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Central circuitries for body temperature regulation and fever

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Nakamura K. Central circuitries for body temperature regulation and fever. *Am J Physiol Regul Integr Comp Physiol* 301: R1207–R1228, 2011. First published September 7, 2011; doi:10.1152/ajpregu.00109.2011.—Body temperature regulation is a fundamental homeostatic function that is governed by the central nervous system in homeothermic animals, including humans. The central thermoregulatory system also functions for host defense from invading pathogens by elevating body core temperature, a response known as fever. Thermoregulation and fever involve a variety of involuntary effector responses, and this review summarizes the current understandings of the central circuitry mechanisms that underlie nonshivering thermogenesis in brown adipose tissue, shivering thermogenesis in skeletal muscles, thermoregulatory cardiac regulation, heat-loss regulation through cutaneous vasomotion, and ACTH release. To defend thermal homeostasis from environmental thermal challenges, feedforward thermosensory information on environmental temperature sensed by skin thermoreceptors ascends through the spinal cord and lateral parabrachial nucleus to the preoptic area (POA). The POA also receives feedback signals from local thermosensitive neurons, as well as pyrogenic signals of prostaglandin E_2 produced in response to infection. These afferent signals are integrated and affect the activity of GABAergic inhibitory projection neurons descending from the POA to the dorsomedial hypothalamus (DMH) or to the rostral medullary raphe region (rMR). Attenuation of the descending inhibition by cooling or pyrogenic signals leads to disinhibition of thermogenic neurons in the DMH and sympathetic and somatic premotor neurons in the rMR, which then drive spinal motor output mechanisms to elicit thermogenesis, tachycardia, and cutaneous vasoconstriction. Warming signals enhance the descending inhibition from the POA to inhibit the motor outputs, resulting in cutaneous vasodilation and inhibited thermogenesis. This central thermoregulatory mechanism also functions for metabolic regulation and stress-induced hyperthermia.
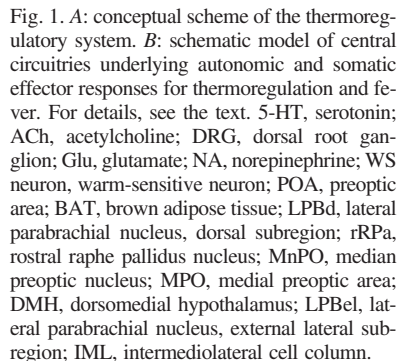
brown adipose tissue thermogenesis; shivering; skin vasoconstriction; sympathetic nervous system

TEMPERATURE WITHIN THE BODY of homeothermic animals, including humans is rigidly regulated through a variety of involuntary thermoregulatory responses, such as shivering and nonshivering thermogenesis, cutaneous vasomotion, sweating, panting, and piloerection. All these physiological responses are controlled by brain mechanisms in an orchestrated manner to optimize the internal thermal environment for appropriate molecular activities and reactions by bioactive proteins, such as enzymes and ion channels. The thermoregulatory system also works for host defense from invading pathogens through elevation of body core temperature, which is called fever. Fever is considered advantageous to the host in surviving bacterial infections, as reported in humans and rabbits (67, 79), although high fevers can have

detrimental effects on the host (9). The febrile rise in body temperature could exert beneficial effects for the host by enhancing immune cell activities (138) and making the internal thermal environment out of the temperature range optimal for pathogen growth (90). Increase in body temperature similar to fever is also observed when animals receive psychological stress. Psychological stress-induced hyperthermia is a fundamental stress response involving sympathetic thermoregulatory effector functions and has some differences in its characteristics from fever (118). A further role of the thermoregulatory system is to regulate energy consumption. Heat production is one of the major outlets to consume energy taken from diet, and, therefore, the thermoregulatory system that is shifted to hypometabolism could cause obesity (71).

