Insulin-like Growth Factor 1-mediated Hyperthermia Involves Anterior Hypothalamic Insulin Receptors*

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The objective is to investigate the role of insulin-like growth factor 1 (IGF-1) in the regulation of core body temperature. Sequencing cDNA libraries from individual warm-sensitive neurons from the preoptic area (POA) of the hypothalamus, a region involved in the central control of thermoregulation, identified neurons that express both IGF-1 receptor (IGF-1R) and insulin receptor transcripts. The effects of administration of IGF-1 into the POA was measured by radiotelemetry monitoring of core temperature, brown adipose tissue (BAT) temperature, metabolic assessment, and imaging of BAT by positron emission tomography of 2-18F-fluoro-2-deoxyglucose uptake combined with computed tomography. IGF-1 injection into the POA caused dose-dependent hyperthermia that could be blocked by pretreatment with the IGF-1R tyrosine kinase inhibitor, PQ401. The IGF-1-evoked hyperthermia involved activation of brown adipose tissue and was accompanied by a switch from glycolysis to fatty acid oxidation as a source of energy as shown by lowered respiratory exchange ratio. Transgenic mice that lack neuronal insulin receptor expression in the brain (NIRKO mice) were unable to mount the full hyperthermic response to IGF-1, suggesting that the IGF-1 mediated hyperthermia is partly dependent on expression of functional neuronal insulin receptors. These data indicate a novel thermoregulatory role for both IGF-1R and neuronal insulin receptors in IGF-1 activation of BAT and hyperthermia. These central effects of IGF-1 signaling may play a role in regulation of metabolic rate, aging, and the risk of developing type 2 diabetes.

Insulin-like growth factor-1 (IGF-1) signaling plays a critical role in a diverse range of functions including normal growth (1), neuroprotection (2), progression of Alzheimer disease (3), tumorigenesis and metastasis (4, 5), and longevity (6–8). Decline in the levels of IGF-1 is associated with significant morbidity in adulthood with an increased risk of developing cardiovascular disease, osteoporosis, neurodegenerative diseases (9), and diabetes (10). Furthermore, administration of IGF-1 to patients with severe insulin resistance or type 2 diabetes results in improved postprandial glucose usage and reduction in HbA1c values (11). However, reduced exposure of tissue to IGF-1 is associated with an extended lifespan (12–14), and partial loss-of-function mutations in the IGF-1 receptor (IGF-1R) gene are overrepresented among centenarians compared with controls (15, 16).

The IGF-1R is widely expressed in many tissues (17–20), including the hypothalamus (17, 19–23) and notably within the preoptic area (POA) (17). Insulin receptors are also expressed in several regions of the hypothalamus, including the arcuate nucleus and the POA (14–26). Both insulin and IGF-1 play an important part in the central control of peripheral metabolism; intracerebroventricular infusion of IGF-1 increases the sensitivity of the liver to insulin (27), whereas intracerebroventricular infusion of insulin results in decreased food intake and body weight (28) and suppresses glucose production independent of circulating levels of insulin (29). Further studies have shown that hypothalamic insulin receptors play an important role in food intake and insulin resistance (30).

Neuronal loss of the insulin receptor in mice results in increased body fat and in plasma insulin and leptin levels (31), suggesting the importance of central insulin receptors in mediating the CNS effects of insulin on energy homeostasis. Mice with inactivation of the Insr gene exhibit normal embryonic development with normal birth weights, but these mutants survive postnatally only for a few days and then die of diabetic ketoacidosis (32, 33). Mice lacking the IGF-1R gene display a severe growth-deficiency phenotype at birth (approximately

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The abbreviations used are: IGF-1, insulin-like growth factor 1; aCSF, artificial cerebrospinal fluid; AUC, area under the curve; BAT, brown adipose tissue; CBT, core body temperature; DMH, dorsomedial hypothalamus; 18F-FDG, 18F-fluoro-2-deoxyglucose; IGF-1R, IGF-1 receptor; IR, insulin receptor; NIRKO, neuronal IR knock-out; POA, preoptic area; RPa, raphe pallidus; UCP, uncoupling protein.
45% of the normal weight) and die of respiratory failure shortly after birth (34). Deletion of the IGF-1R in skeletal muscle in mice results in glucose intolerance and impaired insulin sensitivity (35). The neuronal IGF-1R knock-out shows no metabolic or aging phenotype but a lowered growth hormone level, and the heterozygote exhibits a substantially longer life span (1).

