Central pathway for spontaneous and prostaglandin E₂-evoked cutaneous vasoconstriction

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Submitted 15 February 2008; accepted in final form 1 May 2008


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IN BOTH HUMANS (19, 20, 43) and rats (53) cutaneous vasoconstrictor (CVC) tone regulates heat loss to the environment and contributes importantly to the maintenance of normal body temperature and to the development of fever. The essential role for the generation of PGE₂ in the preoptic area (POA) in the production of fever has led to the use of nano-injections of PGE₂ into the POA to understand the neural pathways controlling thermoregulatory effectors during the febrile response. Similarly, observations of thermal receptor responses elicited by changes in skin (Tsk) or core temperature (Tc) have provided important information on the central neural networks responsible for body temperature homeostasis. Such studies have been pursued most extensively with regard to the sympathetic neural regulation of non-shivering thermogenesis in rodent brown adipose tissue (BAT), a highly metabolic tissue in which heat production contributes significantly to the defense of body temperature in cold environments and to the elevation of body temperature during the febrile response. The neural circuit hypothesized to underlie the cooling-evoked and the PGE₂-evoked stimulation of BAT thermogenesis involves relief, either through activation of the cold afferent pathway or through PGE₂ binding to EP3 receptors, of a tonic GABAergic inhibition from the POA to neurons in the dorsomedial hypothalamus (DMH) and to neurons in the rostral raphe pallidus (rRPa) (23, 29, 35–38, 71). The synergistic effect of a reduced inhibition of BAT sympathetic premotor neurons in the rRPa and the increased excitatory input they receive from disinhibited DMH neurons is hypothesized to result in activation of BAT sympathetic premotor neurons in the rRPa (22, 30, 35, 36, 38) and thus to increases in BAT sympathetic nerve activity (SNA) and BAT thermogenesis.

Although control of cutaneous vasoconstriction and the accompanying changes in heat loss to the environment also have significant roles in the homeostatic regulation of body temperature and in the defended elevation in body temperature that defines fever, the functional organization of the central pathways regulating the CVC sympathetic outflow remains poorly understood. There are several similar qualities in the organization of the neural circuits controlling cutaneous vasoconstriction and BAT thermogenesis and in the responses of the neural outflows controlling their effector functions. Both effectors are regulated by sympathetic neural activity and activation of their respective populations of sympathetic premotor neurons, located principally in the rostral medullary raphe, is sufficient to strongly drive either BAT or tail CVC sympathetic outflows (31, 41, 46). Both sympathetic outflows are strongly modulated by the central respiratory pattern generator (11, 12, 31, 40). PGE₂ injected into the POA activates both sympathetic outflows (23, 63), a response that is specifically dependent on the activation of sympathetic premotor neurons in the rRPa (18, 22). Additionally, neurons in the midbrain rostral ventromedial periaqueductal grey can provide an inhibitory influence on the sympathetic outflow to BAT (47) and on that to arterioles in the rat tail (73). However, several distinctions, likely reflecting differences in the functional organization of their central regulatory circuits, have been observed between the sympathetic outflows controlling BAT and CVC. For example, CVC sympathetic outflow exhibits barosensitivity (15, 42), while BAT SNA does not (31, 33). Neuronal activity in the rostral ventrolateral medulla (RVLM) accounts for a portion of the excitation of tail CVC neurons (41, 46, 64), whereas, neuronal excitation in the RVLM is neither necessary nor sufficient for BAT SNA.
activation and may exert an inhibitory effect on BAT SNA (31). BAT SNA is more sensitive to variations in $T_{SK}$ than to those in $T_C$, while the reverse is true for tail CVC activity (40). These differential responses could reflect fundamental differences in the pathways controlling these thermoregulatory effectors that may also contribute to the absence of a correlation, other than a shared respiratory modulation, between simultaneously recorded, cold-activated rat tail CVC and BAT sympathetic outflows (40).

The parallels between the functional roles of the sympathetic innervation to BAT and to the cutaneous vasculature, coupled with the organizational similarities described above in their central control circuits, have led to the suggestion that the neural circuits that underlie thermoregulatory reflex control of both tissues are likely to be broadly similar. Specifically, it has recently been suggested that the DMH may play an important role in the cold- and febrile-evoked activation of tail sympathetic outflow (6, 7). In the present study, we tested this hypothesis by inhibiting DMH neurons during the activation of tail CVC neurons elicited by application of PGE$_2$ into the POA. We show that although disinhibition of neurons in the DMH does increase the excitation of tail CVC neurons, the activity of DMH neurons is not necessary for tail CVC neuronal activity driven by cool thermal afferents or for the increase in their activity evoked by PGE$_2$ in the POA.

**EXPERIMENTAL 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. Male Sprague-Dawley rats (Charles River, Indianapolis, IN; $n = 25$ rats) weighing 350–600 g were initially anaesthetized with isoflurane (2.5% in 100% O$_2$). A femoral artery and vein and the trachea were cannulated for measurement of arterial pressure, the delivery of drugs, and artificial respiration, respectively. Rats were transitioned to intravenous urethane (500 mg/kg) and chloralose (80 mg/kg), and the depth of anesthesia was monitored by the absence of a hindlimb withdrawal to noxious pinch and by the absence of a corneal reflex. Rats were positioned in a stereotaxic frame with the incisor bar at and by the absence of a corneal reflex. Rats were positioned in a

**RESULTS**

Properties of tail CVC postganglionic neurons. The rat tail CVC neurons recorded in this study showed properties consistent with those previously reported for CVC fiber populations.
Tail CVC neuronal activity was increased in response to skin cooling effected by perfusing the water blanket with cold water. Shortly after obtaining recordings from the axons of tail CVC neurons, when $T_c$ averaged 37.3 ± 0.3°C and at a mean $T_{SK}$ of 36.6 ± 0.7°C, the basal firing rate of CVC neurons was 1.1 ± 0.4 Hz ($n$ = 12 CVC neurons). After 5 min of cooling the trunk skin, $T_{SK}$ fell by −1.2 ± 0.3°C ($T_c$ fell by −0.4 ± 0.1°C), and the tail CVC neurons increased their mean firing rate to 2.7 ± 0.6 Hz.

