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Glucoprivation in the ventrolateral medulla decreases brown adipose tissue sympathetic nerve activity by decreasing the activity of neurons in raphé pallidus

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Madden CJ. Glucoprivation in the ventrolateral medulla decreases brown adipose tissue sympathetic nerve activity by decreasing the activity of neurons in raphé pallidus. Am J Physiol Regul Integr Comp Physiol 302: R224–R232, 2012. First published November 9, 2011; doi:10.1152/ajpregu.00449.2011.—In urethane/α-chloralose anesthetized rats, cold exposure increased brown adipose tissue sympathetic nerve activity (BAT SNA: +699 ± 104% control). Intravenous administration of 2-deoxy-D-glucose (2-DG; 200 mg·ml⁻¹·kg⁻¹) reversed the cold-evoked activation of BAT SNA (nadir: 139 ± 36% of control) and decreased BAT temperature (−1.1 ± 0.2°C), expired CO₂ (−0.4 ± 0.1%), and core temperature (−0.5 ± 0.0). Similarly, unilateral nanoinjection of the glucoprivic agent 5-thioglucose (5-TG; 12 μg/100 nl) in the ventrolateral medulla (VLM) completely reversed the cold-evoked increase in BAT SNA (nadir: 104 ± 7% of control), and decreased TBAT (−1.4 ± 0.3°C), expired CO₂ (−0.2 ± 0.0%), and heart rate (−35 ± 10 beats/min). The percentage of rostral raphé pallidus (RPa)-projecting neurons in the dorsal hypothalamic area/dorsomedial hypothalamus that expressed Fos in response to cold exposure (ambient temperature: 4–10°C) did not differ between saline (28 ± 6%) and 2-DG (30 ± 5%) pretreated rats, whereas the percentage of spinally projecting neurons in the RPa/raphé magnus that expressed Fos in response to cold exposure was lower in 2-DG-pretreated rats (22 ± 6% vs. 42 ± 5%, respectively). The increases in BAT SNA evoked by nanoinjection of bicuculline in the RPa or by transection of the neuraxis at the pontomedullary border were resistant to inhibition by glucoprivation. These results suggest that neurons within the VLM play a role in the glucoprivic inhibition of BAT SNA and metabolism, that this inhibition requires neural structures rostral to the pontomedullary border, and that this inhibition is mediated by a GABAergic input to the RPa.

RVLM: hypoglycemia; 2-DG: metabolism; thermoregulation; CVLM

GLUCOSE IS A CRITICAL METABOLIC substrate for the central nervous system. Decreases in the availability of glucose as a source of metabolic fuel for the central nervous system, such as those occurring during insulin-induced hypoglycemia or administration of glucoprivic agents [e.g., 2-deoxy-D-glucose (2-DG) or 5-thioglucose (5-TG)] elicit integrated counterregulatory responses, which include stimulation of feeding behavior, adrenal medullary secretion of epinephrine, glucagon secretion, corticosterone secretion, and inhibition of insulin secretion. These responses counteract the glucose deprivation by replenishing energy stores, stimulating glycogenolysis, stimulating gluconeogenesis, and conserving existing glucose supplies for use by the central nervous system. Hypoglycemia and glucoprivation also cause hypothermia (13, 23), at least in part by inhibiting sympathetically mediated metabolism in brown adipose tissue (BAT) (11). This neurally regulated decrease in metabolism decreases cellular oxidative demands, reducing the transport of glucose into BAT, which can be a major site of glucose disposal (9), thereby representing another critical counterregulatory response to glucose deprivation. Indeed, prevention of hypothermia during severe hypoglycemia increases mortality (4).

The recent demonstration of BAT in adult humans (8, 10, 29, 45, 47) and the inverse correlation between activity of this tissue and obesity (10, 45, 46) has rejuvenated interest in the physiological regulation of this tissue. Metabolic stimuli clearly play a role in regulating the activity of BAT: feeding induces BAT thermogenesis (3, 36), whereas fasting or food restriction inhibits thermogenesis (35, 43). Furthermore, the nutritional content of the diet can affect the activity of BAT (2, 17, 37). These dietary influences on metabolism in BAT may contribute to the etiology or maintenance of obesity. Despite the obvious importance of understanding how metabolic stimuli influence BAT, the neural circuitry responsible for this regulation is incompletely understood.