The insulin-like growth factor-1 receptor (IGF-1R) and the insulin receptor (IR) are both transmembrane tyrosine kinase receptors that signal through “dimeric” receptors composed of combinations of two insulin half-receptors (IR/IR), two IGF-1 half-receptors (IGF-1R/IGF-1R), or as hybrids composed of IR and IGF-1 half-receptors. In cells that express both the IR and IGF-1R, hybrid receptors form by random association (36, 37). Insulin and IGF-1 can bind to either the dimeric IR or IGF-1R or hybrid receptors, respectively, with varying affinities. For example, IGF-1 has 100 times lower affinity than insulin for the homodimeric insulin receptor, yet it can activate the autophosphorylation of the IR and thus acts as an IR agonist (38). The binding of IGF-1 or insulin to any of the three possible dimeric receptors (IR/IR, IGF-1R/IGF-1R, or IR/IGF-1R) induces activation of tyrosine autophosphorylation of the receptor that initiates the downstream signaling cascade (39, 40).

In this study, we have identified the IR and IGF-1R transcripts in warm-sensitive neurons by sequencing cDNA libraries from individual neurons from the POA. The POA is a nucleus within the hypothalamus that is essential for the regulation of core body temperature (41). The POA sends inhibitory signals through separate outflow pathways to other hypothalamic nuclei, the dorsomedial hypothalamic nucleus (DMH) and raphe pallidus (RPa), to mediate differential inhibitory control of the sympathoexcitatory drive determining brown adipose tissue (BAT) thermogenesis (42). We have recently shown that insulin causes hyperthermia by direct inhibition of these neurons from the POA of the hypothalamus (43). The presence of both IR and IGF-1 receptors in the same warm-sensitive neurons raised the possibility that both insulin and IGF-1 can affect the activity of these GABAergic projection neurons through inhibitory action at the DMH and RPa to mediate a disinhibition of the sympathoexcitatory drive resulting in BAT activation and thus causing hyperthermia (44). To delineate the roles of the IR and of the IGF-1R in the anterior hypothalamic effects of IGF-1, we have examined the IGF-1-mediated hyperthermia both in mice that express IR and IGF-1R and in transgenic mice that lack expression of the neuronal insulin receptor (NIRKO), respectively.

**EXPERIMENTAL PROCEDURES**

*Animals and Procedures—*All procedures were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute and were carried out on 3–4-month-old male C57BL/6 mice. NIRKO mice were a kind gift from Ronald Kahn’s laboratory. Animals were maintained on regular chow (Harlan Teklad LM-485 Diet 7012 (65% carbohydrate kcal), 13% fat, metabolizable energy 3.41 kcal/g). Access to food and water was *ad libitum*, and the light/dark cycle was 12/12 h with lights on at 6:00 a.m.

Telemetry studies were performed as described previously (43). Briefly, male mice were anesthetized with isoflurane (kcal), 13% fat, metabolizable energy 3.41 kcal/g). Access to chow (Harlan Teklad LM-485 Diet 7012 (65% carbohydrate). Animals were maintained on regular

[FIGURE 1. POA microinjection of IGF-1 causes a dose-dependent hyperthermic response. A, profile of dose-dependent effects on CBT of POA injection of IGF-1 (*n* = 6 mice/group) for 6 h after injection (arrow shows time of injection). B, AUC analysis showing the significant increase in temperature with increasing doses of IGF-1 either 1 ng, 5 ng or 10 ng (*p* < 0.05) compared with vehicle control. C, hyperthermic effect of IGF-1 shown not to be due to differences in locomotor activity. D, IGF-1R transcript expressed in the POA, DMH, and RPa nuclei within the hypothalamus. Data are presented as mean ± S.E. (error bars). *p* < 0.05 significantly different from vehicle.]

