Activation of 5-HT$_{1A}$ receptors in raphe pallidus inhibits leptin-evoked increases in brown adipose tissue thermogenesis

Shaun F. Morrison
Neurological Sciences Institute
Oregon Health & Science University
Beaverton, OR 97006

**Running Title:** Raphe 5-HT$_{1A}$ receptors inhibit leptin-evoked BAT thermogenesis

**Correspondence to:**
Dr. Shaun Morrison
Neurological Sciences Institute
OHSU
505 NW 185th Avenue
Beaverton, OR 97006
Phone: (503) 418-2670
FAX: (503) 418-2501
Email: morrisos@ohsu.edu
Abstract

To elucidate the central neural pathways contributing to the thermogenic component of the autonomic response to iv. administration of leptin, experiments were conducted in urethan-chloralose-anesthetized, ventilated rats to address (1) the role of neurons in the rostral ventromedial medulla, including raphe pallidus (RPa), in the leptin-evoked stimulation of brown adipose tissue (BAT) sympathetic nerve activity (SNA) and (2) the potential thermolytic effect of 5-HT₁A receptors on RPa neurons that influence BAT thermogenesis. Leptin (1 mg/kg) administration increased BAT SNA by 1219 % of control, BAT temperature by 2.8°C, expired CO₂ by 1.8 %, heart rate by 90 bpm and mean arterial pressure by 12 mmHg. Microinjection of the 5-HT₁A receptor agonist, 8-OH-DPAT, into RPa resulted in a prompt and sustained reversal of the leptin-evoked stimulation of BAT SNA, BAT thermogenesis and heart rate, with these variables returning to their pre-leptin control levels. Subsequent microinjection of the selective 5-HT₁A receptor antagonist, WAY100635, into RPa reversed the BAT thermolytic effects of 8-OH-DPAT, returning BAT SNA and BAT temperature to the elevated levels following leptin. In conclusion, activation of neurons in RPa, possibly BAT sympathetic premotor neurons, is essential for the increases in BAT SNA, BAT thermogenesis and heart rate stimulated by iv administration of leptin. Neurons in RPa express 5-HT₁A receptors whose activation leads to reversal of the BAT thermogenic and the cardiovascular responses to iv leptin, possibly through hyperpolarization of local sympathetic premotor neurons. These results contribute to our understanding of central neural substrates for the augmented energy expenditure stimulated by leptin.

Keywords
sympathetic nerve activity
hypothermia
temperature regulation
ventromedial medulla
energy expenditure
Introduction

Leptin, an anorexigenic hormone produced by adipose tissue, binds to specific receptors within the central nervous system to influence neuronal networks that govern energy balance as well as those that determine sympathetic tone to cardiovascular targets. In addition to a reduction in appetite and food intake, activation of central leptin receptors leads and an increase in energy expenditure, which, in small mammals and the young of larger mammals occurs, at least in part, through an increase in the sympathetic outflow to brown adipose tissue (BAT) (17) and the resulting stimulation of BAT metabolism and BAT thermogenesis. Although the neural circuits through which leptin alters energy balance remain to be defined, considerable evidence indicates that neurons within the arcuate nucleus of the hypothalamus play a key role in transducing the blood-borne leptin signal (11; 12; 16). Since the neural circuits between the hypothalamic neurons directly influenced by leptin and the spinal sympathetic preganglionic neurons controlling BAT thermogenesis are not fully understood, the goal of this study was to provide information on the medullary neuronal populations mediating the leptin-evoked increase in BAT SNA and thermogenesis.

The rostral medullary raphe nuclei, including raphe pallidus (RPa) and the surrounding ventromedial medulla, have been identified as containing neurons essential for the sympathetic regulation of target tissues, including BAT that are involved in thermoregulation and energy metabolism. Several lines of evidence converge to suggest that the RPa contains sympathetic premotor neurons for BAT (29): neurons in this region project to the thoracic intermediolateral nucleus (23), they are among the earliest medullary neurons infected following pseudorabies virus injections into interscapular BAT (2; 9), their activation leads to large increases in BAT sympathetic nerve activity (SNA) and thermogenesis (25; 29) and they are essential for the increase in BAT SNA and BAT thermogenesis that contributes to the febrile response elicited by prostaglandin E2 (32; 33). In the present study, the hypothesis that RPa neurons play an essential role in mediating the leptin-evoked stimulation of BAT SNA and BAT thermogenesis was tested by determining the effect of an 8-OH-DPAT-mediated inhibition of neurons in RPa on the increase in BAT SNA and BAT temperature following leptin administration. Some of these results have been presented in abstract form (30).

