Introduction
The control of insulin secretion by the islet of Langerhans β-cell is not fully understood. Nutrients, circulating hormones, neural and autocrine (produced by the β-cell itself)/paracrine (produced by other islet cell types) mechanisms all apparently have an influence. Inhibitory mechanisms are important to ensure an equal cell response to stimulatory factors and thus protect individual cells from over-responding and exhaustion damage. Here we concentrate on the roles of the autocrine inhibitory peptides pancreastatin, neuropeptide Y (NPY) and amylin (also known as islet amyloid polypeptide or IAPP) but also discuss the paracrine/neurocrine inhibitors, somatostatin, calcitonin gene-related peptide (CGRP) and galanin. Amylin has also been proposed as a circulating regulator of glucose metabolism. The evidence for this role, particularly in man, will be reviewed. The peptides covered here reflect the emphasis of work in our laboratory and several important inhibitors of insulin secretion are not considered.

Many compounds have been shown to inhibit insulin secretion but a physiological role is only possible if the effect occurs at concentrations which are present at the β-cell and if increased insulin secretion results from blocking the action of the endogenous compound. For autocrine/paracrine effects this can be demonstrated in the isolated islet or isolated perfused pancreas using immunoneutralization or specific receptor antagonists. Neurocrine inhibition, however, often may only be demonstrated in the intact animal where immunoneutralization is problematical and even specific receptor antagonists will affect other related physiological systems.

Autocrine inhibitors of insulin secretion
Pancreastatin is a C-terminally amidated 49-amino-acid peptide [1] derived from cleavage of the secretory granule protein chromogranin A [2]. Immunoreactive pancreastatin is found in plasma and many endocrine tissues, but chromatographic analysis shows that only in the pancreas and thyroid is pancreastatin 1–49 the predominant form [3]. Generally, pancreastatin is observed to inhibit glucose-stimulated insulin secretion in perfused rat pancreas [4], isolated rat islets [5,6] and in vivo in the rat [5]. However, no effect was seen in vivo in the pig [3] or dog [7]. In isolated rat islets the pancreastatin inhibition of glucose-stimulated insulin secretion is not due to hyperpolarization but to a direct inhibition of calcium uptake [8]. Pancreastatin is found in all four islet cell types (glucagon α-cells, insulin β-cells, somatostatin δ-cells and pancreatic polypeptide PP-cells) [3], but has no consistent effect on glucagon (inhibition demonstrated in the perfused rat pancreas [4]) or somatostatin release in the rat [9]. Two problems are apparent when considering a physiological inhibitory role for pancreastatin. First, no receptors for pancreastatin have been demonstrated in islets or any other tissue. Secondly, there are no reports of immunoneutralization of endogenous pancreastatin leading to an increase in insulin secretion.

NPY is a 36-amino-acid C-terminally amidated peptide which is particularly abundant in neuroendocrine tissue. Exogenous NPY is a potent inhibitor of glucose-stimulated insulin secretion [10,11]. Pancreatic NPY has been assumed to be derived from the extrinsic sympathetic innervation. NPY innervation of the islet is sparse and in some species, despite careful investigation, has not been detected [12]. We have demonstrated NPY and NPY mRNA in isolated rat islets and the clonal β-cell lines, HIT-T15 (hamster) and RINm5F (rat) [13]. Islet NPY and NPY mRNA content was unchanged in rats treated with the sympathetic nerve toxin, 6-hydroxydopamine, which depleted neurons of NPY elsewhere. Similarly capsaicin, which causes sensory nerve degeneration, also had no effect on islet NPY. This suggested that islet NPY was not of neural origin. Treatment of rats with dexamethasone (2 mg/kg per day), a synthetic type-II glucocorticoid receptor agonist, for 12 days elicited a reversible increase in NPY content (15-fold) and NPY mRNA (10-fold), demonstrating modulation of islet NPY by endocrine status. NPY mRNA and peptide release have since been shown by others in the rat insulinoma β-cell line, INS-1 [14].

