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Permeability of a Cell Junction During Intracellular Injection of Divalent Cations

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Summary. Divalent cations are microinjected into *Chironomus* salivary gland cells while the cell-to-cell passage of fluorescein (330 dalton) and electrical coupling are monitored. Injections of Ca and Mg that substantially depolarize the cells produce block or marked slowing fluorescein passage, accompanied by electrical uncoupling. Injections of Ca, Mg or Sr that cause little depolarization, and presumably smaller elevation of divalent cation concentration in the cytoplasm, produce block or marked slowing of fluorescein passage with little or no detectable electrical uncoupling. This partial uncoupling may reflect total closure of a fraction of the channels in junctional membrane or partial closure of all channels.

In a wide variety of tissues, the intracellular space is made continuous by cell membrane junctions that provide a path for direct cell-to-cell diffusion of molecules of a certain size range (Loewenstein, 1966, 1975*c*; Furshpan & Potter, 1968). The permeability of these junctions is markedly reduced when Ca^{2+} flux into the cells is excessively high, as in the case of damage to (nonjunctional) cell membrane in Ca-medium (Délèze, 1964, 1970, 1975; Loewenstein, 1966; Loewenstein, Nakas & Socolar, 1967; Oliveira-Castro & Loewenstein, 1971), or when the cytoplasmic Ca^{2+} concentration in the intact cells is elevated in several experimental conditions (Politoff, Socolar & Loewenstein, 1969; Rose & Loewenstein, 1971). This has led to the hypothesis that the junctional membrane permeability is determined by the cytoplasmic Ca^{2+} concentration (Loewenstein, 1967). In the present work we have attempted to test this hypothesis by injecting Ca^{2+} and other divalent cations into the cell, while monitoring cell-to-cell passage of fluorescein and electrical coupling.

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Fluorescein passage, indeed, was found to be depressed; but electrical coupling was depressed only in certain of the Ca^{2+} injections, namely in those producing depolarization. This raised the question of whether the injected Ca^{2+} had spread far enough inside the still coupled cells to reach a critical concentration at the junctions. To resolve this crucial point, we postponed publication of this work (performed in 1971/1972) until techniques for monitoring the intracellular free Ca concentration could be developed. This has now been done and the companion paper describes the complementary results. These, we believe, provide a full explanation of the present findings and show that junctional permeability indeed depends on the cytoplasmic free Ca^{2+} concentration in the junctional locale (Rose & Loewenstein, 1976).

Materials and Methods

Salivary glands of *Chironomus thummi* were isolated in physiological medium (Politoff *et al.*, 1969). Electrical coupling was measured by pulsing inward current (0.4×10^{-7} or 1×10^{-7} A; 0.2 sec duration; ~12/min) into a cell (*I*) and measuring the resulting steady-state displacements of membrane voltage (*V*) in this cell and a contiguous one (*II*) (Fig. 1, *inset*). The ratio V_{II}/V_I (*coupling ratio*) provided a convenient index of the coupling (Loewenstein & Kanno, 1964).

Divalent cations (Ca, 8–30 mM; Mg, 15–30 mM; or Sr, 3–9 mM) were injected into cell *II* as Cl-salt solutions, made isotonic (260 mOsm) by addition of KCl or of a mixture of KCl (2/3 of the required osmolar concentration) and sucrose (1/3). The divalent cation solutions also contained Na fluorescein (2–6 mM) to make visible the outflow from the injection micropipette and to probe junctional permeability (Loewenstein & Kanno, 1964). Pressure pulses, variable in strength, duration and frequency, were applied to the injection micropipettes while electrical coupling and membrane potential were measured. To avoid cell damage by excessive pressure, the injections were begun with pulses, 10/min, of increasing pressure and duration up to injection threshold as evidenced by the sudden appearance of a bright intracellular fluorescence. The pressure pulses were then routinely continued for 20–60 sec in an effort to inject a sufficient volume; but the injections seemed generally effectively limited to the first few pulses, as the divalent cation-containing pipettes tended to clog. The fluorescent pipette meniscus was photographed immediately before and after injection (e.g. M_1 and M_2, Fig. 1) and the injected volume was calculated as that of the corresponding truncated cone or cylinder in the pipette. The height of the cone or cylinder was determined from the distance between the meniscus projections on the horizontal plane as measured on the photographs and from the angle of the micropipette with this plane. It is difficult to assess an error on the volume measurements, but there is no evident argument for a substantial systematic overestimate: evaporation was negligible. However, some underestimate due to a rise in fluid level by capillarity after injection was possible, although controls with pipettes inside the cells showed little, if any, sign of such a rise.

In the series of control injections in which K substituted for the divalent cations, the injected solutions contained besides Na-fluorescein (2–6 mM), either 130 mM KCl (4 injections), 45 mM KCl (1 injection), or a mixture of 45 mM KCl and 170 mM sucrose (3 injections).