Materials and Methods

Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996) and under
protocols approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University. Sprague-Dawley rats (n=8, 240-400 gm) were obtained from Charles River, Inc. Animals were anesthetized intravenously with urethane (0.8 gm/kg) and chloralose (80 mg/kg) after induction with 3% isoflurane in 100% O₂. A femoral artery, a femoral vein and the trachea were cannulated for measurement of arterial pressure, drug injection and artificial ventilation, respectively. Heart rate was derived from the arterial pressure signal. After the animals were positioned prone in a stereotaxic frame with the incisor bar at –3.8 mm and with a spinal clamp on the T₁₀ vertebra, they were paralyzed with D-tubocurarine (0.3 mg initial dose, 0.1 mg/hr supplements) and artificially ventilated with 100% O₂ (50 cycles per minute, tidal volume: 3-4.5 ml). Small adjustments in minute ventilation were made as necessary to maintain basal, mixed-expired CO₂ levels between 3.5-4.5 %. Colonic temperature was maintained at 37.5°C with a thermostatically-regulated heat lamp and heating plate beneath the animal.

Postganglionic SNA to BAT was recorded from the central cut end of a small nerve bundle dissected from the ventral surface of the right interscapular BAT pad after dividing the fat pad along the midline and reflecting it laterally. Nerve activity was recorded with bipolar hook electrodes in a monopolar configuration, filtered (1-300 Hz) and amplified (50k, Cyberamp 380, Axon Instruments). BAT temperature was measured by placing a thermistor (Physitemp, Inc.) beneath the left half of the interscapular BAT pad, which was left intact. BAT SNA, BAT temperature, colonic temperature, expired CO₂, arterial pressure and stimulus trigger pulses were digitized (1 kHz) and recorded on VCR tape (Neurodata) and computer hard drive (Axoscope, Axon Instruments).

Initially, a tungsten microstimulating electrode (30 µm exposed tip) and, subsequently, a microinjection pipette (tip outside diameter: 20µm) were positioned stereotaxically in the RPa. Relative to lambda, the coordinates for the RPa were approximately AP: -3.0 mm, ML: 0.0 mm, DV: -9.5 mm below the dural surface. The optimal dorsoventral site for microinjection into RPa was determined as that yielding the lowest microstimulation threshold (<10 µA) for evoking an excitatory potential on the BAT sympathetic nerve with twin pulses (1 ms duration, 6 ms interpulse interval, 0.4 Hz) applied to RPa. At the end of each experiment, the microinjection pipette was retracted vertically from the RPa, refilled with a 4 % solution (pH 8.0) of fast green dye, lowered to the site of microinjection and dye was electrophoretically deposited (15 µA anodal direct current for 15 minutes). Following perfusion and histological processing, the locations of the microinjection sites in the RPa were plotted on camera lucida drawings of sections through the rostral medulla (38).
During each experiment, BAT SNA, BAT and colonic temperatures, expired CO₂, arterial pressure and heart rate were recorded during a control period of at least 30 minutes and following subsequent intravenous (iv.) administration of murine leptin (Amgen, Inc., 1mg/kg given as an initial bolus of 0.5 mg/kg in 0.5 ml saline followed by an infusion of 0.5 mg/kg in 5 ml over 1 hour (17)). The dose of leptin used in this study has been shown to produce a large and sustained increase in BAT SNA (17) upon which the effects of altering neuronal discharge in RPa could be tested. The effects on leptin-evoked responses of microinjecting the following agents into RPa were determined: (a) saline vehicle (60 nl), applied 10–20 minutes after the onset of the leptin-evoked increase in BAT SNA, (b) the 5-HT₁A receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, Sigma, 10mM, 60 nl), applied 30–60 minutes after the onset of the leptin-evoked increase in BAT SNA, (c) the selective, competitive 5-HT₁A receptor antagonist, WAY-100,635 (Sigma, 10mM, 60 nl), applied 10–20 minutes after the microinjection of 8-OH-DPAT.

