

Regulation of Insulin Secretion by Nerves and Neuropeptides

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Abstract

The pancreatic islets are innervated by sympathetic, parasympathetic and sensory nerves and within the terminals of these nerves several different neurotransmitters are stored, both the classical neurotransmitters, acetylcholine and noradrenaline, and several neuropeptides. The neurotransmitters affect insulin secretion both in a stimulatory and in an inhibitory direction depending on the nervous system activated, the neurotransmitter released and the status of the B cell. This neural regulation of insulin secretion is of importance during various forms of stress as well as during the cephalic phase of food intake.

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Introduction

The pancreatic islets of Langerhans were described by Paul Langerhans in 1869. They are distributed throughout the entire pancreas forming in adults approximately 1% of the pancreatic mass. Each islet comprises a number of cells which are arranged in a central core of the insulin producing B cells (approximately 60% to 80% of all islet cells) surrounded by a mantle zone containing the other cell types. The mantle zone contains the glucagon producing A cells, the somatostatin producing D cells and the pancreatic polypeptide (PP) producing F cells.¹ A key role for the pancreatic islets is to deliver an optimal amount of insulin into the blood stream for carbohydrate metabolism, since insulin facilitates transport of glucose into insulin-dependent cells for storage as glycogen and/or fat, causing a reduction in blood glucose levels. Consequently, when the islets are not functioning properly, an inadequate amount of insulin is produced, which may result in hyperglycaemia and type 2 diabetes.

Multifactorial Regulation of Insulin Secretion

Insulin is synthesized in and secreted from the centrally located B cells in the islets. A variety of factors influence insulin secretion. This results in a degree of insulin secretion which is optimal for the requirement of the body. The most important regulator of the islet B cells is glucose, which stimulates insulin secretion. This results in raised plasma insulin levels which reduce the circulating levels of glucose, thereby closing an insulin-glucose regulatory loop. Insulin secretion is also regu-

lated by other circulating nutrients. Thus, circulating amino acids like arginine and leucine stimulate insulin secretion. Furthermore, free fatty acids stimulate and inhibit insulin secretion under different conditions. These nutrient-induced changes in insulin secretion are of importance for the regulation of the carbohydrate and lipid metabolism, and particularly under conditions of metabolic stress when circulating levels of glucose and amino acids are increased, insulin secretion is increased to allow a peripheral cellular uptake of glucose, amino acids and lipids to restore normal levels in the circulation. Insulin secretion is also regulated by gut hormones. Thus, the hormones GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory polypeptide) both stimulate insulin secretion. This is of importance after food intake, when these hormones are liberated from the gut into the circulation and then cause a humoral stimulation of insulin secretion. This is why an oral ingestion of glucose elicits a much higher insulin secretory response than an intravenous glucose challenge also when the glycaemia is matched: the oral glucose load causes release of gut hormones which potentiate insulin secretion. Insulin secretion is also regulated by nerves innervating the pancreatic islets, which will be summarized in this article. Finally, insulin secretion is also regulated by hormones produced in the other islet endocrine cells, like glucagon (stimulatory) and somatostatin (inhibitory). As a whole, the integrative action on insulin secretion by these various regulatory mechanisms (circulating nutrients, gut hormones, islet nerves, islet hormones) results in an optimal release of insulin for the minute-to-minute

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requirement. An important aspect of the present islet research is to understand how these different regulatory mechanisms integrate to elicit an optimal insulin secretion, and the contribution of these factors to the islet dysfunction which is seen during the development of type 2 diabetes.*

Islet Nerves

Already Paul Langerhans showed that the pancreatic islets are innervated. Today, it is known that autonomic nerves innervate each individual islet. These autonomic nerves are branches of the parasympathetic, sympathetic and sensory nervous systems, and they influence the secretion of all four islet hormones. During the last two decades, it has been shown that besides the classical neurotransmitters (i.e. noradrenaline in sympathetic nerves and acetylcholine in parasympathetic nerves), there are also several neuropeptides acting as signalling substances in nerves present in the islets.^{3,4} Hence, both classical neurotransmitters and neuropeptides may be released from the nerve terminals and affect islet hormone secretion.