The activity of tail CVC postganglionic neurons was modulated by the baroreceptor reflex. The spontaneous and evoked activity of tail CVC neurons was inhibited during the transient increase in mean arterial pressure (MAP) following intravenous injection of phenylephrine (5 μg in 0.1 ml). Five CVC neurons were completely silenced (1.3 ± 0.35 Hz to 0.04 ± 0.04 Hz) at the peak of a phenylephrine-induced rise in MAP of +59 mmHg (peak MAP: 139 ± 17 mmHg). The spontaneous discharge of tail CVC neurons was also modulated over the course of the cardiac cycle. Coincident with the late diastolic phase of the arterial pressure wave, there was a mean decline of 53 ± 8% ($n$ = 6 CVC neurons) from the peak discharge frequency. This level of cardiac cycle modulation has been described as reflecting a “moderate correlation” between unit discharge and arterial pressure (11).

**Bicuculline nanoinjection into the DMH activates tail CVC neurons.** As an initial step in determining the role of DMH neurons in controlling the activation of CVC neurons, we determined whether CVC neuronal activity could be increased by activation of neurons in the DMH. Responses to nanoinjection of bicuculline (50 pmol) into the DMH were measured from 10 tail CVC neurons recorded in six rats. Bicuculline-evoked disinhibition of local inhibition in the DMH increased tail CVC neuronal activity. In the example in Fig. 1, CVC unit activity increased from 1.1 Hz during control to 4.3 Hz at 1 min after bicuculline in the DMH. Within 1 min of the nanoinjection into the DMH, mean tail CVC neuronal discharge increased ($P < 0.05$) from 0.3 ± 0.1 Hz ($T_{SK}$ = 37.0 ± 0.6°C, $T_c$ = 37.2 ± 0.4°C) to a peak level of 2.1 ± 0.8 Hz (Fig. 1B). There was no significant difference between the discharge rate of tail CVC neurons at 1 min and at 10 min after bicuculline nanoinjection into the DMH. In each of four cases, bicuculline nanoinjection into the DMH also increased BAT SNA recorded simultaneously with tail CVC neurons (Fig. 1A). These data indicate the region within the DMH at which bicuculline nanoinjection activates tail CVC neurons overlaps with that eliciting excitation of BAT SNA and BAT thermogenesis (4, 72).

**Transections of the neuaxis.** Following upon descriptions of the effects of transections of the neuaxis on BAT thermogenesis (5, 51, 57) and on the increases in BAT SNA produced by PGE2 in POA (32, 47), we used transections of the neuaxis to obtain a similar, indirect assessment of descending inhibitory and excitatory influences on CVC neuronal activity. Transection of the neuaxis immediately caudal to the POA activates BAT thermogenesis (5, 51), and a subsequent transection immediately caudal to the DMH reverses the increase in BAT SNA (32, 47). To test the hypothesis that the pathways descending from or through the POA and the DMH influencing tail CVC neuronal activity are organized similarly to those that influence BAT thermogenesis, we determined the changes in tail CVC neuronal activity following transections caudal to POA and to DMH. As shown in Fig. 2, transection caudal to the POA (bregma −2 mm) produced a strong and sustained excitation of tail CVC neurons. In this case, the discharge frequencies of two-tail CVC neurons were increased from quiescence induced by maintaining an elevated body temperature to 4.0 Hz and 3.5 Hz. Maximal increases occurring within 5 min after transection and sustained at 10 min after transection (Fig. 2, A and B). At 5 min after transection, mean tail CVC neuronal activity (Fig. 2B) had increased ($P < 0.05$) from 1.0 ± 0.6 Hz to 6.1 ± 1.6 Hz ($n$ = 5 CVC neurons).

Subsequent transections caudal to DMH at bregma −4 mm had no effect ($P > 0.05$) on the increased level of tail CVC neuronal activity (5.9 ± 1.6 Hz before vs. 5.9 ± 1.1 Hz after; Fig. 2B). In the example in Fig. 2A, trace b, mean unit discharge rates were 6.2 and 4.8 Hz after transection caudal to the DMH. There was also no effect ($P > 0.05$) on the elevated level of tail CVC neuronal discharge from a subsequent transection at −6 mm caudal to bregma (6.5 ± 1.9 Hz before vs. 5.5 ± 1.1 Hz after; Fig. 2B). In the example in Fig. 2A, mean unit discharge rates were 4.7 and 4.5 Hz after transection caudal to the rostral ventromedial periaqueductal gray/ventral tegmental area (−6 mm). Transection of the neuaxis caudal to the rPpa markedly attenuated ($P < 0.005$) the evoked activity of each CVC neuron, with mean activity falling from 5.3 ± 1.1 to 0.7 ± 0.2 Hz ($n$ = 5 CVC neurons) following this transection. In the example in Fig. 2A, trace c, mean unit discharge rates were reduced to 1.2 and 0.9 Hz after transection caudal to
Residual activity was eliminated by iv hexamethonium (Fig. 2, A and B).

