GAP JUNCTIONS

Structural Changes after Uncoupling Procedures

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ABSTRACT

The freeze-fracture appearance of rat stomach and liver gap junctions changes after uncoupling procedures such as inhibition of the metabolism of perfusion with hypertonic sucrose. In control stomach, either fixed immediately or kept for 1 h in a well-oxygenated Tyrode's solution at 37°C, most gap junctions between mucous cells contain particles irregularly packed at an average center-to-center spacing of 10.3-10.5 nm. After 1-h treatment with 2,4-dinitrophenol (DNP), at the same temperature and oxygenation, most particles aggregate hexagonally at an average spacing of ~8.5 nm. Similar changes are seen in hypoxic specimens. In control liver, fixed by perfusion, most junctional particles are irregularly packed at an average center-to-center spacing of ~10 nm. Small areas of fairly regular hexagonal packing are occasionally seen, where the average particle spacing is 9.2-9.5 nm. In hypoxic liver, the junctional particles form regular hexagonal packings in which the average center-to-center particle spacing is ~8.5 nm. In liver perfused with hypertonic sucrose-calcium solutions, following EDTA solutions, most junctions are pulled apart. The separated junctional membranes, expected to be highly impermeable, contain particles regularly and tightly packed as in hypoxic or DNP-treated junctions. Preliminary measurements indicate also a possible change in particle diameter, from ~8.6 nm (control) to ~7.7 nm (treated). The structural changes are similar to those previously reported in crayfish and may reflect conformational changes in particle subunits resulting in functional uncoupling.

The intercellular permeability via gap junctions is sharply reduced by treatments which increase the cytoplasmic-free calcium (12, 22, 23).

In the crayfish, changes in the permeability of interneuronal gap junctions were found to parallel changes in the structure of the gap junctions, characterized by a tighter aggregation of the intramembrane particles, a decrease in the width of the junction and gap, and a possible decrease in particle diameter (15, 17, 18). The closely packed, presumably uncoupled, junctions were similar to isolated negatively stained junctions, also believed to be uncoupled. As a hypothesis, it was proposed that the structural changes represent conformational rearrangements in the components of the intramembrane particles, resulting in a reduced permeability of the intercellular channels (18).

The possibility that similar structural changes take place in vertebrate gap junctions was suggested by the observation that in vertebrates, isolated, negatively stained gap junctions contain particles more regularly and tightly packed than the junctions fixed in vivo. To test the possible structural changes in mammalian junctions, rat
stomach and liver were treated with uncoupling procedures and examined by freeze-fracture. A decrease in particle spacing from 10 to 11 nm (control) to ~8.5 nm, an increase in irregularity of particle packing, and possibly a decrease in particle diameter were observed. Preliminary reports on these findings were published previously (15, 17, 18).

MATERIALS AND METHODS
1- to 2-mo-old rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used in all experiments.

Uncoupling Procedures

STOMACH: The rats were sacrificed by brain concussion and the stomach was rapidly dissected out. Specimens of ~0.5 cm² were cut from the stomach body and treated as follows: some specimens were fixed immediately in glutaraldehyde-H₂O₂ (19) and prepared for freeze-fracture (see below). Other specimens were immersed for 1 h in 40 ml of a Tyrode’s solution containing 0.18-5.0 x 10⁻⁴ M 1,4-dinitrophenol (DNP) (Sigma Chemical Co., St. Louis, Mo.) at 37°C with continuous bubbling of a 95% O₂, 5% CO₂ mixture. As a control, some specimens from the same stomach were left for 1 h in Tyrode’s solution at the same temperature and oxygenation.

Other specimens were kept for 1 h in Tyrode’s solution at 37°C in closed bottles (to produce hypoxia). The composition of the normal Tyrode’s solution (in millimolars per liter) was: NaCl 137; KCl 2.7; CaCl₂ 1.8; MgCl₂ 0.5; NaHCO₃ 1.2; Na₂HPO₄ 0.4; glucose 5.6; pH 7.3.

