Brown adipose tissue thermogenesis contributes to fentanyl-evoked hyperthermia

Wei-Hua Cao and Shaun F. Morrison
Neurological Sciences Institute, Oregon Health and Science University, Beaverton, Oregon
Submitted 29 September 2004; accepted in final form 23 November 2004

Cao, Wei-Hua, and Shaun F. Morrison. Brown adipose tissue thermogenesis contributes to fentanyl-evoked hyperthermia. Am J Physiol Regul Integr Comp Physiol 288: R723–R732, 2005. First published December 2, 2004; doi:10.1152/ajpregu.00669.2004.—μ-Opioid receptor activation increases body temperature and affects cardiovascular function. In the present study, fentanyl was administered intravenously [100 μg/kg (300 nmol/kg) iv] and intracerebroventricularly [3.4 μg (10 nmol) in 10 μl icv] in urethane-chloralose-anesthetized, artificially ventilated rats. Increases in brown adipose tissue (BAT) sympathetic nerve activity (SNA) (peak, +326% of control), BAT temperature (peak, +0.8°C), renal SNA (peak, +146% of control), and heart rate (HR; peak, +32 beats/min) produced by intravenous fentanyl were abolished by premanillary transection of the neuraxis but were mimicked by intracerebroventricular administration of fentanyl, which also increased arterial pressure (AP; peak, +12 mmHg). Pretreatment with the opioid antagonist naloxone (100 nmol in 10 μl icv) eliminated the intracerebroventricular fentanyl-evoked responses. Microinjection of glycine (0.5 M, 60 nl) to inhibit local neurons in the rostral raphe pallidus (RPa) selectively reversed the intracerebroventricular fentanyl-evoked increases in BAT SNA and HR, while the fentanyl-evoked excitation in RSNA, the pressor responses, and the tachycardic responses were reversed by inhibition of neurons in the rostral ventrolateral medulla (RVL). Prior inhibition of neurons in the dorsomedial hypothalamus eliminated the intracerebroventricular fentanyl-evoked increases in BAT SNA, BAT temperature, and HR, but not those in RSNA or AP. These results indicate that activation of central μ-opioid receptors with fentanyl can elicit BAT thermogenesis and cardiovascular stimulation through excitation of the sympathetic outflows to BAT, kidney, and heart. Activation of neurons in the rostral RPa and RVL are essential for the increases in BAT thermogenesis and renal sympathoexcitation, respectively, induced by activation of central μ-opioid receptors. BAT thermogenesis could contribute to fentanyl-evoked hyperthermia, particularly in infants where BAT plays a significant role in thermoregulation.

renal sympathetic nerve; heart rate; arterial pressure; μ-opioid receptor; naloxone; raphe pallidus; dorsomedial hypothalamus; rostral ventrolateral medulla

THE ENDOGENOUS OPIOD PEPTIDES and opiate alkaloids affect many physiological processes, including analgesia, neurotransmission, function, food consumption, and temperature regulation (4). Although the direction and magnitude of the effects of opiate drugs on body temperature can depend on many factors, including species, age, route of administration, and ambient temperature, the primary determinants are the opioid receptor selectivity of the compound and the dose administered. Low doses and high ambient temperature favor opioid-induced hyperthermia, whereas high doses and low ambient temperature favor hypothermia (2). Three main classes of opioid receptors, μ, κ, and δ, have been identified (19, 25, 34, 51), each of which can influence thermoregulation. Activation of central μ-opioid receptors with agonists such as morphine, DAMGO, or PL017 produces hyperthermia (1, 48), whereas activation of κ-receptor agonists produces hypothermia (2, 48), and the role of the δ-opioid receptors in thermoregulation remains unclear. In the present study, we sought to determine if thermogenesis in brown adipose tissue (BAT) plays a role in the hyperthermic response to central μ-opioid receptor activation with fentanyl.

Sympathetically regulated thermogenesis in BAT plays a significant role in thermoregulation in small mammals, including the rat, and in human infants, providing metabolic heat production to maintain core temperature in response to a cold environment (10). The central neural circuits regulating BAT sympathetic outflow and thermogenesis (38) include BAT sympathetic premotor neurons in the rostral raphe pallidus (RPa) (5, 11, 41), a site from which large increases in BAT sympathetic nerve activity (SNA) are elicited by local blockade of a tonic GABAergic inhibition (41), and neurons in the dorsomedial hypothalamus (DMH), where a similar disinhibition elicits sympathoexcitatory responses in BAT SNA and in renal SNA (RSNA) accompanied by a rise in BAT temperature and an elevated heart rate (HR) and arterial blood pressure (AP) (12, 17, 24, 46, 58). The increases in BAT SNA and HR elicited by bicuculline microinjection into the DMH are primarily dependent on neurons in the RPa (12, 47), whereas the increases in RSNA and AP are attenuated by inhibition of neurons in the rostral ventrolateral medulla (RVL) (12, 17, 24).

Little is known, however, about the central mechanisms and pathways underlying opiate-evoked effects on thermoregulation and on cardiovascular regulation. The aims of the present study were to determine the effects of central administration of the μ-opioid receptor agonist fentanyl on BAT thermogenesis and on cardiovascular function and to identify the sites of putative sympathetic premotor neurons mediating the opioid-induced alterations in thermoregulatory and cardiovascular function. The results increase our understanding of the central mechanisms eliciting hyperthermia, renal sympathoexcitation, and cardiac stimulation during administration of fentanyl, a frequently used analgesic in surgical settings, including those involving infants where BAT plays a significant role in thermoregulation.

