Leptin reduces glucose transport and cellular ATP levels in INS-1 β-cells

N T Lam1, A T Cheung1, M J Riedel2, P E Light2, C I Cheeseman3 and T J Kieffer1,3

Departments of 1Medicine, 2Pharmacology, and 3Physiology, University of Alberta, Edmonton, Alberta, Canada

(Requests for offprints should be addressed to T J Kieffer, Department of Physiology, 2146 Health Sciences Mall, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z3; Email: tim.kieffer@ubc.ca)

Abstract

Leptin suppresses insulin secretion by opening ATP-sensitive K⁺ (K_{ATP}) channels and hyperpolarizing β-cells. We measured the intracellular concentration of ATP ([ATP]_i) in tumor-derived β-cells, INS-1, and found that leptin reduced [ATP]_i by ~30%, suggesting that the opening of K_{ATP} channels by leptin is mediated by decreased [ATP]. A reduction in glucose availability for metabolism may explain the decreased [ATP]_i, since leptin (30 min) reduced glucose transport into INS-1 cells by ~35%, compared to vehicle-treated cells. The twofold induction of GLUT2 phosphorylation by GLP-1, an insulin secretagogue, was abolished by leptin. Therefore, the acute effect of leptin could involve covalent modification of GLUT2. These findings suggest that leptin may inhibit insulin secretion by reducing [ATP], as a result of reduced glucose availability for the metabolic pathway. In addition, leptin reduced glucose transport by 35% in isolated rat hepatocytes that also express GLUT2, suggesting that glucose transport may also be altered by leptin in other glucose-responsive tissues such as the liver.


Introduction

Leptin, the product of the ob gene, which is produced primarily by adipose tissue, relays information about adipocyte metabolism and body weight to the appetite centers in hypothalamic regions of the brain (Ahima & Flier 2000). Mice that harbor genetic mutations rendering them incapable of expressing functional leptin protein (ob/ob) or leptin receptors (db/db) are characterized by the development of obesity and type 2 diabetes. These mice are hyperphagic and insulin resistant, and have high circulating blood insulin and glucose levels. This phenotype in ob/ob mice is rapidly normalized by leptin treatment (Campfield et al. 1995, Halaas et al. 1995, Pelleymounter et al. 1995, Saladin et al. 1995). While the effects of leptin on satiety and energy expenditure appear to be centrally mediated, leptin action has also been reported in peripheral tissues, such as liver, skeletal muscle, adipose tissue and pancreas (Lee et al. 1996, Tartaglia 1997). The long isoform of the leptin receptor (ObRb) is expressed in pancreatic β-cells (Kieffer et al. 1996), and although the direct effects of leptin on insulin secretion are still controversial (Tanizawa et al. 1997, Leclercq-Meyer & Malaisse 1998, Muzumdar et al. 2003), leptin has been reported to inhibit insulin secretion from isolated islets in ob/ob and wild-type mice and tumor-derived β-cell lines (Emlisson et al. 1997, Fehmann et al. 1997, Kieffer et al. 1997, Kulkarni et al. 1997, Ahren & Havel 1999, Tsiotra et al. 2001).

Leptin has been shown to activate ATP-sensitive K⁺ (K_{ATP}) channels, which play a central role in the control of insulin secretion from β-cells (Harvey et al. 1997, Kieffer et al. 1997, Harvey & Ashford 1998). Closure of K_{ATP} channels by glucose metabolism and a subsequent elevation in ATP relative to ADP, results in the reduction of K⁺ efflux from the cells, leading to depolarization of the plasma membrane and activation of voltage-dependent Ca²⁺ channels (VDCC). The consequent rise in intracellular Ca²⁺ levels results in insulin secretion (Ashcroft & Rorsman 1989). Electrophysiological studies on ob/ob β-cells and on the rat insulinoma cell line, CRI-G1, showed that the application of leptin hyperpolarizes the cells as a result of opening the K_{ATP} channels (Harvey et al.
1997, Harvey & Ashford 1998, Kieffer et al. 1997), thereby inhibiting insulin secretion. Leptin-induced KATP channel activation in the CRI-G1 β-cell line appears to involve signaling through phosphoinoside 3-kinase (PI3K) (Harvey & Ashford 1998, Harvey et al. 2000) and may involve disruption of the actin cytoskeleton (Harvey et al. 2000). Since KATP channels are sensitive to changes in ATP levels (Misler et al. 1986), we sought to determine whether leptin might also open KATP channels by reducing the intracellular concentration of ATP ([ATP]i). Furthermore, since KATP channels are sensitive to the ATP/ADP ratio, which in turn is governed by the availability of glucose for metabolism, we investigated whether leptin could affect glucose availability by reducing glucose transport in INS-1 cells, an insulin-secreting, tumor-derived, pancreatic β-cell line, previously shown to be responsive to leptin (Ahren & Havel 1999).

