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In Vivo Localization of Insulin Binding to Cells of the Rat Pancreas (41827)

CHOITSU SAKAMOTO, JOHN A. WILLIAMS, EILEEN ROACH, AND IRA D. GOLDFINE

Cell Biology Laboratory, Harold Brunn Institute, Mount Zion Hospital and Medical Center, San Francisco, California 94120, and Departments of Physiology and Medicine, University of California, San Francisco, California 94143

Abstract. The uptake of ^{125}I-insulin by rat pancreas was studied *in vivo*. Following fixation and light microscope autoradiography, saturable uptake of ^{125}I-insulin was quantitatively demonstrated on acinar and duct cells but not on blood vessels and islets of Langerhans. Electron microscopy revealed the localization of ^{125}I-insulin to the basolateral cell membranes of acinar and duct cells.

Recent studies have shown that hormones from the islets of Langerhans, especially insulin, in addition to their general metabolic effects, also regulate pancreatic exocrine cells (1, 2). Using isolated pancreatic acini from rats and mice we have shown that ^{125}I-insulin binds to specific high-affinity receptors on the basolateral cell membrane of acinar cells and is subsequently internalized (3). After binding to its receptor, insulin influences several acinar cell functions including sugar transport, protein synthesis, amylase content, and sensitivity to the secretagogue CCK (4–8).

In addition to acinar cells it is not known what other cells of the pancreas have insulin receptors. The present study was designed to evaluate pancreatic insulin receptors *in vivo* and to answer two questions. The first was whether pancreatic acini, ducts, islets, and blood vessels have saturable insulin binding sites *in vivo*. The second was whether the localization of insulin binding to the basolateral domain of the acinar cell plasma membrane previously seen *in vitro* with isolated acini (3) is also seen *in vivo*.

Materials and Methods. Male Sprague–Dawley rats weighing 80–100 g were fasted overnight and anesthetized with 50 mg/kg sodium pentobarbital administered ip. ^{125}I-insulin (120–150 μCi/μg) was prepared as previously described (9) and rapidly injected iv into the external jugular vein in the absence and presence of 300 μg nonradioactive insulin. For tissue distribution studies, 50 ng of ^{125}I-insulin was used, whereas for the autoradiographs 1 μg of ^{125}I-insulin was used. Two and one-half minutes after injection, the aorta was cannulated through the left ventricle, the atria opened, and within 45 sec the animal was perfused by gravity-induced flow with 20 ml ice-cold 0.9% saline followed by perfusion with cold fixative at a rate of 3.1 ml/min by use of a syringe-driven pump. The fixative consisted of 2.7% glutaraldehyde, 0.8% paraformaldehyde, and 80 m*M* sodium cacodylate, pH 7.5 (3). At this time, the pancreas and other organs were removed, weighed, and tissue radioactivity determined.

For autoradiographic studies, the perfused-fixed tissue was minced with a razor blade and fixation continued overnight in the afore-

TABLE I. *In Vivo* Uptake of ^{125}I-Insulin in Rat Pancreas and Other Organs of the Gastrointestinal Tract

Tissue	Insulin uptake (% injected/g tissue)	
	^{125}I-Insulin	^{125}I-Insulin + unlabeled insulin
Pancreas	4.2 ± 0.3	0.7 ± 0.1
Liver	14.7 ± 1.0	2.2 ± 0.2
Stomach		
Fundus	0.6 ± 0.1	0.6 ± 0.1
Antrum	2.8 ± 0.2	1.4 ± 0.2
Intestine		
Duodenum	2.7 ± 0.3	0.8 ± 0.1
Jejunum	2.5 ± 0.2	0.8 ± 0.1
Ileum	3.7 ± 0.5	1.2 ± 0.2

Note. Rats were injected via the external jugular vein with 50 ng ^{125}I-insulin in the absence and presence of 300 μg insulin. After 2.5 min the aorta was cannulated and perfused with 20 ml ice-cold 0.9% saline followed by cold fixative. All values are the mean ± SE of four or five rats.