Spectral analysis was used to determine the amplitude of BAT SNA (42). Throughout each experiment, the autospectra of sequential 4-sec segments of BAT SNA was calculated and the amplitude of BAT SNA in these 4-sec bins was determined as the square root of the sum of the power values (rms power) in the 0-20 Hz frequency range. For control or drug treatment conditions, the level of BAT SNA was the average of the eight, 4-sec BAT SNA values in the 32 seconds of BAT SNA prior to a treatment or in the 32 seconds surrounding the maximal or minimal BAT SNA produced by the treatment.

For determination of plasma catecholamine levels, 1 ml arterial blood samples were taken 10 min prior to the onset of the iv. leptin administration and at the peak of the increase in BAT SNA. Blood was replaced with an equal volume of a 30% solution of rat plasma (Sigma Chemical, Inc.). Blood samples were centrifuged at 3000 rpm for 20 min at 4°C and the plasma was stored at -80°C until the catecholamine assay was performed. Catecholamine levels in plasma were assayed by liquid chromatography with electrochemical detection. The method combined liquid:liquid extraction of catecholamines from plasma with reversed-phase chromatography incorporating a cation-exchange reagent (Plasma Catecholamine Kit, Bioanalytical Systems, West Lafayette, IN) (24; 27). For replicate determinations of a 1.0 ml plasma sample containing 0.25 pmol epinephrine and 1.6 pmol norepinephrine, intra-assay coefficients of variation were 6% and 3%, respectively, and the inter-assay coefficients of variation were 6% and 4%, respectively.
All data are presented as means ± standard error (SE), unless otherwise noted. Statistical differences were assessed with Students, two-tailed, paired t-test and Bonferroni correction (p<0.05).

**Results**

Leptin (1 mg/kg, iv.) was administered to 8 urethane/chloralose-anesthetized rats whose average MAP and HR were 110 ± 4 mmHg and 359 ± 18 bpm, respectively. Under control conditions, with the rat’s core temperature maintained between 36.5 – 37.5 °C, the activity on the sympathetic postganglionic nerve to the interscapular BAT was very low, exhibiting only occasional, low-amplitude bursts and reflecting the absence of either thermoregulatory or metabolic drives for BAT thermogenesis. As illustrated in the example in Figure 1, leptin administration produced increases in the sympathetic outflow to interscapular BAT (peak: +1475 % of control), in BAT temperature (peak: +3.3 °C), in expired CO₂ (peak: +1.9 %), in core temperature (peak: +0.5 °C), in HR (peak: +148 bpm) and in MAP (peak: +29 mmHg). The mean peak values of the BAT thermogenic and cardiovascular parameters following leptin administration are presented in Table 1. Leptin evoked a mean peak increase of +1219 ± 298 % (p<0.001) in BAT SNA which produced a mean peak increase in BAT temperature of +2.8 ± 0.5 °C (p<0.001). These were accompanied by a mean peak increase in expired CO₂ of +1.8 ± 0.3 % (p<0.001), a mean maximum rise in core temperature of +0.4 ± 0.08 °C (p<0.01), a mean peak tachycardia of +90 ± 18 bpm (p<0.01) and a mean pressor response of +12 ± 4 mmHg (p<0.05).

In addition to BAT thermogenesis, leptin administration also stimulated adrenal catecholamine release. Plasma epinephrine, measured at 50 ± 5 min after beginning the iv. leptin infusion, was increased by 101 ± 65% (p<0.05, n=6) from a control level of 1.35 ± 0.40 pmol/ml. Plasma norepinephrine, reflecting both adrenal release and spillover from sympathetic nerve terminals, rose by 179 ± 48% (p<0.05) from a control level of 1.82 ± 0.23 pmol/ml.