We have previously demonstrated that activation of neurons in the ventrolateral medulla (VLM) inhibits BAT sympathetic nerve activity (SNA) and thermogenesis (5). Since this area of the VLM contains neurons that are activated by glucoprivation (34) and that play important roles in counterregulatory responses to glucoprivation (22, 31, 33), the goal of the present study was to determine whether glucoprivation selectively in this area is capable of inhibiting BAT SNA and thermogenesis and to further define the neural pathways involved in this glucoprivic inhibition of thermogenesis.

MATERIALS AND METHODS

All procedures conform to the regulations detailed in the Guide for the Care and Use of Laboratory Animals: 8th Edition (National Research Council, National Academies Press, 2010) and were approved by the Animal Care and Use Committee of the Oregon Health and Science University.

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Acute physiology experiments in anesthetized rats. Male Sprague-Dawley rats (350–450 g, Charles River, Indianapolis, IN) were anesthetized with isoflurane (2–3% in 100% O2) during which time the femoral artery and vein were cannulated. Following cannulation, rats were transitioned from isoflurane to a combination of urethane (750 mg/kg iv) and α-chloralose (60 mg/kg iv) anesthesia. The trachea was cannulated, and the animals were artificially ventilated with 100% O2 (stroke volume: 1 ml/100 g body wt, 50–70 strokes/min) and paralyzed with d-tubocurarine (0.6 mg/rat iv, supplemented thereafter with 0.3 mg when spontaneous respiratory activity was observed). Animals were placed in a stereotaxic frame with the incisor bar positioned 4 mm below the interaural line. A thermocouple inserted in the rectum was used to measure the core temperature ($T_{\text{core}}$), which was maintained at 36.5 ± 0.5°C with a heat lamp and a water-perfused thermal blanket, unless otherwise noted. BAT temperature ($T_{\text{BAT}}$) was recorded using a thermocouple placed in the interscapular BAT. Skin temperature ($T_{\text{skin}}$) was recorded using a thermocouple placed on the hindquarter skin. A sympathetic nerve innervating the right BAT pad was recorded using bipolar hook electrodes as described previously (21). Nerve activity was differentially amplified (10,000 to 20,000 times, CyberAmp 380; Axon Instruments, Union City, CA), filtered (1–300 Hz), digitized, and recorded onto a hard drive using Spike 2 software (Cambridge Electronic Design, UK).

2-DG (200 mg/kg iv) was administered to evaluate the ability of glucoprivation to inhibit BAT SNA and thermogenesis evoked by: 1) cooling of the rat with a water-perfused thermal blanket ($n = 4$); 2) injection of bicuculline (60 nl, 0.5 mM) into the rostral raphe pallidus (RPa; 3 mm caudal to lambda, on midline, 9.8 mm ventral to dura; $n = 4$); or 3) transection of the neuraxis at the pontomedullary border (just caudal to the transverse sinus, ~0.5–1 mm caudal to lambda; $n = 4$). To determine whether local glucoprivation in the VLM inhibits BAT SNA and thermogenesis, the glucoprivic agent, 5-TG (12 μg/100 nl) was nanoinjected into the VLM (3.5–4.5 mm caudal to lambda, 1.8 mm lateral to lambda, 9.5 mm ventral to the dura covering the cerebellum) following cooling of the rat with a water-perfused thermal blanket ($n = 6$) or injection of bicuculline (60 nl, 0.5 mM) into the RPa ($n = 4$). The injection sites in the VLM targeted the approximate center of the region of the VLM that we previously demonstrated to inhibit BAT SNA (5). This region spans the areas defined as the rostral VLM (RVL) pressor area and the caudal VLM std depressor area, with the approximate center of the injection sites located between the most active pressor and depressor areas in a region where many C1 neurons with projections to the forebrain reside (40). Injection sites were verified by localization of fluorescent polystyrene microspheres (Fluo-Spheres, models F8797, F8801, or F8803; Molecular Probes, Eugene OR) included in the injectate (dilution of 1:200).

