GAP JUNCTIONS OCCUR IN VERTEBRATE RENAL PROXIMAL TUBULE CELLS

FREDRIC J. SILVERBLATT and RUTH ELLEN BULGER. From the Departments of Medicine and Pathology, University of Washington, Seattle, Washington 98105

INTRODUCTION

Renal proximal tubular cells are known to be electrically coupled (1). In other tissues, three types of specialized junctions have been implicated in intercellular ionic communication: septate desmosomes (2), gap junctions (3, 4), and tight junctions (5, 6). Of these, only the tight junction has been previously identified in kidney tubular epithelium (7) and, therefore, has been thought to be the site of electrical coupling in this tissue. In this report, we describe the occurrence of gap junctions in the proximal tubule cells of rabbit, fish, and monkey.

MATERIALS AND METHODS

Primary fixation of the tissues was accomplished the following ways. Female New Zealand rabbits were perfused via the aorta with a 1:1 dilution of Karnovsky's fixative with water (8). Small pieces of renal cortex were fixed for an additional 4 hr at 4°C and washed briefly in 0.1 M sodium cacodylate with 7.5% sucrose. Kidney from the aglomerular seahorse, *Hippocampus* sp., was fixed by immersion for 4–6 hr in the same fixative. Kidney from spider monkey, *Ateles geoffroyi*, was perfused with 3% glutaraldehyde in 0.1 M phosphate buffer.

The tissues were subsequently treated in two ways: (*a*) Routine electron microscopy. One-cubic-millimeter blocks were soaked in 1% OsO_4 in 0.1 M *s*-collidine buffer, dehydrated in a graded series of ethanol, and embedded in Epon 812. Additional blocks were soaked in 0.5% uranyl acetate solution in 0.1 M *s*-collidine (pH 6.1) for 90 min prior to dehydration. Sections were stained sequentially with lead tartrate and uranyl acetate. (*b*) Lanthanum hydroxide studies. A colloidal suspension of lanthanum hydroxide was made according to Revel and Karnovsky (9). One-cubic-millimeter blocks of rabbit renal cortex were postosmicated for 90 min in a 1:1:1 mixture of 4% OsO_4, 0.1 M *s*-collidine, and 4% lanthanum hydroxide, and then soaked in uranyl acetate as described above. The tissue was subsequently dehydrated in ethanol and embedded in Epon 812. Lanthanum hydroxide was added to all the alcohols except 100% alcohol. Sections were viewed with and without additional heavy metal staining.

RESULTS AND DISCUSSION

In each species, gap junctions were seen along the lateral cell membranes of proximal tubular profiles (Fig. 1). These plaquelike structures occurred anywhere from the apex to the base. While the width of the intercellular space elsewhere was at least 200 A, in the region of the gap junction it narrowed abruptly to 20 A and the apposing membranes were aligned parallel to one another (Fig. 2). These features were most easily recognized when the tissue had been soaked *en bloc* in uranyl acetate.

In order to characterize these junctions further, blocks of rabbit renal cortex were placed in a suspension of colloidal lanthanum hydroxide. The tracer penetrated the gap and occupied a space wider than the unfilled junction, that is, 55 A rather than 20 A (Fig. 3). In surface views, the lanthanum delineated a closely packed array of particles with repeating periodicity of about 90 A within the junction.

