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Washout Kinetics of Blood Cells from the Perfused Pancreas of Normoglycemic and Diabetic Rats

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ABSTRACT

The aim of the present study was to evaluate vascular compartments within the rat pancreas with compartmental analysis of the outflow of blood cells from the perfused gland in situ. The presence of two vascular compartments requiring approximately 15 and 30 min for emptying, was noted in normoglycemic rats. The pancreas from diabetic rats, in which the islet ß-cells had been destroyed by intravenous injection of streptozotocin 1 or 6 weeks earlier, demonstrated the same outflow characteristics. It is therefore likely that these observations reflect the presence of two vascular compartments within the rat pancreas, possibly representing the islet-acinar vasculature and the ductal vasculature.

INTRODUCTION

The scattered distribution of endocrine pancreatic cells as islets of Langerhans has led to the development of a unique vascular system. The organization differs somewhat between mammalian species, but a common denominator is the presence of one or several afferent arterioles in combination with two types of venous drainage (1,2,23,24). The latter consist either of venules, preferentially in the larger islets, either alone or in combination with an insulo-acinar portal system, i.e serially connected capillary systems located in the islets and exocrine pancreas (5,6,7). The presence of a third serially connected capillary system associated with the ducts has been suggested (13). It has also been proposed that the anatomical organization of islet vasculature is of importance for the paracrine interactions between the

endocrine cells (21).

Blood flow measurements with microspheres have shown that the islets, despite constituting only 1-2% of the pancreatic volume and having a capillary volume per unit length only 1.5 times that of the exocrine pancreas (14,22), receive approximately 10% of whole pancreatic blood flow (9,12). One possibility to further functionally analyze the vascular compartments of the pancreas would be to evaluate the washout kinetics of red blood cells from the gland (cf. 11,20). A compartmental analysis of this outflow enables a kinetic interpretation of the vascular compartments within the rat pancreas. To identify a possible compartment corresponding to the islets, washout kinetics were studied in both normal control animals and in animals rendered diabetic 1 or 6 weeks before the experiments by administration of the drug streptozotocin, which selectively destroys the ß-cells (18).

METHODS

Animals: Male Sprague-Dawley rats weighing approximately 350 g were obtained from a local breeding colony (Biomedical Center, Uppsala, Sweden) and had free access to pelleted food and tap water. Some of the animals were injected intravenously with streptozotocin (a kind gift from Dr. A.Y. Chang, Upjohn Company, Kalamazoo, MI, USA) 1 or 6 weeks before the measurements. All these animals had a blood glucose concentration exceeding 20 mM measured with glucose reagent strips (ExacTech®; Baxter Travenol Inc., Deerfield, IL, USA) at the time of perfusion.

Pancreas perfusions: The pancreas was isolated with a modification (8) of the technique of Loubatières-Mariani et al (15). A catheter was inserted into the aorta and the portal vein was immediately tied off to preclude any outflow of blood from the gland. The pancreas preparation was not removed from the animal but was perfused in situ. The perfusion was made at a rate of 1.0 ml/min, with a medium consisting of Krebs-Ringer bicarbonate buffer supplemented with 10 mM Hepes (Sigma Chmeicals Co., St. Louis, MO, USA) and 2% dextran T70 (Pharmacia Fine Chmeicals, Uppsala, Sweden) and 2% bovine serum albumin (Fraction V; Miles, Slough, UK). The medium was heated to 37^0C before being administered to the pancreas. Immediately after starting the perfusion pump the portal vein was cut and the medium was collected in 30-sec fractions during the first 5 min, and then every 60 sec for 55 min.

