PRELIMINARY NOTES

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Metabolism and the permeability of cell membrane junctions

The ion permeability of the membrane regions of contact between cells (junctional membranes) is labile. It may change from a state of low permeability, similar to that of the non-junctional membrane regions, to a state of permeability several orders of magnitude higher, and *vice versa*[1,2]. In cell junctions of various tissues, the normal state is that of high junctional membrane permeability, the cell interiors thus being in rather free communication throughout the tissue[3]. Recent results suggested the possibility that the maintenance of this state requires continuous extrusion of intracellular Ca^{2+} and Mg^{2+} across the non-junctional membranes against electrochemical gradients, and thus depends on cell metabolism[1]. We have now investigated the effects of cooling and of several metabolic inhibitors and have found that junctional membrane permeability falls when cellular metabolism is inhibited.

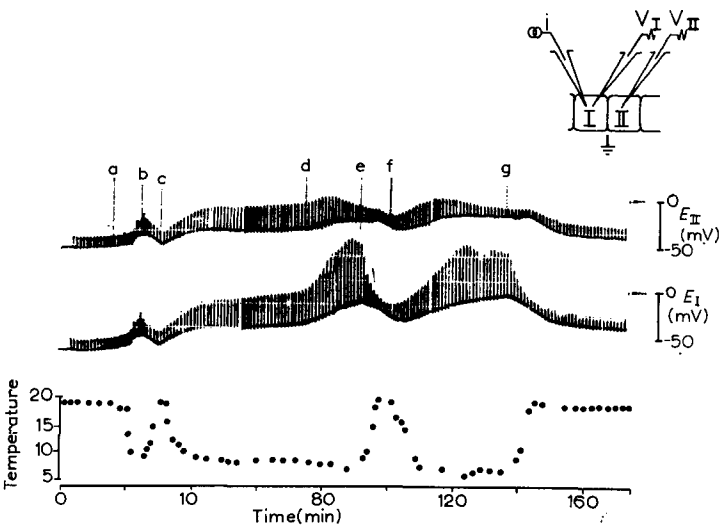

Fig. 1. Depression of junctional membrane conductance (and cell resting potential) by cooling. Two contiguous cells are impaled by 3 microelectrodes (see inset). Temperature of bathing medium, controlled thermo-electrically, is monitored by thermistor and displayed on bottom curve. Upper two curves are continuous storage oscilloscope records. Their baselines (E_I, E_{II}) give cell membrane potentials of cells I and II. Rectangular current pulses ($2 \cdot 10^{-8}$ A, 180 msec) are passed, 1/min, through electrode i. Peaks of corresponding voltage pulses (V_I, V_{II}) are recorded as vertical displacements from curves E_I, E_{II} (baselines). Brief cooling (a-b) is seen to give only cell depolarization (reversible: b-c). Prolonged cooling (c-e) eventually causes fall in junctional membrane conductance, as reflected in the changes of V_I and V_{II}. Warming (e-f) restores junctional conductance and, partially, cell resting potential. On further cooling and warming, phenomena repeat, but without delay. Potentials registered by electrodes on withdrawal from cells into bathing medium are shown by last segments at right (0 mV). V_I, V_{II} magnitudes are on same scale as E_I, E_{II}.

Abbreviation: NEM, *N*-ethylmaleimide.

Salivary glands of fourth-instar larvae of *Chironomus thummi* were isolated into a medium (control medium) of the following composition: KCl, 2 mM; disodium fumarate, 28 mM; $CaCl_2$, 5 mM; magnesium succinate, 7 mM; glutamine, 80 mM; Tris–sulfonic acid, 5 mM; titrated to pH 7.4 with NaOH. In this medium, the cells could be easily kept for several hours without signs of deterioration of resting potential (50–60 mV), junctional and non-junctional membrane conductances, or general morphological appearance. Cellular communication was monitored as described previously[2] by passing electrical current pulses between the inside of a cell and the outside, while recording the associated voltage drops in this cell (V_I) and in a contiguous one (V_{II}) (Fig. 1, inset). For application of chemical agents, a superfusion system was used that allowed continuous exchange of the bathing medium.

The effects of cooling of the cell system are shown in Fig. 1. The cells depolarize as temperature falls. Cellular communication is initially unaffected; but as the temperature is held low (6–8° in this case), junctional membrane conductance eventually starts to decrease, as reflected by the rise of V_I simultaneous with the decline in ratio V_{II}/V_I (see ref. 2 for theory and for methods of computation). Upon warming, the cells repolarize, and junctional membrane conductance increases without delay, re-establishing normal communication between cells. On subsequent cooling, decrease in junctional membrane conductance follows now without delay (Fig. 1).