All specimens were fixed for 2 h by immersion in 3% glutaraldehyde-H₂O₂ buffered to pH 7.2 with 0.1 M Na⁺ cacodylate, washed in 0.1 M buffer for 1 h to overnight, and prepared for freeze-fracture or thin sections.

LIVER: Rat liver was fixed by perfusion as follows: under ether anesthesia, 0.1 ml (100 U) of Na⁺ heparin (Dell Laboratories, Inc., Teaneck, N. J.) was injected into the inferior vena cava. The portal vein was dissected and cannulated with a 20-gauge needle. Both the inferior vena cava and the aorta were cut, and simultaneously, 80 ml of a 3% glutaraldehyde buffer with Na⁺ cacodylate (as previously described) was perfused through the portal vein at room temperature or at 37°C. Specimens were taken from well-fixed regions of the liver and kept in the same fixative for 1 h at room temperature, washed in buffer, and prepared for freeze-fracture or thin sections.

To asphyxiate liver cells, rats were sacrificed by brain concussion and small pieces of liver were dissected out, kept for 1 h in Tyrode’s solution at 37°C in closed bottles, and fixed as previously described. Other specimens were fixed by immersion immediately after dissection.

Attempts to uncouple liver cells by pulling apart the junctional membranes were made by perfusing hypertonic solutions through the portal vein (8). 0.5-2.0 M sucrose, either calcium-free or containing 10 mM CaCl₂, were tested, but all solutions produced inconsistent results and provided rare junctional separations. A great improvement in the frequency of junctional separation was obtained by the following procedure: 30 ml of a solution containing 3 mM EDTA and 155 mM NaCl (pH 7.4) was perfused through the portal vein, followed by 20 ml of a solution containing 155 mM NaCl and 10 mM CaCl₂, 80 ml of a solution containing 1 M sucrose and 10 mM CaCl₂, and 50 ml of a 3% glutaraldehyde in 0.1 M Na⁺ cacodylate, containing 1 M sucrose. Small samples of liver were then kept in the same fixative for 1 h and processed for freeze-fracture.

Electron Microscopy

FREEZE-FRACTURE: Glutaraldehyde-fixed, buffer-washed specimens were immersed in a 5, 10, 20, and 30% series of glycerol solutions in H₂O at 30-60 min to 1-h intervals at room temperature. The species were mounted on aluminum holders, rapidly frozen in liquid Freon-22 (Rohm & Haas Co., Philadelphia, Pa.), and transferred to a Denton freeze-fracture device (Denton Vacuum, Inc., Cherry Hill, N. J.). For platinum evaporation, the Denton electrode was substituted with a modified Fullam electrode (E. F. Fullam, Inc., Schenectady, N. Y.). The platinum electrode was outgassed, before introducing the specimen, by passing a current of about 25 A through it for 30-60 min. Before outgassing, a current of ~40 A was briefly passed through the electrode to melt the platinum. After melting, the platinum accumulated in a small droplet close to the tip of the carbon rod. Care was taken to make certain that the platinum droplet was precisely oriented towards the specimen holder. If the droplet was on the side or the back of the carbon tip, the electrode was removed and the carbon rod rotated until the droplet was oriented facing the specimen holder.

Both the shroud and the specimen holder were cooled with liquid nitrogen before introducing the specimen. The specimens were fractured at -110°C and immediately shadowed with carbon-platinum at 45°C followed by carbon at 90°C. All replicas were made at a vacuum of 1.5 x 10⁻⁷ torr. After carbon evaporation, dry nitrogen was introduced into the bell jar and the replicas were rapidly coated with a drop of 2% collodion in amyl acetate (16).

The specimens were digested in Clorox (Procter & Gamble Co., Cincinnati, Ohio) and the replicas, washed three times in distilled water, were collected on 400-mesh grids. The collodion film was dissolved by a 2-3-min immersion in amyl acetate.