Table 1. Effects of cation injection on coupling and membrane potential

Injected cation	n[a]	Amount injected[b] pmole	Coupling ratio V_{II}/V_I[c]		Cell-to-cell flux of fluorescein	Δ Membrane potential[d] %[c]	
			before injection	1 min after injection		Cell *I*	Cell *II*
Group A							
K	8	32 −133	0.92±0.02	0.90±0.03	normal	−13.9±2.7	−15.9± 3.9
Ca	10	2 − 63	0.90±0.02	0.86±0.02	blocked[e] or slowed	−7.7±3	−12.5± 3.4
Mg	6	5 − 18	0.92±0.02	0.88±0.04	slowed	−11.9±6	−8.3± 6.1
Sr	4	1.5− 12	0.95±0.03	0.91±0.02	slowed	−10.3±5.9	−11.9± 6.2
Group B							
K	1	66	0.95	0.44	blocked	−31.3	−59
Ca	5	6 −132	0.89±0.02	0.26±0.11	blocked[f] or slowed	−42.4±8.9	−76.5±10
Mg	1	33	0.84	0.31	blocked	0	−64.3

[a] Number of cases.
[b] Ranges: lowest and highest value in each group.
[c] Mean with SE.
[d] The change in the membrane potential (at zero current) 1 min after start of injection; mean membrane potentials and SE in 32 experiments were 43 mV±19 (E_I) and 41.9±19 (E_{II}).
[e] In 5 cases.
[f] In 4 cases.

Results

Electrical Coupling

The effects of the divalent cation injections are summarized in Table 1. The results on electrical coupling fell into two groups, depending on the degree of depolarization associated with the injection. In one group (*A*), in which the average depolarization did not exceed 12% of the initial membrane potential, no significant decrease in coupling ratio (*uncoupling*) was detectable over 3–12 min of continuous coupling measurement (Figs. 1–3). In the other group (*B*), in which the depolarization was substantial (in all cases >30%; in most ≧45%), the cells uncoupled. The coupling ratios decreased to a fraction of their initial values (in the Ca injections the ratios decreased on the average by a factor of 3.4) or to below detectable level. In a typical example of this group, shown in Fig. 4*A*, the membrane potential (E_{II}) of the injected cell and the transfer voltage V_{II} fell abruptly at the onset of the Ca injection. Subsequently V_I rose progressively while V_{II} stayed near zero, showing conclusively that

