Leptin Constrains Phospholipase C-Protein Kinase C-Induced Insulin Secretion via a Phosphatidylinositol 3-Kinase-Dependent Pathway

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Leptin-deficient *Lep^{ob}/Lep^{ob}* mice hypersecrete insulin in response to acetylcholine stimulation of the phospholipase C-protein kinase C (PLC-PKC) pathway, and leptin constrains this hypersecretion. Leptin has been reported to activate phosphatidylinositol 3-kinase (PI 3-K) and subsequently phosphodiesterase (PDE) to impair protein kinase A (PKA)-induced insulin secretion from cultured islets of neonatal rats. We determined if PKA-induced insulin secretion was also hyperresponsive in islets from *Lep^{ob}/Lep^{ob}* mice, and if leptin impaired this pathway in islets from these mice. Additionally, the possible role for PI 3-K and PDE in leptin-induced control of acetylcholine-induced insulin secretion was examined. Stimulation of insulin secretion with GLP-1, forskolin (an activator of adenylyl cyclase), or IBMX (an inhibitor of PDE) did not cause hypersecretion of insulin from islets of young *Lep^{ob}/Lep^{ob}* mice, and leptin did not inhibit GLP-1-induced insulin secretion from islets of these mice. Inhibition of PDE with IBMX also did not block leptin-induced inhibition of acetylcholine-mediated insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice. But, preincubation of islets with wortmannin, an inhibitor of PI 3-K activity, blocked the ability of leptin to constrain acetylcholine-induced insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice. We conclude that the capacity of the PKA pathway to stimulate insulin secretion is not increased in islets from young *Lep^{ob}/Lep^{ob}* mice, and that leptin does not regulate this pathway in islets from mice. Leptin may stimulate PI 3-K to constrain PLC-PKC-induced insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice. Exp Biol Med 228:175–182, 2003

Key words: Insulin secretion; *Lep^{ob}/Lep^{ob}* mice; leptin; phosphatidylinositol 3-kinase; phospholipase C-protein kinase C

A mutation in the *Lep^{ob}* gene disrupts leptin synthesis and causes profound obesity in *Lep^{ob}/Lep^{ob}* mice (1). These animals are hyperinsulinemic early in development, before they exhibit elevated food intake (2), decreased metabolic rates (3, 4), or insulin resistance (5). This suggests that leptin might directly regulate insulin synthesis or secretion. Several reports have shown that leptin decreases insulin mRNA abundance in islets and cell lines (6, 7). Other studies have focused on insulin secretion. Leptin inhibits insulin secretion from islets and insulin-secreting cell lines, although there are conflicting reports (reviewed in Ref. 8).

Insulin secretion is stimulated by a variety of signals including nutrients, neurotransmitters, and hormones that interact within the pancreatic islets (9). *Lep^{ob}/Lep^{ob}* mice have elevated plasma insulin concentrations by 2 weeks of age, but their pancreatic islets secrete insulin normally in response to glucose at this age (10, 11). This suggests that neurotransmitters and/or hormones might be responsible for the initial hypersecretion of insulin in young *Lep^{ob}/Lep^{ob}* mice. Glucose-induced insulin secretion is potentiated by stimulation of the phospholipase C-protein kinase C (PLC-PKC) and protein kinase A (PKA) signal transduction pathways (12). Acetylcholine and cholecystokinin activate the PLC-PKC signaling pathway to stimulate insulin secretion (13). Enhanced sensitivity of islets from *Lep^{ob}/Lep^{ob}* mice to this pathway has been suggested as a possible mechanism for their initial development of hyperinsulinemia (10, 11). Islets from 2-week-old, as well as adult, *Lep^{ob}/Lep^{ob}* mice hypersecrete insulin in response to acetylcholine and cholecystokinin, and this hypersecretion of insulin is suppressible by leptin (10, 14). The inhibitory effect of leptin was still present when insulin secretion was stimulated by a PKC

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agonist, phorbol-12-myristate-13-acetate (14, 15), suggesting that this pathway is a possible target for leptin to regulate insulin secretion.

The possibility that the PKA pathway might also be activated to cause hypersecretion of insulin from islets of young *Lep*^{ob}/*Lep*^{ob} mice has not been as extensively investigated as the PLC-PKC pathway in these mice. Leptin was shown to activate phosphodiesterase (PDE) 3B in cultured islets from neonatal rats, to suppress cAMP content of the islets, and to inhibit glucagon-like peptide-1 (GLP-1)-stimulated insulin secretion from these islets (16). This raises the possibility that islets from leptin-deficient mice might exhibit an enhanced rate of insulin secretion when exposed to GLP-1 to activate adenylyl cyclase. The observation that leptin activates PDE in islets from neonatal rats (16) also raises the possibility that the leptin-induced inhibition of PLC-PKC-mediated insulin secretion observed in islets from *Lep*^{ob}/*Lep*^{ob} mice might occur via cross-talk between the PKA and PLC-PKC pathways (11, 17).

