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Leptin and Insulin Secretion

Leptin was the first described of a growing number of hormones produced by adipose tissue (Rajala and Scherer, 2003; Havel, 2004). Leptin is integrally involved in the regulation of energy balance *via* its effects in the central nervous system to inhibit food and enhance energy expenditure. Circulating leptin concentrations are proportional to body adiposity and, consequently, leptin levels are increased in obesity. However, leptin production and its circulating concentrations respond acutely to feeding and fasting, independently of body adiposity. Decrease of leptin during restriction of energy intake appear to play a major role in the adaptation to starvation (Havel, 2004). In addition to its central action to influence energy homeostasis, leptin has diverse effects in a number of peripheral tissues (Margetic et al., 2002). One such effect is its influence on islet function. The interest in potential effects of leptin on islet function and glucose homeostasis originates from earlier findings that the leptin deficient ob/ob mice are characterized not only of obesity but also of insulin resistance, hyperglycemia and severe hyperinsulinemia (Coleman, 1978). In addition, administration of leptin to ob/ob mice reduces the hyperinsulinemia prior to changes in glycemia or body weight. Based on these results, it has been proposed that leptin may have direct actions to inhibit insulin secretion (Kulkarni et al., 1997; Seufart et al.,1999a,b). This idea has been reinforced by studies demonstrating that islet beta cells express the active long isoform of the leptin receptor (Ob-Rb), that Ob-Rb mRNA is present in islet homogenates (by RT-PCR) (Kieffer et al., 1996; Emilsson et al., 1997) and by immunocytochemical localization of Ob-Rb immunoreactivity in islet beta cells (Kieffer et al., 1997).

In studies undertaken to investigate the direct action of leptin on insulin secretion, an inhibitory action of the leptin was demonstrated when isolated rodent islets, perfused rat pancreas preparations, or insulin cell lines were exposed to leptin at concentrations of 1–1000 nmol/l (Chen et al., 1997; Emilsson et al., 1997; Fehmann et al., 1997; Ishida et al., 1997; Kieffer et al., 1997; Kulkarni et al., 1997; Mizuno et al., 1998; Ahrén and Havel, 1999a,b). Leptin was also shown to inhibit insulin secretion in isolated human islets (Kulkarni et al., 1997). In some studies, the inhibitory action of leptin was demonstrated at low, medium or high glucose concentrations (Emilsson et al., 1997; Fehmann et al., 1997; Ishida et al., 1997; Kulkarni et al., 1997; Mizuno et al., 1998). In contrast, other studies reported that leptin did not inhibit glucose-stimulated insulin secretion although it did inhibit insulin secretion induced by other secretagogues, including as acetylcholine or agents that increase intracellular cAMP levels (Chen et al., 1997; Ahrén and Havel, 1999a). In several *in vivo* studies, leptin has been shown to inhibit insulin secretion, not only in the ob/ob mice (Kulkarni et al., 1997; Seufart et al., 1999a,b) but also in normal mice (Kulkarni et al., 1997) and rats (Cases et al., 2001).

It has been suggested that the effect of leptin to inhibit insulin secretion is mediated by hyperpolarization induced by opening of ATP-regulated K^+-channels in beta cells. For example, incubation of single mouse beta cells with leptin, leads to opening of these K^+-channels and this effect is reversed by sulphonylurea, which inhibit ATP sensitive K^+-channels (Kieffer et al., 1997). The hyperpolarization induced by opening of the K^+-channels results in closure of the voltage-sensitive Ca^{2+}-channels which diminishes the uptake of extracellular Ca^{2+} and therefore reduces cytoplasmic concentrations of Ca^{2+}. In accord with this proposed mechanism, leptin has been shown to reduce cytoplasmic Ca^{2+} levels in beta cells (Fehmann et al., 1997; Kieffer et al., 1997; Seufart et al., 1999a,b). The opening of ATP-regulated K^+-channels by leptin has been reported to be blocked by inhibition of PI-3-kinase and by inhibitors of tyrosine phosphatase activity (Harvey and Ashford, 1998; Harvey et al., 2000), suggesting that leptin opens the K^+-channels by tyrosine phosphorylation, most likely at a point prior to activation of PI-3-kinase. This may be similar to leptin's action in hypothalamic neurons where it has also been shown to activate PI-3-kinase (Spanswick et al., 2000).

