Inhibition of glucose-induced insulin secretion by long-term preexposure of pancreatic islets to leptin

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Abstract Here we evaluated the effect of leptin on glucose-induced insulin secretion by normal rat pancreatic islets. We show in perifusion experiments that leptin had no acute effect on the secretory activity of β-cells. However, following preexposure to leptin a pronounced time- and dose-dependent inhibition of both first and second phases of secretion was observed. Maximum inhibition was obtained at 24 h and with 100 nM leptin. This inhibition did not involve a decrease in cellular insulin content. It was also not observed with islets from fa/fa rats. Leptin thus inhibits insulin secretion by a mechanism which requires long-term preexposure to the hormone and which may involve alteration in β-cell gene expression.

Key words: Insulin secretion; Leptin receptor; Obesity; Pancreatic islet

1. Introduction

The obese (ob) gene product encodes a 16 kd protein, referred to as leptin, which is secreted by adipose tissue [1]. Its expression is under the positive control of different hormones and neurotransmitters such as epinephrine, glucocorticoids [2] and insulin [3]. Leptin is a major hormone controlling food intake and energy expenditure and its site of action is thought to be mainly in the hypothalamus [4]. Mutation of leptin gene in ob/ob mice leads to severe obesity and non-insulin-dependent diabetes mellitus (NIDDM). The receptor for leptin (Ob-R) is a single transmembrane protein closely related to the gp130 signal-transduction component of cytokine receptor [5]. This receptor has several spliced isoforms (a–e) [6]. The longer one (Ob-Rb), through its extended cytoplasmic tail, transmits intracellular signals, probably through the activation of the Jack/STAT pathway [7]. This receptor is expressed principally in the brain but also in peripheral tissues including pancreatic islets [8]. Mutations of this receptor in db/db mice [6] or in fa/fa rats [9] are responsible for the development of obesity in these animals.

That leptin is a satiety factor was demonstrated by intraperitoneal or intracerebroventricular injections of recombinant hormone to ob/ob mice [10,11] or to normal or fa/fa rats [12]. These injections led to reduced food intake, decreases in body weight and were correlated with lower plasma insulin levels. The decrease in circulating insulin was however more pronounced in leptin-treated compared to pair-fed ob/ob mice showing identical decreases in body weight [13]. This last finding and the presence of the long form leptin receptor on β-cells [8] suggested that leptin can play a direct role on the control of insulin secretion. The hypothesis of a direct action of leptin on the control of insulin secretion has also been tested using islets from ob/ob mice. In one report, the secretory response to glucose was markedly reduced [14] and in another, the activity of the ATP-dependent K^+ channel was reported to be increased [15], providing a possible explanation for the reduction in secretory activity. Although one report mentions that leptin can induce a similar decrease in secretory activity in normal pancreatic islets compared to islets from ob/ob mice [14], no such effect could be seen when the secretory activity of β-cells was measured in a pancreas perfusion experiment [16]. At present it is thus not clear whether leptin has a real inhibitory effect on insulin secretion by normal islets. Other actions on peripheral tissue have been reported. For instance leptin has been shown to inhibit cortisol secretion from freshly isolated adrenocortical cells [17].

In this report, we determined that long-term, but not acute, exposure of isolated rat islets to leptin produces a time- and dose-dependent inhibition of glucose-induced insulin secretion. This effect was not accompanied by a decrease in islet insulin content. This inhibition was also not observed with islets isolated from fa/fa rats. These data demonstrate an important role of leptin on long-term modulation of insulin secretion. Since insulin stimulates leptin secretion, these data indicate the presence of an inhibitory control mechanism by which leptin negatively modulates insulin secretion.

2. Material and methods

2.1. Materials

Male Sprague-Dawley rats were purchased from Biological Research Laboratories Ltd.; fa/fa rats from IFFA Credo, France; Ficoll Dl-400 from Fluka, collagenase (type IV) from Worthington, murine leptin (GF050) from Chemicon, and bovine serum albumin (BSA, fraction IV RIA grade) from Sigma, antibodies for the insulin radioimmunoassay from Linco Research Inc.

