Distinct Effects of Leptin and a Melanocortin Receptor Agonist Injected Into Medial Hypothalamic Nuclei on Glucose Uptake in Peripheral Tissues

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OBJECTIVE—The medial hypothalamus mediates leptin-induced glucose uptake in peripheral tissues, and brain melanocortin receptors (MCRs) mediate certain central effects of leptin. However, the contributions of the leptin receptor and MCRs in individual medial hypothalamic nuclei to regulation of peripheral glucose uptake have remained unclear. We examined the effects of an injection of leptin and the MCR agonist MT-II into medial hypothalamic nuclei on glucose uptake in peripheral tissues.

RESEARCH DESIGN AND METHODS—Leptin or MT-II was injected into the ventromedial (VMH), dorsomedial (DMH), arcuate nucleus (ARC), or paraventricular (PVH) hypothalamus or the lateral ventricle (intracerebroventricularly) in freely moving mice. The MCR antagonist SHU9119 was injected intracerebroventricularly. Glucose uptake was measured by the 2-[^{3}H]deoxy-D-glucose method.

RESULTS—Leptin injection into the VMH increased glucose uptake in skeletal muscle, brown adipose tissue (BAT), and heart, whereas that into the ARC increased glucose uptake in BAT, and that into the DMH or PVH had no effect. SHU9119 abolished these effects of leptin injected into the VMH. Injection of MT-II either into the VMH or intracerebroventricularly increased glucose uptake in skeletal muscle, BAT, and heart, whereas that into the PVH increased glucose uptake in BAT, and that into the DMH or ARC had no effect.

CONCLUSIONS—The VMH mediates leptin- and MT-II–induced glucose uptake in skeletal muscle, BAT, and heart. These effects of leptin are dependent on MCR activation. The leptin receptor in the ARC and MCR in the PVH regulate glucose uptake in BAT. Medial hypothalamic nuclei thus play distinct roles in leptin- and MT-II–induced glucose uptake in peripheral tissues. ***Diabetes*** **58:2757–2765, 2009**

Leptin is an adipocyte hormone that inhibits food intake and increases energy expenditure (1). The hypothalamus is a principal target of leptin in its regulation of energy metabolism (2–5). The arcuate nucleus (ARC) is the most well characterized of hypothalamic nuclei in terms of its role in the central effects of leptin (2–5). The ARC contains two populations of leptin-responsive neurons: pro-opiomelanocortin (POMC)-expressing neurons, which release the potent anorexic peptide α-melanocyte–stimulating hormone, and neurons that release two potent orexigenic peptides, agouti-related peptide (AgRP) and neuropeptide Y (NPY) (2–5). α-Melanocyte–stimulating hormone activates the melanocortin receptor (MCR), whereas AgRP competitively inhibits this receptor and NPY functionally antagonizes MCR signaling (6). Both sets of neurons project to second-order MCR-expressing neurons within the hypothalamus, including the paraventricular (PVH), ventromedial (VMH), dorsomedial (DMH), and lateral hypothalamus, as well as to other brain regions such as the brain stem (2,4,7,8). Leptin inhibits food intake through reciprocal regulation of POMC and AgRP/NPY neurons in the ARC and consequent activation of MCR in hypothalamic nuclei, including the PVH (5,6,7,9). Mice lacking the melanocortin 3 (MC3R) or 4 (MC4R) receptor show increased adiposity and feeding efficiency (4). Restoration of MC4R expression in certain sets of PVH neurons prevented hyperphagia and reduced body weight in MC4R-null mice (9). In addition to that in the ARC, the leptin receptor Ob-Rb in other hypothalamic nuclei has also been shown to regulate energy intake and adiposity. Neurons positive for steroidogenic factor 1 (SF1; also known as Ad4BP) (10,11) are largely restricted to the VMH in the adult brain. Leptin depolarizes these neurons, and specific ablation of the leptin receptor in SF1-positive cells induced obesity and increased susceptibility to a high-fat diet in mice (12).

The leptin receptor in the brain also regulates glucose metabolism in certain peripheral tissues (13–17). Treatment with leptin ameliorates diabetes in lipodystrophic mice and humans (18,19). Intravenous or intracerebroventricular administration of leptin markedly increased whole-body glucose turnover and glucose uptake by certain tissues in mice without any substantial change in plasma insulin or glucose levels (13). We have also previously shown that microinjection of leptin into the medial hypothalamus, such as into the VMH, but not into the lateral hypothalamus, preferentially increased glucose uptake in skeletal muscle, heart, and brown adipose tissue (BAT) (14–16). Restoration of Ob-Rb expression in the ARC and the VMH of the Ob-Rb–mutated Koletsky rat by adeno-

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virus- or adeno-associated virus–mediated gene transfer improved peripheral insulin sensitivity and reduced plasma glucose concentration (17,20). Ablation of suppressor of cytokine signaling 3 (SOCS3) in SF1-positive cells (10,11) improved glucose homeostasis in mice fed a high-fat diet (21). Furthermore, intracerebroventricular injection of the MCR agonist (MT-II) increased whole-body glucose turnover and expression of GLUT4 in skeletal muscle (22). Ob-Rb in the ARC and the VMH as well as the brain melanocortin pathway are thus implicated in the regulation of glucose uptake in peripheral tissues as well as in energy metabolism. However, little is known about the contributions of the leptin receptor and MCR in individual medial hypothalamic nuclei to regulation of glucose uptake in peripheral tissues, as opposed to their roles in the regulation of food intake and leanness.

We have now examined the acute effects of microinjection of leptin and MT-II into the VMH, ARC, DMH, and PVH, all of which express Ob-Rb, MC3R, and MC4R at a high level (3–7,23–25), on glucose uptake in peripheral tissues of mice in vivo. Our results suggest that the VMH mediates stimulatory actions of leptin and MT-II on glucose uptake in skeletal muscle, heart, and BAT, whereas the leptin receptor in the ARC as well as MCRs in PVH regulate glucose uptake in BAT. The medial hypothalamic nuclei thus appear to play distinct roles in the regulation of glucose uptake in peripheral tissues by leptin and MT-II.