Muscimol in the DMH does not affect the PGE$_2$-evoked increases in tail CVC neuronal activity. Finding that transection of the neuraxis caudal to POA increased CVC neuronal activity but that transection caudal to DMH did not reverse the evoked increase in CVC neuron activity strongly suggests that, contrary to the case for BAT SNA, a descending excitatory pathway from the DMH is not necessary for the activation of CVC neurons during the febrile response to PGE$_2$ in POA. To test this directly, we inhibited neuronal activity bilaterally in the DMH during the increase in CVC neuronal activity produced by PGE$_2$ in POA. Nanoinjection of PGE$_2$ into the POA increased the discharge frequency of tail CVC neurons (Figs. 3–5). The activity of 14 individual tail CVC neurons recorded in 11 rats was increased ($P < 0.005$) from 1.0 ± 0.3 Hz to the rRPa. Residual activity was eliminated by iv hexamethonium (Fig. 2, A and B).

Fig. 2. Effect of sequential transections of the neuraxis on the activity of tail CVC postganglionic neurons. A: top image illustrates the approximate positions of serial (rostral-to-caudal) transections of the neuraxis by vertical lines (labeled as distance caudal to bregma) superimposed on a drawing of the rat brain (44). Traces, time courses of the mean arterial pressure (MAP) and the action potential frequency of two discriminated tail CVC neurons (insets: average of 150 discriminated action potentials; vertical scale: 20 μv; horizontal scale: 2 ms). Phenylephrine (PE)-induced increase in MAP (+90 mmHg from 94 mmHg) elicited a baroreceptor-mediated inhibition of the discharge of the CVC neurons (trace a). Horizontal scale: 10 min. Oscillographic traces of unit action potentials (a–c) are 1 min duration, vertical scale: 20 μv. POA, preoptic area; rRPa, rostral raphe pallidus; Th, thalamus; Dk, nucleus of Darkschewitsch; 3: oculomotor nucleus. B: mean ± SE CVC neuronal discharge frequency following serial transections of the neuraxis. Open hatched bar indicates pretransection firing frequency. *Significant ($P < 0.05$) increase above pretransection level resulting from cut at bregma −2 mm (caudal to the POA). After transections at bregma −4 or −6 mm, mean CVC neuronal activity was different from that after transection at bregma −2 mm. Transection at bregma −13 mm (caudal to the rRPa) significantly reduced CVC neuronal activity (solid bracket: $P < 0.01$). Hexamethonium eliminated CVC neuronal activity (dashed bracket: $P < 0.05$).

Fig. 3. Increase in tail CVC activity evoked by PGE$_2$ into the POA is not blocked by muscimol (MUSC) in the DMH. A: rate histogram of multunit tail CVC neuronal discharge following nanoinjection (arrowhead) of PGE$_2$ into the POA. Traces a–e below the spike frequency histogram illustrate oscillographic records of neuronal activity at designated time points. Bilateral MUSC injections (arrowheads) were made into the DMH and 2 nanoinjections of MUSC (arrowheads) were made into the rRPa. The residual activity remaining after MUSC into the rRPa was eliminated by nanoinjection of MUSC (arrowhead) into the right rostral ventrolateral medulla (RVLM). B: mean ± SE ($n = 14$ CVC neurons) discharge frequency of tail CVC neurons following PGE$_2$ into the POA, MUSC into the DMH, MUSC into the rRPa, and MUSC into the RVLM. White bars represent mean discharge frequency during the 1-min control period prior to each treatment. The control discharge frequencies prior to MUSC into the DMH or into the rRPa were not different ($P > 0.05$) from that recorded 10 min after the PGE$_2$-evoked activation. Significant differences were $^*P < 0.005$ and $^†P < 0.05$, compared with respective control frequencies. Horizontal scale: 10 min for frequency histogram trace in A and 3.5 s for oscillographic traces a–e; vertical scale bar: 20 μv for traces a–e.
1.6 ± 0.3 Hz within 1 min of the nanoinjection of PGE₂ into the POA (Fig. 3B), reaching a peak of 2.9 ± 0.5 Hz (P < 0.005) within 5 min [t-test (1' vs. 5') P < 0.005] and sustained at 3.0 ± 0.5 Hz at 10 min [t-test (5' vs. 10') P > 0.05]. The rate histogram in Fig. 3A was derived from a multiunit recording of CVC neurons and illustrates the PGE₂-evoked increase in discharge frequency from 13 spikes/10 s in control (trace a) to 62 spikes/10 s at 10 min (trace b) after PGE₂. Approximately 20 min after the PGE₂ nanoinjection into the POA, bilateral nanoinjection of muscimol (400 pmol, 200 nl) into the DMH had no significant effect (P > 0.05) on the PGE₂-mediated elevation in the discharge of tail CVC neurons (Figs. 3 and 4). In the example in Fig. 3A, multiunit CVC neuronal discharge frequency was 73 spikes/10 s prior to first nanoinjection of muscimol into DMH and 69 spikes/10 s after bilateral nanoinjections. For the population of individual discriminated tail CVC neurons, mean discharge rate 10 min after the second nanoinjection of muscimol into the DMH was 2.7 ± 0.5 Hz (Fig. 3B), which was not significantly different (P > 0.8) from their mean activity (2.9 ± 0.6 Hz) at 1 min prior to the first nanoinjection of muscimol into the DMH. Muscimol also had no effect on the PGE₂-evoked increases in tail CVC neuronal activity (data not shown) when nanoinjected into sites outside of the dorsal DMH area where bicuculline nanoinjections increased tail CVC neuronal activity (see Fig. 1).

