The adipoinsular axis: effects of leptin on pancreatic β-cells

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Kieffer, Timothy J., and Joel F. Habener. The adipoinsular axis: effects of leptin on pancreatic β-cells. Am. J. Physiol. Endocrinol. Metab. 278: E1–E14, 2000.—The prevalence of obesity and related diabetes mellitus is increasing worldwide. Here we review evidence for the existence of an adipoinsular axis, a dual hormonal feedback loop involving the hormones insulin and leptin produced by pancreatic β-cells and adipose tissue, respectively. Insulin is adipogenic, increases body fat mass, and stimulates the production and secretion of leptin, the satiety hormone that acts centrally to reduce food intake and increase energy expenditure. Leptin in turn suppresses insulin secretion by both central actions and direct actions on β-cells. Because plasma levels of leptin are directly proportional to body fat mass, an increase of adiposity increases plasma leptin, thereby curtailing insulin production and further increasing fat mass. We propose that the adipoinsular axis is designed to maintain nutrient balance and that dysregulation of this axis may contribute to obesity and the development of hyperinsulinemia associated with diabetes.

insulin secretion; diabetes

OVERVIEW

The ob/ob and db/db mouse strains are both characterized by the development of obesity, hyperinsulinemia, and hyperglycemia, a syndrome resembling type 2 diabetes mellitus (28, 29, 43, 62, 63, 79). By a series of parabiosis experiments thirty years ago, in which the circulations of two animals became partially fused (ob/ob with db/db; ob/ob with +/+; db/db with +/+), Coleman (29) predicted that the ob/ob mice could respond to an appetite-suppressing hormone that it could not produce itself, whereas the db/db mice could produce this satiety factor but could not respond to it. The ob gene was identified in 1994 by use of positional cloning techniques; it encodes a 167-amino acid product termed leptin (188), which is comprised of a four-helical bundle similar to that of the long-chain helical cytokine family (97, 187). It was discovered that mutations in the ob gene of ob/ob mice prevent the synthesis of a functional leptin protein (188).

Although leptin is expressed at low levels in gastric epithelium (6), placenta (120), muscle (178), and possibly the central nervous system (46, 113), it is predominantly produced by and secreted from adipose tissue (188). As a consequence of this circumstance, plasma leptin levels are directly proportional to fat mass (33, 36, 42, 77, 104, 118, 121). It now appears that leptin is the long-sought-after hormone that communicates information about adipocyte metabolism and body weight to the appetite centers in the hypothalamic regions of the brain. Treatment of leptin-deficient ob/ob mice with recombinant leptin markedly increases energy expenditure and decreases food intake, body weight, and adiposity (17, 69, 135, 166, 182). The exogenous administration of leptin to mice also attenuates the neuroendocrine responses to food restriction, indicating that leptin may also play an important role in signaling nutritional status during periods of food deprivation (1).

The leptin receptor was first isolated from the mouse by the expression cloning of complementary DNA prepared from the choroid plexus (171). Positional cloning of the leptin receptor gene (db) revealed that it encodes five alternatively spliced forms of mRNAs (110). As had been predicted earlier from the parabiosis experiments, abnormal splicing of the mRNA transcribed from the leptin receptor gene in db/db mice results in a truncated version of the otherwise longer signaling isoform (ObRb) (110). Therefore ObRb, the so-called long form of the leptin receptor, is essential for the weight-reducing effects of leptin. The functions of the other isoforms of the leptin receptor remain unclear. Notably,
however, it is now recognized that leptin receptor isoforms are widely distributed throughout peripheral organs (52, 66, 80, 115). These observations support the concept that leptin has peripheral actions independent of its actions on the central nervous system. In particular, the ObRb leptin receptor is expressed in the pancreatic β-cells that produce insulin, raising the possibility that leptin directly regulates insulin release (94). Because the adipogenic actions of insulin are well known, the idea was postulated that leptin may suppress insulin release as part of a bidirectional adipoin- sular axis (94).

The reader is referred to several comprehensive reviews of leptin biology (5, 19, 32, 56, 61, 170). In this review we present the evidence that supports the existence of an adipoinsonal axis in which leptin suppresses insulin production and secretion. The other limb of the proposed adipoinsonal axis, the stimulation of leptin production and secretion in adipose tissue by insulin, seems to be well established and will only be reviewed briefly. To place the concept of the adipoinsonal axis into a comprehensible body of understanding, it is helpful to describe briefly the biology of the endocrine pancreas and adipose tissue.