Microinjection of the 5-HT₁A receptor agonist, 8-OH-DPAT, into RPa shortly after the leptin-evoked increase in BAT SNA reached peak levels, produced a prompt reversal (Fig. 1) of the leptin-stimulated rise in BAT SNA (maximum reduction: -101 % of the leptin-evoked increase), in BAT temperature (peak reduction: -2.2 °C), in expired CO₂ (maximum fall: -1.4 %) and in HR (peak bradycardia: -129 bpm). Microinjection of 8-OH-DPAT into RPa also resulted in small reductions in MAP (maximum fall: -7 mmHg) and core temperature (decline: -0.1°C), but these were less than the increases following leptin administration. In the absence of further
treatment, the lowered levels of BAT thermogenesis and heart rate elicited by microinjection of 8-OH-DPAT into RPa were sustained for at least 30 minutes. The mean nadir values of the BAT thermogenic and cardiovascular parameters following 8-OH-DPAT microinjection into RPa are presented in Table 1. Application of 8-OH-DPAT to RPa neurons during the sustained thermogenesis stimulated by iv. leptin resulted in a mean maximum fall in BAT SNA of -96 ± 15 % (p<0.01, n=6) of the leptin-evoked increase in BAT SNA, resulting in a mean maximum fall in BAT temperature of -2.1 ± 0.3 °C (p<0.005) from the peak BAT temperature levels evoked by leptin administration. These declines in sympathetically-mediated BAT thermogenesis were accompanied by maximal decreases in expired CO2 of -1.6 ± 0.2 % (p<0.001), in HR of -109 ± 12 bpm (p<0.001) and in MAP of -15 ± 7 mmHg (p<0.05) from the peak levels evoked by leptin. The mean minimum values of BAT SNA, BAT temperature, HR and expired CO2 following microinjection of 8-OH-DPAT into RPa were not significantly different from the control values prior to leptin administration. Microinjection of saline vehicle into RPa had no effect (Fig. 1) on any of the leptin-evoked increases in the parameters measured in these experiments.

To establish the specificity of the effects of 8-OH-DPAT to its activation of 5-HT1A receptors in RPa, the selective, 5-HT1A receptor antagonist, WAY100635, was microinjected into RPa 17 ± 1 minutes after the 8-OH-DPAT microinjection in 5 rats. Microinjection of WAY100635 into RPa produced a prompt reversal (Fig. 1) of the 8-OH-DPAT-evoked inhibition of BAT thermogenesis and reinstated the leptin-evoked activation of BAT SNA (maximum increase: +1628 % of the untreated control prior to leptin) and the accompanying increases in BAT temperature (peak increase: +2.8 °C), in expired CO2 (maximum rise: +1.6 %) and in HR (peak tachycardia: +129 bpm). Microinjection of WAY100635 into RPa also increased MAP (peak: +17 mmHg) and core temperature (peak: +0.7 °C). The mean peak values of the BAT thermogenic and cardiovascular parameters following local microinjection of WAY100635 into RPa in rats treated sequentially with iv. leptin and microinjection of 8-OH-DPAT into RPa are presented in Table 1. Following leptin and 8-OH-DPAT administration, microinjection of WAY100635 into RPa evoked a mean peak increase in BAT SNA of +1156 ± 320 % (p<0.01) of the untreated control SNA prior to leptin, which produced a mean peak increase in BAT temperature of +2.2 ± 0.3 °C (p<0.005). These were accompanied by a mean peak increase in expired CO2 of +1.5 ± 0.2 % (p<0.002), a mean peak tachycardia of +99 ± 21 bpm (p<0.01) and a mean pressor response of +22 ± 7 mmHg (p<0.05). The peak levels of BAT thermogenic and
cardiovascular variables following WAY100635 application to RPa neurons in leptin- and 8-OH-DPAT-treated rats were not different from those initially evoked by the leptin administration.

