Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue

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PROSTAGLANDIN E2 acting within the preoptic area of the hypothalamus has been implicated in the acute phase component of the febrile response. Fever can be elicited by injection of prostaglandin directly into the medial preoptic area (MPA) (11, 27, 28, 31) and blockage of prostaglandin synthesis within the preoptic area of the hypothalamus attenuates fever (16, 25). Furthermore, the prostaglandin E3 receptor is found in the MPA (10, 19, 22) and EP3 receptor knockout mice have an impaired febrile response to systemic pyrogen administration (30). Recent observations suggest that ventromedial medullary neurons, including those in the rostral raphe pallidus (RPa), are an essential site of synaptic integration in the central pathways mediating the thermogenic and cardiovascular components of the febrile response in the rodent model (15, 17, 20). However, the neural pathways responsible for conveying the thermogenic and cardiovascular drive from the MPA to neurons in the RPa are poorly understood. Several lines of evidence suggest that

Madden, C. J., and S. F. Morrison. Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol 286: R320–R325, 2004. First published October 16, 2003; 10.1152/ajpregu.00515.2003.—We determined whether the dorsomedial hypothalamus (DMH) plays a role in the thermogenic, metabolic, and cardiovascular effects evoked by centrally administered PGE2. Microinjection of PGE2 (170 pmol/60 nl) into the medial preoptic area of the hypothalamus in urethane-chloralose-anesthetized, artificially ventilated rats increased brown adipose tissue (BAT) sympathetic nerve activity (SNA; +207 ± 18% of control), BAT temperature (+1.5 ± 0.2°C), expired CO2 (0.9 ± 1%), heart rate (HR; 106 ± 12 beats/min), and mean arterial pressure (22 ± 4 mmHg). Within 5 min of subsequent bilateral microinjections of the GABA A receptor agonist muscimol (120 pmol/60 nl • side) or the ionotropic excitatory amino acid antagonist kynurenic acid (6 nmol/60 nl • side) into the DMH, the PGE2-evoked increases were, respectively, attenuated by 91 ± 3% and 108 ± 7% for BAT SNA, by 73 ± 12% and 102 ± 28% for BAT temperature, by 100 ± 4% and 125 ± 21% for expired CO2, by 72 ± 11% and 70 ± 16% for HR, and by 84 ± 19% and 113 ± 16% for mean arterial pressure. Microinjections outside the DMH within the dorsal hypothalamic area adjacent to the mamillothalamic tracts or within the ventromedial hypothalamus were less effective for attenuating the PGE2-evoked thermogenic, metabolic, and cardiovascular responses. These results demonstrate that activation of excitatory amino acid receptors within the DMH is necessary for the thermogenic, metabolic, and cardiovascular responses evoked by microinjection of PGE2 into the medial preoptic area.

Experimental Procedures

Male and female Sprague-Dawley rats (Charles River, Indianapolis, IN, n = 20) weighing 250–450 g were given ad libitum access to standard rat chow and water in a colony room maintained at 22–23°C and kept on a 12:12-h light-dark cycle. Rats were anesthetized with isoflurane (2–3% in oxygen) and implanted with femoral arterial and venous catheters and transitioned to urethan and chloralose anesthesia (500 and 80 mg/kg iv, respectively) over a 10-min period. To record arterial pressure and HR, the arterial catheter was attached to a pressure transducer. The trachea was cannulated, and the animals were paralyzed with d-tubocurarine (0.5 mg iv, supplemented with 0.1 mg every 1 h) and ventilated (tidal volume: 1 ml/100 g body wt, 60 cycles/min) with 100% oxygen. Mixed expired CO2 was monitored using a capnometer. Colonic temperature was monitored using a copper-constantan thermocouple inserted 6 cm into the rectum, and core body temperature was maintained at 37.5 ± 0.5°C with a heating plate and a heat lamp. Animals were placed in a stereotaxic instrument with the incisor bar positioned 4 mm below the interaural line. For microinjections into the MPA, small portions of the parietal and frontal bones were removed. On the basis of the coordinates at which microinjection of prostaglandin elicited hyperthermia (31), our coordinates for injections into the MPA were 0.0 to 0.3 mm caudal and 0.8 mm lateral to bregma and 8.0 mm ventral to dura. For microinjections into the DMH, small portions of the parietal bones were removed. The coordinates for injections into the DMH were 3.0–3.5 mm caudal to bregma, 0.5–0.7 mm lateral to the midline, and 8.2–9.0 mm ventral to dura. These coordinates correspond to the area at which a microinjection of bicuculline evokes an increase in BAT SNA (6) and an increase in HR (6, 26). Microinjections located within 100 μm of the boundaries of the DMH designated in the atlas of Paxinos and Watson (24) were considered effective DMH injection sites. All other microinjections were considered to be control injections and were analyzed as a separate group. Glass micropipettes (outer tip diameter, 20–30
μm) were used for all microinjections that were given over a 10- to 20-s period using a pressure injection system. All drugs were obtained from Sigma (St. Louis, MO) except isoflurane, which was obtained from Abbott Laboratories (North Chicago, IL).

