Biochemical Pharmacology, Vol. 56, pp. 1339–1346, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S006-2952(98)00194-4

Leptin Regulation of Islet Amyloid Polypeptide Secretion from Mouse Pancreatic Islets

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ABSTRACT. Leptin receptors are expressed in pancreatic β-cells. However, leptin's role in islet hormone secretion is essentially unknown. In the present study, we aimed to elucidate leptin's effect on isolated pancreatic NMRI mouse islets by examining islet amyloid polypeptide (IAPP) and insulin secretion in acute experiments and after 48-hr exposure to leptin (1–100 nM). It was also examined whether a putative effect of leptin was affected by the glucose concentration. Islets were cultured in medium RPMI 1640 + 10% fetal calf serum, and the effects of leptin on islet cell replication, glucose metabolism, and hormone content were subsequently examined. Glucose-stimulated IAPP secretion was reduced both acutely and after 48-hr exposure to leptin, whereas only minor effects were found on insulin release, i.e. an inhibition in islets cultured with 1 nM leptin. An acute inhibitory effect by 10 nM leptin was observed on the ratio of IAPP/insulin release at 5.6–11.1 mM glucose, but this was overcome by 16.7 mM glucose. The islet glucose oxidation rate was enhanced by 1 nM leptin, but decreased at higher concentrations of leptin in acute experiments. In contrast, glucose metabolism was not affected in long-term experiments. Moreover, leptin did not influence islet (pro)insulin synthesis or the cell replication rate after culture. In conclusion, we show that islet IAPP release seems to be more sensitive to leptin than is insulin release. The effect of leptin on islet hormone secretion is dependent on the glucose concentration. The regulation of hormone secretion seems to be dissociated from glucose metabolism, an effect previously described in islets after exposure to certain cytokines. Our data necessarily suggest that a previously proposed negative feedback loop between leptin and insulin can be counteracted by IAPP. BIOCHEM PHARMACOL **56**;10:1339–1346, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. β-cells; insulin secretion; IAPP secretion; leptin; pancreatic islets

The product of the ob gene, leptin, is considered to have a central role in the regulation of adipose tissue. Leptin is exclusively produced in adipose tissue and even though leptin receptors are widespread, some studies indicate that tissues clearly involved in energy metabolism, e.g. hypothalamus and pancreas, have a higher expression of the functional form of the receptor [1]. It is well established that leptin has the ability to decrease food intake and increase energy expenditure by binding to receptors in the hypothalamus. However, a possible effect of leptin on the pancreas is more controversial. The fact that leptin receptors are expressed in pancreatic β-cells has lead to speculations about an adipo-insular feedback loop which controls adipose tissue [2, 3]. There is evidence of such inhibitory action of leptin on insulin release [4, 5]. However, other studies indicate that leptin does not affect insulin secretion [6].

Insulin resistance is a phenomenon with a close correlation to the amount of adipose tissue, but the mechanism behind insulin resistance is obscure. It has been shown that fasting insulin, representing insulin resistance, is a significant determinant of serum leptin concentration, independent of gender, BMI§ or Waist/Hip Ratio, suggesting a role for leptin in insulin resistance [3]. One of the factors also proposed to be involved in insulin resistance is IAPP. IAPP is cosecreted together with insulin from pancreatic β-cells; its physiological role is not fully understood [7, 8, 9]. Both native IAPP extracted from human diabetic pancreas and synthesized IAPP-carboxylate cause resistance to insulin in rat skeletal muscle *in vitro* [10, 11]. IAPP has also been reported to decrease insulin sensitivity *in vivo* [12]. The fact that both IAPP and leptin show a correlation with insulin resistance and that receptors for leptin are found in pancreatic β-cells makes it relevant to investigate whether leptin has any direct effect on IAPP secretion.

In the present study, we have examined the possible role of leptin on insulin release. We have also investigated a putative role for leptin in regulation of IAPP secretion. For this purpose, we isolated pancreatic islets from NMRI mice.

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Received: 19 January 1998; accepted: 18 May 1998.

§ Abbreviations: BMI, Body Mass Index; IAPP, Islet amyloid polypeptide; KRBH, Krebs–Ringer bicarbonate buffer supplemented with 2 mg/mL BSA and 10 mM HEPES; IL-6, Interleukin-6; RIA, Radioimmunoassay.

Hormone release from islets was studied both in acute experiments and after long-term exposure to leptin (1–100 nM). It was also examined whether leptin affected islet cell replication and islet glucose metabolism, insulin synthesis, and hormone content.

MATERIALS AND METHODS

Animals, Islet Isolation, and Culture

Male NMRI mice, aged 12–16 weeks, were purchased from BK-Universal. The animal room had 12-hr light/dark cycles, the temperature was 21° and the humidity was 30–35%. Pancreatic islets were isolated by collagenase digestion (Collagenase A, Boehringer Mannheim) from overnight-fasted mice. After hand-picking using a braking pipette, the islets were cultured for 5–7 days. The islets were kept free-floating in culture medium RPMI 1640 containing 11.1 mM glucose (Sigma Chemical Co.) supplemented with 10% (v/v) FCS (Sigma) at 37° in an atmosphere of humidified air plus 5% CO_2. Medium was changed every second day.