Anatomy of the Islet Autonomic Nerves

The sympathetic nerves innervating the islets have their nerve cell bodies in the coeliac ganglion and in the paravertebral ganglia. The preganglionic nerves passing to the ganglia originate in nerve cell bodies in the hypothalamus. The postganglionic sympathetic nerves pass within the splanchnic nerves and enter the pancreas within the periarterial sheath surrounding the arteries. The parasympathetic nervous system has a different anatomy. Its nerves innervating the pancreatic islets have their nerve cell bodies in intrapancreatic ganglia. The preganglionic parasympathetic nerve fibres originate in the dorsal motor nucleus in the brain

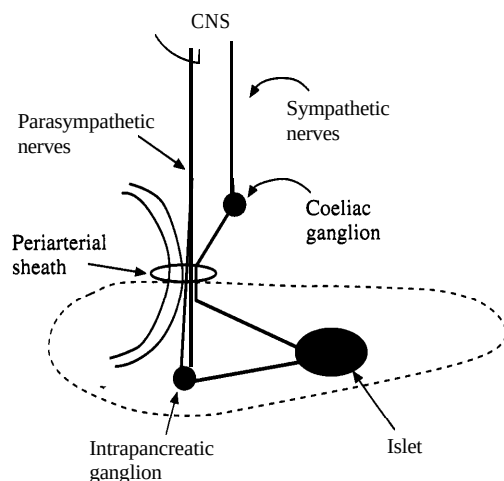


Fig. 1. Schematic view of the innervation of the pancreas with the two main branches of the autonomic nerves (parasympathetic preganglionic nerves and sympathetic postganglionic nerves) entering the pancreas within a periarterial sheath.

stem and pass through the vagal nerves and enter, together with the sympathetic fibres, the pancreas within the periarterial sheath. Within the pancreas, the parasympathetic nerve fibres terminate in intrapancreatic ganglia. Sympathetic nerves entering the pancreas are therefore postganglionic fibres, whereas parasympathetic nerves entering the pancreas are preganglionic (Fig. 1). The pancreatic islets are also innervated by sensory nerves. These nerve fibres pass through the splanchnic nerves and reach the spinal cord, at the level of Th8-Th11, and the nodose ganglion. Within the pancreas, most autonomic nerves innervate the vessels, but some fibres also enter the islets and terminate in close proximity to the islet endocrine cells thereby enabling a direct regulation of islet hormone secretion by the release of neurotransmitters from the nerve terminals.^{3,4}

Neurotransmitters of the Islet Nerves

The classical neurotransmitters in the autonomic nervous system are noradrenaline in sympathetic nerves and acetylcholine in the parasympathetic nerves. Also in the islets, these classical neurotransmitters function as signalling molecules for the two branches of the autonomic nervous system. These classical neurotransmitters activate α_2 - and β_2 -adrenoceptors (noradrenaline) and muscarinic m_3 -receptors (acetylcholine) on the plasma membranes of the islet cells. A series of signalling pathways is then induced in the endocrine cells which affects insulin secretion. In addition to these classical neurotransmitters, the pancreatic autonomic nerve terminals contain a variety of neuropeptides, which might function as co-transmitters together with the classical neurotransmitters.^{3,4} Several lines of evidence support that the neuropeptides function as neurotransmitters in the islets:

- a) the neuropeptides are localized to autonomic nerve terminals within the islets using immunocytochemistry,
- b) the neuropeptides are released from the pancreas upon autonomic nerve activation,
- c) the neuropeptides exert effects on islet hormone secretion in line with the effects of nerve stimulation, and
- d) antagonism of the neuropeptide effects inhibits neurally induced action on islet hormone secretion.

Based on these criteria, evidence has been presented that neuropeptide Y (NPY) and galanin are sympathetic neuropeptides whereas vasoactive intestinal polypeptide (VIP) and gastrin releasing polypeptide (GRP) are parasympathetic neuropeptides. Furthermore, evidence has also accumulated that calcitonin gene-related peptide (CGRP) and substance P (SP) are sensory neuropeptides in the islets. In addition, cholecystikinin (CCK) and pituitary adenylate cyclase activating polypeptide (PACAP) may be important neuropeptides

in the islets, although the exact nature of the nerves producing these neuropeptides remains to be finally established (Fig. 2).^{2,3}

