Junctional Membrane Permeability
Depression by Substitution of Li for Extracellular Na,
and by Long-Term Lack of Ca and Mg;
Restoration by Cell Repolarization

BIRGIT ROSE and WERNER R. LOEWENSTEIN

Cell Physics Laboratory, Department of Physiology,
Columbia University College of Physicians & Surgeons,
New York, New York 10032

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Summary. Substitution of extracellular Na\(^+\) by Li\(^+\) causes depression of junctional membrane permeability in Chironomus salivary gland cells; within 3 hr, permeability falls to so low a level that neither fluorescein nor the smaller inorganic ions any longer traverse the junctional membrane in detectable amounts (uncoupling). The effect is Li-specific: if choline\(^+\) is the Na\(^+\) substitute, coupling is unchanged. The Li-produced uncoupling is not reversed by restitution of Na\(^+\). Long-term exposure (> 1 hr) of the cells to Ca, Mg-free medium leads also to uncoupling. This uncoupling is fully reversible by early restitution of Ca\(^++\) or Mg\(^++\). Coupling is maintained in the presence of either Ca\(^++\) or Mg\(^++\), so long as the total divalent concentration is about 12 mm. The uncoupling in Ca, Mg-free medium ensues regardless of whether the main monovalent cation is Na, Li or choline.

The uncouplings are accompanied by cell depolarization. Repolarization of the cells by inward current causes restoration of coupling; the junctional conductance rises again to its normal level. The effect was shown for Li-produced uncoupling, for uncoupling by prolonged absence of external Ca\(^++\) and Mg\(^++\), and for uncoupling produced by dinitrophenol. In all cases, the recoupling has the same features: (1) it develops rapidly upon application of the polarizing current; (2) it is cumulative; (3) it is transient, but outlasts the current; and (4) it appears not to depend on the particular ions carrying the current from the electrodes to the cell. The recoupling is due to repolarization of nonjunctional cell membrane; recoupling can be produced at zero net current through the junctional membrane. Recoupling takes place also as a result of chemically produced repolarization; restoration of the K gradients in uncoupled cells causes partial recoupling during the repolarization phase.

An explanation of the results on coupling is proposed in terms of known mechanisms of regulation of Ca\(^++\) flux in cells. The uncouplings are explained by actions raising the Ca\(^++\) level in the cytoplasmic environment of the junctional membranes; the recoupling is explained by actions lowering this Ca\(^++\) level.

Previous work has shown that injection of Ca\(^++\) into cells, or excessive influx of Ca\(^++\) through mechanically or chemically produced leaks in cell surface membrane or in junctional seal causes depression of permeability.
of junctional membrane (Loewenstein, Nakas & Socolar, 1967; Loewenstein, 1967b). These results have led to the hypothesis that the permeability of the junctional membrane depends on the amount of Ca bound to it and, hence, on the free Ca concentration in the cytoplasm in contact with this membrane. The permeability is high in the low Ca ++ concentration normally prevailing in cytoplasm (<10^{-6} M); it falls when the Ca ++ concentration rises (Loewenstein, 1966, 1967a).

The present work was prompted by this hypothesis. We studied the junctional permeability of Chironomus salivary gland cells in two experimental situations in which cytoplasmic Ca ++ may be expected to rise: when Li + substitutes for Na + in the external medium, and when the external medium lacks Ca ++. Ca ++ influx is known to rise in the first situation and Ca ++ efflux to fall in both situations in several types of cells (Niedergerke, 1963; Baker, Blaustein, Hodgkin & Steinhardt, 1967; Baker & Blaustein, 1968; van Breemen & van Breemen, 1968). It will be shown that junctional membrane permeability does, in fact, fall markedly in both situations.

In the course of this study, the fall of junctional membrane permeability, which was accompanied by cell depolarization, was found to be reversible by cell repolarization. An examination of this effect in several situations of uncoupling will show that the permeability of junctional membrane depends on the potential across nonjunctional membrane.

Brief accounts of some of the results have already appeared (Rose & Loewenstein, 1969; Rose, 1970).

**Materials and Methods**

**Preparation and Media**

Salivary glands of 10 to 12-day-old Chironomus thummi larvae were isolated as described in the preceding paper (Rose, 1971), and set up in a perfusion chamber. The perfusion system allowed vibration-free exchange of medium; about 96% of the chamber's medium was exchanged within 3 min, as shown by tests with colorant solutions.

The composition of the media is given in Table 1. The control medium approximates hemolymph with respect to content of cations and chloride, pH and osmolarity; it contains some organic components of hemolymph (Politoff, Socolar & Loewenstein, 1969). The cells maintained transparency, high resting potentials and coupling for at least 4 hr in this medium. In the experiments with 2,4-dinitrophenol, this substance was dissolved in control medium in concentrations of 1 to 10 \times 10^{-5} M.

**Electrical Measurements**

Three microelectrodes were inserted into two contiguous cells for measurement of coupling (Fig. 1, top). One electrode served to pass rectangular current pulses (1 to 4 \times 10^{-8} amp; 100 to 200 msec duration; base-line leakage <10^{-11} amp) and the other