For the long-term exposure experiments, leptin (recombinant murine leptin, PeproTech) was then added in the concentration range 1–100 nM for 48 hr, whereupon the islet function was studied. The biologic activity of the leptin preparation was studied after injection intraperitoneally (i.p.) once daily for 5 days (2.5 μg/g body weight) in obese (ob/ob) and lean (+/+) C57BL/6 mice. This resulted in a significant reduction in body weight and food intake.¶

In a separate experimental series, groups of 20 islets were cultured in 2.5 mL of RPMI 1640 containing 10% (v/v) FCS and 1–100 nM leptin. Insulin content in the medium was subsequently measured 3, 18, 24, and 48 hr after addition of leptin, and IAPP content was analyzed after 48 hr. Some experiments were undertaken to study similarities between leptin and IL-6 in islet hormone secretion. Islets were exposed to murine IL-6 (PeproTech); 1 ng/mL or 5 ng/mL for 48 hr, prior to measurement of IAPP and insulin release. In other experiments, the acute effect of leptin (1–100 nM) on islet insulin and IAPP release as well as the glucose oxidation rate was examined; i.e. leptin was present only during the actual incubations. Effects of leptin on islet IAPP and insulin release at different glucose concentrations were studied in other experiments. Concerning the glucose oxidation rate, some experiments were also carried out in the presence of 10 nM leptin during the actual incubation of islets exposed to 10 nM leptin for 48 hr.

Islet IAPP Release, Insulin Release, Islet Hormone, and DNA Content

Groups of 10 islets were transferred in triplicate to glass vials containing 0.25 mL of Krebs-Ringer bicarbonate buffer supplemented with 10 mM HEPES (Sigma) and 2 mg/mL of BSA (fraction V; Miles), hereafter designated as KRBH buffer. During the first hour of incubation at 37° (O_2/CO_2, 95:5), the KRBH medium contained 1.7 mM glucose. The medium was then carefully removed and replaced by 0.25 mL of KRBH supplemented with 16.7 mM glucose and incubated for another hour. When islet hormone secretion was studied at different glucose concentrations, 10 islets per group were incubated in 100 μL of KRBH medium in a 48-well plate for 1 hr.

The insulin and IAPP concentrations in the incubation media were determined by RIA [13, 14]. For the insulin measurements, ^{125}I-labeled porcine insulin (Euro-Diagnostica) was used as tracer, guinea-pig antibovine insulin serum (Miles-Yeda) was used as antibody, and porcine insulin (Novo Industries) was used as standard. The sensitivity of the assay was 0.1 ng/mL of insulin, the inter-assay variation was 7% and the intra-assay variation was 4%. Human IAPP was used for standard and tracer preparations, and a polyclonal rabbit antiserum raised against human IAPP was used as antibody (Peninsula, Belmont). The antibody cross-reacts 100% with both rat and mouse IAPP. The sensitivity of the IAPP assay was 0.125 fmol/tube, and there was no cross-reactivity with calcitonin gene-related peptide. The inter-assay variation was 9.9% and the intra-assay variation 8.2%. The procedure for determination of IAPP has been described in detail elsewhere [14].

After the incubations, the islets were harvested, pooled in groups of 30 and homogenized by sonication in 0.2 mL of redistilled water. Two 50-μL aliquots of the aqueous homogenate were then used for DNA measurement by fluorophotometry [15]. A fraction of the homogenate was mixed with acid-ethanol (0.18 M of HCl in 96% (v/v) ethanol) and insulin and IAPP were extracted overnight at 4°. Insulin and IAPP were measured by RIA as described above.

DNA Synthesis

For estimation of islet cell DNA synthesis, groups of 50 islets, cultured for 48 hr, with or without 100 nM leptin, were exposed during the last 6 hr of culture to 1 μCi/mL [methyl-^{3}H]thymidine (Amersham International). The islets were washed in Hanks' solution containing 10 mM thymidine and disrupted in 0.2 mL of redistilled water by ultrasonication. Duplicate aliquots of the homogenates were precipitated with 10% (w/v) trichloroacetic acid, and the labeled DNA was separated from unbound [^{3}[reset]H-]thymidine by filtering through glass microfiber filers (GF/A 2.5 cm; Whatman). After drying, the radioactivity on the filters was determined by liquid scintillation. In another sample of the homogenate, the islet DNA content was determined by fluorophotometry [15].

(Pro)insulin and Total Protein Biosynthesis

Duplicate groups of 10 islets were incubated at 37° in 100 μL of KRBH containing 16.7 mM glucose and 50 μCi/mL

¶Dr. P–O Carlsson, Dept. of Medical Cell Biology, Uppsala University, personal communication.

