LETTER TO THE EDITOR

**DIRECT VISUALIZATION OF THE HYDROPHILIC CHANNEL
IN THE GAP JUNCTION UNDER AN ELECTRON
MICROSCOPE USING ALKALI-METAL IONS**

The structure and function of the gap junction has become a modern, cell biological problem of extreme interest in view of physiological studies suggesting that the gap junction mediates an intercellular communication, creating metabolic and electrical coupling between adjacent cells (2). The most significant point in the model of the gap junction is a continuous, hydrophilic channel, transversing both junctional membranes, although electron micrographs showing a direct evidence of such a channel are limited. In this report we tried to visualize the channel under an electron microscope using alkali-metal ions such as Cs^+ or Rb^+ served as a physiological isotope for K^+ and an electron microscopic observation revealed dense precipitates within the gap junction, suggesting that the gap junction is involved in the intracellular ion transport.

The ultrastructure of the gap junction has been examined by the thin sectioning technique, negative staining and freeze-replica methods which have shown that it is composed of packed arrangements of 8–9 nm particles, called connexons, which are embedded in the opposing gap junction membranes (1, 4, 7, 12, 13, 17). The connexon is thought to be constructed from protein oligomers arranged in the form of an annulus and delineating a narrow hydrophilic channel through the membrane. The hydrophilic channel has been indicated repeatedly in various models of the gap junction, which is supposed to explain most of the available permeability data, obtained by fluorescence-labeled materials, showing that the channel allows a passage of small molecules up to 800–1200 daltons (11, 14). However, direct electron microscopic evidence showing the existence of a transversing channel has not been attained, although there have been reports indicating stain-penetrated cores of ca. 2 nm in diameter which appear to span the full 15 nm thickness of junctional membrane profiles in the negatively stained gap junction (5). However, alternative explanations of such images have been reported (8).

We now report an electron microscopic study showing direct morphological evidence for the existence of a hydrophilic channel by using alkali-metal ions which pass through the channel physiologically. The experimental approach attempted here takes advantage of the fact that alkali-metal ions such as K^+ , Rb^+ or Cs^+ are accumulated in cells by the same osmotic pressure in that they replace each other in a mole for mole fashion under physiological conditions (9, 10), so that Cs^+ or Rb^+ serves as a physiological isotope for K^+ . In addition, Rb^+ (an atomic weight of 85) and Cs^+ (an atomic weight of 133) are heavy enough to be visualized by electron microscopy (3, 15). However, since the ultracytochemical demonstration of these water-soluble ions involves problems such as the difficulty of visualization and diffusibility of ions, we had to devise the following two techniques to resolve these

FIG. 1. Stereo-pair electron micrographs ($\pm 7^\circ$) showing hydrophilic channels visualized by Method 1.

a. $+7^\circ$ Precipitation of cesium is observed in the cytoplasmic sides of the junctional profile. Some precipitations are seen as narrow bridges crossing two junctional membranes (corresponding arrows).

b. -7° Tilting causes the disappearance of the bridge-like structure.

FIG. 2. Electron micrograph of the isolated gap junction postfixed with osmium tetroxide. It has a pentalamellar structure (three dense strata separated by two light strata).

FIG. 3. Electron micrograph of the incubated gap junction sheets. *En face* view. Electron dense deposits are widely distributed within the gap junction.

difficulties:

Method 1: Mouse myocardial gap junctions isolated by using a modified method of Hertzberg and Gilula (6) were incubated in a cesium chloride or rubidium chloride solution (10–20 mM), buffered to pH 7.4 with 1 mM Tris for 30 min at 37°C and then centrifuged at $\times 20,000$ g for 20 min. Immediately after centrifugation incubated gap junction pellets were quick-frozen with liquid helium by using Eiko RF-10A or with liquid nitrogen by using Eiko RF-2. Freeze-substitution was then carried out by keeping frozen pellets in acetone at -80°C for 1–3 days. Pellets were slowly warmed to room temperature and embedded in Spurr's resin. Ultrathin dry-cut sections were examined without poststaining under a JEM-100C electron microscope using low-irradiation procedures. In many cases stereo-pair pictures were taken by tilting sections ± 7 degrees.

Figs. 1a, b are stereo-pair micrographs, showing the cross-sectional view of isolated myocardial gap junctions incubated with cesium. It does not show the pentalamellar structure as seen routinely in electron microscopic studies carried out with osmium tetroxide (Fig. 2); in particular, no central dense stratum between the external leaflets of the two junctional membranes is prominent. However, electron-dense cesium deposits can be seen mainly at the cytoplasmic side of the gap junctional membranes. In some areas narrow cross-bridges of ca. 1–2 nm in width can be seen traversing two opposing gap junctional membranes (corresponding arrows in Fig. 1a) which disappear in the tilted pair (Fig. 1b). It is likely that these cross-bridges represent cesium retained or precipitated in the hydrophilic channels.

Method 2: After centrifugation, the obtained pellets were resuspended with Tris buffer, pH 7.4, spread directly on the formvar-coated grids, and blotted with filter paper.

Fig. 3 shows the *en face* view of incubated gap junctions with cesium. Electron-dense cesium deposits, ca. 2 nm in diameter, are widely distributed within the gap junction preparation. The distribution of cesium shows regularity and the average center to center spacing is ca. 9–10 nm.

Electrophysiological studies on the diffusion of radioactive potassium along the myocardial cells (16) indicate a high permeability for the gap junction. The electron micrographs presented here provide direct morphological evidence for the presence of Cs^+ or Rb^+ , a physiological isotope for K^+ , in the penetrable hydrophilic channel.

Thanks are due to Ms. Ikuko Ohashi for her technical assistance. This work was supported by Grant-in-Aid for Co-operative Research No. 337001 and also by Grant-in-Aid for Encouragement of Young Scientist Nos. 58770017 and 59770016, the Ministry of Education, Science and Culture, Japanese Government.

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August 21, 1984

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