REFLEXIVE GAP JUNCTIONS
Gap Junctions between Processes Arising from the Same Ovarian Decidual Cell

JOHN C. HERR. From the Department of Anatomy, University of Wisconsin Medical School, Madison, Wisconsin 53706. Dr. Herr's present address is the Department of Anatomy, University of Iowa, Iowa City, Iowa 52242.

The occurrence of true decidual cells within the tunica albuginea of the ovary during pregnancy is a phenomenon reported in no other species but the human female. Patches of these cells are visible to the naked eye upon the ovarian surface at term. At the light microscope level, ovarian decidual cells share many histological features in common with uterine decidual cells (6), although their ultrastructure has not been previously examined and correlated with that of uterine decidua. Ovarian decidua has been long, and perhaps erroneously, viewed as a pathological finding (14) and the frequency of its occurrence little appreciated. Sampling and examination by light and transmission electron microscopy of 30 ovarian biopsies excised in the last trimester of pregnancy has revealed the invariable presence of ovarian decidual tissue, thus establishing these cells as a typical histological feature of the human ovary in late pregnancy. The functional significance of ovarian decidual cells remains enigmatic.

This paper reports a unique aspect of ovarian decidual cell ultrastructure: the presence of gap junctions between processes of the same cell. Gap junctions have been observed between the plasma membranes of closely apposed cells (intercellular gap junctions) in a wide variety of adult animal tissues (4, 8, 9), in embryos (2, 15), and between cells in culture (5, 11, 13). Although direct evidence is still lacking, an extensive literature of circumstantial evidence implicates the gap junctions as the site of electrotonic coupling (1). Observations of gap junctions between portions of the same cell have been reported at least twice: be-

\[\text{Kelly, R. O., and J. F. Fallon. 1975. Ultrastructural analysis of the apical ectodermal ridge during vertebrate limb morphogenesis. I. Human forelimb with special references to gap junctions.} \textit{Dev. Biol.} \text{(In press).}\]
tween areas of the plasmalemmum of human aortic smooth muscle cells (7) and between individual processes of mesangial and lacis cells from the rat kidney glomerulus (12), both cell types being rich in microfilaments. Another intercellular junction, the desmosome, has been reported to occur between processes of the same chick epithelial cell in culture (10).

MATERIALS AND METHODS

Ovarian biopsies were obtained from women after delivery by cesarean section and removal of the placenta at 36–40 wk of pregnancy. The biopsies were taken to include patches of decidua on the ovarian surface. Decidual patches appear to the naked eye as slightly elevated, cream-colored foci, circumscribed by a rich vascular network.

Ovarian biopsies were immediately immersed and diced in fixative at room temperature. Razor blade cuts normal to the ovarian cortex were made to isolate decidual tissue. Karnovsky’s fixative was employed, containing: 2% glutaraldehyde, 2% formaldehyde (prepared from paraformaldehyde), 0.1 M phosphate buffer, pH 7.4 was used. After fixation for 3 h, tissues were rinsed six times in the same buffer for 1 h and postfixed in 1% OsO4 in the same buffer for 2 h. Tissues were rapidly dehydrated in a graded series of ethanols, placed in propylene oxide, and embedded in Epon 812. Thick (1 μm) sections were cut with glass knives and stained with 1% methylene blue or 1% toluidine blue in 1% sodium borate. Areas of decidua were identified in thick sections, and then thin sections (silver to gold) were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome (DuPont Instruments, Sorvall Operations, Newtown, Conn.). Sections were stained with a 50% ethanol solution saturated with lanthanum nitrate (K and K Laboratories, Plainview, N.Y.), in 0.1 M sodium cacodylate buffer, pH 7.4 was used. After fixation for 3 h, tissues were rinsed six times in cacodylate buffer and postfixed in 2% OsO4 in the same buffer for 2 h.

For several biopsies, lanthanum nitrate was added to a modified Karnovsky’s fixative to clearly delineate the elements of gap junctions. A solution of 2.5% glutaraldehyde, 2% formaldehyde, and 1% lanthanum nitrate (K and K Laboratories, Plainview, N.Y.), in 0.1 M sodium cacodylate buffer, pH 7.4 was used. After fixation for 3 h, tissues were rinsed six times in cacodylate buffer and postfixed in 2% OsO4 in the same buffer for 1 h in the same buffer.

For several biopsies, lanthanum nitrate was added to a modified Karnovsky’s fixative to clearly delineate the elements of gap junctions. A solution of 2.5% glutaraldehyde, 2% formaldehyde, and 1% lanthanum nitrate (K and K Laboratories, Plainview, N.Y.), in 0.1 M sodium cacodylate buffer, pH 7.4 was used. After fixation for 3 h, tissues were rinsed six times in cacodylate buffer and postfixed in 2% OsO4 in the same buffer for 2 h.