Functional neuroanatomy. Rats were anesthetized with isoflurane (2–3% in 100% O2) and placed in a stereotaxic frame fitted with a nose cone to maintain isoflurane anesthesia. The fur on the top of the head, neck, and over the interscapular region was shaved and the skin was scrubbed with betadine and then 70% ethanol; an intramuscular injection of penicillin G (40,000 U/kg) was performed. An incision in the skin, and a burr hole was drilled in the skull to permit injection of bicuculline (60 nl, 0.5 mM) into the rostral raphé pallidus (RPa; 3 mm caudal to lambda, on midline, 9.8 mm ventral to dura; $n = 4$) cooling of the rat with a water-perfused thermal blanket ($n = 1$). Upon completion of the experiment, the brain was removed, fixed by perfusion in 4% paraformaldehyde. Brains and spinal cords were removed and postfixed for 2–12 h in 4% paraformaldehyde and then transferred to a 30% sucrose solution at 4°C for at least 1 day at which time they were cut on a microtome (30 μm, 1:6 series) and preserved in cryoprotectant at −20°C until processed for immunohistochemistry.

Immunohistochemistry. Tissue sections were removed from cryo-protectant and washed (twice for 10 min each wash) in phosphate-buffered saline (PBS). Sections were incubated in the antibody dilution solution (PBS containing 0.3% Triton X-100, 2.5 g/l lambda carrageenan, 200 mg/l NaN3, 10 ml/l normal donkey serum) for 2 h at which time they were incubated in the primary antibody solution: goat anti-Ctb (1:20,000 in antibody dilution solution, lot no. 7032A6; List Biological Laboratories) and rabbit anti-c-Fos (dilution 1:5,000, PC38; Calbiochem) for 16 h on a shaker table at room temperature. Two rinses in PBS containing 0.03% Triton X-100 were performed, and then the tissue was incubated in the secondary antibody solution (donkey anti-rabbit Alexa Fluor 488, used at 1: 200, cat. no. A21206; Molecular Probes and donkey anti-goat Alexa Fluor 594, used at 1: 200, cat. no. A11058; Molecular Probes) for 1 h. The tissue was rinsed in PBS (2 × 10 min) and then mounted on slides and coverslipped using Prolong Gold anti-fade reagent (Invitrogen, Eugene, OR).

Data and statistical analyses. BAT SNA, $T_{\text{BAT}}$, $T_{\text{core}}$, $T_{\text{skin}}$, expired CO2, arterial pressure, and stimulus trigger pulses were digitized (Micro3 1401; Cambridge Electronic Design, Cambridge, UK) and recorded onto a computer hard drive for analysis (Spike 2; Cambridge Electronic Design). Spike 2 software (Cambridge Electronic Design) was used to obtain a continuous measure (with 4-s bins) of BAT SNA amplitude. This was accomplished by calculating the root mean square value of the BAT SNA (square root of the total power in the 0.1 to 20 Hz band) from the autospectra of sequential 4-s segments of BAT SNA. The control level of BAT SNA was taken as the mean BAT SNA amplitude during a 2-min period of minimum BAT SNA recorded when the rat was in a warm condition ($T_{\text{core}} > 37°C$) and basal BAT SNA was absent.

All statistics were performed using Systat software (version 10; Cranes Software International, Chicago, IL). Data are expressed as means ± SE. For each variable, statistical comparisons were done between the 30-s period prior to treatment and the 30-s window at the peak or nadir of the treatment-evoked effect. Statistical significance was assessed using a paired t-test, a two-sample t-test, or an ANOVA with repeated measures and post hoc testing as appropriate. Results with $P < 0.05$ were considered significant.

RESULTS

Systemic glucoprivation reverses cold-evoked BAT thermogenic responses. To test the hypothesis that glucoprivation would inhibit the increase in BAT SNA and BAT thermogenesis evoked by cold exposure, the glucoprivic agent, 2-DG was administered intravenously during cold exposure. As illustrated in Fig. 1A, during cold exposure, BAT SNA is characterized by large bursts of activity reflecting the summed action potentials of postganglionic axons in the recorded nerve bundle. The cold-evoked increase in BAT SNA was completely reversed within 5 min of administration of 2-DG (200 mg/kg...
iv) (Fig. 1, A and B). 2-DG administration also significantly decreased $T_{BAT}$, expired $CO_2$, and $T_{core}$ (Fig. 1B).