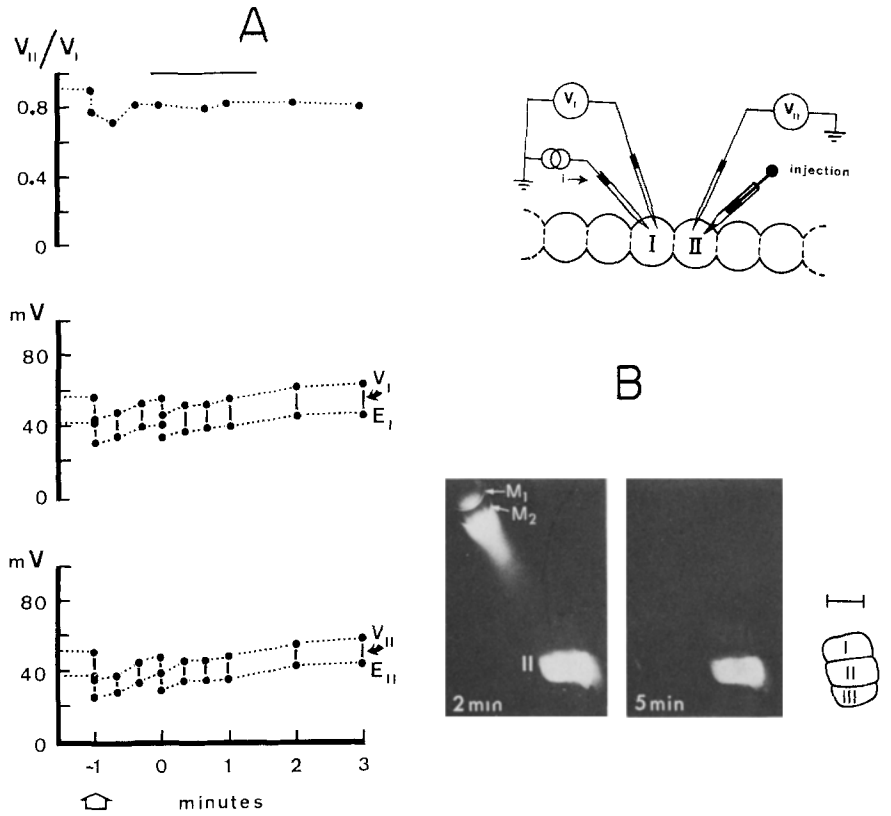

Fig. 1. Partial uncoupling by Ca (block of fluorescein passage). Membrane potential, electrical coupling and cell-to-cell flow of fluorescein during intracellular injection of a solution containing Ca and fluorescein as a tracer. *Upper right inset:* diagram of cell chain with current-passing electrode (*i*), recording electrodes (V_I, V_{II}) and injection micropipette in cells *I* and *II*. (*A*) values of the membrane potentials (E_I, E_{II}) showing also the steady-state displacements (V_I, V_{II}) during test current pulses ($i = 0.4 \times 10^{-7}$ A) and the coupling ratios (V_{II}/V_I), sampled from a continuous record. Bar starting at time 0 marks the injection of 4 pmole Ca in cell *II*. The transient depolarization near -1 min was caused by insertion of the injection micropipette (white arrow); typically the membrane "seals" in the following min. (*B*) Cell-to-cell flow of fluorescein. Darkfield micrographs of the fluorescence of the living cells after injection in cell *II*. At bottom, the times after injection the photographs were taken. Although electrical coupling was maintained (group *A* in Table 1), there was no detectable fluorescein diffusion beyond the boundaries of cell *II*. Superposed at tops are photographs of the fluorescent solution in the micropipette showing the meniscus M_1, M_2 before and after the injection. To the right is a tracing of the outlines of cells *I* and *II* and one neighbor. Calibration in this and subsequent figures, 100 μ

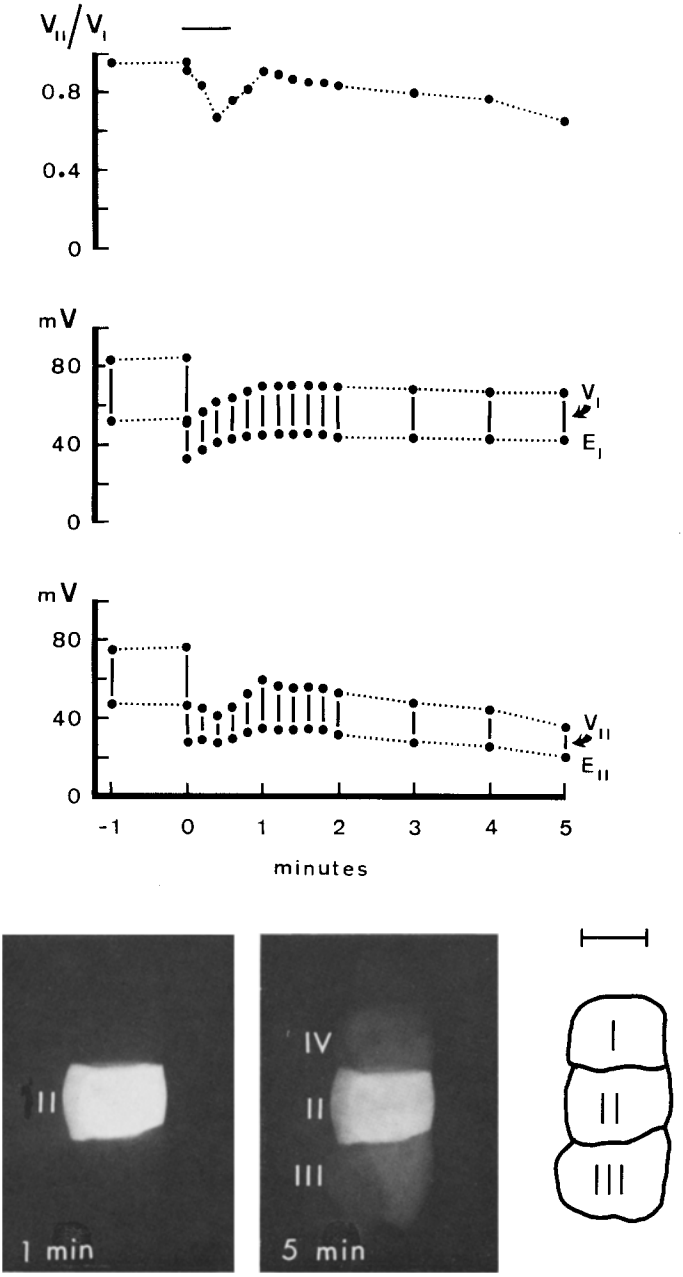

Fig. 2. Partial uncoupling by Ca (slowing of fluorescein passage). Set-up and notation in this and subsequent figures as in Fig. 1. Test current pulses 0.4×10^{-7} A. Bar starting at time 0 marks the injection of an unknown amount of Ca. V_{II}/V_I fell during the injection, but is close to control values between 1 and 4 min (group-*A* experiment). 1 min after the injection, fluorescein is still restricted to the injected cell (*II*). At 5 min a distinct fluorescence appears in the two adjacent cells (*I* and *III*), but the discontinuity in brightness at the boundaries of cell *II* indicates a constraint on fluorescein diffusion

