Role of the Sympathetic Nervous System and Insulin in Enhancing Glucose Uptake in Peripheral Tissues After Intrahypothalamic Injection of Leptin in Rats

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Our previous study demonstrated that microinjection of leptin into the ventromedial hypothalamus (VMH) dramatically increased glucose uptake in the heart, brown adipose tissue (BAT), and skeletal muscles, but not in white adipose tissue (WAT) in conscious unrestrained rats, as assessed in vivo by the 2-[^{3}H]deoxyglucose method. Here we examined the role of the sympathetic nervous system and insulin in enhanced glucose uptake by tissues after hypothalamic leptin injection. Pretreatment with guanethidine significantly suppressed the increased glucose uptake by the tissues in response to leptin injected into the VMH, whereas bilateral adrenal demedullation had no significant effect. Treatment with propranolol but not phenoxybenzamine also decreased significantly enhanced glucose uptake by the tissues. We further examined the interaction of the effects of hypothalamic leptin and insulin administered peripherally by clamping the glucose concentrations at a constant level. When leptin was injected into the VMH and a maximal dose of insulin was administered intravenously, the rates of glucose uptake by the heart, BAT, and skeletal muscles, but not by WAT, markedly increased beyond the values reached by insulin stimulation alone. Surgical sympathetic denervation of BAT abolished the enhancement of glucose uptake in this tissue, decreasing to the level stimulated by insulin alone. These results appear to indicate that leptin in the hypothalamus enhances glucose uptake in certain peripheral tissues through mediation of a β-adrenergic mechanism for the sympathetic nerves innervating the tissues and that central leptin and peripheral insulin have a synergistic role in augmenting tissue glucose uptake. *Diabetes* 48:1706–1712, 1999

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BAT, brown adipose tissue; EDL, extensor digitorum longus; 2-[^{3}H] DG, 2-deoxy-D-[^{3}H]glucose; K_i, rate constant of net tissue uptake of 2-[^{3}H]DG; VMH, ventromedial hypothalamus; WAT, white adipose tissue.

Leptin, the product of the *ob* gene, is a secreted protein hormone that is primarily produced by adipocytes (1,2). Recent studies suggest that leptin informs the brain about the abundance of body fat, thereby allowing the feeding behavior and peripheral metabolism to be coupled with the nutritional state (2–7). In fact, a recessive mutation of the *ob* gene causes severe obesity in *ob*/*ob* mice, and the systemic and intracerebroventricular administration of exogenous leptin reverses its obesity by suppressing appetite and stimulating energy metabolism (8–10).

Recently, we demonstrated that microinjection of leptin into the ventromedial hypothalamus (VMH) markedly increased glucose uptake in the heart, brown adipose tissue (BAT), and skeletal muscles, but not in white adipose tissue (WAT) or skin in unanesthetized rats, without significant changes in plasma insulin levels (11). Kamohara et al. (12) also reported that intravenous or intracerebroventricular administration of leptin significantly increased glucose uptake by certain tissues in mice in the absence of insulin changes: intracerebroventricular infusion of a low dose of leptin resulted in the same range of effects as intravenous administration of a higher dose, suggesting that the effect of peripheral leptin on tissue glucose uptake is due to its central action.

The increased glucose uptake in certain peripheral tissues in absence of a significant change in the insulin concentration suggests that the leptin-mediated glucose uptake in the tissues is not due to the result of an increase in insulin secretion. Rather, it seems likely that some of the effects of leptin are mediated through the sympathetic nerves innervating the tissues. In fact, it was found that the enhanced rate of glucose uptake by BAT in response to leptin injected into the VMH was effectively suppressed by surgical sympathetic denervation of the tissue (11). Furthermore, it has been demonstrated that systemic and intracerebroventricular administration of leptin increases sympathetic nerve activity to BAT and other peripheral tissues (13–15). Our previous studies revealed that the sympathetic neurotransmitter norepinephrine and insulin increased glucose uptake through different mechanisms in cultured brown adipocytes (16) and L6 myocytes (17). Moreover, studies with cultured brown adipocytes have also shown that insulin stimulates translocation of GLUT4 from an intracellular pool to the plasma membrane, whereas norepinephrine increases glucose uptake by the cells through acti-

vation of GLUT1 present in the plasma membrane (18). Thus, it is reasonable to speculate that the mechanism of the enhancement of glucose uptake by peripheral tissues after microinjection of leptin into the VMH may be different from that of insulin. However, it is also possible that hypothalamic leptin causes increased sensitivity of peripheral tissues to insulin and results in increased glucose uptake in these tissues. Indeed, systemic or intracerebroventricular administration of leptin has recently been reported to increase insulin sensitivity and systemic glucose utilization in rats (19–23).

