QUANTITATIVE RADIOAUTOGRAPHIC STUDY ON ^3H -LEUCINE UPTAKE OF PERI- AND TELE-INSULAR ACINAR CELLS OF THE PANCREAS IN NORMAL AND STREPTOZOTOCIN-DIABETIC MICE

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The uptake of ^3H -leucine by peri- and tele-insular acinar cells of the pancreas was analyzed quantitatively 60 min after the intraperitoneal injection of ^3H -leucine in normal and streptozotocin-diabetic mice. A large tissue sample was analyzed and the grain density was counted with a color image analyzer equipped with a computer. The radioactivity was expressed as number of silver grains per acinar cell as well as per unit area of acinar tissue. In normal mice, the radioactivity in peri-insular acinar cells was 43% greater than in tele-insular acinar cells. The intercalated ducts associated with the former also showed a larger number of silver grains. In streptozotocin-diabetic mice, the uptake of ^3H -leucine in peri- and tele-insular acinar cells was almost similar. The estimate of mean acinar cell profile area was 30% larger in the peri-insular acini of normal mice, while no difference was observed between the estimates of peri- and tele-insular acinar cells of diabetic mice.

These results confirm previous reports that the B cell selection plays a certain role in the protein synthesis of pancreatic acinar cells, especially for the cells of peri-insular acini.

The dispersed distribution of the endocrine tissue in the exocrine pancreas has raised the possibility of physiological interaction between these two entities. Since the observation of the halo phenomenon around the islets of Langerhans (11), the concept of insulo-acinar interaction has drawn the attention of many researchers. Morphological differences have been observed between the acinar cells surrounding the islets of Langerhans (peri-insular acini) and those located at a distance from the islets (tele-insular acini): the peri-insular acinar cells are larger and contain more zymogen granules (3, 9, 15). These findings have usually been considered to be due to locally high insulin levels, since these morphological differences are more prominent in obese, hyperinsulinemic mice and disappear after treatment with a B-cell toxin, alloxan (9, 15). Previous quantitative radioautographic and biochemical studies demonstrated an increased rate of incorporation of radioactive amino acids in rat

pancreatic acinar cells after insulin injection (14) or after continuous glucose infusion (2). Isolated pancreatic acini from streptozotocin-induced diabetic rats incorporated more radioactive amino acids into secretory proteins when treated with insulin (12, 20).

As most of previous studies (13, 15) have been conducted a short time (10 min) after the introduction of radioactivity, the aim of the present study was to examine the activity of protein synthesis in peri- and tele-insular acinar cells, 60 min after intraperitoneal injection of ^3H -leucine. The uptake of ^3H -leucine in peri- and tele-insular acinar cells was analyzed in normal and streptozotocin-diabetic mice by means of light microscope quantitative radioautography combined with a computerized image analysis.

MATERIALS AND METHODS

Male ICR mice of 5 weeks old (30 g body weight) kept on normal food pellets and water were used in the study. Experimental diabetes was induced in a group of overnight fasted mice with streptozotocin (60 mg/kg body weight, Sigma Chemical, Co., St. Louis, Mo.,

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U.S.A.) in 0.05 M citrate buffer (pH 4.5) via the tail vein. Another group of mice received 0.05 M citrate buffer (pH 4.5) via the tail vein were used as controls. Glucosuria was checked by Tes-Tape (Eli Lilly and Co., Indianapolis, Ind., U.S.A.). The body weight and glucosuria were recorded before and every other day after the injection. Criteria in the establishment of experimental diabetes were failure of gaining body weight, glucosuria and high blood glucose level.