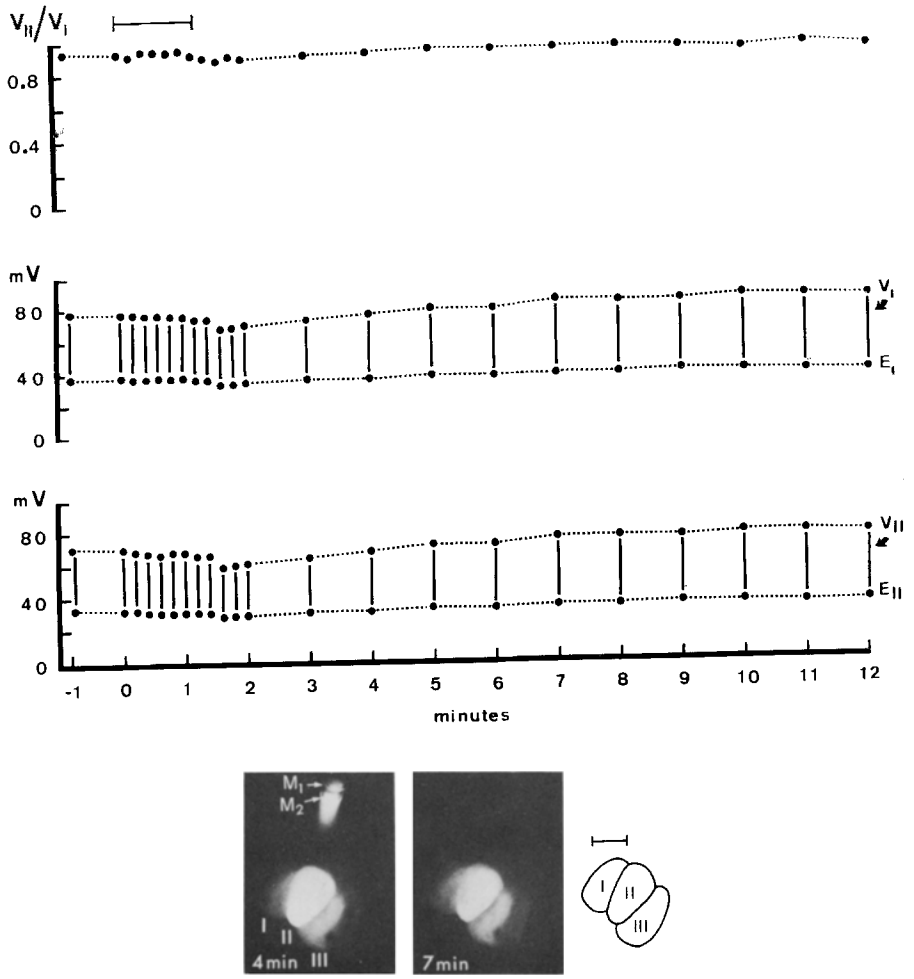

Fig. 3. Partial uncoupling by Mg (slowing of fluorescein passage). Test current pulses 1×10^{-7} A. Bar starting at time 0 marks the injection of 9 pmole Mg in cell *II*. Cells remain electrically coupled (group-*A* experiment). Slight passage of fluorescein to adjacent cells (*I*, *III*) is detectable at 4 min

the junctional membrane conductance had markedly fallen (*See* Loewenstein *et al.*, 1967).

Fluorescein Passage

Cell-to-cell passage of fluorescein was either reduced below detectable magnitude (Figs. 1 and 4) or markedly slowed (Figs. 2 and 3) in all cases of divalent cation injection, including those of group *A* in which the cells

