Reinnervation of the Endocrine Pancreas After Autotransplantation of Pancreatic Fragments in the Spleen of the Dog: A Morphofunctional Study

Mário L.C. Madureira, M.D., A. Adolfo, M.D., J. Dias, M.D., M. Sebe, M.D., H.A. Carvalhais, M.D., and P. von Hafe

Departments of Surgery I and Pathology, University of Oporto School of Medicine, Oporto, Portugal

Secondary diabetes following pancreatectomy in the dog was prevented by autotransplantation of pancreatic fragments in the spleen. The function and composition of the endocrine elements were analyzed by functional studies and by light and electron microscopy at 4, 8, and 24 weeks. During these different periods, splenectomy proved that all the animals were dependent on the transplanted tissue for normoglycemia. The morphologic study of the spleen showed the survival of the intrinsic pancreatic ganglia as well as the presence of an amyelinic, terminal nervous network, with adrenergic varicosities, exclusively related to the endocrine elements.

Nerve endings were identified in close contact with beta, alpha-2, and alpha-1 cells, either isolated in the splenic pulp or forming insular structures with a variable size and composition. No cholinergic varicosities could be identified nor was there any innervation in the ductal and acinar tissue.

From 4 weeks on, Schwann cells could be seen coating large surfaces of the endocrine cells at the same time as they sheathed nervous elements, thus creating true "neuro-insular complexes" with beta and alpha-2 cells. The few ganglia that could only be identified under light microscopy, as well as the ultrastructural characteristics of the innervation, suggest that the sympathetic fibers of the perivascular, trabecular and capsular plexus of the spleen had grown and invaded the inoculated endocrine tissue in the splenic pulp.

This work is part of a Research Program on Transplantation of the Endocrine Pancreas supported by the Gulbenkian Foundation and the Instituto Nacional de Investigação Científica (I.N.I.C.).

Reprint requests: Mário Madureira, M.D., Rua da Constituição 798-3ºE, 4200 Porto, Portugal.

The question of reinnervation of transplanted endocrine pancreas continues to be controversial and, as far as we know, there are no morphological studies that have proven the survival of the intrinsic pancreatic ganglia, nor has the existence of nerve endings in relation with the transplanted endocrine cells been demonstrated [1, 2].

In the rat, after hepatic embolization of isolated islets, a serial ultrastructural study carried out in the 32 days following transplantation made no identification of nerve endings or ganglion cells, but only occasionally identified some amyelinic fibers in the pericapillary space of the transplanted islets [3]. In an identical experimental model, 3 or more months after transplantation, functional studies showed an exaggerated response to stress characterized by an abnormal elevation of blood glucose and glucagon, a marked decrease of glucose tolerance, and a marked inhibition of insulin secretion. These metabolic abnormalities were attributed to hypersensitivity of the endocrine cells to catecholamines as a consequence of the probable absence of adrenergic innervation [4, 5].

It was also demonstrated that, after a meal, the early cephalic phase of insulin secretion was abolished, which in turn led to abnormalities of later insulin secretion and glucose tolerance. These factors were noted 10–14 weeks after the hepatic embolization of isolated islets [5-8] and 22–37 days after the transplantation of 3–4 pancreata in the renal subcapsular space [9]. From these studies it was concluded that transplanted endocrine cells would not be subject to direct vagal control.

Functional studies, however, cannot distinguish between the absence of innervation and imperfect nervous control. The interpretation of abnormalities in insulin secretion and tolerance to glucose is a
relatively complex matter and requires that other factors be taken into account. Of these, one must especially note the heterotopia of the endocrine tissue, the type of venous drainage (systemic, portal, or mixed) [10-12], the composition of the endocrine units [13], the amount of functional beta cells after transplantation [14-16], and the possible alteration in the sensitivity of endocrine cells to the stimulating action of intestinal hormones and nutrients. One should also consider the time that has elapsed between the transplant and the morphological and functional studies, and the characteristics of the innervation of the receptor organ.

In order to clarify the question of reinnervation of the transplanted endocrine units, it seemed essential that new morphological studies be carried out, since ultrastructure is necessary for the precise definition of relations of nervous elements to endocrine cells and between cells themselves.

Material and Methods

Dogs were used for this experimental study. Six animals, subjected to total pancreatectomy and autotransplantation of fragments of the pancreas by inoculation in the spleen, were selected. The pancreatic tissue transplanted corresponded to 80-90% of the gland. It included all of the epithelial elements, and was prepared according to a personal definition of relations of nervous elements to endocrine cells and between cells themselves.

Functional Study

Total pancreatectomy caused severe diabetes with progressive hyperglycemia and death on an average of 7.4 ± 2.8 days later. In the 6 dogs selected for the morphological study, autotransplantation of fragments of the pancreas inoculated in the spleen reestablished preoperative blood glucose levels in all animals after a phase of hyperglycemia.

Four weeks after transplantation, an intravenous glucose overload produced significantly different tolerance curves from those observed in normal controls; the glucose assimilation coefficient (Constant K) was 1.37 ± 0.22, a significant decrease when compared to normal controls (2.182 ± 0.385). All animals presented steatorrhea and a progressive loss of weight. In 2 dogs the weight stabilized and, after 6 months, the Constant was 1.11 ± 0.09 and their weight had increased to 90% of the original level. No animal suffered a spontaneous relapse of hyperglycemia.

At the end of each observation period, serum insulin dosing before (0') and after (15') stimulation with glucose (1 g/kg of body weight per 50%) showed a highly significant gradient between the venae cavae (VC) and the splenic vein (SV), always in favor of the latter (0'—VC:10.5 ± 2.8, SV:48.5 ± 13.8; 15'—VC: 23.7 ± 5.0, SV:119.7 ± 25.5).

A splenectomy performed immediately afterward caused hyperglycemia in all animals. In the animals