We therefore undertook the present study to specify possible roles of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues in response to hypothalamic leptin. We examined if guanethidine treatment, adrenal demedullation, and adrenergic antagonists could block the effects of leptin injected into the VMH. In addition, studies were further undertaken to explore the interaction of the effects of hypothalamic leptin and peripheral insulin by examining whether microinjection of leptin into the VMH could modulate the maximally increased glucose uptake in peripheral tissues in response to insulin.

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats (Nihon Clea, Tokyo) weighing 200–240 g were used. They were housed individually in plastic cages at 25 ± 1°C with lights on from 7:00 A.M. to 7:00 P.M. and were given laboratory diet and water ad libitum. Under pentobarbital anesthesia (50 mg/kg intraperitoneally), rats were stereotaxically implanted with a chronic double-walled stainless steel cannula in the unilateral VMH according to the atlas of Pellegrino et al. (24). The stereotaxic coordinates used were as follows: 5.8 mm anterior to the interaural line, 0.5 mm lateral to the sagittal suture, and 9.5 mm below the surface of the skull. The cannula was then anchored firmly to the skull with acrylic dental cement. Seven days after the implantation of the brain cannula, a silicone cardiac catheter was chronically implanted into the right atrium through the external jugular vein. The rats were repeatedly handled during the 5- to 7-day recovery period to habituate them to the injection and blood sampling procedures. Guanethidine (guanethidine sulfate; Tokyo Kasei, Tokyo) was administered by a single subcutaneous injection at a dose of 100 mg/kg 17–20 h before the experiments. Bilateral adrenal demedullation was carried out by the dorsal approach 1 week before the experiments. After the surgical procedures, the rats with adrenal demedullation were maintained on 1% saline. The absence of adrenal medulla was confirmed at autopsy. Phenoxybenzamine (5 mg/kg) or propranolol (10 mg/kg) was injected twice intraperitoneally, 15 min before and 3 h after leptin microinjection into the VMH.

Correct placement of tips of the cannulas was verified microscopically in brain sections stained with Cresyl violet when the experiments were completed. All groups in the experiments consisted of six rats successfully injected with leptin or saline into the VMH or administered with insulin.

Microinjection of leptin into the VMH and measurement of the rate constant of net tissue uptake of 2-deoxy-D-[^{3}H]glucose. The experiment was started at 9:00 A.M. (lights on from 7:00 A.M.). Foods were removed at 8:00 A.M. and water was only made available to the rats during the experiments. Recombinant murine leptin (50 ng) (Pero Tech EC, London) dissolved in 0.5 µl saline solution was injected into the VMH in conscious unrestrained rats through the implanted brain cannula using the Hamilton microsyringe. Control rats were injected with 0.5 µl of saline into the VMH. Six hours after microinjection into the VMH, each rat was injected with 25 µCi of 2-deoxy-D-[^{3}H]glucose (2-[^{3}H] DG) and 5 µCi of [^{14}C]sucrose (ICN Radiochemicals, Irvine, CA) dissolved in a 0.2-ml saline solution through the cardiac catheter (11). The catheter was then immediately flushed with 0.2 ml of saline.

Blood and tissue samples. Blood samples (0.15 ml) were taken 10 min before the microinjection of leptin or saline into the hypothalamus and were also taken –10, 0, 7, 10, 15, and 20 min after the injection of the tracers; blood taken at each time was replaced with an equivalent volume of saline. As soon as the final blood samples were obtained (20 min after the injection of the tracers), overdose of sodium pentobarbital (100 mg/kg) was injected through the cardiac catheter and the rats were quickly decapitated. Interscapular BAT, heart (ventricle), epididymal, and retroperitoneal WAT, and skeletal muscles (left extensor digitorum longus [EDL] and soleus) were rapidly dissected, weighed, and frozen in liquid nitrogen. The rate constant (K_i) of net tissue uptake of 2-[^{3}H] DG was calculated as described previously (25). When specified, glycogen content of the liver was also determined by the procedure described by Hell et al. (26), which involves alkaline digestion, followed by ethanol precipitation, acid hydrolysis, and enzymatic glucose assay. Plasma samples were analyzed for radioactivities of 2-[^{3}H] DG and [^{14}C]sucrose as well as for glucose concentration (by a specific glucose oxidase method). In addition, the last plasma samples were analyzed for insulin concentrations by enzyme-linked immunosorbent assay (Morinaga Institute of Biological Science, Yokohama, Japan).