RESEARCH DESIGN AND METHODS

Male FVB/N Jcl mice (CLEA Japan, Tokyo, Japan) were studied at 12–16 weeks of age. The animals were housed individually in plastic cages at 24 ± 1°C with lights on from 0600 to 1800 h, and they were maintained with free access to a laboratory diet (Oriental Yeast, Tokyo, Japan) and water. Mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg body mass) and xylazine (10 mg/kg), and a chronic double-walled stainless steel cannula was implanted stereotaxically and unilaterally into the right side of the VMH, ARC, DMH, or PVH or into the lateral ventricle according to the atlas of Franklin and Paxinos (26). The stereotaxic coordinates were AP 1.3 (1.3 mm anterior to the bregma), L 0.3 (0.3 mm lateral to the bregma), and H 5.8 (5.8 mm below the bregma on the surface of the skull) for the VMH; AP 1.6, L 0.2, and H 6.1 for the ARC; AP 1.6, L 0.3, and H 5.5 for the DMH; AP 0.75, L 0.2, and H 4.9 for the PVH; and AP 0.3, L 1.0, and H 2.25 for the lateral ventricle. Cannulas were anchored firmly to the skull. In experiments featuring administration of the MCR antagonist SHU9119, cannulas were implanted both into VMH and intracerebroventricularly.

Three days before determination of tissue glucose uptake, a silicone catheter was implanted into the external jugular vein. Animals were handled repeatedly during the recovery period (2 weeks) after cannula implantation to habituate them to the injection and blood-sampling procedures. Correct placement of the cannula tips was verified microscopically in brain sections in all experiments, with >90% of animals manifesting correct placement. Tyrosine phosphorylation of signal transducer and activator of transcription 3 (STAT3) in each medial hypothalamic nucleus was examined after injection of leptin, as described below. Food was removed just before the administration of the agents. All animal experiments were performed in accordance with institutional guidelines for the care and handling of experimental animals, and they were approved by the ethics committee for animal experiments of the National Institute for Physiological Sciences.

Administration of leptin, MT-II, and SHU9119. Leptin (5 ng) (National Hormones and Pituitary Program, Torrance, CA) or MT-II (10 ng) (Phoenix Pharmaceuticals, Burlingame, CA) dissolved in 0.1 μl physiological saline was injected with the use of a Hamilton microsyringe into the right side of the VMH, DMH, PVH, or ARC of freely moving mice through the unilateral cannula implanted into the corresponding nucleus. The concentrations of leptin (3 μmol/l) and MT-II (100 μmol/l) dissolved in 0.1 μl physiological saline injected into the medial hypothalamic nuclei are at least 10 times higher than those necessary for the maximum activation of leptin receptor and MCRs (27–29). Alternatively, MT-II (3 μg) in 0.5 μl saline was injected into the lateral ventricle. SHU9119 (1 μg) (Phoenix Pharmaceuticals) in 0.5 μl saline was also injected intracerebroventricularly immediately before leptin injection. The dose of MT-II and SHU9119 injected intracerebroventricularly altered food intake significantly (30,31). Control animals received 0.1 μl saline delivered into the various nuclei or 0.5 μl saline delivered intracerebroventricularly, respectively.

Measurement of the rate constant of 2-[^{3}H]deoxy-D-glucose uptake in peripheral tissues. The rate constant of net tissue uptake of 2-[^{3}H]deoxy-D-glucose (2[^{3}H]DG) in peripheral tissues was determined, as described previously (32,33), by injecting a mixture of 6.25 μCi 2[^{3}H]DG (10 Ci/mmol) and 1.25 μCi [^{14}C]sucrose (10 Ci/mmol) (American Radiolabeled Chemicals, St. Louis, MO) through the jugular vein catheter 3 or 6 h after the microinjection of leptin or MT-II. Blood was collected 0, 10, 15, and 20 min after injection of the radioactive tracers. Immediately after collection of the final blood sample (20 min), an overdose of pentobarbital sodium (100 mg/kg) was injected through the jugular vein catheter and the mice were rapidly decapitated. Hypothalamic nuclei were then rapidly dissected as described below and frozen in liquid nitrogen for subsequent immunoblot analysis. Skeletal muscle (soleus, red and white portions of the gastrocnemius, and extensor digitorum longus [EDL]) as well as interscapular BAT, heart, spleen, and epididymal white adipose tissue (WAT) were rapidly dissected, weighed, and assayed for the radioactivity. Plasma samples were also analyzed for glucose (Glucose CII Test; Wako, Osaka, Japan) and insulin (mouse insulin ELISA kit [U-Type]; Shibayagi, Gunma, Japan) concentrations. The rate constant of net tissue uptake of 2[^{3}H]DG was calculated as described previously (32,33). The radioactivity of [^{14}C]sucrose in tissues was used to calculate the 2[^{3}H]DG radioactivity remaining in the extracellular space (33).

Sampling of medial hypothalamic nuclei. Medial hypothalamic nuclei were isolated as described previously (34). The accuracy of the dissection was assessed by measurement of mRNAs for neuropeptides or transcription factors such as corticotropin-releasing factor (CRF) mRNA for the PVH, POMC and NPY mRNAs for the ARC, SF1 mRNA for the VMH, and the absence of these various mRNAs for the DMH. The right side of the PVH, ARC, VMH, or DMH was dissected from a 1-mm-thick sagittal section prepared from the midline of the fresh brain (online appendix supplemental Fig. 1 [available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0638/DC1]).

Immunoblot analysis. Hypothalamic nuclei were homogenized at 4°C in PBS containing 1% Nonidet P-40. The homogenates were centrifuged, and the resulting supernatants (5 μg of protein) were fractionated by SDS-PAGE. Immunoblot analysis was then performed with antibodies (1 μg/ml), including those to the Tyr^{705}-phosphorylated or total forms of STAT3 (Cell Signaling Technology, Danvers, MA), to c-Fos (Santa Cruz Biotechnology, Santa Cruz, CA), or to β-actin (Cell Signaling Technology). Immune complexes were visualized with horseradish peroxidase–conjugated secondary antibodies (Santa Cruz Biotechnology) and enhanced chemiluminescence reagents (GE Healthcare, Tokyo, Japan). Protein bands were quantified using Image J software (National Institutes of Health, http://rsbweb.nih.gov/ij).