(Induction 3–5%, maintenance 1–1.5%) and surgically implanted with radiotelemetry devices (TA-F20; Data Sciences) into the peritoneal cavity for core body temperature (CBT) and locomotor activity measurement. For direct injection into the nuclei within the hypothalamus, mice were implanted with cannulas. Coordinates for the POA cannula were anterior-posterior from bregma = 0.38 mm anterior to the bregma, lateral = midline, ventral = 3.8 mm, cannula 26-gauge, 10-mm length. Coordinates for the DMH were anterior-posterior = 1.58 mm posterior to the bregma, lateral = 0.25 mm; ventral = 4.6 mm from the surface of the brain. Coordinates for the RPa were anterior-posterior = 6.12 mm posterior to the bregma, lateral = 0.0 mm; ventral = 5.8 mm from the surface of the brain. Insulin (11507; Sigma), IGF-1 (R&D Systems) and interleukin 1β (IL-1β) (401-ML-005; R&D Systems) were predissolved in saline and subsequently diluted in artificial cerebrospinal fluid (aCSF). PQ401 (2768; Tocris, Ellisville, MO) was predissolved in 100% ethanol and subsequently diluted in aCSF (final concentration of 5% ethanol). Injections of insulin, IGF-1 (or vehicle, aCSF), or PQ401 (or vehicle, aCSF with 5% ethanol) were
administered via the implanted cannula using an injector (33-gauge, protruding 0.4 mm beyond the tip of the cannula, total length 10.4 mm) connected to plastic tubing and a microsyringe (10 μL) in a volume of 0.5 μL over a period of 5 min to allow diffusion (n = 5 mice/group). Arrow indicates time of injection. A, graph showing the differential effects on CBT of the injection of 10 ng of IGF-1 in the POA, the DMH, and the RPa (circles, POA; triangles, DMH; diamonds, RPa; white, IGF-1; black, control) for 6 h after injection (n = 5 mice/group). Arrow indicates time of injection. B, AUC analysis showing injection of IGF-1 in the POA (p < 0.05) or DMH (p < 0.05) resulting in a significant increase in CBT but no significant effect on temperature after injection to the RPa. Injection of IGF-1 to the POA resulted in a significantly higher elevation in temperature compared with the same dose (10 ng) applied to either the DMH or RPa. C, effects on CBT of DMH injection of IGF-1 (white triangles, 10 ng; gray triangles, 25 ng; black triangles, vehicle) (n = 6 mice/group) for 6 h after injection (arrow shows time of injection). D, AUC analysis showing no significant increase in temperature with increasing doses of IGF-1 applied to the DMH compared with vehicle control. E, effects on CBT of RPa injection of IGF-1 (white diamonds, 10 ng; gray diamonds, 25 ng; black diamonds, vehicle) (n = 5 mice/group) for 6 h after injection (arrow shows time of injection). F, AUC analysis showing no significant increase in temperature with increasing doses of IGF-1 applied to the RPa compared with vehicle control. Data are presented as mean ± S.E. (error bars). *, p < 0.05 significantly different from vehicle.

Figure 2. IGF-1 acts specifically at the POA to elicit a hyperthermic response.

A, graph showing the differential effects on CBT of the injection of 10 ng of IGF-1 in the POA, the DMH, and the RPa (circles, POA; triangles, DMH; diamonds, RPa; white, IGF-1; black, control) for 6 h after injection (n = 5 mice/group). Arrow indicates time of injection. B, AUC analysis showing injection of IGF-1 in the POA (p < 0.05) or DMH (p < 0.05) resulting in a significant increase in CBT but no significant effect on temperature after injection to the RPa. Injection of IGF-1 to the POA resulted in a significantly higher elevation in temperature compared with the same dose (10 ng) applied to either the DMH or RPa. C, effects on CBT of DMH injection of IGF-1 (white triangles, 10 ng; gray triangles, 25 ng; black triangles, vehicle) (n = 6 mice/group) for 6 h after injection (arrow shows time of injection). D, AUC analysis showing no significant increase in temperature with increasing doses of IGF-1 applied to the DMH compared with vehicle control. E, effects on CBT of RPa injection of IGF-1 (white diamonds, 10 ng; gray diamonds, 25 ng; black diamonds, vehicle) (n = 5 mice/group) for 6 h after injection (arrow shows time of injection). F, AUC analysis showing no significant increase in temperature with increasing doses of IGF-1 applied to the RPa compared with vehicle control. Data are presented as mean ± S.E. (error bars). *, p < 0.05 significantly different from vehicle.

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**BAT Temperature Measurement**—An incision was made between the scapulae and the BAT exposed by dissecting the overlying fascia. Blunt dissection was used to form a subcutaneous channel between the two openings. The thermistor tip of the biotelemetry cannula was guided through this channel and sutured to muscle to maintain its position between the left and right lobes of the BAT. The flank wound was closed with Michel clips and the interscapular incision with silk sutures. The position of the thermistor tip in relation to the BAT was confirmed postmortem. BAT temperature was measured using biotelem-
etry probes (DSI Systems, St. Paul, MN), BAT temperature was recorded in at 3-min intervals for 6 h after injection of IGF-1 or vehicle.