In addition to its effects on ATP-regulated K^+-channels, leptin has been shown to inhibit insulin secretion induced by the protein kinase A (PKA) pathway. This was demonstrated in rat insulinoma (INS-1) cells, where leptin markedly inhibited insulin secretion induced by forskolin, glucagon-like peptide-1, pituitary adenylate cyclase activating polypeptide, dibutyryl cAMP or inhibition of phosphodiesterase (Ahrén and Havel, 1999a). The common mechanism of action of these agents is that they all increase cytoplasmic cAMP levels. In contrast, leptin did not inhibit insulin secretion induced by agents without effects on cyclic AMP, such as cholecystokinin or the muscarinic agonist, carbachol (Ahrén and Havel, 1999b). Si-

milarly, in isolated rodent islets, leptin inhibited glucose-stimulated insulin secretion only in the presence of the inhibitor of phosphodiesterase, 3-isobutyl 1-methylxanthine (IBMX), which enhances intracellular cAMP levels (Poitout et al., 1998). In rat islets and in hamster insulinoma cells (HIT-T15), leptin has been shown to activate a subspecies of phosphodiesterase (PDE3B) which reduces the cellular cAMP content through a PI3-kinase sensitive mechanism. In addition, selective inhibition of PDE3B prevents leptin from inhibiting insulin secretion (Zhao et al., 1998). Therefore, leptin may promote the degradation of cellular cAMP by PI3-kinase-induced PDE3B in beta cells. A link between cAMP and closing of K^+-channels has been suggested (Holz and Habener, 1992). It is therefore possible that the inhibitory action of leptin on insulin secretion involves crosstalk with the cAMP pathway leading to opening of K^+-channels.

An important action of leptin in a number of tissues is the stimulation of lipid oxidation resulting in lowering of intracellular lipid stores (Unger et al., 1999). This action might also contribute to its inhibitory action in beta cells, since glucose-stimulated insulin secretion has been shown to be partially mediated by a lipid-derived signal (Prentki and Corkey, 1996; McGarry and Dobbins, 1999). A reduction of the lipid storage in beta cells by leptin might interfere with the actions of lipid-derived signalling to augment or mediate glucose-stimulated insulin secretion thereby leading to diminished insulin secretion. Indeed, leptin has been shown to reduce the lipid content in islets (Koyama et al., 1997; Shimabukuro et al., 1997), but whether this is causally related to inhibition of insulin secretion is not clear.

In summary, evidence from numerous studies suggest that leptin inhibits insulin secretion by a direct action on the beta cells mediated by activation of PI3-kinase, by opening of ATP-regulated K^+-channels, by impairment of the cyclic AMP-PKA-pathway and perhaps by reduction of beta cell lipid content (Kieffer and Habener, 2000). However, the complete picture of the putative beta cell action of leptin is more complex, since there are several reports of results that are inconsistent with these proposed mechanisms. For example, some studies have shown that leptin does not inhibit glucose-stimulated insulin secretion, yet it inhibits insulin secretion stimulated by acetylcholine (Chen et al., 1997) or by agents that increase cAMP levels (Poitout et al., 1998; Ahrén and Havel, 1999a) whereas other studies have shown that leptin inhibits glucose-stimulated insulin secretion without inhibiting insulin secretion induced by sulphonylurea exposure (Kulkarni et al., 1997). In addition, a stimulatory effect of leptin has been reported on insulin secretion from isolated rat islets (Tanizawa et al., 1997) and there are several other studies reporting no effect of leptin on insulin secretion (Leclerq-Meyer and Malaisse, 1997; Karlsson et al., 1998; Leclerq-Meyer and Malaisse, 1998). Although the reason for these divergent results is unclear, one possible explanation is that different experimental conditions, including the type of system used to study insulin secretion, affects the degree of expression of Ob-Rb receptor. Alternatively, the use of different preparations of leptin with varying degrees of biological activity as well as the possibility of concentration-dependent actions of leptin may explain the contradictory results. In order to address these discrepancies, standardized experiments examining the effects of leptin over a wide range of leptin concentrations are necessary. In addition the biological activity of the leptin preparation employed in each study should be assessed.