Materials and methods

Cell culture

INS-1 cells were kindly provided by Prof. Claes Wollheim, Geneva, Switzerland, and Dr Marc Prentki, Montreal, Canada. Cells were cultured at 37 °C in humidified air containing 5% CO2/95% O2 in RPMI 1640 medium, pH 7·4, supplemented with 2 mM L-glutamine, 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, 11 mM glucose and 50 μM β-mercaptoethanol (all reagents from Life Technologies, Grand Island, NY, USA).

Rat hepatocyte isolation and culture

Hepatocytes were isolated by collagenase perfusion of the livers of male Sprague Dawley rats (200–300 g; Biological Sciences Animal Services, University of Alberta, Edmonton, AB, Canada) fed ad libitum, as previously described, with modifications (Seglen 1976). Animal studies were conducted in accordance with and under the approval of the Health Sciences Animal Policy and Welfare Committee, University of Alberta, Edmonton, AB, Canada. In brief, the hepatic portal vein was cannulated and perfused with a Ca2+-free solution, pH 7·4 (142 mM NaCl, 6·7 mM KCl, 10·1 mM HEPES and 5·5 mM NaOH), prior to perfusion with type IV collagenase, pH 7·6 (Sigma-Aldrich Canada, Oakville, ON, Canada). Cells were isolated, trypsin blue (Sigma-Aldrich) stained for viability and then cultured for 24 h in M199 media, pH 7·4 (Sigma-Aldrich), supplemented with 0·2% BSA (Sigma-Aldrich) prior to glucose transport studies.

Measurement of intracellular ATP

INS-1 cells were cultured in 96-well, black Optilux-Iso microplates (Becton Dickinson, Sparks, MD, USA) for 24–48 h in RPMI medium, pH 7·4, supplemented as described above. Cells were washed twice in bicarbonate buffer, pH 7·4 (120 mM NaCl, 4·8 mM KCl, 2·5 mM CaCl2, 1·2 mM MgCl2, 1 mM Na2HPO4, 24 mM NaHCO3 and 1 mg/ml BSA) and maintained in this buffer for 4 h prior to ATP assay. After 3 h and 15 min of incubation, cells were treated with 10 ng/ml recombinant human leptin (PeproTech, Rocky Hill, NJ, USA) for the remaining 45 min. Prior to the ATP assay, cells were challenged with 5 mM glucose in bicarbonate buffer for 1–30 min. Cells were lysed with a somatic cell ATP-releasing reagent (Sigma-Aldrich) and immediately placed on ice. Intracellular ATP was determined by a luciferin/luciferase method using an ATP bioluminescent assay kit (Sigma-Aldrich). Luminescence was determined using the Wallac Trilux 1450 Microbeta liquid scintillation and luminescence counter (Wallac, Turku, Finland). Use of the 96-well microplate limited cross-talk between samples to 0·002%. The instrument was preset to integrate the amount of light produced over a 5s interval without an initial delay. Standard curves generated from ATP standards (Sigma-Aldrich) displayed linearity in the range of 10–12 to 10–8 mol ATP.