Zhao *et al.* (16) showed that the leptin-induced activation of PDE in islets from neonatal rats was mediated by activation of phosphatidylinositol 3-kinase (PI 3-K). Inhibition of PI 3-K activity by wortmannin blocked the ability of leptin to inhibit GLP-1-induced insulin secretion. Other reports have linked the inhibitory effects of leptin on insulin secretion to activation of K^+_{ATP} channels via activation of PI 3-K activity (18, 19). Leptin also functions in other tissues to stimulate PI 3-K and affect glucose transport and glycogen synthesis (20, 21). The possibility that the enhanced acetylcholine potentiation of insulin secretion from *Lep*^{ob}/*Lep*^{ob} mice is linked to PI 3-K activity has, to our knowledge, not been investigated.

The present study was undertaken first to determine if islets from neonatal leptin-deficient *Lep*^{ob}/*Lep*^{ob} mice hypersecrete insulin in response to activation of the PKA signaling pathway, as is observed when the PLC-PKC signaling pathway is stimulated in islets from these mice (10). Next, the effects of leptin on insulin secretion mediated by the PKA signaling pathway in islets from mice were examined. Finally, wortmannin, an inhibitor of PI 3-K (22), was used to investigate the possible role for PI 3-K in the enhanced acetylcholine-induced insulin secretion characteristic of islets from young *Lep*^{ob}/*Lep*^{ob} mice.

Materials and Methods

Animals. *Lep*^{ob}/*Lep*^{ob} and lean mice were obtained from our breeding colony (C57BL/6J-*Lep*^{ob}/+). Care and treatment of the mice was according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985) and local institutional guidelines. Mice were housed in solid-bottom cages with wood shavings for bedding and were maintained at 25°C with a 12:12-hr light–dark cycle (lights on at 0700 hr). They were fed a nonpurified diet (Harlan Teklad Rodent Diet 8640; Madison, WI). Litters were adjusted to 6 pups per litter within a few days after birth. Mice were weaned at 3 weeks of age. Approximately equal numbers of male and female mice were used at 2 and 4 to 5 weeks of age. *Lep*^{ob}/*Lep*^{ob} and lean mice were identified by visual examination of body shape. Phenotype was further confirmed by visualization of abdominal fat pads when pancreatic islets were obtained. Even at 2 weeks of age, *Lep*^{ob}/*Lep*^{ob} mice have approximately three times as much abdominal fat as lean mice (10). Littermate mice were used in selected trials as noted in the table footnotes and figure legends.

Islet Isolation, Insulin Secretion, and Insulin Assay. Pancreatic islets were isolated with collagenase type V (Sigma Chemical, St. Louis, MO) as described previously (10, 23). Isolated islets were selected with the aid of a pipette under a stereoscopic microscope. Similar-sized islets from individual mice (10 islets/dish) were distributed into small (35-mm) black-bottom petri dishes. One dish per animal was assigned to each treatment. In Experiments 3 and 4, islets from two mice were pooled and then aliquoted (10 islets/dish) into each of four petri dishes to be assigned to the four experimental treatments.

Islets were preincubated at 37°C for 30 min under a 95% O_2/5% CO_2 atmosphere in 1 ml Krebs-Ringer bicarbonate buffer (KRB, pH 7.4) containing 0.5 mM glucose and 0.1% bovine serum albumin (BSA, Amoresco, Solon, OH). Islets secreting more than 1 fmole insulin·islet^{-1}·min^{-1} in 0.5 mM glucose were considered damaged during isolation. Data from these islets were excluded. Islets were then incubated in KRB containing 10 mM glucose (or 10 mM glucose + 20 nM wortmannin [Sigma Chemical, St. Louis, MO], as indicated in the legend to Fig. 6) during a second 30-min period. This concentration of glucose (i.e., 10 mM) was selected because the PKA and PLC-PKC signaling pathways function to potentiate glucose-induced insulin secretion (12). Various treatments, as indicated in the figure legends, were then added, and the incubation of the islets in 10 mM glucose continued for 30 min. We demonstrated earlier that insulin secretion from islets of mice remains constant when exposed to 10–20 mM glucose for 60 min (10).

To measure insulin secretion, 0.5 ml of incubation media was collected. Insulin was quantified by an enzyme-linked immunosorbent assay (24).

Experimental Design. *Experiment 1.* Islets from 2-week-old leptin-deficient *Lep*^{ob}/*Lep*^{ob} pups respond normally to glucose but increase insulin secretion more in response to activation of the PLC-PKC pathway than islets from lean littermates (10, 11). This experiment utilized GLP-1 (Sigma Chemical, St. Louis, MO), a peptide that activates adenylyl cyclase via a receptor-mediated process (25, 26); forskolin (Sigma Chemical, St. Louis, MO), a direct activator of adenylyl cyclase (27); and 3-isobutyl-1-methylxanthine (IBMX) (Sigma Chemical, St. Louis, MO), an inhibitor of PDE (28), to determine if insulin secretion mediated by the PKA signal transduction pathway was altered in islets from 2-week-old *Lep*^{ob}/*Lep*^{ob} mice. The concentrations of GLP-1, forskolin, and IBMX utilized are pre-

sented in the Figure 1 legend. In a follow-up trial, 9 nM milrinone (Sigma Chemical, St. Louis, MO), an inhibitor of PDE 3B (29), was used to further characterize the influence of PDE on insulin secretion.