THIN SECTIONS: Specimens from control (1 h in Tyrode’s solution) or DNP-treated stomach, fixed in glutaraldehyde-H₂O₂ and washed in buffer (as previously described), were postfixed for 2 h at room temperature in 2% OsO₄ buffered to pH 7.4 with 0.1 M Na⁺ cacodylate packing, and possibly a decrease in particle diameter were observed. Preliminary reports on these findings were published previously (15, 17, 18).
They were en bloc stained by a 2-h immersion in a saturated solution of uranyl acetate in H₂O, dehydrated in graded alcohol, and embedded in Epon. Thin sections were cut with an LKB Ultrotome microtome (LKB Instruments, Inc., Rockville, Md.) from the region of the mucous cells and stained with uranyl and lead salts as previously described (16).

All specimens were examined with an AEI EM 801 electron microscope. The microscope magnification was standardized before each photographic exposure by eliminating the hysteresis of the lenses. All magnifications were previously standardized with a carbon-grating replica (model 1002, E. F. Fullam Inc.). In all the freeze-fracture micrographs the direction of the platinum shadowing is from the bottom of the micrograph. The fracture faces have been labeled according to a recently revised nomenclature (5), P and E indicating the fracture face of the protoplasmic and exoplasmic leaflet, respectively.

**ATP Assay**

The ATP content was determined on stomach samples frozen either immediately after dissection, or after 1 and 2 h in Tyrode's solution or Tyrode's solution containing 5 x 10⁻³ M DNP. The ATP was assayed using the hexokinase, glucose-6-phosphate dehydrogenase method (10).

**Measurements of Particle Spacings**

In junctions containing hexagonally packed particles, the average center-to-center spacing between adjacent particles was obtained by calculating the mean particle periodicity along each of three rows of particles oriented at 120° to each other and by making a final average of the three means. In junctions containing irregularly packed particles, equal numbers of center-to-center spacings were measured in three directions at 120° to each other and the average spacing was calculated. Because of the variable orientation of the junctions in the tissue, the fracture plane along which some of the junctions are split may be variably tilted to the axis of the electron beam. The tilting, of course, will introduce errors in the measurements of particle periodicity; however, since both the control and treated junctions are subjected to the same artifacts, the errors tend to cancel out.

All measurements were performed on micrographs of junctions enlarged × 123,600 using a ×7 measuring magnifier (Bausch and Lomb Inc., Rochester, N. Y.) equipped with a 20-mm long scale calibrated to a minimum separation of 0.1 nm.

**RESULTS**

**Stomach**

Numerous disk-shaped gap junctions 0.1-1.0 μm in diameter couple adjacent mucous cells (Fig. 1). In control stomach, both the one fixed immediately after dissection (Fig. 2) and the one kept for 1 h in a well-oxygenated Tyrode's solution at 37°C (Figs. 4, 5), the junctional particles are loosely and irregularly packed at an average center-to-center spacing ranging from 9.5 to 12.9 nm.

The great percentage of junctions, 50% in stomach fixed immediately (Fig. 10) and 51.4% in that kept in Tyrode's solution for 1 h (Fig. 11), have an average spacing ranging from 10.0 to 10.9 nm, the mean of this class being 10.33 ± 0.09 and 10.48 ± 0.06 nm, respectively (mean ± 1 SEM). If all the junctions are considered together, the mean spacing is 10.7 ± 0.19 and 10.9 ± 0.115 nm, respectively.

In stomach kept for 1 h at 37°C in well-oxygenated Tyrode's solution containing 5 x 10⁻³ M DNP, the junctional particles are more tightly and
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regularly packed into hexagonal arrays (Figs. 3, 8, 9). The average center-to-center spacing between particles ranges from 7.0 to 10.9 nm (Fig. 12). The greatest percentage of junctions (66%) have an average spacing ranging from 8.0 to 8.9 nm, the mean of this class being 8.55 ± 0.04 nm. If all the junctions are considered together, the mean spacing is 8.8 ± 0.09 nm.

