A Morphological Basis for Intercellular Communication between α- and β-Cells in the Endocrine Pancreas

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Abstract By degranulating β-cells in the islets of Langerhans of the rat with sulfonylurea, it has been possible to distinguish unambiguously α-cells from β-cells in freeze-fracture replicas. In such preparations, we found morphologically typical tight and gap junctions occurring between α- and β-cells. The presence of gap junctions offers indirect evidence that these cells are coupled with one another; coupling may influence the secretory behavior of α- and β-cells maintaining glucose homeostasis within tightly constricted limits.

INTRODUCTION

As is true for cells in most tissues, cells of the islets of Langerhans share intercellular junctions. The first junction recognized in the islet was the desmosome (1) involved in cellular adhesion (2, 3). Recently, the use of the freeze-fracture technique allowed us to demonstrate in the islets of several mammalian species, including human, the presence of two other types of junctions, the tight and the gap junctions (4–6). These two have important physiological properties since tight junctions determine a closure of the intercellular space (1, 2) while gap junctions allow ions and small molecules to cross from one cell to another (intercellular coupling) (2, 3, 7–10). However, because of the heterogeneity of the islet cell population and the limitations of the freeze-fracture technique, it has always been difficult to determine exactly which cell types were sharing tight or gap junctions, except in the normal rat islet. In the normal islet, β-cells form the near totality of its center, and junctions detected by freeze-fracture in this region were attributed with great probability to β-cells (5). Similarly, those junctions situated at the extreme periphery of the islet were related tentatively to α-cells (5). With a new experimental approach, we are now able to present morphological evidence for tight and gap junctions occurring also between α- and β-cells in rat islets.

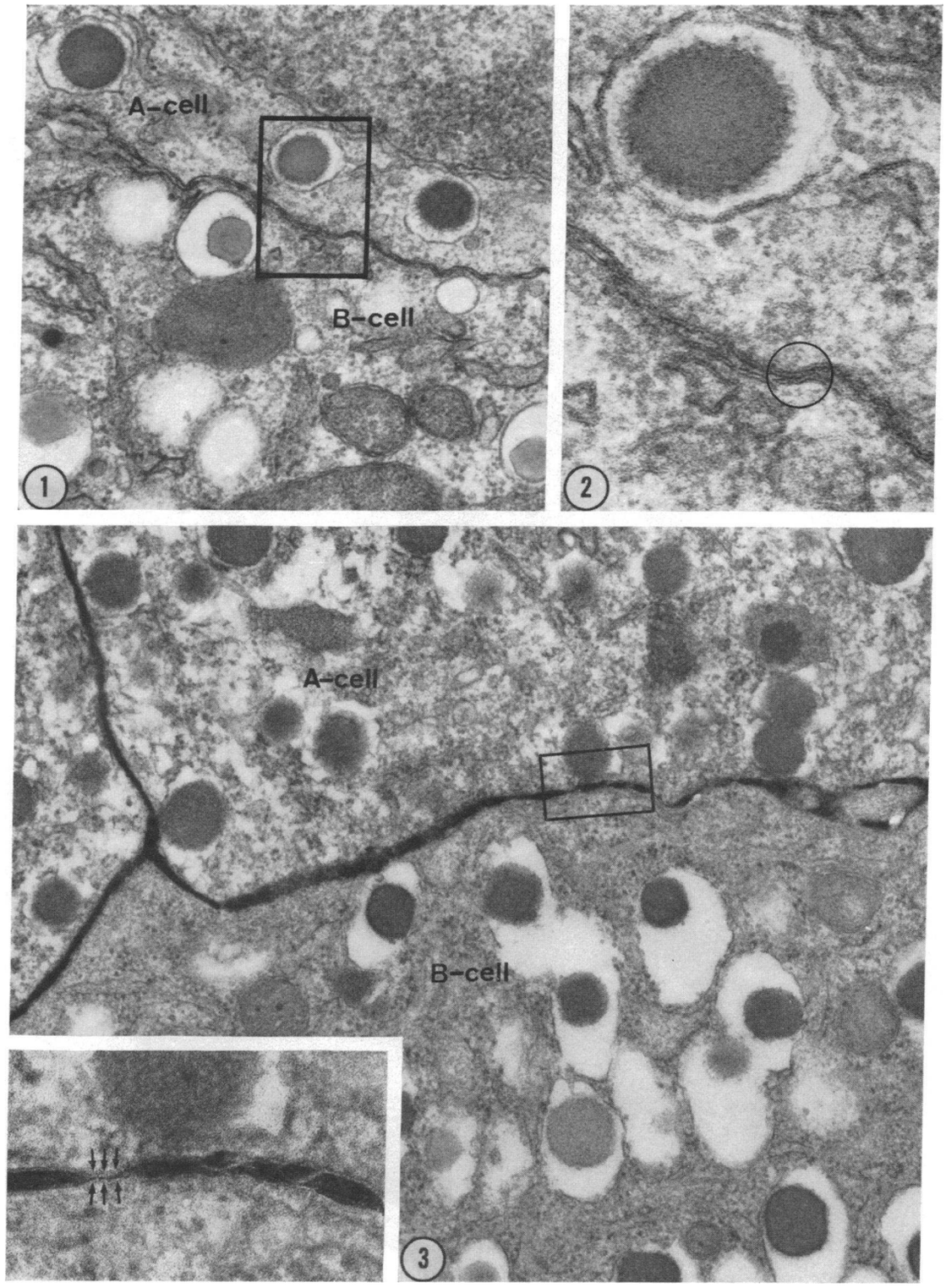
Received for publication 16 April 1975 and in revised form 7 July 1975.