These gap junctions, therefore, appear similar to those described by Revel and Karnovsky in liver and cardiac muscle (9), and by Brightman and Reese in nervous tissue (10). Previously, three types of junctions have been recognized in the renal proximal tubule: a short zonula occlu-

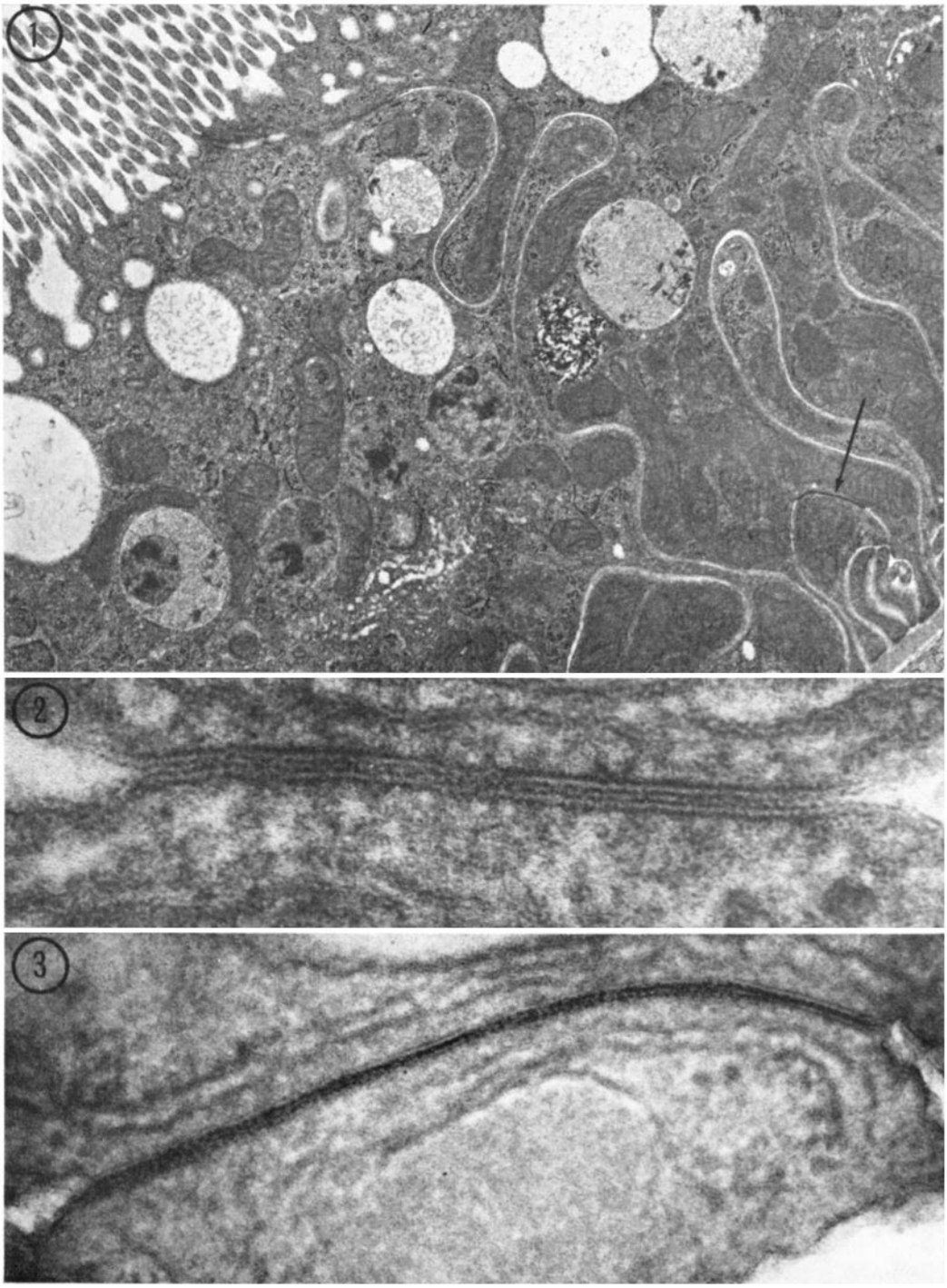

FIGURE 1 Electron micrograph of rabbit proximal tubule showing a cross-section of a gap junction (arrow) near the base of the cell. × 12,500.