As a concept, the thermoregulatory system consists of the three components: sensory afferent part, integration part, and command efferent part (Fig. 1A). The thermoregulatory center in the brain receives information through sensory afferent

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Fig. 1. *A*: conceptual scheme of the thermoregulatory system. *B*: schematic model of central circuitries underlying autonomic and somatic effector responses for thermoregulation and fever. For details, see the text. 5-HT, serotonin; ACh, acetylcholine; DRG, dorsal root ganglion; Glu, glutamate; NA, norepinephrine; WS neuron, warm-sensitive neuron; POA, preoptic area; BAT, brown adipose tissue; LPBd, lateral parabrachial nucleus, dorsal subregion; rRPa, rostral raphe pallidus nucleus; MnPO, median preoptic nucleus; MPO, medial preoptic area; DMH, dorsomedial hypothalamus; LPBel, lateral parabrachial nucleus, external lateral subregion; IML, intermediolateral cell column.

neuronal pathways on environmental temperatures from thermoreceptors in the skin, on visceral temperatures from thermosensory fibers in the abdominal cavity and on central temperatures from thermosensitive neurons in the brain and spinal cord. In case of infection, immune signals delivered to the brain trigger the local production of pyrogenic mediators, which are then sensed by the thermoregulatory center. The information on peripheral and central temperatures, immune signals, and other homeostatic parameters (e.g., osmolarity in tissue fluid) that is delivered to the thermoregulatory center is integrated and then, the center provides command signals to peripheral effectors through efferent neural and neuroendocrine pathways. In many mammals, the preoptic area (POA), which is located in the rostral pole of the hypothalamus, is thought to function as the thermoregulatory center.

Research on the central thermoregulatory circuitries has recently made a great progress by efficiently combining anatomical and physiological approaches using rodents. Identifying neurons expressing c-Fos proteins, a histochemical marker of neuronal activation (140), in brain tissue sections has been a powerful technique to locate candidate neuronal populations that are activated to mediate thermoregulatory afferent or efferent signaling. Trans-synaptic retrograde neural tract tracing from thermoregulatory effectors with pseudorabies virus

has visualized thermoregulatory efferent pathways, as well as neuronal populations involved in the pathways. In vivo electrophysiological recordings of sympathetic nerves and EMG activities controlling thermoregulatory effectors in combination with stereotaxic nanoinjections of drugs into specific brain regions have enabled investigators to functionally analyze the roles of neurons at the injection sites in the development and maintenance of thermoregulatory and febrile responses. These powerful technical driving forces have facilitated our understandings of the central circuitry mechanisms of thermoregulatory and febrile responses, especially shivering thermogenesis in skeletal muscles, nonshivering thermogenesis in brown adipose tissue (BAT) and cutaneous vasomotion. It is an honor to receive the 2010 New Investigator Award of the Neural Control and Autonomic Regulation Section of the American Physiological Society, and in this review, I describe the current understandings of the central circuitry mechanisms of thermoregulation and fever in mammals, incorporating recent important discoveries that have been made since previous reviews (95, 96). On the basis of the accumulated experimental evidence, I propose the model of the fundamental neuronal pathways that regulate sympathetic and somatic thermoregulatory effectors to defend internal thermal homeostasis from environ-

mental heat and cold challenges, as well as to combat invading pathogens through developing fever (Fig. 1B).

Peripheral Thermosensation for Body Temperature Regulation

Changes in environmental temperature have direct and more rapid effects on skin temperature than on temperatures within the body core. When environmental temperature is lowered, skin temperature rapidly falls, whereas brain and rectal temperatures are not affected or slightly increased in rats (17, 78), cats (38), and dogs (51). Therefore, to rapidly initiate thermoregulatory responses before environmental thermal challenges affect body core temperature, feedforward thermal afferent information from skin thermoreceptors needs to be delivered to thermoregulatory command neurons that are located in the POA.