Experiment 2. Leptin has been reported to suppress GLP-1-induced insulin secretion from islets of neonatal rats (16). The ability of murine leptin (PeproTech Inc., Rocky Hill, NJ) to suppress GLP-1-induced insulin secretion from islets of 4-week-old lean mice was therefore examined. See the legend to Figure 2 for concentrations of leptin and GLP-1 used.

Experiment 3. Acetylcholine, via activation of the PLC-PKC signaling pathway, causes hypersecretion of insulin from islets of Lep^{ob}/Lep^{ob} mice, and leptin suppresses this hypersecretion (10, 14). Leptin suppresses PKA activity in neonatal rat pancreatic islets by activating PDE (16). To determine if PDE might, via cross-talk between the PKA and PLC-PKC signal transduction systems (11, 17), influence acetylcholine-induced insulin secretion, we measured acetylcholine-induced insulin secretion in the presence and absence of leptin and IBMX, an antagonist of PDE. Concentrations of acetylcholine, leptin, and IBMX used are presented in the legend to Figure 3.

Experiment 4. Wortmannin, an inhibitor of PI 3-K activity (22), was used to examine the role of PI 3-K activity in acetylcholine-induced insulin secretion from islets of 4- to 5-week-old lean and Lep^{ob}/Lep^{ob} mice. Concentrations of acetylcholine, leptin and wortmannin used are presented in the legends to Figures 4–6.

Statistical Analysis. Data are presented as means ± SE. Data from Experiment 1 and Experiments 2–4 were analyzed by two-way ANOVA and one-way ANOVA, respectively, in conjunction with LSD adjustment. Differences were considered statistically significant at $P < 0.05$.

Results

Similar Rates of PKA-Potentiated Insulin Secretion from Islets of Lean and Lep^{ob}/Lep^{ob} Mice. Islets from 2-week-old lean mice and Lep^{ob}/Lep^{ob} littermates secreted similar amounts of insulin in the presence of 10 mM glucose alone (Fig. 1), consistent with previous studies (10, 11, 30). Addition of 1 μM GLP-1, but not 0.1 μM GLP-1, significantly ($P < 0.05$) potentiated glucose-induced insulin secretion similarly from islets of 2-week-old lean mice and Lep^{ob}/Lep^{ob} littermates (Fig. 1). Stimulation of adenylyl cyclase with 2.5 μM forskolin markedly increased insulin secretion from islets of lean and Lep^{ob}/Lep^{ob} littermates (Fig. 1). Addition of IBMX, an inhibitor of PDE, also increased insulin secretion from islets of both groups of mice (Fig. 1). Milrinone (9 μM), an inhibitor of PDE 3B (29), increased insulin secretion during the 30-min incubation period as much as IBMX did from islets of 4-week-old lean mice (0.64 ± 0.09, 3.40 ±1.12, and 3.77 ± 0.65 fmole·islet^{-1}·min^{-1} in response to 10 mM glucose, glucose plus milrinone, and glucose plus IBMX, respectively, n = 8). Thus, it appears that PDE 3B may be the major PDE