RNA extraction, RT-PCR analysis, and quantitative real-time PCR. Total RNA was isolated from hypothalamic and peripheral tissue with the use of Isogen (Nippon Gene, Wako, Japan), and portions of the RNA (300 ng) were subjected to reverse transcription with an oligo(dT) primer and avian myeloblastosis virus reverse transcriptase (Takara, Shiga, Japan). The resulting cDNA was subjected to the PCR with LA *Taq* (Takara) and primers obtained from Sigma Genosys (Ishikari, Japan). For quantitative real-time PCR, cDNA was amplified using SYBR Green PCR Master Mix with an ABI 7500 real-time PCR system (Applied Biosystems, Tokyo, Japan). Data were normalized by the amount of eukaryotic elongation factor 2 (eEF2) mRNA. The sequences of the primers are shown in supplemental Table 1.

Statistical analysis. Data are presented as means ± SE. Statistical analysis of STAT3 phosphorylation, c-Fos expression, and real-time PCR was performed by Student *t* test, and analysis for other experiments was performed by ANOVA followed by Dunnett test. A P value of <0.05 was considered statistically significant.

RESULTS

Effects of leptin injection into medial hypothalamic nuclei on glucose uptake in peripheral tissues. Microinjection of leptin (5 ng) into the VMH induced a significant increase in the rate constant of 2[^{3}H]DG uptake in the red type of skeletal muscle (soleus and red portion of gastrocnemius), mixed type of skeletal muscle (EDL), BAT, and heart but not in spleen or epididymal WAT (Fig. 1). Glucose uptake in red or mixed skeletal muscle was significantly increased at 6 h after leptin injection (Fig. 1*A*), whereas that in BAT and heart was increased at both 3 and 6 h (Fig. 1*B*). Glucose uptake in the white portion of the gastrocnemius showed a tendency to increase in

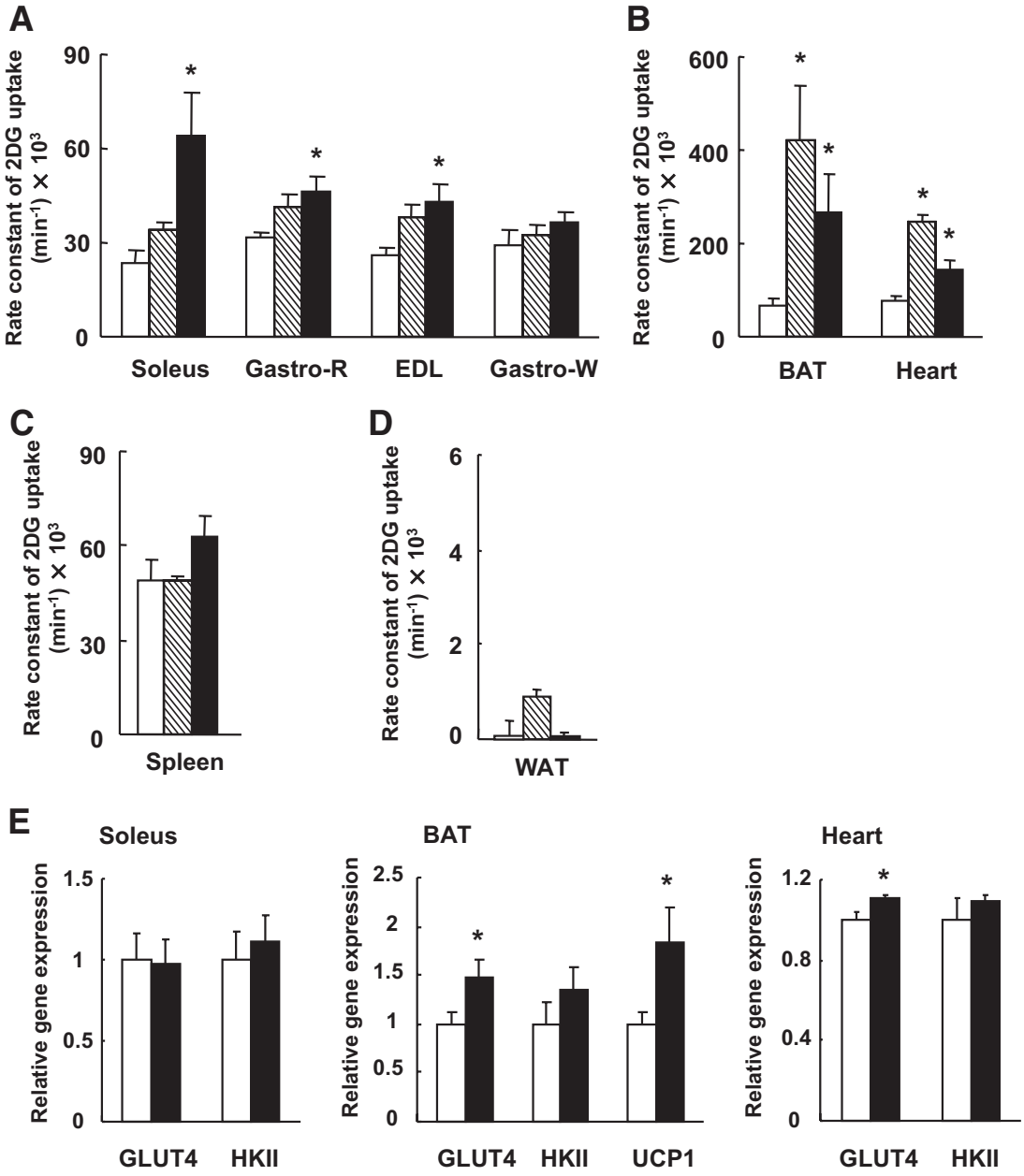

FIG. 1. Effects of leptin injection into the VMH on glucose uptake and gene expression in peripheral tissues. The rate constant of 2[^{3}H]DG uptake was measured in skeletal muscle (*A*), BAT and heart (*B*), spleen (*C*), and epididymal WAT (*D*) at 3 and 6 h after injection of leptin or saline (control) into the VMH of mice. □, Saline VMH (6 h); ▨, leptin VMH (3 h); ■, leptin VMH (6 h). *E*: The mRNA levels of GLUT4, hexokinase II (HKII), and UCP1 in soleus, BAT, and heart were quantified by real-time PCR. Values are normalized by the level of eEF2 mRNA. Gastro-R, red portion of gastrocnemius; Gastro-W, white portion of gastrocnemius. Data are means ± SE for four to seven mice. *$P < 0.05$ vs. the corresponding value for saline-injected controls. □, Saline VMH (6 h); ■, leptin VMH (6 h).

response to leptin, but the change was not statistically significant. Glucose uptake in peripheral tissues at 3 h after saline injection into the VMH did not differ from that apparent at 6 h (data not shown). Injection of leptin into the VMH significantly increased GLUT4 mRNA in BAT and heart but not in soleus muscle at 6 h after leptin injection (Fig. 1*E*). It also increased uncoupling protein 1 (UCP1) mRNA in BAT. Hexokinase II mRNA did not change in soleus, BAT, or heart (Fig. 1*E*).

