Physiological Increase in Plasma Leptin Markedly Inhibits Insulin Secretion In Vivo

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The demonstration of leptin receptors on the pancreatic β-cells suggests the possibility of direct actions of leptin on insulin secretion. In vitro studies on islets or perfused pancreas and β-cell lines produced inconsistent results. We performed an in vivo study to distinctly examine whether leptin has an effect on glucose-stimulated insulin secretion. Young chronically catheterized Sprague-Dawley rats (n = 28) were subjected to a 4-h hyperglycemic clamp study (~11 mmol/l). At minute 120 to 240, rats were assigned to receive either saline or leptin (0.1, 0.5, and 5 μg · kg^{-1} · min) infusion. Leptin decreased plasma insulin levels abruptly, and an approximately twofold decrease in plasma insulin levels compared with saline control was sustained over the 2 h of the study (14.8 ± 5.8 vs. 34.8 ± 2.6 ng/ml with leptin and saline infusion, respectively, P < 0.001). Moreover, a dose-dependent decrease in plasma insulin levels was noted (r = –0.731, P < 0.01). Since milrinone, an inhibitor of cAMP phosphodiesterase (PDE) 3, did not reverse the effect of leptin on glucose-induced insulin secretion, its action may be independent of PDE3. These findings suggest that acute physiological increase in plasma leptin levels acutely and significantly inhibits glucose-stimulated insulin secretion in vivo. The site of leptin effects on insulin secretion remains to be determined. *Diabetes* 50:348–352, 2001

Leptin, a 167-amino acid product of the *ob* gene and predominantly produced by and secreted from adipose tissue (1), plays an important role in the central nervous system's regulation of food intake influencing body weight, energy expenditure, and adiposity (2). Leptin action has also been reported in peripheral tissues such as fat, muscle, and liver (3,4). The demonstration of the long form of leptin receptor (ObRb) mRNA in rat islets (5,6) also supports the possibility of direct actions of leptin on pancreatic β-cells. This last observation, coupled with the critical role of insulin in metabolism, has led to multiple studies of the effect of leptin on insulin secretion mainly in vitro. Leptin clearly reduces insulin secretion in isolated pancreatic β-cells (7,8) and perfused pancreas (7) of *ob/ob* mice that do not produce leptin. However, acute administration of leptin on insulin secretion has produced divergent results in normal rodents. In isolated islets from rat or mouse, or perfused rat pancreas, leptin has been reported to stimulate insulin secretion (9), to have no effect (10–12), and to have biphasic effects (13), depending on dose. However, a number of studies have also demonstrated that leptin inhibits insulin release (14–17). In whole-animal studies, acute leptin administration decreases basal insulin levels, though this may have been due to increased insulin sensitivity (18). One proposed local mechanism for leptin inhibition of insulin secretion is through its activation of phosphodiesterase (PDE) 3B. Static incubation in HIT-T15 cells demonstrated the reversal of leptin effects on insulin secretion by agents such as milrinone, a PDE3 inhibitor (13).

The variable ways in which experiments have been performed make findings difficult to compare and have left many uncertainties regarding optimal models and conditions. This situation led us to design an in vivo study that optimizes the conditions and limits the confounding variables for insulin secretion. The main purpose of this study was to evaluate whether acute increases in plasma leptin levels that are within the physiological range affect glucose-stimulated insulin secretion in conscious rats and whether activation of PDE3 by leptin could be implicated.

RESEARCH DESIGN AND METHODS

Animals. Three-month-old male Sprague-Dawley rats (n = 28) (Charles River Laboratories, Wilmington, MA) were used for this study. Rats were housed in individual cages and were subjected to a standard light (6:00 A.M. to 6:00 P.M.) and dark (6:00 P.M. to 6:00 A.M.) cycle. All rats were fed ad libitum using regular rat diet that consisted of 64% carbohydrate, 30% protein, and 6% fat with a physiological fuel value of 3.3-kcal/g food. One week before the in vivo study, rats were anesthetized by inhalation of methoxyflurane, and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery. This method of anesthesia allows fast recovery and normal food consumption after 1 day. The venous catheter extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch. Recovery was continued until body weight was within 3% of the preoperative weight (~4–6 days). Studies were performed in awake, unstressed, chronically catheterized rats (19,20).