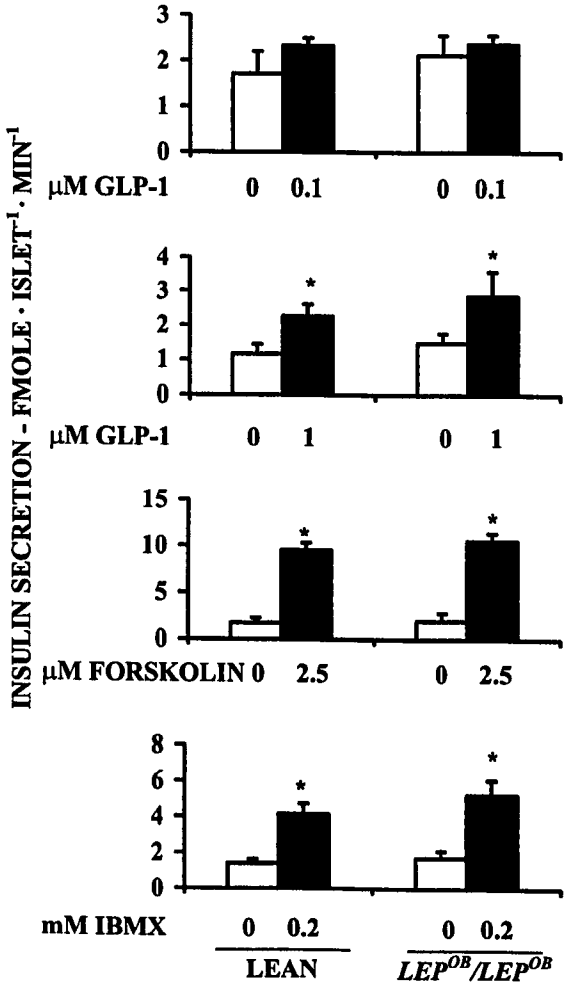

Figure 1. Stimulation of insulin secretion by the protein kinase A signaling pathway in islets from 2-week-old lean mice and Lep^{ob}/Lep^{ob} littermates. Islets from individual mice were incubated in 10 mM glucose for 30 min and then in 10 mM glucose plus 0.1 μM GLP-1 for an additional 30 min. Islets from additional mice were also incubated in 10 mM glucose for 30 min and then in 1 μM GLP-1, 2.5 μM forskolin (43) (an activator of adenylyl cyclase), or 0.2 mM IBMX (43) (an antagonist of phosphodiesterase) for 30 min. Data represent means ± SE for 7–8 mice. * indicates significant ($P < 0.05$) effects of 1.0 μM GLP-1, 2.5 μM forskolin, and 0.2 mM IBMX on insulin secretion, as determined by two-way ANOVA in conjunction with LSD test. Phenotype did not influence insulin secretion.

isoform in islets from mice, as it is in rat islets (16). The similar insulin secretion responses of islets from 2-week-old lean mice and Lep^{ob}/Lep^{ob} littermates to stimulators of the PKA signaling pathway (i.e., GLP-1, forskolin, and IBMX) (Fig. 1) suggest that the increased plasma insulin concentrations observed in these young Lep^{ob}/Lep^{ob} mice (11) are not caused by an increase in PKA-induced insulin secretion.

GLP-1-Potentiated Insulin Secretion Was Not Inhibited by Leptin. Addition of 20 nM leptin to islets from 4-week-old lean mice did not inhibit GLP-1 (1 μM)-induced insulin secretion (data not shown). The concentration of GLP-1 (1 μM) used in this trial may have been too high for leptin to exert an inhibitory action, as noted by Zhao *et al.* (16). Because 0.1 μM GLP-1 failed to stimulate insulin secretion from islets of mice (Fig. 1), a subsequent

trial used an intermediate GLP-1 concentration (i.e., 0.5 μM GLP-1). Insulin secretion was elevated by 0.5 μM GLP-1, but leptin failed to inhibit secretion (Fig. 2). These results, when considered with the observation that leptin deficiency does not enhance PKA-induced insulin secretion (Fig. 1), suggest that the PKA signaling pathway may not be a direct target for leptin action in islets from mice.

Leptin Inhibits Acetylcholine-Potentiated Insulin Secretion in the Presence of IBMX. The possibility that leptin may act via a PDE-dependent signaling pathway (16) to regulate acetylcholine-induced insulin secretion was investigated. As shown previously (10, 14), leptin suppressed 10 μM acetylcholine-potentiated, glucose-induced insulin secretion from islets of 4- to 5-week-old Lep^{ob}/Lep^{ob} mice but not from islets of lean mice (Fig. 3). In the presence of 0.2 mM IBMX, a PDE inhibitor, acetylcholine-induced insulin secretion was further increased in islets from both lean and Lep^{ob}/Lep^{ob} mice. Addition of 0.2 mM IBMX did not affect the ability of 20 nM leptin to suppress acetylcholine-induced insulin secretion from islets of Lep^{ob}/Lep^{ob} mice (Fig. 3). This suggests that the leptin-induced reduction of acetylcholine-potentiated insulin secretion by islets from Lep^{ob}/Lep^{ob} mice is independent of PDE activity.

Inhibition of PI 3-K and Insulin Secretion. To determine if PI 3-K activity affects insulin secretion from islets of mice, we examined insulin secretion in the presence of wortmannin, an inhibitor of PI 3-K. Wortmannin tended ($P = 0.059$, Student's t-test) to increase 10 mM glucose-induced insulin secretion (1.12 ± 0.22 and 1.96 ± 0.32 fmole insulin released·islet^{-1}·min^{-1} in the absence and presence of wortmannin, respectively, $n = 6$) from islets of 4-week-old lean mice. Wortmannin significantly increased acetylcholine potentiation of insulin secretion from islets of 4-week-old lean mice (Fig. 4).