Glucoprivation selectively in the VLM reverses cold-evoked BAT thermogenic responses. To test the hypothesis that glucoprivation selectively in the VLM would inhibit the increase in BAT SNA and BAT thermogenesis evoked by cold exposure, the glucoprivic agent, 5-TG was nano-injected directly into the VLM during cold exposure. Unilateral nano-injection of 5-TG (12 $\mu$g in 100 nl) into the VLM promptly and completely inhibited the cold-evoked increase in BAT SNA and lowered $T_{BAT}$, expired $CO_2$, and heart rate (HR; Figs. 2, A and B). All nano-injection sites were located lateral to the pyramidal tract, ventromedial to the nucleus ambiguus, and from ~11.9 mm to 13.3 mm caudal to bregma (Fig. 2C).

Functional neuroanatomy. To provide insight into the neurocircuitry involved in the 2-DG-induced inhibition of thermogenesis, rats received an injection of a retrograde tracer into the spinal cord or into the RPa/raphé magnus (RPa/RMg) and 1 wk later were treated with saline or 2-DG and then placed in an environmental chamber maintained at 4–10°C for 5 min.
2 h at which time they were killed. Prior to receiving an intraperitoneal injection of either saline or 2-DG, T_{BAT} did not differ between groups (37.0 ± 0.4°C and 37.2 ± 0.5°C, respectively). Thirty minutes of cold exposure resulted in an increase in T_{BAT} in animals that were pretreated with saline (+1.0 ± 0.5°C, n = 6), which was significantly different (P < 0.001) from the decrease in T_{BAT} that was observed in animals pretreated with 2-DG (−0.8 ± 0.5°C, n = 6). By the conclusion of the 2-h cold exposure, T_{BAT} of saline and 2-DG pretreated rats were not significantly different (37.3 ± 0.2°C and 37.3 ± 0.5°C, respectively, P > 0.05).

The area of the spinal cord containing retrograde tracer did not differ between saline and 2-DG-treated rats (Fig. 3D). A representation of the area in which quantifications were performed is shown in Fig. 3A. Counts of retrogradely labeled neurons in the RPa/RMg were performed in the three sections (30 μm, 1:6 series; consecutive sections separated by 150 μm) of the hindbrain that most closely correspond to the area of the RPa from which increases in BAT SNA can be elicited (18, 24). Nanoinjection of retrograde tracers in the spinal cord resulted in labeled neurons in the RPa and RMg. A representative photomicrograph illustrating neurons in the RPa that were labeled with CTb-only, Fos-only, or double-labeled for CTb and Fos is presented in Fig. 3B. The number of retrogradely labeled neurons in the RPa/RMg did not differ between saline and 2-DG treated rats (60 ± 17 and 68 ± 15, respectively; P = 0.741). However, the percentage of retrogradely labeled (spinally projecting) neurons of the RPa/RMg that expressed Fos in response to cold exposure was significantly decreased by pretreatment with 2-DG compared with saline (Fig. 3C).

The diffusion sphere of CTb following injection into the RPa/RMg was similar between rats subsequently receiving saline or 2-DG (Fig. 4A). A cluster of retrogradely labeled neurons was found in the dorsal area of the hypothalamus/dorsomedial hypothalamus (DA/DMH) following nanoinjection of CTb in the RPa/RMg (Fig. 4C). Counts of retrogradely labeled neurons in the DA/DMH were made from the two sections (30 μm, 1:6 series; consecutive sections separated by ~150 μm) of the hypothalamus that most closely correspond to the area shown previously to be required for febrile or cold-evoked activation of BAT SNA and thermogenesis (19, 27) and to provide direct input to the rostral RPa (28, 39, 48). Anatomically this area can be defined as a box extending dorsoventrally from the dorsal edge of the mamillothalamic tract to the ventral edge of the fornix, bordered laterally by the medial edge of the mamillothalamic tracts, and at the rostrocaudal level where the ventral edge of the mamillothalamic tracts is closely aligned with the dorsal aspect of the third ventricle and the compact formation of the DMH is clearly defined, corresponding approximately to bregma −3.2, according to Paxinos and Watson (30) (Fig. 4B). The number of retrogradely labeled neurons in the DA/DMH did not differ