METHODS

General procedures. All procedures conform to the regulations detailed in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University. Experiments were performed on 28 Sprague-Dawley rats (300–400 g) anesthetized with intravenous urethane (800 mg/kg) and α-chloralose (80 mg/kg) after induction with 3% isoflurane in 100% O2 and cannulation of the femoral vein, the femoral artery for measurement of arterial pressure (AP), and the trachea for artificial ventilation. Heart rate (HR) and mean AP (MAP) were obtained from the AP recording with a Gould Biotach and a low-pass filter, respectively. The animals

Address for reprint requests and other correspondence: S. F. Morrison, Neurological Sciences Institute, Oregon Health and Science Univ., 505 NW 185th Ave., Beaverton, OR 97006 (E-mail: morrisos@ohsu.edu).

http://www.ajpregu.org
0363-6119/05 $8.00 Copyright © 2005 the American Physiological Society

R723
were paralyzed with d-tubocurarine chloride (initially 0.6 mg/rat iv; thereafter 0.3 mg/h iv) and then artificially ventilated with 100% O2 at a minute volume of 150–250 ml. End-expiratory CO2 was continuously monitored and maintained between 3.5 and 4.5% by adjusting ventilation volume and rate. Rats were placed prone in a stereotaxic apparatus with the bite bar –12 mm below the interaural line. In some animals, a bilateral transection of the neuraxis was made by passing a spatula through holes in the skull just rostral to the transverse sinus.

These transections enter the brain dorsally between the colliculi and exit ventrally just caudal to the mamillary nuclei. Colonic temperature was maintained at 37°C with a thermostatically controlled heating pad and lamp. BAT temperature was monitored with a thermocouple inserted beneath the left interscapular brown fat pad.

Sympathetic nerve recording. The left postganglionic renal sympathetic nerve was dissected through a retrosphenoidal incision and identified near the renal artery. Postganglionic BAT SNA was recorded from a small nerve bundle dissected from the ventral surface of the right interscapular BAT pad after the pad was divided along the midline and reflected laterally. The central cut end of each nerve was placed on a bipolar hook recording electrode and immersed in mineral oil to avoid drying. The efferent nerve activity was differentially amplified (20,000 times) and filtered between 100 and 3,000 Hz with a Cyberamp 380 (Axon Instruments, Foster City, CA), digitized and recorded onto a computer hard drive using data-acquisition hardware and software (Biopac, CA: Spike2, Cambridge Electronic Design).

Levels of sympathetic nerve discharge were quantified from measurements of integrated SNA (iSNA) determined after full-wave rectification, integration over 1-s time intervals, and subtraction of noise levels determined after ganglionic blockade with trimethaphan (1.0 mg in 0.1 ml saline iv). Changes in iSNA are expressed as a percentage of the pretreatment control level by dividing the mean level of iSNA during the 1 min of the peak or nadir of the response by the mean level of iSNA during the 5 min immediately before the treatment. Student’s t-tests with P < 0.05 was considered to indicate a significant change in the measured variables.

Administration of drugs. Intravenous injections of fentanyl (Sigma, St. Louis, MO) were given in a dose of 300 nmol/kg (100 μg/kg), which is a low, hyperthermic dose (18, 53). Intracerebroventricular (icv) injections of fentanyl [10 nmol (3.4 μg) in 10 μl saline in 60 s] and naloxone (100 nmol in 10 μl saline in 60 s; Sigma, St. Louis, MO) were given into the lateral ventricle using a 100-μl microsyringe connected to an injection cannula (26-gauge tube) whose tip was stereotaxically positioned with respect to bregma at 1.4 mm posterior, 1.5 mm lateral to the midline and 3.0–3.5 mm ventral to the dura. All drugs were dissolved in normal saline, and saline vehicle (150 mM, 10 μl) was given intracerebroventricularily in 6 rats as a control for injectate volume.

Microinjections of drugs into restricted brain regions were accomplished through a stereotaxically positioned micropipette (tip outside diameter: 20 μm) using a pressure-injection apparatus, and the volume of each microinjection was estimated by observing the displacement of the fluid meniscus with the use of a calibrated reticle. Microinjections (30 nmol, 60 nl) of glycine (0.5 M) were made into the rostral RPa (+3.1 mm anterior, 0.0 mm lateral, −2.7 mm ventral to calamus scriptorius) or RVLM (+2.6 mm anterior, 2.0 mm lateral, and −2.5 mm ventral to calamus scriptorius) to provide a reversible inhibition of local neuronal activity during the course of the response to intracerebroventricular fentanyl. Microinjections (120 pmol, 60 nl) of muscimol (2 mM) were made into the DMH (−4.2 mm caudal, 0.6 lateral, and −8.3 mm ventral to bregma) to provide a long lasting inhibition of DMH neurons beginning before intracerebroventricular fentanyl. Microinjections (1 nmol, 100 nl) of naloxone (10 mM) were made into the RVLM to block local opioid receptors.

Histological localization of injection sites. At the end of each experiment, the microinjection pipettes were retracted vertically, refilled with 2% solution of fast green dye, and repositioned at the sites of the microinjections, and dye was electrophoretically deposited (20 μA, anodal direct current for 10 min). The animal was transcardially perfused with normal saline followed by 10% formalin solution. Coronal vibratome sections (100 μm) of the brain stem containing the dye spots were photographed, and the location of the microinjection sites were plotted on drawings from a rat stereotaxic atlas (43).