Experiment with intravenous administration of insulin. In this experiment, five groups of rats were used: *1*) control rats injected with saline in the VMH without insulin administration, *2*) rats injected with leptin in the VMH, *3*) rats injected intravenously with 3 U/kg insulin, *4*) rats administered 30 U/kg insulin, and *5*) rats injected with leptin in the VMH and then administered 3 U/kg insulin. These groups of rats were chronically implanted with silicon catheters in the right external jugular and right femoral veins. At the same time, surgical sympathetic denervation of the interscapular BAT was unilaterally performed by severing five branches of the intercostal nerves entering the right pad of the BAT, as described previously (27). The nerves entering the left pad of the BAT were kept intact. Five or seven days after the operations, leptin was injected into the VMH in free-moving rats, as described above. Then, a bolus of insulin (3 U/kg or 30 U/Kg) was administered through the femoral vein catheter. To maintain the basal glucose levels, an exogenous infusion of glucose (20%) was delivered through the cardiac catheter at variable rates according to instant plasma glucose measurements (Medisafe; Terumo, Tokyo). Blood samples were taken through the femoral vein catheter. The radioisotopes for tracers were injected through the femoral vein catheter 10 min after insulin administration. At 20 min after the injection of tracers, the rats were killed and the K_i values of peripheral tissues were measured as described above.

Statistical analysis. All values are expressed as means ± SE. The effects of the different treatments on all data were evaluated with factorial analysis of variance. When a significant effect was found, these results were further compared with Newman-Keuls multiple range test. The difference was considered significant if $P < 0.05$.

RESULTS

Effects of microinjection of leptin into the VMH on the K_i values of net uptake of 2-[^{3}H] DG in peripheral tissues. In confirmation with our previous report (11), microinjection of leptin into the VMH increased the rate constants of glucose uptake in heart, skeletal muscles, and BAT (Figs. 1–3 and Table 1). In heart muscle, the K_i value of 2-[^{3}H] DG uptake was increased 4.2 times that of the controls (Figs. 1 and 2 and Table 1). The K_i values of soleus and EDL muscles were increased 4.1 and 1.7 times, respectively (Figs. 1 and 2 and Table 1). Thus, the effect of leptin injected into the VMH was more prominent in the slow-twitch oxidative fibers (soleus) than in the fast-twitch glycolytic fibers (EDL) of skeletal muscles. In BAT, the K_i value was increased 3.0 times after hypothalamic injection of leptin (Figs. 1 and 3 and Table 1). However, the K_i values of the epididymal and retroperitoneal WAT did not increase in response to leptin injected into the hypothalamus (Figs. 1 and 3 and Table 1).

Effects of guanethidine treatment and adrenal demedullation. To ensure the role of the sympathetic nervous system in enhancing glucose uptake by certain peripheral tissues after microinjection of leptin into the VMH, we examined the effects of guanethidine treatment and bilateral adrenal demedullation. As shown in Fig. 1, pretreatment with guanethidine abolished the enhanced glucose uptake by heart, soleus, EDL, and BAT in response to hypothalamic microinjection of leptin, although it had no significant effects on the K_i values of these peripheral tissues after hypothalamic injection of saline (Fig. 1). Bilateral adrenal demedullation, on the other hand, did not affect the increased K_i values in response to hypothalamic leptin (Fig. 1). Guanethidine pretreatment and adrenal demedullation had no significant effects on the K_i values of the epididymal and retroperitoneal WAT in either the saline- or leptin-injected group (Fig. 1*E* and *F*).

Effects of adrenergic antagonists. We next examined the effects of adrenergic antagonists on the enhanced glucose