Three weeks after the injection, controls and streptozotocin-diabetic mice were fasted overnight with free access to water. Next morning, each mouse received 4 μ Ci/g body weight of L-leucine-3,4,5- 3 H (specific activity 155 mCi/mM, Sigma Chemical, Co., St. Louis, Mo., U.S.A.) intraperitoneally and was fed for 60 min. Under Nembutal anesthesia, a blood sample was taken from the portal vein, and the mouse was perfused with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) through the left ventricle of the heart. The pancreas was removed, sliced into

small pieces without considering pancreatic regions and left in the same fixative for overnight at 4°C. Islet-containing tissues were dissected with a dissecting microscope and were then briefly washed in 0.1 M sodium cacodylate buffer (pH 7.3). They were postfixed with 1% osmium tetroxide in the same buffer for 2 hr at 4°C. Tissues from each animal were dehydrated in a graded ethanol series and embedded in Araldite.

Using the blood samples taken at the time of sacrifice, blood glucose levels were measured by the glucose-oxidase method using a YSI model 23A glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.). Four control mice confirmed to be normoglycemic and four streptozotocin-injected mice fitted to the diabetic criteria were used for the quantitative study.

Five blocks per mouse were selected at random, and semithin sections (1 μ m-thick) were cut and stained with 1% toluidine blue and examined for the

FIGS. 1a-d. Light micrographs illustrating the steps in quantitating the silver grains with the computerized image analyzer. a. Conventional picture. b. The acinar area of selected field is outlined, and the acinar lumen, centroacinar cells and interacinar tissue are excluded. The number of nuclei was counted. c. The area of the selected field was measured. d. The number of silver grains (shown in white spots) was determined. $\times 680$

presence of islets. Sections containing a single islet were used in the radioautographic study. From each block, three clean glass slides with serial semithin sections were prepared (3–4 sections on each slide). The glass slides were coated with a thin homogeneous bubble-free layer of Konica NR-M2 emulsion (Konica Corporation, Tokyo, Japan) by the dipping method in a dark room, stored in dust-free dark boxes containing silica gel and kept in complete darkness at 4°C. After 4 weeks of exposure, they were developed in D-19 developer for 4 min at 20°C, stained with 0.1% toluidine blue for 10 sec at 45°C and mounted with coverslips. In the quantitative study only one slide per block, technically the best, was selected.

Quantitative measurement was done with a computerized color image analyzer (Model CIA 102, Olympus Co., Tokyo, Japan) fitted with a light microscope at $\times 1000$ -fold magnification. In each slide, three fields of acini in direct contact with an islet of Langerhans were selected (peri-insular acini). The boundary of the acinar area was drawn in each field ex-

cluding the acinar lumen, centroacinar cells and intercellular space, and the acinar area and the number of nuclei were determined (Fig. 1). The number of silver grains was dot-blotted and was expressed per both acinar cell and mm^2 area of acinar tissue. Silver grains on the interacinar space were also counted in each section and used as the background. The tele-insular acini were selected from a distance at furthest from the islet and analyzed by the same procedure. Total fields analyzed for peri- or tele-insular acini in each of the normal and diabetic group amounted to 60. The silver grain densities over the intercalated ducts (number of silver grains per duct) associated with peri- and tele-insular acini were also quantitated in both normal and streptozotocin-diabetic mice.

The cell profile area was calculated from dividing the measured acinar area in the field by the number of nuclei.

Data from peri-insular or tele-insular region were processed separately in each animal and then averaged to produce overall estimates (mean $\pm$ standard error)

FIGS. 2a–c. A low magnification picture of normal mouse pancreas (a) showing an islet (arrow) and an example of peri- and tele-insular acini (rectangular areas P and T) which is shown at a higher magnification in b and c, respectively. The grains are mainly seen over the supranuclear region, but some grains are also present in the basal part of the acinar cell. The number of grains in the peri-insular acinar cells (b) looks more numerous than in the tele-insular acinar cells (c).

a. $\times 400$, b, c. $\times 1,200$

of each region in normal or diabetic group. Statistical treatment of data was done with Student's '*t*' test.