Counting of erythrocytes: The medium samples from the perfusions were kept in a refrigerator (+4^{0}C) and the number of erythrocytes in the samples was counted within 24 h from the perfusion. Within this time period no significant hemolysis affecting the counting of red blood cells took place. The counting was made by first mixing the erythrocytes evenly in the sample with a whirl mixer, and then transferring the sample to a Bürker-chamber. During the first 5 min the collected samples had to be diluted 10-1000 times. At least 25 areas in 3 different samples from each tube were counted in each perfusate. Since no erythrocytes remained in the medium from 30 min and onwards only one sample was taken at these latter time points.

Statistical computations: The number of erythrocytes was plotted on a logarithmic scale as a function of time. The curve was then resolved into its exponential components with a computerized program according to a previously described theory (19).

RESULTS

A total of 5 pancreata were excluded from the experiments due to technical failures of the perfusion. The outflow of erythrocytes from the perfused glands showed a rapid decrease during the first min, and this period was excluded from the mathematical calculations. In control animals (Figure 1A), animals diabetic for 1 week (Figure 1B) or 6 weeks (Figure 1C) the logaritmic representation of blood cell outflow as a function of time could be separated into two straight lines, corresponding to the exponential components of the original curve. The equations for each of the lines are given in the figure legends. The values after 30 min are not given, since virtually no erythrocytes were present in the perfusate after that time point. The intercept with the abscisse, which represents the time required to empty the compartment, consisted of one rapid (13-15 min) and one slow (27-30 min) phase in all groups of animals. No differences in the time required to empty the compartment were seen between the experimental groups (Figure 1A-C).

DISCUSSION

The present findings of an initial very rapid decrease in number of blood cells in the effluent from the rat pancreas agree with corresponding studies in the spleen (11,20). It is likely that these observations represent the emptying of large venous