2.2. Islet isolation

Sprague-Dawley rats were killed, their bile duct cannulated with a 21-gauge needle, and the pancreas was distended with a solution containing 2 mg/ml collagenase. Each pancreas then was incubated in tissue culture flask at 37°C and the islets isolated by the method of Gotoh et al. [18].

2.3. Islet culture

After isolation, islets were maintained at 15–20 islets/ml in RPMI 1640 medium (11 mM glucose), supplemented with 10% fetal calf serum, 10 mM HEPES, pH 7.4, 1 mM sodium pyruvate. After one day the medium was changed to RPMI containing 5.6 mM glucose and the same addition as above, in the absence or presence of different concentrations of leptin.

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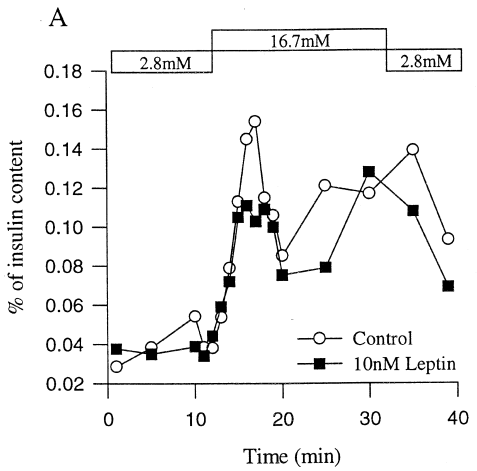
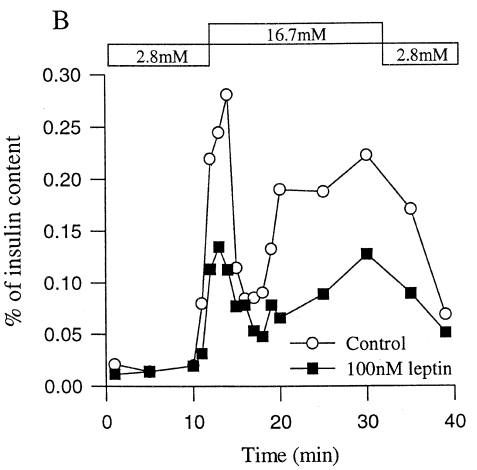
2.4. Islets perifusion

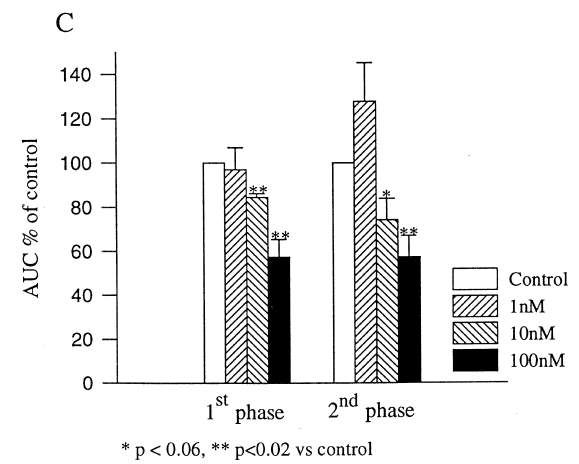
Batches of 10 islets were placed in a perifusion chamber. The perifusion buffer was a Krebs-Ringer solution containing 0.5% BSA, and the flow rate was adjusted at 1 ml/min. Perifusion experiments consisted in a 35 min equilibration period in the presence of 2.8 mM glucose, a 20 min perifusion in the presence of 16.7 mM glucose, and a 15 min perifusion in the presence of 2.8 mM glucose. Fractions were collected every minute. Insulin was then quantitated by radioimmunoassay using rat insulin as a standard.

2.5. Quantitative analysis

Results are presented as mean ± S.E.M. Statistical differences were analysed by Student's *t* test.