METHODS

For freeze-etching studies, islets isolated by collagenase digestion were used (11). Two types of preparations were examined: (*a*) islets of control rats and (*b*) islets of rats treated for 3 days with a sulfonylurea (glibenclamide; 0.5 mg i.p. twice daily). About 100 isolated islets of each preparation were collected and centrifuged so as to form a pellet. The pellet was briefly fixed with phosphate-buffered glutaraldehyde and freeze-fractured (12) in a Balzers BAF 301 freeze-fracture device (Balzers High Vacuum Corp., Balzers, Liechtenstein). For thin-section electron microscopy, two different methods were used: in some cases, single isolated islets were fixed with phosphate-buffered glutaraldehyde, postfixed in osmium tetroxide, and block-stained with uranyl acetate before alcohol dehydration and Epon embedding. In other cases (tracing experiment), pieces of pancreas were fixed in a glutaraldehyde solution containing lanthanum hydroxide (13) as the electron-dense tracer. Lanthanum diffuses in the extracellular space and is retained in several places so as to appear as black deposits in thin sections. Freeze-fracture replicas and thin sections were examined in a Philips EM 300 electron microscope (Philips Electronic Instruments, Eindhoven, The Netherlands).

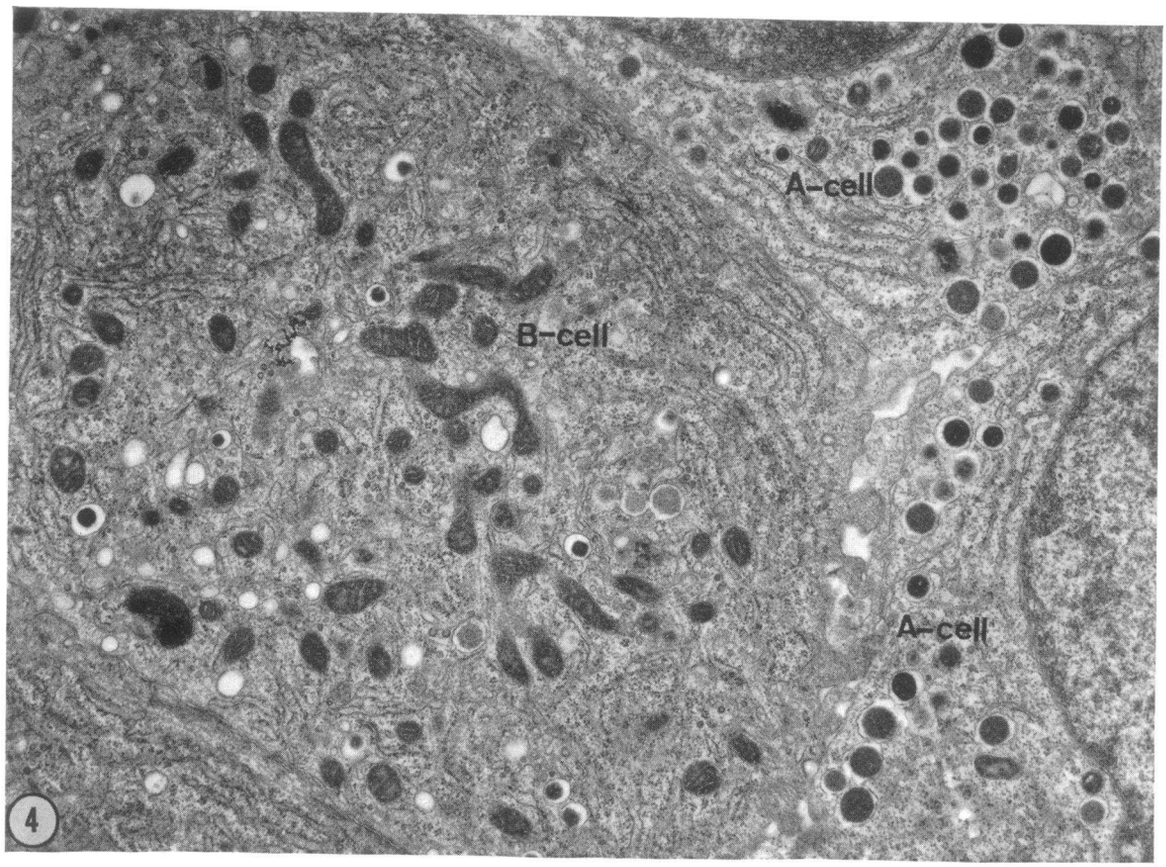
RESULTS

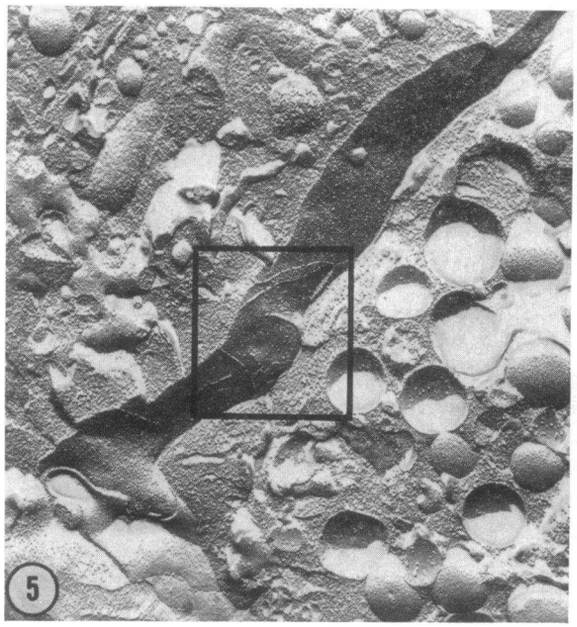
Morphologic evidence suggesting the presence of tight and gap junctions can be found in conventional thin sections (Figs. 1 and 2), but it is more easily obtained in preparations in which the intercellular space contains lanthanum. In such specimens, the intercellular space appears as a black line of variable width (about 15–20 nm) which is interrupted or narrowed in some

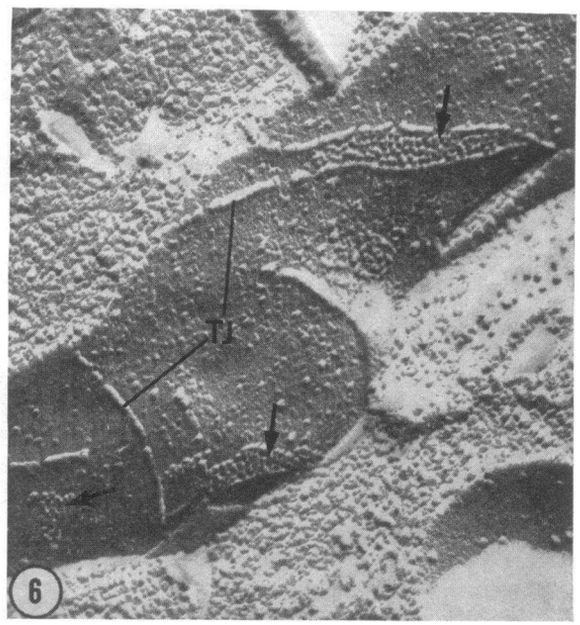


FIGURES 1 and 2 Isolated islets from normal rat, stained en bloc with uranyl acetate. The junction between an α- and a β-cell present in the framed area of Fig. 1 is shown at a higher magnification in Fig. 2. In the encircled area, the outer leaflets of the adjacent cell membranes undergo fusion, resulting in a pentalaminar structure, characteristic of a tight junction. Fig. 1, × 38,000; Fig. 2, × 124,000.

