SHORT COMMUNICATION

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Stability of electrical coupling in leech giant nerve cells: Divalent cations, propionate ions, tonicity and pH

Recent work from this laboratory has shown that sequestering of divalent cations causes, under certain conditions, complete functional uncoupling of epithelial cell membrane junctions[1,2]. Moreover, preliminary results on Retzius nerve cells (leech) have led to the conclusion that such sequestering leads to uncoupling in these cells too[3]. A fuller study now reveals that in the nerve cells, unlike epithelial cells, divalent cation sequestering causes, at most, a small change in junctional resistance.

Ganglia of the leech *Hirudo medicinalis* were isolated into saline. Three microelectrodes were inserted into the bodies of two adjacent Retzius nerve cells. One electrode served to pass rectangular current pulses of $1 \cdot 10^{-8}$ A into one cell, and the other two electrodes served to record the resulting voltages in this cell (V_I) and simultaneously in the adjacent one (V_{II}) (Fig. 1, inset). The electrical arrangement and general procedure were essentially as described before[3], except for an improved system of superfusion which reduced mechanical disturbances to the preparation.

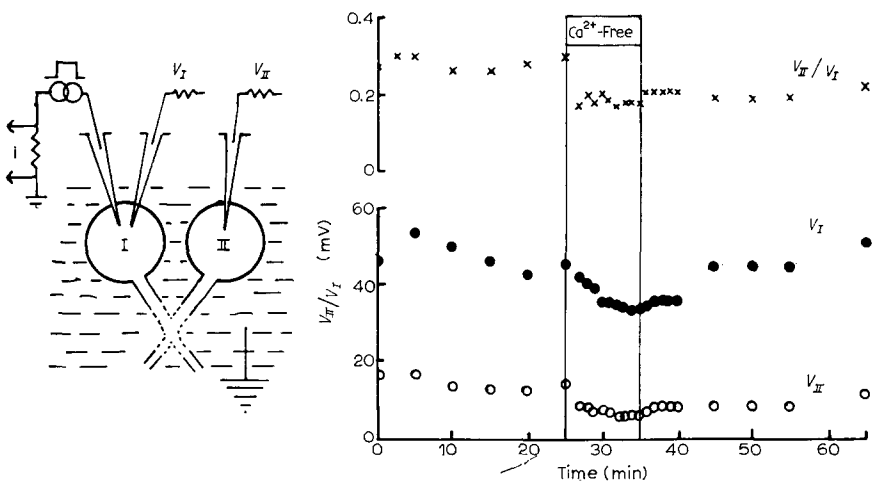

Fig. 1. Effects of Ca^{2+}-free medium on electrical cell coupling. Current pulses ($I = 1 \cdot 10^{-8}$ A) are passed between interior and exterior of cell I. The resulting voltage drops are recorded simultaneously in cell I (V_I) and cell II (V_{II}); the lower ordinate gives the corresponding resistive voltages. Vertical lines enclose the period in Ca^{2+}-free medium.

With this system, microelectrodes could generally be kept inside the cells throughout the experiments; variations in cell input resistances were thereby minimized. The following superfusion media were used:

(a) Control medium (normal saline), in mM: NaCl, 113.5; KCl, 4.3; $CaCl_2$, 1.8; maleic acid, 10; glucose, 10; Tris buffer, 10; NaOH, approx. 10, adjusted to pH 7.4.

(b) Calcium-free medium: normal saline Ca^{2+}-free.

(c) Chelating media: normal saline with disodium ethylenediamine tetraacetate (EDTA)–$CaCl_2$ or ethylene glycol-bis-(β-aminoethyl ether)-*N'*,*N*-tetraacetic acid (EGTA)–$CaCl_2$ mixtures, giving free Ca^{2+} concentrations ranging from $5 \cdot 10^{-7}$ to 10^{-4} M (total EDTA or EGTA was $2.9 \cdot 10^{-3}$ to $3 \cdot 10^{-3}$ M); and Ca^{2+}-free saline with EDTA (total EDTA, $3 \cdot 10^{-3}$ M).

(d) Anisotonic media: normal saline *plus* sucrose to give osmolarities 2 times normal, and normal saline reduced in NaCl to give osmolarities 0.5 times normal.

(e) Alkaline media: normal saline buffered to pH 10 with glycine.

(f) Propionate medium: NaCl of normal saline replaced by equimolar sodium propionate.