* p < 0.06, ** p<0.02 vs control

3. Results

In a first attempt at evaluating the effect of leptin on insulin secretion, rat islets were perifused in the presence or absence of different concentrations of leptin. No significant effect on the secretory pattern could be observed, even in the presence of 100 nM leptin (not shown). We thus evaluated whether preexposure of islets to leptin for 24 h would lead to a more pronounced inhibitory effect. As shown in Fig. 1, keeping the islets for 24 h in the presence of 10 or 100 nM leptin, led to a significant decrease in first and second phases of insulin secretion, even though leptin was not present during the perifusion experiment. As summarized in Fig. 1C, the inhibitory effect was dose-dependent with a maximum inhibition of first and second phase by 43 ± 8% and 44 ± 10%, respectively, obtained with 100 nM leptin. The time-dependence of this effect is shown in Fig. 2. A significant decrease in first (16 ± 0.3%), but not second, phase insulin secretion was already observed after 6 h of exposure to 100 nM leptin. This effect was maximal at 24 h and was not further increased after a 48 h incubation period (not shown).

The insulin content of leptin-treated islets was not significantly different from control islets (77.4 ± 8.3 ng/islet vs. 64.4 ± 3.4 ng/islet in the presence and absence of leptin, respectively; $P > 0.3$). Insulin mRNA levels were also unchanged (not shown).

Finally, to determine whether the leptin effect was dependent on the presence of a fully active leptin receptor, we evaluated the secretory response of islets from fa/fa rats preincubated for 24 h in the presence or absence of 100 nM leptin. Fig. 3 shows that leptin did not modify the islet secretory response of these islets.

4. Discussion

Previous studies have described acute effects of leptin on inhibition of insulin release [14] or on activation of the ATP-sensitive K^+ channels, a key link between glucose metabolism, membrane depolarization and insulin secretion [15]. These effects were however obtained with islets obtained from ob/ob mice which may have an hypersensitive leptin receptor because they have never been previously exposed to the hormone owing to the absence of active leptin in these mice. No acute effects of leptin on insulin secretion by perfused pan-

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Fig. 1. Leptin inhibition of glucose-induced insulin secretion. A: Rat islets were maintained in the presence or absence of 10 nM leptin for 24 h before the perifusion experiment. Groups of ten islets were then hand picked and their secretory response to glucose evaluated in perifusion experiments in the presence of the indicated concentrations of glucose. Insulin content of the ten control islets was 0.845 μg and 0.755 μg for the leptin-treated islets. Leptin was absent from the perifusion experiment. This is a representative experiment out of a total of three. B: Same as in A but with islets pretreated with 100 nM leptin. Insulin content of the ten control islets was 0.603 μg and 0.679 μg for the leptin-treated islets. C: Quantitation (area under the curve, AUC) of first and second phase insulin secretion by islet treated or not with the indicated concentrations of leptin. The results are expressed as mean ± S.E.M. for $n = 3$. The effect of 1 nM leptin was not significant. 10 nM leptin reduced first and second phase secretion by 15 ± 2% and 25 ± 10%, respectively. In the presence of 100 nM leptin, first phase was reduced by 43 ± 8% and second phase by 44 ± 10%.

creas of normal rats were however observed using leptin at a dose of 1 nM [16].

In the present study we demonstrate that leptin has a profound time- and dose-dependent inhibitory effect on insulin secretion. A minimum of 6 h exposure to the hormone is required for a significant effect on first phase secretion to be observed and a maximal effect on both first and second phases is observed at 24 h. The fact that prolonged exposure to leptin is required for the inhibitory effect to be observed suggests that inhibition is not the result of a direct interaction between the intracellular leptin and glucose signalling pathways but, rather, that the leptin effect is mediated through a change in β-cell gene expression. These results are thus different from those obtained with islets from ob/ob mice which showed an acute inhibitory effect on insulin secretion. The bases for this difference is not known but may result from the fact that ob/ob islets are hypersensitive to the action of leptin having never been exposed previously to the action of this hormone. Alternatively a species-specific difference in sensitivity to leptin may also be possible. In support of a long-term effect of leptin mediated by changes in gene expression are the studies by Unger et al. which demonstrated that leptin reduces the esterification and increases the oxidation of fatty acids [19]. The increase in fatty acid oxidation can be attributed, at least in part, to an increase in the expression of carnitine palmitoyltransferase and acetyl CoA oxidase, and to a decrease in the expression of acetyl CoA carboxylase [20]. As it has been described that elevated levels of free fatty acid inhibit insulin secretion by a mechanism dependent on their mitochondrial oxidation [21], the above data suggest that the inhibitory effect of leptin may be mediated by its stimulation of fatty acid catabolism. Alternatively, leptin may modify the expression of other genes whose products may be essential in different steps of the glucose signalling mechanism and of insulin granule exocytosis.