The Endocrine Pancreas

The endocrine pancreas consists of circumscribed clusters of endocrine tissue (islets of Langerhans) imbedded in the exocrine tissue of the pancreas. The islets are composed of at least four major phenotypically specific hormone-producing cells: the α-, β-, δ-, and PP-cells that produce glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. The islets are highly vascularized, with a rate of blood flow similar to that of the brain, and thus are designed to respond rapidly to changes in nutrients in the bloodstream that signal the islet cells to secrete, or not to secrete, hormones. The hormones insulin, glucagon, and somatostatin clearly have important physiological actions in the maintenance of blood glucose levels and the metabolism of glucose and other nutrients, such as amino acids and lipids. Insulin and glucagon exert opposing actions on nutrient metabolism and are secreted in a reciprocal fashion. Insulin is an anabolic hormone whose functions are manifested during the fed state. Insulin is secreted in response to meals and acts on its key target tissues, skeletal muscle, liver, and adipose tissue, to stimulate the uptake of glucose and to store calories predominantly in the form of glycogen and fat. Gluca-

Regulation of Leptin Production by Insulin

Because leptin is produced and secreted from adipocytes, and the relative body mass of most individuals is determined to a large extent by the fat mass, circulating leptin levels correlate directly with body mass index (BMI) (33, 36, 42, 77, 104, 118, 121). Those individuals with higher BMIs typically have a higher adipose tissue mass and therefore higher levels of circulating
leptin. This direct relationship between BMI and plasma leptin levels has been verified by correlating circulating leptin to body fat content (42, 104, 118). It is worth noting that the regression between body weight or body fat content and circulating leptin is not complete, in the range of 0.7–0.8, with a correlation, or r², of 0.5–0.6 (3), indicating that other factors must also be involved in leptin regulation. Furthermore, plasma leptin levels and leptin gene expression increase at night and decrease acutely during fasting (33, 98, 118, 150, 160). These observations cannot be adequately accounted for by corresponding changes in body fat content. In this regard a role for nutrients, hormones, and neurotransmitters in the regulation of leptin expression and secretion is emerging. For example, leptin production by white adipose tissue is stimulated by insulin and cortisol and attenuated by β-adrenergic agonists, CAMP, and thiazolidinediones.

Insulin has been considered to be a potential regulator of leptin, because plasma insulin concentrations decrease during fasting and increase after refeeding in parallel with plasma leptin levels. Current evidence suggests that insulin plays a chronic role in the regulation of leptin gene expression and production by white adipose tissue. Hyperinsulinemia increases plasma leptin levels and gene expression in white adipose tissue after many hours in rodents and humans (34, 99, 100, 119, 149, 150, 174, 176). Plasma leptin levels and leptin mRNA are also increased in subjects with insulinoma, and plasma leptin levels return to normal after removal of the insulinoma (35, 138). Insulin deficiency induced by streptozotocin results in diminished plasma leptin levels and leptin mRNA, and this suppression is rapidly reversed by treatment with insulin (11, 78, 86, 117, 134, 162). This action of insulin appears to be mediated, at least in part, directly at the level of adipocytes, because insulin also increases leptin secretion and mRNA levels in vitro (8, 64, 70, 99, 111, 129, 143, 148, 164, 177, 185), perhaps due to increased glucose transport and metabolism (126). A candidate transcription factor, adipocyte determination differentiation dependent factor 1/sterol regulatory element binding protein 1 (ADD1/SREBP1), linking changes in insulin levels to ob gene expression in mice, was recently reported (96). ADD1/SREBP1 expression increases upon treatment of adipocytes with insulin, and the increased ADD1/SREBP1 transactivates the leptin gene (96). Undoubtedly other transcription factors, including members of the C/EBP and PPAR families, are involved in the regulation of leptin gene expression by hormones such as insulin (47, 85). For additional reviews on the regulation of leptin production, see the following references (3, 31, 83, 85).

RATIONAL FOR PROPOSING AN ADIPOINSULAR AXIS

The hypothesis for the presence of an adipoinsular axis, or feedback loop (see Fig. 1) between adipose tissue and the endocrine pancreas (94), was generated based on the existence of literature on the leptin-deficient or -resistant ob/ob or db/db mouse, respectively. Studies spanning three decades have reported that in both of these obese, diabetic mouse models, hyperinsulinemia precedes the development of both obesity and insulin resistance (22, 25, 30, 43, 55, 62, 63, 68, 114). It seemed reasonable that, if in the absence of a functional leptin signal, the first metabolic abnormality observed is hyperinsulinemia, then perhaps in normal circumstances leptin acts to curtail insulin release. Physiologically such a pathway would be important in the regulation of fat deposition. Insulin is a major adipogenic hormone. Therefore, as fat stores increase, rising plasma leptin concentrations would reduce insulin levels, thereby directing less energy to the formation of adipose tissue. In the event that adipose stores diminish, falling plasma leptin levels would permit increased insulin production, thereby resulting in the deposition of additional fat. Therefore, it was postulated that leptin might be considered as a thermostat or set point to appropriately regulate insulin levels according to the extent of adiposity (94). Such a putative feedback loop must be superimposed on the well-established enteroinsular axis, whereby insulin secretion is acutely regulated during meals by way of the secretion of intestinal incretin hormones.

