PRELIMINARY NOTES

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Depression of junctional membrane permeability by substitution of lithium for extracellular sodium

The present experiments were prompted by the hypothesis that the high permeability of junctional membranes is a consequence of the low concentration of free Ca^{2+} in cytoplasm[1]. Junctional membrane permeability has been found to fall under experimental conditions in which cytoplasmic Ca^{2+} concentration rises or may be expected to rise: when Ca^{2+} is microinjected into cells[2], when Ca^{2+} enters cells through leaks in nonjunctional surface membrane or in perijunctional insulation[2,3], or when cell energy metabolism is inhibited[4,5]. Here we examine junctional membrane permeability under another experimental condition of this kind, when Li^+ substitutes for Na^+ in the extracellular medium. Such substitution is known to produce increase in cytoplasmic Ca^{2+} concentration in several cell systems[6–8].

Salivary glands of *Chironomus thummi* (fourth instar) were isolated into a flow chamber which allowed continuous or intermittent superfusion. The time required for exchange of 99% of the chamber medium was of the order of 3 min or less. The delays of the effects given below refer to the start of an exchange. Junctional communication was monitored as described before[2,5] by passing electric current pulses (100 msec duration) at the rate of 1/min between the inside of a cell and the outside, while recording the resulting membrane voltages in this cell (V_I) and, simultaneously, in an adjacent one (V_{II}) (Fig. 1, inset). The current-passing and voltage recording microelectrodes were inside the cells throughout an experiment. The control medium had the following composition: NaCl, 28 mM; disodium succinate, 28 mM; $CaCl_2$, 5 mM; magnesium succinate, 7 mM; KCl, 2 mM; glutamine, 80 mM; *N*-tris(hydroxylmethyl)methyl-2-aminoethane sulfonic acid, 5 mM; titrated to pH 7.40 with NaOH[5]. In this medium, cell resting potentials (30–60 mV) and junctional coupling (V_{II}/V_I, nearly unity) were well maintained over periods longer than 5 h. In the substitution media, NaCl and sodium succinate of the control medium were replaced mole for mole by LiCl and lithium succinate, or by choline chloride and choline succinate. The Ca-, Mg-free Na medium contained 38 mM NaCl, 38 mM disodium succinate; and the Ca-, Mg-free Li medium, 38 mM LiCl, and 38 mM dilithium succinate, to give the same ionic strength as the control medium (KCl, glutamine, and buffer at concentrations as in control medium).

Fig. 1A illustrates an experiment in which Na^+ of the control medium is replaced by Li^+. Some 100 min after the replacement, junctional membrane conductance starts to fall, as reflected by the rise in V_I simultaneous with the fall in the ratio V_{II}/V_I (see ref. 2 for theory and methods of computation). After 3 h in the Li medium, junctional membrane conductance has fallen so low that junctional communication

between the cells is interrupted (junctional uncoupling). The degree of junctional uncoupling of the example illustrated is quite typical of 13 cases. (The cells start to depolarize within 10 min of Li^+ substitution. The resting potentials decline progressively to near 0, or in some cases, reverse sign over a period of 2 h. The absolute values and the rates of decline of resting potentials, about equal in adjacent cells while they are coupled, often diverge upon uncoupling.) Replacement of Na^+ by choline produced no uncoupling in 2 trials of 2.5 and 3 h duration (Fig. 1D).