**Discussion**

These data provide the first demonstration that neurons in the rostral ventromedial medulla, including the RPa, play a critical role in the stimulation of the BAT thermogenic and heart rate responses to iv. administration of leptin in the rat. This conclusion was reached using the specific 5-HT_{1A} receptor agonist, 8-OH-DPAT, to hyperpolarize local neurons in RPa (3; 4), potentially reducing their responsiveness to activation of leptin-sensitive pathways arising from the arcuate and caudal dorsomedial nuclei of the hypothalamus(11; 12; 16). The specific 5-HT_{1A} receptor antagonist, WAY-100635 reversed the BAT sympathoinhibitory effect of 8-OH-DPAT, as well as the falls in BAT temperature, expired CO2 and heart rate. The ability of a microinjection of 8-OH-DPAT into RPa to inhibit BAT SNA extends to thermoregulation, the initial suggestion (18) that the RPa is one of the central sites of 5-HT_{1A} receptors whose activation is responsible for the changes in autonomic function that occur with the clinical use of 5-HT_{1A} receptor agonists in the treatment of depression and anxiety. Similarly, the absence of an available 5-HT_{1A} receptor-mediated inhibitory input to RPa neurons may contribute to the exaggerated hyperthermic and heart rate responses to stress in 5-HT1A receptor knockout mice (37).

The finding that microinjection of 8-OH-DPAT into RPa resulted in a dramatic fall in leptin-stimulated levels of BAT SNA, BAT temperature and expired CO2 (providing an index of acute changes in oxidative metabolism, likely reflecting that in BAT and heart) indicates the existence of 5-HT_{1A} receptors on neurons in the RPa that influence the sympathetically-driven thermogenic component of the increase in energy expenditure produced by leptin. The rostral ventromedial medulla, including the RPa, is hypothesized to be the site of sympathetic premotor neurons controlling lipid metabolism and thermogenesis in BAT (29; 31). This conclusion is based on (a) the demonstration of direct projections from neurons in RPa to the thoracic intermediolateral nucleus containing sympathetic preganglionic neurons (1; 23), (b) the identification of RPa as one of the earliest sites of retrogradely infected neurons following pseudorabies virus injections into BAT (2; 9; 34) and (c) the large increases in BAT SNA and thermogenesis produced uniquely by activation of medullary neurons in RPa, either with local microinjection of the GABA_A receptor antagonist, bicuculline (29), or of the glutamate receptor...
agonists, NMDA or kainic acid (25). Additionally, interruption of the activity of neurons in RPa eliminates the stimulation of BAT SNA (32) and BAT thermogenesis (33) during the acute febrile response produced by central administration of PGE$_2$ in anesthetized rats and elicits a marked reduction in core temperature in awake rats maintained at room temperature (44). Thus, it seems likely that the reversal of the leptin-stimulated increase in BAT thermogenesis by microinjection of 8-OH-DPAT into RPa results from an inhibition of the discharge of BAT sympathetic premotor neurons located there. The most direct explanation for our results is that leptin activates hypothalamic neurons which, through pathways yet to be elucidated, activate BAT sympathetic premotor neurons in the RPa which, in turn, stimulate BAT thermogenesis. Alternatively, a certain level of tonic activity in BAT sympathetic premotor neurons in the RPa may be necessary to facilitate the excitation of BAT sympathetic preganglionic neurons by other descending pathways that are activated by leptin, such as the cocaine- and amphetamine-regulated transcript (CART)-containing neurons in the retrochiasmatic area and lateral arcuate nucleus (13).

While the data presented here do not indicate whether 8-OH-DPAT microinjected into RPa binds to 5-HT$_{1A}$ receptors located on BAT sympathetic premotor neurons or on an antecedent population of local excitatory interneurons, they do suggest that the well-described hypothermic effect of 5-HT$_{1A}$ receptor agonist administration (22; 28) could occur, at least in part, through a reduction in sympathetically-regulated heat production effected by inhibition of the raphe sympathetic premotor neurons controlling the sympathetic activation of thermogenic tissues. Although, Berner, et al. used a similar experimental design to show that injections of 8-OH-DPAT into the raphe magnus inhibited the increases in oxygen consumption and shivering normally elicited by cooling the preoptic/anterior hypothalamus (5), the large injection volumes used in their study would not distinguish 8-OH-DPAT-mediated effects on neurons in raphe magnus from those on neurons in neighboring RPa. The demonstration that 8-OH-DPAT inhibits the normal, cold-evoked increase in cutaneous sympathetic vasoconstrictor tone (35) suggests that an increase in heat loss also contributes to 5-HT$_{1A}$ receptor-evoked hypothermia. Interestingly, sympathetic premotor neurons regulating cutaneous blood flow are also postulated to be in the same RPa region of the ventromedial medulla (6; 7; 39), providing a potential substrate for the cutaneous heat loss contribution to 5-HT$_{1A}$ receptor-mediated hypothermia that would parallel that revealed here for a reduction in thermogenesis.