Postganglionic BAT sympathetic nerve activity (SNA) was recorded from the central cut end of a nerve bundle isolated from the ventral surface of the right intercapular fat pad after dividing it along the midline and reflecting it laterally. The nerve bundle was placed on bipolar hook electrodes and covered in mineral oil. Nerve activity was filtered (1–300 Hz) and amplified (20,000–50,000) with a Cyberamp 380 (Axon Instruments, Union City, CA). The BAT temperature was monitored with a copper-constantan thermocouple (Physitemp, Clifton, NJ) inserted beneath the intact left interscapular fat pad. Physiological variables were digitized (Digidata 1320A, Axon Instruments) and recorded onto a personal computer hard drive using AxoScope acquisition software (Axon Instruments).

Rats received a microinjection of PGE2 (170 pmol/60 nl) into the MPA followed 15–20 min later by bilateral microinjections into the DMH of either the GABA_A agonist muscimol (120 pmol/60 nl –1 side; n = 6), the nonselective ionotropic EAA receptor antagonist kynurenic acid (6 nmol/60 nl –1 side; n = 10) or saline (60 nl/side; n = 4). The dose of kynurenic acid used in the present study was effective in blocking PGE2-evoked BAT thermogenesis when microinjected into the RPA (13).

At the conclusion of each experiment, the DMH and MPA microinjection sites were marked by retracting the microinjection pipettes vertically, filling them with 2% fast green dye, repositoning them to the approximate dorsoventral coordinates of the microinjection sites, and electrophoretically (20-μA anodal direct current for 10 min) depositing the dye. Rats were perfused transcardially with a 10% paraformaldehyde solution. The brains were removed, postfixed for 12–24 h, and sectioned at 60 μm. Brain sections containing fast green dye deposits were mounted on slides and photographed using a digital microscope camera (Kodak DC 220). Photomicrographs were downloaded to a personal computer and assembled using Adobe Photoshop software; only brightness, sharpness, and contrast were adjusted. The locations of microinjection sites were plotted on atlas drawings (24).

BAT SNA amplitude was derived from autospectral analysis using Datapac 2000 software (Run Technologies). For each experimental condition, the average autospectrum of a 101.6-s BAT SNA data segment was computed by dividing the data into 20 equal segments, computing the autospectrum for each of these 5.08-s segments, and then averaging these individual autospectra (i.e., the average power at each frequency value is the mean of the powers at that frequency value in the 20 individual autospectra). The root mean square amplitude of the BAT SNA for each experimental condition was taken as the square root of the total power in the 0.19- to 20-Hz band of the averaged autospectrum. Resting (baseline) values were obtained from the data during the 2-min period immediately before microinjection of PGE2 into MPA. Response values of BAT SNA were obtained from the data during the 2-min period of peak change in BAT SNA after microinjection of PGE2 into MPA. All statistics were performed using Systat software (version 10, SPSS). Values are expressed as means ± SE. Statistical significance was assessed using a two-way repeated measures ANOVA with drug treatment as the grouping variable and time as the repeated measure. Significant group-by-time interactions were followed by ANOVA tests at each time point with Fisher’s least significant difference post hoc testing where appropriate. Significance level was set at P < 0.05.