Sympathetic Nerves

Effects Of Sympathetic Nerves

The direct influences of the autonomic nerves on insulin secretion have been examined by the use of electrical activation of the nerves in animal models. In dogs, sympathetic nerve stimulation is accomplished by isolating the mixed autonomic nerves entering the pancreas around a pancreatic artery. The isolated nerve fibres are then placed in bipolar electrodes and stimulated electrically.⁵ This procedure activates both the preganglionic parasympathetic and the postganglionic sympathetic fibres. To achieve a selective sympathetic nerve stimulation, the parasympathetic nerves are blocked by infusing a ganglionic antagonist, like hexamethonium, which inhibits the pancreatic ganglia. This method allows therefore a selective sympathetic activation. Insulin levels are then analysed in the corresponding pancreatic vein, and by simultaneously analysing the pancreatic blood flow and also determining the arterial insulin levels, a good estimate of the insulin secretory rate is achieved by multiplying blood flow by the arteriovenous concentration difference of insulin

across the pancreas. The effect of such a selective sympathetic stimulation is inhibition of basal insulin secretion.⁵ Another method for the study of the influences of sympathetic nerve stimulation is intravenous administration of 6-hydroxydopamine (6-OHDA). This amine is actively taken up by the amine pump in the sympathetic nerve terminals, and causes a depletion of the naturally occurring neurotransmitter, noradrenaline. Therefore, a chemical "sympathectomy" is induced by 6-OHDA. This has been verified in a study of the rat pancreas, where it was demonstrated that 2 days after intravenous administration of 6-OHDA, noradrenaline was depleted from nerves in the pancreatic islets.⁶ This resulted in increased baseline insulin levels in rats,⁶ thereby supporting that sympathetic nerves are of importance as inhibitory factors of insulin secretion. A similar finding has been obtained after surgical section of the splanchnic nerves in rats.⁷ Hence, both by activating the sympathetic nerves electrically and preventing the nerves from action, it has been concluded that the function of the sympathetic nerves is to inhibit insulin secretion.

Mediator of the Islet Sympathetic Nerves

The inhibitory action of sympathetic nerve stimulation on insulin secretion might be mediated by any of the potential neurotransmitters produced by the sympathetic neurons and stored in the nerve terminals close to the insulin producing B cells. At least three different neurotransmitters may be considered, noradrenaline, NPY and galanin. All these neurotransmitters are localized to sympathetic nerve terminals within the pancreas, they are released into the pancreatic venous effluent during sympathetic nerve stimulation and they all inhibit insulin secretion through a direct action on the islet B cells.^{3,4} The potential contribution by these neurotransmitters to the effects of sympathetic nerve stimulation may be examined by locally infusing them directly into the pancreatic artery. This results in a high local concentration of the neurotransmitters close to the insulin producing cells. Such studies have shown that local infusion of noradrenaline or NPY is unable to inhibit basal insulin secretion in the dog, whereas infusion of galanin has a profound inhibitory effect.^{3,4} In contrast, noradrenaline inhibits glucose- and arginine-stimulated insulin secretion. This suggests that in the dog, galanin is a likely explanation for the sympathetic nerve-induced inhibition of basal insulin secretion whereas noradrenaline is a candidate for mediating sympathetically-induced inhibition of stimulated insulin secretion, for example during hyperglycaemia. The finding that noradrenaline does not inhibit basal insulin secretion might be surprising, considering its well known inhibitory action on stimulated insulin secretion, which is executed via α_2 -adrenoceptors.³ However, a failure of noradrenaline to inhibit sympathetically-induced inhibition of basal insulin secretion is confirmed by another