An anatomical substrate for the effects on BAT SNA and thermogenesis seen with microinjection of 8-OH-DPAT into RPa is found in immunocytochemical studies localizing 5-
HT₁A receptor binding sites on neurons in the RPa and the parapyramidal regions of the ventromedial medulla (20; 43) and in particular on raphespinal neurons projecting to the intermediolateral cell column, including a significant number of 5-HT-containing neurons in these regions (19). These results coupled with the demonstration in dorsal raphe, that 5-HT₁A receptor activation hyperpolarizes both 5-HT- immunopositive and non-5-HT neurons (21), preclude any conclusion on the specific role of 5-HT-containing neurons in RPa in the regulation of BAT thermogenesis to be drawn from the 8-OH-DPAT-induced reduction in BAT SNA found in the present study. That 5-HT neurons in RPa might contribute to activation of BAT SNA is supported by the finding that some of the neurons in RPa are infected at short survival times after injection of the retrograde tracer, pseudorabies virus, into interscapular BAT were tryptophan hydroxylase-immunoreactive (9), that serotonergic neurons in the RPa of freely-moving cats increase their discharge during exposure to a low ambient temperature (26) and that cold-exposure increases 5-HT synthesis, metabolism and utilization in the rat thoracic spinal cord (36). Electron microscopic examination of labeled 5-HT₁A receptors in the dorsal raphe indicated an exclusive somatodendritic location (40) suggestive of a role in mediating serotonergic effects on neuronal firing rather than transmitter release. Whether a similar localization of 5-HT₁A receptors occurs in medullary RPa and parapyramidal neurons remains to be determined.

Regarding a role for RPa neurons in mediating the increased energy expenditure stimulated by leptin, anatomical experiments using induction of c-fos to identify neural pathways activated by leptin administration (14; 15) did not reveal activation of neurons in the RPa region of the medulla. Although the reason for this discrepancy is unclear, it may be that the appropriate RPa neurons do not express c-fos (however, see (8; 29)), that the number or distribution of RPa neurons expressing c-fos in response to leptin was not remarkable or that activity in RPa neurons projecting to BAT sympathetic preganglionic neurons is only necessary in a permissive role to observe an increase in BAT SNA following leptin administration.

Microinjection of 8-OH-DPAT into RPa reversed the increase in heart rate resulting from the iv administration of leptin. This result indicates that the activity of neurons in RPa region is necessary not only for the leptin-evoked increase in BAT thermogenesis, but also for the stimulation of cardiac sympathetic outflow mediating the accompanying tachycardia. Coupled with the demonstrations that activation of RPa neurons elicits a sympathetically-mediated tachycardia (10), that neuronal activity in RPa is required for the tachycardic component of the response to central administration of PGE₂ (32) and that the air-jet stress-induced increase in heart rate and that following disinhibition of neurons in the dorsomedial hypothalamus are
mediated, at least in part, by neurons in the RPa (41; 45), the current finding is consistent with
the proposal that RPa contains a population of cardiac sympathetic premotor neurons (10) that do
not contribute to resting cardiac sympathetic tone or resting heart rate(44), but rather mediate the
increases in heart rate that comprise an important component of the cardiovascular support for
the responses to a variety of thermoregulatory, metabolic and stress-related challenges.