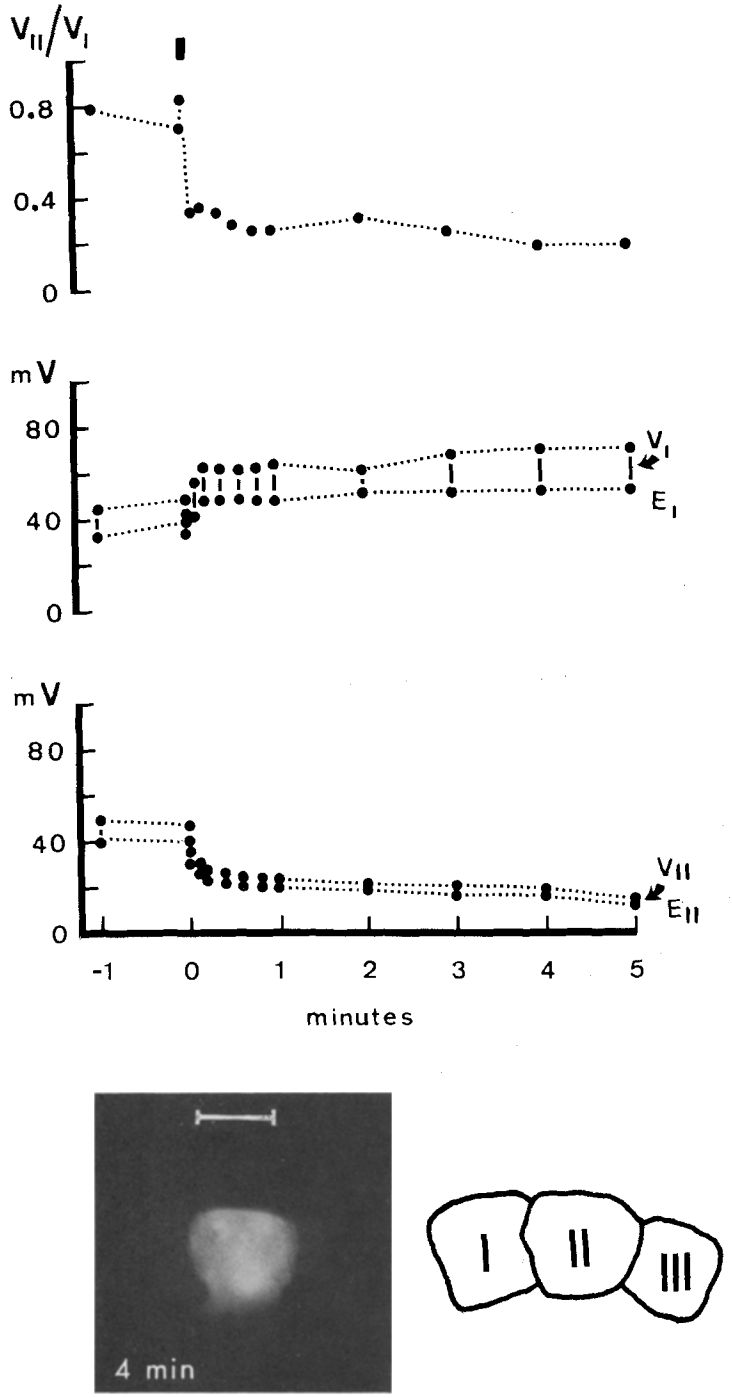

Fig. 4. General uncoupling by Ca. Test current pulse 0.4×10^{-7} A. Bar at time 0 signals the sudden injection of an unknown amount of Ca in cell *II*. The injection is followed by a substantial depolarization of cell *II* and by electrical uncoupling as shown by the decrease of V_{II} and the rise of V_I, and by the divergence of E_I and E_{II} (group-*B* experiment). Fluorescein is confined to the injected cell

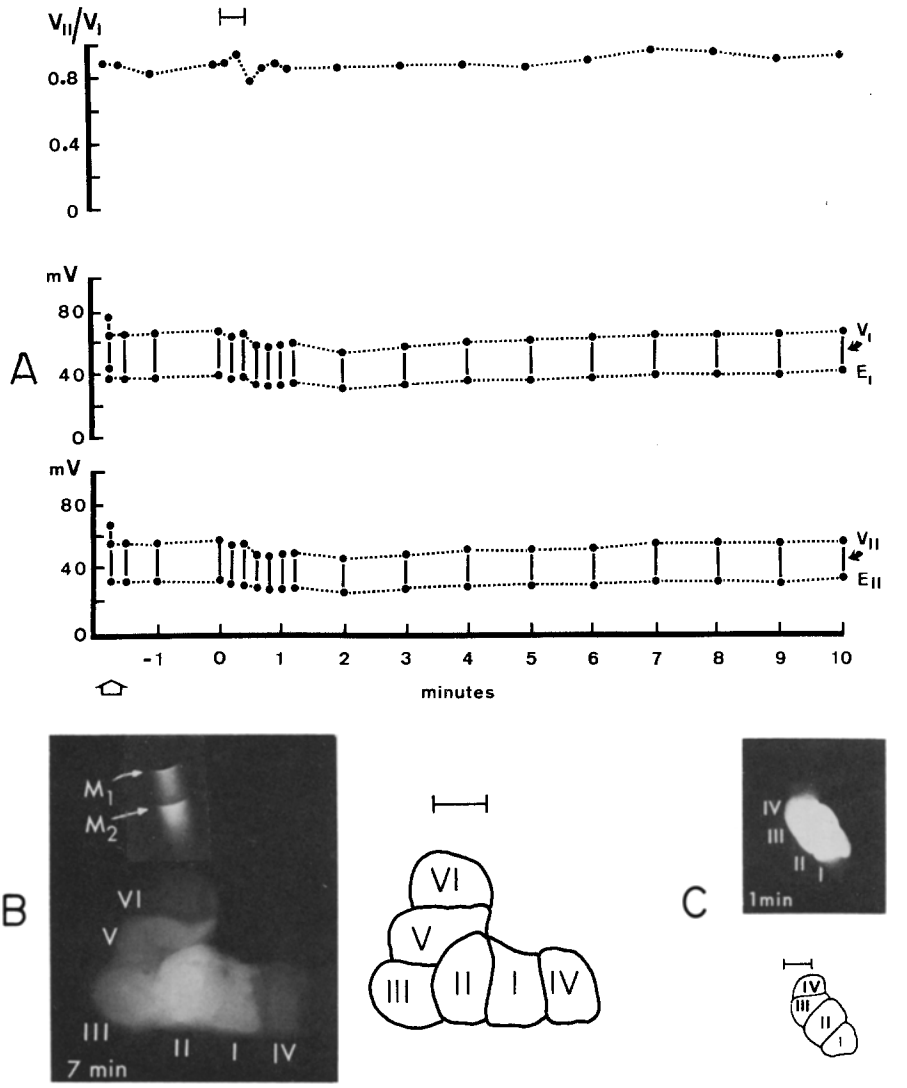

Fig. 5. Control injections of K. White arrow at start of plot indicates insertion of the injection micropipette in cell *II*. Bar marks the injection period of a solution containing 130 mM KCl and 2.5 mM fluorescein. (*A*) values of *E*, *V* and V_{II}/V_I ($i = 1 \times 10^{-7}$ A). (*B*) darkfield micrograph from the same experiment showing the cell-to-cell passage of fluorescein. (*C*) darkfield micrograph from a similar experiment showing uniform distribution of the injected fluorescein 1 min after injection

