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What is This?
Morphological Changes of Intercellular Junctions in the Rat Submandibular Gland Treated by Long-term Repeated Administration of Isoproterenol

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Long-term repeated administration of isoproterenol (IPR) 2 mg/100 g bw, once daily for ten days, resulted in morphological changes in the intercellular junctions of rat submandibular glands, which were investigated by means of the freeze-fracture technique. A significantly increased number of tight-junctional strands was present. These junctional strands extended much deeper toward the basal membrane than those in normal acinar cells. The basal frontier strands that branched from the networks of tight junctions were elongated and had either free-endings or terminal loops, which were more frequently observed in the IPR-treated acinar cells than in untreated acinar cells. Some of the strands of tight junctions were connected to small gap junctions. The diameters of gap junctions were not significantly different from those of control acinar cells. However, smooth areas devoid of particles were found intermingling with the usual packed particles in irregularly shaped small gap junctions. There was no significant difference between the desmosomes of IPR-treated and untreated acinar cells, in terms of either morphology or distribution. These changes in junctional morphology in the IPR-treated acinar cells resemble those seen in salivary glands during development, and in some experimental conditions including tumorous changes.


Introduction.

It is well established that long-term repeated administration of isoproterenol (IPR), a β-adrenergic agonist, gives rise to an enlargement of both the parotid and submandibular glands (Selye et al., 1961; Yamashina and Barka, 1974), and that the enlargement of the salivary glands is caused by an increase in the content of DNA, RNA, and protein (Barka, 1968; Robinovitch et al., 1977; Barka and Van der Noen, 1976). Our previous study showed that the secretory granules of rat submandibular glands, treated by long-term administration of IPR, contained peculiar lamellar inclusions (Inoue et al., 1980).

The morphology of intercellular junctions of salivary glands has been investigated in adult rat sublingual glands (Shimoto et al., 1980, 1984), in developing submandibular glands of rats (Shimoto et al., 1981), and in rat parotid glands (Simson and Bank, 1984). More recently, Mazariegos et al. (1984), using tracer and freeze-fracture techniques, investigated the alteration of tight junctional permeability in the parotid gland after a single injection of IPR. However, little is known about the morphology of the junctions of salivary glands under different experimental conditions. Particularly, morphological changes of intercellular junctions and functional alterations in the submandibular acinar cells induced by long-term repeated administration of IPR are by no means clear.

The purpose of the present study was to demonstrate the morphological changes of intercellular junctions after chronic administration of IPR, and to discuss the potential effects on the paracellular permeability and cell-to-cell coupling of the acinar cells in the IPR-treated submandibular gland.

Materials and methods.

Fifty male Sprague-Dawley rats (Charles River Japan, Inc., Kanagawa, Japan), weighing about 200 g each, were used. The animals were injected intraperitoneally with 2 mg of IPR (Sigma Chemical Co., St. Louis, MO) per 100 g of body weight once daily (at 6:00 pm) for ten consecutive days, according to the method of our previous report (Inoue et al., 1980). Twenty-four hours after the final administration of IPR, the animals were killed by cervical dislocation. As a control, 15 untreated animals were used. The submandibular glands obtained from both IPR-treated and untreated animals were immediately removed and fixed by immersion in the following fixative procedures:

For thin sectioning, tissue fragments were processed by two different procedures:

(1) Modified Karnovsky's (1965) fixative — Tissues were fixed in 0.12 mol/L Sorensen's phosphate buffer containing 2% paraformaldehyde and 2.5% glutaraldehyde, pH 7.4, for two hr at room temperature and then post-fixed in 1% phosphate-buffered osmium tetroxide at 4°C. Staining of tissue blocks was performed in 2% uranyl acetate in 10% alcohol prior to dehydration.

(2) Lanthanum impregnation — Tissues were fixed for two hr in 0.1 mol/L cacodylate-buffered 2% glutaraldehyde containing 1% lanthanum hydroxide (TAAB Laboratory Equipment Limited, Berks, England), pH 7.3, and finally post-fixed with 1% osmium tetroxide in the same buffer for one hr. After dehydration in a graded series of alcohols, the tissues were embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

For freeze-fracture replicas, small fragments of samples were fixed in modified Karnovsky's fixative, as described above, for three hr and infiltrated by 10, 20, 30, and 40% glycerol diluted with 0.12 mol/L Sorensen's phosphate buffer (30 minutes each passage). The fixed specimens were frozen rapidly in liquid Freon 22, cooled in liquid nitrogen, and then fractured at −100°C and replicated with either platinum or carbon in a freeze-etching apparatus (JFD-7000, JEOL, Tokyo, Japan). Replicas were cleaned by successive washings with 10% sodium hypochlorite and distilled water. Thin sections and replicas were examined in a JEM-100CX electron microscope.

