

Leptin Inhibits Insulin Secretion by Activation of Phosphodiesterase 3B

Allan Z. Zhao,* Karin E. Bornfeldt,† and Joseph A. Beavo*

*Department of Pharmacology and †Department of Pathology, University of Washington, Seattle, Washington 98195

Abstract

The molecular signaling events by which leptin exerts its functions *in vivo* are not well delineated. Here, we show a novel leptin signaling mechanism that requires phosphoinositide 3-kinase (PI 3-kinase)-dependent activation of cyclic nucleotide phosphodiesterase 3B (PDE3B) and subsequent suppression of cAMP levels. In pancreatic β cells, leptin causes the activation of PDE3B, which leads to marked inhibition of glucagon-like peptide-1–stimulated insulin secretion. The effect of leptin is abolished when insulin secretion is induced with cAMP analogues that cannot be hydrolyzed by PDE3B. Selective inhibitors of PDE3B and PI 3-kinase completely prevent the leptin effect on insulin secretion and cAMP accumulation. The results demonstrate that one of the physiological effects of leptin, suppression of insulin secretion, is mediated through activation of PDE3B and suggest PDE3B as a mediator of leptin action in other tissues. (*J. Clin. Invest.* 1998, 102:869–873.) Key words: cAMP • diabetes • phosphoinositide 3-kinase • β cell • glucagon-like peptide-1

Introduction

Initially discovered for its central role in hypothalamic regulation of food intake, leptin is also known to influence insulin actions in peripheral tissues, such as fat and liver (1–4). Leptin deficiencies in both mice and humans led to obesity and diabetic syndromes including hyperinsulinemia (1, 5). The cause of the hyperinsulinemia in leptin deficiencies may be attributed to either peripheral tissue insulin resistance or lack of a direct inhibitory control of insulin secretion, or a combination of both. Recent reports have shed some light on the molecular actions of leptin. For example, in response to leptin, transcription factors, such as STAT3, can be activated in the presence of a long form of leptin receptor (OB-Rb)¹ (6). Both OB-Rb and a short form of the leptin receptor (OB-Ra) can induce tyrosine phosphorylation of insulin receptor substrate-1 (7, 8). However, the exact signaling events underlying leptin's func-

tions in most peripheral tissues and the central nervous system remain to be defined.

In the presence of elevated blood glucose, cAMP stimulates insulin secretion from pancreatic β cells (9). The steady-state levels of intracellular cAMP are regulated by the activities of both adenylyl cyclases and phosphodiesterases (PDEs) (10). Collectively, nine families of PDEs have been identified (11, 12). As a member of the type 3 PDE family, PDE3B is known to mediate many of the effects of insulin in adipocytes and hepatocytes by reducing cAMP (13). This process is dependent on activation of phosphoinositide 3-kinase (PI 3-kinase) (13). We have reported recently that PDE3B is also highly expressed in the pancreatic β cells and plays an important role in mediating the inhibitory effect of IGF-1 on insulin secretion (14). In this report, we show that leptin inhibits glucose- and glucagon-like peptide-1 (GLP-1)-potentiated insulin secretion, and that the molecular signaling mechanism requires the PI 3-kinase–dependent activation of PDE3B.

Methods

Cell cultures. The preparation and culture of neonatal rat monolayer pancreatic islets has been described previously (14). This system has been well characterized, and shows phasic insulin secretion in response to glucose and also allows easy access of secretagogues and hormones to a monolayer of islet cell clusters. In general, the islet cells were preincubated in KRB containing 1.6 mM glucose and 0.1% BSA before the leptin (recombinant mouse leptin; PeprTech, Inc., Rocky Hill, NJ) or GLP-1 (Peninsula Laboratories Inc., Belmont, CA) treatment. The duration of hormonal treatment was 30 min, and each condition was studied in triplicate. The HIT-T15 cells were cultured in Ham's F12 medium supplemented with 15% (vol/vol) heat-inactivated FBS. Static incubation of HIT cells was performed in a manner similar to that used for the rat pancreatic islet monolayer cells. However, the HIT cells were preincubated in 0 mM glucose.