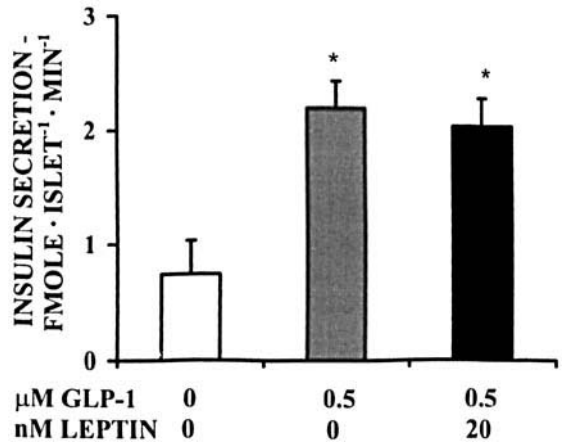

Figure 2. Leptin did not affect GLP-1-induced insulin secretion. Islets from 4-week-old lean mice (two dishes of 10 islets/dish per mouse) were incubated in 10 mM glucose for 30 min followed by 10 mM glucose + 0.5 μM GLP-1 ± 20 nM leptin (8) for 30 min. Data represent means ± SE for 7 mice. * indicates that 0.5 μM GLP-1 significantly ($P < 0.05$) increased insulin secretion, as determined by one-way ANOVA in conjunction with LSD. Leptin did not influence GLP-1-induced insulin secretion.

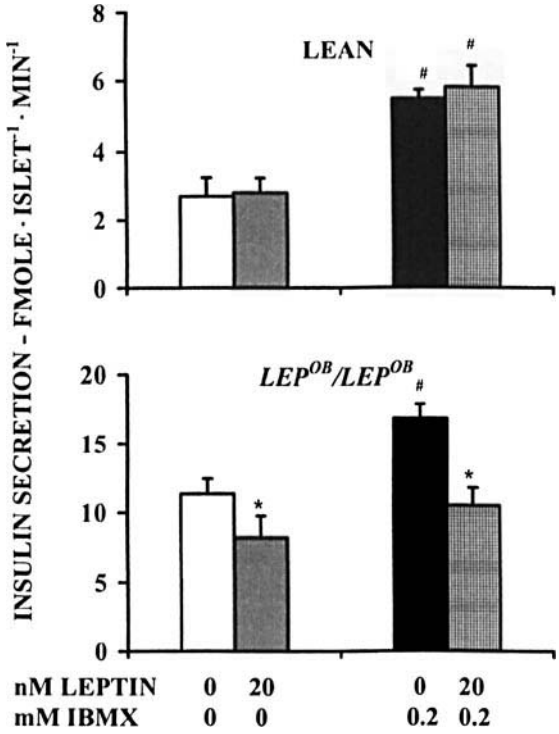

Figure 3. Leptin inhibited acetylcholine-induced insulin secretion from islets of Lep^{ob}/Lep^{ob} mice, but not from islets of lean mice, in the absence or presence of IBMX. Islets from 4- to 5-week-old lean and Lep^{ob}/Lep^{ob} mice (four dishes of 10 islets/dish obtained from two mice) were incubated in 10 mM glucose for 30 min. Insulin secretion averaged 0.74 ± 0.17 and 4.93 ± 0.42 fmole·islet^{-1}·min^{-1} in lean and Lep^{ob}/Lep^{ob} mice, respectively. All islets were then exposed to 10 mM glucose plus 10 μM acetylcholine ± 20 nM leptin and ± 0.2 mM IBMX for 30 min as indicated in the figure. Data represent means ± SE for 5–7 observations (each observation contained islets pooled from two mice). * indicates a significant effect of leptin on acetylcholine-induced insulin secretion in the absence or in the presence of IBMX, and # indicates a significant stimulatory effect of IBMX on insulin secretion, as determined by one-way ANOVA in conjunction with LSD test ($P < 0.05$).

In another trial, 20 nM wortmannin was again shown to stimulate acetylcholine-induced insulin secretion from islets of lean mice (Fig. 5). In contrast, wortmannin did not stimulate acetylcholine-induced insulin secretion from islets of Lep^{ob}/Lep^{ob} mice (Fig. 5).

Consistent with the finding in Figure 3, leptin suppressed acetylcholine-potentiated insulin secretion from islets of Lep^{ob}/Lep^{ob} mice but not from islets of lean mice (Fig. 5). However, in the concurrent presence of wortmannin, leptin suppressed acetylcholine-potentiated insulin secretion from islets of both Lep^{ob}/Lep^{ob} and lean mice (Fig. 5).

Wortmannin inhibits PI 3-K in pancreatic islets with a time lag of as long as 20 min (31, 32), whereas leptin inhibits acetylcholine-induced insulin secretion within 3 min (14). Thus, the more rapid-onset actions of leptin to block insulin secretion may have prevented the stimulatory effects of wortmannin on insulin secretion (Fig. 5).

To determine if leptin would inhibit acetylcholine-induced insulin secretion from islets of Lep^{ob}/Lep^{ob} mice when PI 3-K was first inhibited, islets were preincubated