RESULTS

Intravenous injections of fentanyl. As illustrated in the example in Fig. 1A, intravenous administration of fentanyl [300 nmol/kg (100 μg/kg)] produced an activation of BAT SNA, an increase in BAT temperature, and a stimulation of RSNA. Intravenous fentanyl caused initial precipitous falls in HR and AP that were followed by a tachycardia and a pressor response. The time courses of the mean responses to intravenous fentanyl administration are illustrated in Fig. 1C, and the values are presented in Table 1. Transections of the neuraxis between 6 and 8 mm caudal to bregma (from midcolliculi dorsally to just caudal to the mamillary nuclei ventrally) increased the basal levels of BAT SNA, RSNA (see absolute levels in Table 1), and resting HR. Whereas intravenous administration of fentanyl in the intact animal produced a sympathetically mediated thermogenic and cardiovascular stimulation, Fig. 1B shows that after separation of the hypothalamus from the brain stem, intravenous fentanyl depressed BAT SNA and RSNA and reduced AP and HR. The time courses of the mean responses to intravenous fentanyl after transection are illustrated in Fig. 1C, and the mean changes in these variables are given in Table 1. These sympathoinhibitory and cardiovascular depressor responses to intravenous fentanyl in premamillary-transected rats were eliminated by bilateral microinjection of naloxone into the rostral ventrolateral medulla (RVLM). The time courses of the mean responses to intravenous fentanyl in transected rats after microinjection of naloxone into RVLM are illustrated in Fig. 1C, and the values are presented in Table 1.

Intracerebroventricular injections of fentanyl. To assess whether the effects of intravenous fentanyl arose from central or peripheral opioid receptors, fentanyl [10 nmol (3.4 μg) in 10 μl] was administered intracerebroventricularily in 11 rats. As shown in Fig. 2A, intracerebroventricular administration of fentanyl elicited sympathetic, thermogenic, and cardiovascular excitations similar to those seen with intravenous administration of fentanyl: fentanyl elevated BAT SNA, BAT temperature, and RSNA and, after initial falls, increased MAP and HR. No changes in any of these variables occurred after microinjection of the same volume of saline into the lateral ventricle (data not shown). As shown in the mean time courses of the changes evoked by intracerebroventricular fentanyl (Fig. 2C), the onset and peak of the increases in BAT SNA lagged those of the increases in RSNA. Both sympathetic responses were sustained for 20–30 min. Intracerebroventricular injection of fentanyl produced significant (P < 0.01, n = 11) mean maximal increases in BAT SNA of +336 ± 45% of control, in RSNA of +149 ± 14% of control, in BAT temperature of +0.9 ± 0.1°C, in MAP of +18 ± 3 mmHg from an average MAP of 95 ± 4 mmHg, and in HR of +39 ± 6 beats/min from a mean resting HR of 381 ± 12 beats/min.

To determine whether the thermogenic and cardiovascular sympathoexcitatory responses to intracerebroventricular fentanyl were specific for stimulation of opioid receptors, we examined the effect of intracerebroventricular fentanyl 10 min
after intracerebroventricular pretreatment with the nonselective opioid receptor antagonist naloxone (100 nmol in 10 µl) in 5 rats. Although intracerebroventricular administration of naloxone was without effect on any of the measured variables, pretreatment with intracerebroventricular naloxone completely blocked the sympathoexcitatory responses produced by intra-cerebroventricular fentanyl (Fig. 2B). The mean time courses of the sympathetic, thermogenic, and cardiovascular variables following intracerebroventricular fentanyl after pretreatment with intracerebroventricular naloxone are shown in Fig. 2C and indicate that there were no differences in any of the measured variables between the control values following naloxone pretreatment and those at the times after intracerebroventricular fentanyl where peaks occurred in non-pretreated animals.

Role of RPa neurons in the BAT thermogenic effects evoked by intracerebroventricular fentanyl. To determine if sympathetic premotor neuron populations in the RPa and in the RVLM might mediate the BAT thermogenic, renal, and cardiovascular sympathoexcitatory responses induced by intracerebroventricular fentanyl, we used microinjections (30 nmol, 100 nl) of naloxone (Nlx, 10 mM) into the rostral ventrolateral medulla (RVLM). Note the elimination of the sympathoinhibitory and cardiovascular depressor responses to iv fentanyl after microinjection of naloxone into the RVLM.

Table 1. Effect of premamillary transection on the sympathetic, thermogenic, and cardiovascular responses to intravenous fentanyl