TABLE 1. IAPP and insulin release from mouse pancreatic islets exposed acutely to different concentrations of leptin

Leptin exposure (nM)	IAPP release (fmol/10 islets × hr)	Insulin release (pmol/10 islets × hr)	Ratio IAPP/insulin (%)
0	129 ± 12.6	6.3 ± 0.6	2.3 ± 0.2
1	132 ± 17.8	6.5 ± 0.5	2.2 ± 0.2
10	103 ± 11.7**	6.2 ± 0.4	1.8 ± 0.2*
100	117 ± 12.2	5.8 ± 0.5	2.3 ± 0.3

Islets were isolated from NMRI mice and cultured for 6–7 days in medium RPMI 1640 + 10% fetal calf serum. Then, triplicate groups of 10 islets were incubated in KRBH buffer supplemented with 1.7 mM glucose for 1 hr (not shown). During the second hour, the islets were incubated in medium supplemented with 16.7 mM glucose and different concentrations of leptin. IAPP and insulin concentrations were measured by RIA. Values are means ± SEM for 13–15 different islet preparations. * and ** denote $P < 0.05$ and $P < 0.01$ versus control, using Student's paired *t*-test.

of L-[4.5-^{3}H]leucine (Amersham) in an atmosphere of humidified air plus 5% CO_2. After 2 hr, the islets were washed in buffer containing nonradioactive leucine (10 mM) and sonicated in 200 μL of redistilled water. The amount of labeled (pro)insulin was determined by an immunoabsorption technique [16], and the total protein biosynthesis was measured in trichloroacetic acid precipitates of islet homogenate.

Islet Glucose Oxidation Rate

Triplicate groups of ten islets were transferred to glass vials containing 100 μL of KRBH supplemented with D-[U-^{14}C]glucose (Amersham) and nonradioactive glucose to a final glucose concentration of 16.7 mM glucose (spec. act. 0.5 mCi/mM), incubated for 90 min at 37° (O_2/CO_2, 95:5), and the islet glucose oxidation rates measured as described elsewhere [17].

Statistical Analysis

Every experiment represented different mouse islet donors. In each experiment, the IAPP and insulin secretion was calculated as mean of the values obtained from the three incubation vials and considered as one separate observation. Means ± SEM were subsequently calculated, and groups of data were compared, using Student's paired *t*-test. When multiple comparisons were performed, the data were compared by ANOVA, including Fisher's protected least statistical difference test, using StatView (Abacus Concepts).

RESULTS

Acute Effects of Leptin on Pancreatic Islet Function

All groups of islets released more insulin and IAPP at 16.7 mM glucose compared to the preceding 1-hr incubation at 1.7 mM glucose. The basal insulin and IAPP release did not differ between the groups (data not shown). The range of glucose-stimulated insulin release (16.7/1.7 mM glucose) was also similar in the groups exposed to different leptin concentrations: control, 938% ± 142%; 1 nM leptin, 1143% ± 198%, 10 nM leptin, 1016% ± 138%; 100 nM leptin, 886% ± 111%. Thus, 1–100 nM leptin did not affect the insulin release at 16.7 mM glucose (Table 1). However, there was a decline in IAPP release from pancreatic islets exposed to 10 nM leptin, but this was not observed at the highest IAPP concentration. There was also a decline in the IAPP/insulin secretion ratio when islets were incubated with 10 nM leptin plus 16.7 mM glucose, but no such effect was seen for the other leptin concentrations.

In a separate series of experiments, the acute effects of leptin (10 nM) on IAPP and insulin release were studied at different glucose concentrations (Fig. 1). Increases in glucose concentration resulted in augmented IAPP/insulin ratios (Fig. 1c). This stimulation was counteracted by 10 nM leptin, and only islets incubated with the maximal glucose concentration showed an increase in the IAPP/insulin ratio compared to islets incubated at 1.7 mM glucose. Islet IAPP or insulin release *per se* was not significantly altered by increases in glucose concentration irrespective of the presence of 10 nM leptin or not (Fig. 1a and b).

Leptin altered the islet glucose metabolism in a bimodal way: at 1 nM there was an increase in the glucose oxidation rate, whereas at 10 and 100 nM leptin there was a clear inhibition (Fig. 2).

Long-term Effects of Leptin on Pancreatic Islet Function

In these experiments, the islets were cultured with 0–100 nM leptin for 48 hr and then functionally examined in the absence of leptin. None of the leptin concentrations affected either IAPP release or insulin release during the first hour of incubation at 1.7 mM glucose (data not shown). Both IAPP and insulin release from islets cultured with either 1, 10, or 100 nM leptin, as well as the controls, was markedly increased upon stimulation with 16.7 mM glucose compared to 1.7 mM glucose (data not shown). Glucose-stimulated IAPP release was, however, significantly reduced in cultures supplemented with 10 and 100 nM leptin (Table 2). A significant reduction in glucose-stimulated insulin release was found at 1 nM leptin but not at higher concentrations (Table 2). Despite the changes in

