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Quantitative Electron Microscopic Autoradiography of Insulin, Glucagon, and Somatostatin Binding Sites on Islets

Abstract. *After monolayer cultures of rat islets were exposed to [^{125}I]insulin, [^{125}I]glucagon, and [^{125}I]tyrosinyl somatostatin, specific autoradiographic grains associated with each radioactively labeled ligand were found on B, A, and D cells. The density of labeling of the B, A, and D cells with each labeled ligand correlated well with the known actions of the three hormones on each of the islet cells.*

The islets of Langerhans consist of a heterogeneous population of endocrine cells (*1*). Adjacent islet cells appear to influence each other by direct, local interactions. Evidence for such interactions, which are also called paracrine (*2, 3*), is derived both from morphological studies, showing a nonrandom distribution of the different endocrine cells within a given islet (*1, 4*), and from functional studies, showing that the B, A, and D cells can each influence the secretion of the other two cell types (*5–9*). Furthermore, observations that insulin and somatostatin can themselves inhibit B- and D-cell function, respectively (*6, 10, 11*), suggest that at least these two islet cells are capable of autoregulation by an ultrashort loop feedback mechanism.

The mechanism underlying the paracrine interaction between the adjacent islet cells is poorly understood, but is believed to be mediated by specific cell surface receptors for each hormone. Attempts to characterize these receptors have been made in the case of insulin and somatostatin by means of direct binding studies with whole rat islets and the appropriate radioactive ligands (*12*). By this method, however, one cannot identify binding sites on any given type of islet cell. To overcome this problem and to gain further insight into the functional interactions among the B, A, and D cells, we have studied, by quantitative electron microscopic autoradiography, the distribution of radioactively labeled insulin, glucagon, and somatostatin on B, A, and D cells in monolayer cultures of dissociated rat islets.

Monolayer cultures of dissociated islet cells from the pancreases of 3-day-old Wistar rats were established in 35-mm plastic petri dishes. Such cultures are rich in B, A, and D cells (*13*). The medium consisted of minimum essential medium with 10 percent heat-inactivated fetal calf serum (MEM-FCS). The glucose concentration was maintained at 16.5 m*M* until day 5 of culture when it was reduced to 5.5 m*M*; labeling experiments were then conducted on day 6. Porcine monocomponent insulin (Novo Industrie, Denmark), porcine glucagon (Novo), and synthetic tyrosinyl somatostatin (Tyr-S-14) (Bachem Fine Chemicals) were radioiodinated by the chloramine-T technique (*14*) and purified by gel filtration (Sephadex G-50 superfine for [^{125}I]insulin, Sephadex G-25 superfine for [^{125}I]Tyr-S-14), or ion exchange chromatography with QAE Sephadex for [^{125}I]glucagon), as described (*15*). The specific activities of the three ligands were as follows: [^{125}I]insulin, 87 μCi/μg; [^{125}I]glucagon, 390 μCi/μg; and [^{125}I]-Tyr-S-14, 753 μCi/μg.

Before conducting the labeling experiments we discarded the maintenance media and washed the cells with 2 ml of MEM-FCS. Paired petri dishes were then incubated at 37°C for 5 minutes and 60 minutes with 1 ml of medium (MEM-FCS with 4.5 m*M* glucose) containing radioiodinated hormones at the following concentrations: [^{125}I]insulin, 5.05 n*M*; [^{125}I]glucagon, 0.5 n*M*; [^{125}I]Tyr-S-14, 0.83 n*M*. These concentrations were at least 10 to 100 times greater than the expected concentrations of endogenous insulin, glucagon, and somatostatin secreted under the conditions of the experiment. Control cells were incubated with the same concentrations of labeled hormones in the presence of excess (0.3 to 0.6 μ*M*) unlabeled hormones.