<table>
<thead>
<tr>
<th></th>
<th>Intact (n = 13)</th>
<th>Pre-tranX (n = 8)</th>
<th>Post-tranX, Post-Nlx-RVLM (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IBAT SNA (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100%</td>
<td>+32% ± 68%</td>
<td>100%</td>
</tr>
<tr>
<td>Fentanyl iv</td>
<td>(1.0 ± 0.2)</td>
<td>(4.0 ± 0.7)</td>
<td>(2.3 ± 0.7)</td>
</tr>
<tr>
<td><strong>BAT temp, °C</strong></td>
<td>34.3 ± 0.3</td>
<td>+0.8 ± 0.2</td>
<td>34.3 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>34.0 ± 0.3</td>
<td>(1.3 ± 0.4)</td>
<td>(1.0 ± 0.3)</td>
</tr>
<tr>
<td>Fentanyl iv</td>
<td>(9.7 ± 2.1)</td>
<td>(20.8 ± 3.5)</td>
<td>(18.5 ± 2.7)</td>
</tr>
<tr>
<td><strong>iRSNA (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100%</td>
<td>+146 ± 19%</td>
<td>100%</td>
</tr>
<tr>
<td>Fentanyl iv</td>
<td>(11.9 ± 2.5)</td>
<td>(20.8 ± 3.5)</td>
<td>(18.5 ± 2.7)</td>
</tr>
<tr>
<td><strong>MAP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>98 ± 5</td>
<td>+8 ± 3</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>Fentanyl iv</td>
<td>(98 ± 5)</td>
<td>(98 ± 5)</td>
<td>(98 ± 5)</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td>31 ± 2</td>
<td></td>
<td>421 ± 19</td>
</tr>
<tr>
<td>Control</td>
<td>433 ± 19</td>
<td></td>
<td>(11 ± 11)</td>
</tr>
<tr>
<td>Fentanyl iv</td>
<td>(11 ± 11)</td>
<td></td>
<td>(11 ± 11)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Intravenous fentanyl (fentanyl iv) values are peak changes; nerve activities [integrated brown adipose tissue (IBAT) sympathetic nerve activity (SNA) and integrated renal SNA (iRSNA)] are expressed as % of pre-fentanyl control values for each of the three conditions: intact; transection of the neuraxis immediately caudal to the mamillary bodies (tranX); and tranX followed by microinjection of naloxone (Nlx) into the rostral ventrolateral medulla (RVLM) (Post-tranX, Post-Nlx-RVLM). Values in parentheses are nerve activity values in μV/s. Response values for each animal were obtained as the change in the mean value of the variable during the 1-min interval surrounding the peak of the response. Temp, temperature; MAP, mean arterial pressure; HR, heart rate. Significantly different response amplitude from Post-tranX transection without Nlx in the RVLM: *P < 0.05, †P < 0.01.
(60 nl) of glycine (0.5 M) to inhibit local neurons in RPa or in RVLM (in separate experiments) during the course of the sympathetic responses to intracerebroventricular fentanyl. Glycine was microinjected into RPa at a mean delay of 12 min following completion of the intracerebroventricular fentanyl. As illustrated for the RPa in Fig. 2A, microinjection of the saline vehicle into either RPa or RVLM had no effect on any of the responses to intracerebroventricular fentanyl.

As illustrated in the example in Fig. 3A, ~15 min following the end of the fentanyl injection into the lateral ventricle, at a time when the increases in thermogenic, renal, and cardiovascular sympathoexcitatory variables produced by intracerebroventricular fentanyl were well developed, microinjection of glycine into the RPa produced a prompt and complete reversal of the fentanyl-evoked increase in BAT SNA. The fentanyl-evoked level of BAT SNA just before glycine microinjection (299 ± 59% of control; n = 6) was not different from that (302 ± 33% of control; n = 11) at the same time after intracerebroventricular fentanyl in control animals that received either saline or no injections into RPa (Table 2). Five minutes later, however, the BAT SNA level in the control group was 380 ± 47% of control, while 5 min after the microinjection of glycine into RPa, the BAT SNA was reduced (P < 0.01) to 115 ± 6% of control. Subsequent to this reduction in BAT SNA, microinjection of glycine into RPa appeared to foreshorten the fentanyl-evoked rise in BAT temperature (Fig. 3A).

Role of RPa neurons in the cardiovascular sympathoexcitatory effects evoked by intracerebroventricular fentanyl. Microinjection of glycine into RPa also reversed the fentanyl-evoked tachycardia (Table 2): in the example in Fig. 3A, 5 min after the microinjection of glycine into RPa, HR had fallen by 41 beats/min, eliminating the fentanyl-evoked increase of 34 beats/min. In contrast to the effect of microinjection of glycine into RPa, the time course and amplitude of the fentanyl-evoked increase in RSNA appeared unaltered (Fig. 3A) by microinjection of glycine into RPa.

Fig. 2. Thermogenic and cardiovascular sympathoexcitatory responses to intracerebroventricular (icv) administration of fentanyl and the effects of icv pretreatment with naloxone. A: icv fentanyl [time 0, 10 nmol (3.4 μg) in 10 μl] evoked increases in iBAT SNA (peak: +267% of control), Tbat (peak: +0.5°C from 34.3°C), iRSNA (peak: +206% of control), AP (peak: +25 mmHg from 96 mmHg), and HR (peak: +56 beats/min from 438 beats/min). Microinjection (60 nl) of saline vehicle into rostral raphe pallidus (RPa) during the icv fentanyl-evoked responses had no effect on any of the measured variables. B: after icv pretreatment with naloxone (100 nmol in 10 μl), icv fentanyl failed to elicit changes in any of the measured variables: the latencies to the peak response values in A, the corresponding values in B are iBAT SNA, 101% of control; Tbat, 34.3°C from a control of 34.4°C; iRSNA, 111% of control; AP, 81 mmHg from a control of 84 mmHg; HR, 503 beats/min from a control of 505 beats/min. C: time courses of the mean control responses to icv fentanyl (●, n = 11) and after icv pretreatment with naloxone (○, n = 5). Note the elimination of the sympathoexcitatory and cardiovascular responses to icv fentanyl after icv pretreatment with naloxone.
the control group was 227 ± 13% of control, while 5 minutes after the microinjection of glycine into RPa, the RSNA was 163 ± 10% of control, indicating a more rapid (P < 0.05) decline of the fentanyl-evoked increase in RSNA. The effect of microinjection of glycine into RPa on the fentanyl-evoked increase in MAP was variable, and the group data (Table 2) indicate that the mean value of MAP at 5 min following microinjection of glycine into RPa was not significantly different from that at the peak of the response. Figure 3A illustrates one of the cases in which the fentanyl-evoked increase in MAP was reduced at 5 min following microinjection of glycine into RPa. The effects of microinjection of glycine into RPa on the responses to intracerebroventricular fentanyl are presented in Table 2.