To demonstrate whether the DMH sites where muscimol nanoinjections were ineffective in reducing the PGE₂-mediated increases in tail CVC neuronal activity were indeed those that reversed the PGE₂ in the POA- or skin cooling-evoked increases in BAT SNA (23, 36, 38, 71), simultaneous recordings of tail CVC neurons and BAT SNA were accomplished in three cases. These experiments were complicated by the differential TC/TSK thresholds for these two thermoregulatory effectors (40, 42) and the interaction of TC and their sensitivities to mediators of the febrile response (50, 59). Thus, increasing the TC above 37.5°C to reduce the high discharge frequency of tail CVC neurons present at TC below 37.5°C, and thereby allow observation of a marked increase in tail CVC neuronal discharge in response to PGE₂ in the POA (Fig. 3A), frequently resulted in a nearly complete elimination of the BAT response to PGE₂ in the POA (Fig. 4B). Illustrated in Fig. 4A is a case in which spontaneous tail CVC neuronal activity was high at a TC of 37.5°C, resulting in only a modest increase in discharge frequency (peak increase: +37%) following PGE₂ in the POA, and in which BAT SNA exhibited a robust response (+4,300%) to PGE₂ in the POA at a TC of 37.5°C. Bilateral
nanoinjections of muscimol into the DMH promptly terminated the PGE$_2$-evoked increase in BAT SNA (−97% of the premuscimol level at 5 min after muscimol into the DMH) and BAT temperature, but had little effect on that in the tail CVC neuron (−9% of the premuscimol level at 5 min after muscimol in the DMH). In the case illustrated in Fig. 4B, spontaneous tail CVC neuronal activity, which was reduced by maintaining a T$_C$ of 37.5°C, markedly increased in discharge frequency (peak increase: +3.2 Hz) following PGE$_2$ in the POA; however, the BAT SNA response to PGE$_2$ in the POA was nearly completely inhibited at 37.5°C. A subsequent reduction in T$_{SK}$ and T$_C$ resulted in an increase in BAT SNA (peak: +870% of control), likely a combination of the effect of the PGE$_2$ in the POA and of the thermoregulatory response to stimulation of cutaneous cool afferents. Bilateral nanoinjections of muscimol into the DMH nearly reversed the increase in BAT SNA (−85% of the premuscimol level at 5 min after bilateral muscimol into the DMH), but had no effect on the increase in tail CVC neuronal activity (−3% of the premuscimol level at 5 min after muscimol in the DMH). Although in this example, there was a slight increase in T$_{SK}$ during the BAT SNA response, this was insufficient to affect BAT SNA amplitude by comparison to the dramatic reductions in BAT SNA time-locked to the bilateral deliveries of muscimol into the DMH (Fig. 4B). In the three experiments in which BAT SNA could be simultaneously increased with tail CVC neuronal activity, bilateral muscimol nanoinjections into the DMH reduced (P < 0.05) the stimulated (PGE$_2$ in POA and/or skin cooling) BAT SNA to 11 ± 8% of the premuscimol level at 5 min after bilateral muscimol into DMH, but did not alter the PGE$_2$-evoked activation of tail CVC neuronal activity (94 ± 12% of the premuscimol level at 5 min after bilateral muscimol into the DMH).

**Muscimol into the DMH has no effect on spontaneous, temperature-sensitive tail CVC discharge.** Having determined that DMH neuronal discharge was not required for the activation of tail CVC neurons by PGE$_2$ in the POA, we sought to determine whether DMH neurons play a role in the temperature-sensitive drive maintaining CVC neuronal discharge, also thought to be mediated through POA (17, 64). For six tail CVC neurons from five rats, bilateral nanoinjections of muscimol into the DMH were made prior to the nanoinjection of PGE$_2$ into the POA (Fig. 5). At T$_C$ and T$_{SK}$ of 36.7 ± 0.6°C and 36.2 ± 0.8°C, respectively, the mean basal firing rate of the tail CVC neurons was 1.9 ± 0.5 Hz (Fig. 5, A and D). Bilateral nanoinjections of muscimol (400 pmol each in 200 nl) into the DMH had no significant effect (P > 0.1) on the spontaneous tail CVC neuron discharge (2.4 ± 0.5 Hz after muscimol into the DMH; Fig. 5, A and D). In the example in Fig. 5A, the mean CVC neuronal discharge frequency was 4.1 Hz prior to and 4.7 Hz at 5 min following bilateral muscimol nanoinjections into DMH.

Tail CVC neuron activity remained sensitive to elevations in T$_{SK}$ and T$_C$ following muscimol into the DMH. Ten minutes after bilateral muscimol into the DMH, the rats were warmed to increase T$_C$ to 38.3 ± 0.2°C (T$_{SK}$ = 38.1 ± 0.3°C), which resulted in a fall (P < 0.05) in mean tail CVC neuron activity (Fig. 5, B and D) to 1.1 ± 0.3 Hz from the level of spontaneous activity at 10 min after bilateral muscimol into the DMH (Fig. 5D, WARMING). Subsequent nanoinjection of PGE$_2$ into the POA increased (P < 0.05) tail CVC neuron activity (Fig. 5C) to 2.7 ± 0.6 Hz at 5 min after PGE$_2$ nanoinjection (Fig. 5D).