In support of the hypothesis that leptin may directly suppress insulin release from pancreatic β-cells are the observations that the administration of leptin for 5–30 days, either via injection, osmotic mini-pump, or gene therapy, lowers plasma insulin levels in ob/ob mice (112, 128, 135, 151, 166, 182). In a recent study, a single injection of leptin reduced plasma insulin levels in ob/ob mice by ~75% after only 24 h (152). Furthermore, a significant reduction in plasma insulin levels occurs within 10 min after the administration of leptin to ob/ob mice (103). This acute fall in plasma insulin
levels is accompanied by a concomitant increase in plasma glucose levels. By 2 h after a leptin injection, the plasma glucose excursion after an oral glucose challenge was significantly increased relative to saline-treated animals (71). Therefore, the acute reduction in plasma insulin levels after leptin treatment does not appear to be secondary to either diminished body weight or a fall in plasma glucose concentrations. Notably, however, leptin treatment for longer periods of time appears to normalize plasma glucose levels in hyperglycemic rodents (69, 72, 112, 128, 135, 151, 152, 166, 182). This effect of leptin appears to be mediated by an increase in insulin sensitivity at the levels of the liver and peripheral tissues (9, 13, 26, 92, 147, 154, 163, 179). Thus reductions in circulating insulin levels after several hours or days of leptin treatment likely result from both increased insulin sensitivity and a direct leptin-mediated reduction in insulin secretion.

The autonomic nervous system likely contributes to the inhibitory effect of leptin on insulin secretion. The administration of leptin in vivo inhibits neuropeptide Y (NPY) gene expression (151, 166) and secretion (166), which in turn could result in reduced insulin secretion by either inhibiting the parasympathetic or activating the sympathetic nervous system. In support of this notion, insulin levels are reduced by one-half in ob/ob mice deficient in NPY (45). Direct evidence has been obtained for the regulation by leptin of sympathetic inputs to pancreatic ß-cells (123). In vagotomized rats, leptin significantly suppresses the increase in plasma insulin levels after an intravenous glucose injection. However, the effect of leptin in vagotomized rats is abolished after chemical sympathicectomy (123). Therefore, it appears that leptin can regulate insulin secretion through the autonomic nervous system. However, there is now abundant evidence to support the hypothesis that leptin also directly modulates insulin secretion by actions on leptin receptors on pancreatic ß-cells.

DETECTION OF LEPTIN RECEPTORS IN ISLETS

There are multiple isoforms of leptin receptors (60, 110, 170). Although the short isoform of the receptor (ObRa) is capable of signaling (15, 127), the long isoform (ObRb) is currently believed to convey most of the physiological actions of leptin. The db/ db strain of mice has a genetic defect that results specifically in the absence of expression of ObRb, whereas other isoforms of the receptor are not altered (21, 27, 110). Given that the resultant phenotype closely resembles that of the leptin-deficient ob/ ob mice, including the presence of hyperinsulinemia, it seems probable that the actions of leptin on pancreatic islets are mediated largely by ObRb. This concept is substantiated by the observation that leptin has no insulin-lowering action either in vivo or in vitro in db/ db mice, whereas leptin lowers plasma insulin levels in ob/ ob mice studied in parallel (44, 103, 151).

To support the hypothesis of direct actions of leptin on pancreatic ß-cells, evidence must be provided for the presence of leptin receptors on these cells. On the basis of results of tissue surveys, it is often argued that the only tissue where ObRb is found in high abundance is the hypothalamus, raising doubts as to the possibility of peripheral actions of leptin (60, 170). It is important to consider, however, that pancreatic islets comprise only 1–2% of the entire pancreas; the majority of the pancreas consists of an exocrine organ in which large quantities of digestive enzymes are synthesized. Therefore, pancreatic islet RNA represents a minor fraction of total pancreatic RNA. Yet of 21 tissues examined by sensitive RNase protection assay, total pancreas appears to have the most abundant proportion of ObRb mRNA relative to ObRa mRNA of all the peripheral tissues examined, and it is only second in amount to the brain and hypothalamus overall (11% ObRb vs. 18% and 36%, respectively) (66). Thus, just as the proportion of ObRb mRNA is considerably enriched in hypothalamic preparations compared with total brain, the same is true of islet ObRb mRNA compared with total pancreas. Messenger RNA for ObRb has been found in relatively high abundance in murine pancreatic islets but was not detected in whole pancreas by RNA blot hybridization because of the limited sensitivity of the technique used and the dilution of islet-specific RNA by exocrine pancreas RNA (44).

By use of a probe common to all leptin receptor isoforms, leptin receptor mRNA was detected in rat islets in greater abundance than was found in total brain (94). Leptin receptor mRNA was also observed by RT/PCR in rat islets and the tumor-derived ß-cell line ßTC3 (94). Expression of a functional protein was confirmed by the observation that ßTC3 cells bind 125I-labeled leptin (94). These initial observations support the hypothesis of the existence of an adipoinsular axis. The presence of leptin receptors in pancreatic islets has since been confirmed by a variety of techniques. Although shorter isoforms of the leptin receptor may predominate, clearly ObRb is expressed in pancreatic ß-cells (44, 51, 89, 95, 101, 103, 107, 133, 136, 157, 169, 189). The distribution of leptin receptors within rat pancreatic islets was examined using antisera generated against an extracellular epitope of the leptin receptor. Leptin receptor immunoreactivity was observed on the majority of ß- and ð-cells, but not on glucagon-producing a-cells (95). Similar results were obtained using leptin tagged with the fluorescent compound iodocarbocyanine (Cy3) (95). The function of leptin receptors expressed on somatostatin-secreting ð-cells remains to be determined.