Injection of maximal dose of leptin into the ARC induced a small but significant increase in the rate constant of 2[^{3}H]DG uptake in BAT at 6 h after injection, but it had no effect on glucose uptake in skeletal muscle, heart, spleen, or WAT (Fig. 2). Injection of leptin into the DMH or PVH had no effect on glucose uptake in peripheral tissues (Fig. 2). Glucose uptake in peripheral tissues after saline injection into the DMH or PVH did not differ from that apparent after saline injection into the ARC (data not shown). Plasma glucose and insulin concentrations were not affected by leptin injection into the VMH or other hypothalamic nuclei (supplemental Table 2), consistent with previous observations (13,15).

To determine whether leptin activated Ob-Rb in the hypothalamic nuclei, we examined the tyrosine phosphorylation of STAT3 in tissue samples therefrom. RT-PCR analysis confirmed that the isolated PVH, ARC, and VMH specimens were enriched in CRF mRNA, POMC and NPY mRNAs, and SF1 mRNA, respectively, and that the DMH

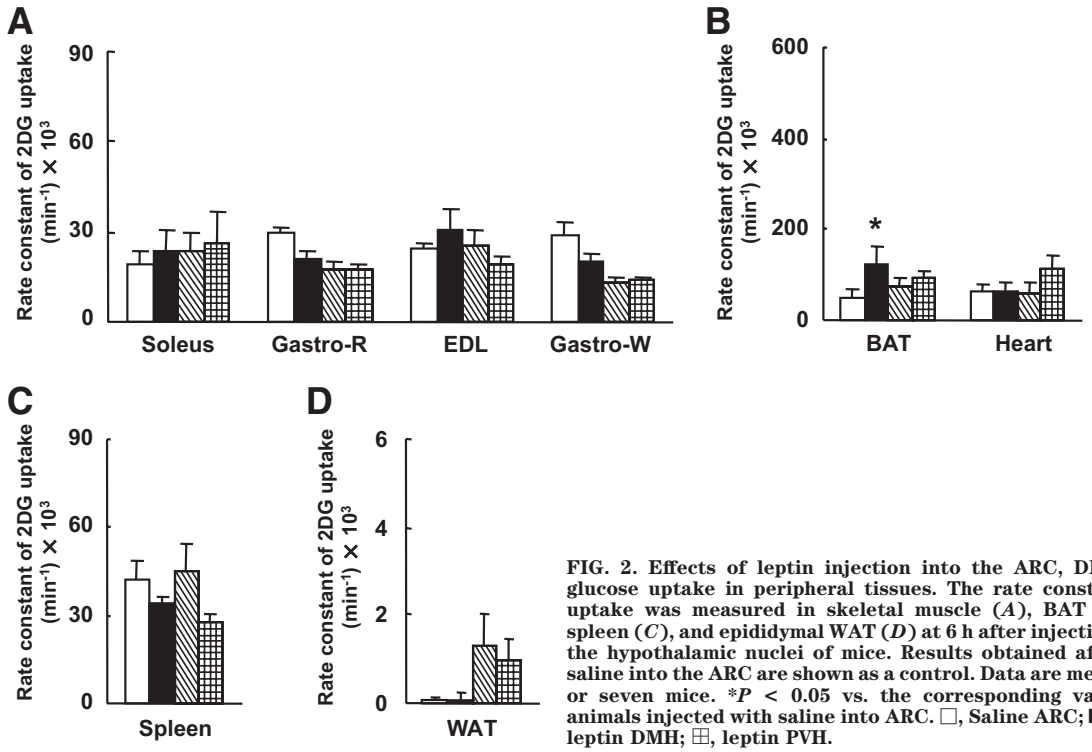

FIG. 2. Effects of leptin injection into the ARC, DMH, or PVH on glucose uptake in peripheral tissues. The rate constant of 2[^{3}H]DG uptake was measured in skeletal muscle (*A*), BAT and heart (*B*), spleen (*C*), and epididymal WAT (*D*) at 6 h after injection of leptin into the hypothalamic nuclei of mice. Results obtained after injection of saline into the ARC are shown as a control. Data are means ± SE for six or seven mice. *$P < 0.05$ vs. the corresponding value for control animals injected with saline into ARC. □, Saline ARC; ■, leptin ARC; ▨ leptin DMH; ⊞, leptin PVH.

was largely devoid of these mRNAs (Fig. 3*A*). The medial hypothalamic nuclei were thus accurately dissected. Microinjection of leptin into the VMH, DMH, or PVH preferentially increased the tyrosine phosphorylation of STAT3 to the similar extent in the corresponding nucleus at 6 h after injection (Fig. 3*B–D*). These results confirmed that leptin was correctly injected into these various hypothalamic nuclei. Injection of leptin into the ARC increased the tyrosine phosphorylation of STAT3 in the DMH as well as in the ARC (Fig. 3*E*), possibly as a result of leakage of leptin into the DMH through the surface of the injection cannula, given that the ARC cannula passed through the DMH. Together, these results suggested that the VMH is a key target of leptin in its regulation of glucose uptake in skeletal muscle, heart, and BAT, whereas the leptin receptor in the ARC mediates stimulation of glucose uptake in BAT.

We next examined whether leptin injection into the VMH might increase neuronal activity in other hypothalamic nuclei by measuring expression of the transcription factor c-Fos. Leptin injection into the VMH significantly increased c-Fos expression in the ARC as well as in the VMH at 6 h after injection (Fig. 3*F*). Leptin injection into the VMH increased the phosphorylation of STAT3 in the nucleus preferentially at 1 h after the injection, similar to that at 6 h. However, it did not increase c-Fos expression in any nucleus at 1 h (supplemental Fig. 2), as described previously (35). Leptin injection into the VMH did not increase POMC mRNA in the ARC at 6 h after injection (supplemental Fig. 2).