RESULTS

In the streptozotocin-treated mice, a significant glucosuria was noticed 24 hr after the injection of the drug and persisted throughout the experiment, while the control mice never showed glucosuria. Blood samples taken from the portal vein at the time of sacrifice showed higher glucose levels in the streptozotocin-treated mice (517 ± 33.7 mg/dl versus 93 ± 6.4 mg/dl for the control mice, $p < 0.001$). In the control mice a significant gain in body weight was noticed within a 3 week period of follow-up (29.8 ± 0.09 g initial body weight versus 35 ± 0.22 g body weight after 3 weeks, $p < 0.001$), while a small but significant loss in body weight was noticed in the diabetic mice (29.9 ± 0.08 g initial body weight versus 28 ± 0.54 g weight 3 weeks after streptozotocin injection, $p < 0.025$).

Light microscope examination of the pancreatic

acinar tissue showed that most of the silver grains were confined to the supranuclear region of the cell, mainly over the zymogen granules (Figs. 2, 3). A lower density of grains was also detected in the basal part of the cell (ergastoplasm), and very occasionally, a few grains were present over the nuclei. The radioactive labels were also seen in acinar lumina and intercalated ducts. The background density in all sections examined was minimum.

In normal mice, the density of silver grains in the peri-insular acinar cells was apparently greater than that observed in the tele-insular acinar cells (Fig. 2). However, in streptozotocin-diabetic mice, both peri- and tele-insular acinar cells exhibited similar patterns of radiolabelings (Fig. 3).

To confirm these histological observations, we quantitated the number of silver grains in the peri- and tele-insular acini and their associated intercalated ducts (Tables 1 and 2). In the normal mice, the number of silver grains per acinar cell was approximately 43% greater in the peri-insular acini (18.87 ± 1.17 versus 10.98 ± 0.47 for tele-insular acini,

FIGS. 3a-c. A low magnification picture of streptozotocin-diabetic pancreas (a) showing an islet (arrow) and peri- and tele-insular acini (rectangular areas P and T) which are shown at a higher magnification in b and c, respectively. The density of silver grains looks similar in peri-insular (b) and tele-insular acini (c).

a. $\times 400$, b, c. $\times 1,200$

TABLE 1. *Number of silver grains in peri- and tele-insular acinar cells of normal mice*

	Mice	Number of silver grains/cell	Number of silver grains/mm ² acinar area	Number of silver grains/mm ² background area	Cell profile area (μm ²)
Peri-insular acini	(1)	18.98 ± 1.65 ^e	50.43 ± 2.55 ^c	0.97 ± 0.19	371 ± 21.0 ^d
	(2)	15.73 ± 0.70 ^e	44.23 ± 1.48 ^a	0.71 ± 0.09	357 ± 14.4 ^e
	(3)	22.31 ± 1.97 ^e	47.58 ± 2.58 ^b	0.59 ± 0.08	462 ± 20.3 ^e
	(4)	18.47 ± 0.79 ^e	41.16 ± 0.99 ^d	0.64 ± 0.06	449 ± 16.2 ^e
	Mean (n=4)	18.87 ± 1.17 ^e	45.85 ± 1.74 ^d	0.73 ± 0.07	410 ± 23.1 ^e
Tele-insular acini	(1)	10.86 ± 0.91 ^e	41.21 ± 1.73 ^c	0.97 ± 0.19	265 ± 20.5 ^d
	(2)	10.37 ± 0.74 ^e	39.80 ± 1.98 ^a	0.71 ± 0.09	262 ± 14.7 ^e
	(3)	12.53 ± 0.59 ^e	39.15 ± 1.65 ^b	0.59 ± 0.08	320 ± 6.60 ^e
	(4)	10.15 ± 0.47 ^e	35.39 ± 1.23 ^d	0.64 ± 0.06	289 ± 12.4 ^e
	Mean (n=4)	10.98 ± 0.47 ^e	38.89 ± 1.08 ^d	0.73 ± 0.07	284 ± 11.7 ^e
% Difference between means		43%	15%	—	30%

Data are presented (mean ± standard error) separately for each animal (n = 15 fields) and for the whole group of animals (n = 4). The estimates are statistically different between peri- and tele-insular acini at p < 0.1^a, p < 0.025^b, p < 0.01^c, p < 0.005^d, and p < 0.001^e, respectively.