**Fig. 5.** Bilateral MUSC into the DMH has no effect on the spontaneous or PGE$_2$-evoked activity of tail CVC neurons. A–C: sequential segments of the activity recorded from a single tail CVC neuron. A: spontaneous discharge frequency (T$_C$, 37.4°C) was unaffected by bilateral MUSC (arrowheads) into the DMH. B: increasing T$_C$ reduced the tail CVC neuronal discharge frequency to 1.6 Hz. MUSC into the DMH (arrowhead) had no effect on tail CVC neuronal discharge. C: 10 min after the second MUSC into the DMH (repeated portion of B), PGE$_2$ into the POA increased the tail CVC neuronal discharge frequency. D: mean ± SE (n = 6 CVC neurons) discharge frequency of tail CVC neurons following bilateral MUSC into the DMH. White bars represent the mean control discharge frequency during the 1 min prior to MUSC into the DMH. Increasing T$_C$ (WARMING) reduced the tail CVC neuronal discharge frequency. *P < 0.05 compared with 10 min after MUSC in the DMH. **Significant (P < 0.05) increase in tail CVC neuronal discharge frequency compared with before PGE$_2$ into the POA.
In the example in Fig. 5C, PGE$_2$ into the POA increased the tail CVC neuron activity from 1.8 to 4.6 Hz within 1 min. The peak discharge rates of tail CVC neurons at 5 and 10 min following PGE$_2$ into the POA of rats that had received prior bilateral nanoinjections of muscimol into the DMH (Fig. 5D) were not different ($P > 0.8$) from those at 5 and 10 min following PGE$_2$ into the POA of naïve rats (Fig. 3B).

**Muscimol in the rRPa attenuates the PGE$_2$-evoked activity of tail CVC neurons.** Having established that neurons in the same DMH locus as those required for BAT SNA responses were not necessary for tail CVC responses, we sought to determine whether the activity of neurons in the rRPa, a locus of sympathetic premotor neurons for CVC drive (2, 39, 41, 58), is necessary for the activation of tail CVC neurons evoked by PGE$_2$ into the POA by observing the effect on tail CVC neuronal discharge ($n = 9$ CVC neurons) of muscimol into the rRPa subsequent to PGE$_2$ into the POA. These rats had received prior bilateral nanoinjections of muscimol into the DMH that were without effect on the neuronal activation elicited by PGE$_2$ into the POA (Figs. 3 and 4). One minute prior to nanoinjection of muscimol into the rRPa, tail CVC neuronal discharge (3.2 ± 0.7 Hz) was not different ($P > 0.4$) from that at 10 min after the prior nanoinjections of PGE$_2$ into the POA (3.4 ± 0.7 Hz). Nanoinjection of muscimol (200 pmol in 100 nl) into the rRPa reduced tail CVC neuronal discharge (Figs. 3A and 4, A and B) to 1.8 ± 0.4 Hz ($P < 0.001$, Fig. 3B) at 1 min and to 1.2 ± 0.4 Hz at 5 min after muscimol into the rRPa, a decrease of more than 60%. In the example in Fig. 3A, CVC unit discharge frequency was reduced from 7.0 Hz prior to the first nanoinjection of muscimol into rRPa, to 3.2 Hz after the second nanoinjection. In Fig. 4, A and B, CVC neuronal firing rate at 5 min after muscimol in rRPa was reduced by 38% and by 100%, respectively, from the premuscimol levels.

**Muscimol into the RVLM eliminated tail CVC neuron activity remaining after muscimol into the rRPa.** Since both rRPa and RVLM contain sympathetic premotor neurons controlling CVC neurons (41, 58), we sought to determine whether the RVLM was the source of the residual drive supporting CVC neuronal discharge after inhibition of the rRPa. We assessed the role of neurons in the RVLM (41) as the potential source of the activity remaining in five tail CVC neurons after stimulation by PGE$_2$ into the POA and the subsequent reduction following muscimol into the rRPa, by subsequently applying muscimol (200 pmol in 100 nl) into the RVLM. Unilateral nanoinjection of muscimol into the right RVLM eliminated the residual tail CVC neuronal discharge (Fig. 3A): the mean discharge frequency of 1.3 ± 0.4 Hz prior to muscimol into the RVLM was reduced ($P < 0.001$) to 0 ± 0.0 Hz at 1 min after muscimol into the RVLM (Fig. 3B, $n = 5$ CVC neurons in 5 rats). The discharge of tail CVC neurons in animals that did not receive muscimol into the RVLM was eliminated by ganglionic blockade with intravenous hexamethonium (Fig. 4A).

**Muscimol into the rRPa prior to PGE$_2$ into the POA eliminates spontaneous tail CVC activity and prevents its PGE$_2$-evoked activation.** In four different tail CVC neurons recorded at $T_c = 37.8 ± 0.3^\circ C$ and $T_{SK} = 37.1 ± 0.5^\circ C$, we observed that muscimol into the rRPa completely inhibited ($P < 0.001$) their spontaneous discharge (Fig. 6, mean discharge frequency: 1.6 ± 0.1 Hz at 1 min prior to muscimol into rRPa, 0 ± 0.0 Hz at 5 min after muscimol into rRPa). Both subsequent nanoinjection of PGE$_2$ into the POA and subsequent transsections of the neuraxis caudal to the POA and to the DMH failed to evoke increases in tail CVC neuronal activity (Fig. 6).