**RESULTS**

**Inhibition of neurons within DMH after PGE2 in MPA.** Under resting conditions in urethane-chloralose-anesthetized rats, BAT SNA was typically low with only a few, small-amplitude bursts being recorded every minute. Resting values for BAT temperature, expired CO2, HR, and mean arterial pressure before microinjection of PGE2 into the MPA are shown in Table 1. Unilateral microinjection of PGE2 into the MPA produced a dramatic increase in BAT SNA and significant (P < 0.01) increases in BAT thermogenesis, expired CO2, HR, and mean arterial pressure (Fig. 1A). These responses were rapid in onset, with initial increases beginning within minutes and typical peak responses occurring within 20 min of the microinjection of PGE2. As illustrated in Fig. 1, the PGE2-evoked responses were also long lasting: in rats receiving subsequent microinjections of saline vehicle into the DMH, the augmented levels of BAT SNA, BAT temperature, and expired CO2 remained within 20% of peak values for at least 40 min. In the control response to microinjection of PGE2 illustrated in Fig. 1A, PGE2 produced peak increases in BAT SNA of 209%, in BAT temperature of 1.0°C, in expired CO2

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<th>Effect of saline, muscimol, or kynurenate into DMH on the cardiovascular, metabolic and thermogenic responses to PGE2 into MPA</th>
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Values are means ± SE (saline, n = 4; Musc, n = 5; Kyn, n = 5; Kyn outside the DMH, n = 5). Values for physiological variables are provided under resting (baseline) conditions, at the peak response within 20 min of the microinjection of PGE2 into the medial preoptic area (MPA) and at the minimum level within 5 min after subsequent bilateral microinjections of saline, muscimol (Musc), or kynurenic acid (Kyn) into the dorsomedial hypothalamus (DMH) or surrounding areas. Note, one animal received an injection of Musc outside the DMH; the response in this animal was similar to those in rats receiving an injection of Kyn outside the DMH. However, because this was the only animal receiving this treatment it was excluded from analysis. BAT, brown adipose tissue; SNA, sympathetic nerve activity. *P < 0.05, increase compared with the baseline value; †P < 0.05, compared with the saline control response.
of 0.8%, in HR of 136 beats/min, and in mean arterial pressure of 22 mmHg. Table 1 contains the mean values of the responses to microinjection of PGE2 into the MPA. Bilateral microinjection of saline vehicle into the DMH had no effect on the levels of the PGE2-evoked responses in any of the thermogenic or cardiovascular variables (Fig. 1A, Table 1).

Bilateral microinjections of the GABA_A receptor agonist muscimol into the DMH significantly reduced the levels of BAT SNA, BAT temperature, expired CO2, HR, and mean arterial pressure from those produced by PGE2 administration into the MPA to levels that were not different from those under control conditions before PGE2 administration (Fig. 1B). BAT

Fig. 1. Reversal of the PGE2-evoked increases in brown adipose tissue (BAT) thermogenesis and heart rate (HR) by microinjection of the GABA_A receptor agonist muscimol or the excitatory amino acid (EAA) receptor antagonist kynurenate (Kyn) into the dorsomedial hypothalamus (DMH). Microinjection of PGE2 into the medial preoptic area (MPA) (open arrows) increased BAT sympathetic nerve activity (SNA), BAT temperature, expired CO2, heart rate, and mean arterial pressure. A: microinjection of saline vehicle into the DMH (filled arrow) had no effect on any of the measured variables. In different rats, bilateral microinjections of muscimol (B) or kynurenate (C) into the DMH (filled arrows) completely reversed the PGE2-evoked responses. Vertical scale bar represents peak-to-peak BAT SNA of 80 μV in A, 80 μV in B, and 250 μV in C. bpm, Beats/min.
SNA began to decrease immediately after the microinjections of muscimol into DMH, with maximum reductions in BAT SNA occurring within 11 ± 4 min. In the example in Fig. 1B, BAT SNA was reduced from a peak response level of +235% of control to a nadir of +1% of control. Similarly, the increases in BAT temperature, expired CO₂, HR, and mean arterial pressure evoked by PGE₂ administration were reversed by microinjections of muscimol into the DMH (Fig. 1B), returning to pre-PGE₂ levels within 9 ± 2, 6 ± 1, 14 ± 4, and 8 ± 2 min, respectively, of the blockade of EAA receptors in DMH. As shown in Fig. 1B, microinjection of muscimol into the DMH reduced BAT temperature from a peak of 1.5°C above the resting control level to a nadir of +0.9% below the resting control level, HR from a maximum increase of +148 to +18 beats/min, and mean arterial pressure from a peak of 51 mmHg above the resting control level to −8 mmHg below the resting control level. Table 1 provides the mean values of these variables after microinjections of muscimol into the DMH.