Effect of intracerebroventricular injection of an MCR antagonist on glucose uptake in peripheral tissues induced by injection of leptin into the VMH. We next examined the role of MCRs in glucose uptake in peripheral tissues induced by injection of leptin into the VMH (Fig. 4). Injection of the MCR antagonist SHU9119 (1 μg) into the lateral ventricle (intracerebroventricularly) abolished the increase in 2[^{3}H]DG uptake in peripheral tissues normally apparent at 6 h after the injection of leptin into the VMH. Injection of SHU9119 alone did not affect glucose uptake in peripheral tissues. Plasma glucose and insulin levels were also not changed in response to intracerebroventricular injection of SHU9119 (supplemental Table 2).

Effects of intracerebroventricular injection of an MCR agonist on glucose uptake in peripheral tissues. We tested the effects of intracerebroventricular injection of the MCR agonist MT-II on glucose uptake in peripheral tissues (Fig. 5). The intracerebroventricular injection of MT-II (3 μg) increased 2[^{3}H]DG uptake in BAT, heart, and all types of skeletal muscle, including the white portion of the gastrocnemius, but not in spleen or epididymal WAT, at 3 or 6 h after injection. Glucose uptake in peripheral tissues at 3 h after saline injection did not differ from that apparent at 6 h (data not shown). The intracerebroventricular injection of MT-II increased the plasma glucose level at both 3 and 6 h after injection (supplemental Table 2). These results thus suggested that intracerebroventricular injection of MT-II promotes glucose production as well as glucose uptake in certain peripheral tissues. In contrast, plasma insulin concentration did not change after intracerebroventricular injection of MT-II, despite the associated hyperglycemia, suggesting that MT-II inhibits insulin secretion from pancreatic β-cells.

Effects of MT-II injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues. Finally, we examined the effects of direct injection of MT-II into the individual medial hypothalamic nuclei on glucose uptake in peripheral tissues (Fig. 6). Microinjection of MT-II (10 ng) into the VMH increased 2[^{3}H]DG uptake in BAT, heart, and all types of skeletal muscle but not in spleen or epididymal WAT. In contrast, injection of MT-II into the PVH increased glucose uptake only in BAT, and that into the DMH or ARC did not affect glucose uptake in any of the peripheral tissues examined. Plasma glucose and insulin levels did not change in response to injection