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Fig. 3. A: line drawing indicating the area in which quantification of retrogradely labeled and Fos-positive neurons were evaluated (dashed box). B: representative photomicrograph from a rat pretreated with saline of cold-evoked Fos expression (green) and retrogradely labeled RPa neurons following an injection of cholera toxin subunit b (CTb; red) into the spinal cord. Arrowheads indicate double-labeled (Fos-positive, CTb-positive) cells. Note the entire area of quantification is not represented by this high-power photomicrograph. Scale bar is 50 μm. C: bar graph depicting the % spinally projecting raphé neurons that contain Fos in response to cold exposure in saline (n = 4) or 2-DG (n = 4) pretreated rats. Values are means ± SE. *P < 0.05 compared with saline control group. D: photomicrograph showing a CTb injection site in the forth thoracic segment (T4) of the spinal cord (right hemisection) and an illustration of the maximal spread of retrograde tracer injections in all 8 cases (left hemisection); red lines indicate injection sites of rats that received subsequent 2-DG treatment; green lines indicate injection sites of saline treated rats.
BAT thermogenic responses evoked by blockade of GABA<sub>A</sub> receptors in RPa are resistant to inhibition by glucoprivation. To test the hypothesis that glucoprivation would inhibit the increase in BAT SNA and BAT thermogenesis evoked by disinhibition of neurons in the RPa, the glucoprivic agent 2-DG was administered intravenously or 5-TG was nanoinjected in the VLM ~4 min after nanoinjection of bicuculline in the RPa. Compared with saline control injections, systemic administration of 2-DG did not significantly affect the increase in BAT SNA or T<sub>BAT</sub> evoked by nanoinjection of bicuculline in the RPa (Fig. 5, A and B). Similarly, the bicuculline-evoked increases in BAT SNA and T<sub>BAT</sub> were not affected by nanoinjection of 5-TG in the VLM (Fig. 5, C and D) at sites indistinguishable from those that effectively inhibited cold-evoked activity (Fig. 2C).

BAT thermogenic responses evoked by pontomedullary transection are resistant to inhibition by glucoprivation. To determine whether neural structures rostral to the medulla are necessary for glucoprivic inhibition of BAT thermogenesis, the effect of 2-DG administration on the increase in BAT SNA and thermogenesis evoked by transection of the neuraxis at the pontomedullary border was assessed. As we have previously demonstrated (5), transection of the neuraxis at the pontomedullary border increased BAT SNA, T<sub>BAT</sub>, expired CO<sub>2</sub>, HR, and MAP (Fig. 6). Following transection, all recorded variables remained elevated for the duration of the experiment (at least 30 min, up to >2 h). Intravenous administration of 2-DG did not significantly reduce any of the recorded variables (Fig. 6B).

**DISCUSSION**

The major novel findings of the present studies are that 1) glucoprivation selectively within the VLM completely reverses cold-evoked BAT SNA and thermogenesis; 2) glucoprivation-evoked inhibition of BAT SNA requires neural circuits rostral to the pontomedullary border; and 3) glucoprivation-evoked inhibition of BAT SNA involves inhibition of neurons in the RPa, possibly via activation of GABA<sub>A</sub> receptors on sympathetic premotor neurons for BAT.

We previously reported that activation of neurons in the VLM is capable of inhibiting BAT SNA and thermogenesis; however, the physiological context in which this neural circuit is active was not experimentally addressed (5). The present study provides data to suggest that conditions of reduced glucose availability increase the activity of this neural circuit. Specifically, glucoprivation restricted to the VLM completely inhibited cold-evoked BAT SNA and thermogenesis.

In earlier studies, glucoprivation in the lateral hypothalamus produced ~25% reduction in BAT SNA (12) and induced a modest hypothermia (41), although systemic administration of 2-DG completely inhibited BAT SNA (11), as in the present study (Fig. 1). The ventromedial hypothalamic nucleus has also been implicated in counterregulatory responses to hypoglycemia (16), and the VMH has been suggested to play a role in the regulation of BAT SNA (49), although a role of the VMH in glucoprivic inhibition of BAT SNA has not been demonstrated. Nonetheless these results are consistent with glucoprivic sensitivity in both hypothalamic and VLM neurons that can influence BAT SNA and thermogenesis; however, the mechanisms underlying their respective contributions to glucoprivation-induced inhibition of BAT...
thermogenesis during physiological conditions of reduced glucose availability await further study.