**Sequencing mRNA from Individual Warm-sensitive Cells**—Warm-sensitive neurons were identified by electrophysiology and isolated as described in detail (43). The cytoplasm was extracted from individual warm-sensitive neurons and amplified using three rounds of linear antisense RNA (aRNA) amplification (46), after which they were made into Illumina GA II competent libraries and sequenced. Alignment of Illumina sequencing reads to mouse genes was accomplished as follows. Specific read coverage was performed using Bowtie (47) version 0.9.8 using the default parameters on the Mouse July 2007 assembly.

**Gene Expression Analysis**—Total RNA was extracted from tissue with Purelink total RNA purification system (Invitrogen) following the manufacturer’s protocol. Total RNA was reverse-transcribed using an iScript cDNA synthesis kit (Bio-Rad). PCR was carried out using iTaq SYBR Green supermix (Bio-Rad) on an MJ Research Chromo4 Real Time PCR system (Bio-Rad). mRNA of uncoupling protein 1 (UCP-1) was measured with primers 5’-CGACTCAGTCCAAGATACTTCTTC-3’ and 5’-GCCGGCTGAGATCTTGTTTC-3’ and normalized to β-actin, 5’-GCTTCTTTGCAGCTCCCTCGT-3’ and 5’-ATATCGTCATCCATGCGAC-3’. Amplification of the IGF-1R was performed using 2× Hot start Supermix Taq (Bio-pioneer). 30 cycles of amplification were performed using the following conditions: 30 s at 95 °C, 30 s at 60 °C, 1 min at 72 °C with primers IGF-1R-F: aggtctgggaaccatttgtg and IGF-1R-R: tccatcgagccttttgactt.

**Statistics**—Individual pairwise comparisons were performed using Student’s t test. Multiple comparisons were made using a one-way ANOVA followed by a Tukey post hoc test. Area under the curve (AUC) analysis was performed using GraphPad Prism 5 software. All results are expressed as means ± S.E. A p value < 0.05 was considered significant.

**RESULTS**

Illumina sequencing of cDNA libraries individual, electrophysiologically identified, warm-sensitive neurons from the POA identified a subpopulation of neurons that express both IGF-1R and IR transcripts. Read counts are the number of times that any of the 42-base sequences map back to the mRNA of interest. When normalized for the mRNA length of the IR and
the IGF-1R, these read counts are a reflection of the abundances of the corresponding mRNAs. Read counts show that the IR mRNA is present at 5-fold higher level than that encoding the IGF-1R in the cell that expresses both transcripts. Read count analysis also shows that there is variability in gene expression between cells with one warm-sensitive neuron expressing both mRNAs (read counts, IGF-1R 4, IR 30), and another cell studied expressed neither mRNA. Both the IR and IGF-1R mRNAs were likewise present in the pooled mRNA samples (four distinct heat-sensitive neuronal cell somas, read counts for IGF-1R 4, IR 15). The presence of the IR and IGF-1R transcripts in the same neurons facilitates the possibility that the gene products of the IR and IGF-1R genes can form hybrid receptors in the same neuron.

To determine whether the IGF-1R expression leads to functional signaling receptors, we administered IGF-1 into the POA in mice implanted with a radiotelemetric device in the peritoneum and measured CBT. The initial increase in temperature (60-min duration) observed in all groups, including the vehicle-treated mice, is due to the stress-induced hyperthermia after the injection. IGF-1 injection into the POA caused a significant dose-dependent hyperthermic response (Fig. 1, A and B) that was not due to changes in locomotor activity (Fig. 1C) between vehicle and IGF-1 injected mice, respectively. The POA sends inhibitory signals through other hypothalamic nuclei, the DMH and the RPa to the BAT where thermogenesis occurs. The IGF-1R is expressed in the POA and in the projection areas of the warm-sensitive GABAergic neurons: the DHM and RPa nuclei of the hypothalamus (Fig. 1D).

The administration of IGF-1 to the POA had the largest hyperthermic effect compared with microinjection of IGF-1 to either the DMH, where there was a late onset hyperthermic response, or the RPa where the effect on core body temperature was less pronounced compared with the other two regions studied (Fig. 2, A and B). For example, 2.5 h after the administration of IGF-1 the average core body temperature was 2.02 °C greater than when vehicle was applied to the POA, whereas the change in core temperature after microinjection of IGF-1 to the DMH or the RPa relative to vehicle was 0.82 °C and 0.65 °C, respectively. A trend of increased hyperthermic effect with higher doses of IGF-1 administered to the DMH was observed (Fig. 2, C and D) for approximately 3 h after injection, but no dose-dependent effect was observed when applied to the RPa (Fig. 2, E and F).