Stomach treated with lower concentrations of DNP (0.18-1.0 × 10⁻³ M) also display a significant increase in junctions with hexagonally packed particles at an average center-to-center spacing of ~8.5 nm. In addition, several junctions display intermediate characteristics (Figs. 6, 7); they contain tightly and regularly packed particles at the periphery, but loosely and irregularly packed particles towards the center.

Similar changes are seen in junctions of hypoxic stomach. Tight junctions and desmosomes (Fig. 1) are apparently unaffected by these treatments. In some cells of DNP-treated or hypoxic stomach, random intramembrane particles also show a tendency to aggregate as observed in crayfish ganglia after DNP treatment as well (18).

Control and DNP-treated junctions, examined in cross sections, do not show appreciable differences. Although in several measurements of the overall junctional thickness the DNP-treated junctions seemed 0.5-1.0 nm thinner than controls, preliminary optical diffraction experiments did not confirm these data consistently. The overall thickness of the junctions is 17.0-18.5 nm and the gap 2.0-3.0 nm.

ATP assays were made on control and DNP-treated stomach. Stomach kept for even 2 h in well-oxygenated Tyrode's solution at 37°C maintains the same ATP content as stomach frozen immediately after dissection from the animal. In stomach treated for 1 h with 5 × 10⁻³ M DNP, the ATP decreases to 59% of the control value and after 2 h of treatment is down to 35% of control.

Liver

In liver rapidly fixed by perfusion either at room temperature or at 37°C, all the gap junctions are characteristically similar to control junctions of the stomach. Particles on P face and pits on E face (Fig. 13) are most often aggregated without obvious order at an average center-to-center spacing of ~10 nm. Small regions of fairly regular hexagonal packing are occasionally seen, especially in the largest junctions. In these regions the average center-to-center particle spacing is 9.2-9.5 nm (this value represents the average of the particle spacing along the three axes of the hexagonal array.)

Liver samples made hypoxic by a 1-h immersion in Tyrode's solution at 37°C, in closed bottles, display junctions with particles very regularly packed into hexagonal arrays at an average center-to-center spacing of ~8.5 nm (Fig. 14). In these junctions, the regularity of the pattern is always more pronounced on the E face (Fig. 14). In both the liver (Figs. 14, 17) and the stomach (Fig. 9), the regularity of the hexagonal particle packing is frequently interrupted by smooth, particle-free areas. In liver samples fixed by immersion immediately after dissection, several junctions are already changed, displaying tightly packed, hexagonal particle arrays.

Observing at high magnification the particle arrays of control (Fig. 15) and treated (Fig. 16) junctions, one has the impression that the particles differ in size, the control appearing larger than the treated. A statistical analysis of the mean particle diameter measured along an axis perpendicular to
FIGURES 10-12. Histograms of frequencies (in percentiles) of average particle spacing, measured on freeze-fractured gap junctions between mucous cells of rat stomach. The junctions are distributed among six classes of 10 nm each. On the abscissa are the mean values of the classes.

**FIGURE 10**  Control samples fixed immediately after dissection. 18% of the gap junctions are in the 9.5 nm class (9.77 ± 0.09 nm, mean ± 1 SEM), 50% in the 10.5 nm (10.33 ± 0.09 nm, mean ± 1 SEM), 14% in the 11.5 nm (11.3 ± 0.17 nm, mean ± 1 SEM), 18% in the 12.5 nm (12.2 ± 0.2 nm, mean ± 1 SEM). 807 spaces were measured in 22 junctions. All the junctions considered together give an average spacing of 10.7 ± 0.19 nm, mean ± 1 SEM.