Assays for cAMP and insulin. Immediately after hormonal treatment, the HIT cells were dissolved and incubated in ice-cold 5% TCA overnight. The cAMP assay was carried out using a radioimmunoassay kit from DuPont/NEN (Cambridge, MA). The concentration of insulin secreted to the medium was measured with a radioimmunoassay kit from Linco Research (St. Charles, MO).

Immunoprecipitation, PDE assay, and PI 3-kinase assay. HIT-T15 cells were treated with leptin and/or GLP-1 for 30 min in the presence of 11.1 mM glucose. The immunoprecipitation of PDE3B enzyme as well as the assay of the PDE activity were carried out according to a previously published protocol, with 1 μ M cAMP as substrate (15). The PDE activity is adjusted according to the quantity of

Address correspondence to Joseph A. Beavo, Department of Pharmacology, Box 357280, University of Washington, School of Medicine, Seattle, WA 98195-7280. Phone: 206-543-4066; FAX: 206-685-3822; E-mail: beavo@u.washington.edu

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1. **Abbreviations used in this paper:** 8-Br-cAMP, 8-bromo-cAMP; GLP-1, glucagon-like peptide-1; N⁶-MB-cAMP, N⁶-monobutyryl-cAMP; OB-Ra, short form of leptin receptor; OB-Rb, long form of leptin receptor; PDE, phosphodiesterase; PI 3-kinase, phosphoinositide 3-kinase; PKA, protein kinase A.

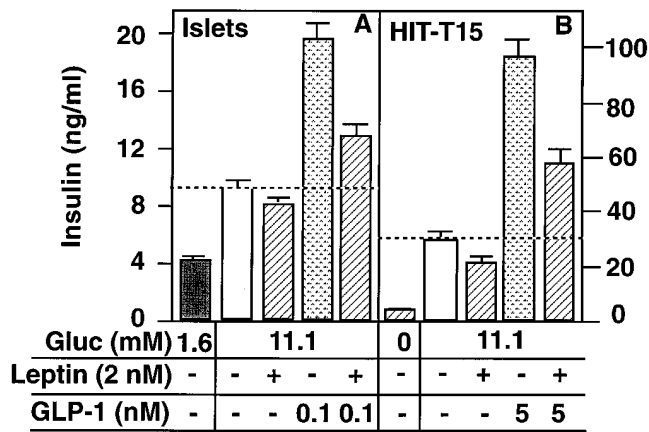


Figure 1. Leptin attenuates glucose- and GLP-1-stimulated insulin secretion from rat pancreatic islets (A) and a β cell line, HIT-T15 (B). The dotted lines represent the insulin secretion levels as stimulated by 11.1 mM glucose (Gluc). Data are the means \pm SEM with quadruplets for the islet cell, and triplicates for the HIT cells.

immunoprecipitated PDE3B as shown on Western blot, and expressed as picomoles (hydrolyzed cAMP) per minute per unit of PDE3B. The PI 3-kinase complex was precipitated with an anti-p85 antibody (Transduction Laboratories, Lexington, KY). The PI 3-kinase assay and subsequent TLC were carried out according to a standard protocol (Avanti Polar Lipids, Alabaster, AL) with phosphatidylinositol as substrate in the presence of [γ - 32 P]ATP.

Adenylyl cyclase assay. HIT cells cultured in Ham's F12 medium were incubated with 2 μ Ci/ml of [3 H]adenine overnight. The cells were then washed once in KRB, then continued to be cultured in the KRB containing 0.1% BSA and no glucose for 1 h before the hormonal treatment. After 30 min of hormonal treatment, the medium was decanted and the cells were immediately dissolved in ice-cold 5% TCA solution and the soluble nucleotides were separated by ion-exchange chromatography. The activity of the adenylyl cyclase is expressed as a ratio of [3 H]cAMP to total ATP, ADP, and AMP pool.

