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The Blood Flow to the Islets of Langerhans in Different Regions of the Rat Pancreas (42572)

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Abstract. Pancreatic blood flow (PBF) and islet blood flow (IBF) have been determined separately in two different regions of the rat pancreas, namely, that perfused by the superior mesenteric artery (SMA) and that perfused by the coeliac artery (CA). Although there were no differences in either the PBF or the IBF between these two regions, the IBF, when expressed as a percentage of PBF, was significantly higher in the region perfused by the SMA. It is concluded that glucose regulation of the blood flow to the islets and exocrine parenchyma is similar in the two regions perfused by SMA and CA. In the SMA region the percentage of blood supply to the islets, in relation to the acinar part, is, however, somewhat higher than in that of the CA region.

Depending on their embryologic origin, the cellular composition of the pancreatic islets varies so that islets originating in the ventral pancreatic bud contain a higher proportion of PP-cells and a lower proportion of A-cells than those originating in the dorsal bud (1, 2). The islets rich in PP-cells are furthermore located entirely in that part of the pancreas perfused by the superior mesenteric artery (SMA), while A-cell-rich islets predominate in the part of the gland perfused by the coeliac artery (CA) (3).

Previous studies have suggested that in the PP-cell-rich islets insulin release and insulin biosynthesis have an attenuated response to glucose (4, 5). Differences in hormone release have also been observed between islets from the SMA-perfused parts of the pancreas and from the CA-perfused parts (3). The redox states of cytochromes *in vivo,* in both the endocrine and exocrine parts of the gland, are also different between the SMA- and CA-perfused parts of the pancreas (6). These studies suggest that the anatomic location of an islet may influence its cellular composition and function. The present study was an attempt to evaluate to what extent the structure and function of islets in different pancreatic parts relate to differences in the rate of blood perfusion through these parts.

Materials and Methods. A total of 14 male Sprague–Dawley rats, weighing approximately 350 g and with free access to tap water and pelleted food (Ewos Type R3; Ewos, Anticimex, Södertälje, Sweden) were used in the experiments. The animals were anesthetized with an ip injection of thiobutabarbital sodium (Inactin Byk; Byk Gulden, Konstanz, FRG) and heparinized (heparin 5000 IU/ml; Lövens Läkemedel AB, Malmö, Sweden; 200 IU iv). Polyethylene catheters (i.d. approximately 0.30 mm) were inserted into the left ventricle of the heart and into the lower part of the abdominal aorta. After the introduction of the catheters the mean arterial blood pressure and body temperature were monitored throughout the experiments.

The animals were then divided into two groups, seven animals in each; one of the groups received an iv injection of 1 ml of a 30% (w/v) solution of D-glucose and the other 1 ml of 0.9% (w/v) sodium chloride. Five minutes after injection whole pancreatic blood flow and islet blood flow were measured with the aid of the microsphere technique, as previously described in detail (7). Briefly, 1–1.5 $\times$ 10^5 nonradioactive microspheres (New England Nuclear Corp., Boston, MA) with a diameter of 10.2 $\pm$ 0.6 μm (mean $\pm$ SD) were injected via the intracardiac catheter. Starting 5 sec before this injection, and continuing for 60 sec, an arterial reference sample was withdrawn into a preweighed tube at a rate of approximately 0.60 ml/min. The samples were weighed to confirm the withdrawal rate and then stored at +4°C to await further processing (see below). Arterial blood samples were obtained immediately after the injection of the microspheres and later analyzed for glucose content by a glucose–oxidase method (Glucose

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Analyzer 2; Beckman Instruments, Fullerton, CA) and for insulin content by radioimmunoassay (8).

The abdominal cavity was opened using a V-shaped (point downward) incision. The intestines were moved to the right side of the animal to expose the abdominal aorta and vena cava, which were gently rubbed with dry gauze to remove connective tissue, and to expose the origin of the coeliac artery and the superior mesenteric artery. A ligature was placed around the coeliac artery and tightened. Immediately 1 ml of a 2% (w/v) solution of neutral red (Kebo Grave AB, Stockholm, Sweden), dissolved in physiologic saline, was injected directly into the vena cava. The pancreas and the adrenal glands were removed 5 min later and the pancreas was carefully divided into one portion stained with neutral red and one nonstained, corresponding to the different arterial blood supplies. The organs were weighed and further processed by a freezing technique to visualize both pancreatic islets and microspheres (9). The number of microspheres in the reference sample was determined by transferring the sample to glass microfiber filters and counting them in transmitted light.

The blood flow values could then be calculated according to the formula

$$Q_{org} = \frac{N_{org} \times Q_{ref}}{N_{ref}},$$

where Q_{org} = organ blood flow (ml/min), Q_{ref} = withdrawal rate of reference sample (ml/min), N_{org} = number of microspheres in the organ, and N_{ref} = number of microspheres in the reference sample.