Skin thermoreceptors are situated strategically to detect changes in environmental temperature. Also, skin temperature should be affected by body core temperature, especially when recruitment of heat from internal body to the skin is facilitated by increased skin blood flow through cutaneous vasodilation. Therefore, the thermosensory neural pathways from skin thermoreceptors to the POA are thought to mediate the feedforward signaling to elicit thermoregulatory responses to thermal disturbances from the environment (Fig. 1A) (64), as well as, to some extent, feedback signaling to return information on the thermal state of internal body to the POA (181). However, the effect of body core temperature on skin thermosensation would be small when skin blood flow is attenuated due to cutaneous vasoconstriction. Furthermore, other feedback sensory mechanisms (e.g., the one mediated by thermosensory POA neurons; see below) seem to more faithfully reflect changes in body core temperature. Therefore, the significance of the feedback mechanism through skin thermoreceptors in thermoregulation has not been determined. This review describes the role of the thermosensory afferent signaling from the skin in eliciting feedforward thermoregulatory responses to thermal disturbances from the environment.

The molecular entity of cutaneous thermoreceptors that are responsible for initiating thermoregulatory afferent signaling has not been determined. However, recent studies have suggested potential roles of the transient receptor potential (TRP) family of cation channels in temperature sensation required for thermoregulation. Among the TRP family channels, which sense a broad physiological range of temperatures, TRPM8 is activated by modest cooling ($< 27°C$) (89, 131) and distributed in the peripheral free endings of primary somatosensory C fibers in the skin (7, 89, 131). Mice with a genetic deficiency in TRPM8 display a reduced ability to sense innocuous cold temperatures but can maintain their basal body core temperatures in a thermoneutral environment (7). However, in a cold environment (10°C), TRPM8-deficient mice exhibit reduced activation of BAT thermogenesis and lowered body core temperature compared with wild-type mice (165). Application of menthol, which activates TRPM8, to the skin of wild-type mice evokes warm-seeking behavior, as well as cold-defensive, physiological responses, including BAT and shivering thermogenesis and cutaneous vasoconstriction (166). These findings indicate that TRPM8 plays a role in the sensation of environmental cooling that is required for eliciting feedforward cold-defensive responses.

TRPV3 and TRPV4 are the TRP channels that are activated by innocuous warm temperatures. Either TRPV3 or TRPV4 is abundantly expressed in keratinocytes in skin epidermis, but low in somatosensory ganglia (45, 132). ATP released from heat-activated keratinocytes is proposed as a diffusible factor to transmit thermal information detected by TRPV3 in keratinocytes to sensory nerves (85). Although mice lacking TRPV3 exhibit strong deficits in behavioral responses to innocuous and noxious heat (91), their phenotypes related to thermoregulation have not been reported. TRPV4-deficient mice show altered behaviors in selecting innocuous warm temperatures but exhibit intact circadian body temperature fluctuations and can defend their body core temperatures, even in a cold (4°C) or warm (35°C) environment indistinguishably from wild-type mice (73, 74). The lack of obvious thermoregulatory phenotypes in these TRP channel-deficient animals might be due to redundant peripheral and central sensory mechanisms that are inherent in the thermoregulatory system to secure the maintenance of thermal homeostasis.

There are several findings suggesting a role of TRPV1 in thermoregulatory and metabolic functions. TRPV1 is a TRP channel that can be activated by a noxious range of heat ($>43°C$), by protons (pH ≤ 5.9), or by capsaicin (24, 173). Peripheral or central administration of capsaicin induces hypothermia (54, 62), and administration of TRPV1 antagonists induces hyperthermia (40, 162) by both increasing metabolism and reducing heat loss from the body surface, likely though antagonizing TRPV1 located within abdominal viscera (157). These findings suggest that tonic activation of peripheral TRPV1, effected by nonthermal stimuli at body temperatures below the threshold for TRPV1 activation, provides afferent signals to reduce body temperature. TRPV1-deficient mice exhibit no obvious deficit in the basal maintenance of body temperature or in temperature responses to thermal challenges (39, 58, 163). However, these mice exhibit lower metabolism, enhanced skin vasoconstriction, higher locomotor activity, and preference for a lower ambient temperature (39). Intriguingly, many of these mice become overweight with age (39). Therefore, TRPV1 appears to be implicated in the regulation of body temperature and metabolism, although a few phenotypes in these mice are inconsistent with the effects of TRPV1 antagonists. Further studies will be required to determine the cells that express the TRPV1 channels responsible for the phenotypes in TRPV1-deficient mice and for the effects of TRPV1 ligands.