Results

Previous studies with an RT-PCR approach (16) have revealed the presence of both OB-Rb and OB-Ra in pancreatic islets. However, we can detect by Western blot analysis only a 110-kD protein that represents the dominant form of leptin recep-

tor in β cells (data not shown). Such an immunoreactive band is consistent with OB-Ra, although we cannot rule out the possibility of low expression of OB-Rb in these cells. In a rat pancreatic islet culture, leptin only mildly suppressed glucose-stimulated insulin secretion (Fig. 1 A). However, leptin has a significant inhibitory effect on GLP-1-potentiated insulin secretion. When applied at or near physiological concentrations, e.g., 0.1 nM (17, 18), GLP-1 potentiated insulin release by \sim 2.5-fold over the glucose (11.1 mM) effect and \sim 6-fold over the baseline insulin release (glucose 1.6 mM), similar to the magnitude of insulin secretion in response to GLP-1 stimulation in vivo (18). Leptin markedly suppressed this GLP-1 effect (Fig. 1 A). When we performed similar studies on a pancreatic β cell line, HIT-T15 (Fig. 1 B), leptin was also able to attenuate the glucose- as well as GLP-1-stimulated insulin secretion from this cell line (Fig. 1 B), indicating a direct effect of leptin on the β cells.

Somewhat surprisingly, we found that the concentration effect of leptin on insulin secretion is biphasic, with the maximal effect achieved at \sim 2 nM (Fig. 2, A and B). Higher concentrations of leptin are less efficient in suppressing insulin secretion. Such leptin dosage curves are probably not an artifact of a particular preparation of leptin, as another source of leptin (a gift from Zymogenetics, Seattle, WA) prepared in a different expression system also yielded a very similar curve (data not shown). Nor is the biphasic effect unique to a specific β cell line, as the pancreatic islet culture (Fig. 2 A), the HIT-T15 cells (Fig. 2 B), and the β -Tc cells (data not shown) all showed a similar type of dose-response effect. The leptin concentrations used in our study are within the physiological range as determined in previous studies, which varies from \sim 0.2 nM to as high as 3 nM in rodents and 5 nM in humans (19–21). Taken together, our data indicate that such physiologically relevant concentrations of leptin suppress insulin secretion, but higher levels of leptin will be less effective. The biphasic dose-response of leptin may also explain why some of the recent studies using high concentrations of leptin failed to see any significant effect on GLP-1-stimulated insulin secretion (22).

Since GLP-1 potentiates insulin secretion by elevating cAMP levels in the β cells, we tested whether the inhibitory effect of leptin on insulin secretion was due to a reduction of cAMP accumulation. In the presence of elevated glucose concentrations, GLP-1 (5 nM) elevated the cAMP level in the HIT cells about threefold (Fig. 2 C); leptin was able to sup-

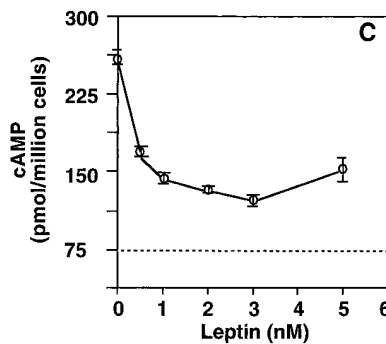
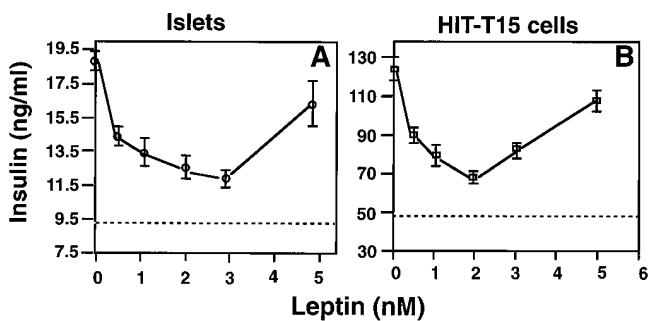