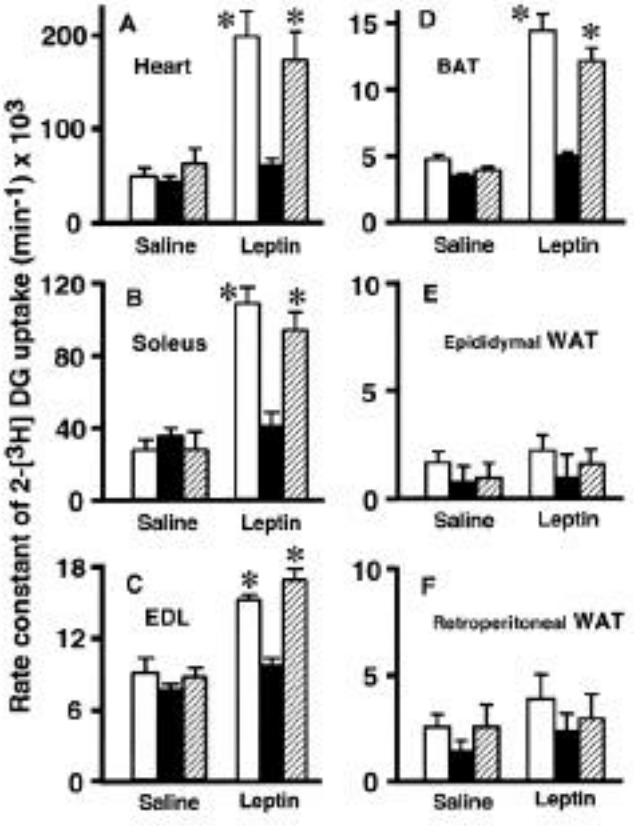

FIG. 1. Effects of guanethidine and adrenodemedullation on the K_i values of the 2-[^{3}H] DG uptake in heart (*A*), soleus (*B*), EDL (*C*), BAT (*D*), epididymal WAT (*E*), and retroperitoneal WAT (*F*) in response to leptin injected into the VMH. In the guanethidine-treated group, a single dose (100 mg/kg) of guanethidine sulfate was administered subcutaneously 17–20 h before the experiments. In the adrenodemedullated group, bilateral adrenal medullae were removed surgically 1 week before the experiments. Another group of rats was used as the one without pretreatment. Leptin or saline was injected into the VMH in conscious unrestrained rats through a chronically implanted cannula. Six hours after injection of leptin or saline into the VMH, each rat was injected with 25 µCi 2-[^{3}H] DG and 5 µCi [^{14}C]sucrose, and the animal was killed as soon as the final blood sample was obtained (20 min). The K_i values of tissue 2-[^{3}H] DG uptake were assessed as described in METHODS. Results are the means ± SE for six rats. *Significantly different from respective controls without injection of leptin at $P < 0.01$. □, Without pretreatment; ■, guanethidine treatment; ▨, adrenodemedullation.

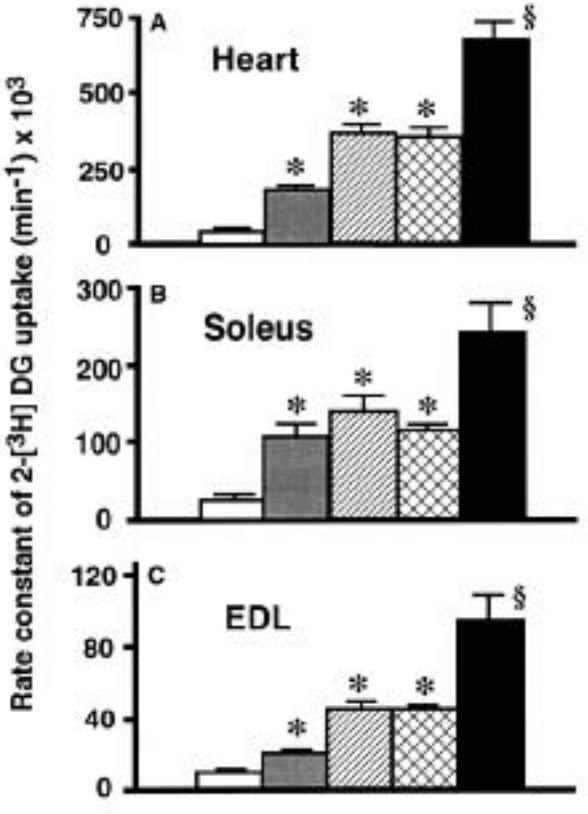

FIG. 2. Interaction of hypothalamic leptin and peripheral insulin on the K_i values of 2-[^{3}H] DG uptake in heart (*A*), soleus (*B*), and EDL (*C*). Five groups of rats were investigated: *1*) control rats with saline injected into the VMH without insulin administration (□), *2*) rats with leptin injected into the VMH (▩), *3*) rats administered 3 U/kg insulin intravenously (▨), *4*) rats administered 30 U/kg insulin (▩), and *5*) rats with leptin injected into the VMH and then administered 3 U/kg insulin (■). In the insulin-treated groups, an exogenous infusion of glucose was delivered through the cardiac catheter at variable rates according to instant plasma glucose measurements to maintain the basal glucose levels. The K_i values of tissue 2-[^{3}H] DG uptake were assessed as described in METHODS. Other experimental conditions are as in Fig. 1. Results are the means ± SE for six rats. *Significantly different from controls at $P < 0.01$. §Significantly different from the value of the insulin-treated group (3 U/kg) at $P < 0.01$.

uptake by peripheral tissues in response to hypothalamic leptin. As shown in Table 1, the β-adrenergic antagonist propranolol effectively suppressed the increase in K_i values of heart, EDL, soleus, and BAT in response to hypothalamic leptin. However, the α-adrenergic antagonist phenoxybenzamine did not affect appreciably the increased K_i values in the tissues in response to hypothalamic leptin. Treatment with phenoxybenzamine or propranolol did not change the K_i values of the epididymal and retroperitoneal WAT in either the saline- or leptin-injected group.