stayed well-coupled electrically. However, the intracellular diffusion of fluorescein appeared unchanged; the spatial extent and the rate of fluorescein diffusion within the injected cell was not noticeably altered. In groups *A* and *B*, fluorescein diffusion appeared always to be restricted to the injected cell for at least the first 2 min after injection (with Ca

injections, for at least the first 4 min). In the next 5 min, a rather weak fluorescence appeared in 1 to 4 adjacent cells in 50% of the experiments of group *A* and 15% of group *B*, but there was then at all times an obvious discontinuity in brightness at the junctional cell boundaries (Figs. 2 and 3). This discontinuity and the slowness of the fluorescence spread across the junction contrasted sharply with the pattern of cell-to-cell spread of fluorescein when divalent cations were absent from the injected solution. Then the fluorescein was seen to cross the junctional boundary within a few seconds of the injection, and the fluorescence intensity appeared continuous across that boundary, falling off gradually with distance from the injection site (Loewenstein & Kanno, 1964; Oliveira-Castro & Loewenstein, 1971). Thus, in the presence of divalent cations, junctional diffusion of the fluorescein molecule seems to be severely constrained.

KCl Injection

Electrical coupling was generally normal and fluorescein passage unconstrained when K substituted for the divalent cations in the injected solution (except for a small amount of Na, K was then the only injected cation; *see* Materials and Methods). In 8 experiments in which little depolarization occurred during the injection, the electrical coupling remained unchanged (Table 1) and fluorescein spread visibly to the adjacent cells within a few seconds, distributing itself rather continuously across the cell boundaries (Fig. 5).

In the one experiment exhibiting a marked depolarization, the coupling ratio fell from 0.95 to 0.44 and fluorescein passage was blocked. This effect may be attributed to Ca^{2+} released from intracellular stores, because it was associated with substantial depolarization which is generally not produced by K injection. As will be shown in the following paper, injection of KCl in sufficient amount can cause the release of Ca^{2+} from intracellular stores, and this release is invariably accompanied by depolarization because an elevation of cytoplasmic $[Ca^{2+}]$ produces an increase in nonjunctional membrane conductance (Rose & Loewenstein, 1976).

Discussion

General Uncoupling

Uncoupling for small inorganic ions. The discrepant results of the divalent cation injections on electrical coupling in the experiment groups *A* and *B* were at first puzzling: electrical uncoupling ensued

only when the injection produced a substantial depolarization. Taken alone, this result seemed to suggest that the divalent cations had no unique action on junctional membrane permeability and that the depolarization might be a primary cause of the uncoupling, particularly since depolarization by outward current can induce uncoupling (Socolar & Politoff, 1971) and repolarization by inward current can reverse it under certain conditions (Rose, 1970). Yet the results of other experiments in which various cations were made to enter the cell from an infinite external reservoir, swamping the cell interior, indicated a rather specific action of divalent cations on junctional permeability (Oliveira-Castro & Loewenstein, 1971). Moreover, experiments in which the cells were depolarized by exposure to extracellular medium of high K concentration showed that depolarization does not suffice for uncoupling (Rose & Loewenstein, 1971).

All these results could, however, be explained if we assume (*i*) that the divalent cations in the experiments of group *A* somehow did not reach the junctions and (*ii*) that depolarization, at least under some conditions, leads to substantial elevation of cytoplasmic Ca^{2+} concentration.

Notion *i* may at first glance seem far-fetched because the injected Ca and Mg quantities were high (Table 1). The intracellular concentrations at the junctions should indeed have been sufficient for uncoupling were the cells passive receptacles, and the divalent cations, like other ions, free to diffuse in the cytoplasm[1]. However, in respect to Ca^{2+}, Mg^{2+}, and Sr^{2+}, the cells are all but passive. These ions – and Ca^{2+} particularly fast – are sequestered by mitochondria acting like high-capacity sinks (*cf.* Lehninger, Carafoli & Rossi, 1967; Pressman, 1970). Moreover, these ions are bound by cytoplasmic protein (Baker & Schaepfler, 1976). Therefore, in spreading through the cytoplasm, the concentrations of the injected divalent cations fall off more steeply with distance than in simple diffusion (Harris, 1957; Hodgkin & Keynes, 1957; Blaustein & Hodgkin, 1969; Baker & Crawford, 1972). In fact, it has just been shown (Rose & Loewenstein, 1975*a*, *b*) that the Ca^{2+} diffusion restriction in *Chironomus* salivary gland cells is so severe that an increase in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), produced by Ca injection similar to those we have used here, is restricted to the immediate vicinity of the