**Role of RVLM neurons in the sympathoexcitatory effects evoked by intracerebroventricular fentanyl.** Bilateral microinjections of glycine into the RVLM (mean: −11 min after intracerebroventricular fentanyl) produced a prompt and complete reversal of the fentanyl-evoked increase in RSNA (Fig. 3B). The fentanyl-evoked level of RSNA just before glycine microinjection (216 ± 14% of control) was not different from that (224 ± 12% of control) at the same time after intracerebroventricular fentanyl in control animals that received saline or no injection into RVLM (Table 2). However, 5 min later, the RSNA level in the control group was 227 ± 13% of control, while 5 min after the microinjections of glycine into the RVLM, the RSNA was reduced (P < 0.01) to 54 ± 7% of control. As illustrated in Fig. 3B, bilateral microinjections of glycine into the RVLM resulted in a marked reduction in MAP and a reduction in HR, reversing both the fentanyl-evoked pressor response and tachycardia (Table 2). The fentanyl-evoked level of BAT SNA (see Table 2) just before glycine microinjection (336 ± 56% of control) was not different from that (302 ± 33% of control) at the same time after intracerebroventricular fentanyl in control animals that received saline or no injection into RVLM. Five minutes later, the BAT SNA level in the control group was 380 ± 47% of control, whereas 5 min after the microinjections glycine into RVLM, the BAT SNA was 309 ± 71% of control, which was not different from the value in the control group at this comparable time point. In the example in Fig. 3B, bilateral microinjection of glycine into RVLM reduced BAT SNA from the fentanyl-evoked level but did not affect the fentanyl-evoked rise in BAT temperature (Table 2). Thus, although microinjection of glycine into RVLM could attenuate the fentanyl-evoked increase in BAT SNA in some animals, it did not significantly alter the mean time course of the fentanyl-evoked responses in BAT SNA or BAT temperature. The effects of microinjections of glycine into RVLM on the responses to intracerebroventricular fentanyl are presented in Table 2.

**Role of neurons in the dorsomedial hypothalamus in the sympathetic responses to intracerebroventricular fentanyl.** To determine whether neurons in DMH play a role in mediating the thermogenic BAT or the cardiovascular responses induced by intracerebroventricular fentanyl, we used bilateral microinjections (each 120 pmol in 60 nl) of the GABAA agonist muscimol (2 mM) to inhibit local neurons in DMH before the intracerebroventricular administration of fentanyl. Microinjection of muscimol into DMH significantly lowered MAP and HR but was without effect on basal levels of BAT SNA, BAT temperature, or RSNA (Table 3). Intracerebroventricular fentanyl, delivered 11 ± 3 min after microinjections of muscimol into DMH, failed to elicit the increases in BAT SNA, BAT temperature, or HR that were observed in untreated rats, while the fentanyl-evoked increases in RSNA and MAP and the initial fentanyl-evoked falls in MAP and in HR were preserved (compare Figs. 4A and 2A; compare Tables 2 and 3; and see Fig. 4B).

**Histological localization of microinjection sites in the rostral RPa and RVLM.** At the end of each experiment, each microinjection pipette was refilled with a 1% solution of fast green dye, positioned at the sites of the microinjections, and dye was electrophoretically deposited. Composite plots and representative histological sections containing dye deposits showing the locations of glycine microinjections in the rostral RPa and in the RVLM and of muscimol microinjections into DMH are shown in Fig. 5. The glycine microinjection sites in the RVLM (Fig. 5A) were all ventral to the nucleus ambiguus and immediately caudal to the facial nucleus, in a region containing sympathetic premotor neurons whose activity is essential for the maintenance of normal vasoconstrictor sympathetic tone and AP (8, 16, 44). The glycine microinjection...
sites in the RPa (Fig. 5B) were localized in the ventral midline medulla at a level of the rostral RPa extending from the RVLM to the middle of the facial motor nucleus and correspond to the locations of putative sympathetic premotor neurons controlling thermogenesis in BAT (11, 41). The muscimol microinjection sites in the DMH (Fig. 5C) were localized in the dorsal aspect of the DMH, at and rostral to the compact division of the DMH, overlapping the region of DMH from which BAT, renal, and cardiac sympathetic responses can be elicited (12, 17, 24, 58), the DMH area shown to contain neurons necessary for the BAT thermogenic responses to application of PGE2 into the medial preoptic area (29, 57), and a region of the DMH containing neurons with direct projections to the RPa (23, 46) and the RVLM (17).