The superfusion periods with the various test media were generally 10 min. A few experiments were also done with each medium for periods up to 30 min with essentially the same results.

When the nerve cells were in Ca^{2+}-free medium, their input resistances fell slightly, as shown by a reduction in V_I and V_{II}, but there was no significant change in coupling ratio (V_{II}/V_I) (Fig. 1). In Ca^{2+}-free chelating media, both input resistance and coupling ratio fell markedly. They rose again during return to control medium. In chelating media with free Ca^{2+} concentrations above 10^{-5} M, the input resistance

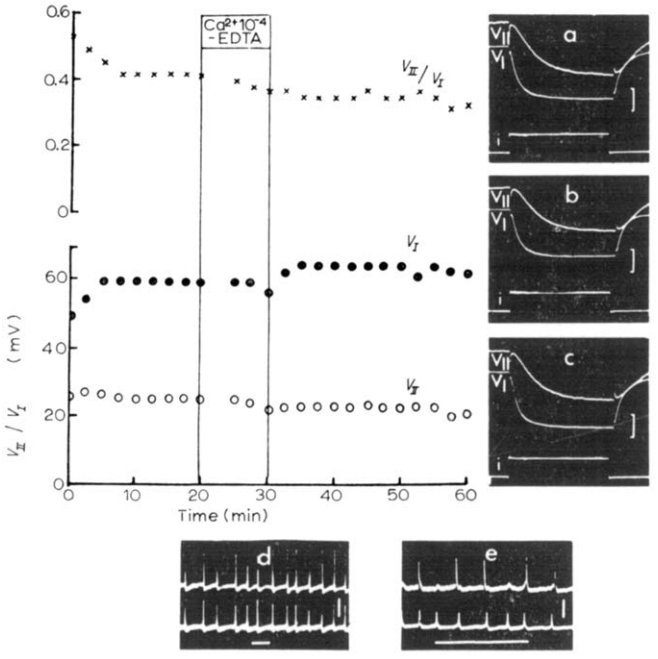

Fig. 2. Effects of chelating medium. Between the vertical lines the cell system is in chelating medium containing $2.9 \cdot 10^{-3}$ M EDTA and $3 \cdot 10^{-3}$ M Ca^{2+}, giving 10^{-4} M free [Ca^{2+}]. Insets are samples of oscilloscope records of the membrane current ($I = 1 \cdot 10^{-8}$ A; 100 msec duration) and membrane voltages (V_I and V_{II}) before (a), during (b) and after (c) superfusion with chelating medium. Voltage calibration: V_I, 25 mV; V_{II}, 10 mV. (d) Samples of spontaneous electrical activity before and (e) after superfusion. Calibration: 10 mV, 1 sec.

and coupling ratio changed little (Fig. 2). Upon return to the control medium, the final coupling ratio was generally lower than that before chelator treatment: V_I was slightly greater and V_{II} smaller than before treatment (Fig. 2). This may possibly reflect a rise in junctional resistance which, estimated on the basis of a simple model of symmetric resistive elements[3] amounts, at most, to a 2-fold rise. However, such a rise, if at all significant, was not sufficient to cause functional uncoupling; synchrony of nerve impulses in the cells persisted in most cases. In a few cases, such as illustrated in Fig. 2e, there may have been partial failure of synchrony. This, however, is not necessarily due to a change in junctional resistance; it may have been due to an increased excitation threshold. (Resting potentials fell in some experiments by as much as 20 %, while in others they stayed constant.)

Treatment with anisotonic or propionate media had no detectable effects on cell coupling. Alkaline media produced effects similar to those of Ca^{2+}-free chelating media.

The coupling in Retzius nerve cells appears to be rather stable in regard to the various treatments above. This contrasts with the behaviour of epithelial and sponge cells[1,4]. Chironomous salivary gland cells, for instance, are readily uncoupled by chelating, hypertonic or alkaline media. With free Ca^{2+} concentrations of 10^{-4} M, uncoupling of these cells in such media is so complete that the junctional membranes, normally highly permeable, become nearly as impermeable as the non-junctional membranes[1,2]. The coupling stability in the Retzius nerve cells contrasts also with that of the crayfish septate axons which, more recently, were shown to uncouple in propionate media[5]. The question is now whether the difference in stability reflects basic differences in junction processes in these various cells. To answer this, one would like to have information on the coarse and fine structure of the Retzius cells, including information on the location of the junctional with respect to the cell body and on possible diffusion barriers.

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