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 Periodically aliquots were withdrawn for labelling.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 In other experiments cycloheximide (0.5 μg/ml) was added to the cell suspension at about 6 h and aliquots were withdrawn for labelling as before.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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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.

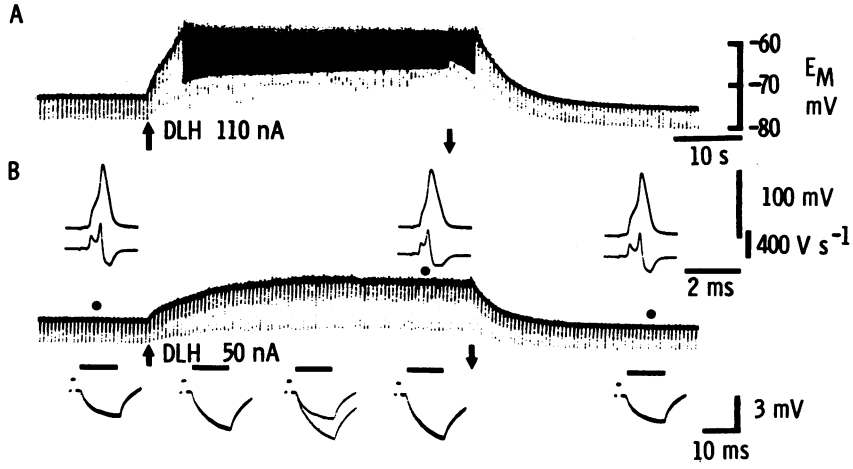

Figure 1 Responses of a hamstring motoneurone to iontophoresed DLH in a nembutal anaesthetized cat. The membrane potential (E_m) was displayed on a mingograph—the traces being modulated by the after-hyperpolarizations of antidromically evoked or DLH induced action potentials and by short hyperpolarizing current pulses. *A.* An unbalanced dose (110 nA) of DLH reversibly depolarized the cell. At 15 mV depolarization the cell fired repetitively at a high frequency. *B.* A lower dose (50 nA) of DLH caused a depolarization of 10 mV, insufficient to evoke firing. Conductance measuring pulses (3 nA, 10 ms) are shown below and were averaged during the periods shown by the bars. The conductance pulse recorded before DLH application is superimposed on the pulse recorded during DLH application in the middle record. Antidromic action potentials and their first derivatives were recorded at the times indicated by the filled circles.

DLH reversibly depolarized the cell which led to repetitive firing with little adaptation (Figure 1A). At a dose insufficient to cause firing the DLH depolarization was accompanied by a marked decrease in G_m (Figure 1B). During the DLH induced depolarization the excitatory post-synaptic potentials (e.p.s.ps) changed differently from neurone to neurone: an increase in amplitude; an increase in the time constant of decay; a combination of these changes or, less commonly, no change. Both inhibitory post-synaptic potentials (i.p.s.ps) and after-hyperpolarizations showed stable increases in size.

Glutamate at low doses caused a depolarization accompanied by either: a small decrease, no change or a small increase in G_m. At higher doses glutamate produced a plateau of depolarization during which the conductance gradually rose until the membrane was virtually short-circuited—as has been similarly seen by Zieglgängsberger & Puil, 1973. Glutamate has previously been seen to depolarize with little or no change in G_m (Bernardi, Zieglgängsberger, Herz & Puil, 1972; Steinberg, Altmann & ten Bruggencate, 1974). The results from one cat indicate that aspartate essentially acts in the same way as glutamate.

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