Interaction with insulin. We further examined the interaction between the effects of hypothalamic leptin and peripheral insulin on glucose uptake by the tissues. When 3 U/kg of insulin was administered intravenously, the rates of glucose uptake in BAT, heart, skeletal muscles, and WAT were markedly increased (Fig. 2 and 3): in the insulin-treated group (3 U/kg), the K_i values of the heart, soleus, and EDL increased 8.3, 5.3, and 5.3 times that of the controls, respectively. When the K_i values in the insulin-treated group (3 U/kg) were compared with those in the leptin-injected group, the K_i value of soleus muscle was similar in both groups, whereas the K_i value of BAT was more markedly increased by the insulin treatment (3 U/kg) than by leptin injection (3 times versus 50 times that of the controls) (Figs. 2*B* and 3*A*). The K_i values of the epididymal and retroperitoneal WAT were also increased 10 and 13 times, respectively (Fig. 3*B*).

When 10 times the dose of insulin (30 U/kg) was given intravenously, there were no greater increases in the K_i values of tissues beyond the values reached by 3 U/kg of insulin (Figs. 2 and 3). This result indicates that administration of 3 U/kg insulin produces the maximal effects of insulin on tissue glucose uptake, as reported previously (28). However, when 3 U/kg insulin was given to rats after hypothalamic microinjection of leptin, the K_i values of the heart, skeletal muscles, and BAT markedly increased over the values reached by insulin alone. In the heart, soleus, and EDL, the K_i values in the group treated with insulin (3 U/kg) plus lep-

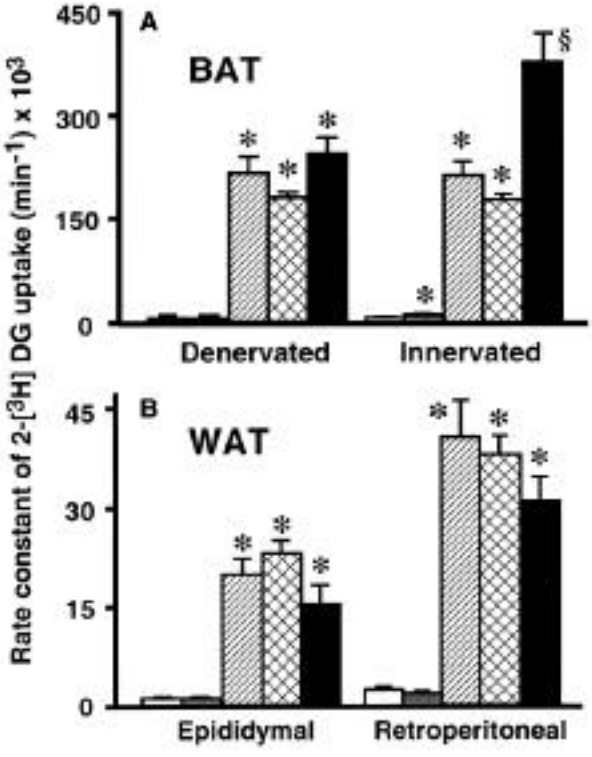

FIG. 3. Interaction of hypothalamic leptin and peripheral insulin on the K_i values of 2-[^{3}H] DG uptake in BAT (*A*) and epididymal and retroperitoneal WAT (*B*). Five groups of rats were investigated: *1*) control rats with saline injected into the VMH without insulin administration (□), *2*) rats with leptin injected into the VMH (▩), *3*) rats administered 3 U/kg insulin intravenously (▨), *4*) rats administered 30 U/kg insulin (▩), and *5*) rats with leptin injected into the VMH and then administered 3 U/kg insulin (■). Other experimental conditions are as in Fig. 2. Results are the means ± SE for six rats. *Significantly different from controls at $P < 0.01$. §Significantly different from the value of the insulin-treated group (3 U/kg) at $P < 0.01$ in the innervated side of BAT.

tin was 1.8, 1.8, and 2.3 times, respectively, the values from insulin treatment alone (Fig. 2). Similarly, the K_i value of the innervated side of BAT was 1.8 times the value obtained with insulin treatment alone (Fig. 3*A*). However, the K_i value of the denervated side of this tissue was not enhanced over the value obtained with insulin treatment alone (Fig. 3*A*). Injection of leptin into the VMH did not enhance the maximally increased K_i values of the epididymal and retroperitoneal WAT after insulin treatment. In fact, leptin injection into the hypothalamus rather suppressed the K_i values of WAT stimulated maximally by insulin (Fig. 3*B*).