DISCUSSION

The major findings of this study are 1) that stimulation of central μ-opioid receptors by intracerebroventricular fentanyl produced sympathoexcitatory responses to icv administration of fentanyl; and 2) that the BAT thermogenic and the tachycardic responses to intracerebroventricular fentanyl are mediated through activation of neurons in the DMH and, in turn, putative sympathetic premotor neuron populations in RPa, while the renal sympathetic and pressor responses to intracerebroventricular fentanyl are dependent principally upon activation of sympathetic premotor neuron populations in the RVLM, but not on activation of neurons in the DMH. Additionally, inhibition of RVLM neuronal activity can reduce the fentanyl-evoked increase in HR. These sympathoexcitatory responses are produced by activation of opioid receptors since the sympathoexcitatory responses can be completely abolished by pretreatment with the opioid receptor antagonist naloxone.

Central or peripheral administration of μ-opioid receptor agonists produces hyperthermic responses in the rat (14, 22, 45, 48, 54, 55), in conjunction with an increase in oxygen consumption (22). The results of the present study are the first to identify BAT thermogenesis as a potential sympathetic

Fig. 3. Effects of microinjection of glycine (Gly) into populations of sympathetic premotor neurons in raphe pallidus (RPa) or RVLM on the thermogenic and cardiovascular sympathoexcitatory responses to icv administration of fentanyl. A: icv fentanyl [time 0, 10 nmol (3.4 μg) in 10 μl] evoked increases in IBAT SNA (peak: 326% of control), Tbat (peak: +0.5°C from 34.2°C), iRSNA (peak: 212% of control), AP (peak: +12 mmHg from a resting MAP of 94 mmHg), and HR (peak: +34 beats/min from a baseline of 336 beats/min). Microinjection (30 nmol, 60 nl) of glycine (Gly, 0.5 M) into rostral RPa reversed the increases in iBAT SNA (5 min post-glycine: 98% of pre-fentanyl control), AP (5 min post-glycine: 93 mmHg), and HR (5 min post-glycine: 329 beats/min) but not that in iRSNA (5 min post-glycine: 157% of pre-fentanyl control). There was a gradual decline in Tbat (8 min post-glycine: 34.4°C). B: icv fentanyl (time 0) evoked increases in iRSNA (peak: 444% of control), Tbat (peak: +1.7°C from 34.4°C), iRSNA (peak: 220% of control), AP (+10 mmHg from a resting MAP of 105 mmHg), and HR (+114 beats/min from a baseline of 315 beats/min). Bilateral microinjections (30 nmol, 60 nl) of glycine (0.5 M) into the RVLM reversed the increases in iRSNA (5 min post-glycine: 56% of pre-fentanyl control) and AP (5 min post-glycine: 73 mmHg) but not those in iBAT SNA (5 min post-glycine: 275% of pre-fentanyl control), Tbat (8 min post-glycine: 36.0°C), or HR (5 min post-glycine: 379 beats/min).
or intracerebroventricular administration of morphine in awake rats, but the rise paralleled that in core temperature and was not accompanied by an increase in oxygen uptake or in BAT GDP binding. The basis is unclear for the differences between these results and those of both the present study showing a centrally evoked, μ-opioid-evoked increase in BAT sympathetic outflow and that of Handler et al. (22) indicating an increased oxygen consumption accompanying the hyperthermia evoked by central μ-opioid administration in awake rats. The finding that μ-opioid receptor stimulation decreases heat exchange (22) and that tail temperature falls during the hyperthermia induced by morphine in the rat (15) suggests that an increased sympathetic cutaneous vasoconstriction also contributes to the hyperthermic response.

Our data indicated a stimulation of sympathetically mediated BAT thermogenesis by the central administration of the μ-opioid agonist fentanyl that is dependent on the activation of neurons in the DMH. These results, in combination with the demonstration that microdialysis of μ-opioid agonists (55) or microinjection of morphine (31) directly into the preoptic anterior hypothalamus (PO/AH) increases core temperature and that μ-opioid agonists reduce the tonic activity and temperature sensitivity of warm-sensitive neurons in the PO/AH (27, 56), are consistent with a model (38) in which a reduction in PO/AH warm-sensitive neuronal discharge would increase BAT sympathetic outflow and BAT thermogenesis through disinhibition of neurons in DMH. A similar mechanism has been proposed for the stimulation of BAT thermogenesis during the febrile response to application of PGE2 into the

Table 3. Effect of microinjection of muscimol into DMH on the sympathetic, thermogenic, and cardiovascular responses evoked by icv fentanyl

<table>
<thead>
<tr>
<th></th>
<th>Pre-muscimol into DMH</th>
<th>Post-muscimol into DMH</th>
<th>Icv Fentanyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iBAT SNA</td>
<td>100%</td>
<td>139 ± 23%</td>
<td></td>
</tr>
<tr>
<td>(1.0 ± 0.3)</td>
<td>(0.8 ± 0.1)</td>
<td>(1.1 ± 0.2)</td>
<td></td>
</tr>
<tr>
<td>BAT temp, °C</td>
<td>34.2 ± 0.1</td>
<td>34.4 ± 0.1</td>
<td>+0.04 ± 0.04</td>
</tr>
<tr>
<td>(34.4 ± 0.1)</td>
<td>(34.4 ± 0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iRSNA</td>
<td>100%</td>
<td>226 ± 24%</td>
<td></td>
</tr>
<tr>
<td>(8.1 ± 1.0)</td>
<td>(9.3 ± 1.7)</td>
<td>(20.7 ± 4.6)</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>89 ± 3</td>
<td>83 ± 2*</td>
<td>+16 ± 4‡</td>
</tr>
<tr>
<td>(99 ± 6)</td>
<td>(100 ± 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>423 ± 13</td>
<td>400 ± 11*</td>
<td>+3 ± 16</td>
</tr>
<tr>
<td>(430 ± 11)</td>
<td>(403 ± 11)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Fentanyl icv values are the integrated SNA values expressed as a percentage of pre-fentanyl [i.e., Post-muscimol microinjection into dorsomedial hypothalamus (DMH)] control values for iBAT SNA and iRSNA and the changes from pre-fentanyl (Post-muscimol into DMH) control levels for the other variables. Means of the actual values are given below in parentheses (iBAT SNA and iRSNA values in parentheses are in μV/s). For each animal, the fentanyl icv response values were obtained as the mean value of the variable during the 1-min interval of maximum response. Statistically different from pre-muscimol control value: *P < 0.01. Statistically different from pre-fentanyl control value: †P < 0.05, ‡P < 0.01.