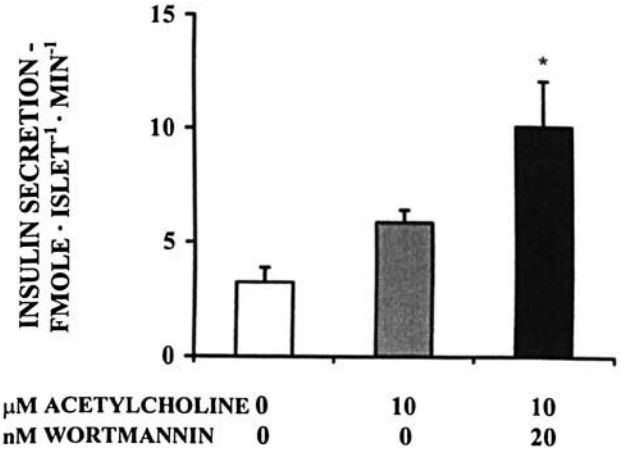

Figure 4. Inhibition of PI 3-K with 20 nM wortmannin (20, 32, 41, 42) increased acetylcholine-induced insulin secretion. Islets from 4- to 5-week-old lean mice (two dishes of 10 islets/dish per mouse) were incubated in 10 mM glucose for 30 min and then in 10 mM glucose plus 10 μM acetylcholine ± 20 nM wortmannin for 30 min. Data represent means ± SE for six mice. Significant ($P < 0.05$) effect of wortmannin on acetylcholine-induced insulin secretion, indicated with an *asterisk*, as determined by one-way ANOVA in conjunction with *post-hoc* LSD test.

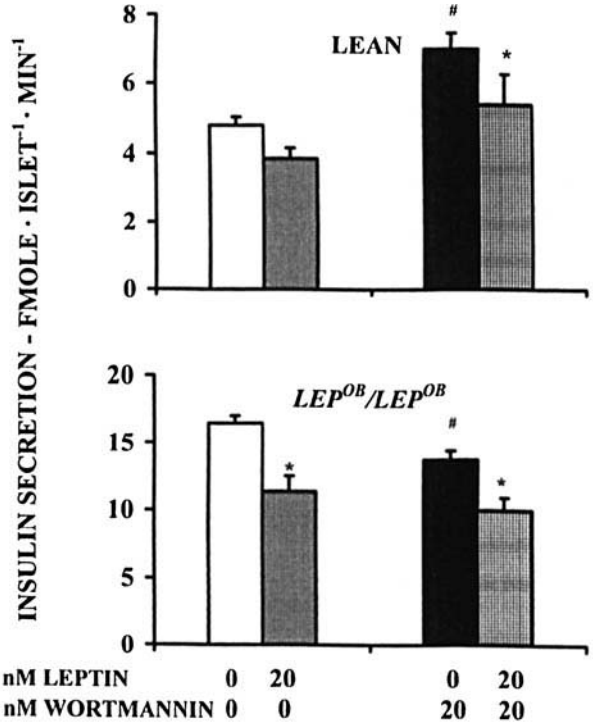

Figure 5. Wortmannin stimulated acetylcholine-induced insulin secretion from islets of lean, but not Lep^{ob}/Lep^{ob}, mice, and acetylcholine-induced insulin secretion was lower in islets simultaneously co-exposed to leptin and wortmannin than in islets exposed to wortmannin alone. Islets from 4- to 5-week-old lean and Lep^{ob}/Lep^{ob} mice (four dishes of 10 islets/dish obtained from two mice) were incubated in 10 mM glucose for 30 min. Glucose-induced insulin secretion averaged 2.02 ± 0.40 and 6.40 ± 0.83 fmole·islet^{-1}·min^{-1} for lean and Lep^{ob}/Lep^{ob} mice, respectively. All islets were then simultaneously exposed to 10 mM glucose plus 10 μM acetylcholine ± 20 nM leptin and ± 20 nM wortmannin for 30 min, as indicated in the figure. Data represent means ± SE for 5–6 observations (each observation contained islets pooled from two mice). # indicates significant ($P < 0.05$) effect of wortmannin on acetylcholine-induced insulin secretion, and * indicates significant ($P < 0.05$) inhibitory effect of leptin on insulin secretion, as determined by one-way ANOVA in conjunction with *post-hoc* LSD test.

with wortmannin for 30 min before incubation with acetylcholine ± leptin. Under these conditions, wortmannin completely blocked the ability of leptin to inhibit acetylcholine-induced insulin secretion (Fig. 6).

Discussion

The present study was undertaken to further examine the basis for hypersecretion of insulin from islets of leptin-deficient Lep^{ob}/Lep^{ob} mice. We conclude that the capacity for PKA-induced insulin secretion is not elevated in islets of young Lep^{ob}/Lep^{ob} mice, but insulin secretion associated with modulation of the PI 3-K signal transduction pathway is altered in these mice. Wortmannin, an inhibitor of PI 3-K, stimulated acetylcholine-induced insulin secretion from islets of lean mice but not from islets of Lep^{ob}/Lep^{ob} mice. This suggests that PI 3-K might be inactive in islets from Lep^{ob}/Lep^{ob} mice and that this low PI 3-K activity might contribute to their hypersecretion of insulin. Leptin, an activator of PI 3-K (16), inhibited the acetylcholine-induced hypersecretion of insulin from islets of Lep^{ob}/Lep^{ob} mice, and this effect of leptin was blocked in islets preincubated with wortmannin.