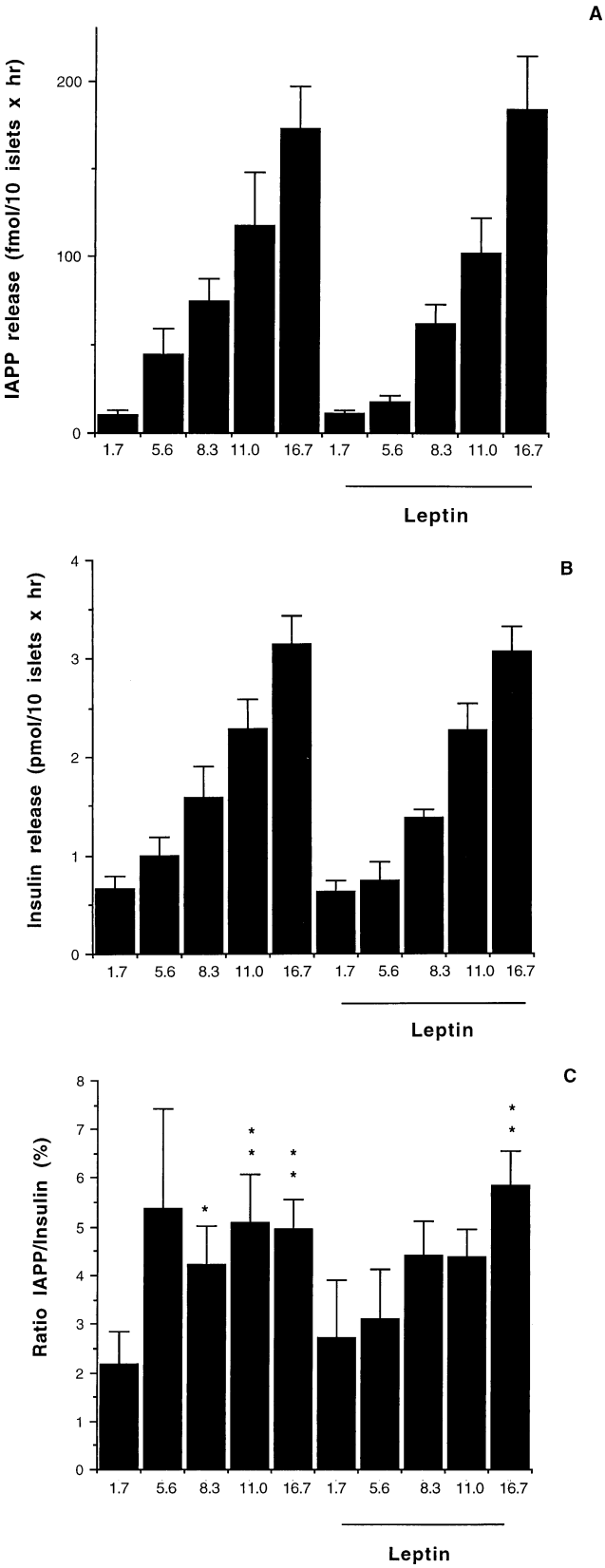

FIG. 1. Effect of leptin (10 nM) at different glucose concentrations on islet IAPP release (A), insulin release (B), and ratio IAPP/insulin (C). Data were collected after 1-hr incubation in KRBH. Values are means ± SEM for seven observations. * and ** denote $P < 0.05$ and $P < 0.01$ versus corresponding islets incubated at 1.7 mM glucose, using Student's paired *t*-test.

IAPP secretion, there was no statistically significant change in the IAPP/insulin secretion ratio.

During the culture period, there was a progressive accumulation of insulin in the media after 3, 18, 24, and 48 hr in all the different groups (Fig. 3). After 48 hr the medium insulin was higher in the islets cultured with 100 nM leptin compared to the corresponding control group. The IAPP content in the culture medium was slightly higher 48 hr after addition of 100 nM leptin (control: 2.4 ± 0.4 pmol/10 islets/2.5 mL vs 100 nM leptin: 2.8 ± 0.3 pmol/10 islets/2.5 mL; $P < 0.05$, using Student's paired *t*-test). Exposure to leptin for 2 days, however, did not significantly alter the islet DNA, IAPP, or insulin content (Table 3).

In accordance with the unaffected insulin content, culture with leptin (100 nM) did not change the islet (pro)insulin biosynthesis rate (control: 15.0 ± 1.4 kdpm/10 islets × 2 hr vs 100 nM leptin: 15.4 ± 1.3 kdpm/10 islets × 2 hr; $n = 15$; $P > 0.05$) or the total protein biosynthesis rate (control: 72.8 ± 6.6 kdpm/10 islets × 2 hr vs 100 nM leptin: 80.7 ± 5.5 kdpm/10 islets × 2 hr; $n = 15$; $P > 0.05$). Moreover, islet cell replication, as measured by the tritiated thymidine incorporation rate, was not affected by leptin (control: 1133 ± 119 dpm/μg of DNA × 6 hr vs 100 nM leptin: 1240 ± 138 dpm/μg of DNA × 6 hr; $n = 16$; $P > 0.05$). The glucose oxidation rate was similar to the controls after prolonged exposure to leptin, although there was a tendency for a decline at 10 nM ($P = 0.069$) (Fig. 2).