Hyperglycemic clamp study. To demonstrate the in vivo effect of leptin on insulin secretion, all rats were subjected to 4-h moderate hyperglycemia (~11 mmol/l) ~6 h postprandially. Briefly, 25% glucose was infused intravenously to raise the plasma glucose concentration acutely to ~11 mmol/l. The glucose infusion rate was then varied to maintain the plasma glucose concentration at this level for 240 min. The first 120 min of the clamp study was maintained before the infusion of either saline or leptin to avoid the confounding effects of the acute increases in insulin levels during the first phase of insulin secretion and achieve similar levels of glucose-stimulated plasma insulin levels.

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FFA, free fatty acid; GIR, glucose infusion rate; MCR, metabolic clearance rate; ObRb, long-form splice variant of the leptin receptor; PDE, phosphodiesterase.

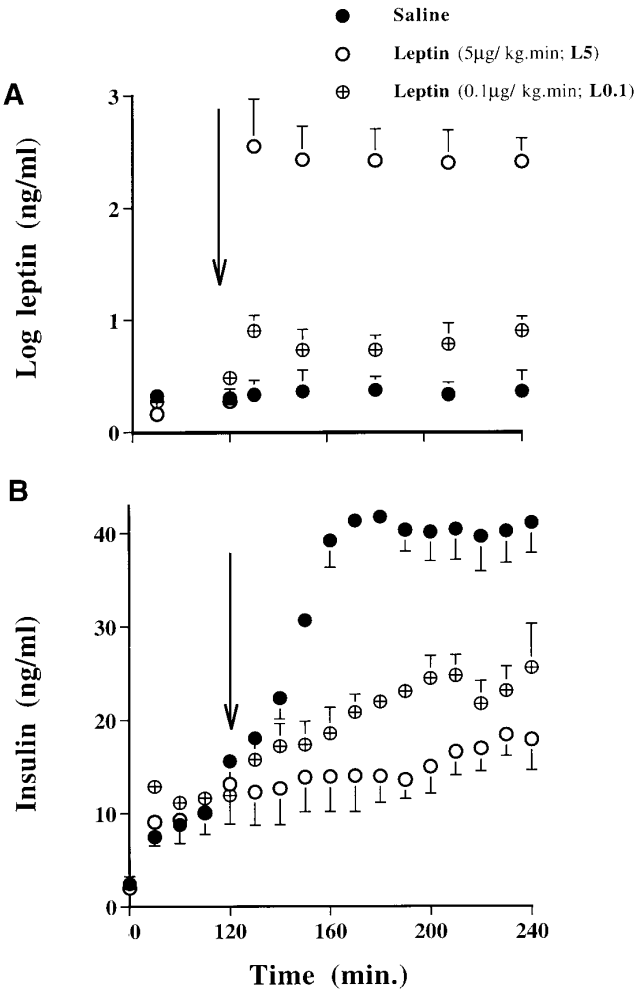

FIG. 1. Effect of acute leptin infusion on plasma insulin levels. Log plasma leptin (*A*) and plasma insulin (*B*) levels were measured during hyperglycemic clamp (~11 mmol/l) study from 0 to 240 min in rats receiving intravenous infusion at minute 120 to 240 (arrow) of saline (control; 1 ml/h) or leptin (L0.1; 0.1 µg · kg^{-1} · min and L5; 5 µg · kg^{-1} · min).

Leptin study. At minute 120, rats were assigned to receive either saline (1 ml/h, n = 5, control) or leptin infusion for an additional 2 h. Leptin was infused as primed (0.5, 2.5, and 25 µg · kg^{-1} · min for 2 min) continuous infusion at 0.1 (L0.1; n = 4), 0.5 (L0.5; n = 4), and 5 µg · kg^{-1} · min (L5; n = 4), respectively, to determine the dose-response effect on plasma insulin levels.

Milrinone study. At minute 120 to 240, either milrinone (1, 3, and 28 µg · kg^{-1} · min, n = 7) or milrinone (28 µg · kg^{-1} · min) + leptin (5 µg · kg^{-1} · min, L5) (n = 4) was infused. Plasma samples for insulin were obtained at 10-min intervals throughout the study. Samples were also obtained for determination of plasma leptin and free fatty acid (FFA) concentrations. A solution (1:1 vol/vol) of ~3.0 ml fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml) was infused at a constant rate throughout the study to prevent volume depletion and anemia. At the end of the clamp study, rats were killed using 60 mg pentobarbital sodium/kg body wt i.v.

The study protocol was reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA) and plasma insulin by radioimmunoassay using rat insulin standards. Plasma leptin was assayed using the Linco leptin assay kit (Linco Research, St. Charles, MO). Plasma nonesterified fatty acid concentrations were determined by an enzymatic method with an automated kit according to the manufacturer's specification (Waco Pure Chemical Industries, Osaka, Japan).

