaCl, 142; KCl, 8·6; $CaCl_2$, 2; $MgCl_2$, 8·5; glucose, 34; HEPES, 8·6; plus NaOH to take the pH to 7·0. By suitable manoeuvring, it is possible to isolate Malpighian tubules into drops of saline under liquid paraffin (mineral oil) so that the properties of the single cells can be investigated. Fig. 2 shows the experimental arrangement used. The uppermost length of the upper tubule is immersed in a drop of saline containing 5-hydroxytryptamine (5-HT) at 10^{-6}M which stimulates it to secrete fluid into the lumen (Maddrell, Pilcher & Gardiner, 1969; Maddrell, Pilcher & Gardiner, 1971). This fluid passes down the tubule and through the lumen of the part bathed in a smaller drop, which contains a radioactive test substance, gathering as it does so any of the substance entering the tubule from this second drop. The fluid is collected from a cut in the wall of the tubule downstream from the smaller drop, and can be assayed for its content of the radioactive substance whose transport into the lumen is being measured. The part of the tubule in the second drop is arranged so that it contains a single upper cell surrounded by cells of the lower tubule (Fig. 2) or a control length of lower tubule. In either case, the length of tubule in the test drop contains about 100 cells.

The arrangement described was used to investigate the ability of single upper cells to transport either sodium ions or the organic anion p-aminohippuric acid (PAH). Normal upper cells transport sodium-rich fluid into the lumen at very high rates when stimulated with 5-HT at concentrations above 10^{-7}M (Maddrell, 1980). They also transport PAH into the lumen (Maddrell, Gardiner, Pilcher & Reynolds, 1974) but at a very low rate unless the tubules are taken from *Rhodnius* fed a few days previously, in which case a high rate of PAH transport is induced (Maddrell & Gardiner, 1975). Lower tubule cells do not transport either sodium ions or PAH (Maddrell & Phillips, 1975).

RESULTS

Transport of $^{22}Na^+$ into the lumen

The rate of arrival of $^{22}Na^+$ into the lumen of a length of tubule with a single upper cell in it was measured and compared with the behaviour of a control length without such a cell. For these experiments a modified saline was used where the concentration of Na^+ was reduced to 4 mM and the concentration of K^+ was correspondingly increased. Under these conditions, any inward transport of sodium-rich fluid shows up more clearly. In tubules with a single upper cell, the rate of entry of $^{22}Na^+$ into the lumen was low when the bathing drop contained no 5-HT, but rapidly increased (Fig. 3) to a rate of $5{\cdot}0 \pm 1{\cdot}0$ pmol min^{-1} ($n = 8$) when the drop was replaced by one containing 5-HT at 10^{-6} M. This rate was not significantly different from the $6{\cdot}0 \pm 0{\cdot}9$ pmol min^{-1} $cell^{-1}$ ($n = 20$) at which upper cells from above the upper/lower boundary transported sodium ions into the lumen under the same conditions. The rate decreased to a low level again if the single cell was moved to a position just outside the bathing drop (Fig. 3).