efferent mechanism contributing to the hyperthermic effect of μ-opioids. In an earlier study (52), BAT temperature was found to increase during the hyperthermia elicited by systemic or intracerebroventricular administration of morphine in awake rats, but the rise paralleled that in core temperature and was not accompanied by an increase in oxygen uptake or in BAT GDP binding. The basis is unclear for the differences between these results and those of both the present study showing a centrally evoked, μ-opioid-evoked increase in BAT sympathetic outflow and that of Handler et al. (22) indicating an increased oxygen consumption accompanying the hyperthermia evoked by central μ-opioid administration in awake rats. The finding that μ-opioid receptor stimulation decreases heat exchange (22) and that tail temperature falls during the hyperthermia induced by morphine in the rat (15) suggests that an increased sympathetic cutaneous vasoconstriction also contributes to the hyperthermic response.

Our data indicated a stimulation of sympathetically mediated BAT thermogenesis by the central administration of the μ-opioid agonist fentanyl that is dependent on the activation of neurons in the DMH. These results, in combination with the demonstration that microdialysis of μ-opioid agonists (55) or microinjection of morphine (31) directly into the preoptic anterior hypothalamus (PO/AH) increases core temperature and that μ-opioid agonists reduce the tonic activity and temperature sensitivity of warm-sensitive neurons in the PO/AH (27, 56), are consistent with a model (38) in which a reduction in PO/AH warm-sensitive neuronal discharge would increase BAT sympathetic outflow and BAT thermogenesis through disinhibition of neurons in DMH. A similar mechanism has been proposed for the stimulation of BAT thermogenesis during the febrile response to application of PGE2 into the
PO/AH (7, 28, 32, 42), a response involving an increase in the sympahtoexcitatory drive to BAT that is also dependent on activation of DMH neurons (29). An interaction of /H9262-opioid receptors and PGE2-sensitive thermogenic effects in the PO/AH is further supported by the demonstration that lipopolysaccharide-evoked fever, which is dependent on production of PGE2 in the PO/AH, can be blocked by a selective /H9262-opioid receptor antagonist (6). Anatomically, among the wide distribution of /H9262-opioid receptors in the CNS, both /H9262-opioid receptor immunoreactive fibers and neurons are located within the PO/AH (30, 37) but also within other regions potentially involved in thermoregulatory control, including the DMH, paraventricular nucleus, and the periaqueductal gray. Thus, while several pieces of indirect evidence support a potential site of action of fentanyl in the PO/AH to increase BAT thermogenesis, this hypothesis will await further investigation.

The results of the present study extend currently available evidence supporting the hypothesis that functionally distinct sympathoexcitatory outflows, such as that to BAT, which mainly regulates the level of lipid metabolism and heat production, and that to the kidney, which primarily controls renal blood flow, are controlled by separate sympathoexcitatory premotor pathways (33, 39). This is suggested first by the basal levels of sympathetic discharge: at normothermia and normal AP, BAT SNA has a very low level of activity, whereas RSNA exhibits tonic, baroreceptor reflex-modulated bursts of activity reflecting an active population of vasoconstrictor sympathetic premotor neurons. Further support for this hypothesis comes from the finding that inhibition of neurons in the RPa rapidly reversed the fentanyl-evoked increase in BAT SNA with little change in the time course of the RSNA response, while inhibition of neurons in RVLM produced the opposite effect. These results parallel those reported for the BAT and renal sympathoexcitatory responses to activation of DMH neurons: BAT SNA increases are dramatically reduced by inhibition of RPa neurons (12), while RSNA activation is primarily dependent on neuronal activity in the RVLM, presumably renal sympathetic premotor neurons (12, 17, 24). In addition, in the present study, the fentanyl-evoked increase in RSNA began and reached peak levels earlier than that in BAT SNA (RSNA started within 1 min and peaked within 5 min, while BAT SNA usually started between 2 and 3 min and peaked ~10 min after intracerebroventricular fentanyl).