The signaling mechanism of the IGF-1 on POA neurons was examined by pretreatment with the IGF-1R tyrosine kinase inhibitor PQ401 that inhibits IGF-1R autophosphorylation (48). Pretreatment with PQ401 before IGF-1 (10 ng in 0.5 μl), both administered to the POA 30 min apart, showed significant attenuation of the IGF-1-induced increase in core body temperature (p < 0.05) (Fig. 3, A and C). A similar attenuated hyperthermic response to IGF-1 by PQ401 pretreatment is observed when the temperature of the BAT is measured (Fig. 3, B and D). Therefore, the in vivo effects of PQ401 are mediated at the tyrosine kinase of the IGF-1R but may also be accounted for in part by a slight inhibition of autophosphorylation of the IR because PQ401 is not entirely selective for the IGF-1R (48). As expected, PQ401 did not block the hyperthermic effects of
the well known pyrogen IL-1β (data not shown), and thus its ability to block the IGF-1 hyperthermia is not a general antipyretic effect.

We have examined whether the IGF-1-induced hyperthermia also involves the neuronal IRs, some of which are co-expressed with IGF-1R in the same neurons, making it likely that hybrid IGF-1R/IRs are expressed by these neurons. We tested the effects of IGF-1 injection into the POA in NIRKO mice that lack neuronal IR expression and thus cannot present hybrid receptors. NIRKO mice show a significantly impaired hyperthermic response to IGF-1 compared with the response seen in wild-type mice (Fig. 4, A and B). These results clearly indicate that the functional expression of the IRs by CNS neurons is required for the full IGF-1-mediated hyperthermic response. As expected, NIRKO mice do not show a hyperthermic response to the POA microinjection of insulin as observed in wild-type mice (Fig. 4, C and D) (43).

Indirect calorimetry measurements show that POA injection of IGF-1 was followed by an increase in oxygen consumption (Fig. 5A) and an increase in carbon dioxide production (Fig. 5B). The respiratory exchange ratio is lowered in IGF-1-treated mice compared with vehicle, indicating a switch from glucose metabolism to elevated fatty acid utilization (Fig. 5C).

BAT is an important thermogenic organ that is involved in control of body temperature (49). Interscapular temperature was measured with an indwelling thermister after infusion of IGF-1 or vehicle into the DMH as well as after IGF-1, vehicle, or insulin was injected to the POA. Interscapular BAT local temperature was significantly elevated after insulin or IGF-1 injection to the POA compared with injection of IGF-1 to the DMH or vehicle control injections (Fig. 6A). In addition, a significant increase in UCP-1 transcript expression was observed in response to IGF-1 injection (Fig. 6B). UCP-1 found in the mitochondria of BAT mediates the generation of heat by nonshivering thermogenesis. Furthermore, IGF-1 injected into the POA in rats induced an elevation of 18F-FDG uptake in BAT which provides an indirect measure of the activation of the BAT adipocytes and resultant thermogenesis (Fig. 6, C and D).

DISCUSSION

Single-cell amplification of cDNA by the linear amplification methodology (46) followed by sequencing of the cDNA library provides a powerful unbiased technique to determine the receptor repertoire of individual cells. In this study we identified the simultaneous expression of the IGF-1R and IR in cDNA libraries from discrete single warm-sensitive neurons that form part of the thermoregulatory center in the POA of the hypothalamus. In some individual neurons or small pools of the warm-sensitive neurons IGF-1R and IR mRNAs were both present within the same cell. These data using cDNA sequences of individual neurons to demonstrate that the same neuron expresses the two mRNAs of interest are a novel more sensitive way to prove co-existence of mRNAs than by double in situ hybridization. The number of sequence hits bears a relationship to the abundance of the mRNA, and in these warm-sensitive cells the IR mRNA is present at an ~4–7 times higher level than the mRNA encoding the IGF-1R; nevertheless, the simultaneous presence of both mRNAs suggests the possibility of random IR/IGF-1R formation. Although data are not yet available for the mouse, hybrid receptors have previously been observed in brain extracts where Bailyes et al. (36) showed by radioligand binding assays that 55% of the total IGF-1R protein in the rabbit brain was found in hybrid receptor formation.