0037-9727/84 $1.50

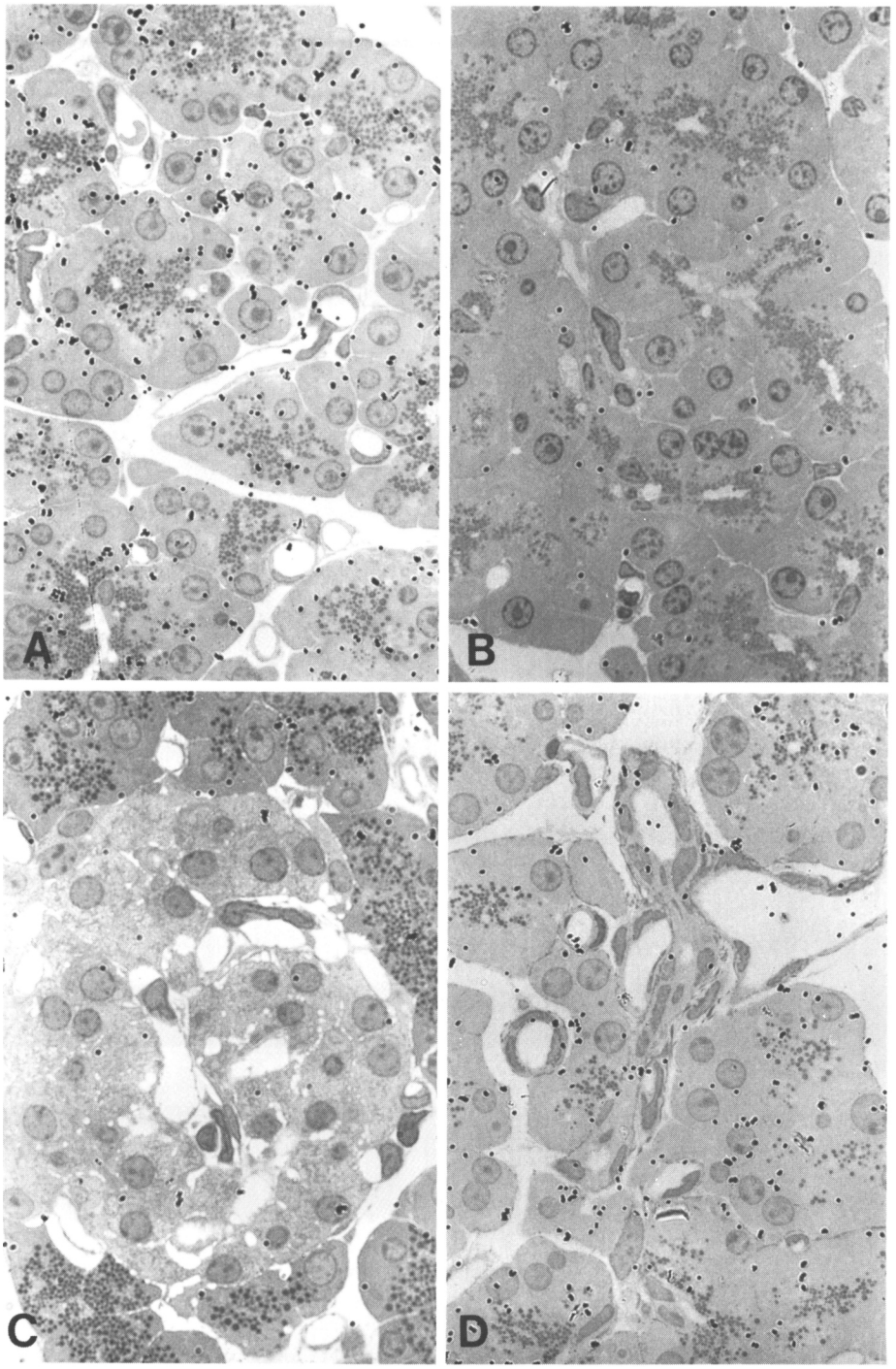

FIG. 1. Light autoradiographs of rat pancreas following the *in vivo* injection of ^{125}I-insulin. (A) Acinar cells, (B) acinar cells with 300 ng nonradioactive insulin, (C) islet of Langerhans, (D) pancreatic duct. ×756.

mentioned fixative. Tissue blocks were post-fixed 90 min in 2% OsO_4 in 80 m*M* sodium cacodylate, pH 7.4, dehydrated, and embedded in Epon 812 resin. Thick sections, 0.5 μm thick, were cut with glass knives and stained with PAS–iron hematoxylin. Sections were then coated with Kodak NTB-3 emulsion diluted 1:1 with water, exposed for 4 weeks at 4°C, and developed with Kodak D-19 developer. Sections were photographed using a Zeiss Photomicroscope II equipped with a 63× planapochromat objective and printed at a final magnification of 882.

For electron microscopy, pale gold thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, coated with carbon and then Ilford L-4 emulsion by the "flat substrate" technique (10). Grids were exposed for 8 weeks at 4°C, developed for 2 min with Kodak D-19 developer, and fixed for 2 min with Kodak rapid fixer without hardener. Sections were photographed with a Zeiss 10C electron microscope at 60 kV using a primary magnification of 5000 and then photographically enlarged 2.5 times.