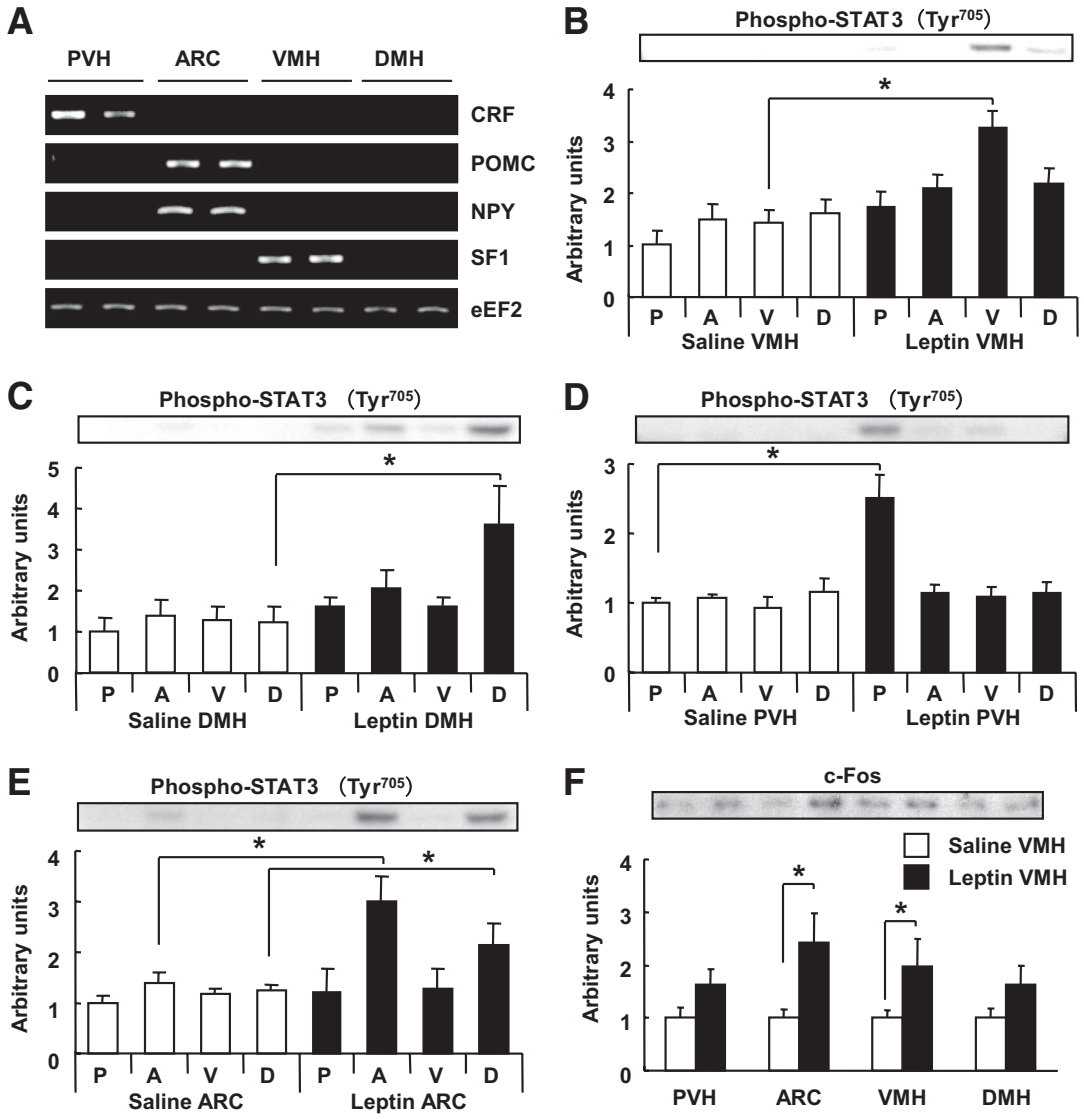

FIG. 3. Effects of leptin injection into medial hypothalamic nuclei on tyrosine phosphorylation of STAT3 and c-Fos expression. *A*: RT-PCR analysis of CRF, POMC, NPY, SF1, and eEF2 (loading control) mRNAs in the PVH, ARC, VMH, and DMH. Data are from two animals in a representative experiment. *B–E*: Immunoblot analysis of the phosphorylation of STAT3 on Tyr^{705} in the medial hypothalamic nuclei at 6 h after leptin injection into the VMH (*B*), DMH (*C*), PVH (*D*), or ARC (*E*). Leptin was injected into the right side of the hypothalamic nuclei, and the same side of the PVH (P), ARC (A), VMH (V), and DMH (D) was collected. Injection of saline into the VMH was used as a control. Figure shows separate saline controls for each nucleus. Representative blots of Tyr^{705}-phosphorylated STAT3 are shown in the *upper panels*, and quantitative data (means ± SE) from four to six mice are shown in the *lower panels*. The amount of Tyr ^{705}-phosphorylated STAT3 was normalized by that of total STAT3, and the normalized values were expressed relative to the corresponding value for the PVH of control mice. *$P < 0.05$ for the indicated comparisons. *F*: Immunoblot analysis of c-Fos expression in the medial hypothalamic nuclei after leptin injection into the VMH. The right side of the PVH, ARC, VMH, and DMH was collected at 6 h after injection of leptin or saline into the same side of the VMH. A representative blot is shown in the *upper panel*, and quantitative data (means ± SE) from four to six mice are shown in the *lower panel*. The amount of c-Fos was normalized by that of β-actin, and the normalized values were expressed relative to the corresponding value for the saline-injected control mice. *$P < 0.05$.

of MT-II into any of the hypothalamic nuclei (supplemental Table 2).

DISCUSSION

Leptin is a physiologically and clinically important hormone that regulates glucose metabolism in peripheral tissues. We have previously shown that microinjection of leptin into the VMH and nearby medial hypothalamic area preferentially increased glucose uptake in skeletal muscle, heart, and BAT (14–16). In the present study, we found that activation of the leptin receptor specifically in the VMH, as detected by measurement of the tyrosine phosphorylation of STAT3, resulted in a marked increase in glucose uptake in those peripheral tissues, similar to the effects of intracerebroventricular or peripheral administration of leptin (13,36). In contrast, injection of leptin into the ARC increased glucose uptake only in BAT. Injection of leptin into the DMH or PVH had no effect on glucose uptake in any of the peripheral tissues examined. The present data thus suggest that the leptin receptor in the VMH and ARC regulates glucose uptake in different peripheral tissues.

Our present results further indicate that MCR activation is necessary for the increase in glucose uptake in peripheral tissues induced by injection of leptin into the VMH. The intracerebroventricular injection of SHU9119 thus abolished the effect of leptin injected into the VMH on peripheral glucose uptake, whereas intracerebroventricu-

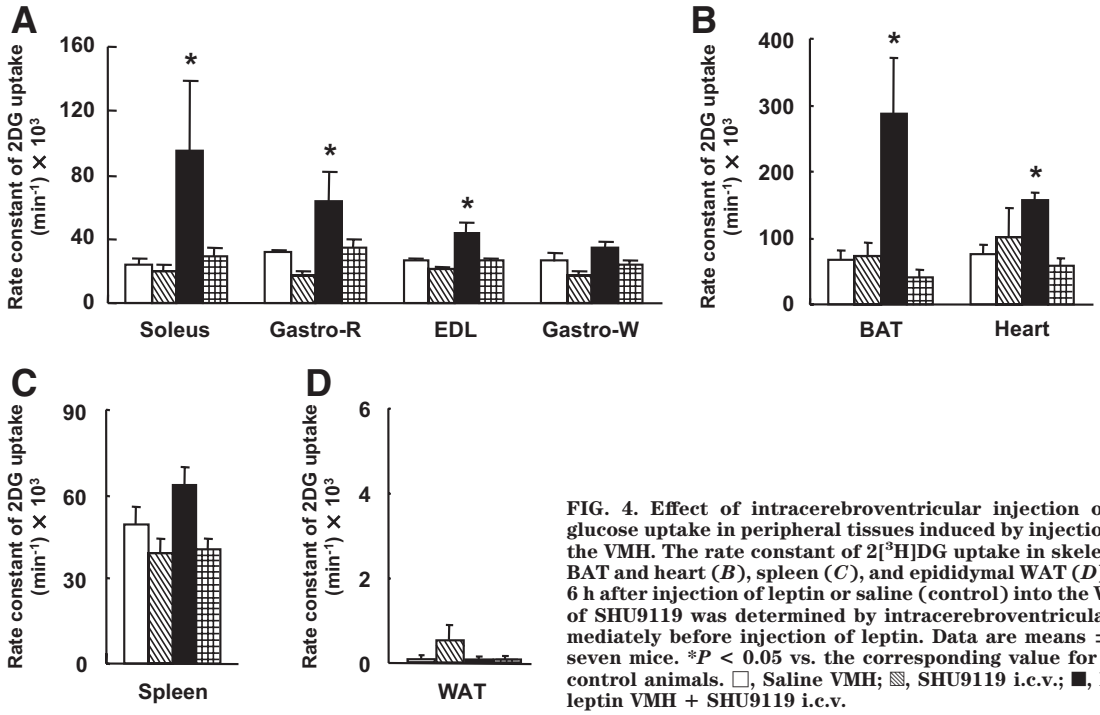

FIG. 4. Effect of intracerebroventricular injection of SHU9119 on glucose uptake in peripheral tissues induced by injection of leptin into the VMH. The rate constant of 2[^{3}H]DG uptake in skeletal muscle (*A*), BAT and heart (*B*), spleen (*C*), and epididymal WAT (*D*) was measured 6 h after injection of leptin or saline (control) into the VMH. The effect of SHU9119 was determined by intracerebroventricular injection immediately before injection of leptin. Data are means ± SE for six or seven mice. **P* < 0.05 vs. the corresponding value for saline-injected control animals. □, Saline VMH; ▨, SHU9119 i.c.v.; ■, leptin VMH; ⊞, leptin VMH + SHU9119 i.c.v.

lar injection of MT-II increased glucose uptake in peripheral tissues. Moreover, injection of MT-II into VMH, but not that into the DMH or ARC, increased glucose uptake in skeletal muscle, heart, and BAT, whereas injection of MT-II into the PVH increased glucose uptake preferentially in BAT. The VMH expresses abundantly MC3R as well as MC4R (23,25). Both MCRs in the VMH and PVH may play an important role in the regulation of glucose uptake in peripheral tissues.