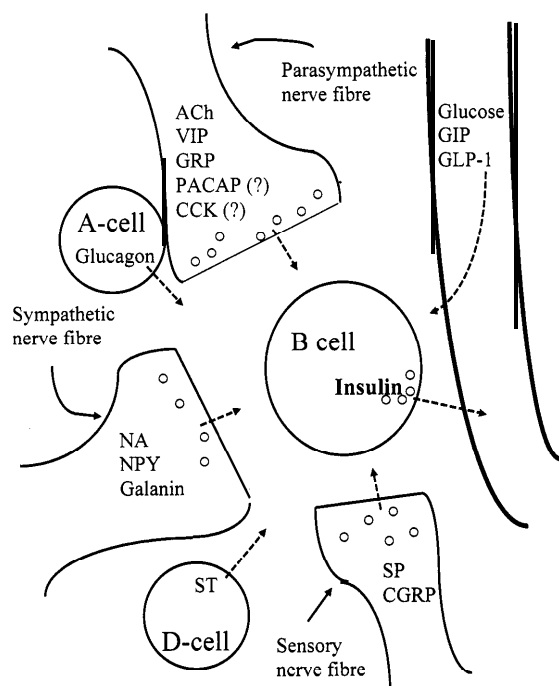


Fig. 2. Schematic view of an islet insulin-producing B cell innervated by parasympathetic fibres [neurotransmitters: acetylcholine (ACh), vasoactive intestinal polypeptide (VIP), gastrin releasing polypeptide (GRP) and perhaps pituitary adenylate cyclase activating polypeptide (PACAP) and cholecystokinin (CCK)], by sympathetic fibres [neurotransmitters: noradrenaline (NA), neuropeptide Y (NPY) and galanin] and by sensory nerve fibres [neurotransmitters: substance P (SP) and calcitonin gene-related peptide (CGRP)]. In addition, the activity of the B cell is regulated by blood borne factors [glucose, gastric inhibitory polypeptide (GIP) and glucagon-like peptide -1 (GLP-1)] and by peptides secreted from neighbouring A cells (glucagon) and D cells (ST: somatostatin).

set of experiment, showing that combined α - and β -adrenergic blockade is unable to counteract the inhibitory action of sympathetic nerve stimulation of basal insulin secretion.⁸ Therefore, the sympathetically induced influences on islet hormone secretion seem to be at least partially non-adrenergic and not mediated by noradrenaline. It might thus be anticipated that noradrenaline mediates the inhibitory action of sympathetic nerves under conditions of stimulated insulin secretion, whereas sympathetically-induced inhibition of basal insulin secretion is more likely mediated by a neuropeptide, e.g. galanin. Possible species differences need also to be considered, however, since for example galanin poorly inhibits insulin secretion in humans. To conclude, activation of the sympathetic nerves inhibits insulin secretion and this action seems to be mediated by noradrenaline and galanin. The relative contributions of these neurotransmitters might differ, however, in different species and under various conditions. A main difference is that noradrenaline seems to mediate inhibition of stimulated insulin secretion, whereas galanin seems to mediate inhibition of basal insulin secretion. It should be emphasized here that activation of β_2 -adrenoceptors stimulates insulin secretion. Therefore, catecholamines may exert a dual action on insulin secretion, through either of the two subtypes of adrenoceptors (α_2 - and β_2 -types) on the B cells. For noradrenaline, the α_2 -mediated inhibition of insulin secretion predominates.

Parasympathetic Nerves

Effects of Parasympathetic Nerves

The effects of parasympathetic nerve activation on insulin secretion may be studied by isolating and electrically activating the vagus nerve within the thorax below the heart and at the same time sample blood from the pancreatic vein.⁹ This results in a marked stimulation of insulin secretion. Another approach is to induce neuroglycopenia by the administration of 2-deoxy-glucose.¹⁰ This glucose analogue competes with glucose metabolism in the cells at the level of the glucose phosphorylation process. This creates a state of intracellular glucose deficiency which in the hypothalamus results in activation of the parasympathetic nerves. In the mouse, this has been demonstrated to markedly stimulate insulin secretion.¹⁰ Hence, in contrast to the inhibition of insulin secretion seen after sympathetic nerve activation, parasympathetic nerve activation stimulates insulin secretion.

Mediator Of the Islet Parasympathetic Nerves

Both acetylcholine and the cholinergic agonist, carbamoyl choline or carbachol, stimulate insulin secretion.³ It has therefore been thought that acetylcholine is the mediator of vagally stimulated insulin secretion. This is supported by studies demonstrating that atro-

pine, which inhibits the muscarinic receptors activated by acetylcholine, counteracts both the parasympathetically-induced insulin secretion in the dog⁹ and the 2-deoxy-glucose-induced insulin secretion in the mouse.¹⁰ However, in the pig, vagally-induced insulin and glucagon secretions are not inhibited by atropine,¹¹ suggesting that a non-cholinergic involvement exists following vagal nerve activation of islet hormone secretion in some species. Studies on the nature of this non-cholinergic involvement have proposed that VIP and GRP might be implicated. Thus, both these potential neuropeptides are localized to nerve terminals of parasympathetic nerves within the islets and they are released from the pancreas during vagal nerve activation.^{3,4} Furthermore, both VIP and GRP potently stimulate insulin secretion. However, whether these two neuropeptides actually participate as mediators of the vagal effects on islet function remains to be established.