Effects on liver glycogen, plasma glucose, and insulin concentrations. Microinjection of leptin into the VMH did not change the plasma glucose concentration, as described previously (11,12). The enhancement of glucose uptake by BAT, heart, and skeletal muscles in response to leptin injected into the VMH without possible change in plasma glucose level suggests that hypothalamic leptin increases glucose output from the liver. In fact, the content of liver glycogen decreased significantly after microinjection of leptin (the saline-injected group: 47.7 ± 2.0 mg/g [n = 6], the leptin-injected group: 40.0 ± 3.0 mg/g [n = 6], $P < 0.05$), as reported previously (12). Likewise, no significant differences were observed in the plasma glucose concentrations after pretreatment with guanethidine, adrenodemedullation, or administration of adrenergic antagonists (data not shown).

When insulin was administered intravenously, the plasma glucose concentration was kept at a steady-state level by glucose infusion during the experiments. The averages of plasma glucose concentration during the experiments were as follows: 109 ± 5 mg/dl in the controls, 105 ± 5 mg/dl in the leptin-injected group, 107 ± 4 mg/dl in the insulin-treated group (3 U/kg), 108 ± 6 mg/dl in the insulin-treated group (30 U/kg), and 103 ± 5 mg/ml in the insulin-treated (3 U/kg) plus leptin-injected group, respectively. The average rates of glucose infusion during the experiments were 14 ± 1 mg · kg^{-1} · min^{-1} in the insulin-treated group (3 U/kg), 15 ± 1 mg · kg^{-1} · min^{-1} in the insulin-treated group (30 U/kg), and 16 ± 2 mg · kg^{-1} · min^{-1} in the insulin-treated (3 U/kg) plus leptin-injected group, respectively. There were no significant differences among the glucose infusion rates in these groups.

Microinjection of leptin into the VMH also did not change the plasma insulin concentration by itself, as reported previously (11,12). In addition, there were no significant changes in the plasma insulin levels after pretreatment with guanethi-

TABLE 1
Effects of α- and β-adrenergic antagonists on the K_i values of 2-[^{3}H] DG uptake in peripheral tissues in response to leptin injected into the VMH

K_i of 2-[^{3}H] DG uptake [(min^{-1}) × 10^3]	Without pretreatment		Treatment with phenoxybenzamine		Treatment with propranolol	
	Saline	Leptin	Saline	Leptin	Saline	Leptin
Heart	47.4 ± 9.6	196.9 ± 27.9†	67.4 ± 10.6	165.7 ± 14.4†	51.3 ± 5.2	62.0 ± 4.2
Soleus	26.2 ± 5.7	108.2 ± 9.7†	33.2 ± 5.3	100.2 ± 3.7†	32.6 ± 4.3	38.4 ± 3.4
EDL	8.9 ± 1.3	15.1 ± 0.5*	11.4 ± 0.5	16.8 ± 0.9*	8.5 ± 0.8	11.0 ± 1.7
BAT	4.7 ± 0.4	14.3 ± 1.2†	5.6 ± 0.5	12.3 ± 0.6†	4.8 ± 0.4	5.3 ± 0.3
epiWAT	1.6 ± 0.3	2.3 ± 0.5	1.9 ± 0.2	1.2 ± 0.3	1.0 ± 0.2	1.4 ± 0.3
rWAT	3.5 ± 0.4	4.3 ± 1.0	4.1 ± 0.9	3.8 ± 0.7	2.5 ± 0.5	2.4 ± 0.5

Data are means ± SE for six rats in each group. The α-adrenergic antagonist phenoxybenzamine (5 mg/kg) or the β-adrenergic antagonist propranolol (10 mg/kg) was injected twice intraperitoneally 15 min before and 3 h after microinjection of leptin into the VMH. Other rats were used as the group without pretreatment. Leptin or saline was injected into the VMH in conscious unrestrained rats through a chronically implanted cannula. The K_i values of tissue 2-[^{3}H] DG uptake were assessed as described in METHODS. Other experimental conditions are as in Fig. 1. The tissues measured include heart (left ventricle), soleus, EDL, interscapular BAT, epididymal WAT (epiWAT), and retroperitoneal WAT (rWAT). *, †Significantly different from the respective controls with saline injected into the VMH at $P < 0.05$ and $P < 0.01$, respectively. There were no statistical differences among control values in three saline-injected groups.

dine, adrenodemedullation, or administration of adrenergic antagonists (data not shown). In the experiment with insulin administration, plasma insulin concentrations of the last blood samples were as follows: 0.90 ± 0.10 ng/ml in the controls, 0.92 ± 0.08 ng/ml in the leptin-injected group, 1.20 ± 0.11 ng/ml in the insulin-treated group (3 U/kg), 5.30 ± 0.11 ng/ml in the insulin-treated group (30 U/kg), and 1.12 ± 0.10 ng/ml in the insulin-treated (3 U/kg) plus leptin-injected group. Thus, administration of 30 U/kg insulin markedly increased the plasma insulin level of the last blood sample ($P < 0.01$). Because plasma insulin concentration exponentially decreases within 30 min after a single injection of insulin (28), it is likely that the plasma insulin level greatly increased just after bolus injection of insulin at 3 and 30 U/kg.