ACTIONS OF LEPTIN ON PANCREATIC ISLETS

Insulin Secretion

The effects of leptin on insulin secretion have been studied in several different experimental settings, with variable and sometimes apparently conflicting results. The pancreatic islets (ß-cells) of ob/ ob mice have been particularly useful in investigating the effect of leptin on insulin secretion, probably as a consequence of upregulation of the leptin receptor signaling in these leptin-deficient mice. In contrast, the ß-cells of normal rodents are chronically exposed to leptin and thus may...
be relatively less responsive under acute experimental conditions. The variable ways in which experiments have been performed often make the findings difficult to compare, and many uncertainties remain regarding optimal models and conditions. Other problematic issues remain, such as the considerable time delay for the action of leptin on β-cells (76, 95) and uncertainties regarding the stability/bioactivity of various leptin solutions. Leptin circulates bound to one or more proteins, which may alter its bioavailability (40, 84, 161). In one study (103), it was suggested that the presence of bovine serum albumin or fetal calf serum may diminish or even obliterate the effects of leptin. Furthermore, it remains unclear why relatively high doses of leptin are required to induce weight loss (17, 69, 135, 166). In β-cells, leptin appears to exhibit a biphasic dose-response curve. In rat islets ~ 2 nM leptin maximally suppresses insulin release, whereas high concentrations are ineffective (133, 189). Until these issues have been resolved, it will be difficult to reconcile all of the observations discussed below.

The application of leptin (6.25–100 nM) for 1–2 h to pancreatic islets isolated from ob/ob mice reduces insulin secretion from ~13 to almost 80% (44, 95). Leptin also significantly reduces insulin release from the perfused pancreas of ob/ob mice (44), a standard, reliable model to assess islet function. Notably, in ob/ob islets, the suppressive effect of leptin may be reduced at higher glucose levels and is absent in the presence of the incretin hormone GLP-1 (24, 95). One explanation for this observation is that leptin acts primarily during fasting to dampen insulin secretion and that this tonic inhibition is overcome by the nutrient and incretin signals (e.g., glucose and GLP-1) that accompany feeding (95). Leptin attenuates insulin release from ob/ob islets stimulated by either acetylcholine or the phorbol ester phorbol-12-myristate 13-acetate (PMA), both activators of protein kinase C (PKC) (24). At concentrations as low as 2.5 nM, leptin partially constrained acetylcholine-induced insulin secretion from islets of ob/ob mice, with an IC50 (effective concentration) of ~5 nM. The finding that leptin curtails acetylcholine-induced insulin secretion may in part explain why islets from leptin-deficient ob/ob mice have enhanced sensitivity to PKC-stimulated insulin secretion (22, 23).

In contrast to β-cells of leptin-deficient ob/ob mice, the β-cells in normal rodents are continually exposed to leptin and therefore may be expected to be less sensitive. Overall, the reports regarding the effect of leptin on insulin secretion in normal rodents are conflicting. There were no significant effects of leptin (0.625–100 nM) on insulin release from isolated rat or mouse islets at glucose concentrations ranging from 1.7 to 16.7 mM (93, 136) or the perfused rat pancreas with 8.3 mM glucose and leptin at either 1.0 nM (107) or 10.0 nM (108, 109). In one study, leptin (1 nM) stimulated insulin release from isolated rat islets incubated in 5 mM glucose (169). However, in the majority of studies, leptin (1–20 nM) significantly reduced insulin release from the perfused rat pancreas and isolated rat or mouse islets in the presence of either low (2.8–3.3 mM) (51, 123), normal (5.5–8.0 mM) (87, 103), or high (10.0–20.0 mM) glucose (44, 51, 123, 132, 133, 144). There are no clear answers for these disparate observations.

Insulin secretion stimulated by agents that elevate cellular concentrations of cAMP, such as glucose + IBMX, a phosphodiesterase inhibitor, or forskolin and GLP-1, is inhibited by leptin during both static incubations and perfusions of mouse or rat islets (49, 136, 189). These observations suggest that in normal rodents, leptin antagonizes cAMP signaling. Leptin also suppresses the elevations of cellular cAMP levels that occur in response to exposure of β-cells to GLP-1 or to forskolin (189). A mechanism proposed to explain this action of leptin on cAMP levels is a phosphoinositide 3-kinase (PI 3-kinase)-dependent activation of phosphodiesterase 3B (PDE3B) and subsequent reduction of the intracellular cAMP (189). Notably, leptin restraints insulin secretion from both mouse ob/ob islets (24) and rat islets (132) induced by PMA, a PKC activator. Therefore, it appears that leptin may antagonize insulin secretion from β-cells by interacting with both cAMP-dependent protein kinase A (PKA) and PKC signaling pathways.

Studies of cultured human islets in vitro indicate that they respond to leptin in a manner similar to that of mouse and rat islets. Leptin suppresses insulin secretion from human islets at concentrations as low as 0.01 nM (48, 103, 153). Thus it appears from these findings in studies of human islets that humans are likely to have a functional adipoinferior axis.