Several approaches were used to determine if PKA-induced insulin secretion was abnormally elevated in islets from leptin-deficient Lep^{ob}/Lep^{ob} mice. First, we observed that the GLP-1 concentrations required to stimulate insulin secretion from islets of mice (i.e., 0.5–1.0 μM GLP-1, Figs. 1 and 2) was higher than reported concentrations of GLP-1 required to stimulate insulin secretion from islets of rats [i.e., 0.1 nM GLP-1 (16)]. These results agree with earlier reports that mouse islets are less responsive to forskolin or IBMX than rat islets (33) and that mouse islets do not exhibit a rising second-phase insulin secretion response to GLP-1 (34). Islets from leptin-deficient Lep^{ob}/Lep^{ob} mice were not more responsive to GLP-1 than islets from lean mice. Likewise, forskolin and IBMX failed to induced greater insulin secretion from islets of Lep^{ob}/Lep^{ob} mice than from islets of lean mice (Fig. 1). These data suggests that PKA-induced insulin secretion is not altered in islets from young Lep^{ob}/Lep^{ob} mice, even though islets from older Lep^{ob}/Lep^{ob} mice do hypersecrete insulin in response to forskolin (35). Likewise, islets from young Lep^{ob}/Lep^{ob} mice do not hypersecrete insulin in response to glucose (Fig. 1), whereas islets from older Lep^{ob}/Lep^{ob} mice are very hyperresponsive to glucose (10, 14). We conclude that leptin deficiency does not directly target glucose- and PKA-induced insulin secretion pathways in mouse islets but rather targets a pathway associated with the PLC-PKC signaling pathway, which is altered very early in the development of Lep^{ob}/Lep^{ob} mice (10, 11). The hyperelevations in glucose-induced and PKA-induced insulin secretion noted in adult Lep^{ob}/Lep^{ob} mice appear to be secondary compen-

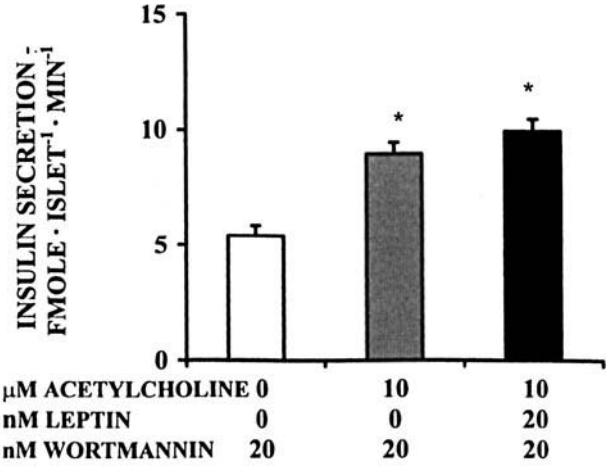

Figure 6. Preincubation of islets from *Lep^{ob}/Lep^{ob}* mice with wortmannin blocked the ability of leptin to inhibit acetylcholine-induced insulin secretion. Islets from 4- to 5-week-old *Lep^{ob}/Lep^{ob}* mice (two dishes of 10 islets/dish per mouse) were incubated in 10 mM glucose plus 20 nM wortmannin for 30 min and then incubated in 10 mM glucose + 10 μM acetylcholine + 20 nM wortmannin ± 20 nM leptin for an additional 30 min, as indicated in the figure. Data represent means ± SE for six mice. * indicates a significant ($P < 0.05$) stimulatory effect of acetylcholine on insulin secretion in the presence of wortmannin, as determined by one-way ANOVA in conjunction with *post-hoc* LSD test. Leptin failed to influence insulin secretion.

satory responses to prolonged hyperphagia and other consequences of leptin deficiency in these mice.

In contrast to the observation in cultured islets from neonatal rats, where leptin inhibited GLP-1-induced insulin secretion (16), leptin did not influence GLP-1-induced insulin secretion from islets of mice (Fig. 2). This failure of leptin to inhibit GLP-1-induced insulin secretion from islets of mice is consistent with our observation that GLP-1-induced insulin secretion is not elevated in islets from young leptin-deficient *Lep^{ob}/Lep^{ob}* mice. If this pathway was a primary target for leptin action, leptin deficiency would be expected to enhance GLP-1-induced insulin secretion. Leptin activates PI 3-K, which leads to activation of PDE 3B and inhibition of GLP-1-induced insulin secretion from islets of neonatal rats (16). Although we were unable to demonstrate a similar linkage in islets from mice, it is possible that leptin-induced activation of PDE 3B might lead to inhibition of acetylcholine-induced insulin secretion via cross-talk between PKA and PLC-PKC signal transduction pathways (11, 17). We thus used IBMX to inhibit PDE 3B and then determined whether leptin would inhibit acetylcholine-induced insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice (Fig. 3). Leptin was as effective in inhibiting acetylcholine-induced insulin secretion in the presence of IBMX as in its absence. It appears that leptin functions to inhibit PLC-PKC-induced insulin secretion independent of PDE regulation. Others have also reported inhibitory effects of leptin on glucose-induced insulin secretion in the presence of IBMX or milrinone, a specific PDE 3B inhibitor (36, 37). These observations further support our conclusion that leptin does not act via the PKA pathway to inhibit insulin secretion from islets of mice.