Patch-clamp experiments

The perforated patch technique was used to measure whole-cell currents from INS-1 cells while maintaining the intracellular signaling environment intact. Amphotericin-B (Sigma) was dissolved in dimethylsulfoxide (DMSO) (40 mg/ml) and diluted in the pipette solution immediately before use to yield a final concentration of 80 μg/ml. Pipettes were back-filled with this amphotericin-containing solution. The pipette solution consisted of the following: 10 mM KCl, 130 mM Kaspartate, 10 mM HEPES, 1·4 mM MgCl2, 1 mM EGTA and 10 mM glucose. The pH of the solution was

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adjusted to 7.4 with KOH. Patch pipettes were pulled using borosilicate glass (G85150T, Warner Instrument Corp., Hamden, CT, USA) to yield pipettes with a tip resistance of 2–6 MΩ when filled with pipette solution. Once a 4Ω seal was obtained, series resistance was monitored. A stable perfusion access resistance of less than 20 MΩ was deemed acceptable. All whole-cell experiments were performed on single cells and recorded in voltage-clamp mode using an Axopatch 200B amplifier and Clampex 8.0 software (Axon Instruments, Foster City, CA, USA). 

KATP channel current was elicited using a series of 480 ms voltage ramps from −90 mV to −30 mV, holding at −70 mV. Currents were sampled every 5 s at 2 kHz. Cells were initially perfused with control solution containing the following: 140 mM NaCl, 10 mM HEPES, 1 mM CaCl2, 1.4 mM MgCl2, 5 mM KCl and 10 mM glucose. Intracellular ATP levels were depleted using a chemically induced metabolic inhibition solution (MI) prepared by replacing glucose with 4 mM NaCN and 5 mM 2-deoxy-glucose (2-DOG) in the control solution. Regeneration of intracellular ATP was achieved by superfusing the cells with control solution. Control cell experiments were performed in the absence of leptin in all solutions. Experimental cells were exposed to 10 ng/ml leptin for approximately 2–3 min prior to MI. Leptin was present in both control and MI solutions. The time required for KATP channel currents to recover 50% of the ATP-mediated inhibition relative to peak current obtained during MI versus that obtained in the control period was analyzed.

Measurement of glucose transport

Cells were cultured in 60 mm dishes (Becton Dickinson) to a confluency of ~80%. Cells were washed twice in a buffered solution, pH 7.4 (5.4 mM KCl, 1.8 mM CaCl2, 1.2 mM MgSO4, 10 mM HEPES, 137 mM NaCl and 0.2% BSA), and fasted in the absence of glucose and serum for 2 h. Cells were treated with 10 ng/ml leptin for approximately 2–3 min prior to MI. Leptin was present in both control and MI solutions. The time required for KATP channel currents to recover 50% of the ATP-mediated inhibition relative to peak current obtained during MI versus that obtained in the control period was analyzed.

32P loading and immunoprecipitation

Cells were serum starved for 2 h in RPMI media, pH 7.4, prior to being transferred into phosphate-free RPMI containing 10 µg/ml protease and phosphatase inhibitor cocktail (Sigma-Aldrich), 1 mM PMSF (Sigma-Aldrich) and 0.1% BSA, for metabolic labeling with 32P (as orthophosphate; Amersham Pharmacia Biotech). Cells were labeled in 0.5 mCi 32P per 10^6 cells for a period of 2 h, at which point cells were treated with 10 or 100 ng/ml recombinant human leptin for 30 min and/or with 10 nM GLP-1 7–36 NH2 (Peninsula Laboratories, Belmont, CA, USA) for 10 min. Cells were lysed in lysis buffer (50 mM HEPES, 1% Triton-X, 2 mM EDTA, 200 mM NaF, 10 mM Na2P4O7, 1 mM PMSF, 1 mM Na3VO4 and 10 µg/ml protease inhibitor cocktail), harvested and immunoprecipitated with 5 µg affinity purified rabbit GLUT2 antibody (Alpha Diagnostic Intl., San Antonio, TX, USA) and protein A conjugated protein A/G (Pierce Chemical Company, Rockford, IL, USA) overnight at 4°C. Immunoprecipitates were washed three times with lysis buffer and then boiled in Laemmli buffer. The resulting supernatants were run on a 10% SDS–PAGE gel. The gel was dried for the determination of the level of radioactivity of GLUT2 by phosphoimaging (STORM 840, Amersham Pharmacia Biotech). Densitometric values were determined with Molecular Dynamics ImageQuant software (Amersham Pharmacia Biotech).