The effects of leptin on insulin secretion have also been investigated in various β-cell lines derived from islet cell tumors (insulinomas). The results obtained in different insulinoma cell lines have been contradictory and less consistent than those of studies in cultured islets and the perfused pancreas. In mouse MIN6 cells and hamster HIT-T15 cells, murine leptin increased insulin secretion (157, 169). In contrast, in other studies, leptin (2 nM) inhibited both glucose and GLP-1-stimulated insulin secretion from HIT-T15 (189), rat RIN5AH, rat RINm5F, and mouse βTC6 cells (103). The inhibition of insulin secretion by leptin in βTC6 cells was observed at 10 min and achieved a maximum by 60 min. In the rat insulinoma cell line INS-1, leptin significantly reduced insulin secretion stimulated by agents that increase intracellular content of cAMP (i.e., GLP-1), but not insulin secretion stimulated by activators of PKC (i.e., carbachol) (2, 49). These latter observations suggest that, in the INS-1 cell line, the cAMP-PKA signal transduction pathway is a target by which leptin may inhibit insulin secretion. A proliferative response to leptin was observed in RINm5F cells and mouse MIN6 cells, as measured by [3H]thymidine incorporation (89, 168). Notably, however, incubation of mouse islets with 100 nM murine leptin for 48 h had no effect on islet cell replication, as assessed by the same [3H]thymidine incorporation assay (93). Thus leptin
responses in transformed cell lines may not necessarily reflect the response of native β-cells.

**Insulin Gene Expression**

In a few studies, leptin had no effect on insulin biosynthesis (93, 144), and in one other study (157) leptin (unreported source) treatment increased preproinsulin mRNA levels in HIT-T15 cells. However, leptin has been shown to suppress preproinsulin mRNA expression in isolated rat islets (103, 133), in mouse βTC6 cells (103), in ob/ob mouse islets, in the rat pancreatic β-cell line INS-1 (152), and in human islets (153). When ob/ob mice are injected intraperitoneally with murine leptin (1 µg/g body weight), steady-state levels of preproinsulin mRNA are reduced by 40% after 24 h compared with vehicle-treated animals (152). Preproinsulin mRNA levels are also reduced by 50% in isolated ob/ob islets incubated with leptin (6.25 nM) for 24 h, suggesting that this action of leptin is mediated at least in part by a direct interaction with receptors on β-cells (152). In studies of human islets, human leptin (6.25 nM) evokes a time-dependent decrease in preproinsulin mRNA levels in the presence of 11.1 mM glucose, but not 5.6 mM glucose (153); by 24 h, the level of preproinsulin mRNA declines to 25% of the initial value. GLP-1 stimulates transcription of the rat insulin gene and increases preproinsulin mRNA levels in β-cells (41, 50). Remarkably, leptin (6.25 nM) inhibits GLP-1-stimulated expression of preproinsulin mRNA in human islets incubated in conditions of both 5.6 and 11.1 mM glucose (153). At 16 h, GLP-1-stimulated preproinsulin mRNA levels are inhibited by 80%.

Incubation of INS-1 cells with leptin (0.625 nM or 6.25 nM) for 16 h significantly reduces preproinsulin mRNA expression when incubated at 25 mM glucose, but not 5.6 or 11.1 mM glucose (152). These findings indicate a dependence of leptin-mediated reductions of preproinsulin mRNA expression on glucose augmentation. However, as with human islets, the stimulation of preproinsulin mRNA expression by GLP-1 (10 nM) is also inhibited by leptin (6.25 nM) at both 5.6 and 11.1 mM glucose (152). Of note, there was no effect of leptin on steady-state levels of preproinsulin mRNA after a 6-h incubation.

The effect of leptin on the transcriptional activity of the insulin gene promoter has been examined. A reporter vector expressing the luciferase gene under control of 410 bp of the rat insulin I gene promoter was transfected into INS-1 cells. Leptin (6.25 nM) inhibited reporter gene expression at 25 mM glucose by 50%, but no such inhibition was observed at 5.6 or 11.1 mM glucose (152). However, the induction of transcriptional activity by GLP-1 (10 nM) in the presence of 11.1 mM glucose was nearly completely inhibited by leptin. These observations indicate that activation of transcription by either GLP-1 or high glucose (25 mM) restores the inhibitory actions of leptin on rat insulin I promoter activity at a glucose concentration (11.1 mM) in which leptin alone fails to reduce insulin promoter activity (152).

**MECHANISM OF LEPTIN ACTION ON β-CELLS**

The leptin receptor gene encodes multiple splice variants of the leptin receptor (ObRa–ObRf), which closely resemble the class I cytokine receptors (110, 170, 171). The longest form of the leptin receptor, ObRb, contains intracellular consensus motifs for interaction with cytoplasmic protein kinases, known as JAKs (Janus kinase), and protein transcription factor kinase substrates of the signal transducers and activators of transcription (STAT) family. Leptin appears to interact with homo-oligomerized ObRb (10, 183, 184), resulting in activation of JAK2 (65), which phosphorylates a number of substrates, including ObRb. When phosphorylated, ObRb serves as a scaffold for recruitment of STATS 1, 3, 5, and 6 are all reportedly activated by leptin (10, 66, 125, 158, 175). Signaling capabilities have also been reported for short (ObRa) isoforms of the leptin receptor (15, 127). However, because leptin has no effect on islets isolated from db/db mice, which harbor mutations resulting in aberrant mRNA that encodes only a shortened form of ObRb (21, 110), the direct effects of leptin on β-cells are likely mediated via ObRb.