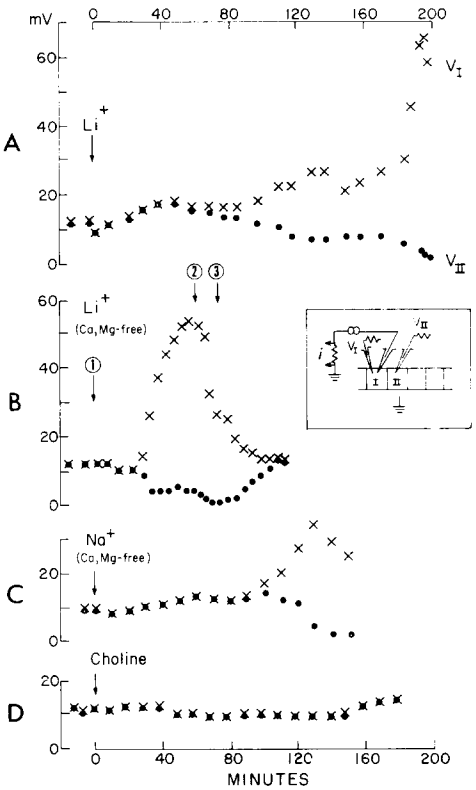

Fig. 1. Effects of various media on junctional coupling. Three microelectrodes are inserted into two contiguous cells (I, II, inset). The middle electrode pulses current ($I = 2 \cdot 10^{-8}$ A, inward) between cell interior and the grounded bathing medium, and the other electrodes record the resulting voltages inside the two cells (V_I, V_{II}). Plotted are the peak values of V_I (×) and of V_{II} (●). The first arrows on each graph indicate change from control to test medium. A. Li medium (Li^+ substitutes for Na^+). B. 1, Ca-, Mg-free Li medium; 2, change to Ca-, Mg-free Na medium; 3, control medium. Note that junctional re-coupling, *i.e.*, recovery to original V_{II}/V_I, ensues only after 3; the fall in V_I between 2 and 3 (starting shortly before 2) is not accompanied by rise in V_{II}. C. Ca-, Mg-free Na medium. D. Choline medium (choline$^+$ substitutes for Na^+).

Junctional uncoupling ensues also in a Li medium free of Ca and Mg. Typically, uncoupling is then already complete within 15–50 min (8 cases) (Fig. 1B). Early return to control medium restores junctional coupling to normal level within 3–30 min (3 cases) (Fig. 1B, 3). No restoration of coupling is produced in Ca-, Mg-free Na medium (Fig. 1B, 2). In fact, exposure to this medium alone leads to junctional uncoupling in 100–160 min (4 cases) (Fig. 1C)[5].

A possible explanation of the results is in terms of the calcium hypothesis, namely that the depression in junctional membrane permeability, in the various media, is mediated by an elevation of the cytoplasmic Ca^{2+} level. The obvious sources of Ca^{2+} here are the mitochondria and the Ca-containing media themselves. Both are large Ca^{2+} reservoirs from which the cytoplasm is normally shielded by membranes and energized transport. We are guided in this explanation by the following facts: (1) Mitochondria (liver), in K^+ concentrations approximating normal cytoplasmic levels, unload much of their Ca^{2+} upon addition of Na^+ or Li^+; the unloading increases with increasing Na^+ or Li^+ concentration[9]. (2) Ca^{2+} efflux through nonjunctional membrane decreases (nerve[7]; and/or Ca^{2+} net influx rises: heart muscle[6], nerve[7,8]) when Li^+ (or choline) substitutes for Na^+ in the extracellular medium. (3) Ca^{2+} efflux (though not necessarily net efflux) decreases in Ca-free extracellular medium (heart[10] and smooth muscle[11], nerve[12]). (4) Net accumulation of Na^+, and, presumably also of Li^+, through nonjunctional membrane (nerve[13,14], erythrocytes[15], plant cells[16], skeletal muscle[17]) increases in Ca-, Mg-free extracellular medium.

The primary factors causing junctional uncoupling, in this interpretation, are decrease in Ca^{2+}-storing capacity of mitochondria (or of other intracellular structures) in the presence of Na^+ or Li^+, and failure in Ca^{2+} extrusion through nonjunctional membrane. The two factors in concert may cause the cytoplasmic Ca^{2+} level to rise above the safety margins for junctional coupling; neither factor alone appears to be sufficient to do so under the present experimental conditions, as suggested by the fast re-coupling upon changing from Ca-, Mg-free Li medium to control medium, and by the persistence of junctional coupling in choline medium.

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