To investigate if islets become less sensitive to leptin during prolonged culture in the presence of the peptide, additional experiments were carried out with 10 nM leptin present both during culture and incubation. With this experimental design, leptin failed to inhibit the islet glucose oxidation rate (leptin: 253.3 ± 37.3 vs control: 278.4 ± 46.4 pmol/10 islets × 90 min; $P > 0.05$: $n = 10$).

Since the leptin and IL-6 receptors have structural similarities, we also tested the effects of IL-6 on IAPP secretion. Exposure for 48 hr to 5 ng/mL murine IL-6 significantly reduced glucose-stimulated IAPP and insulin release compared to noncytokine treated islets, whereas the IAPP/insulin ratios were nonaltered (Table 4). No significant effect on hormone secretion was seen at 1 ng/mL of IL-6.

DISCUSSION

In the present study, we found that glucose-stimulated IAPP secretion was reduced both acutely and after long-term exposure to leptin. Islet glucose metabolism was enhanced at 1 nM leptin but decreased at higher concentrations of leptin in acute experiments, whereas glucose metabolism was not affected in long-term experiments. According to our data, the changes observed in IAPP secretion did not seem to directly correlate to changes in glucose metabolism. Leptin did not influence islet (pro)insulin synthesis or the cell replication rate. The concentrations of leptin tested in our study were chosen according to the work by Emilsson *et al.*, in which they reported a

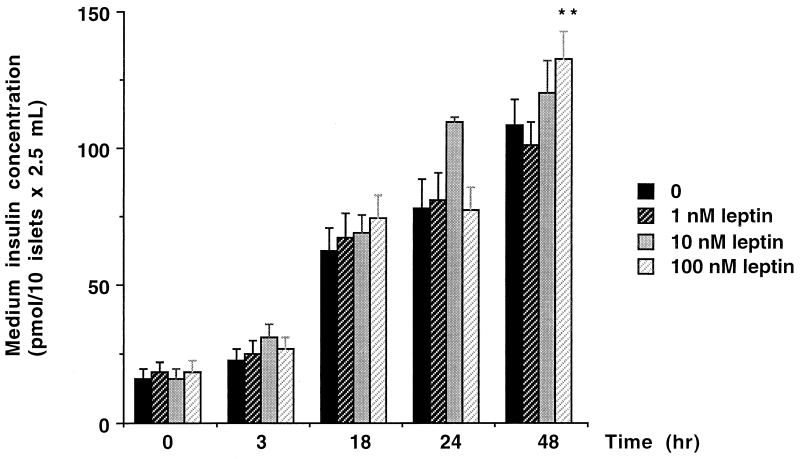

FIG. 2. Effect of leptin on medium insulin accumulation during culture. Islets were kept in culture for 48 hr and medium insulin concentration was measured at different time points after leptin addition. Values are means ± SEM for 12 observations. ** denotes $P < 0.01$ versus corresponding control, using Student's paired *t*-test.

maximal inhibition of insulin release in islets from lean mice and ob/ob mice at 100 nM leptin, and a 50% inhibition of the maximal insulin response at 10 nM leptin [4]. Moreover, 1 nM leptin may be considered to be within the physiological range in humans [18].

The results reported in the literature concerning an effect of leptin on insulin secretion are somewhat divergent. Some studies report the lack of effect of leptin on insulin release. Thus, in the isolated perfused rat pancreas, 1–10 nM leptin failed to affect insulin secretion [19]. In addition, in a study by Chen *et al.*, glucose-stimulated insulin release in isolated islets from ob/ob mice and lean mice were not affected by 100 nM leptin, while leptin seemed to specifically regulate the PhospholipaseC/ProteinkinaseC-induced insulin release [20]. In contrast, leptin has also been suggested to dose-dependently stimulate insulin secretion at a low glucose concentration when studied in a pancreatic β-cell line (MIN 6) and in rat pancreatic islets [21]. Emilsson *et al.* found a dose-dependent inhibition of insulin release in ob/ob mice and lean mice with 1–100 nM leptin [4]. Physiological concentrations of murine leptin inhibited insulin secretion in human islets, rat islets, and insulinoma cells and suppressed insulin mRNA expression in rat islets and insulinoma cells in a study by Kulkarni *et al.* [5]. Furthermore, there have been suggestions that leptin has the ability to activate ATP-sensitive K^+-channel activity and thereby inhibit insulin release from islets through decreased Ca^{2+} influx [22]. If this is the case, it cannot be excluded that a high glucose concentration could partly overcome an inhibitory action by leptin on islet hormone secretion. In our study, this could be supported by the observation that 10 nM leptin failed to reduce the IAPP/insulin ratio acutely at 17 mM glucose, whereas an inhibitory effect was seen for glucose in the range of 5.6 to 11.1 mM glucose.