FIGURE 2 Electron micrograph of a gap junction from rabbit proximal tubule showing the abrupt narrowing of the lateral intercellular space to form the 20 A gap between apposing cell membranes. Stained *en bloc* with uranyl acetate. × 269,000.

FIGURE 3 Electron micrograph of gap junction from rabbit proximal tubule in a piece of tissue treated with colloidal lanthanum hydroxide suspension. Electron-opaque material is seen within the gap and delineates a pattern characteristic of this junction. × 158,400.

dens (or tight junction), a zonula adhaerens (or intermediary junction), and an occasional macula adhaerens (or desmosome) (7). Regions of membrane fusion other than the zonula occludens have been reported by Maunsbach in the proximal tubule of the rat after dehydration with acetone (11). These structures may not represent true junctions, however, as "labile tight junctions" occur with some techniques of specimen preparation (10), especially if dehydration has been accomplished exclusively with acetone (12). Tisher et al. (13) illustrated a structure in human proximal tubules which they referred to as a nexus region. Although the techniques then used did not resolve the characteristic seven-layered array, in retrospect this structure was most likely a gap junction.

The occurrence of gap junctions in the proximal tubules in such widely separated groups as fish, rodents, and primates suggests that these junctions may be prevalent in the kidneys of vertebrates. Although the exact functional significance of the gap junctions in vertebrate proximal tubules is unknown, they may serve as sites of intercellular communication in this tissue.

This work was supported in part by United States Public Health Service grants AM-1000 and AM-10922. The AEI-6B electron microscope was purchased by grant GM-13543. Dr. Bulger is the recipient of Research Career Development Award K4-AM-9878.

Received for publication 13 March 1970.

REFERENCES

1. WINDHAGER, E. E., E. L. BOULPAEP, and G. GIEBISCH. 1967. Electrophysical studies on single nephrons. *Proc. 3rd Int. Congr. Nephrol.* S. Karger, Basel. 1967. **1**:35. (as cited by Giebisch, G. 1969. *Nephron.* **6**:260).
2. LOEWENSTEIN, W. R., and Y. KANNO. 1964. Studies on an epithelial (gland) cell junction. I. Modification of surface membrane permeability. *J. Cell Biol.* **22**:565.
3. BENNETT, M. V. L., P. B. DUNHAM, and G. D. PAPPAS. 1967. Ion fluxes through a "tight junction." *J. Gen. Physiol.* **50**:1094.
4. PAYTON, B. W., M. V. L. BENNETT, and G. D. PAPPAS. 1969. Permeability and structure of junctional membranes of an electrotonic synapse. *Science (Washington).* **166**:1641.
5. SMITH, T. G., F. BAUMANN, and M. G. F. FUORTES. 1965. Electrical connection between the visual cells in the ommatidium of *Limulus*. *Science (Washington).* **147**:1446.
6. LASANSKY, A. 1967. Cell junctions in ommatidia of *Limulus*. *J. Cell Biol.* **33**:365.
7. FARQUHAR, M. G., and G. E. PALADE. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* **17**:375.
8. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* **27**:137A. (Abstr.)
9. REVEL, J. P., and M. J. KARNOVSKY. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *J. Cell Biol.* **33**:67.
10. BRIGHTMAN, M. W., and T. S. REESE. 1969. Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.* **40**:648.
11. MAUNSBACH, A. B. 1966. Influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. I. Comparison of different perfusion fixation methods of glutaraldehyde, formaldehyde and osmium tetroxide fixative. *J. Ultrastruct. Res.* **15**:242.
12. JOHNSTON, P. V., and B. I. ROOTS. 1967. Fixation of the central nervous system by perfusion with aldehydes and its effect on the extracellular space as seen by electron microscopy. *J. Cell Sci.* **2**:377.
13. TISHER, C. C., R. E. BULGER, and B. F. TRUMP. 1966. Human renal ultrastructure. I. Proximal tubule of healthy individuals. *Lab. Invest.* **15**:1357.