Paralleling the effects produced by inhibiting neurons in DMH with microinjections of muscimol, blockade of EAA receptors in DMH with kynurenic acid subsequent to microinjection of PGE₂ into MPA also reversed the increases in BAT SNA, BAT temperature, expired CO₂, HR, and mean arterial pressure produced by PGE₂ administration. BAT SNA began to decrease immediately after microinjections of kynurenic acid into DMH, with maximum reductions in BAT SNA occurring within 8 ± 2 min. In the example in Fig. 1C, BAT SNA was reduced from a peak response level of +336% of control to a nadir of +8% of control. Similarly, the increases in BAT temperature, expired CO₂, HR, and mean arterial pressure evoked by PGE₂ administration were reversed by microinjections of kynurenic acid into the DMH (Fig. 1C), returning to pre-PGE₂ levels within 9 ± 2, 7 ± 2, 11 ± 2, and 7 ± 2 min, respectively, of the blockade of EAA receptors in DMH. As shown in Fig. 1C, microinjection of kynurenic acid into the DMH reduced BAT temperature from a peak of 0.9°C above the control level to −0.5°C below the resting level, expired CO₂ from a peak of +0.9% to −0.2% below resting level, HR from a maximum increase of +70 to +19 beats/min, and mean arterial pressure from a peak of 6 mmHg above the resting control to −4 mmHg below the resting control level. Table 1 provides the mean values of these variables after microinjections of kynurenic acid into the DMH.

The locations of the microinjection sites targeting the DMH and the MPA are shown in Fig. 2. The approximate centers of the injection sites targeting the DMH were located in an area extending dorsally from the dorsal aspect of the ventromedial hypothalamus (VMH) to just medial to the mamillothalamic tracts. The majority of injection sites that were capable of completely reversing the PGE₂-evoked response were located within or just dorsal to the borders of the DMH [according to the atlas of Paxinos and Watson (24)]. Injection sites located more dorsally, near the level of the mamillothalamic tract, or those located ventral to DMH, within the VMH, were less effective at reversing the PGE₂-evoked responses than those located within the DMH (Fig. 2, Table 1). The approximate centers of the microinjections of PGE₂ into the MPA were located primarily dorsal and medial to the anteroventral preoptic nucleus within the boundaries of the MPA (24). These microinjection sites are in the same locations as those previously reported (31) to be thermogenically responsive to prostaglandin.

Fig. 2. Location of microinjection sites targeting the DMH and MPA. Top: representative photomicrographs illustrating the fast green dye deposits (arrows) at the sites of microinjections within the area of the DMH (A) and MPA (B). Bottom: locations of the microinjection sites for rats receiving injections of saline (diamonds), muscimol (circles), or kynurenate (squares) plotted on atlas drawings of the rat hypothalamus (bregma −3.5 and −0.26 mm, respectively; 24). Open symbols represent sites at which injections attenuated the PGE₂-evoked brown adipose tissue sympathetic nerve response by <60%, whereas filled symbols represent sites at which injections attenuated this response by >85%.
The present study is the first to demonstrate that ionotropic EAA receptor-mediated activation of neurons in the DMH is necessary for the increases in BAT SNA and thermogenesis as well as those in expired CO2, HR, and mean arterial pressure evoked by PGE2 administration into the MPA of anesthetized rats. The present data are consistent with, and extend, an earlier study in which inhibition of DMH neurons indicated a critical role of DMH neurons in mediating the increase in core body temperature and the tachycardia evoked by central administration of PGE2 (32).