Terminology and calculations. To express insulin sensitivity, metabolic clearance rate (MCR) of glucose was determined to account for glucose uptake in a variety of nonmaximal stimulations of insulin levels. Thus, the MCR of glucose was calculated as follows: MCR = glucose infusion rate (GIR) divided by 2 plasma insulin.

Statistical analysis. The significance of group differences was evaluated by the two-sample t test. Pearson correlation coefficients were calculated to estimate the linear relationship between variables. All values are presented as means ± SE. All statistical analyses were performed using SPSS.

RESULTS

Leptin studies. Before the study, body weights (305 ± 10 g), plasma glucose (8 ± 0.2 mmol/l), insulin (2.4 ± 0.4 ng/ml), leptin (1.5 ± 0.1 ng/ml), and FFA (0.6 ± 0.04 meq/l) levels were similar in all groups. During the clamp study, glucose levels were maintained at similar levels (~11 mmol/l), and insulin levels continued to increase throughout the saline (control) study and tended to reach a plateau after minute 160 of hyperglycemia (Fig. 1), as previously demonstrated (21). With variable infusion rates of leptin (0.1, 0.5, and 5 µg · kg^{-1} · min), a dose response could be assessed because averaged plasma leptin levels during the clamps were 6 ± 1, 29 ± 5, and 261 ± 4 ng/ml (P < 0.01 between all). These levels were demonstrated 10 min after the primed infusion of leptin. Plasma insulin levels (averaged for the final 120 min of the clamps) were abruptly and significantly decreased by leptin and an approximately

TABLE 1
Metabolic characteristics during leptin study

	Leptin			
	Saline (control) (1 ml/h)	Low dose (LL) (0.1 µg · kg^{-1} · min)	Moderate dose (ML) (0.5 µg · kg^{-1} · min)	High dose (HL) (5 µg · kg^{-1} · min)
n	5	4	4	4
Glucose (mmol/l)	11.0 ± 0.4	11.5 ± 0.4	11.0 ± 0.4	11.2 ± 0.2
Leptin (ng/ml)	2.0 ± 0.3	6.1 ± 2.0*	29.3 ± 10.0†	261.7 ± 8.0‡
Insulin (ng/ml)	34.8 ± 2.6	20.0 ± 0.9†	17.0 ± 4.4†	14.8 ± 5.8§
FFA (meq/l)	0.40 ± 0.14	0.49 ± 0.16	0.46 ± 0.05	0.41 ± 0.14
GIR (mg · kg^{-1} · min)	64 ± 11	43 ± 10*	46 ± 5*	44 ± 11*
MCR (GIR · ng^{-1} · ml^{-1})	2.6 ± 0.8	2.4 ± 0.8	3.0 ± 0.2	3.4 ± 1.3

Data are means ± SE. *P < 0.05 vs. control; †P < 0.01 vs. control; §P < 0.001 vs. control; ‡P < 0.001 vs. all groups. Sprague-Dawley rats underwent a 4-h hyperglycemic (~11 mmol/l) clamp study. After 120 min of hyperglycemia, saline or leptin infusion was started from minute 120 to 240. Plasma glucose, leptin, insulin, FFA levels, GIR, and MCR were averaged over the last 90 min of the study.

twofold decrease was sustained until the termination of the study (14.8 ± 5.8 vs. 34.8 ± 2.6 ng/ml, with leptin and saline infusion, respectively, $P < 0.001$) (Table 1 and Fig. 1). Moreover, decrease in plasma insulin levels occurred earlier with L5 than with L0.1 (insulin levels at 10 min of leptin infusion: 12 ± 3 vs. 18 ± 1 ng/ml with L5 vs. saline compared with insulin levels at 20 min 17 ± 2 vs. 22 ± 2 ng/ml with L0.1 vs. saline, respectively, $P < 0.05$) (Fig. 1). Intermediate results were obtained at plasma leptin levels of ~29 ng/ml (Table 1). Figure 2 further demonstrates leptin's dose-response effect and the significant inverse correlation between leptin and plasma insulin levels ($r = –0.731$, $P < 0.01$). Of note, ~70% of leptin inhibition occurred with the lowest infusion rate that increased leptin levels by only approximately threefold. Leptin infusion significantly decreased GIR by ~30% in all groups compared with saline ($P < 0.05$). However, when GIR was further expressed in terms of the MCR of glucose (GIR/plasma insulin), this remained unchanged in all groups compared with saline (Table 1). These data indicate no change in insulin sensitivity during this short experimental period.