1 With a diffusion constant of, for instance, 1.2×10^{-6} $cm^2 s^{-1}$ (Hodgkin & Keynes, 1957) and a rate constant for nonjunctional Ca extrusion not in excess of 2×10^{-3} min^{-1} (Baker, 1972), it can be shown with the aid of standard equations for random-walk diffusion that the Ca concentration at the junction would be of the order of $10^{-3} - 10^{-2}$ M in the various trials, 1 min after injection, well above the uncoupling threshold.

injection micropipette (even when the Ca in the injection is buffered). When the injection was made into the cell center, raising the $[Ca^{2+}]_i$ within a radius of 5 μ above 10^{-4} M, the $[Ca^{2+}]_i$ at the cell junction – on the average 50 μ away from the center – stayed below 10^{-5} M, the threshold concentration for electrical uncoupling. This was so unless $[Ca^{2+}]_i$ at the cell center was seen to rise particularly rapidly during injection.

Thus, it seemed indeed possible that the injected divalent cations in the experiments of group *A* did not reach the cell junction in concentrations sufficient for uncoupling. The factor determining the uncoupling in group *B* may simply have been the degree of eccentricity of the injection site, the sequestering rate, or the degree of saturation of the sequestering capacity. To resolve this crucial point, one needs to know the divalent cation concentration at the junction during the injection. The means for obtaining this information were developed over the past three years, and the experiments are described in the companion paper (Rose & Loewenstein, 1976). The results show that uncoupling occurs when the $[Ca^{2+}]_i$ in the junctional domain rises above about 5×10^{-5} M and only then. They reveal furthermore that the depolarization is a byproduct of a sufficient $[Ca^{2+}]_i$ elevation at nonjunctional membrane.

Uncoupling for fluorescein. Cell-to-cell passage of fluorescein was hindered in all cases of electrical uncoupling (group *B*, Table 1), as is to be expected if the small inorganic ions, the carriers of the electrical current, and the larger fluorescein anion (300 dalton) pass through the same junctional membrane channels (*see* Loewenstein, 1966 and 1975*a*, for a definition of the junctional channel unit). That in some Ca injections fluorescein passage was slowed, whereas in others it was sensibly blocked, may be a reflection of the number of junctional channels closed by Ca^{2+} action. It may also reflect differences between the rates with which fluorescein and Ca reach sufficient concentration at the junction; that is, slowing, rather than blockage, ensues when fluorescein gets to the junction before Ca^{2+} attains a concentration there sufficient for channel closure. This is a distinct possibility, because the slowing in the group *B* experiments occurred in the case with the largest injection volume (132 pmoles) and because fluorescein, unlike Ca^{2+}, is not avidly sequestered in the cytoplasm (see Fig. 2 of Rose & Loewenstein, 1975*b*).

Selective Uncoupling?

Of great interest is the finding that fluorescein cell-to-cell passage is hindered by divalent cation injection even when the cells stay coupled electrically (group *A*, Table 1). This may mean that the patency or the

charge of the junctional membrane channels changes gradually with divalent cation concentrations; that is, the effective channel bore is reduced even at the low cytoplasmic divalent cation concentrations in the junctional locale in the group *A* experiments. A sufficient reduction in the actual patency of the channel would be more limiting for the 300-dalton fluorescein than for the much smaller inorganic ions; or a shift toward negativity of channel fixed charges (resulting from a conformational change) could discriminate against the fluorescein anion. An alternative interpretation is that the effect of the divalent cations is an all-or-none channel closure, the proportion of the channels closing being a direct function of divalent cation concentration. The first interpretation, that of a truly selective uncoupling according to molecular size or charge, has the more interesting physiological implications, but the second is not excluded because the sensitivity of the fluorescein method for detecting closure of a fraction of the channels may be greater than that of the electrical method. The (electrical) coupling ratio is relatively insensitive to the proportion of channels open, except in the low range. For instance, if we consider the simple case of a coupled symmetrical cell pair, where the coupling ratio is given by $V_2/V_1 = r_o/(r_o + r_c)$, and take the nonjunctional membrane resistance (r_o) to be 10 MΩ and the junctional membrane resistance (r_c) to be 50 kΩ (Loewenstein *et al.*, 1967; Rose, 1971), then closure of 95% of the junctional channels would give only a 10% reduction in coupling ratio.

In view of the uncertainty about which of these two explanations applies here, we will refer to the phenomenon as *partial* uncoupling.