Localization of nanoinjection sites in the DMH, the rRPa, and the RVLM. The anatomical locations of the muscimol nanoinjection sites targeting the DMH were confirmed histologically in 12 rats (Fig. 7, A and B). The nanoinjection sites, at which muscimol was ineffective in altering the PGE$_2$-elevated tail CVC discharge in experiments involving simultaneous recordings of a tail CVC neuron and BAT SNA, were located among those that were similarly ineffective in experiments in which only tail CVC neurons were recorded. The sites of the bilateral muscimol nanoinjections in the DMH were located within the subregions of the DMH: 1) at which bicuculline nanoinjection elicited a large increase in CVC neuronal activity (present study, Fig. 1, 2) that contain neurons necessary for the expression of PGE$_2$- and skin cooling-evoked increases in BAT SNA (7, 23, 36, 38, 71), and 3) at which bicuculline nanoinjections elicit increases in BAT SNA as well as in renal SNA and heart rate (4, 13, 32, 52).

The anatomical locations of the muscimol nanoinjection sites targeting the rRPa were confirmed histologically in 13 rats (Fig. 7, C and D). The rRPa injection sites were located within the region of the rostral ventromedial medulla containing putative sympathetic premotor neurons necessary for activation of cutaneous vasoconstriction (39, 41, 58) and BAT thermogenesis (3, 29, 30, 34, 35).

The anatomical locations of the muscimol nanoinjection sites targeting the RVLM were confirmed histologically in four rats (Fig. 7C). The RVLM injection sites were located within the subregion of the RVLM containing putative CVC sympathetic premotor neurons (58) and implicated in the control of CVC neuronal activity (41, 46), as well as visceral vasoconstriction (31).

**DISCUSSION**

Our results demonstrate that although activation of neurons in the DMH is a potential source of the excitatory drive to increase the activity of rat tail CVC neurons and reduce
contributes to an increase in TC (19, 43, 53, 67). Our finding which heat retention mediated by cutaneous vasoconstriction response, a component of the acute phase immune response in discharge (41).

eurons is also dependent on the discharge of neurons in the component of the PGE2-mediated activity of most tail CVC evoked activity of rat tail vasoconstrictor nerves (18). A similar treatments on the sympathetic outflow to BAT, the POA and the rRPa yielded a sustained increase in tail CVC that transections of the neuraxis at several points between the on the activity of neurons in the DMH, as well as our finding that inhibition of neuronal discharge in the DMH. In contrast, the activity of neurons in the rRPa, presumably sympathetic premotor neurons for cutaneous vasoconstriction (2, 18, 46, 58), contributes significantly to basal (i.e., thermoregulated) CVC neuronal discharge and to the increase in CVC neuronal discharge evoked by PGE2 into the POA. The latter is consistent with the demonstration that GABA nanoionjection into the rRPa attenuates spontaneous and intracerebroventricular PGE1-evoked activity of rat tail vasoconstrictor nerves (18). A component of the PGE2-mediated activity of most tail CVC neurons is also dependent on the discharge of neurons in the RVLM, consistent with the existence of a descending excitatory pathway from the RVLM influencing tail CVC neuronal discharge (41). The discovery that the TSK and Tc-driven and the PGE2 in the POA-evoked discharge of CVC neurons are not dependent on the activity of neurons in the DMH, as well as our finding that transections of the neuraxis at several points between the POA and the rRPa yielded a sustained increase in tail CVC neuronal discharge are in marked contrast to the effects of similar treatments on the sympathetic outflow to BAT, the other principal, sympathetically-regulated, cold defense effector. In naïve animals, brain transections immediately caudal to the POA (5) or in the vicinity of the pontomedullary junction (51, 57) dramatically increase BAT thermogenesis, while transections made in the region caudal to the DMH but rostral to the pontomedullary junction have no effect on basal levels of BAT thermogenesis in normothermic animals (51) and reverse PGE2 in the POA-mediated increases in BAT SNA (32, 47). These results, in combination with studies involving nanoionjection of muscimol into the DMH that show the dependence of cold defense and febrile excitation of BAT SNA on the activity of neurons in the DMH (23, 32, 36, 38, 71) and the demonstration that the POA sends descending GABAergic projections to the DMH (38) and to the rRPa (35) have suggested a model for the cold defense and febrile activations of BAT sympathetic outflow. In this model, skin or core cooling or the interaction of PGE2 with EP3 receptors in the POA act to relieve a tonic inhibition from the POA to intermediate sympathetic excitatory neurons in the DMH and to sympathetic premotor neurons in the rRPa. This disinhibition of sympathetic premotor neurons in rRPa and their augmented excitatory drive from disinhibited DMH neurons are postulated to account for the increases in BAT thermogenesis (29, 35, 36, 38). Indeed, both cold exposure and LPS administration increase fos expression in neurons in the DMH (3, 54, 69). Our data reveal a differential dependence of BAT SNA and tail CVC activity on the discharge of neurons in the DMH.

For the regulation of CVC neuronal activity, the finding that transections caudal to the POA increase CVC neuronal discharge and that this activation remains unabated during subsequent transections made at levels between the POA and the rRPa is consistent with a major inhibitory regulation of CVC sympathetic premotor neurons in the rRPa by a long descending inhibitory input from the POA and with CVC sympathetic premotor neurons in the rRPa receiving their principal excitatory drive from neurons in the medulla. These findings support
the view that PGE$_2$-mediated febrile and cold-defense increases in CVC activity arise principally from the relief of the descending GABAergic inhibition from the POA to CVC sympathetic premotor neurons in the rRPa. In contrast to the pathway regulating BAT SNA, the absence of an effect of inhibition of the DMH neurons on either the cold-defense or febrile activations of tail CVC neurons suggests that neither on-going activity in DMH neurons nor an increase in their activity due to disinhibition is a required source of the excitatory drive to the CVC sympathetic premotor neurons mediating these responses. Rather, these data are consistent with a model in which the requisite source of excitation of the sympathetic premotor neurons regulating cutaneous vasoconstriction and heat loss to the environment resides in the medulla, or potentially in a complement of membrane channels supporting their spontaneous discharge.