DISCUSSION

The present study corroborates that microinjection of leptin into the VMH preferentially increases glucose uptake by the heart, skeletal muscles, and BAT through mediation of a β-adrenergic mechanism of the sympathetic nerves innervating the tissues. Furthermore, when leptin was injected into the VMH together with intravenous administration of insulin, the rates of glucose uptake by these tissues increased synergistically. These results suggest that the leptin-induced activation of the VMH-sympathetic nervous system and insulin have cooperative roles in the regulation of glucose uptake by certain peripheral tissues.

Several lines of evidence have been interpreted as indicating that the VMH is part of the sympathetic neural output from the hypothalamus (29). Indeed, the present study indicates that leptin's action on the VMH was effectively suppressed by pretreatment with guanethidine in terms of the enhanced glucose uptake in the heart, skeletal muscles, and BAT (Fig. 1). Because guanethidine does not inhibit secretion of epinephrine from the adrenal medulla (30,31) and does not affect brain norepinephrine (31), this result suggests that the increased rates of glucose uptake in the tissues by hypothalamic leptin are mediated by the sympathetic nerves innervating the tissues. In fact, bilateral adrenodemedullation did not inhibit the increased rate of glucose uptake by the tissues. These results are in agreement with our previous observations: electrical and chemical stimulation of the VMH neurons preferentially increases glucose uptake in BAT, the heart, and skeletal muscles (25), and the enhanced glucose uptake in these tissues upon VMH stimulation is inhibited almost completely by guanethidine pretreatment but not by adrenodemedullation (32). Furthermore, it has also been reported that systemic or intracerebroventricular administration of leptin increases sympathetic nerve activity to BAT and other peripheral tissues including the hind limb (14,15), similar to the effects seen after electrical stimulation of the VMH (33).

In the present study, the enhancement of glucose uptake in certain peripheral tissues was shown to be mediated by a β-adrenergic mechanism (Table 1). Although it is possible that the effect of propranolol is mediated at a site of the central nervous system as well as peripheral tissues, the effects of guanethidine and adrenodemedullation suggest that the β-adrenergic mechanism of norepinephrine released from the sympathetic nerves innervating the peripheral tissues is involved in the enhanced glucose uptake in response to hypothalamic leptin. In fact, it has been proven that the β_3-adrenergic agonist BRL35135A increases the rates of glucose uptake in vivo in BAT, the heart, and skeletal muscles, but not in WAT in rats (34,35). It has also been shown that BRL37344, the active metabolite of BRL35135A, increases glucose uptake in isolated soleus muscles directly (34,36). Furthermore, studies with primary culture of brown adipocytes (16) and L6 myocytes (17) have demonstrated that physiological concentrations of norepinephrine and the β_3-adrenergic agonist enhance glucose transport into these cells in vitro. Because the stimulatory effect of norepinephrine on cultured brown adipocytes was mimicked by the β_3-adrenergic agonist at concentrations two orders lower than norepinephrine (16), these results indicate that the β_3-adrenoceptor or its subtype may take part in the norepinephrine-induced increase in glucose uptake by the cells. Recently, the β_3-adrenoceptor has been shown to have low affinity for the natural ligands, norepinephrine and epinephrine, which led to the suggestion that the β_3-adrenoceptor responds only to much higher concentrations of catecholamines, like that in the synaptic cleft upon activation of the sympathetic nerve (37). In contrast, the β_1- and β_2-adrenoceptors may respond to the circulating catecholamines as well as to those after sympathetic nerve stimulation (37). If the β_3-adrenoceptor or its subtype mediates the increased glucose uptake in certain peripheral tissues in response to VMH leptin, these characteristics of the receptor may account for the present results that pretreatment with guanethidine but not adrenodemedullation effectively suppress the increased rate of glucose uptake by the tissues in response to hypothalamic leptin. Further studies are needed to clarify if the β_3-adrenoceptor or its subtype is indeed involved in the increased glucose uptake by these tissues in response to hypothalamic leptin.