POMC neurons in the ARC receive strong excitatory input from the dorsomedial region of the VMH (37). The restoration of Ob-Rb expression in the VMH by adeno-associated virus–mediated gene transfer in Koletsky rats increased the amount of POMC mRNA in the ARC (20). We have now shown that injection of leptin into the VMH increased c-Fos expression in the ARC without an effect on STAT3 phosphorylation in the ARC. Furthermore, the effect of leptin injected into the VMH on glucose uptake in peripheral tissues was found to be dependent on MCRs in the brain. We therefore propose that stimulation of VMH neurons by leptin results in activation of a set of POMC neurons in the ARC and thereby increases glucose uptake in skeletal muscle, heart, and BAT. MCRs in the VMH and PVH contribute to the upregulation of glucose uptake in

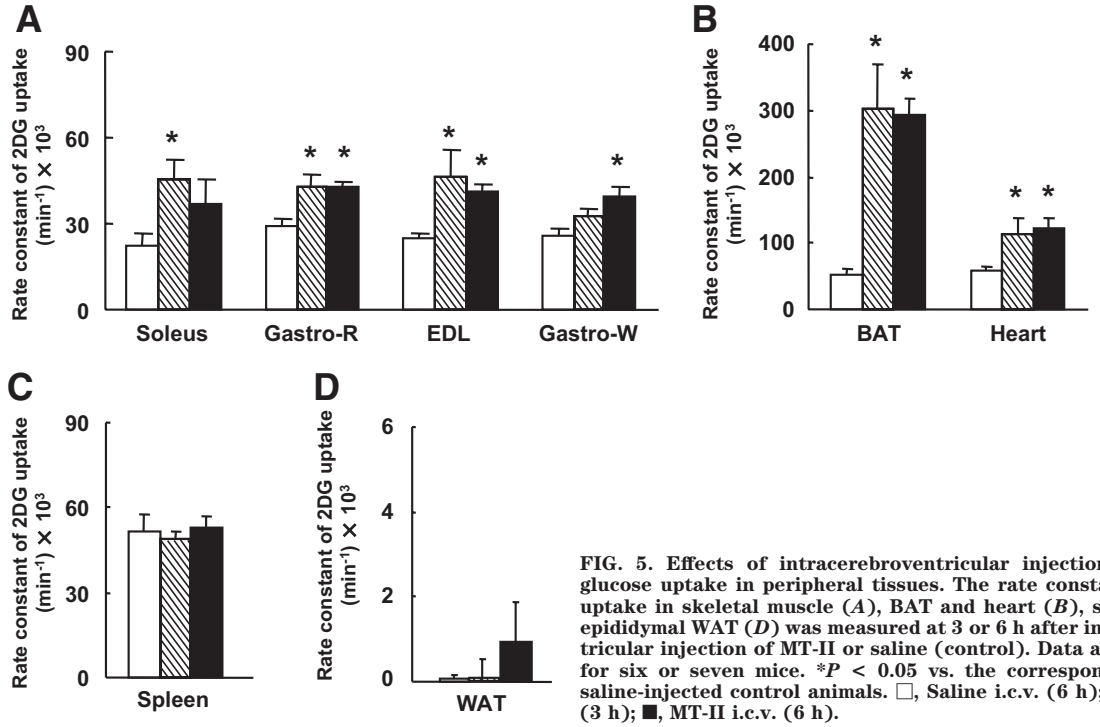

FIG. 5. Effects of intracerebroventricular injection of MT-II on glucose uptake in peripheral tissues. The rate constant of 2[^{3}H]DG uptake in skeletal muscle (*A*), BAT and heart (*B*), spleen (*C*), and epididymal WAT (*D*) was measured at 3 or 6 h after intracerebroventricular injection of MT-II or saline (control). Data are means ± SE for six or seven mice. **P* < 0.05 vs. the corresponding value for saline-injected control animals. □, Saline i.c.v. (6 h); ▨, MT-II i.c.v. (3 h); ■, MT-II i.c.v. (6 h).