Abbreviations used: CGRP, calcitonin gene-related peptide; NPY, neuropeptide Y.
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Recently, we have assessed the role of endogenous islet NPY on insulin secretion from isolated perifused rat islets [15]. The use of isolated islets enables paracrine effects to be measured free from neural influence. However, the commonly used static islet incubation technique is compromised as the build-up of paracrine/autocrine factors in the incubation medium can invalidate the control incubations. Therefore, we have used the perifused rat islet preparation where endogenous factors are continually removed from the islet. NPY release from control islets perifused with 2.8 mM glucose (low glucose) was reduced by 62% when perifusate glucose was raised to 20 mM (high glucose). Basal insulin secretion was increased 4-fold when glucose was raised to 20 mM. In islets from dexamethasone-treated rats, NPY release was 6-fold increased by high glucose. Exogenous NPY (100 nM) decreased insulin secretion by 45%, but the critical experiment was to determine the effect of endogenous NPY. This was performed by immunoneutralization of NPY in perifused islets using a rabbit polyclonal antiserum compared with non-immune serum. In control islets insulin release was increased 3-fold with NPY antiserum in the presence of low (basal) glucose and increased 2-fold in the presence of high (stimulated) glucose (Figure 1). NPY immunoneutralization in perifused islets from dexamethasone-treated rats also increased insulin secretion by 2-fold with low and high glucose. Thus islet NPY appears to exert a tonal control on insulin secretion, which is likely to be physiologically important.

Amylin is a highly conserved 37-amino-acid C-terminally amidated peptide with a 45% sequence similarity to CGRP. It was originally purified from the amyloid deposits of a human insulinoma and islets from type-II diabetic patients. Amylin is expressed in the β-cell where it is co-stored and released with insulin (for a review see [16]). It is now accepted that amylin, at least in high concentrations, inhibits glucose-stimulated insulin secretion [17-19]. Rat islets produce considerable amounts of amylin (1% of insulin secretion) and this may explain the high concentrations (10⁻⁵M) needed to demonstrate effects in static islet incubations [18,19]. Indeed much lower concentrations (75 pM to 75 nM) were effective in the perfused rat pancreas [20] and perfused rat islet (100 nM) [17]. In man, infusion of amylin up to a plasma concentration of 2.2 nM also attenuated glucose-stimulated insulin secretion [21].

The receptors mediating the effect of amylin on insulin secretion remain uncharacterized. Typical CGRP receptors detected using 125I-CGRP will bind amylin at high concentrations, typically with a 5-200-fold lower potency [22]. CGRP also inhibits insulin secretion and may act via the same CGRP receptors. However, CGRP receptors are most often associated with G-protein activation of adenylate cyclase and increased intracellular cyclic AMP, which would paradoxically be expected to increase
insulin secretion. We have reported an amylin-prefering receptor in rat lung [23]. This receptor also binds CGRP but at a 10–100-fold lower affinity. It is not yet known whether this receptor is expressed on rat β-cells. This receptor is G-protein coupled, but appears not to be linked to adenylate cyclase activation. A third G-protein-coupled receptor showing a higher affinity for amylin than CGRP but also binding the related peptide salmon calcitonin (which the previously mentioned receptors do not) is found in the rat nucleus accumbens [24]. Again the presence of this receptor in the islet has not been established.

The CGRP C-terminal fragment CGRP(8–37) acts as a CGRP-receptor antagonist in a number of systems [25]. We synthesized the equivalent amylin fragment, amylin(8–37) [17], and showed that this fragment bound to both CGRP and amylin receptors [22]. We then tested the ability of the fragment to antagonize amylin effects in the perifused rat islet [17]. These experiments clearly demonstrated that the inhibition of insulin secretion by exogenous amylin was prevented by amylin (8–37) (Figure 2) and further that the antagonist alone augmented insulin secretion by 50%. This suggests that endogenously produced amylin continuously restrains insulin secretion. Infusion of amylin(8–37) into anaesthetized rats enhanced arginine-stimulated insulin secretion by 100%, again implying a tonal control of insulin secretion by amylin [26]. Similar results were also reported in anaesthetized rats using the CGRP-receptor antagonist CGRP(8–37) [27]. Both CGRP(8–37) and amylin(8–37) are antagonists of CGRP and amylin effects and the whole animal experiments may therefore include an inhibition of CGRP neuroendocrine effects, but taken together with the perfusion data these studies indicate that, at least in the rat, amylin is a physiological inhibitor of insulin secretion.

Thus, amylin and NPY appear to be good candidates for powerful autocrine inhibitors of insulin secretion. Further characterization of the receptor subtypes mediating the effects of both of these peptides will be important in defining their intracellular mechanisms and developing specific antagonists.

**Figure 2**

**Time course of the effect of amylin and amylin(8–37) on insulin secretion from perifused rat islets**

Perifusate glucose concentration was 2.8 mM from 0 to 40 min and 160 to 200 min and 8 mM from 40 to 160 min with the addition of 0.2 μM carbachol. 40 μM amylin(8–37) was added from 80 to 120 min to treated chambers (●), and no peptide to control chambers (○). Results are the means of the log mean values (n = 6). Reproduced with permission from [17].

