## An attempt to measure the lifetime of sodium channels in transporting epithelia

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Recently it has proved possible to use [$^{14}$C]-amiloride as a reversible site label for sodium entry sites in cells isolated from transporting epithelia (Cuthbert & Shum, 1975). This communication reports an attempt to determine the lifetime of sodium entry sites in isolated cells prepared from toad bladders (*Bufo marinus*). This parameter is of interest, particularly when considering the effects of aldosterone, which we have shown previously causes an increase in the number of binding sites (Cuthbert & Shum, 1975).

Suspensions of epithelial cells were prepared as described previously and binding of amiloride measured at a concentration of 50 nM. At this concentration non-specific binding accounts for only 20% of the total displaceable label.

In initial experiments cells were suspended in a nutrient salt solution (containing 1.1 mEq/l $Na^+$) with penicillin G (1000 u/ml) and streptomycin (0.5 mg/ml) and incubated with gentle shaking at room temperature for periods of up to 144 hours. Periodically aliquots were withdrawn for labelling. The slopes of the regression lines relating the number of binding sites with respect to time were not significantly different from zero. In other experiments cycloheximide (0.5 μg/ml) was added to the cell suspension at about 6 h and aliquots were withdrawn for labelling as before. The times taken for the binding site density to fall to half the original values were 60, 57, 32 and 61 h in four separate experiments.

Cell suspensions which had been exposed to aldosterone (50 nM) for 4 h were then treated with cycloheximide (0.5 μg/ml) and labelled with amiloride. Compared with control suspensions derived from the same tissues, but not exposed to aldosterone, there was a significant ($p < 0.001$) increase in the number of amiloride binding sites. The mean increase in 5 experiments was 50%.

The time course of the decline in the density of binding sites in aldosterone and cycloheximide treated suspensions was identical to that of non-hormone treated cells, suggesting that pre-existing and aldosterone induced sites have similar lifetimes. Provided that amiloride binding sites can be equated with sodium entry sites then this result has consequences for the duration of the aldosterone response, provided sodium entry and not other energetic factors (see for example Sharp & Leaf, 1966) remains the rate determinant of transport.

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**References**

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## DL-Homocysteate-induced motoneurone depolarization with membrane conductance *decrease*

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The non-physiological ion DL-homocysteate (DLH), applied by microiontophoresis, has been used widely to excite cells for extracellular recording in neuropharmacological studies. It has been of particular value for investigations of putative inhibitory transmitters (Curtis, 1965; Engberg & Ryall, 1966). It has been supposed that the action of DLH and other excitatory amino acids is accompanied by an increase in membrane conductance (Curtis, 1970; Curtis, Duggan, Felix, Johnston, Tebecis & Watkins, 1972). We have shown that the DLH induced depolarization of motoneurones is accompanied by a *decrease* of conductance.

DLH 0.3 M, L-glutamate 1 M, L-aspartate 1 M, (all at pH 8) were applied from the six iontophoretic barrels of a coaxial electrode assembly to lumbar motoneurones of seven cats (four pentobarbitone, three decerebrate).

Membrane conductance ($G_m$) was measured by passing current pulses through the screened central recording electrode.

Typical responses to DLH are shown in Figure 1.