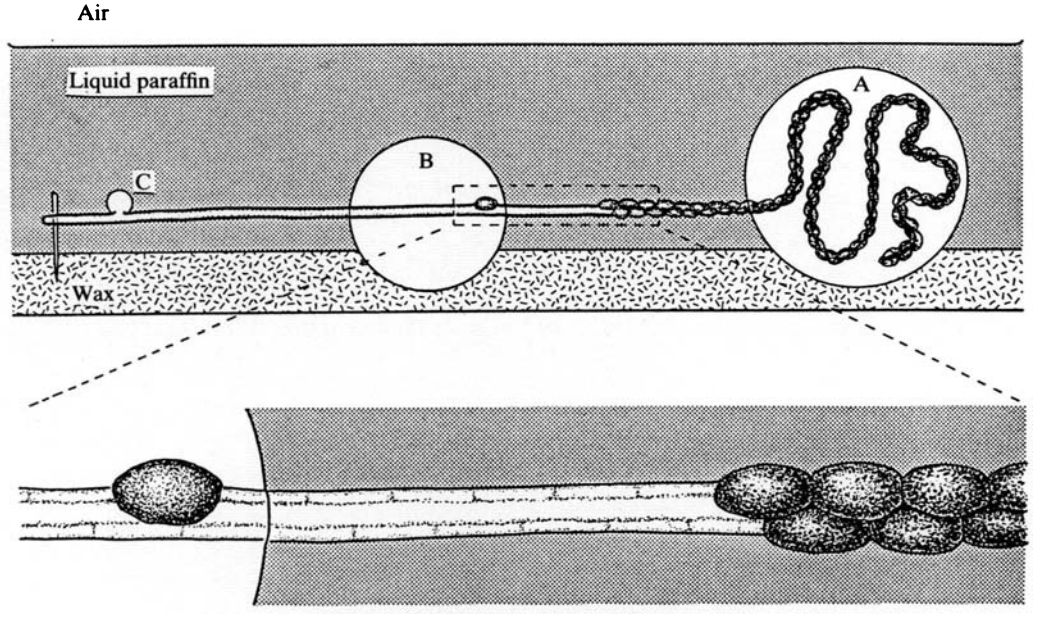

Fig. 2. Diagrammatic representation of the experimental arrangement used to follow the transporting activity of single upper Malpighian tubule cells. Most of the upper, fluid-secreting region of the tubule is placed in a 100 μl drop, A, held in position in a depression in the wax base. The lower tubule is arranged so that the boundary between it and the upper tubule is a short distance upstream from a 20 μl test drop, B. As shown, this allows the single upper cell, whose activity is being investigated, to be situated just within the test drop. The test drop contains the radioactive test substance being used. Fluid passing down the lumen is collected from a cut in the wall of the tubule at C. The lower drawing shows, in close-up, the upper/lower boundary and the position of the single upper cell.

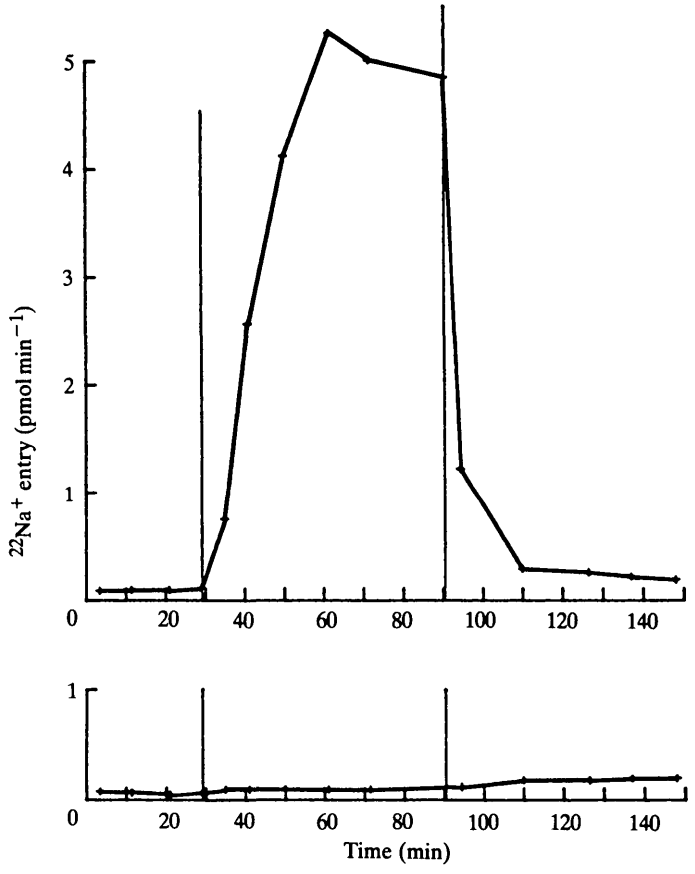

Fig. 3. $^{22}Na^+$ transport into the lumen of lower tubules. In one (upper graph), a single upper cell was included in the length of tubule in the test drop; in the other (lower graph), there were only lower cells. At time = 29 min (first vertical line), the bathing drop was replaced with one containing 10^{-6}M 5-HT in addition. At time = 91 min (second vertical line), a similar short length of tubule was pulled out of each test drop; in the case of the tubule described by the upper graph, this was enough to move the single upper cell to a position just outside the test drop.