At the end of the incubation period the radioactive media were removed, the petri dishes were washed three times with MEM in phosphate-buffered saline (PBS), and the cultures were fixed with 2.5 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer at room temperature for 4 hours. The fixed cells were then dehydrated with ethanol, embedded in Epon, and sectioned. High resolution autoradiography was performed by coating the sections with a thin emulsion of Ilford L4 according to Caro *et al.* (*16*) and then incubating them at 4°C for 4 to 5 weeks. After development with Microdol X the sections were examined in a Phillips 301 electron microscope. Magnifications were calibrated with a grating replica containing 2160 lines per millimeter. To determine the percentages of islet cells labeled, eight separate well-defined clusters of islet cells were first identified and the total number of B, A, and D cells within each cluster counted at low magnification. Individual B, A, and D cells containing autoradiographic grains were then counted separately at high magnification, and the number of labeled cells of each type was expressed as a percentage

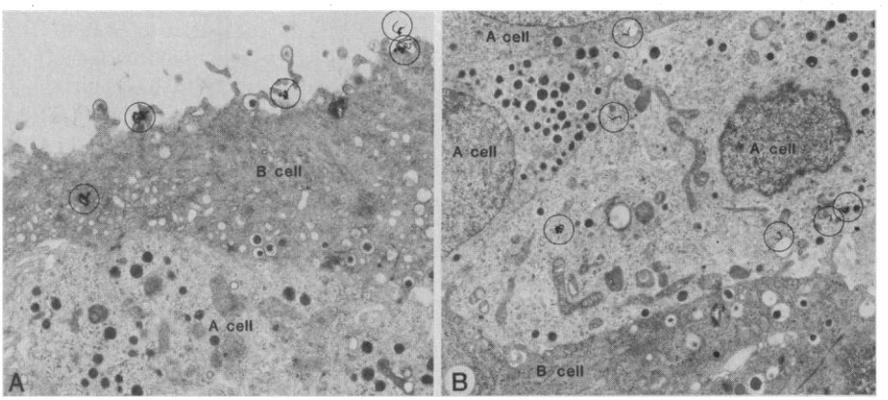

Fig. 1. (A) Monolayer culture labeled with [^{125}I]insulin for 60 minutes at 37°C. The field shows part of a B cell with several autoradiographic grains distributed over its periphery (encircled). The neighboring A cell is not labeled (×16,000). (B) Monolayer culture labeled with [^{125}I]Tyr-S-14 for 60 minutes at 37°C. The field shows the preferential association of autoradiographic grains (encircled) with A cells. The B cell at the bottom of the picture is unlabeled (×12,000). These two examples represent particularly heavily labeled cells for illustrative purposes.

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of the total number of cells for that type. The total number of autoradiographic grains evaluated for each radioactive ligand was 1804 for [^{125}I]Tyr-S-14, 1389 for [^{125}I]insulin, and 1064 for [^{125}I]glucagon. For statistical analysis of the data we used the Student's *t*-test.

The examples of labeled islet cells shown in Fig. 1 illustrate the distribution of autoradiographic grains. Grains were localized over the cell surface as well as over the cytoplasm: internalization of the ligand occurred in a time-dependent and ligand-dependent fashion as observed in other systems binding polypeptide hormones [for a review, see (*17*)]. Control sections incubated with labeled and excess unlabeled hormone contained virtually no demonstrable autoradiographic grains.

The percentage of B, A, and D cells labeled with each of the three radioligands was identical after 5 and 60 minutes of incubation. For quantitative analysis, two separate islet cell clusters per petri dish were evaluated and the data for 5 and 60 minutes were pooled. Figure 2 shows that all three types of islet cells are significantly labeled with each of the three radioligands utilized. Furthermore, the absence of autoradiographic grains when incubations were carried out with excess unlabeled hormone indicates that the labeling corresponds to specific binding sites. Maximum labeling with [^{125}I]-insulin was associated with B cells, 60 percent of which contained autoradiographic grains, whereas 35 percent of A cells and 22 percent of D cells were so labeled. The [^{125}I]insulin labeling of B cells was significantly higher than that of A cells which in turn was higher than that of D cells. Specific labeling of B, A, and D cells with [^{125}I]glucagon was also demonstrated. The highest percentage of islet cells labeled with [^{125}I]glucagon were again the B cells, 40 percent of which contained autoradiographic grains, compared to 31 percent of the D cells and only 15 percent of the A cells. [^{125}I]Glucagon-labeled B and D cells were significantly more numerous than [^{125}I]glucagon-labeled A cells. All three types of islet cells were specifically labeled with [^{125}I]Tyr-S-14, but the A cells showed the highest density of labeling with this radioligand; 75 percent of the A cells, 37 percent of the B cells, and 33 percent of the D cells were labeled with [^{125}I]Tyr-S-14.

