



Karolinska Institutet

Rolf Luft Research Center for Diabetes and Endocrinology
Department of Molecular Medicine and Surgery
Karolinska Institutet, Stockholm, Sweden

Regulation of human pancreas hormone secretion by autonomic innervation

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
Institutet offentligen försvaras i Rolf Luft Auditorium, L1:00,
Karolinska Universitetssjukhuset Solna

Torsdagen den 12 januari 2012, kl 09.00

av

Rayner Rodriguez-Diaz

MSc

Huvudhandledare:

Prof. Per-Olof Berggren
Karolinska Institutet
Institutionen för molekylär medicin och
kirurgi

Fakultetsopponent:

Prof. Herbert Y. Gaisano
University of Toronto
Division of Fundamental Neurobiology

Bihandledare:

Doc. Ingo Leibiger
Karolinska Institutet
Institutionen för molekylär medicin
och kirurgi

Betygsnämnd:

Doc. Mia Phillipson
Uppsala Universitetet
Institutionen för Medicin
Enheten för Medicinska Cellbiologi

Alejandro Caicedo PhD
University of Miami
Department of Medicine

Prof. Lennart Brodin
Karolinska Institutet
Institutionen för Neurovetenskap

Doc. Alexander Chibalin
Karolinska Institutet
Institutionen för fysiologi och farmakologi

Stockholm 2012

ABSTRACT

Diabetes mellitus is a silent killer doing away with one person every 10 seconds. We speak of diabetes when the organism cannot control the right level of glucose in the blood. The hormones insulin and glucagon secreted by the islets of Langerhans are the major players maintaining glucose homeostasis. In the living organism, the function of the islets is orchestrated by their interaction with other organs through the vasculature and with the nervous system. Most of our current knowledge of islet biology has been obtained by using mouse models, but caution is needed, as mice are not simply small humans. Indeed, recent studies have revealed that the cell composition and architecture of the human islet are different from that of mouse islets. Thus, other important features such as nervous regulation of islet function may also be different.

The work in this thesis aimed to identify the role of innervation for islet function. Our hypothesis is that autonomic and paracrine signals are involved in islet function and that the relative role of these components varies among species. To identify the sympathetic and parasympathetic components of innervation as well as their cellular targets we used immunohistochemical staining of human and mouse pancreatic sections. In contrast to mouse, human islets are devoid of parasympathetic innervation. Instead, human alpha cells possess the machinery for exocytosis of acetylcholine, the major parasympathetic neurotransmitter. Our findings suggest that human islets depend less on neural cholinergic input than mouse islets. Alpha cells secrete acetylcholine as a paracrine signal priming the human beta cell to respond optimally to subsequent increases in glucose concentration. In addition, noradrenergic fibers contact few endocrine cells in the human islet and preferentially innervate smooth muscle cells of the islet vasculature. This suggests that sympathetic innervation regulates hormone secretion by controlling the blood flow rather than modulating endocrine cell function directly.

By taking advantage of our recently developed noninvasive anterior chamber of the eye imaging platform we were able to study the role of innervation in the maintenance of glucose homeostasis *in vivo*. We studied the process of reinnervation and revascularization of intraocular islet grafts and showed that islets orchestrate the process of engraftment to restore their original microenvironment. Islet grafts from two different mouse strains and human xenografts showed innervation patterns similar to those in pancreatic sections *in situ*. Islet grafts displayed the characteristic fenestrae of the pancreatic vascular endothelium independently of the origin of the new vessels. In addition, the model allowed controlling the fraction of the graft vasculature that is contributed by the donor islet endothelial cells to the point that the original donor vasculature of the islet is restored. Recording graft function while manipulating the eye's neural input through the pupillary light reflex revealed functional differences in parasympathetic innervation between the two mouse strains. The eye platform also allowed us to follow cell dynamics during immune responses, which will enable investigations aimed at clarifying the role of innervation in the pathogenesis of autoimmune diabetes.

To study human islet biology *in vivo* we further adapted the eye model by transplanting human islets into the eye of diabetic immune compromised mice. Human xenografts reversed diabetes and tightly controlled plasma glucose concentrations. Moreover, our results provided the first real time monitoring of revascularization and blood flow inside human islets and graft function could be modulated by local drug administration. Our findings establish a "humanized" mouse model to investigate human islet biology *in vivo* that will allow addressing how nervous input affects endocrine function or blood flow in human islets. The physiological relevance of the anterior chamber of the eye model is further underscored by the therapeutic potential as a novel transplantation site to treat type 1 diabetic patients.