A quantitative account of the freeze-fracture material was made on 130 randomly-chosen tight junctions, 60 gap junctions, and 60 desmosomes in both IPR-treated and untreated acinar cells, respectively. The number of strands and the depth of the tight junctions were estimated. For the measurement, high-power electron micrographs of the junctions magnified directly at X 10,000 or X 20,000 were taken, enlarged, and printed at a final magnification of X 30,000. Two lines crossing at right angles with the most luminal strand in each junction were drawn on the printed micrographs. Strands crossing these drawn lines were counted as the number of strands. Distances between the most luminal and abluminal strands on the drawn
lines were measured as the depth of the junction. In total, 130 points of tight junctions were estimated in both IPR-treated and untreated acinar cells. Furthermore, the numbers of strands with free-endings and terminal loops found per 10 μm width of junctional membrane were determined. The diameters of either gap junctions or desmosomes were measured. Maximal and minimal diameters were estimated on each junction; thus, a total of 120 diameters was measured in IPR-treated and untreated glands, respectively. The results were expressed as mean values and standard error of means (SEM). Data were analyzed by Student’s t test.

Results.

(1) Intercellular junctions in the untreated acinar cells. — Three types of intercellular junctions — tight junctions, gap junctions, and desmosomes — were observed in acinar cells of the untreated rat submandibular gland. In thin sections from these animals, tight junctions were recognized as focal fusions of the adjacent plasma membranes, while in the lanthanum-treated specimens the tracer did not infiltrate beyond the point of focal fusion (Fig. 1). In freeze-fracture replicas, the tight junctions were detected at the boundary between luminal and lateral plasma membranes. They appeared as continuous belt-like networks of ridges on the PF face and as grooves on the EF face. The junctions were shallow and consisted of from one to six strands that were occasionally interrupted (Fig. 2). The average number of strands was 3.17 ± 0.09. The depth of the junctions ranged from 0.04 to 0.53 μm, with an average of 0.21 ± 0.01 μm. The number of strands with free-endings per 10 μm width varied from 0 to 2, with an average of 0.15 ± 0.03, and those with terminal loops from 0 to 1, with an average of 0.02 ± 0.01.

On freeze-fracture replicas of the acinar cells, gap junctions appeared as closely packed particles measuring from 8 to 10 nm in diameter on the PF face and as small complementary pits on the EF face (Fig. 3). The diameter of gap junctions in acinar cells of unstimulated submandibular glands varied from 0.2 to 1.1 μm, with a mean value of 0.51 ± 0.03 μm.

Desmosomes were found on the lateral plasma membranes of the acinar cells. Their freeze-fracture images could be characterized by clusters of particles measuring from 8 to 11 nm in diameter on both the PF and EF faces (Fig. 2). The diameters of desmosomes in unstimulated submandibular glands varied from 0.15 to 0.24 μm, with a mean value of 0.21 ± 0.03 μm.

(2) General aspects of the IPR-treated acinar cells. — Repeated administration of IPR for 10 days caused a significantly reduced increase in body weight at the level of p < 0.02 and an increase in weight of submandibular glands at the level of p < 0.001 (Table 1). In contrast, the ratio of the wet weight of the submandibular glands to body weight was roughly doubled, because of hypertrophy and hyperplasia of the acinar cells of the submandibular gland. In the enlarged acinar cells, abundant membrane-bound secretory granules containing peculiar lamellar inclusions filled the cytoplasm (Fig. 4).

(3) Intercellular junctions in the IPR-treated acinar cells. — Three types of intercellular junctions were also observed between the acinar cells in chronically IPR-treated animals.
crease in diameters the long-term administration of

(a) Tight junctions

With lanthanum impregnation of the experimental animals, focal fusions revealing tight junctions were observed by negative contrast. They increased in number and extended much deeper toward the basal membrane compared with control acinar cells. The tracer passed through (or around) one or more abluminal fusions; however, it was arrested at a more luminal level (Fig. 5).

On freeze-fracture replicas, tight junctions were observed as more complicated networks of branching and anastomosing ridges or interconnected grooves in IPR-treated acinar cells (Fig. 6). The junctions were composed of from 2 to 10 interlinked strands, with an average of 5.50 ± 0.18, and the depths of the junctions ranged from 0.10 to 1.19 μm, with an average of 0.53 ± 0.03 μm. Moreover, the numbers of strands with free-endings (Fig. 8) varied from 0 to 5, with an average of 1.29 ± 0.04, and those with terminal loops (Fig. 7) from 0 to 2, with an average of 0.23 ± 0.02. As shown in Table 2, long-term administration of IPR resulted in a significant increase in all parameters measured, at the level of p < 0.001.