A brief review of intracellular signaling pathways within pancreatic β-cells is required to interpret possible pathways involved in leptin signaling. Insulin secretion from pancreatic β-cells is controlled in part by the activity of K\textsubscript{ATP} channels. Closure (inactivation) of K\textsubscript{ATP} channels in response to glucose or other insulin secretagogues depolarizes β-cells, resulting in the activation of voltage-dependent Ca\textsuperscript{2+} channels, a rise in cytosolic calcium concentration ([Ca\textsuperscript{2+}]\textsubscript{i}), and insulin secretion (4). Glucose metabolism within β-cells also results in an elevation of fatty acyl-CoA esters, which synergize with rises in [Ca\textsuperscript{2+}]\textsubscript{i} to cause induction of insulin release (139). Glucose-induced insulin secretion is further potentiated by hormone-mediated elevation of the intracellular second messengers cAMP/PKA (81) and phospholipase C/PKC (186). Thus there are multiple potential sites for leptin signaling to influence insulin secretion.

Clues to the cellular mechanisms by which leptin suppresses insulin release come from earlier studies utilizing ob/ob and db/db mouse islets. Notably, over two decades ago it was reported that islets from db/db mice are partially depolarized, even in the absence of glucose (122). This circumstance is associated with elevated basal insulin release and unresponsiveness to further elevation of the glucose concentration (122). The K\textsuperscript{+} permeability of both ob/ob and db/db islets is relatively insensitive to changes in glucose (12, 59, 145), yet this insensitivity does not appear because of a failure of the cells to express functional K\textsubscript{ATP} channels (58, 102). Because these phenotypes both result from the absence of leptin signaling, it was postulated that leptin may normally open (activate) K\textsubscript{ATP} channels on
β-cells and thereby hyperpolarize the membrane. Such a mechanism would be consistent with the observations that leptin reduces insulin secretion (95).

Patch-clamp electrophysiological analyses on both normal and ob/ob mouse β-cells show that the application of leptin (1–10 nM) to single mouse β-cells (95) or the CRI-G1 cells (75, 76) consistently results in hyperpolarization, consistent with an inhibitory action of leptin on insulin secretion. The effect of leptin is delayed by 10–15 min, similar to that observed previously for potassium channel activation by Janus kinase-associated prolactin receptors in Chinese hamster ovary cells (140). Leptin also increases membrane conductance, consistent with the opening (activation) of ATP-sensitive K channels (KATP channels). The activation of KATP channels by leptin is further supported by the observations that the leptin response is sensitive to the application of the sulphonylureas tolbutamide and glibenclamide, selective blockers of KATP channels. Leptin-induced increases in membrane conductance and the resulting hyperpolarization are immediately and completely reversed by the application to β-cells of tolbutamide (100 µM) or glibenclamide (0.5 µM) (73, 75, 76, 95). Notably, in single ob/ob mouse β-cells, the increase in conductance is biphasic in nature (95). The reversal potential associated with the increase in cell conductance in response to leptin is characteristic of the activation of a potassium current (75, 76, 95).

A confirmation of the KATP channel as the molecular target for action of leptin in β-cells was obtained from single-channel recordings. Application of leptin (1–10 nM) induces an increase in the activity of single potassium channels that are sensitive to ATP and sulphonylureas (76, 95). Leptin increases the KATP open channel probability with no significant effect on open times (95). Notably, the activation of KATP channels in CRI-G1 cells by leptin is reversed by nanomolar concentrations of insulin (74). This effect is specific for leptin, because insulin fails to prevent opening of KATP channels by diazoxide (74). These observations suggest that leptin suppresses insulin secretion during fasting, when ambient insulin levels are low (74). However, during feeding, the resultant increases in insulin levels inhibit the opening of KATP channels by leptin and prevent the inhibition of insulin secretion. Such a model could explain in part the apparent resistance to leptin actions observed in obese individuals who are typically hyperinsulinemic, even during fasting.

Given the important role of KATP channels in determining [Ca2+]i in β-cells, it is anticipated that application of leptin to β-cells results in a decrease in [Ca2+]i. A fall in [Ca2+]i occurs in both mouse and human single β-cells after application of leptin (6.25 nM) (95, 153). Notably, this fall in [Ca2+]i is reversed by co-incubation of the β-cells with 20 mM glucose and GLP-1 (10 nM) (95). A 15-min preincubation of INS-1 cells with leptin reduces the subsequent rise in [Ca2+]i in response to either forskolin (1 µM) or GLP-1 (10 nM) (49). Leptin (10 nM) also attenuated glucose-induced elevations in [Ca2+]i in mouse islets (51). Of note, leptin also hyperpolarizes glucose-receptive hypothalamic neurons via opening (activation) of KATP channels (165). Therefore, the KATP channel may function as the molecular end point of the leptin signaling pathway in both hypothalamic neurons and pancreatic β-cells.