**Paracrine/neurocrine inhibitors of insulin secretion**

Somatostatin is produced by the islet δ-cells and acts in a paracrine manner to inhibit β-cell secretion, both basally and in response to glucose, glucagon and sulphonylureas. Treatment of isolated rat islets with somatostatin antisera increases insulin and glucagon release [28]. This is not an effect of circulating somatostatin since neutralization of this alone with antisera does not affect plasma insulin or glucagon levels [29]. Receptors for somatostatin are present on α-, β- and δ-cells [30]. The inhibitory effects of somatostatin on insulin secretion are pertussis toxin-sensitive and linked (probably via Ga1-1, Ga2-2 and Ga3) to inhibition of calcium influx and adenylate cyclase [31].

CGRP occurs in sensory, extrinsic, intra-pancreatic nerves innervating islets and blood vessels and is thought to be a neurocrine inhibitor of insulin secretion acting through a similar mechanism to amylin [32]. Rat CGRP has α and β forms which are the products of separate genes and differ by only one amino acid. We have shown that αCGRP is the predominant form in the central nervous system and sensory innervation and βCGRP is the major component of the gut intrinsic innervation [33]. CGRP-like immunoreactivity occurs in the islet, but capsaicin treatment, which would be expected to deplete all αCGRP, only accounted for half of the activity in the whole pancreas. We have
now demonstrated, using specific radioimmunoassay and Northern blotting, that islet CGRP is locally produced βCGRP [13]. Thus CGRP could also be involved in autocrine/paracrine actions.

Galanin is present in extrinsic adrenergic nerves innervating the islet [34]. It is a potent inhibitor of insulin secretion in many species including dog, rat, mouse and pig. This inhibition is mediated by the recently cloned G-protein-linked Gal-R1 receptor [35]. One of the species where galanin effects on insulin secretion have not been characterized is man. We infused high doses of galanin (150 pmol/kg per min) with no effect on plasma glucose or serum insulin [36]. The mechanism of galanin-mediated inhibition of insulin secretion is complex, involving effects on Ca2+- and K+-ATP-channels, inhibition of adenylate cyclase and events late in stimulus secretion coupling [31,34]. These effects may in turn require activation of up to four G-proteins by the same receptor [31]. Clearly the delineation of β-cell second messenger systems activated by galanin is an interesting problem.

**Amylin as a circulating regulator of glucose metabolism**

Amylin has been shown in vitro to cause 'insulin resistant' effects on glucose metabolism in skeletal muscle and liver. In the isolated rat soleus muscle amylin decreases insulin-stimulated glycogen synthase and glycogen content, activates glycogen phosphorylase and increases lactate output. These effects (for a review see [37]) are thought to be due to non-competitive antagonism of insulin by amylin in this tissue. Amylin has been proposed to act as a circulating regulator of glucose metabolism being particularly important in the insulin resistance seen in type-II diabetes where amylin secretion is increased. In vivo, amylin injection increases blood glucose possibly via increased muscle lactate output stimulating hepatic gluconeogenesis [38]. However, in these experiments 25 nmol of amylin, leading to an initial plasma concentration of 33 nM, were used, which is far in excess of physiological concentrations (2–20 pM). Injection of amylin at 500 pmol into rats and infusion of amylin at 500 pmol/kg per min (plasma concentration, 8.7 nM) into rabbits had no effect on glucose metabolism [39]. In the same paper we reported that CGRP (which mimics many of the in vitro effects of amylin on glucose metabolism) at 500 pmol was effective in increasing blood glucose. Much lower concentrations (200 pM) of amylin increased hepatic glucose output in the glucose-clamped rat model [40], but even here levels are 10-fold greater than those in normal plasma. In man, we infused amylin to achieve plasma concentrations in excess of 1 nM and found no effect on glucose tolerance [41]. We have repeated these experiments to achieve concentrations in excess of 2 nM and observed no effect on glucose metabolism, either using a glucose tolerance test [21] or the hyperinsulinaemic-euglycaemic clamp [42]. In transgenic mice overexpressing amylin to give 15-fold normal plasma concentration no hyperglycaemia was observed [43]. In a patient with a malignant pancreatic tumour expressing 400-fold control amylin levels a euglycaemic insulin clamp showed normal glucose disposal where glucose tolerance tests showed reduced insulin secretion [44]. Thus we conclude, at least in man, that the effects of amylin as a circulating hormone are less important than as an autocrine factor in inhibiting insulin secretion.

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