Grain distribution was quantitated by counting grains over various cell types or compartments giving partial credit when a grain overlay the boundary between two cell types. The same micrographs were then analyzed for relative volume density of each compartment by means of a 1-cm square grid overlay (11). By measuring total print area, the area occupied by each cell type was determined and the grain density obtained by dividing the number of grains over a particular cell type by the area occupied by that cell.

Results. In initial studies, ^{125}I-insulin was injected into rats to demonstrate that saturable ^{125}I-insulin uptake could be observed in the pancreas. Following *in vivo* washing and fixation, 4.2% of the total injected radioactivity was taken up per gram of pancreas, and this value was reduced to 0.7% of total when an excess of nonradioactive insulin was included (Table I). The liver which has been known for several decades to be a major site of insulin uptake *in vivo* (12) took up three times more insulin per gram of tissue, but the ratio of total to nonsaturable uptake (6:1) was similar. Other organs in the GI tract also showed saturable insulin uptake, but both the percentage taken up per gram of tissue, and the ratio of total to nonsaturable uptake were lower. The finding of saturable localization of ^{125}I-insulin in stomach and intestine is similar to the previous report of Bergeron *et al.* (13).

The pancreas from rats injected with ^{125}I-insulin was used to prepare light microscope autoradiographs to localize which cells in the pancreas bound insulin. As shown in Fig. 1 and quantitated in Table II, the highest density of silver grains was observed over acinar cells and blood vessels. When excess nonradioactive insulin was added, the grain density over acinar cells was markedly reduced, but the grain density over blood vessels was not. Thus saturable uptake occurred on acinar cells, but not on blood vessels. Pancreatic duct cells also took up insulin in a saturable manner but to a lesser extent than did acini cells. Silver grains were observed over interlobular and intralobular duct cells as well as centroacinar cells. Pancreatic islets showed low levels of uptake which did not change when excess insulin was present.

Electron micrographs of acini (Fig. 2, upper panel) indicated that the silver grains were predominantly over the basolateral plasma membrane. In contrast, no grains were observed over the luminal plasma membrane. A few grains were greater than 2 μm from the nearest plasma membrane, consistent with internalization of the hormone. Silver grains observed over duct cells (Fig. 2, lower panel)

TABLE II. DENSITY OF INSULIN BINDING DETERMINED BY LIGHT AUTORADIOGRAPHY OVER VARIOUS CELL TYPES IN THE RAT PANCREAS

	Insulin grain density (grains/$\mu m^2 \times 10^{-3}$)	
Cell type	^{125}I-Insulin	^{125}I-Insulin + unlabeled insulin
Acinar	25.1	3.4
Duct	11.3	5.1
Islet	3.8	3.3
Blood vessel	21.5	23.9
Total grains counted	2417	644

Note. The localization of autoradiographic grains and relative area of each compartment were determined on 10 micrographs from each of two rats injected with ^{125}I-insulin alone or ^{125}I-insulin plus 300 μg unlabeled insulin. A second experiment showed a similar distribution of grains between cell types.