By what mechanisms might leptin open KATP channels and thereby lower [Ca2+]i and inhibit insulin secretion? Dialysis with AMP-PNP, a nonhydrolyzable analog of ATP that does not serve as a substrate for protein kinases, prevents leptin activation of KATP channels, indicating that phosphorylation or dephosphorylation is involved (75). The serine/threonine-specific protein phosphatase inhibitors okadaic acid (50 nM) and cyclosporin A (1 µM) fail to block activation of KATP channels by leptin (75). In contrast, whole cell dialysis with the tyrosine phosphatase inhibitor orthovanadate (500 µM) prevents the actions of leptin, suggesting that protein phosphorylation of tyrosine, but not serine/threonine, residues underlies actions of leptin in CRI-G1 cells. Furthermore, inhibition of tyrosine kinase activity by application of genistein (10 µM), tyrphostin B42 (10 µM), or herbimycin A (500 nM) mimics the leptin-induced hyperpolarization and increase in K+ conductance (75). Therefore, the mechanism underlying the opening of KATP channels by leptin may involve inhibition of tyrosine kinases or activation of tyrosine phosphatases and subsequent tyrosine dephosphorylation. Because the opening of KATP channels by leptin is blocked by wortmannin (1–10 nM) and LY-294002 (10 µM), inhibitors of PI 3-kinase, the mechanism underlying leptin activation of KATP channels appears to involve dephosphorylation of cytosolic proteins before activation of PI 3-kinase (74).

Further support for the concept that leptin activates PI 3-kinase is provided by the findings that treatment of insulinoma HIT-T15 cells with murine leptin (2 nM) for 30 min results in a threefold activation of PI 3-kinase (189). PDE3B, which reduces the cellular content of cAMP, is also activated by leptin. Thus, in HIT-T15 cells, leptin (1–5 nM) suppresses the elevation of cAMP induced by GLP-1 (5 nM) (189). This suppression is a PI 3-kinase-dependent process, because the addition of the PI 3-kinase inhibitors wortmannin (20 nM) or LY-294002 (2 µM) abolishes the activation of PDE3B by leptin and the inhibitory effect of leptin on glucose- and GLP-1-stimulated insulin secretion from the HIT cells (189). Furthermore, treatment of HIT cells with either miRlnone (1 µM) or exonimone (1 µM), selective inhibitors of type 3 PDE, completely blocked the inhibitory effect of leptin on glucose- or GLP-1-potentiated insulin secretion. These findings suggest that the inhibitory actions of leptin on insulin secretion are primarily mediated through the PI 3-kinase-dependent activation of PDE3B and a subsequent reduction of the intracellular cAMP (189). Because cAMP potentiates insulin secretion in part by closing (inactivating) KATP channels (81, 82), a reduction of cAMP by leptin is consistent with the activation of KATP channels by leptin.

Another mechanism by which leptin may open KATP channels is through lipid metabolism in β-cells. It has been proposed that leptin increases the storage of
triglycerides in adipocytes to maintain a constant level of intracellular triglycerides in nonadipocytes, such as β-cells (173). Culture of isolated islets with leptin depletes the content of triglycerides and increases the oxidation of free fatty acids (101, 156). This lipopgenic action of leptin is not observed in islets isolated from leptin-resistant fa/ fa Zucker diabetic fatty rats but is reestablished upon the restoration of expression of the leptin receptor (ObRb) in the islets (155, 180, 181). This action of leptin appears to be dependent on formation of fatty acyl-CoA. Remarkably, long-chain acyl-CoA esters (LC-CoA), the metabolically active form of free fatty acids, bind to and open K_{ATP} channels in pancreatic β-cells (16, 105). Therefore, it is possible that elevations in LC-CoA within β-cells after exposure to leptin result in activation of K_{ATP} channels and thereby inhibition of insulin secretion.

The inhibitory effects of leptin on preproinsulin gene expression appear to be independent of the activation of K_{ATP} channels. Diazoxide, which also opens K_{ATP} channels, has no effect itself on preproinsulin mRNA levels in INS-1 cells and does not alter the suppressive effect of leptin (152). Furthermore, inhibition of insulin promoter activity in INS-1 cells at 25 mM glucose was detected in both the presence and absence of K_{ATP} channel activation by diazoxide (152). These findings indicate that inhibition of preproinsulin gene expression by leptin is independent of the opening of K_{ATP} channels and suggests that the molecular mechanisms underlying inhibition of insulin secretion and preproinsulin mRNA by leptin are mediated by different intracellular signaling pathways.