The adrenals were used as a control organ to confirm adequate mixing of the microspheres and when the flow values differed by more than 10% between the two adrenal glands the animals were excluded from the study.

Statistical computations. All values are given as means ± SEM. Probabilities (P) of statistically significant differences between experimental groups were determined with Student's t test.

Results. Neutral red produced a distinct red color in those parts of the pancreas perfused by the SMA. This region was delineated by a thin marginal zone with pinkish appearance, which made it possible to estimate that the SMA perfused approximately one-third of the pancreas (503 ± 31 mg; n = 14), while the coeliac artery perfused two thirds (1021 ± 79 mg; n = 14) of the gland.

Application of the freeze–thawing technique to visualize the islets amplified the difference between the neutral red-stained and the unstained pancreatic parts, in that the larger islets (>50 μm) in the SMA-perfused part were stained intensely red (Fig. 1).

Glucose significantly increased the serum insulin concentrations and both the PBF and the IBF values of the intact pancreas were also increased (Table I). When the effects of glucose on the PBF and IBF in the SMA- and the CA-perfused regions were compared the only difference was that IBF, expressed as a fraction of PBF, was increased in the SMA-perfused part of the gland, while the weight-corrected values for IBF and PBF were similar (Table II). The blood flow values of the adrenal glands were similar (0.12 ± 0.01 and 0.13 ± 0.02 ml/min for the right and left gland, respectively)

TABLE I. SERUM GLUCOSE AND SERUM INSULIN CONCENTRATIONS, AND WHOLE PANCREATIC BLOOD FLOW AND ISLET BLOOD FLOW 5 MIN AFTER AN IV INJECTION OF 1 ml OF 0.9% SALINE OR 30% GLUCOSE

	Control animals (7)	Glucose-injected animals (7)
Serum glucose concentration (mmole/l)	10.7 ± 1.0	26.9 ± 0.8**
Serum insulin concentration (ng/ml)	2.06 ± 0.36	6.16 ± 0.58**
Pancreatic blood flow (ml/min × g pancreas)	0.47 ± 0.06	0.76 ± 0.11*
Islet blood flow (μl/min × g pancreas)	55 ± 6	160 ± 25**
Islet blood flow (% of pancreatic blood flow)	11.8 ± 0.9	22.1 ± 2.8**

Note. All values are given as means ± SEM. The number of experiments in each group is given within parentheses.
* $P < 0.01$ compared to the control animals.
** $P < 0.001$ compared to the control animals determined by Student's unpaired t test.

TABLE II. WHOLE PANCREATIC BLOOD FLOW AND ISLET BLOOD FLOW IN THE REGIONS SUPPLIED BY THE SUPERIOR MESENTERIC ARTERY AND COELIAC ARTERY 5 MIN AFTER AN iv INJECTION OF 1 ml OF 0.9% SALINE OR 30% GLUCOSE

	Coeliac artery		Superior mesenteric artery	
	Control (7)	Glucose (7)	Control (7)	Glucose (7)
Pancreatic blood flow (ml/min × g pancreas)	0.48 ± 0.06	0.79 ± 0.14§	0.45 ± 0.07	0.72 ± 0.10§
Islet blood flow (μl/min × g pancreas)	52 ± 6	158 ± 29§§	61 ± 8	172 ± 22§§
Islet blood flow (% of pancreatic blood flow)	10.9 ± 0.7	20.0 ± 0.7§§	13.9 ± 1.1*	24.3 ± 1.6*,§§

Note. All values are given as means ± SEM. The number of experiments in each group is given within parentheses.
* $P < 0.05$ compared to the corresponding value in the part perfused by the coeliac artery determined by a paired t test.
§ $P < 0.01$ compared to control animals.
§§ $P < 0.001$ compared to control animals.

and thereby indicated an even mixing of the microspheres.

Discussion. The present technique with neutral red injection combined with closure of one of the pancreatic arteries provides a simple and reliable means of separating the pancreatic regions perfused by the SMA and CA. Indeed, the weight of each of these anatomic parts is in good accordance with previously published results (3). The values for IBF and PBF in the present study correspond well with our previous findings (10). The previous finding of similar blood flow values in three arbitrarily chosen parts of the pancreas (10) nevertheless contrasts to the present finding of a significantly higher fractional islet