The effect of leptin is concentration-dependent and has been observed in the presence of 10 and 100 nM. That these concentrations are supraphysiological may indicate that leptin is not very stable in in vitro culture over the time course of our experiments and since the effect requires a long treatment period, high concentrations must be used to observe the inhibitory effect. That leptin has no effect on the secretory activity of fa/fa rat islets, which have a non-functional receptor, strongly argues in favor of a hormone-specific effect.

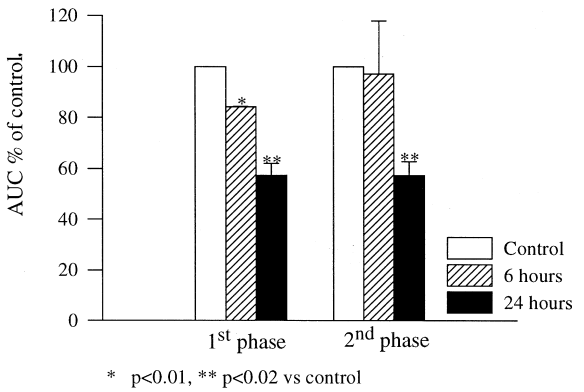

Fig. 2. The effect of recombinant leptin is time-dependent. Islets were incubated in absence or presence of 100 nM leptin for 6 and 24 h before the perifusion experiment was carried out as in Fig. 1. Data are expressed as mean ± S.E.M. for $n = 3$. Data for 24 h time point are the same as in Fig. 1. A 6 h incubation led to a decrease in first phase (16 ± 0.3%) but not in second phase of secretion.

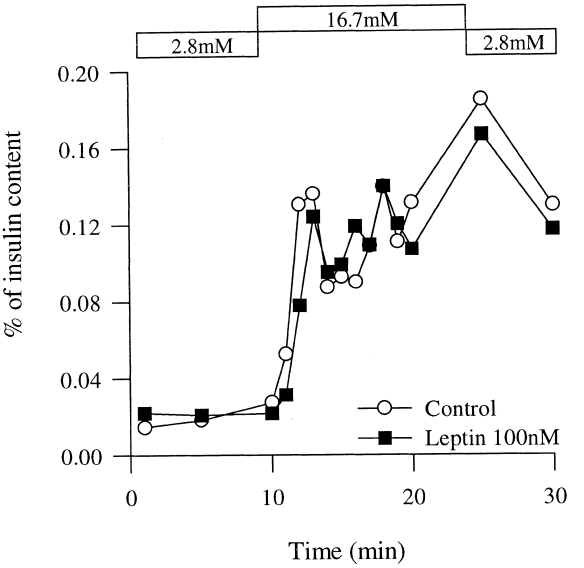

Fig. 3. Leptin does not affect the glucose-induced insulin secretion by fa/fa rat isolated islets. Islets from fa/fa rats were kept in culture for 24 h in the presence of 100 nM leptin. The perifusion experiment was then carried out as in Fig. 1. Insulin content of the ten untreated fa/fa islets was 1.25 μg and 1.31 μg for the leptin-treated fa/fa islets. This perifusion is representative of two experiments. No significant effect of leptin on insulin secretion could be observed.

Our present demonstration of a pronounced chronic effect of leptin on reducing glucose-stimulated insulin secretion suggests the existence of a feedback inhibitory loop regulating leptin and insulin secretion. Leptin secretion by adipocytes has been shown to be increased by insulin [3]. Our present data show that leptin can reduce glucose-induced insulin secretion, thereby providing a feedback inhibitory mechanism to lower its own secretion. By which exact mechanism leptin inhibits insulin secretion is not yet known. Although alteration in lipid metabolism may be a cause for the secretory impairment, modification of other genes involved in glucose metabolism, plasma membrane electrical activity, or fusion of secretory granules with the plasma membrane may also be involved. Also, elucidation of the function of this inhibitory feedback mechanism in normal physiology and its possible alteration in, for instance, obese hyperinsulinemic patients who are known to be hyperleptinemic, should be an important goal of future studies.

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