Activation of STAT proteins in β-cells after leptin treatment is clearly a candidate pathway for the mechanism of action of leptin on insulin gene expression. Treatment of RINm5F cells and isolated rat islets with leptin induces the binding of STATs to a labeled oligonucleotide containing a high-affinity binding element for STAT1 and STAT3 (124). This binding is not altered by treatment with plasminogen activator (PMA, a PKC activator; 100 nM) but is reduced by treatment with acetycholylne (10 µM), ionomycin (calcium ionophore; 1 µg/ml), forskolin (adenylyl cyclase activator; 10 µM), and IBMX (cAMP phosphodiesterase inhibitor; 50 µM). The activation of STAT3 is suggested by the observation that treatment of rat pancreatic islets with leptin produces an increase in the tyrosine phosphorylation of STAT3 (124). Similarly, in MIN6 cells, leptin (5 nM) increases tyrosine phosphorylation of Stat1 and Stat3, but not Stat5 (168). Leptin also increases the transcriptional activation of reporter plasmids containing STAT3 binding elements, but not the STAT5 consensus element, in RINm5F cells (124). These observations implicate STAT3 as a potential mechanism by which leptin regulates gene expression in β-cells. Furthermore, the STAT3 pathway appears to be antagonized by elevations of [Ca^{2+}]_i and cAMP.

That STAT proteins may mediate the inhibition of insulin gene expression after leptin treatment was shown by DNA-binding assays in which sequences between −307 and −410 bp of the rat insulin I gene promoter bind multiple protein complexes contained in nuclear extracts from leptin-treated ob/ob islets. One of the complexes contained STAT5b, which formed on a previously described consensus STAT binding site (152). Although STAT proteins are generally considered to be activators of gene transcription, they may also be inhibitory, depending on the promoter context and cell type. For example, STAT5b stimulated by prolactin induces the β-casein promoter but inhibits the interferon regulatory factor-1 promoter (116).

In addition to activating a STAT pathway within pancreatic β-cells, the leptin receptor appears to be coupled to a mitogen-activated protein kinase (MAPK) pathway in MIN6 and RINm5F cells (124, 168). The phosphorylation of MAPK in response to leptin was
observed with 0.3 nM leptin and was maximal with 5 nM leptin (168). However, the activation of MAPK by leptin may be limited to tumor-derived β-cell lines, because treatment of rat islets with concentrations of leptin up to 100 nM did not result in activation of MAPK (124). Please see Fig. 2 for a summary of leptin signaling pathways within pancreatic β-cells.

**CONCLUDING REMARKS**

Leptin is an important controller of food intake and energy expenditure by its actions on receptors located in regions of the hypothalamus that regulate feeding behavior. There is now growing evidence that leptin also acts to suppress insulin production from pancreatic β-cells. Because insulin is adipogenic and increases the expression of leptin, there is a bidirectional feedback loop between adipose tissue and pancreatic islets, termed the adipoinious axis. The majority of evidence in support of an adipoinsular axis has come from studies in rodents or cell lines, but there is also evidence to suggest that a similar pathway exists in humans. Given the well-known direct relationship between increased adiposity (leptin) and increased insulin levels (7, 88, 137, 167), it has been questioned whether leptin inhibits insulin release in humans (18). If it did, why would obese hyperleptinemic individuals be hyperinsulinemic?

It is generally believed that hyperinsulinemia is simply a compensatory response to insulin resistance (20, 38, 39, 53, 67, 90, 91, 131, 142, 172). Hyperglycemia is believed to occur when β-cells can no longer compensate adequately for insulin resistance, resulting in the development of diabetes. However, many recent studies challenge this dogma because of the observations that hyperinsulinemia appears to precede decreases in insulin sensitivity and perhaps even the development of obesity in humans, thus arguing against the belief that obesity-induced insulin resistance fully explains the development of type 2 diabetes (54, 106, 130, 159). Hyperinsulinemia promotes adipogenesis and may initiate a cycle of increasing insulin resistance, compensatory hyperinsulinemia, and a progression to the diabetic state.

In obese individuals, sustained elevated levels of plasma leptin are proposed to uncouple leptin actions on its receptors in the hypothalamus, thereby attenuating signal transduction pathways that exert the effects of the hormone on satiety and energy expenditure (14, 57). Given that leptin suppresses insulin secretion by autonomic outputs originating in hypothalamic circuitry, breakdown of the central leptin signal may contribute to hyperinsulinemia. By analogy to leptin actions on the hypothalamus, in conditions of increasing adiposity and prolonged elevated plasma leptin levels, the leptin signaling system in pancreatic β-cells may become unresponsive. Such a putative loss of leptin reception by β-cells could result in dysregulation of the adipoinsular axis and a corresponding failure to suppress insulin secretion, resulting in chronic hyperinsulinemia. Additionally, the hyperinsulinemia exacerbates the obesity by increasing adipogenesis and increasing leptin production. This positive feedback loop of leptin desensitization at both the hypothalamic and pancreatic β-cells may result in hyperphagia and hyperinsulinemia, respectively, and thus may be an important factor in the pathogenesis of obesity-associated diabetes mellitus. Furthermore, it has recently been proposed that such dysregulation of the adipoinsular axis may occur independently of obesity in subjects who are susceptible to the development of diabetes (88). Thus investigation of the adipoinsular axis dysregulation hypothesis may lead to the identification of determinants of both human obesity and diabetes.

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