In the present study, IAPP release seemed to be more sensitive, acutely, than insulin release, giving rise to a decline in IAPP/insulin ratios. Regarding effects of leptin on insulin secretion, we found only minor effects, i.e. an inhibition in islets cultured at 1 nM but not at other concentrations. It is possible that an inhibitory effect of leptin on IAPP release could contribute to regulation of the glucose homeostasis under certain conditions. Recently, Kamohara *et al.* showed that leptin increased glucose uptake in skeletal muscle and brown fat tissue [23]. This could also, in an indirect way, be achieved by a lowered IAPP secretion, since IAPP might inhibit glucose uptake [11]. When considering islet hormone secretion, it could be envisaged that an inhibitory effect of leptin on IAPP release counteracts an inhibitory effect of IAPP on stimulated insulin release [24, 25]. If so, such a feedback loop for insulin release could, hypothetically, contribute to higher serum insulin levels in subjects with hyperleptinemia. This means that a negative feedback loop between leptin and

TABLE 2. IAPP and insulin release from mouse pancreatic islets exposed to different concentrations of leptin for 48 hr

Leptin exposure (nM)	IAPP release (fmol/10 islets × hr)	Insulin release (pmol/10 islets × hr)	Ratio IAPP/insulin (%)
0	69.0 ± 10.5	8.2 ± 2.0	1.1 ± 0.2
1	59.8 ± 10.4	6.2 ± 1.4*	1.5 ± 0.3
10	50.2 ± 7.5*	6.1 ± 1.4	1.1 ± 0.2
100	47.6 ± 6.7*	6.7 ± 1.5	1.3 ± 0.3

Islets were isolated from NMRI mice and cultured for 6–7 days in medium RPMI 1640 + 10% fetal calf serum. The islets were then exposed to the different concentrations of leptin for 48 hr. Triplicate groups of 10 islets were subsequently incubated in KRBH buffer supplemented with 1.7 mM glucose for 1 hr (not shown), and 16.7 mM glucose during a second hour. IAPP and insulin concentrations were measured by RIA. Values are means ± SEM for 14 different islet preparations. * denotes $P < 0.05$ versus control, using Student's paired -test.

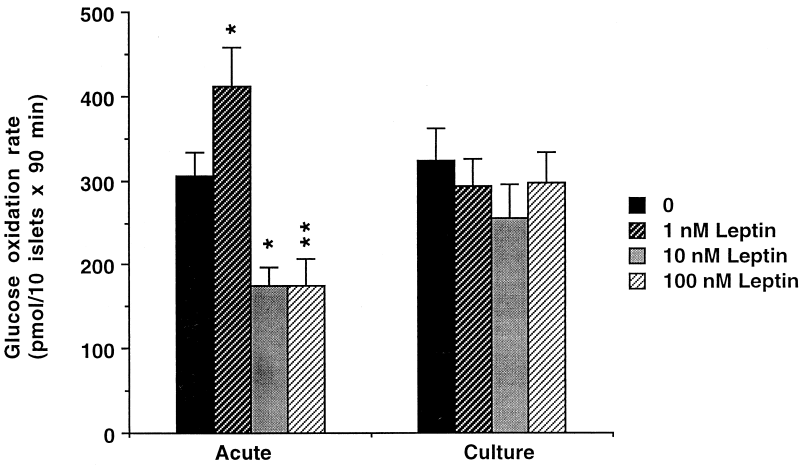

FIG. 3. Effect of leptin on the islet glucose oxidation rate. Islets were either studied acutely in the presence of leptin or after culture with leptin. In the latter case, leptin was not present during the actual incubations. Glucose oxidation rates were measured with the addition of D-[U-^{14}C]glucose and nonradioactive glucose to a final concentration of 16.7 mM glucose. Values are means ± SEM for 8–14 observations. * and ** denote $P < 0.05$ and $P < 0.01$ versus corresponding control, using Student's paired *t*-test.

insulin release as proposed by some authors [4, 22, 5] could be counteracted by lowered IAPP levels.

At 1 nM leptin, we observed a significant increase in the islet glucose oxidation rate in acute experiments. Interestingly, a stimulatory effect by leptin on glucose metabolism has also been seen *in vivo* in mice [23]. The two highest leptin concentrations tested in our study had an inhibitory effect on islet glucose oxidation. Since the leptin receptor shows structural similarities to other receptors, especially by sharing the gp 130 signal-transducing component, it can't be excluded that some of the effects observed with the highest concentrations of leptin might be mediated by signaling through other receptors [26]. The receptor for IL-6 is one of the receptors with the closest similarity to the leptin receptor. Thus, it could also be of interest that we found an increased medium insulin concentration after 48-hr culture with the highest concentration of leptin, an effect previously seen after culture with IL-6 [27]. Moreover, in our study, IL-6 (5 ng/mL), significantly reduced IAPP and insulin release with regard to basal hormone release after 48-hr exposure. The IAPP/insulin ratios were not altered. Thus, our finding suggests that leptin at high concentrations may affect islet function through signaling via the IL-6 receptor.