Our data could also be consistent with a PGE$_2$-evoked and cooling-driven increase in CVC neuronal discharge arising from inhibition of POA neurons that excite neurons that are located between the POA and the rRPa and that inhibit CVC sympathetic premotor neurons in the rRPa. Several observations suggest that a population of such neurons may exist in the rostral periaqueductal gray (PAG). Neurons in the rostral PAG receive input from neurons in the POA that are activated (increased Fos expression) by environmental warming, but not cooling (68). Environmental warming also increases Fos expression in the rostral PAG (69) and chemical excitation of neurons within the rostral PAG increases both tail temperature and blood flow (73), presumably due to inhibition of CVC sympathetic outflow. Together, these data suggest that warm-sensitive neurons in the POA could send excitatory projections to CVC sympathoinhibitory neurons in the rostral PAG. Further studies would be required to determine whether rostral PAG neurons exert an inhibitory influence on tail CVC sympathetic premotor neurons in the rRPa and whether they are required for thermal and febrile activation of cutaneous vasoconstriction.

The present data add to the body of evidence suggesting a differential regulation of thermoregulatory effectors mediated by effector tissue-specific pathways from the POA (17, 49, 55). The finding that inhibition of neurons in the DMH did not affect the responses of tail CVC activity to either increased $T_C$ or to application of PGE$_2$ into the POA is in stark contrast to the elimination of the PGE$_2$-evoked and skin cooling-evoked increases in BAT SNA and BAT thermogenesis following muscimol nanoinjection into the DMH (7, 23, 36, 38, 71). However, while the contrasting effects [sustained increase in tail CVC activity shown here, no increase in BAT SNA (32)] of brain transections placed immediately caudal to the DMH suggest a differential regulation of tail CVC activity and BAT SNA by a tonically active descending inhibitory pathway through this region, our data do not rule out the possibility that tail CVC and BAT sympathetic premotor neurons in the rRPa share a common inhibitory input from the POA (but only those for BAT require activity in DMH neurons). The absence of coherence between the skin cooling-evoked discharges in BAT SNA and in tail CVC SNA (40) is consistent with the existence of separate populations of sympathetic premotor neurons controlling these thermoregulatory motor outputs and with an absence of synchronizing communication between the circuits that determine the burst discharge of these two populations of sympathetic premotor neurons. The current data provide new information on the differences within central pathways, particularly the sympathoexcitatory neurons in the DMH, which could contribute to the lack of nonrespiratory-related coherence between BAT SNA and tail CVC discharge.

It is doubtful that the failure of muscimol to inhibit evoked tail CVC neuronal activity can be attributed to failure to target the DMH appropriately or to a lack of efficacy of muscimol. That the DMH was appropriately targeted is demonstrated by the large activation of tail CVC neurons immediately following bicuculline into the DMH (Fig. 2). Additionally, a positive control for both DMH localization and muscimol efficacy is provided by our finding that when BAT SNA and tail CVC neuronal activity were both increased in response to PGE$_2$ (or to the combination of PGE$_2$ and reduced body temperature), tail CVC neuronal activity was unaffected by bilateral muscimol into the DMH while the discharge in BAT SNA was eliminated. In contrast to the muscimol nanoinjections into the DMH, those into the rRPa were effective at attenuating the PGE$_2$-evoked increases in tail CVC neuronal activity and at completely inhibiting spontaneous tail CVC neuronal discharge. These results demonstrate the effectiveness of the muscimol and that the DMH injection sites were in a region containing neurons capable of exciting tail CVC sympathetic premotor neurons in the rRPa as well as those essential for the elaboration of PGE$_2$-evoked increases in BAT thermogenesis.

Our experiments were conducted in anesthetized animals, and chloralose, one component of our anesthetic regimen, may have lowered the threshold temperature and reduced the response dynamics of thermoregulation in our rats (10). Thus, we expect that the thermoregulatory balance point temperature (48) and the response gains might be lower in our experiments than they would be in unanesthetized rats, but such differences would not affect the validity of our conclusions regarding the thermoregulatory pathways that we have investigated. It is possible, however, that anesthesia could mask the contribution of other brain regions to the febrile response. In this regard, experiments in awake animals have suggested a role for neurons in the hypothalamic paraventricular nucleus in the febrile response (14, 21, 56, 74) as well as a role for norepinephrine in the POA (8) and for neurons in the locus coeruleus (1) in the elaboration of fever. Additionally, the present study employed nanoinjections of PGE$_2$ into the POA to activate descending fever-promoting pathways, and it remains to be determined whether the pathways derived using this stimulus also mediate the febrile responses to intravenous lipopolysaccaride or polyinosinic-cytidylic acid.