**FIGURE 11**  Control samples, kept for 1 h in Tyrode’s solution. 6.6% of the junctions are in the 9.5 nm class (9.8 ± 0.07 nm, mean ± 1 SEM), 51.4% in the 10.5 nm (10.48 ± 0.06 nm, mean ± 1 SEM), 31% in the 11.5 nm (11.37 ± 0.08 nm, mean ± 1 SEM), 11% in the 12.5 nm (12.46 ± 0.2 nm, mean ± 1 SEM). 1,581 spaces were measured in 45 junctions. All the junctions considered together give an average spacing of 10.9 ± 0.115 nm, mean ± 1 SEM.

**FIGURE 12**  Samples kept for 1 h in 5 × 10⁻³ M DNP. 6.4% of the gap junctions are in the 7.5 nm class (7.86 ± 0.03 nm, mean ± 1 SEM), 66% in the 8.5 nm (8.55 ± 0.04 nm, mean ± 1 SEM), 10.6% in the 10.5 nm (10.28 ± 0.15 nm, mean ± 1 SEM). 1,201 spaces were measured in 47 junctions. All the junctions considered together give an average spacing of 8.8 ± 0.09 nm, mean ± 1 SEM.

the shadowing direction gave values of 8.58 ± 0.09 and 7.66 ± 0.08 nm for control and treated particles, respectively (mean ± 1 SEM; P = <0.0001).

Attempts were made to uncouple liver cells by mechanically pulling apart the junctional membranes by perfusion with hypertonic solutions through the portal vein as done by Goodenough and Gilula (8). Various hypertonic sucrose and NaCl solutions containing Ca⁺⁺ or free of Ca⁺⁺ were tested, but all produced inconsistent results. A great improvement in the frequency of junctional separations was obtained by perfusing EDTA solutions before sucrose-Ca⁺⁺ solutions.

In junctions in which the membranes are completely pulled apart (Fig. 17), particles and pits are always closely packed at a center-to-center spacing of ~8.5 nm and form regular hexagonal arrays. Interestingly, in these junctions some of the particles fracture with the external leaflet so that they appear on the E face (Fig. 17).

Junctions in which the process of membrane separation is still incomplete at the time of fixation display interesting features (Figs. 18, 19). Here, the particles and pits are closely and regularly packed at the periphery of the junctions but more and more loosely and irregularly packed towards the center. Observing closely the arrangement of the pits in Figs. 18 and 19, one can detect, from the periphery to the center of the junction,
Figure 13 Freeze-fracture replica of control gap junction from rat liver, fixed by perfusion. The fracture steps down from face E (E) to face P (P). Particles and pits are most often irregularly packed at an average center-to-center spacing of ~10 nm. Regions of regular hexagonal packing are occasionally seen. Here the average spacing is 9.2–9.5 nm. × 159,800.

Figure 14 Freeze-fracture replica of a gap junction from rat liver. The sample was kept for 1 h in Tyrode's solution at 37°C in a closed bottle. The junction is fractured in a step-like fashion. Particles on face P (P) and pits on face E (E) are organized in a regular hexagonal array at an average center-to-center spacing of ~8.5 nm. The arrows point to islands of nonjunctional membrane regions trapped among the hexagonal packing. Some of these regions contain random intramembrane particles. × 159,800.
roughly three regions: a peripheral one containing a regularly hexagonal array of pits at 8.2–8.5 nm center-to-center; an intermediate one in which the pits are still hexagonally packed, but less regularly, at a center-to-center spacing of 9.0–9.2 nm; and a central one in which the pits are irregularly packed at an average center-to-center spacing of 9.2–9.5 nm. Junctions with these features show membrane separations only at the periphery, while in central regions the integrity of the junction is still apparent (Fig. 18).

DISCUSSION

This study confirms previous data on structural changes with functional uncoupling in crayfish gap junctions (15, 17, 18) and extends them to include vertebrate junctions.