The fundamental neurocircuitry responsible for regulating BAT SNA is well-established (25). The sympathetic premotor neurons for BAT are located in the rostral ventromedial medulla, including the RPas (26). Neurons in the DA/DMH are necessary for febrile and cold-evoked BAT thermogenesis (19, 27, 50), and a cluster of neurons in this area has been reported

Fig. 5. Increase in BAT SNA and thermogenesis evoked by bicuculline (Bic) in RPAs is resistant to inhibition by glucoprivation. 
A: representative example of the effect of saline or 2-DG on Bic-evoked thermogenesis. B: group data of the 30-s averages of BAT SNA and TBAT from 2 min prior to nanoinjection of Bic in RPAs (dashed line at time 0) through 30 min postinjection. Saline (○, n = 4) or 2-DG (●, n = 4) was injected intravenously 4 min after Bic nanoinjection (solid line). Values are means ± SE. C: representative example of the effect of saline or 5-TG in the VLM on Bic-evoked thermogenesis. D: group data of the 30-s averages of BAT SNA and TBAT from 2 min prior to nanoinjection of Bic in RPAs (dashed line at time 0) through 20 min postinjection for rats receiving a nanoinjection of saline (○, n = 4) or 5-TG (●, n = 4) in the VLM (solid line). Values are means ± SE. There were no significant differences between the Bic-evoked responses in saline- and 2-DG- or 5-TG-treated groups.

Fig. 6. Glucoprivation does not inhibit BAT SNA and thermogenesis following transection (TransX) of the neuroaxis at the level of the rostral medulla. Transverse transection of the brainstem between the pons and the medulla (dashed lines) induces a large increase in BAT SNA and thermogenesis, as well as increases in HR and AP. Subsequent intravenous administration of 2-DG (200 mg·ml⁻¹·kg⁻¹, solid line) does not reverse the transection-evoked increases. *P < 0.05 compared with the value prior to pontomedullary transection.
to be the only significant population of RPa-projecting cells that express Fos in response to cold exposure (48). These data suggest that the DA/DMH is the predominant source of cold-evoked excitatory input to the BAT sympathetic premotor neurons in RPa. In the present study, the percentage of RPa-projecting neurons in the DA/DMH that expressed Fos in response to cold exposure did not differ between saline and 2-DG pretreated rats. These data demonstrate that 2-DG administration does not prevent cold-evoked activation of the DA/DMH neurons with projections to the RPa and suggest that the glucoprivation-evoked inhibition of BAT thermogenesis occurs downstream of these neurons. In contrast, the percentage of spinally projecting neurons in the RVLM, including the RPa and RMg, that express Fos in response to cold exposure was decreased in rats pretreated with 2-DG compared with rats pretreated with saline. These data together with the present observation of a lack of effect of 2-DG on the cold-evoked activation of DA/DMH neurons with projections to the RPa suggest that glucoprivation-induced inhibition of BAT SNA and thermogenesis impinges on the fundamental neurocircuitry regulating BAT at the level of the BAT sympathetic premotor neurons in the raphé (Fig. 7).

The precise neurocircuitry responsible for the glucoprivation-induced inhibition of BAT sympathetic premotor neurons in RPa is unclear; however, several observations from the present study provide insight into this pathway. First, glucoprivic inhibition of BAT SNA requires neurocircuitry rostral to the medulla (i.e., activation of BAT SNA evoked by transection at the pontomedullary border was not inhibited by administration of 2-DG). In addition, the increase in BAT SNA and thermogenesis evoked by blockade of GABA_A receptors in RPa is resistant to inhibition by glucoprivation, consistent with this inhibition being mediated by activation of a GABAergic input to BAT sympathetic premotor neurons in the RPa (Fig. 7). Considering these data, it is tempting to speculate that glucoprivation excites a GABAergic input to RPa that originates from a region rostral to the medulla. One such pathway could include neurons of the PVH. In this light, it is interesting to note that neurons in the PVH are activated by glucoprivation (32), and activation of neurons in the PVH inhibits BAT SNA, likely via GABAergic inhibition of neurons in the RPa (20). Since the PVH does not contain GABAergic neurons (42) and glucoprivation fails to increase the number of Fos-containing cells in the PVH with projections to the RPa (present study), it is highly unlikely that neurons of the PVH provide a direct GABAergic input to RPa; but instead the neurons of the PVH may excite a GABAergic input to RPa, the location of which remains unknown. Alternatively, glucoprivation could increase the activity of excitatory neurons rostral to the medulla that drive medullary GABAergic interneurons, perhaps even local GABAergic interneurons in the RPa.