TABLE 2. *Number of silver grains in peri- and tele-insular acinar cells in streptozotocin-diabetic mice*

	Mice	Number of silver grains/cell	Number of silver grains/mm ² acinar area	Number of silver grains/mm ² background area	Cell profile area (μm ²)
Peri-insular acini	(1)	16.04 ± 1.11	41.53 ± 2.44	0.69 ± 0.06	390 ± 35.7
	(2)	19.60 ± 2.46	41.85 ± 2.57	0.88 ± 0.13	449 ± 34.4
	(3)	16.73 ± 1.04	35.75 ± 2.07	0.94 ± 0.12	480 ± 30.6
	(4)	11.33 ± 1.02	27.52 ± 1.71	0.84 ± 0.05	419 ± 33.4
	Mean (n=4)	15.93 ± 1.48	36.66 ± 2.90	0.84 ± 0.05	434.5 ± 16.8
Tele-insular acini	(1)	14.85 ± 1.23	46.08 ± 2.84	0.69 ± 0.06	322 ± 16.8
	(2)	15.80 ± 0.92	39.16 ± 1.58	0.88 ± 0.13	412 ± 25.1
	(3)	16.45 ± 1.37	37.72 ± 2.09	0.94 ± 0.12	438 ± 33.5
	(4)	10.97 ± 0.62	28.40 ± 1.23	0.84 ± 0.05	395 ± 25.4
	Mean (n=4)	14.52 ± 1.06	37.84 ± 3.15	0.84 ± 0.05	392 ± 21.5

Data are presented (mean ± standard error) separately for each animal (n = 15 fields) and for the whole group of animals (n = 4). Statistically, no differences were observed between the estimates of the peri- and tele-insular areas.

p < 0.001), and the number of grains per mm² area of acinar tissue was 15% higher in the peri-insular acini (45.85 ± 1.74 versus 38.89 ± 1.08 for tele-insular acini, p < 0.005) (Table 1). In the streptozotocin-diabetic mice, the estimates of silver grains were similar in both peri- and tele-insular acini (15.93 ± 1.48 grains per peri-insular acinar cell versus 14.52 ± 1.06 grains per tele-insular acinar cell; and 36.66 ± 2.90 grains per mm² peri-insular area versus 37.84 ± 3.15 grains per

mm² tele-insular area) (Table 2).

In the normal mice, the mean number of silver grains per intercalated duct in the peri-insular area was higher than that of the tele-insular area (13.62 ± 0.29 grains versus 7.27 ± 0.30 grains, p < 0.001). In the diabetic mice, on the other hand, the mean number of radiolabelings in the intercalated duct was almost equal in both peri- and tele-insular areas (11.50 ± 0.95 grains versus 9.70 ± 0.36 grains).

In normal mice, the mean acinar cell profile area was 30% larger in the peri-insular acini ($410 \pm 23.10 \mu\text{m}^2$ versus $284 \pm 11.70 \mu\text{m}^2$ for tele-insular acini, $p < 0.001$) (Table 1). In streptozotocin-diabetic mice, no difference was recorded in the mean cell profile area estimates between peri- and tele-insular acini ($434.50 \pm 16.80 \mu\text{m}^2$ versus $392 \pm 21.50 \mu\text{m}^2$) (Table 2).

DISCUSSION

The phenomenon of zymogen haloes around the islets of Langerhans has been well documented in the pancreas (9, 15, 22): the cells of peri-insular acini being larger in size with bigger load of zymogen granules than the cells of tele-insular acini. In our previous morphometric study on rats (3), we confirmed that peri-insular acinar cells of normal rats were approximately 20% larger in size and their zymogen granule content was twice as much as in the tele-insular acini. In the streptozotocin-induced diabetic rats, no haloes were observed histologically, and no differences were recorded quantitatively between peri- and tele-insular acinar cells. We concluded that these morphological differences in the pancreatic acinar cells were caused by B cell secretion.