Figure 2. Leptin concentration effects on GLP-1-stimulated insulin secretion from rat pancreatic islets (A) or HIT-T15 cells (B), and on GLP-1-stimulated cAMP in HIT-T15 cells (C). The insulin or cAMP value at 0 nM leptin represents the effect of GLP-1 alone. A maximum inhibition of insulin secretion was achieved at \sim 2 nM of

leptin. The dotted lines represent the insulin secretion or cAMP levels as stimulated by high glucose (11.1 mM). The cell culture, experimental protocols, and insulin assays are described in Methods. Data are the means \pm SEM with quadruplets for the islet cell, and triplicates for the HIT cells.

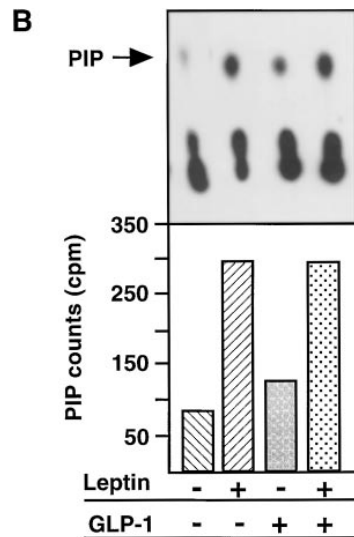
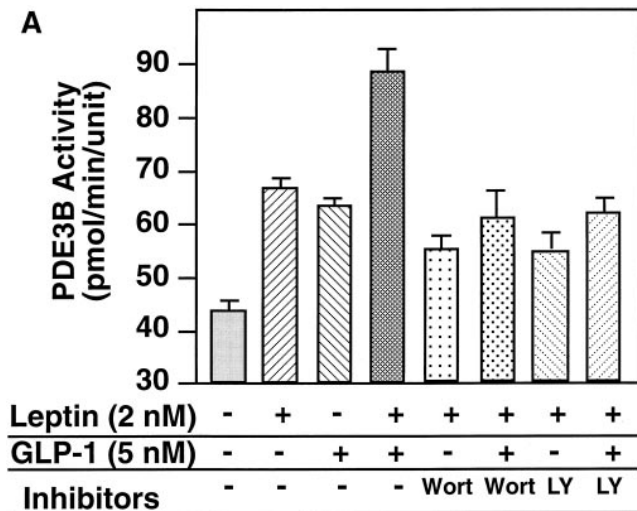


Figure 3. (A) Activation of PDE3B by leptin in pancreatic β cells, and the effects of PI 3-kinase inhibitors on the activation of PDE3B by leptin. (B) Activation of PI 3-kinase by leptin in pancreatic β cells. The concentration of the PI 3-kinase inhibitors are: wortmannin (Wort) = 20 nM; LY294002 (LY) = 2 μ M. PIP, Phosphatidylinositol-3-phosphate.

press the elevation of cAMP level in a dose-dependent manner (Fig. 2 C). Leptin also mildly reduced cAMP levels in the presence of high glucose but in the absence of GLP-1 (see Fig. 4). The extent of reduction of cAMP by leptin was comparable to the reduction of insulin secretion. These data indicate that leptin antagonizes GLP-1-stimulated cAMP accumulation in the β cells which in turn decreases insulin secretion.

We investigated whether the leptin-mediated reduction of cAMP is primarily due to an increase of a PDE activity. Two major types of PDE activities exist in the pancreatic β cells, PDE4 and PDE3B (14, 23). While the PDE4 activity remained unchanged after leptin addition (data not shown), PDE3B activity in HIT cells was elevated \sim 50% by the treatment with leptin alone, or GLP-1 alone (Fig. 3 A). The GLP-1 effect on PDE3B is presumably due to the increase of cAMP, and is consistent with the fact that protein kinase A (PKA) can modestly activate PDE3B (13). The activation of PDE3B by leptin is even greater in the presence of GLP-1 (approximately two-