Membrane protein extraction

Cells grown on 100 mm plates (Becton Dickinson) were placed on ice and collected by scraping and centrifugation. Cells were resuspended in lysis buffer (10 mM Tris and 1 mM PMSF), and homogenized with a Dounce homogenizer. The homogenate was centrifuged at 7500 g for
5 min at 4 °C. The pellet was discarded and the supernatant was centrifuged at 35 000 g for 20 min at 4 °C in a Beckman ultracentrifuge. Subsequently, the supernatant was discarded, and the pellet was resuspended in lysis buffer and again homogenized with a Dounce homogenizer and ultracentrifuged at 35 000 g for 20 min at 4 °C. The supernatant was aspirated and the remaining pellet was resuspended in phosphate-buffered saline containing 0·5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS) and 1 mM PMSF for 30 min at 4 °C. The lysate was centrifuged at 13 000 g for 5 min at 4 °C, and the supernatant fraction was collected for determination of protein concentration by DC protein assay (Bio-Rad Laboratories Canada Ltd, Mississauga, ON, Canada).

Western blot
Protein samples were run on 10% SDS–PAGE gel and transferred overnight at 4 °C to a nitrocellulose membrane (Osmonics, Westborough, MA, USA). The membrane was blocked in TBS-Tween, pH 7·4 (20 mM Tris, 150 mM NaCl and 0·1% Tween 20), containing 5% nonfat dry milk. Immunoblot was performed using affinity-purified rabbit GLUT2 antibody (Alpha Diagnostic) and rabbit IgG conjugated to HRP (Amersham Pharmacia Biotech). Protein was visualized by enhanced chemiluminescence (ECL) Western blotting system (Amersham Pharmacia Biotech). The chemiluminescent signal was captured on Kodak BioMax MR film (Eastman Kodak, Rochester, NY, USA).

Statistical analysis
The significance of group differences was evaluated by ANOVA analysis with Statview software (SAS Institute, Cary, NC, USA), and values are presented as means± standard error. Electrophysiological data were evaluated for statistical significance using the unpaired Student’s t-test. P<0·05 was deemed significant.

Results
Effects of leptin on [ATP]i in INS-1 cells
INS-1 cells were pretreated with leptin (10 ng/ml) for 45 min and then challenged with 5 mM glucose for 1–30 min. In the basal state (no glucose challenge), there was no difference in ATP levels between vehicle- and leptin-treated cells. In the presence of 5 mM glucose, [ATP]i in INS-1 cells rose ~1·5-fold within 1 min of glucose stimulation (P<0·0001, n=4, 0 vs 1 min) and appeared to reach a plateau of ~2·2-fold after 10 min (P<0·0001, n=4, 0 vs 10 min) (Fig. 1). In contrast to controls, [ATP]i did not increase within 1 min (P=0·8, n=4, 0 vs 1 min) and rose to ~1·5 fold only after 5 min in the presence of leptin (P=0·003, n=4). ATP levels did not appear to reach a plateau of ~2·2-fold until 15–20 min of glucose stimulation in leptin-treated cells. Therefore, [ATP]i is significantly reduced in the presence of leptin in the first 10 min of glucose stimulation (P<0·03, n=4, control vs leptin-treated cells at 1, 5 and 10 min).