In the rat, as in the crayfish, uncoupling procedures result in a tighter and more regular packing of the intramembrane particles at the gap junctions and possibly in a decrease in particle size. In both cases, uncoupled junctions are similar to isolated, negatively stained junctions, also believed to be uncoupled by the cell disruption during tissue homogenization. In the rat, unlike the crayfish, a decrease in junction and gap thickness was not detected, either because it does not take place or because it is too small to be measured. In fact, changes smaller than $\pm 1$ nm could not be reliably measured in sections, because of variables such as section thickness, staining, and junctional orientation, which are difficult to control.

In the rat, the functional uncoupling was not monitored electrophysiologically; therefore, it is only assumed that the treatments employed result in cell uncoupling. The assumption, however, is
reasonable on the basis of previous evidence for uncoupling by metabolic inhibitors (17, 18, 20–23), in relation to a decrease in ATP content and a consequential rise in intracellular free calcium (22, 23). A rapid decrease in ATP is also expected in liver and stomach subjected to normothermic ischemia (as a 1-h immersion in Tyrode's solution at 37°C in closed bottles). In tissues of fast aerobic metabolism, in fact, the ATP content has been found to decrease as low as ~10% of control value within 2 min of normothermic ischemia (9, 13). Consistent with these data is the observation of junctions with closely packed particles in liver samples fixed by immersion as early as a few seconds after the death of the animal.

After treatments with hypertonic sucrose solutions, uncoupling occurs as the junctional membranes are pulled apart and the continuity of the intercellular channels is interrupted. In this case, together with junctional membrane separations the channels are expected to close as soon as extracellular calcium leaks into the cell.

Evidence for the low permeability of junctional membranes pulled apart comes from electrophysiological studies on gap junctions between crayfish lateral giant axons (1) and between mammalian cardiac cells (2) in which junctional membranes, pulled apart either by substitution of chloride with less permeable anions (1) or by treatment with hypertonic sucrose solutions (2), were found to acquire high electrical resistivity.

Moreover, if this were not the case, one would expect sucrose to leak into the cell through the open channels, abolishing the osmotic gradient and ending the cell shrinkage process and the tendency for other junctions to pull apart. Therefore the tight particle packing (~8.5 nm spacing) seen in separated junctional membranes is consistent with their expected low permeability. An increase in the regularity of particle packing was also
noticed by Goodenough and Gilula (7) in junctions of mouse liver separated by hypertonic sucrose. Curiously, in junctions which have been only partially pulled apart (at the periphery), regularity and tightness of packing decreases from the periphery to the center. This could be explained by the earlier exposure of peripheral regions to the extracellular medium.

Control junctions from the stomach most often contain particles arranged without obvious regularity, while in control liver small regions of fairly regular hexagonal packing are not infrequent. A reason for this could be the better preservation of membrane integrity in the liver, due to fixation by vascular perfusion, greater compactness of the tissue, and larger junctional size. In both tissues, hexagonally packed particles are at a minimum center-to-center spacing of 9.2–9.5 nm in controls and ~8.5 nm in treated junctions. There is, therefore, at least a 0.7–1.0-nm decrease in particle spacing between the two hexagonal arrays.

Since at the same time the particles may shrink by ~0.9 nm, one would think that the change in particle size could be enough to account for the change in particle spacing. However, some displacements in interparticle components are likely to take place, since in controls the average particle spacing is greater than 10 nm. Moreover, the data on changes in particle size should be accepted with reservations (see below), and will need to be confirmed by other methods.

Junctions with intermediate features, more often seen at low DNP concentrations, could be those in which the process of particle aggregation was not yet completed at the time of fixation. The particles at the periphery may aggregate faster because the lipids around them are easily displaced to the perijunctional regions, while lipids of central regions are probably somewhat trapped by the tightly packed peripheral particles. Trapped lipids are indeed seen as regions of smooth fracture surfaces scattered among tightly packed particles (Figs. 9, 14, 17), even after the hexagonal packing process has reached its latest stage.

