A Comparative Study on the Intercellular Canalicular System and Intercellular Junctions in the Pancreatic Islets of Some Rodents*

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Summary. Intercellular junctions were studied in pancreatic islets of some rodents by electron microscopy of thin sections and freeze-fracture-replicas. Junctions between islet cells can be classified into three types: macula ocludens, a combination of the macula ocludens and gap junction, and gap junction. The first and second types are mainly located on the cell membrane near the intercellular canaliculus, while the third type is present independently of it. The development of these types of junctions varies considerably among animal species: in mice, guinea pigs and hamsters, the second and third types are frequent, but the first type is rarely seen. In Mongolian gerbils, diminutive elements of the three types are infrequently present. In rats, only the third type is developed.

In addition to intercellular communication through gap junctions, the first and second types of junctions may incompletely discriminate the intercellular canalicular lumen, and regulate the microenvironment of the islet cell.

Although much evidence has been gathered on the ultrastructure of the pancreatic islet, only a few research groups have studied the junctions between islet cells (ORCI and PERRELET, 1977; KATAOKA et al., 1982). Intercellular junctions of the typical epithelium are composed of the junctional complex (zonula occludens, zonula adherens, and macula adherens) and large discoid gap junctions. In the islets, on the other hand, small maculae ocludentes and gap junctions are scattered. The macula occludens may modify the movement of extracellular fluid and delimitate the microenvironment, and the gap junction mediates the metabolic, ionic and electrical coupling of the cells (ORCI and PERRELET, 1977).

The cell membranes adjoining neighboring islet cells usually run parallel to each other with minimal intervening intercellular space. While the presence of larger intercellular spaces covered by microvillous projections or digitations have been noted, they have not attracted the attention of investigators (PICTET and RUTTER, 1972). FUJITA et al. (1981) and KATAOKA et al. (1982) found the occurrence of microvillous spaces which actually formed a labyrinth of the intercellular canalicular system, and

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Fig. 1. a. Electron micrograph of a mouse islet showing intercellular canaliculi (arrows). Ca blood capillary. The arrow head indicates an opening of the intercellular canaliculus to pericapillary space, and the arrow B the basal body of a cilium. b and c. Fascia adherens-like junction (b, arrow) and the fusion of the outer leaflets of adjoining cell membranes (c, arrow) between B cells of the rat (b) and the mouse (c). A felt-like substance is accumulated on the inner aspect of the membranes at the junctions. The arrow head indicates an exocytotic figure, C intercellular canaliculus. a: ×5,000, b: ×43,000, c: ×61,000
suggested the role of the system as an efficient pathway for tissue fluid in mice, dogs and Mongolian gerbils.

In this study, the distribution of intercellular junctions was examined by electron microscopy of thin sections and freeze-fracture-replicas in relation to the intercellular canaliculi in the islet of several rodents.

**MATERIALS AND METHODS**

Five species of animals, ICR mice (30-50 g body weight), Wistar rats (200-350 g and 50-70 g), guinea pigs (300-400 g), golden hamsters (70-80 g) and Mongolian gerbils (50-80 g), were used for this study. Anesthetized animals were perfused through the left ventricle of the heart with a fixative containing 2% glutaraldehyde and 0.1M cacodylate buffer (pH 7.3) at room temperature. The pancreas was removed and sliced into small blocks in the same fixative. Tissue blocks containing the islets were collected by dissection under a binocular microscope.

**Examination on thin sections**: Islet-rich tissue blocks were postfixed in 1% osmium tetroxide, dehydrated by alcohol, and embedded in Araldite. Thin sections were doubly stained with uranyl acetate and lead citrate.

**Freeze-fracture study**: Islet-rich tissue blocks were immersed in a 30% glycerine solution. They were then placed on a copper specimen holder, and frozen in Freon 22 cooled by liquid nitrogen. Freeze-fracture-replicas were made in a vacuum evaporator (JEOL, 4C type) combined with a freeze-fracture apparatus (JEOL, IEE-FED-B 3 type), and cleaned with sodium hypochlorite.

**RESULTS**

**General structure of the pancreatic islet observed in thin sections** (Fig. 1)

In the pancreatic islet of these animal species, polyhedral B cells accounted for an overwhelming majority. A small number of A and D cells were mainly located in the peripheral region of the islet, or less frequently scattered among B cells.

An elaborate labyrinth of the intercellular canalicular system was seen between islet cells of the pancreas in all animals examined. The canaliculus communicated with the pericapillary space through the thin basal lamina of the islet. Many microvilli and a cilium projected from the islet cell into the canalicular lumen.