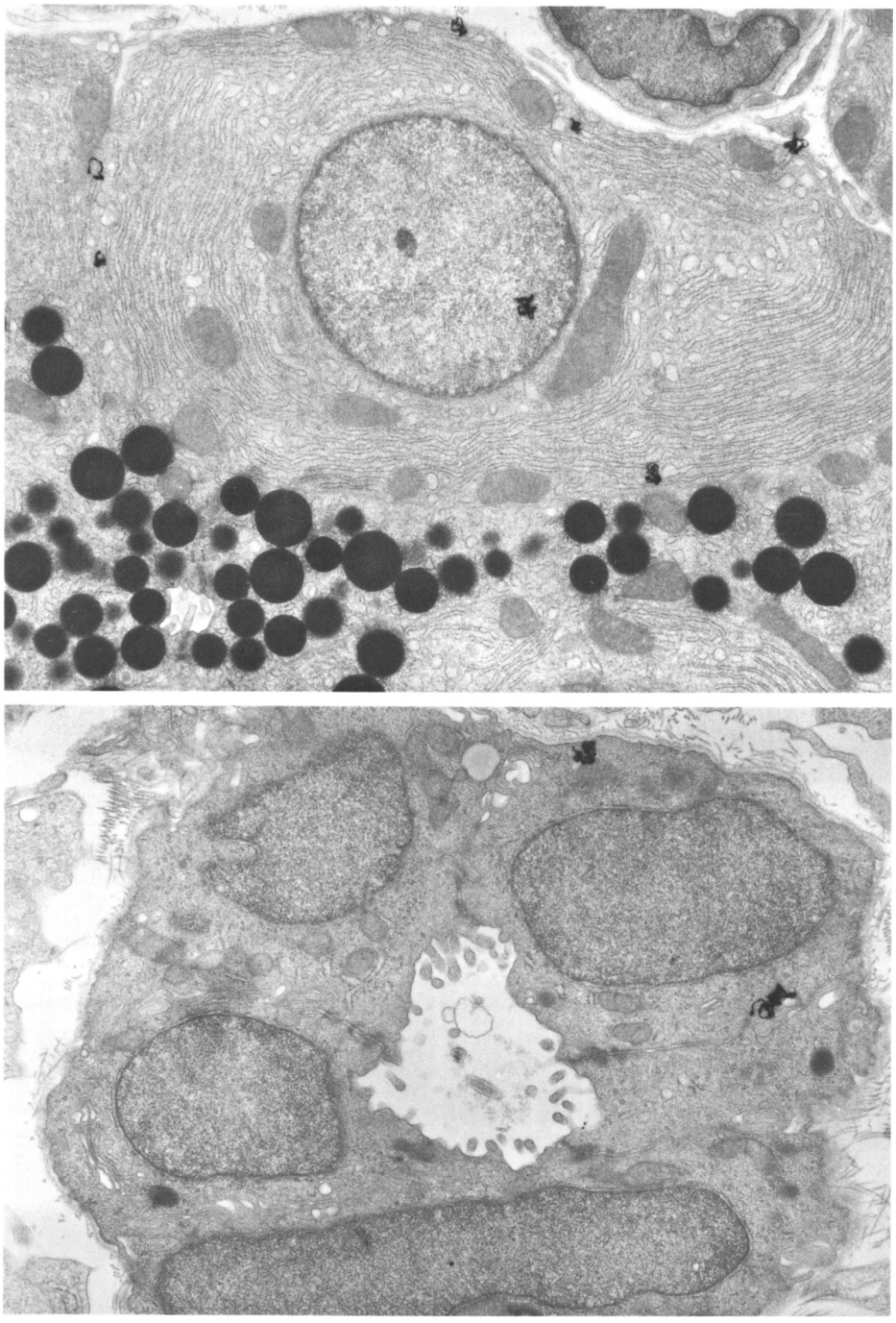

FIG. 2. Electron microscope autoradiographs of rat pancreas injected with ^{125}I-insulin. Upper panel, acinar cells. ×8500. Lower panel, pancreatic duct. ×10,500.

were localized over both the basal and the lateral membranes but not over the luminal membrane.

Discussion. Acinar cell insulin receptors were originally described on isolated pancreatic acini of mice and rats, a preparation

which consists almost entirely of acinar cells (4, 14). An autoradiographic study of acini *in vitro* localized this binding to the basolateral membrane of acinar cells, and also demonstrated that the bound insulin was subsequently internalized (3). The occupancy of pancreatic acinar insulin receptors by insulin correlates with insulin stimulation of both glucose uptake and protein synthesis (5, 8). These studies indicate, therefore, that these insulin receptors have functional significance.

Subsequently, Bergeron *et al.* (15) have confirmed the existence of insulin receptors on the plasma membrane acinar cells by an *in vivo* autoradiographic study, but this group did not quantitate the localization of insulin on other cell types such as islets or ducts. In the present *in vivo* study, we observed saturable uptake of insulin on both the basolateral membranes of acinar cells, and the basolateral membranes of duct cells. In contrast, both the marked uptake of insulin on blood vessels and a low uptake on the islets of Langerhans were not saturable.

To our knowledge there are no known direct effects of insulin on pancreatic ductal function. Grossman and Ivy (16) reported that the diabetes-inducing agent, alloxan, brought about vacuolization of canine duct cells. However, in a recent study in rats, streptozotocin-induced diabetes did not lead to alteration in secretin-stimulated pancreatic juice flow, a presumed ductal function (17). The presence of saturable insulin binding to ductular cells now seen *in vivo* suggests a possible regulation of these cells by insulin. A portal blood system is known to connect the islets and the exocrine portion of the pancreas, and most likely plays a role in the regulation of acinar cell function (6). Insulin released from the islets of Langerhans could, therefore, regulate duct function as well as acinar cell function.

Two previous *in vitro* studies have identified the presence of binding sites for insulin on rat islet tissue. Employing ^{125}I-insulin and isolated islets from rat pancreas, Verspohl and Ammon (18) reported that high-affinity receptors (Kd, 0.5 n*M*) were present on beta cells. With EM autoradiographs of cultured monolayers of pancreas from 3-day-old rats (a preparation consisting primarily of beta cells) Patel *et al.* (19) reported specific uptake of ^{125}I-insulin on alpha, beta, and delta cells. In the present *in vivo* study in rats, we did not observe specific uptake of ^{125}I-insulin on islets. It is possible, therefore, that there may be receptors for insulin on islet cells. However, *in vivo* these receptors are relatively fewer than those on acinar and ductal cells, and thus are not readily detected in the intact pancreas.