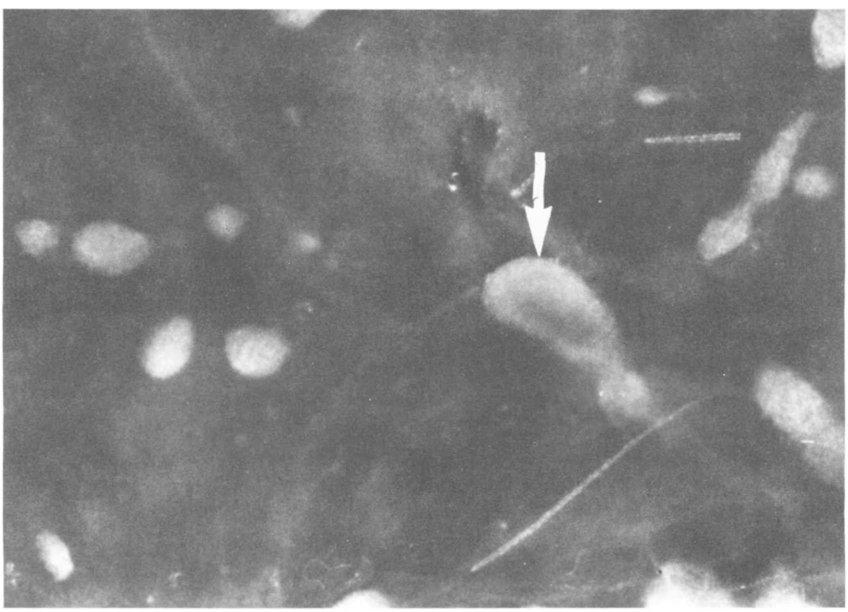

FIG. 1. Dark-field micrograph of a piece of the pancreas which contains a large islet stained with neutral red (arrow) and nonstained islets (right part of the picture). Magnification 160×.

blood flow (fIBF) in the SMA-perfused region of the pancreas. The reason for this discrepancy is probably that the subdivision of the gland in the former study failed to account for the functional and morphologic differences in the islet organ referred to above. The reason for this higher fIBF in the SMA region is unknown, but it is worthy of note that this pancreatic region also differs with respect to the islet cellular composition from the region perfused by the CA. Both fIBF, whole IBF, and whole pancreatic blood flow (PBF) were significantly stimulated by glucose, which is in good accordance with previous results (10, 11). When the SMA- and CA-perfused regions of the pancreas were compared it was found that the flow values increased in a similar fashion, with the retention of the higher fIBF in the SMA-irrigated part after glucose administration. This may seem surprising in view of the higher metabolic activity of the glucagon-rich islets perfused predominantly by the CA (4). It has recently been shown, however, that the stimulatory action of glucose on IBF originates in the central nervous system (12) and would therefore not be influenced by local, intrapancreatic factors. However, it remains unknown at the present why the SMA-perfused part should be preferentially stimulated. It may be suggested that a higher flow may contribute to the vagally stimulated release of PP observed after a meal (13), since it is known that the IBF is also stimulated via a vagal mechanism (12).

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1. Baetens D, Malaisse-Lagae F, Perrelet A, Orci L. Endocrine pancreas: Three-dimensional reconstruction shows two types of islets of Langerhans. Science **206**: 1323–1325, 1979.
2. Malaisse-Lagae F, Stefan Y, Cox J, Perrelet A, Orci L. Identification of a lobe in the adult human pancreas rich in pancreatic polypeptide. Diabetologia **17**:361–365, 1979.
3. Jarousse C, Jeanrenaud B. Functional differences of the endocrine pancreas due to specific blood supply. Diabetologia **23**:177, 1983.
4. Trimble ER, Halban PA, Wollheim CB, Renold AE. Functional differences between rat islets of ventral and dorsal pancreatic origin. J Clin Invest **69**:405–413, 1982.
5. Trimble ER, Renold AE. Ventral and dorsal areas of rat pancreas: Islet hormone content and secretion. Amer J Physiol **240**:E422–E427, 1981.
6. Kanno T, Matsumoto T, Kawahara J, Kuriyama H. Functional discrimination between the regions perfused with blood flow via the superior mesenteric artery and via the coeliac artery in the rat pancreas. Biomed Res **6**:55–58, 1985.
7. Jansson L, Hellerström C. A simple technique for the visualization of the pancreatic islets and its application to measurements of islet blood flow with the microsphere method. In: Larner J, Pohl SL, Eds. Methods in Diabetes Research. Washington, John Wiley & Sons Inc., Vol. 1:pp333–342, 1984.
8. Heding LG. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. Diabetologia **8**: 260–266, 1972.
9. Jansson L, Hellerström C. A rapid method of visualizing the pancreatic islets for studies of islet capillary blood flow using nonradioactive microspheres. Acta Physiol Scand **113**:371–374, 1981.
10. Jansson L, Hellerström C. Stimulation by glucose of the blood flow to the pancreatic islets of the rat. Diabetologia **25**:45–50, 1983.
11. Jansson L. The blood flow to the pancreas and the islets of Langerhans during an intraperitoneal glucose load in the rat. Diabetes Res **1**:111–114, 1984.
12. Jansson L, Hellerström C. Glucose-induced changes in the pancreatic islet blood flow mediated by central nervous system. Amer J Physiol **251**:E644–E647, 1986.
13. Schwartz TW. Pancreatic polypeptide: A hormone under vagal control. Gastroenterology **85**:1411–1425, 1983.

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