PAH transport

From a bathing drop containing ^{3}H-labelled PAH at a concentration of 0·05 mM, relatively little PAH crossed into the lumen of a length of tubule composed entirely of lower cells or with a single upper cell among them when the tubule was taken from an unfed insect. However, with a lower tubule taken from an insect fed a blood meal 2–5 days earlier and having a single upper cell in it, the rate of PAH entry was noticeably higher (Fig. 4). It was rapidly reduced by treatment with 0·5 mM-probenecid, a drug that blocks transport of organic anions such as PAH (Despopoulos, 1965). Alternatively, the PAH entry could be returned to control levels by moving the single upper cell to a position just outside the bathing drop (Fig. 4). The rates of PAH transport by non-inhibited, induced single upper cells in lengths of lower tubule were not significantly different from

those achieved by upper cells from above the upper/lower boundary; single cells in lower tubules transported $0{\cdot}033 \pm 0{\cdot}005$ pmol PAH min^{-1} ($n = 8$), upper cells from upper tubules from the same insects transported $0{\cdot}031 \pm 0{\cdot}004$ pmol PAH min^{-1} $cell^{-1}$ ($n = 8$) in control experiments.

DISCUSSION

These results show that it is possible to measure, in a tubular epithelium, transport activities of *single* epithelial cells spatially isolated from other cells of the same type. In the Malpighian tubules of *Rhodnius* at least, the transporting ability of such isolated cells is not different from that of similar cells that are in contact with one another. The cells of *Rhodnius'* tubules, unlike most of the others in the body, do not divide during larval growth (Maddrell, Lane, Harrison & Gardiner, 1985). The single cells therefore remain isolated during several months when profound changes occur elsewhere in the insect. That they nonetheless maintain their function shows that they do not depend for this on contact with other similar cells. Several interesting questions arise. For example, what is the pattern of cell–cell coupling between cells of the upper tubule, between cells of the lower tubule, between upper and lower cells either side of the upper/lower boundary

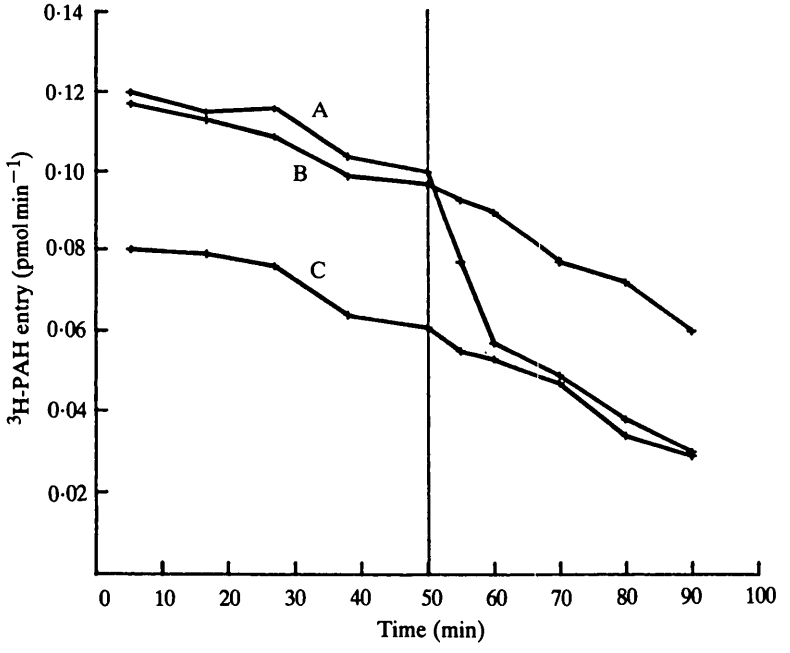

Fig. 4. ^{3}H-PAH transport into the lumen of lower tubules from 5th stage insects fed 4 days previously. In two cases, A and B, a single upper cell was included in the length of tubule in the test drop; in the other, C, there were only lower cells. At time = 50 min (vertical line), similar short lengths of tubules A and C were pulled out of the test drop; tubule B was undisturbed. In A, this treatment removed the single upper cell to a position just outside the test drop.

and between single isolated upper cells and their lower cell neighbours? How in early development do errors in cell positioning arise? Are isolated upper cells identical in ultrastructure to normal upper cells and are they identical to them in all aspects of their transporting abilities, including such potentially subtle differences as differences in dose–response relationships towards stimulants and inhibitors and in the time course of induction and so on? Research now in progress is attempting to answer these questions.

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