Milrinone study. Plasma insulin levels increased promptly upon infusion of milrinone during the first 10 min (insulin: 31 ± 6 vs. 18 ± 1 ng/ml, with milrinone and saline respectively, $P < 0.01$) (Fig. 3). Surprisingly, the stimulatory effect of milrinone lasted only for 30 min and was subsequently followed by a decrease in plasma insulin levels. This may be explained by a direct effect of milrinone on insulin secretion, glucose uptake, or other unrelated pathway by which milrinone may influence glucose homeostasis. Co-infusion of milrinone with L5 also resulted in an abrupt increase in insulin secretion during the first 10 min (insulin: 27 ± 6 ng/m vs. saline; $P < 0.01$) (Fig. 3), suggesting an initial milrinone action. However, there was an acute decrease in plasma insulin levels during the next 10 min, eventually reaching levels similar to those noted with leptin infusion alone (insulin: 17 ± 3 vs. 13 ± 4 ng/ml, with milrinone + L5 and L5, respectively) (Fig. 3).

DISCUSSION

This study demonstrates that acute increases in plasma leptin that are in a physiologically relevant range suppress glucose-stimulated insulin secretion in a dose-dependent manner.

Many in vitro studies on islets, perfused pancreas, and cell lines demonstrated that leptin had an inhibitory effect on glucose-stimulated insulin secretion (14–17), presumably through the ObRb receptor. However, leptin also has an important role in the central regulation of food intake and energy expenditure and possibly peripherally through autonomic output from the hypothalamus (14,24). Thus, the effects of leptin on β-cell secretion may also work centrally. Also, leptin may have other indirect effects on the function and action of other hormones associated with the control of insulin secretion (14).

Thus, in vivo studies may be most appropriate to examine the overall effect of leptin on insulin secretion. This study was designed to optimize the in vivo model of moderate hyperglycemia that will allow a valid interpretation of the short-term in vivo effect of physiological increases in plasma leptin levels on insulin secretion. Two hours of sustained hyperglycemia were allotted before the infusion of leptin or saline to avoid the confounding effects of the acute rise in insulin levels during the first phase of insulin secretion and to obtain similar levels of insulin release in individual rats before the infusion of leptin or saline at minute 120. Thereafter, the clamp study was performed for an additional 2 h with either saline or leptin to examine the ability of leptin to suppress insulin secretion. A 4-h study was conceived to be adequate and appropriate since glucose-stimulated insulin secretion was previously shown to continue to rise during most of this time period (21).

This study also demonstrated that the inhibitory effect of leptin on insulin secretion was immediate (~5–10 min after infusion) and dose dependent (Fig. 2). We have previously

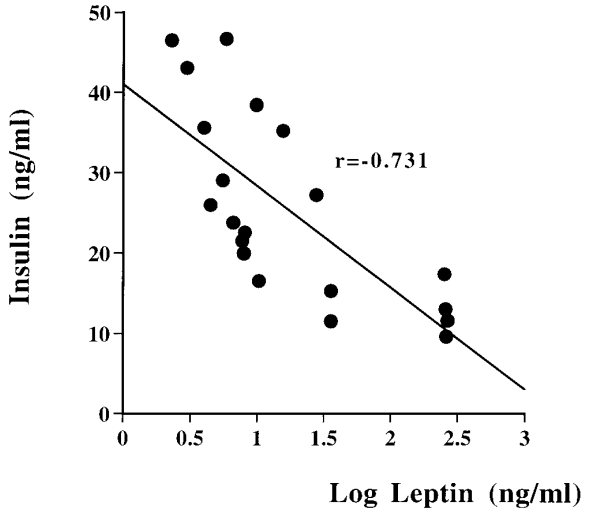

FIG. 2. Dose-response effects of leptin on plasma insulin levels. Plasma insulin and leptin levels are expressed as mean values from 180 to 240 min ($P < 0.01$).