It has been suggested that the RPAs contains the sympathetic premotor neurons responsible for driving the discharge in the sympathetic nerves innervating BAT (15, 17, 18). This suggestion is based on the observations that neurons within the RPAs are labeled by injections of pseudorabies virus into BAT (3, 4, 23), that neurons within the RPAs project directly to the intermediolateral cell column (2, 14), that BAT SNA can be driven by disinhibition of neurons within the RPAs (18), and that inhibition of RPAs neurons (17, 20) or blockade of EAA receptors within the RPAs (15) prevents the sympathetically mediated increase in BAT thermogenesis evoked by central administration of PGE2. Taken together with the results of the current study, these observations support a model for the central activation of BAT thermogenesis during the acute phase of fever in which PGE2 acts within the MPA to produce an EAA-mediated increase in the discharge of neurons in DMH which, in turn, drive sympathetic outflow to BAT via activation of neurons within the RPAs.

Our data do not allow discrimination between a direct activation of DMH neurons by glutamatergic neurons in MPA and an “indirect” activation that might occur through disinhibition of DMH neurons, allowing the influence of an active EAA input to be revealed. However, it is noteworthy that increases in BAT temperature can be produced either by disinhibition of neurons within the DMH using microinjections of the GABA_4 receptor antagonist bicuculline or by transection of the neuraxis between the MPA and the DMH (7, 33). Both of these observations are consistent with the existence of a tonically active inhibitory input from the MPA to the DMH, although this has yet to be directly tested. Although the present study reveals an EAA input to neurons of the DMH, the source of this excitatory drive remains to be determined.

Whether the thermogenic output of the DMH influences sympathetic premotor neurons of the RPAs directly, as suggested previously (32), or whether this influence occurs via a multisynaptic pathway, remains unknown. Regarding the latter, a marked reduction in the cardiovascular responses to disinhibition of DMH neurons results from inhibition of neurons in the dorsolateral periaqueductal gray (9) and increases in BAT temperature can be elicited from the lateral region of the caudal periaqueductal gray (8). In the present study, many of the DMH sites at which microinjections of muscimol or kynurenic acid reversed the PGE2-evoked responses were within the dorsal region of the DMH, shown anatomically to contain retrogradely labeled cells after tracer injections into RPAs (12). However, in a significant number of animals, equally effective DMH microinjections were made into sites outside of the dorsal-most aspect of the DMH and the area just dorsal to the DMH (the area that contains neurons that project directly to the RPAs). In the latter cases, the possibility that the injectate diffused to the RPAs-projecting cells of the dorsal region of the DMH cannot be ruled out. It is also interesting to note that in the present study injection of kynurenic acid into the VMH did not reverse the PGE2-evoked responses. Several studies have suggested that the VMH plays a role in BAT thermogenesis elicited from the preoptic area of the hypothalamus (1, 13); however, Zaretskaia et al. (33) convincingly argued that these studies, which employed high doses and large injection volumes into the VMH, were likely to have had their inhibitory effects by diffusion of the injectate to the DMH.

As we discussed previously (15), we interpret the PGE2-evoked increases in BAT temperature to be secondary to the increase in BAT SNA. Consistent with this interpretation is our observation that the time course of the increase in BAT temperature always followed and paralleled that of the BAT SNA. Similarly, we consider the increase in expired CO2 after PGE2 administration to be secondary to the increased metabolism in BAT and cardiac muscle associated with the PGE2-evoked thermogenesis and tachycardia, respectively. Given that the increase in HR evoked by disinhibition of neurons within the RPAs is mediated by an increase in cardiac SNA (5) and that PGE2-evoked tachycardia is mediated by neurons within the RPAs (15, 17), we suggest that PGE2-evoked tachycardia is mediated by an increase in cardiac sympathetic outflow. It is likely that the PGE2-evoked increase in HR is associated with an increase in cardiac output that may contribute to the increase in mean arterial pressure, although we cannot rule out a role of increased vascular resistance in this response.

In summary, the present study demonstrates that activation of EAA receptors within the DMH is required for the thermogenic, metabolic, and cardiovascular effects resulting from microinjection of PGE2 into the MPA. Further studies will be required to determine the source(s) of the EAA inputs responsible for the excitation of the neurons within the DMH that are involved in thermogenic and tachycardic responses.

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GRANTS

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