In summary, therefore, the present study demonstrates the existence of saturable insulin binding sites on pancreatic acinar cell basolateral membranes. Moreover, these values also demonstrate binding of insulin to ductular epithelia. The data, therefore, provide further support for a functional islet-exocrine relationship in which exocrine pancreatic function is regulated by islet hormones.

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1. Williams JA, Sankaran H, Korc M, Goldfine ID. Receptors for cholecystokinin and insulin in isolated pancreatic acini: Hormonal control of secretion and metabolism. Fed Proc **40**:2497–2502, 1981.
2. Goldfine ID, Williams JA. Receptors for insulin and CCK in the acinar pancreas: relationship to hormone action. Int Rev Cytol **85**:1–38, 1983.
3. Goldfine ID, Kriz BM, Wong KY, Hradek G, Jones AL, Williams JA. Insulin action in pancreatic acini from streptozotocin-treated rats. III. Electron microscope autoradiography of ^{125}I-insulin. Amer J Physiol **240**:G69–G75, 1981.
4. Korc M, Sankaran H, Wong KY, Williams JA, Goldfine ID. Insulin receptors in isolated mouse pancreatic acini. Biochem Biophys Res Commun **84**:293–299, 1978.
5. Korc M, Iwamoto Y, Sankaran H, Williams JA, Goldfine ID. Insulin action in pancreatic acini from streptozotocin-treated rats. I. Stimulation of protein synthesis. Amer J Physiol **240**:G56–G62, 1981.
6. Saito A, Williams JA, Kanno T. Potentiation of cholecystokinin-induced exocrine secretion by both exogenous and endogenous insulin in isolated perfused rat pancreata. J Clin Invest **65**:777–782, 1980.
7. Otsuki M, Williams JA. Direct modulation of pancreatic CCK receptors and enzyme secretion by insulin in isolated pancreatic acini from diabetic rats. Diabetes **32**:241–246, 1983.
8. Williams JA, Bailey AC, Preissler M, Goldfine ID. Insulin regulation of sugar transport in isolated pancreatic acini from diabetic mice. Diabetes **31**:674–682, 1982.
9. Goldfine ID, Smith GJ. Binding of insulin to isolated nuclei. Proc Natl Acad Sci USA **73**:1427–1431, 1976.

10. Salpeter MM, Bachman L. Electron microscope autoradiography. In Hyatt MA, ed. Principles and Techniques of Electron Microscopy: Biological Applications. New York, Van Nostrand Reinhold, Vol II:pp 221–278, 1972.
11. Weibel ER. Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol **26**:235–302, 1969.
12. Lee ND. Studies on insulin labeled with ^{131}I. Ann NY Acad Sci **70**:94–108, 1957.
13. Bergeron JJM, Rachubinski R, Searle N, Borts D, Sikstrom R, Posner BI. Polypeptide hormone receptors in vivo: Demonstration of insulin binding to adrenal gland and gastrointestinal epithelium by quantitative radioautography. J Histochem Cytochem **28**:824–835, 1980.
14. Sankaran H, Iwamoto Y, Korc M, Williams JA, Goldfine ID. Insulin action in pancreatic acini in streptozotocin-treated rats. II. Binding of ^{125}I-insulin to receptors. Amer J Physiol **240**:G63–G68, 1981.
15. Bergeron JJ, Rachubinski R, Searle N, Sikstrom R, Borts D, Bastian P, Posner BI. Radioautographic visualization of in vivo insulin binding to the exocrine pancreas. Endocrinology **107**:1069–1080, 1980.
16. Grossman MI, Ivy AC. Effect of alloxan upon external secretion of the pancreas. Proc Soc Exp Biol Med **63**:62–63, 1946.
17. Sofranková A, Dockray GJ. Cholecystokinin- and secretin-induced pancreatic secretion in normal and diabetic rats. Amer J Physiol **244**:G370–G374, 1983.
18. Verspohl EJ, Ammon HPT. Evidence for presence of insulin receptors in rat islets of Langerhans. J Clin Invest **65**:1230–1237, 1980.
19. Patel YC, Amherdt M, Orci L. Quantitative electron microscope autoradiography of insulin, glucagon, and somatostatin binding sites on islets. Science **217**:1155–1156, 1982.

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