Sensory Nerves

The endocrine pancreas is also innervated by sensory, afferent, nerves. These sensory nerves contain CGRP and substance P as neurotransmitters.^{3,4} The influence of these sensory nerves on islet function is not known, since such studies are difficult to undertake. One approach has been the use of the neurotoxin capsaicin. When administered to rodents, either in the neonatal period or in the adult animal, capsaicin destroys the sensory nerves. By treating neonatal mice with capsaicin, it has been demonstrated that CGRP and substance P are partially or completely depleted from islet nerves after treatment with capsaicin, indicating that this treatment indeed works also in the endocrine pancreas.¹² Such treatment has been found to increase the glucose tolerance, in that a more exaggerated glucose elimination was evident in capsaicin-treated mice than in control mice.¹³ This suggests that the sensory nerves impair glucose tolerance. The mechanism underlying this action of capsaicin has been studied in more detail, and it has been shown that insulin secretion is potentiated in capsaicin-treated mice indicating that the sensory nerves impair glucose elimination by inhibiting insulin secretion.¹³ This is in analogy with some action of the sensory neurotransmitters, CGRP and substance P which in several studies have been found to inhibit insulin secretion.^{2,3} The sensory islet nerves may therefore be of importance for optimal glucose tolerance.

CCK and PACAP Nerves

The two neuropeptides, CCK and PACAP, have been localized to nerve terminals in pancreatic islets, although the nature of these nerves has yet to be defined. Both CCK and PACAP stimulate insulin secretion.^{14,15} Therefore, the two neuropeptides exert similar action as acetylcholine, VIP and GRP (i.e. parasympathetic

neurotransmitters), which might suggest that they have a role as parasympathetic neurotransmitters. However, conclusive evidence for such a hypothesis is lacking at present.

Islet Mechanisms of the Neurotransmitters

Cell Biology Of Islet B Cells

When the islet nerves are activated, the neurotransmitters are released and they thereafter diffuse a short distance to the islet B cells. They activate specific receptors located at the B cell membrane and initiate a series of cell biological events ultimately leading to a change in the rate of exocytosis of the insulin containing secretory granules, seen as inhibition or stimulation of insulin secretion. The most important regulator of insulin secretion is glucose, the mechanism of which has been elucidated in detail (Fig. 3).¹⁶ Glucose which enters the cells is phosphorylated into glucose-6-phosphate, and is further metabolized whereby ATP is formed. Increased ATP closes specific ATP-regulated K^+ channels in the plasma membrane. This reduces the outflow of K^+ from the B cells which in turn depolarizes the B cells and opens the L-type voltage-sensitive Ca^{2+} channels. This causes a massive inflow of Ca^{2+} from the extracellular space, which raises the cytoplasmic Ca^{2+} concentra-

tion.

Glucose also stimulates adenylate cyclase, which induces formation of cyclic AMP and activation of protein kinase A (PKA). Furthermore, glucose also stimulates the hydrolysis of membraneous phosphoinositides through the activation of phospholipase C (PLC). This yields the formation of inositol-1,4,5-trisphosphate (IP_3) and diacyl glycerol (DAG). IP_3 diffuses into the cytoplasm and induces liberation of Ca^{2+} from intracellular Ca^{2+} storage sites, which further increases the cytoplasmic concentration of Ca^{2+} . Furthermore, DAG activates protein kinase C (PKC). Finally, glucose has also been shown to stimulate the formation of arachidonic acid through activation of phospholipase A_2 (PLA_2). All these events induced by glucose (increase of cytoplasmic Ca^{2+} and activation of PKA, PKC and PLA_2) stimulate the exocytosis of insulin containing secretory granules. The molecular basis of these distal events are less well characterized and is the subject of intense research at present. The neurotransmitters which are released during activation of the autonomic nerves stimulate or inhibit insulin secretion by influencing the signal mechanisms initiated by glucose. Hence, the neurotransmitters function as modulators, adjusting and optimizing the insulin secretion in accordance with the requirement as assessed by the centra governing the autonomic nervous system. These biological processes are studied mainly by using isolated islets or cultured insulin producing cell lines.

Neurotransmitters inhibiting the action of glucose (noradrenaline via α_2 -adrenoceptors, NPY and galanin) may counteract the ionic movements initiated by glucose through hyperpolarizing the plasma membrane thereby preventing the uptake of Ca^{2+} through voltage-sensitive Ca^{2+} channels. They may also inhibit the formation of cyclic AMP by inhibiting adenylate cyclase, and they may act on the exocytotic machinery to inhibit the extrusion of secretory granules.² Furthermore, neurotransmitters stimulating insulin secretion may execute such an effect by stimulating adenylate cyclase thereby inducing formation of cyclic AMP (VIP, PACAP, catecholamines via β_2 -adrenoceptors), by stimulating PLC (CCK, GRP, acetylcholine) or by stimulating PLA_2 (CCK, acetylcholine). Hence, the neurotransmitters may interact with one or several of the mechanisms induced by glucose thereby modulating the insulinotropic action of the sugar enabling an optimal release of insulin.