Tissues were rapidly dehydrated in a graded series of ethanols, placed in propylene oxide, and embedded in Epon 812. Thick (1 μm) sections were cut with glass knives and stained with 1% methylene blue or 1% toluidine blue in 1% sodium borate. Areas of decidua were identified in thick sections, and then thin sections (silver to gold) were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome (DuPont Instruments, Sorvall Operations, Newtown, Conn.). Sections were stained with a 50% ethanol solution saturated with uranyl acetate followed by lead citrate (0.23 g in 50 ml 0.2% NaOH), and examined with a Philips 200 electron microscope.

RESULTS AND DISCUSSION

Numerous filopodial processes of varying diameter and length project from the surface of ovarian decidual cells, giving the cell a scalloped appearance. Such an ultrastructural image as seen in Fig. 1 is typical of the distal tips of large pseudopodia, where projections of cytoplasm are seen folding upon one another. Gap junctions are seen binding adjacent processes together (arrows). In Fig. 2, where the tip of a pseudopodium abuts upon the basal lamina of the germinal epithelium, gap junctions are observed between filopodia.

More common in ovarian decidual tissue than these narrow filopodia are processes of a peduncular shape (Fig. 3), which project through a 50–100-nm thick external lamina and which contain an electron-opaque, membrane-bounded secretory product within their expanded tips. This secretory product is seen free in the extracellular space in the form of granules 30–50 nm in diameter (Figs. 3 and 8). Peduncles are observed surrounding, partially (Fig. 4) or entirely (Figs. 5 and 6), other processes with annular gap junctions binding the processes together. When viewed as a composite, Figs. 4, 5, and 6 suggest that one process fits within another as a peg and socket: the three-dimensional structure of the gap junction is calyculate rather than macular in these cases.

Observations of ovarian decidual cells preserved with lanthanum in the fixative clearly confirm the presence of gap junctions. Hexagonal arrays with center-to-center subunit spacing 8–10 nm are evident in sections cut en face, and lanthanum is observed trapped within the 2–4 nm gap between the membranes when seen in cross section (Figs. 7 and 8). It is important to note in Fig. 8 that gap junctions bind peduncles whose secretory granules are evidently being released into the extracellular space.

In this study, gap junctions were observed at the periphery of ovarian decidual cells in every biopsy examined. Fortuitous thin sections through the long axes of two adjacent cell processes have occurred in a dozen instances and confirm, unambiguously, that the gap junctions exist between processes arising from the same cell. Due to the tortuosity of the processes, the more common image (2–3 junctions/cell/section) is of gap junctions at the distal tips of processes seemingly unconnected to the cell soma (as in Figs. 3 and 8). Usually a protuberance from the cell soma in the immediate vicinity of the process and a thickening of the plasmalemma where the section has passed obliquely through the membrane of the process are interpreted as indicating origin of a given process and thus that the gap junctions are indeed between processes of the same cell and not between processes of two different cells. Final clarification of the frequency of such gap junctions awaits morphometric and serial section analysis, which is beyond the scope of this communication. Although
**Figure 1** Distal tip of a pseudopodium of an ovarian decidual cell. Numerous filopodia project from the cell's surface, and gap junctions are seen between adjacent processes (arrows). Scale: 0.5 μm. × 25,800. *Inset:* × 39,000.

**Figure 2** Shows gap junctions (arrows) between decidual cell filopodia in the region of the basal lamina (bl) of the germinal (peritoneal) epithelium (ge). The thickened basal lamina results from a fusion of the external lamina surrounding decidual cells with the basal lamina of the germinal epithelium. Scale: 0.5 μm. × 28,700.
no intercellular gap junctions were observed in examination of some 400 micrographs, the possibility that they exist, as they do in mouse uterine decidua (3), cannot be discounted.

It is generally accepted that gap junctions provide means for intercellular communication by ionic and molecular coupling. The value of ionic and molecular coupling between adjacent processes of the same cell via a gap junction is difficult to understand since cytoplasmic continuity already exists between them. The presence of gap junctions between peduncles which contain secretory product and are apparently releasing that product may indicate that these gap junctions play some role in coordinated merocrine secretion. In order to distinguish intercellular gap junctions from gap junctions occurring between processes of the same cell, it seems appropriate to ascribe to the latter the term, reflexive gap junctions.

SUMMARY

Adjacent processes on ovarian decidual cells were shown by electron microscopy to form gap junctions with one another. Micrographs of tissues preserved with lanthanum included in the fixative confirm the hexagonal array and 2-4 nm gap which characterize gap junctions. It is suggested that these gap junctions may play a role in the process of merocrine secretion from the peduncular processes of ovarian decidual cells. The term reflexive gap junction is introduced to describe gap junctions between adjacent processes from the same cell.