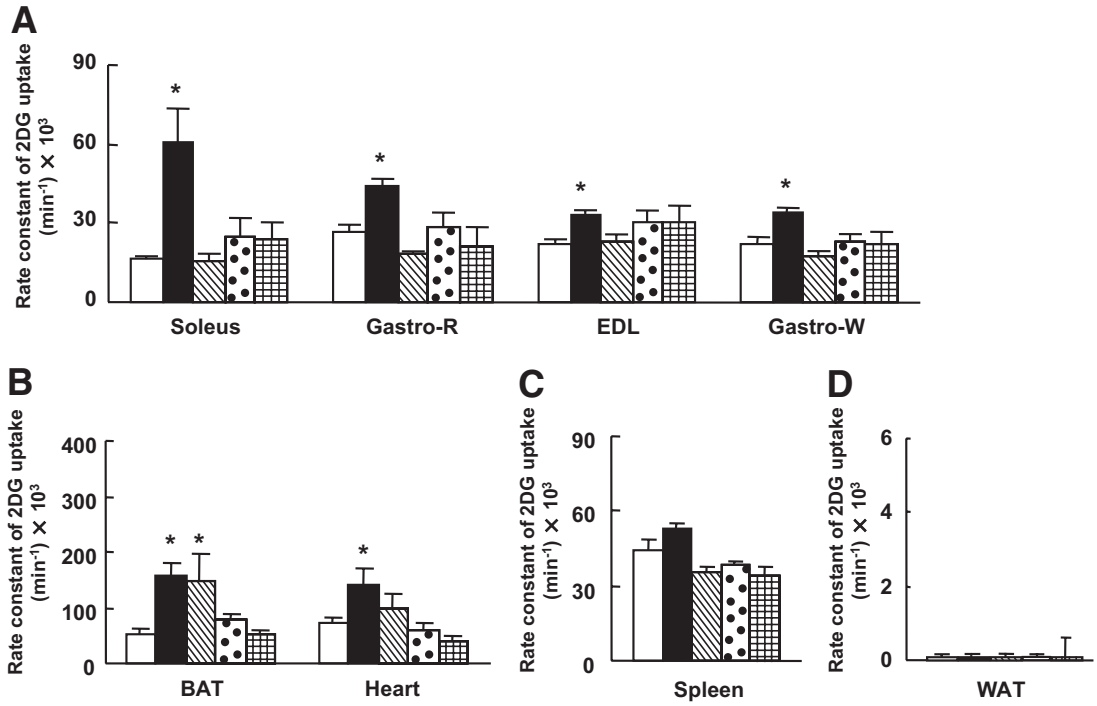

FIG. 6. Effects of MT-II injection into VMH, PVH, DMH, or ARC on glucose uptake in peripheral tissues. The rate constant of 2[^{3}H]DG uptake in skeletal muscle (*A*), BAT and heart (*B*), spleen (*C*), and epididymal WAT (*D*) was measured at 6 h after the injection of MT-II into the individual hypothalamic nuclei. Injection of saline into VMH was used as a control. Data are means ± SEM for six or seven mice. *$P < 0.05$ vs. the corresponding value for saline-injected control animals. □, VMH; ■, MT-II VMH; ▨, MT-II PVH; ⊡, MT-II DMH; ⊞, MT-II ARC.

peripheral tissues induced by injection of leptin into the VMH. Although c-Fos expression did not increase in the PVH in response to leptin injection into the VMH, this may have been due to the operation of γ-aminobutyric acid (GABA)-mediated neurotransmission in the PVH (6). The mechanism by which injection of leptin into the ARC increased glucose uptake specifically in BAT remains unclear. One possible explanation for this observation is that the injection of leptin into the ARC activated a selective set of POMC neurons in the ARC that regulate glucose uptake in BAT alone, with POMC neurons being abundant in both the anterior and posterior regions of the ARC (38). It is also possible that POMC neurons in the ARC that regulate glucose uptake in skeletal muscle and heart require excitatory input as well as leptin for their full activation.

The intracerebroventricular injection of MT-II increased glucose uptake in BAT to a markedly greater extent than did the maximum dose of injection of MT-II into the VMH or PVH. MC4R-expressing neurons in several regions of the brain stem as well as in the hypothalamic nuclei connect polysynaptically with interscapular BAT (39). Direct injection of MT-II into the raphe pallidus induces a thermogenic response in interscapular BAT (40). Glucose uptake in BAT might thus be regulated by MCRs in multiple brain regions. To date, there is little histological evidence of a connection between VMH neurons and interscapular BAT, although injection of leptin into the VMH increases plasma catecholamine levels more effectively than does that into other hypothalamic regions (41). We previously showed that injection of leptin into the medial hypothalamus increased glucose uptake in peripheral tissues through the activation of sympathetic nerves (14,15). Injection of leptin into the medial hypothalamus also increased insulin sensitivity in peripheral tissues by a β-adrenergic mechanism (14). Whereas the molecular mechanism remains elusive, a direct effect of β-adrenergic receptors expressed in peripheral tissues as well as a β-adrenergic receptor–dependent increase in blood flow appear to contribute to leptin-induced glucose uptake in these tissues.

The DMH is an important hypothalamic nucleus in the regulation of thermogenesis in BAT (42). Injection of a GABA type$_A$ ($GABA_A$) receptor antagonist into the DMH thus increased thermogenic activity in BAT (42). Moreover, injection of a $GABA_A$ receptor agonist into the DMH blocked sympathetic, thermogenic, and cardiovascular responses induced either by injection of prostaglandin E_2 into the medial preoptic area (43) or by skin cooling (44). Both Ob-Rb and MC4R are abundant in the DMH (7). Furthermore, DMH neurons, including those expressing MC4R, connect polysynaptically with interscapular BAT (39). However, we have now shown that injection of leptin or MT-II into the DMH did not increase glucose uptake in the peripheral tissues examined. Although we cannot exclude the possibility that injection of leptin or MT-II activated only a subset of DMH neurons expressing Ob-Rb or MCR, our results suggest that the leptin receptor and MCR in the DMH have other roles, such as the regulation of food intake or modulation of BAT thermogenesis in response to cold stimuli.

Skeletal muscle is a central player in glucose homeostasis in mammals. Our present results indicate that the VMH regulates glucose uptake in skeletal muscle as well as in heart and BAT, accompanying increased GLUT4 mRNA in BAT and heart. Thus, increased expression of GLUT4 may involve the acute increase in glucose uptake in BAT and heart in response to leptin, while other mechanism might be involved in the leptin-induced glucose uptake in skeletal muscle. Intracerebroventricular injection of MT-II has

been shown to increase GLUT4 mRNA expression in skeletal muscle at 24 h after the injection (22). Gene expression in skeletal muscle in response to leptin and MT-II may require >24 h.

The present results showed that injection of leptin or MT-II in some medial hypothalamic nuclei increased glucose uptake in certain peripheral tissues, while it did not alter plasma glucose level. These results suggest that leptin and MT-II stimulates hepatic glucose production as well as glucose utilization in peripheral tissues. The result was supported by the previous report (13) showing that leptin increased plasma glucose turnover in mice at 6 h after the injection. Moreover, Gutierrez-Juarez et al. (45) has shown that intracerebroventricular infusion of MCR agonist increased hepatic glucose production in lean rats at 6 h after the start of the infusion, while it inhibited hepatic glucose production at 7 days (46). These results suggest that the effects of leptin and melanocortin receptor agonist on hepatic glucose production alter time dependently.

Recent study suggests that MT-II seems to be a promising agent to bypass leptin resistance and to improve energy homeostasis in diet-induced obese mice (47). We have shown that intracerebroventricular injection of MT-II but not leptin activates AMP-activated protein kinase in skeletal muscle in diet-induced obese mice (48). The PVH and VMH may be target sites of MT-II to improve glucose metabolism in these mice.

Together, our results suggest that the VMH plays a key role in leptin- and MT-II–induced glucose uptake in skeletal muscle, heart, and BAT, whereas the leptin receptor in the ARC and MCRs in the PVH regulate glucose uptake in BAT. The medial hypothalamic nuclei thus appear to play distinct roles in the regulation of glucose uptake in peripheral tissues by leptin and MT-II.

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