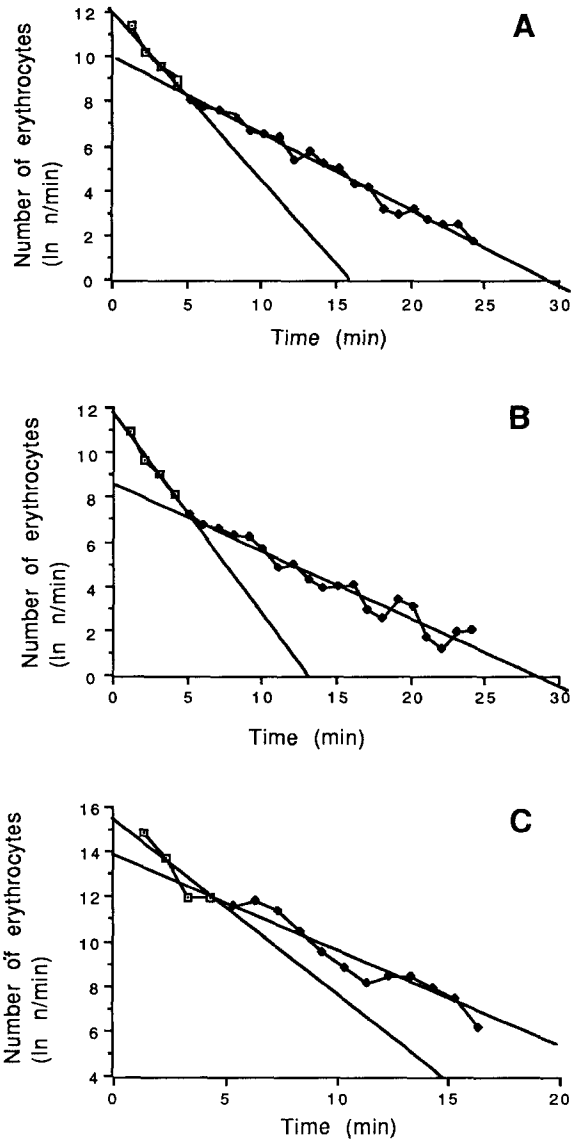

Figure 1: *The number of erythrocytes (n) at different time points in the effluent collected from perfused pancreata of normoglycemic control rats (A; n = 5) or hyperglycemic streptozotocin-treated rats 1 wk (B; n = 5) or 6 wks (C; n = 4) after administration. Note that the number of erythrocytes is given in a logarithmic scale. The equations of the regression lines are* **A**:y = 12.01 - 0.76 x (R = 0.98) which intercepts the ordinate at 15.8 min, and y = 9.91 - 0.34 x (R =0.99) which intercepts the ordinate at 29.2 min; **B**: y = 11.58 - 0.89 x (R = 0.99) which intercepts the ordinate at 12. 9 min, and y = 8.47 - 0.30 x (R = 0.97) which intercepts the ordinate at 27.9 min; **C**: y = 15.54 - 1.05 x (R = 0.95) which intercepts the ordinate at 14.8 min, and y = 13.95 - 0.48 x (R = 0.96) which intercepts the ordinate at 29.2 min.

and arterial blood vessels, and therefore do not represent a vascular compartment per se. When excluding this, two distinct vascular compartments could nevertheless be discerned in the control animals, requiring approximately 15 and 30 min respectively to empty. The possibility that these compartments represented islets and exocrine parenchyma was considered in similar experiments in animals where the ß-cells had been destroyed by streptozotocin (SZ) 1 or 6 weeks earlier. The reason to chose two time points was that the possibility that the vasculature would remain intact 1 wk after administration of SZ could not be excluded. That a degeneration of both the islet cells and their vasculature is complete 6 weeks after multiple injcetions of SZ (16,17) or after single injections of alloxan or SZ (3,4,10) has been previously demonstrated in mice. However, no change in the outflow curves of the blood cells could be seen after SZ administration. The presence of two vascular compartments in the perfused diabetic rat pancreas is therefore likely to represent two vascular compartments in the exocrine pancreas.

The presence of the insulo-acinar portal system, i.e. the serial connections of islet capillaries to a second capillary system in the exocrine pancreas (7) can be invoked to explain why any influence of the islet vasculature is not seen in the present experiments. If the first capillary system, i.e. that in the islets, degenerates in response to the islet destruction caused by SZ, the second acinar system is likely to remain intact. This would leave the outflow characteristics unaltered or only marginally affected. Of crucial importance in this context is to what extent such a portal system is present within the rat pancreas. According to several studies the larger islets in rats have mainly a direct venous outflow, without any intervening capillary system in the exocrine pancreas, whereas the smaller islets have a portal system (2,23). It is therefore likely that when the larger islets, which receive the major part of the islet blood flow (14), degenerate the majority of the previous islet blood flow empties directly into the veins or is diverted into exocrine blood capillaries. The presently used technique measures the capillary volumes within the different vascular compartments of the rat pancreas. Although the islet blood flow is approximately 10% of whole pancreatic blood flow (9,12), the capillary volume per unit length in the islets is only 1.5 times that of the exocrine microvessels (22), i.e approximately 2-3% of the whole pancreatic capillary volume. It may therefore be that a reduction of islet capillary volume caused by the SZ-induced destruction of islets is too small to be detected with the presently used technique. The most

probable explanation for the presence of two blood flow compartments within the rat pancreas is that they represent two divisions within the exocrine pancreas. Possible candidates would be the pancreatic ducts and the exocrine acini respectively (cf. 14).