Leptin activates PI 3-K in a number of tissues including pancreatic islets (16, 18, 19). We used wortmannin, an inhibitor of PI 3-K, to determine if inhibitory effects of leptin on acetylcholine-induced insulin secretion were mediated by a PI 3-K–linked pathway. First, we determined if wortmannin-induced inhibition of PI 3-K would stimulate acetylcholine-induced insulin secretion from mouse islets (Figs. 4 and 5). Zawalich and Zawalich (32) had earlier shown that wortmannin increased carbachol-induced insulin secretion from islets of rats, although conflicting reports have been published (38). Wortmannin stimulated acetylcholine-induced insulin secretion from islets of lean mice but not from islets of *Lep^{ob}/Lep^{ob}* mice (Fig. 5). These findings parallel the report of Zawalich and Zawalich (32), where wortmannin stimulated carbachol-induced insulin secretion from islets of Sprague-Dawley or lean Zucker rats but not from islets of leptin- and insulin-resistant Zucker fatty rats. The failure of wortmannin treatment to increase insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice suggests that PI 3-K activity might be inherently low in these islets. This low PI 3-K activity might possibly explain why islets from *Lep^{ob}/Lep^{ob}* mice hypersecrete insulin in response to acetylcholine (Fig. 7).

The observation that leptin suppresses acetylcholine-induced insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice but not from islets of lean mice (10, 14; Fig. 5) is consistent with the noted effects of wortmannin on insulin secretion from these islets (Fig. 5). That is, leptin would be predicted to lower insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice, where PI 3-K activity is predicted to be low, and would be expected to be less effective in islets from lean mice, where PI 3-K activity is predicted to be high based on the insulin secretion responsiveness to wortmannin (Fig. 5).

When we simultaneously coadministered wortmannin

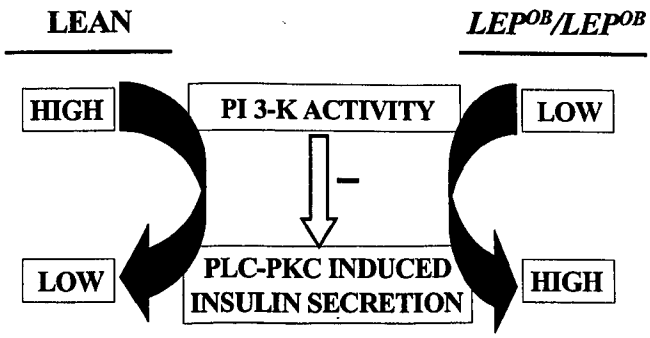

Figure 7. PI 3-K is proposed to inhibit PLC-PKC induced insulin secretion (32; Figs. 4 and 5). The high PLC-PKC-induced insulin secretion characteristic of *Lep^{ob}/Lep^{ob}* mice may be associated with low PI 3-K activity in their islets. Wortmannin, an inhibitor of PI 3-K, would thus not be expected to increase insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice, and leptin, an activator of PI 3-K, would be expected to constrain PLC-PKC-induced insulin secretion from islets of *Lep^{ob}/Lep^{ob}* mice. Data from islets of *Lep^{ob}/Lep^{ob}* mice are consistent with this prediction (Figs. 5 and 6). Conversely, wortmannin would be expected to increase PLC-PKC-induced insulin secretion from islets of lean mice, and leptin would be expected to be ineffective because PI 3-K activity in islets from lean mice is predicted to be high.

and leptin to islets from Lep^{ob}/Lep^{ob} and lean mice, insulin secretion was lower than when wortmannin alone was administered (Fig. 5). These results might be explained by the time frame of wortmannin and leptin actions. It takes 10–20 min for wortmannin to fully inactivate PI 3-K (31, 32), whereas leptin acts rapidly (14, 39). Presumably leptin rapidly activated PI 3-K in islets from Lep^{ob}/Lep^{ob} mice and initially maintained a high level of activity of PI 3-K in islets from lean mice (Figs. 5 and 7). As a result of this, insulin secretion from islets of Lep^{ob}/Lep^{ob} and lean mice exposed simultaneously to leptin and wortmannin was lower than insulin secretion from islets exposed to wortmannin alone (Fig. 5). But when islets from Lep^{ob}/Lep^{ob} mice were first preincubated with wortmannin to inhibit PI 3-K, subsequent addition of leptin failed to suppress acetylcholine-induced insulin secretion (Fig. 6). This finding supports our conclusion that leptin deficiency might elevate insulin secretion secondary to lowered stimulation of PI 3-K activity (Fig. 7). Studies are now needed to directly evaluate PI 3-K activity in islets from Lep^{ob}/Lep^{ob} mice and to determine the mechanism whereby activation of PI 3-K might target components of the PLC-PKC signal transduction pathway to regulate insulin secretion.

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