Other than the cell surface facing the canaliculi, the adjoining islet cell membranes are usually smooth and parallel to each other. Fascia adherens-like thickening and desmosome-like structures were often seen on the adjoining membranes near the canaliculus, and a felt-like substance adhered along the cytoplasmic side of these junctions. Occasionally, the outer leaflets of the adjoining membranes were fused in this region. Only one figure was seen indicating exocytotic release of the secretory granule content of the B cell throughout this study. It occurred on the smooth adjoining membrane in the rat.

**Types of intercellular junctions observed in freeze-fracture-replicas** (Fig. 2-5)

When the fracture plane is split along the islet cell membrane, three portions of it can be identified: a microvillous membrane facing the intercellular canaliculus, a relatively flat membrane adjoining neighboring cells and a membrane in front of the pericapil-
Fig. 2. A large junction (arrow) consisting of a coarse mesh of tight junctional ridges and associated gap junctional particles near the intercellular canaliculus (C), which continues towards the upper left. Mouse islet. ×20,000. Inset: An enlargement of the junctional area. The arrow heads indicate desmosome-like accumulations of particles. ×55,000
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Intercellular junctions are seen on the adjoining cell membrane. They are classified into three types: the macula occludens, a combination of macula occludens and a gap junction, and a gap junction.

The macula occludens, or the focal tight junction, consists of anastomosing short tight junctional ridges on the P-face, or furrows on the E-face. Small maculae occludentes are usually located on the flat adjoining cell membrane along the intercellular canaliculus, which is identified by the presence of fractured microvilli. Irregular accumulation of membrane particles, like desmosomes, are often seen within or near maculae occludentes.

When a small cluster of gap junctional particles is associated with tight junctional ridges, we refer to it as a combination of the macula occludens and gap junction. Like a simple macula occludens, this type of the junction is also present on the flat adjoining cell membrane near the intercellular canaliculus, and desmosome-like aggregations of particles are frequently associated with it.

Small aggregations of gap junctional particles, not associated with tight junctional ridges, are also present. The shape of the aggregation can be round, strand-like, star-shaped, etc. The size is also variable, but does not exceed the typical discoid gap junction of the epithelium. Gap junctions are usually scattered on the flat adjoining cell membrane apart from the intercellular canaliculus, but also sometimes present close to it like a macula occludens with or without gap junctional particles.

Comparison of the intercellular junction among animal species

Comparison in the development of the three types of intercellular junctions among different animal species is summarized in Table 1.

The maculae occludentes without the associated gap junctional particles are infrequent in most animals. The second type, the combination of tight junctional ridges

Fig. 3. A combination of macula occludens and gap junctions (left) and small gap junctions (upper center) in the islet of a guinea pig. ×76,000
Fig. 4. Low magnification electron micrograph of a freeze-fracture-replica of the rat islet. When the fracture face passes along the intercellular canaliculus (C), it is identified by rows of fractured microvilli (arrows Mv). Many small gap junctions (arrows) are seen on the P-face of the flat portion of the membrane. ×14,000

Table 1. Intercellular junctions in the islets of various animals

|                | Macula occludens | Combination of macula occludens and gap junction | Gap junction |
|----------------|------------------|-------------------------------------------------|--------------|
| Mouse          | −                | ++                                              | ++           |
| Rat            | −                | −                                               | #            |
| Guinea pig     | +                | #                                               | +            |
| Hamster        | −                | +                                               | #            |
| Mongolian gerbil| +                | +                                               | +            |

The development of the junction is arbitrarily graded from − to ++
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with gap junctional particles, is the usual form. The macula occludens is not seen in the rat islet.

The development of the total gap junctions, with or without associated tight junctional ridges, does not differ so greatly among these animals except for the Mongolian gerbil, which has only diminutive elements of intercellular junctions.

DISCUSSION

This study demonstrated the presence of three types of intercellular junctions in the pancreatic islet of various rodents, i.e., the macula occludens, the combination of the macula occludens and a gap junction, and the gap junction. The macula occludens, with or without associating gap junctional particles, has been regarded as remnants of the tight junction (DECKER and FRIEND, 1974), an association of the tight junction with gap junctions (ORCI and PERRELET, 1977), or the focal tight junction (ISHIMURA and FUJITA, 1979). As in a previous report (KATAOKA et al., 1982), we prefer the term, macula occludens, to distinguish it from the zonula occludens, the typical belt-like tight junction in the epithelium. In addition to the pancreatic islet cells (ORCI and PERRELET, 1977; KATAOKA et al., 1982), the presence of the macula occludens has been reported on adrenocortical cells (FRIEND and GILULA, 1972), peritoneal mesothelial cells (SIMIONESCU and SIMIONESCU, 1977), renomedullary interstitial cells (MAJACK and LARSEN, 1980), neuroepithelial cells during amphibian neurulation (DECKER and FRIEND, 1974), developing thytoid cells in the chick embryo (ISHIMURA and FUJITA, 1979), chorionic trophoblasts (BARTELS and WANG, 1983) and in the pancreatic acinar cell tumor (IWANJ et al., 1982).