In conclusion, the present results indicate the importance of activation of neurons in the
rostral ventromedial medulla, including RPa, in the leptin-evoked increase in BAT SNA, BAT
thermogenesis and heart rate. These data provide further evidence supporting a model in which
BAT sympathetic premotor neurons in RPa are the final common medullospinal pathway
mediating the excitation of BAT sympathetic preganglionic neurons regulating metabolic
activity, energy consumption and thermogenesis in BAT. This study also suggests a significant
role for 5-HT1A receptors in the RPa region in mediating the hypothermic effects in several
species, including rat and human, of 5-HT1A receptor agonist treatment. Elucidating the local
circuitry and sources of inputs to thermogenic neurons in the RPa region will further our
understanding of the function of this brain region critical to control of sympathetically-regulated
effectors in thermoregulation and energy expenditure.
Acknowledgements

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References


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Figure Legends

Figure 1. Inhibition of leptin-evoked increase in BAT thermogenesis by activation of 5-HT1A receptors in rostral raphe pallidus (RPa). Leptin administration (1 mg/kg, iv, 20 minutes prior to beginning of traces) elicits increases in brown adipose tissue sympathetic nerve activity (BAT SNA, top trace: oscillographic record, second trace: rms power, 4 sec bins), BAT temperature (third trace), expired CO2 (fourth trace), heart rate (fifth trace), and arterial pressure (bottom trace, arrhythmia during BAT sympathoexcitation in this case). Microinjection of saline (60 nl) into RPa does not affect any of the variables, * indicates blood sample for catecholamine determination. Microinjection of 5-HT1A receptor agonist, 8-OH-DPAT, into RPa abolishes leptin-evoked BAT sympathoexcitation and reverses increases in BAT temperature, expired CO2 and heart rate. Subsequent microinjection of the selective 5-HT1A receptor antagonist, WAY100635, into RPa reversed the 8-OH-DPAT-mediated inhibitory effects and reinstated the leptin-evoked stimulation of BAT thermogenesis. Vertical calibration is 200µV for BAT SNA (top trace).

Figure 2. Microinjection sites of 8-OH-DPAT and WAY100635 in RPa region of the rostral ventromedial medulla. Histological section (panel A) and atlas drawing (38) approximately 11.6 mm caudal to bregma. Arrow in panel A indicates location of fast green dye spot in RPa. Filled circles in panel B indicate locations of dye spots marking the injection sites in the RPa region of the ventromedial medulla in 8 rats.
TABLE 1. Effects on BAT thermogenic and on cardiovascular variables of iv. leptin and subsequent microinjections into RPa of 8-OH-DPAT and WAY100635.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (8)</th>
<th>LEPTIN (8)</th>
<th>8-OH-DPAT RPa (6)</th>
<th>WAY100635 RPa (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT SNA (% control)</td>
<td>100</td>
<td>1319 ± 298</td>
<td>124 ± 43 †</td>
<td>1518 ± 512</td>
</tr>
<tr>
<td>BAT Temp (°C)</td>
<td>33.7 ± 0.6</td>
<td>36.5 ± 0.3</td>
<td>34.4 ± 0.4 †</td>
<td>36.5 ± 0.3</td>
</tr>
<tr>
<td>Exp CO₂ (%)</td>
<td>3.0 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>3.3 ± 0.4 †</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>Core Temp (°C)</td>
<td>36.7 ± 0.09</td>
<td>37.1 ± 0.10</td>
<td>36.8 ± 0.10 *</td>
<td>37.1 ± 0.05</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>359 ± 18</td>
<td>449 ± 15</td>
<td>330 ± 24 †</td>
<td>424 ± 22</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>109 ± 5</td>
<td>123 ± 3</td>
<td>109 ± 6 *</td>
<td>131 ± 3</td>
</tr>
</tbody>
</table>

Mean ± sem of the peak values of brown adipose tissue (BAT) thermogenic and cardiovascular variables in untreated control rats and following iv. leptin (1mg/kg), subsequent microinjection of 8-OH-DPAT into the rostral raphe pallidus (RPa), and, in the case of 5 rats receiving 8-OH-DPAT, subsequent microinjection of WAY100635 into RPa. The numbers of animals in each group are shown in parentheses. BAT sympathetic nerve activity (BAT SNA) is always expressed as percent of the untreated control level. * indicates significantly (p<0.05) less than leptin-evoked level, † indicates significantly (p<0.005) less than leptin-evoked level.
FIGURE 1 TOP
Morrison, S.F.
Activation of 5-HT_{1A} receptors...
Figure 2
Morrison, S. F.
Activation of 5-HT1A receptors