FIGURE 3 Pancreas treated with lanthanum hydroxide, which delineates the intercellular space in black. The area outlined by the rectangle is shown at high magnification in the inset. At places indicated by arrows, the intercellular space between an α- and a β-cell appears considerably narrowed (presumably a gap junction). Fig. 3, × 31,500; inset, × 102,000.







FIGURES 4–6 Islets isolated from sulfonylurea-treated rats.

FIGURE 4 Part of the periphery of an islet showing a poorly granulated β-cell neighboring two well granulated α-cells.

FIGURE 5 Freeze-etch replica of a similar area. The fracture process has split the plasma membrane between two cells tentatively identified as β- and α-cells on the basis of their content in secretory granules.

FIGURE 6 Higher magnification of the framed area in Fig. 5. One can see the linear ridges or fibrils, characteristic of tight junctions (TJ), and the aggregates of particles, characteristic of gap junctions (arrows). Fig. 4, $\times$ 13,000; Fig. 5, $\times$ 25,000; Fig. 6, $\times$ 92,000.

places (Fig. 3). The interruptions of the black line indicate the presence of tight junctions (closure of the intercellular space due to the fusion of the outer leaflets of the adjacent plasma membranes) (2, 3) while narrowing of the intercellular space is compatible with gap junctions (in these junctions, the outer leaflet of the adjacent plasma membranes come very close to one another and determine a narrow slit, or gap, bridged by subunits) (2, 3). When junctions are small, which is the case in the islets (see Figs. 1–3), it is always difficult to distinguish unambiguously between a fusion or a gap, and one has to rely on the freeze-fracture technique. With this technique each of the junctions, no matter how small, shows a specific pattern in the fractured membranes.[1] In contradistinction to thin-sectioning, however, freeze-fracturing (being a replication technique) does not allow one to distinguish between the different cell types of the islet, which are identified mainly by the electron density and the shape of the core of their secretory granules. In order to distinguish α- and β-cells in freeze-fracture replicas, we used one indirect approach, namely degranulation of the β-cells by sulfonylurea treatment (15), which leaves mainly α-cells filled with secretory granules (Fig. 4). Our assumption was that if morphological specializations characterizing tight and gap junctions were found between granulated and degranulated cells, their presence could be considered as indirect, yet reasonably safe evidence for the occurrence of tight and gap junctions between α- and β-cells. Figs. 5 and 6, a freeze-fracture replica from a rat islet treated with sulfonylurea, clearly show that both tight and gap junctions do occur between α- and β-cells.

DISCUSSION

As stated in the Introduction, the terms *tight* and *gap* junctions have not only a morphological meaning but a functional one as well; in the case of the islet cell membrane differentiations, we are of course aware that functional evidence for tight and gap junctions is entirely lacking, since it is only in a very few selected preparations that morphology could be directly related to function (for example, demonstration of closely packed particles *and* recording of electrotonic coupling as in reference 9). However, there seems to be sufficient cross evidence in this field (for review, see references 2 and 3) between a given structure and a given function to allow us to assume that the junctions described morphologically in islet cells do probably represent functional tight and gap junctions. One peculiarity of these junctions is that they are diminutive forms of the more elaborate ones (for example those found in liver cells) which have provided the basis for a classification. While the role of the small tight junctions in islets is fully open at present, the presence of gap junctions between α- and β-cells justifies the speculation that the coupling of α- and β-cells through gap junctions could account for certain observed phenomena of islet function not readily explained in terms of responses to external stimulation alone. For example mounting physiological evidence suggests that α- and β-cells release precisely titrated quantities of secretory products that have opposed effects and yet maintain extracellular glucose concentration within a tightly constricted range during major changes in glucose turnover (16). The question as how the net secretory output emerging from the total islet mass is so precisely titrated to maintain nutrient homeostasis might, in part, be answered by the existence of intercellular coupling.

[1] Tight junctions are seen as ridges or fibrils in the cytoplasmic leaflet of the freeze-fractured membrane while gap junctions are seen as aggregates of globular subunits or particles in the same membrane face (14).

ACKNOWLEDGMENTS

We thank M. Sidler-Ansermet, M. Bernard, and I. Bernard for excellent technical assistance.

This work was supported by the Fonds National Suisse de la Recherche Scientifique (grants 3.8081.72, 3.0310.73, and 3.553-75), the National Institutes of Health (grant AM 02700–15), and a grant-in-aid from Hoechst Pharmaceuticals, Frankfurt Hoechst, Germany.

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