During amphibian neurulation, the zonula occludens, which joins contiguous neuroepithelial cells, fragmentizes into solitary plaque-like remnants as the neural groove deepens (DECKER and FRIEND, 1974). In the acinar cell tumor, where tumor cells grow

Fig. 5. A group of gap junctions of various shapes and sizes in a rat islet. × 66,000

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as disarrayed cords and do not form acinar structures, disrupted tight junctional
strands are randomly distributed over the entire plasmalemma (Iwani et al., 1982).
These two cases of the maculae occludentes are noteworthy when considering the
formation of the islet cell junction during histogenesis of the islet, since endocrine cells
may lose their junctional complex joining them to neighboring exocrine cells prior to
budding off from the exocrine tissue (Pictet and Rutter, 1972). On the other hand,
the focal tight junction on developing thyroid cells gives rise to zonula occludens seal-
ing the follicular lumen by successive divisions of the cells (Ishimura and Fujita, 1979).
A typical zonula occludens in the epithelium encircles the cytoplasm containing
abundant felt work of cytoskeletal elements just beneath the cell apex bearing micro-
villi. Maculae occludentes on peritoneal mesothelial cells (Simionescu and Simionescu,
1977) and chorionic trophoblasts (Bartels and Wang, 1983) are present on the lateral
cell membrane near the microvillous free surface. In the pancreatic islet, as shown in
this and the previous study (Kataoka et al., 1982), the maculae occludentes are present
on the adjoining cell membranes in the vicinity of the microvillous intercellular cana-
lliculus, and the underlying cytoplasm of this region contains felt work of cytoskeletal
elements. These findings suggest that cytoskeletal elements beneath the microvillous
surface may play some roles in the assembling or sustaining of tight junctional com-
ponents in the membrane.
Contradictory findings were obtained between Orci et al.'s (1973) and our present
studies on the intercellular junctions of rat islet cells: Orci et al. (1973) revealed maculae
occludentes on control (normal) islet cells, while we could not find them in the present
study. This contradiction may possibly be ascribed to the different specimen prepara-
tion methods between the two groups: Orci et al. (1973) isolated islets by collagenase
digestion prior to fixation instead of our perfusion fixation in situ. Since pronase
induces extensive development of tight junctional strands on islet cells (Orci et al.,
1973), it may be reasonable to suspect that a similar effect was caused by the collagen
ase preparation or proteolytic enzymes released by pancreatic exocrine cells during the
isolation. We believe that the maculae occludentes are very rare on the rat islet cell
in situ.
As to the functional significance of the macula occludens, we agree with Orci and
Perrelet (1977) suggesting that it delimitates the microenvironment by selectively
opening or closing certain membrane areas to substance diffusing into the extracellular
fluid. Moreover, the presence of the maculae occludentes along the intercellular cana-
lliculus suggests that they partially bound the intercellular canaliculus from the rest of
the intercellular space between islet cells and modify the microenvironment of the two
compartments by affecting the flow of extracellular fluid. However, the boundary may
be very incomplete and the flow of extracellular fluid between the two compartments
is more or less maintained, since the junction is never belt-like.
The presence of the gap junction, the site of metabolic, ionic and electrical coupl-
ing of the cells, and correlation of the gap junction development with the B cell func-
tion were reported in the rat islet (Orci and Perrelet, 1977; Meda et al., 1979, 1980).
In the present study, gap junctions were present on adjoining membranes of islet cells
in all animals examined.
The ultrastructure of the intercellular canaliculus and the distribution of intercel-
lular junctions suggest the possibility that three portions of the islet cell membrane, i.e.
the first portion facing the intercellular canaliculus, the second portion adjoining
neighboring cells and the third portion facing the pericapillary space, have different
roles in the receptosecretory function of the cell (Fujita et al., 1981; Kataoka et al.,
1982). In the present study, the single exocytotic figure of the granule release was found on the adjoining cell surface. Further studies are required to prove the functional significance of each portion of the membrane, as well as alterations in the membrane structure including intercellular junctions in different functional states of the cells.

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