fold, Fig. 3 A), suggesting an additive effect between the two hormones in activating PDE3B. Since the activation of PDE3B by insulin has been shown to be mediated by a PI 3-kinase-dependent process in adipocytes (13), we examined the ability of leptin to activate PI 3-kinase in pancreatic β cells. Cultured HIT cells were treated with leptin for 30 min, and the PI 3-kinase complex was immunoprecipitated for assay of its activity. Leptin was able to activate PI 3-kinase by about threefold (Fig. 3 B). The extent of activation of PI 3-kinase by leptin alone was similar to that by leptin and GLP-1 together (Fig. 3 B). Addition of low concentrations of the PI 3-kinase inhibitors, wortmannin or LY294002, to this system abolished the activation of PDE3B by leptin (Fig. 3 A), implying that the activation of PDE3B by leptin could be mediated through activation of PI 3-kinase.

If the reduction of insulin secretion by leptin is due to a PI 3-kinase-dependent activation of PDE3B, then selective inhibitors of PDE3B or PI 3-kinase would be predicted to block

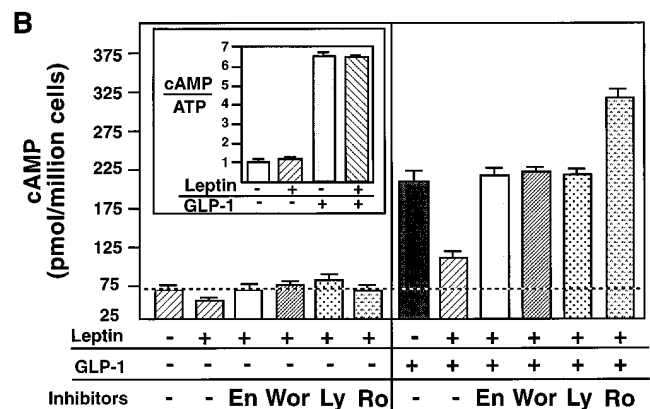
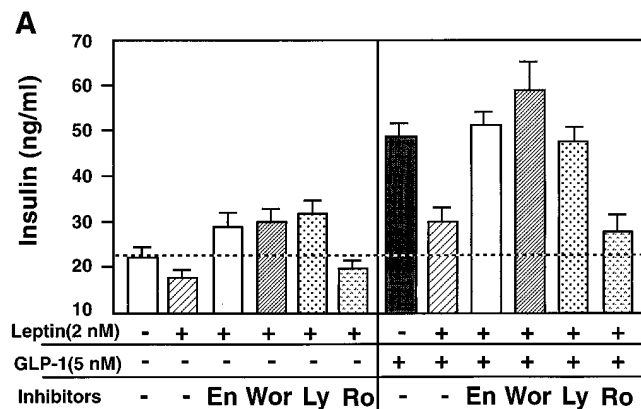


Figure 4. The influence of pharmacological inhibitors on insulin secretion (A) and cAMP (B). Both PDE3B and PI 3-kinase inhibitors reversed the leptin inhibitory effects on insulin secretion and cAMP levels in HIT-T15 cells. A selective PDE4 inhibitor, rolipram, elevated total cAMP levels, but did not block the leptin effect on insulin secretion. The abbreviations and concentrations of pharmacological agents are as follows: PDE3B inhibitor, enoximone (En) at 1 μ M; PI 3-kinase inhibitors, wortmannin (Wor) at 20 nM, and LY294002 (Ly) at 1 μ M; PDE4 inhibitors, rolipram at 3 μ M (Ro). Data are means \pm SEM, triplicates. The leptin and GLP-1 concentrations are 2 and 5 nM, respectively. The small insert indicates that leptin has no direct influence on the adenyl cyclase activity of pancreatic β cells in the presence or absence of GLP-1. The data are the means \pm SEM with triplicates.