(b) Gap junctions

In IPR-treated acinar cells (Fig. 9), the sizes of gap junctions varied from 0.1 to 1.6 μm in diameter, with a mean value of 0.52 ± 0.04 μm. There was no significant difference in the diameters of the gap junctions between control and experimental glands (Table 3). However, smooth areas devoid of particles intermingling with the usual packed particles were found in the gap junctions (Fig. 10). The frequency, size, and shape of the gap junctions in the IPR-treated acinar cells were more irregular than in control acinar cells. Small and very infrequent gap junctions, which consisted of loosely-aggregated particles, were also seen (Fig. 11). Small gap junctions associated with tight-junctional strands were often detected on the lateral plasma membrane close to the lumen (Fig. 12).

(c) Desmosomes

In IPR-treated acinar cells (Fig. 6), the diameters of desmosomes varied from 0.18 to 0.31 μm, with a mean value of 0.27 ± 0.06 μm. There was no significant difference between IPR-treated and untreated glands, in either the morphology or distribution of desmosomes (Table 4).

Discussion.

Tight junctions. — In this study, we found that morphological changes of intercellular junctions were induced in the acinar cells of the IPR-treated rat submandibular gland. Our results on normal glands confirmed the observations of Shimono et al. (1980, 1981, and 1984), who investigated intercellular junctions of normal rat salivary glands by means of either tracer methods or freeze-fracture replications. They observed that in acinar cells of the rat sublingual gland, tight junctions formed continuous belts around the luminal regions of the plasma membranes and were composed of from one to four, occasionally interrupted, sealing elements, and the depths of the tight junction were about 0.28 μm (Shimono et al., 1980).

With respect to numbers of sealing elements and the depths of the junctions, our present study showed that the junctions in normal submandibular glands were similar to those of sublingual glands (Table 2). Shimono et al. (1981) have also demonstrated the formation process of tight junctions in developing submandibular glands of the rat. They observed proliferation of the elongated basal frontier strands branching from the networks and recognized strands with both free-endings and terminal loops. They suggested that morphological changes of
Fig. 5 — Lanthanum passes through (or around) the focal fusions (arrowheads), but its penetration is stopped at the most luminal one. The focal fusions of the tight junctions extended much deeper toward the basal side compared with untreated glands. Lu, Lumen.

Figs. 6-12 — Freeze-fracture micrographs of acinar cells of IPR-treated submandibular glands. PF, P fractured face; EF, E fractured face.

Fig. 6 — Tight junctions are deeper and composed of many anastomosing strands; branching from networks can be seen. Lu, Lumen; Sg, Secretory granule; Ds, Desmosome.

Fig. 7 — Tight junctional strands with terminal loop (asterisk) are observed.

Fig. 8 — Tight junctional strands with free-endings (arrowheads) are deeper, composed of five or more anastomosing strands. Lu, Lumen.
Figs. 9-11 — Freeze-fracture images of gap junctions in IPR-treated acinar cells. The sizes and shapes of the junctions are more irregular. However, there is no significant difference in mean value of the diameter of the junction between control and experimental glands.

Fig. 9 — Huge gap junction of 1.6 μm in diameter appears as closely packed particles on the PF face and as small complementary pits on the EF face.

Fig. 10 — Irregularly shaped gap junction with smooth area (arrows) devoid of particles can be seen.

Fig. 11 — Small gap junction, 0.1 μm in diameter, consisting of about 20 particles.

Fig. 12 — Small gap junction (asterisk) associated with tight-junctional strands is detected. L, Lumen.
TABLE 1
WET WEIGHT OF SUBMANDIBULAR GLANDS AND BODY WEIGHT OF CONTROL AND EXPERIMENTAL RATS

<table>
<thead>
<tr>
<th></th>
<th>Control Rats</th>
<th>Experimental Rats</th>
<th>Student’s t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>220 – 345</td>
<td>186 – 356</td>
<td>**</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>269 ± 10.6</td>
<td>229 ± 11.4</td>
<td></td>
</tr>
<tr>
<td>Gland weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.40 – 0.61</td>
<td>0.55 – 1.29</td>
<td>*</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.48 ± 0.02</td>
<td>0.72 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.001; ** p < 0.02.