A further possibility, that the fluorescein and inorganic ions take altogether different routes seems unlikely in view of several other findings: (*i*) A broad variety of colorant and fluorescent tracer substances which do not permeate nonjunctional membrane pass, like fluorescein, rapidly from cell to cell (Kanno & Loewenstein, 1966; *cf.* Loewenstein, 1975*c*; I. Simpson, B. Rose, H. Wiegandt & W. R. Loewenstein, *unpublished; see also* Payton, Bennett & Pappas, 1969; Johnson & Sheridan, 1971; Imanaga, 1974). (*ii*) Cell-to-cell passage of fluorescein is blocked whenever the junctional passage of small inorganic ions is blocked by experimental elevation of $[Ca^{2+}]_i$ (Loewenstein, 1967; Oliveira-Castro & Loewenstein, 1971; Rose & Loewenstein, 1971). (*iii*) Certain cell strains which are genetically incapable of junctional passage of small inorganic ions are also incapable of fluorescein passage. When these strains are rendered capable of fluorescein passage by genetic manipulation, they become also capable of small-ion passage (Azarnia, Larsen & Loewenstein, 1974).

One further possibility, that of selective uncoupling of the giant cell junction will be discussed. The *giant* salivary gland cells, the cells which we inject into and record from, are coupled to each other directly via their common junctions and indirectly via their junctions with 2 or 3 *flat* cells spanning the lumen. Because the flat cells are very thin, it is difficult, except under favorable conditions, to demonstrate the presence of fluorescein inside them; yet they provide a path for close electrical coupling between the giant cells (Rose, 1971). Hence, if the giant cell junctions were blocked to the passage of both inorganic and fluorescein ions in the group-*A* experiments, and the electrical coupling were mediated by the flat cell junctions alone, fluorescein cell-to-cell passage might not have been seen in the flat cells. However, the possibility of a selective uncoupling of this kind seems unlikely because with the quantities (and rates) of the fluorescein injections in the group-*A* experiments the fluorescein should have become visible in the adjacent giant cells had the flat cell junctions been open to the passage of this molecule. Although the fluorescein is generally not visible in the flat cells themselves, these cells provide normally a good path for fluorescein passage between giant cells; fluorescein transfer between giant cells via flat cell junctions has been demonstrated with fluorescein injections comparable to the present ones, even where the giant cells were farther apart (Rose, *personal communication*). Besides, there are no obvious reasons why the giant cell junction should behave differently from the flat cell junction in the conditions of the group-*A* experiments. Both kind of junctions are anatomically and functionally similar (Rose, 1971), and their topography is such that in any given divalent cation injection, regardless of its centricity, flat cell junctions are as close or closer to the injection site than one of the two giant cell junctions of the injected cell.

General uncoupling is shown in the following paper to require a $[Ca^{2+}]_i$ in the junctional domain $\gtrsim 5 \times 10^{-5}$ M (Rose & Loewenstein, 1976). Partial uncoupling thus must take place at even lower $[Ca^{2+}]_i$, presumably in the range 10^{-7} to 5×10^{-5} M. This points up a danger in the studies of junctional permeability which was hitherto not apparent. The $[Ca^{2+}]_i$, normally $< 10^{-7}$ (Baker, 1972), can rise to this range as the result of experimental manipulations; Ca^{2+} leakage from the medium through an insufficiently sealed membrane region of microelectrode insertion, or depression of cell energy metabolism (on which the $[Ca^{2+}]_i$ ultimately depends) by an inadequate external medium, are only two of several possible causes of artifactual uncoupling.

In this light, we shall discuss a recent report by Van Venrooij, Hax,

Schouten, Denier van der Gon and van der Vorst (1975) that the junctional permeability for fluorescein and other similar-sized fluorescent molecules is low in salivary gland cells of *Drosophila hydeii* (end third-instar stage). Their sample photographs of intracellular fluorescence distribution on which the permeability calculations are based show some fluorescence transfer, but with sharp discontinuities in intensity at the cell boundaries. With molecules of the size of fluorescein, this is typical of partial uncoupling in *Chironomus* salivary gland cells with maintained electrical coupling (*see* Results). Furthermore, in comparing the results of Van Venrooij *et al.*, with those obtained with salivary gland cells of *Drosophila fulvimacula flavorepleta* (early and mid third-instar stage) (Loewenstein & Kanno, 1964), which are quite similar in dimension, configuration and grouping to those of *D. hydeii*, one is struck by the difference in the extent of the fluorescein spread along the major gland axis. In the experiments on *D. hydeii*, the injected fluorescein did not spread detectably beyond two cell junctions, whereas in those on *D. fulvimacula* it spreads, even with short injections, through >20 junctions (in fact, often throughout nearly the entire gland). Thus, Van Venrooij *et al.* may have worked unwittingly on a partially uncoupled cell system. The uncoupling may have been an experimental artifact or an uncoupling occurring naturally during development between the mid and end third-instar stage.

Slack and Palmer (1969) made the interesting discovery that there is no detectable fluorescein transfer between electrically coupled cells of the early *Xenopus* embryo. This has been observed also in various other embryos (Bennett, Pappas & Spira, 1972; Tupper & Saunders, 1972; but *see also* Sheridan, 1971). This partial uncoupling may be an important physiological mechanism in embryonic development, namely in the establishment and fixation of cell differentiation (Loewenstein, 1968; 1975*b*). Since it bears at least a superficial resemblance with the present partial uncoupling produced by Ca^{2+} injection, it may be rewarding to invest future effort in studying the $[Ca^{2+}]_i$ in such embryo cells.

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