When leptin was injected into the VMH and insulin was administered intravenously, the rates of glucose uptake by the heart, skeletal muscles, and BAT, but not by WAT, further increased over the values reached by insulin stimulation alone (Figs. 2 and 3). These results are consistent with recent reports that systemic or central infusion of leptin increases insulin sensitivity measured as whole-body glucose utilization in the presence of hyperinsulinemia at clamped constant glucose (19–21,23). More strictly, because intrahypothalamic injection of leptin increased the maximally stimulated effect of insulin on tissue glucose uptake under normoglycemic conditions, the results of the present study suggest that the responsiveness of certain tissues to insulin is augmented by central leptin. In addition, the exaggerated enhancement of glucose uptake by BAT in response to insulin and hypothalamic leptin was observed only in the innervated side of the tissue, indicating that synergistic action of the two factors is dependent on intact sympathetic innervation of the tissue (Fig. 3*A*). Norepinephrine and insulin have been shown to increase additively glucose uptake by brown adipocytes in primary culture and by L6 myocytes (16,17). Furthermore, different mechanisms are thought to operate in the effects of insulin and norepinephrine; whereas insulin stimulates translocation of GLUT4 from an intracellular pool to the plasma membrane, norepinephrine causes activation of GLUT1 present in the plasma membrane (18). In line with this, studies in vivo also showed that the apparent functional activity of glucose transporters was increased with the plasma membrane vesicles prepared from the heart and BAT after electrical stimulation of the VMH for 30 min, without significant alteration of either the number of glucose trans-

porters in the plasma or microsomal membranes (38,39). In contrast, insulin treatment increased the number of glucose transporters in the plasma membrane and decreased those in the microsomal membrane, but it had no significant effect on the functional activity of glucose transporters measured in vitro with the plasma membrane vesicles (38,39). Hence, it can be expected that the enhanced glucose uptake in some peripheral tissues in response to VMH leptin might be mediated by an insulin-independent mechanism like that seen after electrical stimulation of the VMH. Indeed, the stimulatory effects of insulin and hypothalamic leptin on the rate of glucose uptake were roughly additive in soleus muscle and in the heart (Fig. 2*A* and *B*). However, the effects of hypothalamic leptin and peripheral insulin on glucose uptake were apparently synergistic in BAT (Fig. 3*A*). These results can be interpreted as supporting the notion that hypothalamic leptin and peripheral insulin act cooperatively in enhancing glucose uptake by some tissues, rather than exerting their effects independently. Presumably, subacute stimulation by hypothalamic leptin for 6 h through the sympathetic nerves may cross-talk to the insulin-signaling pathways and improve the response of glucose uptake in some tissues to insulin. However, it is also possible that the synergistic effect of insulin and hypothalamic leptin is partly mediated by a central action of insulin. It has been reported that euglycemic hyperinsulinemia stimulates sympathetic efferent activity in peripheral tissue (40,41). Furthermore, the effects of hypothalamic leptin and insulin could conceivably be related, at least in part, to a change in blood flow. Because it has been reported that electrical stimulation of the VMH increases the regional blood flow of BAT and skeletal muscles (42), a possible opening of the capillary beds may contribute to the glucose uptake observed in these tissues after microinjection of leptin into the VMH.

Injection of leptin into the VMH did not change the plasma glucose level, while glucose uptake by BAT, the heart, and skeletal muscles was enhanced markedly. It is conceivable that microinjection of leptin into the VMH might increase glucose turnover by stimulating glucose output from the liver, as described previously (11,12). In fact, analysis of liver glycogen revealed that hypothalamic leptin decreased the content of liver glycogen significantly. This seems incompatible with the previous reports of a euglycemic-hyperinsulinemic clamp study showing that systemic or intracerebroventricular leptin infusion enhanced the insulin-induced suppression of glucose output from the liver (19,23,43). The inconsistency might be explained on the basis of different experimental protocols, e.g., in the present study, leptin was injected into the VMH and a bolus of insulin was administered intravenously. We need further investigations to measure the actual rate of hepatic glucose production after microinjection of leptin into the VMH.

In summary, this study provides evidence that microinjection of leptin into the VMH increased glucose uptake by the heart, skeletal muscles, and BAT, but not by WAT, through a β-adrenergic mechanism of the sympathetic nerves innervating the tissues. Moreover, intrahypothalamic administration of leptin was found to enhance the responsiveness of tissue glucose uptake maximally stimulated by insulin. Elucidation of this mechanism, though it remains to be established, may have major implications for the role of hypothalamic leptin in the regulation of glucose utilization in peripheral tissues under condition of insulin resistance, such as in obesity and diabetes.

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