this leptin effect. Indeed, wortmannin (at 20 nM) or LY294002 (at 1 μ M) completely blocked the inhibitory leptin effect on glucose- and GLP-1-stimulated insulin secretion from the HIT cells (Fig. 4 A), and these inhibitors also prevented the leptin-induced reduction in cAMP (Fig. 4 B). Milrinone and enoximone have been characterized as highly selective pharmacological inhibitors of type 3 PDE with at least 50-fold selectivity over other types of PDEs ($IC_{50} = 0.3 \mu$ M) (11). When 1 μ M of milrinone or enoximone (three times their IC_{50}) was applied to the HIT cells, it elevated the cAMP level as expected. In addition, these reagents completely blocked the inhibitory effect of leptin on glucose- or GLP-1-potentiated insulin secretion (Fig. 4 A). As a control, we also applied a selective PDE4 inhibitor, rolipram (3 μ M, $\sim 3 \times IC_{50}$) (11), to the β cells. Interestingly, the PDE4 inhibitor also elevated the overall cAMP level, but it failed to prevent the leptin inhibitory effect on insulin secretion (Fig. 4, A and B), suggesting that there are distinctive pools of cAMP mediating different physiological functions of the β cells, and that the leptin effect on insulin secretion is specifically mediated by PDE3B. Such data are also consistent with recent findings that the GLP-1 stimulatory effect on insulin secretion requires anchoring of PKA to specific subcellular regions of the β cells (24). Taken together, our data show that the leptin inhibitory action on insulin secretion is primarily mediated through activation of PDE3B and a subsequent reduction of the intracellular cAMP. The activation of PDE3B is mediated by PI 3-kinase or possibly by a related kinase inhibited by wortmannin and LY294002 (25).

To further confirm that the reduction of cAMP and insulin secretion by leptin is primarily due to activation of PDE3B rather than inhibition of an adenylyl cyclase, we used two different cAMP analogues to bypass the requirement of adenylyl cyclase activity, both of which can act as PKA agonists (26). Whereas 8-bromo-cAMP (8-Br-cAMP) is susceptible to hydrolysis by PDE3B, N^6 -monobutyl-cAMP (N^6 -MB-cAMP) is resistant to hydrolysis by this isozyme due to the covalent modification at N^6 position (26). Both cAMP analogues promoted insulin secretion in a concentration-dependent manner (Fig. 5, A and B). However, leptin had a negative effect only on the 8-Br-cAMP-stimulated, but not the N^6 -MB-cAMP-stimulated, insulin secretion (Fig. 5). Furthermore, a direct measurement of adenylyl cyclase activity did not show any significant changes in the leptin-treated β cells (Fig. 4 B, inset). These data indicate that the leptin effect on insulin secretion is mostly mediated through activation of PDE3B, rather than inhibition of an adenylyl cyclase, in the β cells. These data also make it unlikely that the leptin effect is mediated by a target downstream of cAMP, such as a substrate of PKA, since leptin would not have had a discriminatory effect on insulin secretion induced by different cAMP analogues in that case.

Discussion

It is worth noting that a recent study has shown that leptin suppresses glucose-stimulated insulin secretion from the pancreatic islets of *ob/ob* mice (22). Modulation of an ATP-sensitive K^+ channel or inhibition of Ca^{2+} influx was suggested to contribute to this leptin inhibitory effect (22). It has been postulated that cAMP potentiates insulin secretion by inhibiting ATP-sensitive K^+ channels through PKA phosphorylation of the channels and/or by activation of Ca^{2+} channels (9). Therefore, we believe that the reduction of cAMP by leptin is consis-