The large increase in tail CVC activity elicited by bicuculline nanoinjection into the DMH suggests that disinhibition of CVC sympathoexcitatory neurons in the DMH is involved in eliciting an as yet unidentified, CVC response. One such response may be the increase in CVC discharge and reduction of skin blood flow evoked by certain types of stress. Psychological stress has been shown to stimulate cutaneous vasoconstriction in rats (66), rabbits (70) and humans (26). Neurons in the DMH have been postulated to play an essential role in the psychologically-evoked activation of sympathetic outflow (6, 27). The activity of neurons in the DMH is required for restraint stress-evoked tachycardia (28, 61), but not for that evoked by contextual fear (9). The role of DMH neurons in
mediating the increases in tail CVC activity in response to various stress paradigms remains to be determined.

Muscmicoll nanoinjection into the rRPa eliminated spontaneous tail CVC discharge and completely prevented the increase in tail CVC neuronal activity when muscmicoll was delivered prior to PGE2 in the POA, but not when nanoinjected during the PGE2-evoked tail CVC activation. These results are consistent with the earlier demonstration that GABA into the ventromedial medulla, including the rRPa, abolished spontaneous SNA to the rat tail, but only attenuated the increased tail SNA evoked by intracerebroventricular administration of PGE1 (18). Spinal administration of serotonin can evoke increases in rat tail SNA after spinal transection (25) and blockade of spinal serotonin receptors attenuates evoked sympathetic outflow to the cutaneous vessels in the rabbit ear (39).

Nanoinjection of serotonin into the spinal intermediolateral nucleus produces a long-lasting potentiation of NMDA-evoked increases in BAT SNA, even at doses of serotonin that are subthreshold for evoking increases in BAT SNA (24). Thus, serotonin may produce a long-lasting increase in the excitability of sympathetic preganglionic neurons (45), including those regulating CVC. We hypothesize that PGE2 into the POA elicits a release of spinal serotonin, potentially from neurons in the rRPa (3, 60, 65), which does not occur to a significant degree under conditions of spontaneous CVC discharge and which potentiates the excitability of CVC sympathetic preganglionic neurons to inputs such as those from the rRPa and from the RVLM (41). If this is the case, nanoinjection of muscmicoll into the rRPa prior to PGE2 in the POA could prevent the activation of neurons in the rRPa, including those that release spinal serotonin. However, application of muscmicoll into the rRPa after PGE2 administration in the POA could eliminate the descending glutamatergic excitation from rRPa, but serotonin would already have increased the excitability of CVC sympathetic preganglionic neurons to other premotor inputs, such as that from the RVLM (41). Serotonergic potentiation of a drive to CVC preganglionic neurons from RVLM-spinal neurons would thus be consistent with the elimination of the residual activity of tail CVC neurons by muscmicoll nanoinjection into the RVLM (Fig. 3).

Overall, these data reveal that although the DMH contains neurons whose connections enable them to elicit increases in tail CVC neuronal activity, activation of neurons in the DMH is not necessary for the stimulation of CVC neuronal discharge and the decreased skin blood flow that contributes to the increase in body temperature during fever. In contrast, febrile activation of CVC sympathetic outflow requires the activity of neurons in the rRPa, likely the sympathetic premotor neurons located there. Our results are consistent with the existence of a descending inhibitory pathway from the POA that regulates the discharge of CVC sympathetic premotor neurons in the rRPa. We also suggest that the potentiation of CVC preganglionic neuronal excitability by serotonin contributes to the increase in tail CVC sympathetic outflow evoked by PGE2 in the POA and that such a potentiation of CVC preganglionic neuronal responses to their spinal inputs may determine the effectiveness of descending excitation from the RVLM in influencing tail CVC neuronal activity. These findings support the concept that the coordinated responses of distinct central circuits regulating different thermoregulatory effectors underlie the maintenance of body temperature homeostasis and the PGE2-driven deviation from homeostasis during fever.

Perspective and Significance

Increasing evidence has, for many years, supported the potential for central neurons to selectively activate the autonomic outflows to different effector target tissues and thus for central autonomic networks to exert the differential control of effectors that comprises the patterned responses we observe to homeostatic challenges. The present study reveals an aspect of the central thermoregulatory circuit controlling cutaneous vasoconstriction that differs markedly from that controlling thermogenesis: a differential role for neurons in the DMH in the elaboration of cold defense and febrile activations of these two sympathetic outflows.

One of the principal differences among thermoregulatory effectors is their temperature thresholds for activation, which seem to be inversely related to their energetic costs: cutaneous vasoconstriction, requiring little energy expenditure, is activated at a relatively high body temperature compared with BAT thermogenesis with a greater metabolic cost and a lower temperature threshold for activation. Our finding that cutaneous vasoconstriction may be regulated by preoptic inhibitory neurons that project to the rostral ventromedial medulla, in contrast to those that regulate BAT, which are hypothesized to project to the DMH, is consistent with differential temperature thresholds for activation of these effector systems since neurons in the POA are expected to play a significant role in determining the temperature threshold characteristics of the responses of different thermal effectors.

Differences in the functional organization of the central circuits controlling these thermoregulatory effectors may also relate to their different roles in nonthermoregulatory responses. The cutaneous vascular bed has a large capacity, and its resistance characteristics can play a role in cardiovascular homeostasis in contrast to the sympathetic outflow to BAT, which is not regulated by the baroreceptor reflex. The considerable ability of BAT to consume lipids during thermogenesis implicates its sympathetically-regulated energy expenditure in overall metabolic homeostasis and regulation of energy stores.

ACKNOWLEDGMENTS

This research was supported by National Institute of Neurological Disorders and Stroke Grant NS-40987. We thank Brad Sugden for preparation of histological material and Dr. Kazuhiro Nakamura for critical review of the manuscript.

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