Effects of leptin on whole-cell KATP channel currents in INS-1 cells
We examined the effects of ATP depletion/regeneration on the activation and subsequent recovery of whole-cell KATP channel currents in intact INS-1 cells. Intracellular ATP was depleted using a solution containing no glucose and the metabolic inhibitors sodium cyanide and 2-deoxyglucose (MI solution). Perfusion of INS-1
cells with the MI solution resulted in the rapid development of large whole-cell currents. These currents were inhibited by application of the K\textsubscript{ATP} channel-specific sulfonylurea drug tolbutamide (100 µM; Fig. 2A), as well as by reperfusion with a control solution containing 10 mM glucose (Fig. 2B). Increases in tolbutamide-sensitive current were recorded within 1–2 min of metabolic inhibition, indicating a reduction in intracellular ATP levels and a corresponding increase in K\textsubscript{ATP} channel current. In cells treated with leptin (10 ng/ml), the time required for ATP levels to regenerate sufficiently to inhibit 50% of the MI-induced K\textsubscript{ATP} channel current was significantly increased, from 212.14 ± 21.52 s (n=7 cells) to 483.75 ± 54.86 s (n=8 cells) (P<0.01, Fig. 2C).

**Effects of leptin on glucose transport in INS-1 cells**

Glucose transport was assessed by measuring the uptake of \(^3\)H-3-O-MG, a transported, but non-metabolizable hexose substrate. Transport of 3-O-MG was a saturable process with increasing exposure time to the substrate, reaching a maximum by 4 min (3.2 ± 0.2 nmol/µg protein) (Fig. 3A). The rate of 3-O-MG uptake remained linear within the first 45 s. Leptin (≥1 ng/ml) dose-dependently reduced the rate of glucose transport in INS-1 cells. After 15 s, 3-O-MG uptake in cells pretreated with 10 ng/ml leptin for 30 min reached only 64 ± 3% (P<0.0001, n=5) of the level reached by vehicle-treated cells (Fig. 3B).
effect of leptin on glucose transport was acute with a significant reduction by 20 min of leptin treatment (33 ± 11%, \( P=0.03, n=4 \)) (Fig. 3C).

### Transporter regulation by leptin and GLP-1

GLUT2 is the primary glucose transporter responsible for shuttling glucose into \( \beta \)-cells (Johnson et al. 1990). To determine whether the acute effect of leptin on glucose transport may be due to changes in the phosphorylation of the transporter, we assessed changes in phosphorylation of GLUT2. Western blot analysis showed no difference in GLUT2 membrane expression levels following either GLP-1 or leptin treatment for 30 min (Fig. 4A). To examine changes in the phosphorylation state of GLUT2, INS-1, cells were labeled metabolically with \( ^{32}P \) and then treated with GLP-1 and/or leptin, followed by GLUT2 immunoprecipitation. Treatment of cells with GLP-1 (10 nM) for 10 min increased GLUT2 phosphorylation by \( \sim 1.5 \)-fold. While leptin alone had no effect on GLUT2 phosphorylation, preincubation with leptin (10 or 100 ng/ml) for 30 min completely abolished GLP-1-stimulated phosphorylation of GLUT2 (Fig. 4B).

### Effects of leptin on glucose transport in isolated hepatocytes

Since hepatocytes express GLUT2 similarly to INS-1 cells, we investigated whether the effects of leptin on glucose transport in INS-1 cells could be mimicked in rat hepatocytes. In rat hepatocytes treated with leptin (10 ng/ml) for 15 min, glucose transport was 66 ± 2% \( (P=0.01, n=4) \) of vehicle-treated cells (Fig. 5). This finding suggests that leptin might specifically target the GLUT2 transporter in both \( \beta \)-cells and hepatocytes.