In addition to these direct actions of leptin on beta cell function, leptin may affect insulin secretion indirectly through activation of the sympathetic nervous system. Leptin is known to activate the sympathetic nervous system (Haynes et al., 1997a,b; Tang-Christensen et al., 1999) and activation of the sympathetic nervous system is well known to inhibit insulin secretion (Ahrén, 2000). Evidence that leptin can inhibit insulin secretion *via* SNS activation is provided by a study showing that chemical sympathectomy induced by administration of 6-hydroxydopamine prevents leptin from inhibiting insulin secretion in vagotomized rats (Mizuno et al., 1998). Similarly, leptin increases circulating insulin levels in fasted mice by an action that is prevented by chemical sympathectomy (Ahrén and Havel, 1999b). Therefore, leptin has the potential to inhibit insulin secretion both *via* a direct effect on the beta-cell and indirectly by activation of the sympathetic nervous system. Indirect actions of leptin to inhibit insulin secretion *in vivo* have also been suggested by results of an experiment demonstrating inhibition of insulin secretion when leptin was infused in rats in the presence of an inhibitor of PDE3B (Cases et al., 2001).

In addition to its acute effects to inhibit insulin secretion from beta cells, leptin has also been shown to inhibit proinsulin gene expression and beta cell proliferation in both rodent and human islets (Kulkarni et al., 1997; Pallett et al., 1997; Seufart et al., 1999a,b). Since diazoxide does not affect this effect of leptin, the inhibition of proinsulin gene expression by leptin appears to be independent of its effect on ATP-regulated K^+-channels (Seufart et al., 1999a,b). It may be mediated through activation of JAK (janus kinase)-STAT (signal tranducers and activators of transcription) signalling pathways. Accordingly, tyrosine phosphorylation of STAT is induced by leptin in rodent islets and in clonal insulin cells (Morton et al., 1999; Seufart et al., 1999a,b) and a protein complex bound to the insulin gene promoter and extracted from the nucleus after leptin stimulation of ob/ob islets has been found to contain STAT (Seufart et al., 1999a). However, whether leptin inhibits insulin secretion under all conditions is not known, since stimulatory effects have been observed in fetal rat islet cells (Islam et al., 2000) and in insulinoma cells (Tanabe et al., 1997).

Insulin secretion is increased as a compensatory response to insulin resistance (Ahrén and Pacini, 2004). In type 2 diabetes, however, insulin secretion is impaired and cannot meet the increased demand caused by the insulin resistance. Therefore, type 2 diabetes can be viewed as a defect in the ability of the islets to adequately compensate for reduced insulin sensitivity. One mechanism by which islet function is compromised in diabetes may be islet lipotoxicity due to excess accumulation of lipids in islets and/or beta cells (McGarry and Dobbins, 1999). The action of leptin to reduce intracellular lipid content may therefore be relevant to its actions on insulin secretion in diabetes. For example, the diabetic Zucker fatty rat, which has a defect affecting the function of long form of the leptin receptor, has a high beta cell content of lipids. When beta cells from these animals are transfected with the long for leptin receptor (OB-Rb) and treated with leptin, islet lipid content is reduced and the impairment of insulin secretion is ameliorated (Shimabukuro et al., 1998; Wang et al., 1998a,b). Similarly, administration of leptin to ob/ob mice for 7 days was found to reduce the lipid content of the islets and this was accompanied by an improvement of glucose-stimulated insulin secretion (Khan et al., 2001). Thus, it is reasonable to hypothesize that resistance to this action of leptin could contribute to islet dysfunction in diabetes.

In conclusion, there is evidence that leptin is involved as a regulator of islet function, primarly as an inhibitor of insulin secretion. The effects of leptin on insulin secretion appears to be mediated by several signalling pathways and may also involve alteration of the beta cell/islet lipid content.

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