These studies show that by means of electron microscopic autoradiography one can identify specific binding sites for insulin, glucagon, and somatostatin on pancreatic islet cells, and measure the relative distribution of the binding sites

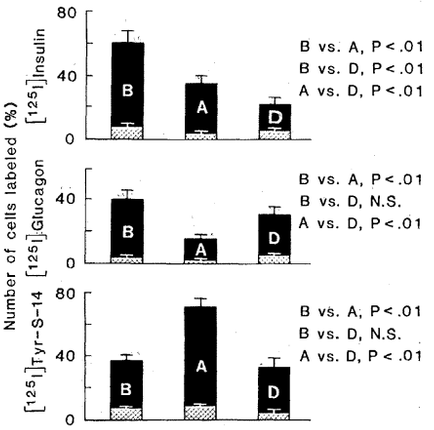

Fig. 2. Percentages of B, A, and D cells labeled with [^{125}I]insulin, [^{125}I]glucagon, and [^{125}I]Tyr-S-14 in the absence (■) or presence (▨) of excess unlabeled hormones. A total of eight islet cell clusters was analyzed for each radioactive ligand. N.S., not significant.

for the three radioactive ligands on B, A, and D cells.

The receptor densities for insulin, glucagon, and somatostatin on the B, A, and D cells as evaluated by autoradiography in the present study show a remarkable correlation with the known actions of the three hormones on each of the islet cells. Thus insulin at circulating concentrations within the physiological range is a well-recognized inhibitor of A and B cells and may have a lesser (inhibitory or stimulatory) effect on D cells at high concentrations such as those that may occur locally within the islets (*5–7*). Glucagon stimulates both B and D cells but has not been reported to regulate A-cell function (*8*). Tetradecapeptide somatostatin [somatostatin-14 (S-14)] is a potent inhibitor of insulin and glucagon (*9*). The A cells, however, appear to be 50 times more sensitive to S-14 inhibition than the B cells (*18*), a fact that correlates well with the present demonstration of significantly greater labeling of A cells with [^{125}I]Tyr-S-14 compared to B cells. Biologically active but immunologically inactive analogs of S-14 have also been reported to inhibit endogenous D-cell function (*11*). This observation and the reported B-cell inhibitory effect of insulin, coupled with our finding of the association of [^{125}I]Tyr-S-14 with D cells and of [^{125}I]insulin with B cells provide considerable evidence that at least these two islet cells are capable of autoregulation.

The difference in the percentages of B, A, and D cells labeled with a given radioactive ligand could be due to differences in the affinity of the receptor for the particular ligand, or to the number of receptors on a given cell type, or both. In recent studies of direct binding of [^{125}I]insulin and [^{125}I]Tyr^{1}-S-14 to whole rat islets (*12*), the binding of [^{125}I]insulin correlated well with B-cell function, and thus appeared to be related in part to this cell type, whereas the islet cell type associated with [^{125}I]Tyr^{1}-S-14 binding was not determined. These reports highlight the difficulty of estimating the affinity and concentration of a given islet hormone receptor as it pertains to a known islet cell type because of the heterogeneity of islet cells; they also emphasize the need for suitable techniques for preparing pure populations of normal B, A, and D cells for undertaking direct binding studies. Quantitative electron microscopic autoradiography provides one alternative approach to investigating islet hormone receptors on individual islet cells.

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