The HR and AP responses to either systemically or centrally administered fentanyl were biphasic, consisting of an early precipitous fall, followed by a slower, sustained tachycardia and pressor response. The central mechanism underlying the early bradycardia remains unclear, although the finding that fentanyl disinhibits cardiac vagal premotor neurons in the nucleus ambiguous (20) supports a potent role for vagal nerve activation. The subsequent tachycardia was eliminated by inhibition of either RPa neurons or neurons in RVLM (see Table 2). RVLM is a potential source of cardiac sympathetic premot-

Fig. 5. Histological localization of microinjection sites in RVLM, RPa, and DMH. A, top: centers (●) of 1 of the 2 glycine microinjections made into the RVLM of 11 rats plotted on a drawing (adapted from Ref. 43) of the brain stem at bregma −11.80 mm. A, bottom: histological section containing a dye deposit (arrow) marking a representative glycine microinjection site in the RVLM. B, top: centers (●) of the glycine microinjections made into the RPa of 6 rats plotted on a drawing of the brain stem at bregma −11.30 mm. B, bottom: histological section containing a dye deposit (arrow) marking a representative glycine microinjection site in the RPa. C, top: centers (●) of 1 of the 2 muscimol microinjections made into the DMH of 10 rats plotted on a drawing of the hypothalamus at bregma −3.30 mm. C, bottom: histological section containing a dye deposit (arrow) marking a representative muscimol microinjection site in the DMH. PrH, nucleus prepositus hypoglossal; MVe, medial vestibular nucleus; Sol, nucleus of the solitary tract; Amb, nucleus ambiguous; SpV, spinal trigeminal nucleus; IO, inferior olivary nucleus; py, pyramidal tract; 7, facial motor nucleus; LPGi, lateral paragigantocellular nucleus; RMg, nucleus raphe magnus; 3V, third ventricle; 4V, fourth ventricle; mt, mammillothalamic tract; f, fornix; MtTu, medial tuberal nucleus; VMH, ventromedial hypothalamic nucleus; DA, dorsomedial hypothalamic area; Re, nucleus reuniens thalami; ME, median eminence.
tor neurons (50) that regulate a variety of cardiac functions (9) and maintain cardiac SNA under resting conditions (12). That the RPa contains cardiac sympathetic premotor neurons is suggested by the anatomic finding of retrogradely infected cells in RPa after viral injections into the heart or stellate ganglion (26, 50), the cardiac sympathoexcitatory and tachycardia evoked from RPa after inhibition of neurons in the RVLM (13), and the effectiveness of inhibition of RPa neurons, but not those in RVLM, to reduce the tachycardia evoked by disinhibition of neurons in the DMH (12, 17, 46, 47). The potential interaction between these two populations of sympathetic premotor neurons influencing cardiac rate in determining the response to fentanyl, particularly whether the elimination of the fentanyl-evoked tachycardia by inhibition of RVLM neurons resulted from a disfacilitation of cardiac sympathetic preganglionic neurons, remains to be determined.

Although disinhibition of DMH neurons increases RSNA (12, 17), inhibition of neuronal activity in the DMH did not eliminate the renal sympathoexcitatory response to central µ-opioid administration, whereas the BAT thermogenic and the tachycardic responses were abolished. This finding sug-
gests a difference in the fentanyl-sensitive, sympathoexcitatory pathways antecedent to the sympathetic premotor neurons for BAT SNA in the RPa and for the RSNA in the RVLM. Indeed, our finding that premamillary transection eliminated all of the sympathoexcitatory responses to fentanyl indicates that the fentanyl-sensitive site(s) for the sympathoexcitatory responses in both sympathetic nerves is within or rostral to the hypothalamus. The cardiovascular sympathoinhibitory, depressor, and bradycardic responses to fentanyl after premamillary transection appear to be mediated by µ-opioid receptors in the vicinity of the RVLM, since they were eliminated by microinjections of naloxone into the RVLM. µ-Opioid receptors have been localized in the RVLM (3, 21, 35), and sympathoinhibitory responses accompanying their stimulation in the RVLM have been described (21, 36, 49). From our data, it appears that in the intact animal, the cardiovascular sympathoexcitatory effects elicited from fentanyl binding to µ-opioid receptors in a site rostral to the brain stem predominate over the cardiovascular sympathoinhibitory effects elicited from fentanyl stimulation of µ-opioid receptors in the RVLM. The mechanism through which this occurs is unclear. The low level of activity on the BAT sympathetic nerve in the absence of large thermogenic bursts in BAT SNA is synchronized to the cardiac cycle, is regulated by the RVLM sympathetic premotor neurons, and thus appears to be vasoconstrictor in function (40). This conclusion is supported by the finding in the present study that following transection, the responses in the small-amplitude BAT SNA paralleled those in RSNA: both were increased by transection, both were inhibited by fentanyl, and both were insensitive to fentanyl after microinjection of naloxone into the RVLM.

In conclusion, the findings of the present study demonstrate that central administration of a µ-opioid receptor agonist produces sympathoexcitatory responses in BAT SNA and RSNA accompanied by an elevation in BAT temperature, a tachycardia, and a pressor response. The BAT thermogenic and tachy-cardiac responses are selectively dependent on activation of neurons in the DMH. The results support the hypothesis that the activity of sympathetic premotor neurons in the rostral RPa plays a critical role in regulating the sympathetic outflow to BAT mediating thermogenic activation and to the heart mediating tachycardia during activation of BAT thermogenesis, while the activity of sympathetic premotor neurons in the RVLM drives the sympathoexcitatory outflow to cardiovascular targets such as the renal blood vessels and to the heart under resting conditions. Increased cardiac sympathetic tone and vasoconstrictor sympathetic outflows such as RSNA could contribute to µ-opioid-induced cardiovascular responses, while augmented BAT SNA and BAT thermogenesis could contribute to fentanyl-evoked hyperthermia, particularly in infants where BAT plays a significant role in thermoregulation.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grants DK-57838, DK-20378, and NS-40987.

REFERENCES