The establishment of the expression of cDNA encoding IGF-1R and IR was followed by in vivo experiments that examined the functional expression of IGF-1R, using POA injections of IGF-1 and radiotelemetry to measure changes in the core body temperature. We have found that IGF-1 injection into the POA causes dose-dependent hyperthermia and an increase in thermogenic activity in BAT. Furthermore, it is likely that at the high POA concentrations of IGF-1 used in the microinjection experiments may also activate hybrid receptors as well as IR homodimers. To examine whether the IGF-1-induced hyperthermia involves the neuronal IR, we have tested the effects of IGF-1 in wild-type and NIRKO mice, respectively. The results indicate that the functional expression of the IR is required for

![FIGURE 5. POA injection of IGF-1 increases fatty acid utilization and BAT activity.](image-url)
the full IGF-1 mediated hyperthermic response. The full hyperthermic response observed after IGF-1 administration to the POA of wild-type mice but not of NIRKO mice, could thus be due to either the effect of IGF-1 acting at the IGF-1R homodimers (as seen in NIRKO mice) or through action at the hybrid receptor (IR/IGF-1R) or at the IR homodimer, with the wild-type mice expressing all three receptor dimers and the NIRKO mice only the IGF-1R, respectively. The inhibition of the IGF-1-evoked hyperthermia with the IGF-1R tyrosine kinase inhibitor PQ401 suggests that the hyperthermic effect of IGF-1 is mediated largely through IGF-1R and hybrid receptors of IR and IGF-1-R, which are known to be expressed abundantly in the brain (36).

In the present study and in previous recent studies, we have shown that the IR protein is functional in POA neurons as insulin injection to the POA mediates a specific hyperthermic effect (43). With the POA IGF-1R-mediated hyperthermia described here we now extend the diverse central roles of IGF-1 to include temperature regulation. Interestingly, transgenic growth hormone mice with high levels of IGF-1 have a 0.5 °C higher temperature than their wild-type mice and are resistant to the development of diet induced obesity (53). Furthermore, growth hormone KO mice display a reduced core body temperature (0.4 °C less than wild type littermates) (54). The CBT differences observed in these mice could be explained by the hyperthermic effects of IGF-1 outlined in this paper.

These central effects of IGF-1 on CBT are important not only in the context of metabolic regulation as with insulin, but also in the context of IGF-1 effects on aging, as longevity is observed at lower IGF-1 signaling levels whereas caloric restriction attenuates the age-related decline in brain IGF-1 with the effect of extending lifespan (50). Furthermore, caloric restriction, the best documented method of lifespan expansion, also results in decreasing CBT (51). Thus, it is possible that IGF-1 is a key

FIGURE 6. POA injection of IGF-1 increases BAT activity. A, changes in interscapular BAT temperature, measured with an indwelling thermister, after injection of vehicle or IGF-1 (10 ng) to the POA or DMH (squares, POA; triangles, DMH; white, IGF-1; black, vehicle; gray, insulin). Data represent the mean ± S.E. (error bars) of the temperature at each time point for each group. B, real time PCR analysis of UCP-1 transcript expression 90 min after IGF-1 injection to the POA. UCP-1 expression was normalized to β-actin and expressed as a percentage of vehicle control. C, 2-h profile of PET/CT of 18F-FDG uptake in rats injected with vehicle or 10 ng of IGF-1 in the POA. Top panel, representative PET/computed tomography. Box squared in dotted lines is the area investigated following treatment with IGF-1 or vehicle shown in lower panel (n = 2/group). The anatomical position of BAT is indicated with an arrow. D, graph showing 2-h profile quantification of 18F-FDG uptake into BAT following IGF-1 treatment as indicated. Data are presented as mean ± S.E (error bars). *, p < 0.05 significantly different from vehicle.
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mediator of this CBT response. The link between lowered CBT and increased lifespan has previously been described in transgenic mice that overexpress UCP-2 in hypocretin neurons (Hcrt-UCP2) (52).

The ability of IGF-1 to activate BAT via IGF-1R in the POA is of particular importance as we increasingly recognize the functional role of BAT in adult human metabolism (45). The thermogenic capacity of BAT makes it an attractive therapeutic target for antiobesity and antidiabetic therapies through increasing energy expenditure. The observation that lowered IGF-1 signaling is associated with heightened risk of diabetes (10) may be closely linked to the role of IGF-1, possibly in the POA neurons, in regulation of BAT activity.

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