The synthesis, segregation and transport of exportable proteins have been widely explored in the pancreatic acinar cells (5, 10): the radiolabels are mainly localized in the basal part of the cell 10 min after the administration of radioactive amino acids, transported to the Golgi complex within 30 min, and over the zymogen granules by 60 min with some labels seen in the acinar lumen.

Kramer and Tan (15), and Kramer and Poort (13) studied the incorporation of ^3H -leucine in pancreatic peri- and tele-insular acinar cells of normal and pilocarpine-stimulated rats 10 min after its intravenous injection: paraffin sections were coated by the stripping method, the grain density was measured from a relatively small tissue sample, and a higher uptake of ^3H -leucine was recorded in the peri-insular acini. These authors (13, 15) have related their findings to the influence of high insulin levels on the larger amount of proteins synthesized in the peri-insular acini. However, by their study, the possibility that the increased incorporation of ^3H -leucine in peri-insular acini caused by faster protein synthesis in these acini is not ruled out. To explore further, the present study examined the uptake of ^3H -leucine in the peri- and tele-insular acinar cells by quantitative radioautography in normal and streptozotocin-diabetic mice 60 min after an intraperitoneal injection

of ^3H -leucine. The radiolabels were mostly localized over the zymogen granules, and some radioactivity was also detected in the intercalated ducts, as the animals were sacrificed after feeding. By quantitating the silver grains in the peri- and tele-insular acini as well as in their associated intercalated ducts, we had further information on the activity of protein synthesis in these acini during the experiment. In the normal mice, peri-insular acinar cells were more active in ^3H -leucine incorporation than tele-insular cells, and their associated intercalated ducts had more silver grains. Streptozotocin treatment resulted in an even uptake of ^3H -leucine by peri- and tele-insular acinar cells with equal density of silver grains in their associated intercalated ducts. Based on these results, we have agreed with the previous authors (13, 15) that the cells of peri-insular acini are more active in protein synthesis than the cells of tele-insular acini, due to exposure of the former cells to high concentrations of insulin, which are caused by the blood flow directly from the islet capillaries toward peri-insular acini (4, 16-19).

Adler and Kern (2) elevated the circulating endogenous insulin levels of rats either by continuous glucose infusion or by the application of a potent anti-diabetic drug, glibenclamide. An increased rate of protein synthesis was biochemically and quantitatively detected after pancreatic lobules were removed and incubated with ^3H -leucine. Increased incorporation of radioactive amino acids into the secretory proteins also was demonstrated in the incubated pancreatic fragments obtained from the rats previously received subcutaneous injections of insulin (14). Insulin increased the incorporation of ^{35}S -methionine into total cellular proteins, as well as into digestive enzymes such as amylase, trypsinogen, ribonuclease and lactate dehydrogenase, in isolated pancreatic acini from streptozotocin-diabetic rats (12). Other studies (7, 24) have illustrated the specific binding of insulin to receptors on the pancreatic acinar cell membrane. In addition, an anabolic effect of insulin on protein synthesis has also been demonstrated in several other tissues including adipose tissue, liver and brain (8, 21, 23).

Similarly to our previous morphometric observation on rats (3), the present study also showed that the mean acinar cellular area of peri-insular acini was 30% larger than tele-insular acini in normal mice. This can be explained by their different amount of stored secretory proteins and partly by the different amount of other cellular organelles. On the other hand, no such difference was recorded in the diabetic mice.

All of previous reports together with the present study focus on the stimulant effects of insulin on the

protein synthesis in pancreatic acinar cells. However, other hormones and peptides, including glucagon, somatostatin and pancreatic polypeptide are also secreted by islet cells which may have certain roles in regulating pancreatic exocrine function. Studies on the effect of glucagon, somatostatin and pancreatic polypeptide have shown an inhibitory regulation of the pancreatic exocrine function (1, 6, 25), although this regulation is not so well established, and little is known concerning the site of action of these hormones.

In conclusion, our present results indicate that pancreatic peri-insular acinar cells are larger in size and more active in protein synthesis than the tele-insular acinar cells, probably due to the influence of insulin.

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