Both acutely and after long-term exposure, leptin's effects on glucose metabolism seemed to be dissociated from that on insulin release, as the latter mainly was unaffected. Indeed, a dissociation between the islet glucose oxidation and insulin secretory rates have frequently been found after exposure to various cytokines [27–30]. In contrast to the findings in the acute experiments, leptin did not change the islet glucose oxidation rate after long-term exposure. It could not be excluded that the lack of response to leptin after culture could be due to a degradation of leptin during tissue culture. However, this does not seem probable when the altered long-term IAPP secretion by leptin is taken into account. One contribution to the lack of response to leptin after 48 hr in culture might be that islets become less sensitive to leptin during culture with leptin present. This may be reflected here by the fact that leptin (10 nM) failed to inhibit glucose metabolism in islets exposed to leptin (10 nM) for 48 hr. Other possibilities could be that leptin becomes bound to serum proteins during culture with FCS or that the metabolic effects of leptin on the islet cells are transient.

In conclusion, we show that islet IAPP release seems to be more sensitive to leptin than is insulin release. The effect of leptin on islet hormone secretion was dependent on the glucose concentration. The regulation of hormone secretion seems to be dissociated from glucose metabolism, an effect previously described in islets after exposure to certain cytokines. Our data necessarily suggest that a

TABLE 3. IAPP, insulin and DNA contents in mouse pancreatic islets exposed to different concentrations of leptin for 48 hr

Leptin exposure (nM)	IAPP content (pmol/μg DNA)	Insulin content (pmol/μg DNA)	DNA content (μg/10 islets)
0	8.7 ± 1.1	327 ± 46.0	0.19 ± 0.01
1	9.8 ± 1.0	391 ± 61.8	0.19 ± 0.01
10	8.4 ± 1.1	382 ± 63.1	0.19 ± 0.01
100	8.3 ± 0.8	371 ± 55.5	0.21 ± 0.02

Islets were isolated from NMRI mice and cultured for 6–7 days in medium RPMI 1640 + 10% fetal calf serum. Islets were first incubated for 1 hr in 1.7 nM glucose and then for a second hr in 16.7 mM glucose and then pooled into groups of 30, and homogenized by sonication in 0.2 mL of redistilled water. Two 50-μL aliquots of the aqueous homogenate were then used for DNA measurement by fluorophotometry. Another fraction of the homogenate was mixed with acid–ethanol, and IAPP and insulin were extracted overnight at 4°. IAPP and insulin were later determined by RIA. Values are means ± SEM for 11–12 observations.

TABLE 4. IAPP and insulin release from mouse pancreatic islets exposed to different concentrations of IL-6 for 48 hr

IL-6 exposure (ng/mL)	IAPP release 16.7/1.7 (%)	Insulin release 16.7/1.7 (%)	Ratio IAPP/insulin 16.7 (%)
0	452 ± 49	594 ± 104	0.74 ± 0.09
1	429 ± 55	973 ± 491	0.74 ± 0.15
5	283 ± 25**	369 ± 63*	0.71 ± 0.13

Islets were isolated from NMRI mice and cultured for 6–7 days in medium RPMI 1640 + 10% fetal calf serum. The islets were then exposed to the different concentrations of murine IL-6 for 48 hr. Triplicate groups of 10 islets were subsequently incubated in KRBH buffer supplemented with 1.7 mM glucose for one hour, and 16.7 mM glucose during a second hour. IAPP and insulin concentrations were measured by RIA. Values for IAPP and insulin release are expressed as relative stimulation at 16.7 mM glucose compared to 1.7 mM glucose. The fourth column gives the molar ratio of IAPP/insulin secretion at 16.7 mM glucose. Values are means ± SEM for 10 different islet preparations. * and ** denote $P < 0.05$ and $P < 0.01$, using Student's paired *t*-test.

previously proposed negative feedback loop between leptin and insulin may be counteracted by IAPP.

The skilfull technical assistance of Margareta Engkvist, Ing–Britt Hallgren, and Astrid Nordin is gratefully acknowledged. The study was supported by grants from the Swedish Medical Research Council (12X-8273, and 12P-10739), the Novo Nordic Fund, the Swedish Diabetes Association, the Family Ernfors Fund, and the Juvenile Diabetes Foundation International.