Physiology

The inhibitory influence of sympathetic nerve activation on insulin secretion is of physiological importance during various forms of stress. Examples of these conditions are bleeding, hypovolaemia, burns, diseases and psychological stress. The sympathetic nervous system is also activated during exercise. During such conditions, a goal of the metabolism is the preservation of hypergly-

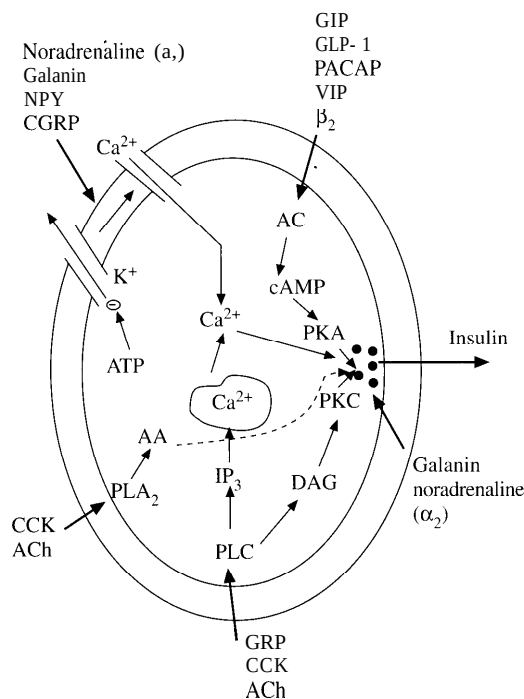


Fig. 3. Schematic view of signal transduction pathways involved in the modulation of insulin secretion by noradrenaline, galanin, neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), gastric inhibitory polypeptide (GIP), glucagon-like peptide -1 (GLP-1), pituitary adenylate cyclase activating polypeptide (PACAP), vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), gastrin releasing polypeptide (GRP) and acetylcholine (ACh). For explanation see text. ATP: adenosine triphosphate, AC: adenylate cyclase, cAMP: cyclic adenosine monophosphate, PKA: protein kinase A, PKC: protein kinase C, DAG: diacyl glycerol, IP_3 : inositol-1,4,5-trisphosphate.

caemia to maintain the delivery of glucose to the brain. This is accomplished by inhibition of insulin secretion and a simultaneous stimulation of glucagon secretion, which results in low circulating levels of insulin and high circulating levels of glucagon. This will simultaneously increase hepatic glucose production and inhibit peripheral glucose uptake which will preserve hyperglycaemia. These effects are executed by the increased activity in the sympathetic nervous system, and within the islets, all three neurotransmitters, noradrenaline, NPY and galanin, might contribute to the actions. The relative contribution of these neurotransmitters for the responses remains to be established. However, considering the above-mentioned difference in action between these neurotransmitters, it maybe hypothesized that the neuropeptides are of importance under baseline conditions, whereas noradrenaline inhibits insulin secretion during hyperglycaemia.

Parasympathetic nerve activity stimulates both the secretion of insulin and glucagon, thereby increasing the glucose turnover rate in the body during maintenance of normoglycaemia by both stimulating hepatic glucose production and peripheral glucose uptake. This is of importance when increased glucose turnover is required in combination with avoidance of hyperglycaemia. One such condition is after food intake when increased insulin levels are of importance to meet the metabolic challenge executed by the absorption of food constituents. Consequently, the parasympathetic nerves have been shown to be of physiological importance during the cephalic phase after food intake, when both insulin and glucagon secretion are stimulated. For example, atropine has been demonstrated to inhibit the early insulin response to a food challenge. These actions may be executed by acetylcholine, VIP and/or GRP, but CCK and PACAP may also contribute. A current important issue for research within this field is therefore to establish the mechanism of the islet responses during the cephalic phase of food intake and establish the relative contribution by the various neurotransmitter candidates.

Summary

The islets of Langerhans are richly innervated by sympathetic, parasympathetic, peptidergic and sensory nerves, and much is known today on the effects of stimulatory manipulations, of blockade methods and of the proposed neurotransmitters on islet function. How-

ever, we are still at the beginning of understanding the potential physiological contribution of these nerve-islet interactions, and still far from understanding the possible involvement of these interactions in alterations in diseases e.g. obesity and diabetes.

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