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**Figure 3** (A) 3-O-MG transport as a function of time in INS-1 cells. Cells were exposed to 5 mM \([^{3}H]-3\)-O-MG for up to 4 min. Time 0 uptake was assessed by pretreating cells with 20 µM cytochalasin B for 15 min prior to glucose uptake. The radioactivity remaining in these lysates was considered background and was subtracted from all time points. Results are expressed as mean±S.E.M. (B) Dose response of leptin on glucose transport in INS-1 cells. Prior to exposure to \([^{3}H]-3\)-O-MG for 15 s, INS-1 cells were treated with leptin for 30 min (values compared to 0 ng/ml; \*\( P<0.0001, n=5 \)). (C) Time response of leptin on glucose transport in INS-1 cells. Prior to exposure to \([^{3}H]-3\)-O-MG for 15 s, INS-1 cells were treated with leptin for the durations indicated (values compared to 0 min; \*\( P<0.05, n=4 \)). Values in B and C are presented as mean±S.E.M.
Leptin has been reported to activate K\textsubscript{ATP} channels in \(\beta\)-cells by signaling through the PI3K pathway (Harvey & Ashford 1998, Harvey et al. 2000) and by mediating the downstream disruption of the actin cytoskeleton (Harvey et al. 2000). From this present study, we propose that leptin may also target the glucose metabolic pathway, reducing the elevation in ATP levels that normally results from glucose metabolism, and thereby activating K\textsubscript{ATP} channels. Under basal conditions, the [ATP]\textsubscript{i} was not different between vehicle and leptin-treated cells. However, leptin attenuated glucose-induced increases in [ATP]\textsubscript{i} by \(\sim 25\%\) within the first 10 min of glucose stimulation. Gradually lowering the ATP/ADP ratio in mouse islets by increasing concentrations of respiratory chain and glycolytic inhibitors has been shown to inhibit insulin secretion progressively by opening K\textsubscript{ATP} channels (Detimary et al. 1994). We have tested the ability of INS-1 cells to regenerate ATP and therefore increase the ATP/ADP ratio after a brief period of metabolic inhibition. K\textsubscript{ATP} currents were rapidly elicited during metabolic inhibition and reduced by inhibitor washout and subsequent reperfusion with glucose. Leptin significantly prolonged K\textsubscript{ATP} channel activity following metabolic inhibition, an observation that may be attributed to a delayed increase in the ATP/ADP ratio as observed in this study. Therefore, our finding that leptin attenuates the production of intracellular ATP from glucose metabolism and as a result prolongs the activity of K\textsubscript{ATP} channels may explain, in part, the reduction of glucose-stimulated insulin secretion observed in the presence of leptin (Harvey et al. 1997, Kieffer et al. 1997, Cases et al. 2001).

The observation that leptin reduces glucose-stimulated, but not basal, [ATP]\textsubscript{i} suggests that...
Leptin inhibits a substrate-driven pathway that normally results in the production of ATP. Leptin potentially reduces ATP concentrations in β-cells by increasing the activity of uncoupling proteins (UCPs). Zhou et al. (1997) demonstrated that overexpression of leptin in mice by adenoviral delivery resulted in a sixfold increase in the levels of UCP-2 mRNA in islets. An elevation of UCP-2 mRNA in islets resulted in a sixfold increase in the levels of GLUT2 mRNA was also observed in vitro in islets cultured with recombinant leptin (Zhou et al. 1997). Alternatively, given that changes in glucose availability give rise to changes in [ATP]i, leptin potentially decreases [ATP]i by reducing glucose transport into β-cells. Indeed, in our study, we found that leptin dose-dependently reduced glucose transport in INS-1 cells. Although a reduction of glucose transport is proposed to translate to a reduction in [ATP]i, it is generally accepted that the rate of glucose transport greatly exceeds the rate of glucose phosphorylation by glucokinase. Therefore, glucose metabolism, and not transport, is the rate-limiting step (Meglasson & Matschinsky 1986). However, it has been argued that when the expression or function of glucose transporters is significantly reduced, the access of glucose to glucokinase may be limiting, and therefore the rate of glucose transport can be indicative of the rates of glucose metabolism and insulin secretion (Orci et al. 1990, Thorens et al. 1990, Unger 1991). In our studies, we measured glucose transport at 0.5 mM (data not shown) and 3-0-MG, substrate concentrations below the level (>5.5 mM) that would normally render glucokinase as the rate-limiting step of glucose metabolism (Malaisse et al. 1990, Matschinsky 1990). Therefore, leptin-mediated reductions in glucose transport may translate to less substrate available for metabolism to ATP.