TABLE 2
CHARACTERISTICS OF TIGHT JUNCTIONS IN CONTROL AND EXPERIMENTAL RATS

<table>
<thead>
<tr>
<th></th>
<th>Control Rats</th>
<th>Experimental Rats</th>
<th>Student’s t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of strands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1 – 6</td>
<td>2 – 10</td>
<td>*</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>3.17 ± 0.09</td>
<td>5.50 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Depth of junction (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.04 – 0.53</td>
<td>0.01 – 1.19</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.21 ± 0.01</td>
<td>0.53 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Number of strands with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>free-endings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0 – 2</td>
<td>0 – 5</td>
<td>*</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.15 ± 0.03</td>
<td>1.92 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Number of strands with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>terminal loops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0 – 1</td>
<td>0 – 2</td>
<td>*</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.02 ± 0.01</td>
<td>0.23 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.001.

TABLE 3
DIAMETERS OF GAP JUNCTIONS IN CONTROL AND EXPERIMENTAL RATS

<table>
<thead>
<tr>
<th></th>
<th>Control Rats</th>
<th>Experimental Rats</th>
<th>Student’s t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of junction (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.2 – 1.1</td>
<td>0.1 – 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.51 ± 0.03</td>
<td>0.52 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

There was no significant difference in the diameters of the gap junctions between control and experimental glands. However, size and shape in the experimental acinar cells were more irregular.

TABLE 4
DIAMETER OF DESMOSOMES IN CONTROL AND EXPERIMENTAL RATS

<table>
<thead>
<tr>
<th></th>
<th>Control Rats</th>
<th>Experimental Rats</th>
<th>Student’s t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of desmosomes (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.15 – 0.24</td>
<td>0.18 – 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.21 ± 0.03</td>
<td>0.27 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

NS = not significant.

junctional strands revealing these free-endings and terminal loops indicate newly formed strands. Therefore, we envisage that strands with free-endings and terminal loops observed in our material indicate signs of de novo formation of the tight junction and are induced by the long-term administration of IPR. It has been found that morphological changes of the junctions are produced by some experimental conditions or tumorous changes. Robenek et al. (1980), for instance, studied the formation and turnover of tight junctional strands of rat liver, after chronic administration of the carcinogen N-nitrosomorpholinite (NNM). They found that NNM produced an extensive proliferation of the tight junction in the rat liver. Inoue et al. (1984) also found tight junctions appearing similar to those described above in acinic cell carcinoma arising from minor salivary glands. Recently, Mazariigos et al. (1984) studied the permeability of junctional complexes using ultrastructural tracers and also the freeze-fracture appearances of junctional structures of rat parotid glands from one to four hr after a single injection of IPR. They found increased irregularity of the tight-junctional networks, free-endings often extending basally from this network, and double elements. However, they found no obvious appearance to account for the increased permeability that had occurred. In our studies, there was an increase either in the number of strands or in their apical-basal depth. Furthermore, strands with free-endings and terminal loops extending from the network were frequently observed after chronic administration of IPR. This suggests that the permeability barrier at the level of the acinar cells becomes tighter during the hyper trophy and hyperplasia of the acinar cells, since Claude and Goodenough (1973) found an association between the degree of tightness and the number of strands present.

Gap junctions. — In addition to classic gap junctions of acinar cells, some consisting of small clusters of particles associated with tight junctions were occasionally observed in IPR-treated glands. A similar combination of two different types of junctions has been observed in many tissues (Schiller and Taugner, 1979; Murphy and Rogers, 1980; Robenek et al., 1980; Shimono et al., 1980) and has been considered to
be a stage in the formation of gap and tight junctions. It is suggested that an aggregation of particles forming a gap junction may increase in size and frequency during the course of development (Shimono et al., 1980). Furthermore, we observed irregularly-shaped gap junctions with smooth areas lacking particles, intermingled with areas of packed particles. Similar gap junctions have been observed in many tissues (Albertini and Anderson, 1975; Shimono and Clementi, 1977; Perrachia, 1977; Shibata et al., 1980; Iiguchi et al., 1984), and this peculiar junction is regarded as one type of gap junction seen during developmental stages. However, it is difficult to determine whether these irregularly-shaped gap junctions can facilitate the function of cell-to-cell coupling (Petersen, 1980).

The morphological changes of tight and gap junctions in the acinar cells of submandibular glands treated by long-term administration of IPR resemble those seen in salivary glands during development, some experimental conditions, and tumorous changes. These results suggest that both tight and gap junctions are dynamic entities, and their structure is the result of an equilibrium between association and dissociation of specific intramembranous particles (Shimono and Clementi, 1979; Meldolesi et al., 1978).

REFERENCES