A significant feature of the result is the delay (frequently several hours) in the initial permeability change at the junctional membranes (12 experiments). This suggests that the permeability change is the result of a critical change in a metabolically-dependent concentration of a cellular constituent. One would then expect that suitable chemical inhibitors of metabolism can produce the change in permeability too. This is in fact the case. Treatment of the cell system with 2,4-dinitrophenol or *N*-ethylmaleimide (NEM), each confirmed in 5 experiments, causes marked decline in junctional membrane conductance.

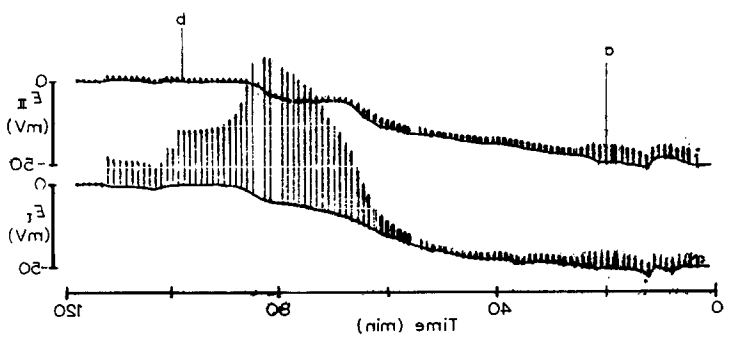

Fig. 2. Depression of junctional membrane conductance by NEM. Set-up and notation as in Fig. 1, except that temperature is 20° throughout. In control medium (to left of a), cells I, II display normal ratio V_{II}/V_I. a, control medium replaced by medium containing 10^{-5}–10^{-4} M NEM in addition. Junctional membrane conductance falls markedly, as shown by changes in V_I and V_{II}. b, return to control medium fails to reverse the effect (reduction in V_I here reflects increased conductance of non-junctional membrane of cell I; see ref. 2). Right-end portions of baseline curves give electrode potentials on withdrawal into bathing medium.

Fig. 2 illustrates this for the case of NEM. Some 30–40 min after application of this sulfhydryl reagent (10^{-5}–10^{-4} M), a decrease in junctional membrane conductance is apparent. Cell depolarization, on the other hand, develops without delay. At higher concentrations of NEM (up to $5 \cdot 10^{-3}$ M, the highest used), the change in junctional conductance develops sooner and at a time when cell resting potential is still fairly high (up to 45 mV). With concentrations of NEM ranging down to about 10^{-5}–10^{-4} M the conductance change is not reversed by washing the cell system in control medium. Effects essentially similar, both in intensity and in time course, were obtained with 2,4-dinitrophenol (10^{-4} M).

Ouabain (4 experiments) caused no significant effects on junctional membrane conductance during 2 h of application, even at the relatively high concentration of 10^{-4} M. The cells, however, depolarize and the non-junctional conductance rises by a factor of 2 to 7. Sodium azide (10^{-3} M) (2 experiments) produced no detectable reduction in junctional membrane conductance in 1 h.

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Cell Physics Laboratory,
Department of Physiology,
Columbia University,
College of Physicians and Surgeons,
New York, N.Y. (U.S.A.)

A. POLITOFF
S. J. SOCOLAR
W. R. LOEWENSTEIN

1 W. R. LOEWENSTEIN, *Develop. Biol.*, 15 (1967) 503.
2 W. R. LOEWENSTEIN, M. NAKAS AND S. J. SOCOLAR, *J. Gen. Physiol.*, 50 (1967) 1865.
3 W. R. LOEWENSTEIN, *Conference on Biological Membranes: Recent Progress, Ann. N.Y. Acad. Sci.*, 137 (1966) 441.

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Evidence for a special type of bicarbonate transport in the isolated colonic mucosa of *Bufo arenarum*

The ability of the colonic mucosa to transport Na^+, *in vivo* and *in vitro*, from mucosa to serosa, against its electrochemical gradient, has been demonstrated in mammalia (CURRAN AND SCHWARTZ[1], COOPERSTEIN AND BROCKMAN[2]) and amphibia (USSING AND ANDERSEN[3], COOPERSTEIN AND HOGBEN[4]). In *Rana catesbeiana*[4], the Na^+ transport does not explain the whole short circuit current and the ionic species responsible for the difference observed has not been identified. The present report demonstrates the existence of at least two different components of the short circuit current in the isolated colonic mucosa of the South American toad *Bufo arenarum*.