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REFERENCES

1. Bonner-Weir, S.: The microvasculature of the pancreas, with special emphasis on that of the islets of Langerhans. Anatomy and functional implications. In: The pancreas. Biology, pathobiology and disease 2nd (ed. V.L. Go, E.P. DiMagno, J.D. Gardner, E. Lebenthal, H.A. Reber & G.A. Scheele), pp. 759-768. Raven Press Ltd, New York, 1993.
2. Bonner-Weir, S. & Orci, L.: New perspectives on the microvasculature of the islets of Langerhans in the rat. Diabetes 31:883-889, 1982.
3. Brunfeldt, K., Hunhammar, K. & Skouby, A.P.: Studies on the vascular system of the islets of Langerhans in mice. Acta Endocrinol 29:473-480, 1958.
4. Bunnag, S.C., Warner, N.E. & Bunnag, S.: Microcirculation of islets of Langerhans in alloxan diabetes of mice. Bibl Anat 9:169-175, 1967.
5. Fujita, T.: Insulo-acinar portal system in the horse pancreas. Arch Histol Jap 35:161-171, 1973.
6. Henderson, J.R.: Why are the islets of Langerhans? Lancet II:469-470, 1969.
7. Henderson, J.R. & Daniel, P.M.: A comparative study of the portal vessels connecting the endocrine and exocrine pancreas, with a discussion of some functional implications. Q J Exp Physiol 64:267-275, 1979.
8. Jansson, L.: Flow distribution between the endocrine and exocrine parts of the isolated rat pancreas during perfusion in vitro with different glucose concentrations. Acta Physiol Scand 126:533-538, 1986.
9. Jansson, L. & Hellerström, C.: Stimulation by glucose of the blood flow to the pancreatic islets of the rat. Diabetologia 25:45-50, 1983.
10. Kivisaari, L.: The effect of experimental pancreatitis and diabetes on the microvasculature of the rat pancreas. Scand J Gastroenterol 14:689-695, 1979.
11. Levesque, M.J. & Groom, A.C.: Washout kinetics of red cells and plasma from the spleen. Am J Physiol 231:1665-1671, 1976.
12. Lifson, N., Kramlinger, K.G., Mayrand, R.R. & Lender, E.J.: Blood flow to the rabbit pancreas with special reference to the islets of Langerhans. Gastroenterology 79:466-473, 1980.
13. Lifson, N. & Lassa, C.V.: Note on the blood supply of the ducts of the rabbit pancreas. Microvasc Res 22:171-176, 1981.
14. Lifson, N., Lassa, C.V. & Dixit, P.K.: Relation between blood flow and morphology in islet organ of rat pancreas. Am J Physiol 249:E43-E48, 1985.

15. Loubatières, A., Mariani, M.M., Ribes, G., de Malbosc, H. & Chapal, J.: Etude expérimentale d'un nouveau sulfamide hypoglycémiant particulièrement actif, le HB 419 ou glibenclamide. I. Action bêtacytotrope et insulino-sécrétrice. Diabetologia 5:1.-10, 1969.
16. Papaccio, G. & Chieffi-Baccari, G.: Alterations of islet microvasculature in mice treated with low-dose streptozocin. Histochemistry 97:371-374, 1992.
17. Papaccio, G., Chieffi-Baccari, G., Mezzogiorno, V. & Esposito, V.: Capillary area in early low-dose streptozocin-treated mice. Histochemistry 95:19-21, 1990.
18. Rerup, C.C.: Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22:485-518, 1970.
19. Robertson, J.S.: Theory and use of tracers in determining transfer rates in biological systems. Physiol Rev 37:133-154, 1957.
20. Song, S.H. & Groom, A.C.: Storage of blood cells in spleen of the cat. Am J Physiol 220:779-784, 1971.
21. Stagner, J.I. & Samols, E.: The vascular order of islet cellular perfusion in the human pancreas. Diabetes 41:93-97, 1992.
22. Svensson, A.M., Jansson, L. & Hellerström, C.: The volume and area of the capillaries in the endocrine and exocrine pancreas of the rat. Histochemistry 90:43-46, 1988.
23. Thiel, A.: Untersuchungen über das Gefäss-system des Pankreasläppchens bei verschiedenen Säugern mit besonderer Berücksichtigung der Kapillärknäuel der Langerhansschen Inseln. Z Zellforsch 39:339-372, 1954.
24. Wharton, G.K.: The blood supply of the pancreas, with special reference to that of the islands of Langerhans. Anat Rec 53:55-81, 1932.

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