The acute response (20 min) of leptin on glucose transport suggests that these changes are unlikely to be mediated by changes in GLUT2 protein levels. Furthermore, unlike GLUT4, GLUT2-mediated glucose transport is not thought to be regulated by translocation between intracellular vesicles and the plasma membrane (Thorens et al. 1993). In agreement, our Western blot analysis of membrane proteins from leptin-treated INS-1 cells showed approximately equal abundance of GLUT2. Therefore, changes in protein levels do not appear to explain the leptin-mediated reductions in glucose transport. It has been postulated that GLUT2 activity can be affected by phosphorylation of the transporter (Thorens et al. 1996). Both forskolin and GLP-1 have previously been shown to induce protein kinase A-dependent phosphorylation of GLUT2 on specific serine and threonine residues of the carboxyl-terminal tail of the transporter, thus altering transport activity (Thorens et al. 1996). Although changes in the phosphorylation state of GLUT2 is one mechanism by which glucose transport activity may be altered, leptin on its own did not alter GLUT2 phosphorylation in our study. However, leptin reduced the GLP-1 stimulated increase in GLUT2 phosphorylation, suggesting that leptin may act as an antagonist to GLP-1. The decrease in phosphorylation could result from leptin reducing ATP levels and thereby causing reduced production of cAMP upon GLP-1 treatment. Antagonistic actions of leptin in the presence, but not absence, of GLP-1, have also been observed at the levels of insulin expression (Seufert et al. 1999) and release in β-cells (Ahren & Havel 1999).

Through its actions on the β-cell, leptin plays a key role in the regulation of glucose homeostasis by reducing insulin release. The liver has been implicated as an additional target for leptin regulation of glucose metabolism. In vivo, leptin reduces high circulating blood glucose levels (Campfield et al. 1995, Halaas et al. 1995, Pelleymounter et al. 1995, Stephens et al. 1995), and central or i.v. infusion of leptin to rats enhances hepatic insulin sensitivity and increases whole-body glucose utilization (Kamohara et al. 1997, Sivitz et al. 1997, Barzilai et al. 1999, Chinoookoswong et al. 1999). The presence of leptin receptors on hepatocytes supports the observations of leptin action on the gene expression of gluconeogenic and glycogenic enzymes and of leptin interaction with the insulin-signaling cascade in hepatic cell lines (Cohen et al. 1996, Rossetti et al. 1997, Wang et al. 1997, Liu et al. 1998, Aiston & Agius 1999, Nemecz et al. 1999, Szanto & Kahn 2000). Since liver and β-cells express GLUT2, we determined whether leptin might also diminish glucose transport in hepatocytes. Similar to effects observed in β-cells, 3-0-MG levels in leptin-treated cultured rat hepatocytes were significantly reduced compared to controls. Since 3-0-MG is nonmetabolizable, any changes in cellular concentrations reflect changes in transport. Given that a reduction in glucose transport into hepatocytes is a reflection of a
reduced intrinsic activity of the transporter, glucose transport out of hepatocytes is proposed to be similarly lowered by leptin. Therefore, in vivo, a combination of effects on glucose transport and on gluconeogenic and glycogenolytic pathways by leptin may culminate in an overall result of reduced hepatic glucose output, thereby lowering blood glucose levels.

In summary, we have identified a proximal pathway by which leptin may reduce insulin secretion in pancreatic β-cells. The inhibition of insulin secretion by leptin might, in part, explain the reduction in plasma insulin when leptin is administered to hyperinsulinemic ob/ob mice. Interestingly, leptin-mediated reductions in glucose transport may also occur in hepatocytes. This common mechanism of action in β-cells and hepatocytes could contribute to the reduction of both the plasma insulin and glucose observed in vivo following leptin treatment. Further studies are required to determine the exact mechanism by which leptin reduces glucose transport.

Acknowledgements

Funding support from Canadian Institutes of Health Research (CIHR) to TJK is gratefully acknowledged. TJK was supported by Alberta Heritage Foundation for Medical Research (AHFMR) and CDA scholarships and a Juvenile Diabetes Research Foundation career development award. NTL was supported by Natural Sciences and Engineering Research Council scholarships and an AHFMR studentship. ATC was supported by AHFMR and CDA fellowships. PEL is an AHFMR scholar and a CIHR new investigator. Operating funds (to PEL) were provided by the CDA in honour of the late Gordon M Stevenson.

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Received in final form 16 December 2003
Accepted 29 December 2003