Transection of the neuraxis at the pontomedullary border increased not only BAT SNA and thermogenesis but also increased arterial pressure and HR. These data suggest that there is a population of neurons rostral to the pontomedullary border that provides tonic inhibition to cardiac sympathetic premotor neurons, possibly those located in the RPa. Indeed neurons in the RPa are capable of markedly increasing cardiac SNA (6, 38). Alternatively, the transection-evoked increase in HR observed in the present study could be mediated by

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Fig. 7. Schematic diagram summarizing proposed models of the neurocircuitry responsible for glucoprivic inhibition of BAT thermogenesis. Glucoprivation activates neurons in the VLM, which, in turn, drive a GABAergic input to sympathetic premotor neurons for BAT located in the rostral RPa. Glucoreponsive neurons of the VLM may directly activate a GABAergic input to the RPs (pathway 2) and/or this neural circuit may involve the activation of neurons in the paraventricular hypothalamus (PVH; pathway 1), which inhibit BAT SNA, likely via GABA release in the RPa (20). The location of the GABAergic neurons that project to the RPa and are activated by glucoprivation is unknown (7), though these neurons are likely to be located rostral to the level at which the transection (TransX) was made in the present study (dotted line) since BAT SNA evoked by the transection was resistant to glucoprivic inhibition. The neural circuit depicted in gray represents the fundamental neural circuitry responsible for the increase in BAT SNA evoked by cooling (for a review see Ref. 25). Distances (mm from bregma) are given in the lower right of each schematic section. VII, facial nucleus; Ach, acetylcholine; IML, intermediolateral cell column; NE, norepinephrine; POA, preoptic area; R, recording electrode.
excitation of cardiac sympathetic premotor neurons located in other regions, such as the RVLM, or by the withdrawal of resting vagal tone. The increase in AP following the pontomedullary transection could be a reflection of increased cardiac output associated with the elevated HR. Alternatively, total peripheral resistance could have been increased by activation of sympathetic vasomotor neurons of the RVLM in response to removal of a tonically active inhibitory input. Transections of the neuraxis at levels rostral to the medulla have been reported to have no effect on arterial pressure or SNA (1). The apparent discrepancy between the observation of the present study and that of Alexander (1) could be explained by differences in the precise levels of the transections and the resulting balance between the control of excitatory and inhibitory inputs.

Conclusions. The present study demonstrates that glucoprivation in the VLM inhibits BAT SNA and thermogenesis, and that neural circuitry located rostral to the medulla is required for glucoprivic inhibition of BAT thermogenesis. In addition, the present data suggest a role for GABAergic inputs to the sympathetic premotor neurons in the RPa in the glucoprivic inhibition of BAT SNA and thermogenesis.

Perspectives and Significance

The endogenous activity of BAT has been suggested to play a role in obesity and diabetes (44, 45), although some controversy surrounds this issue and the role of the endogenous activity of BAT in energy homeostasis has been questioned (15). Nonetheless, it is clear that 1) adult humans possess BAT, 2) the level of BAT energy expenditure is determined principally by the central neural circuits controlling BAT sympathetic outflow, and 3) activation of BAT can have a significant impact on energy expenditure and glucose homeostasis. Therefore, a more complete understanding of the neural circuits involved in the sympathetic regulation of BAT, including metabolic influences on this regulation, such as that provided by the present studies, might suggest therapeutic targets for obesity and diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.J.M. conception and design of research; C.J.M. performed experiments; C.J.M. analyzed data; C.J.M. interpreted results of experiments; C.J.M. prepared figures; C.J.M. drafted manuscript; C.J.M. edited and revised manuscript; C.J.M. approved final version of manuscript.

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