References

1. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH and Skoda RC, Defect STAT signalling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci USA* **93:** 6231–6235, 1996.
2. Kieffer TJ, Heller RS and Habener JF, Leptin receptors expressed on pancreatic β-cells. *Biochem Biophys Res Commun* **224**: 522–527, 1996.
3. Zimmet PZ, Collins VR, de Courten MP, Hodge AM, Collier GR, Dowse GK, Alberti KG, Tuomilehto J, Hemraj F, Gareeboo H, Chitson P and Fareed D, Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius. Mauritius NCD Study Group. *Int J Obes Relat Metab Disord* **22**: 171–177, 1998.
4. Emilsson V, Liu YL, Cawthorne MA, Morton NM and Davenport M, Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* **46**: 313–316, 1997.
5. Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghatei MA, Withers DJ, Gardiner JV, Bailey CJ and Bloom SR, Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, *in vivo*, in mice. *J Clin Invest* **100**: 2729–2736, 1997.
6. Leclercq-Meyer V, Considine RV, Sener A, and Malaisse WJ, Do leptin receptors play a functional role in the endocrine pancreas? *Biochem Biophys Res Commun* **229**: 794–798, 1996.
7. Clark A, Islet amyloid: An enigma of type 2 diabetes. *Diabetes/Metab Rev* **8**: 117–132, 1992.
8. Cooper GJS, Amylin compared with calcitonin gene-related peptide: Structure, biology, and relevance to metabolic disease. *Endocr Rev* **15**: 163–201, 1994.
9. Westermark P, Johnson KH, O'Brien TD and Betsholtz C, Islet amyloid polypeptide—a novel controversy in diabetes research. *Diabetologia* **35**: 297–303, 1992.
10. Cooper GJ, Leighton B, Dimitriadis GD, Parry Billings M, Kowalchuk JM, Howland K, Rothbard JB, Willis AC and Reid KB, Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc Natl Acad Sci USA* **85**: 7763–7766, 1988.
11. Leighton B and Cooper GJ, Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle *in vitro*. *Nature (London)* **335**: 632–635, 1988.
12. Sowa R, Sanke T, Hirayama J, Tabata H, Furuta H, Nishimura S and Nanjo K, Islet amyloid polypeptide amide causes peripheral insulin resistance *in vivo* in dogs. *Diabetologia* **33**: 118–120, 1990.
13. Heding LG, Determination of total serum insulin (IRI) in insulin-treated patients. *Diabetologia* **8**: 260–266, 1972.
14. Stridsberg M, Wilander E, Öberg K, Lundqvist G and Eriksson B, Islet amyloid polypeptide-producing pancreatic islet cell tumor. A clinical and biochemical characterization. *Scand J Gastroenterol* **27**: 381–387, 1992.
15. Hinegardner R T, An improved fluorometric assay for DNA. *Anal Biochem* **39**: 197–201, 1971.
16. Halban PA, Wollheim CB, Blondel B and Renold AE, Long-term exposure of isolated pancreatic islets to mannoheptulose: Evidence for insulin degradation in the β-cell. *Biochem Pharmacol* **29**: 2625–2633, 1980.
17. Andersson A, and Sandler S, Viability tests of cryopreserved endocrine pancreatic cells. *Cryobiology* **20**: 161–168, 1983.
18. Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, Ma Z, Vignati L, Bowsher R, Herzog D and Klibanski A, Serum leptin levels in women with anorexia nervosa. *J Clin Endocrin Metab* **81**: 3861–3863, 1996.
19. Leclercq-Meyer V and Malaisse WJ, Failure of leptin to counteract the effects of glucose on insulin and glucagon release by the perfused rat pancreas. *Diabetologia* **40** (Suppl.1): A175, 1997. (abstract).
20. Chen NG, Swick AG and Romsos DR, Leptin constrains acetylcholine-induced insulin secretion from pancreatic islets of ob/ob mice. *J Clin Invest* **100**: 1174–1179, 1997.
21. Tanizawa Y, Okuya S, Ishihara H, Asano T, Yada T and Oka Y, Direct stimulation of basal insulin secretion by physiological concentrations of leptin in pancreatic beta cells. *Endocrinology* **138:** 4513–4516, 1997.
22. Kieffer TJ, Heller RS, Leech CA, Holz GG and Habener JF, Leptin suppression of insulin secretion by the activation of ATP-sensitive K^+ channels in pancreatic β-cells. *Diabetes* **46**: 1087–1093, 1997.
23. Kamohara S, Burcelin R, Halaas JL, Friedman JM and Charron MJ, Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature (London)* **389**: 374–377, 1997.
24. Ghatei MA, Datta HK, Zaidi M, Bretherton–Watt D, Wimalawansa SJ, MacIntyre I and Bloom SR, Amylin and amylin-amide lack an acute effect on blood glucose and insulin. *J Endocrinol* **124**: R9–R11, 1990.
25. Ohsawa H, Kanatsuka A, Yamaguchi T, Makino H and Yoshida S, Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. *Biochem Biophys Res Commun* **160**: 961–967, 1989.
26. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT and Deeds J, Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83:** 1263–1271, 1995.
27. Sandler S, Bendtzen K, Eizirik DL and Welsh M, Interleukin-6 (IL-6) affects insulin secretion and glucose metabolism

of rat in pancreatic islets *in vitro*. *Endocrinology* **126**: 1288–1294, 1990.
28. Sandler S and Welsh N, IL-10 stimulates rat pancreatic islets *in vitro*, but fails to protect against IL-1. *Biochem Biophys Res Commun* **195**: 859–865, 1993.
29. Sandler S and Sternesjö J, Interleukin 4 impairs rat pancreatic islet function *in vitro* by an action different to that of interleukin 1. *Cytokine* **7**: 296–300, 1995.
30. Sternesjö J and Sandler S, IL-13 counteracts suppression induced by IL-1β of glucose metabolism but not of insulin secretion in rat pancreatic islets. *Autoimmunity* **26:** 153–159, 1997.