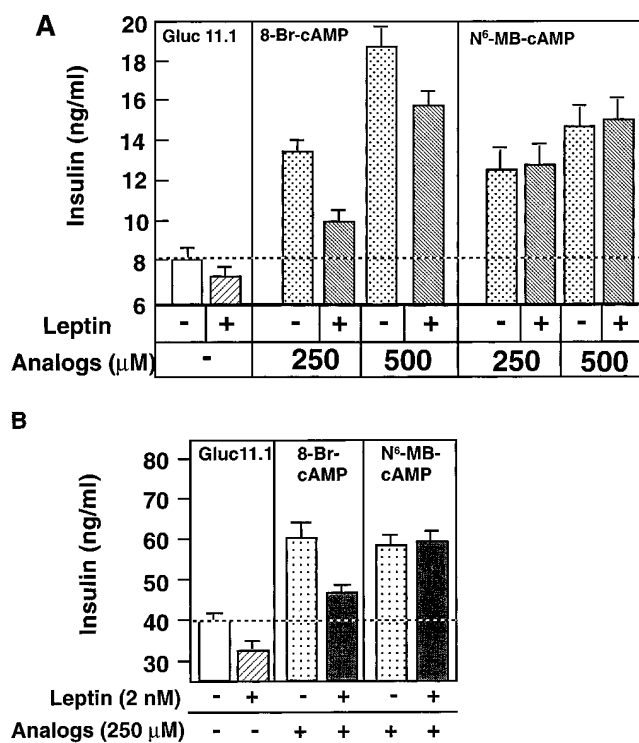


Figure 5. Differential effects of leptin on cAMP analogue-stimulated insulin secretion from rat pancreatic islets (A) and HIT-T15 cells (B). 8-Br-cAMP and N^6 -MB-cAMP were applied with or without 2 nM leptin to either the rat pancreatic islet culture or the HIT-T15 cells. The dotted lines represent the secreted insulin level in the presence of 11.1 mM glucose alone (*Gluc 11.1*). Data are the means \pm SEM with quadruplets, triplicates for the islet and HIT cells, respectively.

tent with the results of leptin influencing the K^+ and/or Ca^{2+} current in pancreatic β cells. However, these same studies also reported the failure of leptin to inhibit GLP-1-stimulated insulin secretion where very high concentrations of GLP-1 (e.g., 10 nM) were applied to the pancreatic islets (22). Such high concentrations of GLP-1 are far beyond physiological ranges, and will maximize the insulin secretion (greater than eightfold higher than the 16.7 mM glucose effect) and presumably synthesis of cAMP (Zhao, A.Z., and J. Teague, unpublished observations). Under such conditions, we found that leptin, even when applied at an optimal concentration, had an insignificant effect on the total cAMP content, and hence the GLP-1-stimulated insulin secretion (data not shown).

Overall, the data presented here suggest that the inhibitory effect of leptin on insulin secretion represents a homeostatic control by one of the primary insulin targets, i.e., adipose tissue, and that this effect is primarily regulated at the level of cAMP through activation of PDE3B. PDE3B is also known to be important in mediating insulin's suppression of glycogenolysis in hepatocytes and lipolysis in adipocytes through reduction of cAMP (13). Therefore, the data presented in this study suggest that insulin and leptin share at least in part common signaling pathways. We expect that regulation of cAMP by leptin through activation of PDE3B most likely will not be unique to the pancreatic β cells. For example, we have found recently that leptin can also activate PDE3B in primary rodent hepatocytes (manuscript in preparation). Such results are con-

sistent with recent reports that leptin, when infused into rodents, reduces glycogenolysis in liver (4). In addition, we mapped the expression of PDE3B in the mouse central nervous system through in situ hybridization. Of particular interest is the expression of PDE3B in the arcuate nucleus and lateral hypothalamus (data not shown), where cAMP may play a role in appetite control (27, 28). Currently, we are investigating the potential functional roles of PDE3B in mediating leptin-regulated food intake.

Finally, insulin resistance in peripheral tissues has been considered to be one of the major contributing factors to hyperinsulinemia, a hallmark of obesity and many forms of type 2 diabetes. Considering the high levels of serum leptin in obese people and the biphasic dosage effect of leptin on insulin secretion, it should be very interesting to further investigate whether leptin resistance of the β cell might also be a major contributing factor to hyperinsulinemia in obese subjects. Furthermore, PDE3B and the signaling components leading to the activation of PDE3B may potentially be useful targets for pharmaceutical intervention in the treatment of diabetes and obesity.

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