The author is indebted to Drs. John W. Anderson and Louis B. Curet for advice and assistance in obtaining specimens and for providing working space. The author thanks Dr. Harland W. Mossman for his encouragement and knowledge of ovarian decidua and Drs. David B. Slautterback, John F. Fallon, Bruce H. Lipton, and Paul M. Heidger for critical examination of this manuscript.

This study was supported by National Institutes of Health grant 5 T01 GM00723.

Received for publication 11 August 1975, and in revised form 22 December 1975.

REFERENCES

1. BENNETT, M.V.L. 1973. Function of electrotonic junctions in embryonic and adult tissues. *Fed. Proc.* 32:65.
2. BENNETT, M. V. L., and J. P. TRINKAUS. 1970. Electrical coupling between embryonic cells by way of extracellular space and specialized junctions. *J. Cell Biol.* 44:592.
3. FINN, C. A., and A. M. LAWN. 1967. Specialized junctions between decidual cells in the uterus of the pregnant mouse. *J. Ultrastruct. Res.* 20:321.
4. FRIEND, D. S., and N. B. GILULA. 1972. Variations in tight and gap junctions in mammalian tissues. *J. Cell Biol.* 53:758.
5. GILULA, N. B., O. R. REEVES, and A. STEINBACH. 1973. Metabolic coupling, ionic coupling, and cell contacts. *Nature (Lond.)*, 235:262.
6. ISRAEL, S. L., A. RUBENSTONE, and D. R. MERANZE. 1954. The ovary at term. I. Decidual-like reaction and surface cell proliferation. *Obstet. Gynecol.* 3:399.
7. IWAYAMA, T. 1971. Nexus between areas of the surface membrane of the same arterial smooth muscle cell. *J. Cell Biol.* 49:521.
8. MATTER, A. 1973. A morphometric study on the nexus of rat cardiac muscle. *J. Cell Biol.* 56:690.
9. MCNUTT, N. S., and R. S. WEINSTEIN. 1970. The ultrastructure of the nexus. A correlated thin-section and freeze-cleave study. *J. Cell Biol.* 47(2, Pt. 2): 135 a. (Abstr.).
10. OVERTON, J. 1974. Selective formation of desmosomes in chick cell reaggregates. *Dev. Biol.* 39:210.

Figure 3 Peduncular processes containing a membrane-bounded electron-opaque secretory product. The secretory product is evident free in the extracellular space (large arrow). The peduncles project through a 50-100-nm thick external lamina. The small arrow indicates a gap junction between peduncles. Scale: 1.0 μm. × 17,800.

Figure 4 One peduncle partially surrounding another process with an annular gap junction binding the two together. Scale: 0.38 μm. × 34,000.

Figure 5 Shows an annular gap junction of one peduncle entirely surrounding another process (asterisk). Note the membrane-bounded secretion product. Scale: 0.56 μm. × 23,000.

Figure 6 Several peduncles in longitudinal section. The gap junctions (arrow) at their tips appear to correlate with such annular gap junctions as seen in Figs. 4 and 5. Figs. 4, 5, and 6 suggest that the three-dimensional shape of such gap junctions is calyculate. Scale: 0.55 μm. × 27,000.
FIGURE 7 Gap junctions cut both in cross section and en face at the periphery of an ovarian decidual cell. Hexagonal array is delineated by lanthanum deposits. Unstained. Scale: 0.25 μm. × 89,700.

FIGURE 8 Lanthanum within gap junctions between adjacent peduncles. Secretory granules appear extracellularly (arrow). Lanthanum fixation, unstained. Scale: 0.5 μm. × 42,575.
11. PINTO DA SILVA, P., and N. B. GILULA. 1972. Gap junctions in normal and transformed fibroblasts in culture. Exp. Cell Res. 71:393.
12. PRICAM, C., F. HUMBERT, A. PERRELET, and L. ORCI. 1974. Gap junctions in mesangial and lacis cells. J. Cell Biol. 63:349.
13. REVEL, J. P., A. G. YEE, and A. J. HUDSPETH. 1971. Gap junctions between electrotonically coupled cells in tissue culture and in brown fat. Proc. Natl. Acad. Sci. U. S. A. 68:2924.
14. Rewell, R. E. 1972. Extra uterine decidua. J. Pathol. 105:219.
15. TRELSTAD, R. L., J. P. REVEL, and E. D. HAY. 1967. Tight junctions between cells in the early chick embryo as visualized with the electron microscope. Dev. Biol. 16:78.