As previously mentioned, the data on changes in particle diameter should be accepted with reservations because of the difficulty in detecting the very edge of the particles in tightly packed arrays, which makes the measurements of those particles less certain than those of more loosely packed particles, and because of possible variations in surface contamination and replica thickness which may affect the particle size. Some confidence, however, is given by the observation that particles located at the very edge of the junctions, where the particle edges can be defined more precisely, are similar in size to the other particles. Moreover, possible variations in replica thickness and contamination do not seem to significantly affect the particle diameter, since negligible changes in the mean diameter were detected between similar particle populations of different replicas. On the other hand, particles which are spread apart are likely to accumulate more platinum than those closely packed, hence the former may have, in the replica, a larger apparent diameter.

Regarding the mechanism governing junctional changes following uncoupling treatments, one can only speculate at present. As mentioned for crayfish gap junctions, an intriguing hypothesis visualizes the process as a Ca++-triggered conformational change in particle subunits, resulting in particle shrinkage, tighter particle aggregation, and functional uncoupling.

Figure 18 Freeze-fracture replica of a gap junction from a liver perfused with a hypertonic sucrose-calcium solution following EDTA. The junctional membranes are pulled apart only at the periphery, where they are separated by a gap of variable size. Towards the center the membranes are closely apposed, as indicated by the small step between face E (E) and face P (P) (top-center of micrograph). Notice that the regularity of packing decreases from the periphery to the center. The line marked by the arrowheads limits the region of hexagonal packing (periphery) from that of irregular and looser packing (center). × 98,840.

Figure 19 Particular of Fig. 18 (upper right corner) at higher magnification. The regularity and tightness of packing of both pits and particles decreases from peripheral (right) to central (left) junctional regions. Where the pits are hexagonally packed (right), the average center-to-center spacing varies from a minimum of 8.2 nm at the very periphery to a maximum of 9.2 nm in more central regions. Where the pits are irregularly packed (left), the average center-to-center spacing is 9.2–9.5 nm. P = face P; E = face E. × 164,840.

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A correlation between increased intracellular Ca++ and functional uncoupling has been well established in invertebrates (22, 23). In mammals, uncoupling has been produced by Ca++ injection in heart muscle cells (6). Still hypothetical, however, are the molecular events leading to uncoupling and the action of Ca++ if any, on junctional components. Indirect evidence for the presence of Ca++ receptors on the cytoplasmic side of gap junctions is offered by the affinity of these regions for ionic lanthanum (14), which is known to compete with Ca++ for Ca++-binding sites in membranes (4, 24). In addition, electron-opaque deposits, possibly made up of insoluble calcium salts, have been described at gap junctions fixed with glutaraldehyde-calcium solutions (11).

The binding of Ca++ to the junctions could lead to conformational changes in the intramembrane particles, resulting in a decreased intercellular permeability. The change in particle size could reflect conformational changes in their protein components, and the particle aggregation could result from modifications in surface charges as a consequence of particle rearrangements. In any event, it should be kept in mind that the junctional change could be a phenomenon that parallels functional uncoupling and that plays little part in its mechanism. Moreover, the findings of the uncoupling effects of glutaraldehyde fixation (3) should restrain attempts to establish a precise correlation between structure and permeability of junctional membranes. For a discussion of the latter point, see a preceding paper (18).

In conclusion, treatments of mammalian tissues with agents known to produce functional uncoupling, such as inhibition of the metabolism or perfusion with hypertonic solutions, result in structural changes in the membranes of gap junctions characterized by a tighter and more regularly hexagonal aggregation of the intramembrane particles and, possibly, a decrease in particle size. These data confirm previous findings on structural changes with functional uncoupling in crayfish gap junctions (15, 17, 18).

The author wishes to thank Dr. Richard Connelt for his collaboration in assaying ATP, Lillian Peracchia for her technical assistance and diagrams, and Maria E. Fernandez-Jaimovich for her technical assistance.

This research was supported by grants from the National Institutes of Health (1 ROI GM 20113 and 1 PO1 NS 10981).

Received for publication 27 July 1976, and in revised form 22 October 1976.

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