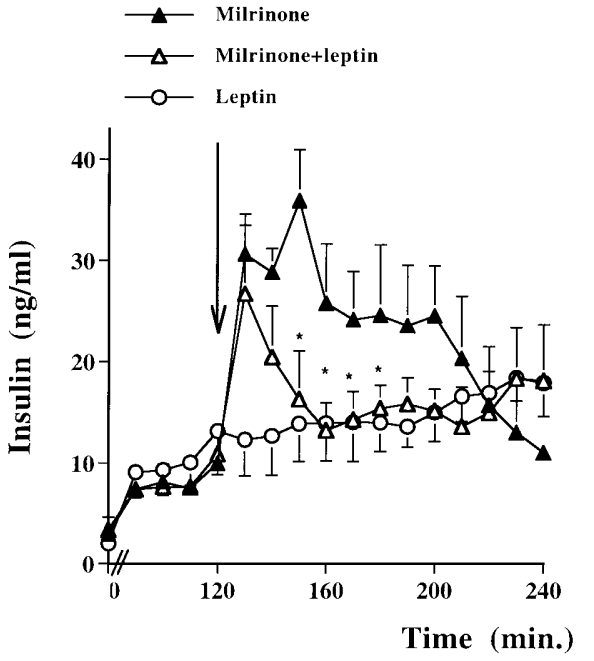

FIG. 3. Effect of milrinone, leptin, and milrinone with leptin on plasma insulin levels. Plasma insulin levels were measured during a hyperglycemic clamp (~11 mmol/l) study from 0 to 240 min in rats receiving intravenous infusion at minute 120 to 240 (arrow) of milrinone (28 µg · kg^{-1} · min), leptin (L5; 5 µg · kg^{-1} · min), or milrinone + leptin (28 µg · kg^{-1} · min + 5 µg · kg^{-1} · min). *$P < 0.01$ vs. milrinone alone.

determined that leptin has no effect on the metabolic clearance rate of insulin (4). This abrupt inhibition of insulin secretion with leptin treatment is highly suggestive that this effect is solely due to leptin action. The physiological plasma leptin levels achieved to significantly suppress insulin secretion are at levels found in mild obesity (25) and with impaired glucose tolerance (26). Whereas Kieffer and Habener (27) proposed that the chronic hyperleptinemic state of obesity may lead to a relative insulin-deficient state (the adipoinsular axis hypothesis), this short-term study cannot make valid implications regarding the effects of chronic hyperleptinemia on insulin secretion. Hyperleptinemia for 14 days in vivo and 3 days in vitro in normal and *fa/fa* rats suppressed insulin secretion due to its lipopenic effect on islet cells (28,29). These observations (with leptin administration for several days) were different from our study in which leptin effects had a rapid onset (within minutes), and without changes in markers of lipid homeostasis.

Clues to direct cellular mechanisms by which leptin suppresses insulin release come from earlier studies from *ob/ob* and *db/db* mouse islets because both phenotypes result from the absence of leptin signaling. One of the leading proposed mechanisms is through a reduction of cAMP by leptin. This proposed mechanism was further supported when milrinone, a selective inhibitor of PDE3, completely blocked the inhibitory effect of leptin on glucose- or glucagon-like peptide 1–potentiated insulin secretion in vitro (13). In an effort to examine whether leptin action on glucose-induced insulin secretion is through the activation of PDE3, we infused high doses of milrinone alone and milrinone with high doses of leptin. The interpretation of these data is limited, because milrinone increased insulin secretion initially, but with time insulin secretion decreased (Fig. 3). This could have been due to a downregulation of its own effect on insulin secretion through the cAMP pathway. However, if we limit our discussion to the first 60 min of milrinone infusion, leptin inhibited milrinone-induced insulin secretion, an effect not readily explained by leptin activation of PDE3 (13).

Our study does not clearly demonstrate the site of leptin action on insulin secretion. Although it was previously assumed from in vitro studies that the metabolic effects of leptin are mediated locally through its tissue receptors, such a notion had been challenged. In fact, many of leptin's effects were demonstrated both centrally and peripherally. For example, it was demonstrated that marked and acute hyperleptinemia modulated hepatic gene expression of the gluconeogenic enzyme PEPCK and the rate of gluconeogenesis in vivo by intracerebroventricular and intravenous routes (4). The same results were demonstrated in liver cell line (30), suggesting that the efferent pathway of leptin from the hypothalamus can influence peripheral metabolism. Combined with negative results from examining the PDE3 system, this example cautions us from concluding that the major effect of leptin on insulin secretion is directly through its receptor on the β-cells.

Finally, chronic hyperleptinemia in obesity is proposed to uncouple leptin action on its receptor in the hypothalamus, thereby attenuating signal transduction pathways that exert resistance to the hormone on satiety and energy expenditure and metabolism (2). If leptin resistance extends to the effect of leptin on insulin secretion, then high leptin levels may not contribute to the modulation of insulin secretion. However, this hypothesis can be supported only by further studies on obese leptin-resistant rodents compared with lean controls.

In summary, this is the first study designed to demonstrate the acute effects of physiological increases in plasma leptin levels on the inhibition of glucose-stimulated insulin secretion in vivo. Although our findings link hyperleptinemia and decreased insulin secretion, the role of chronic effects of hyperleptinemia with obesity leading to the transition to diabetes requires further studies.

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