In addition to the skin, cold and warm receptors are contained among the splanchnic and vagus nerve afferent fibers distributed in the abdomen and their responses to temperature changes are similar to those of cutaneous thermoreceptors (46, 136). Consistently, various TRP channels, including the ones described here, are expressed in vagal afferent neurons (194). However, how abdominal thermal information contributes to thermoregulatory functions is mostly unknown. In contrast to the abdomen, existence of thermoreceptors in the thoracic cavity is uncertain. Nerve recordings in afferents from the thoracic region identified atrial baro-afferents and lung stretch afferents, but none of them was sensitive to cooling (46), whereas, in a different study, an elevation of intrathoracic

temperature ($> 39.2°C$) increased activity of pulmonary C-fibers (139).

Cutaneous Thermosensory Afferent Pathways to the POA

Environmental cooling and warming signals sensed by cutaneous cool and warm receptors, respectively, activate separate primary somatosensory neurons that innervate neurons in the spinal and trigeminal (medullary) dorsal horns (Fig. 1*B*). The dorsal horn harbors separate populations of spinothalamic projection neurons that are activated by innocuous skin cooling (COOL cells) (29) and by innocuous skin warming (WARM cells) (4). The cutaneous thermosensory signals mediated by these spinothalamic neurons are further relayed to the primary somatosensory cortex, and this spinothalamocortical signaling mediates perception and discrimination of skin temperatures (Fig. 1*B*) (27, 28). However, this ascending somatosensory pathway does not mediate the thermosensory signaling required for eliciting feedforward involuntary thermoregulatory responses. Lesions of the thalamic areas that mediate the spinothalamocortical signaling eliminate EEG responses in the primary somatosensory cortex evoked by changes in skin temperature, but do not affect autonomic thermogenic responses to skin cooling (105). Consistently, even after removal of the neocortex, dorsal hippocampus, and most of the striatum, the animals still maintain the ability to initiate a metabolic increase in response to skin cooling (130). Therefore, the afferent neural pathways mediating cutaneous thermosensory signals for involuntary thermoregulation are distinct from the spinothalamocortical pathway mediating perception and discrimination of cutaneous thermosensation (Fig. 1*B*).

Recent attempts of functional neuroanatomy combining retrograde neural tracing and c-Fos immunohistochemistry successfully provided a clue to identify the afferent neural pathways for feedforward thermosensory signaling to the thermoregulatory center, POA (104, 105). These studies first localized neurons that project to the POA and that were activated in response to cold or warm exposure of animals; they potentially include neurons that directly deliver cutaneous thermosensory signals to the POA. For this purpose, a retrograde neural tracer was injected into a midline portion of the POA, including the median preoptic nucleus (MnPO), in rats (Fig. 2*A*) to label neuronal populations that project their axons to the POA. Subsequently, the rats were exposed to a cold (4°C), warm (36°C), or control (24°C) environment. Their brain tissues were then subjected to immunohistochemical visualization of neurons that were labeled with the tracer and that expressed c-Fos. These experiments revealed that such double-labeled neurons are densely localized in the lateral parabrachial nucleus (LPB; Fig. 2, *B* and *C*) (104, 105). Within the LPB, POA-projecting neurons that are activated by cutaneous cooling are localized in the external lateral subregion (LPBel) and those that are activated by cutaneous warming are localized in the dorsal subregion (LPBd) (Fig. 2, *B* and *C*) (104, 105). This finding was further supported by in vivo unit recording experiments, in which POA-projecting neurons in the LPBel and LPBd increased their firing rates in response to skin cooling and warming, respectively (Fig. 2, *D* and *E*) (104, 105). These results indicate that separate populations of neurons located in these two adjacent LPB subregions mediate cutaneous cool and warm signaling to the POA through their direct projections

(Fig. 1*B*). The LPB is a sensory mediating region that receives massive projections from the dorsal horn (8, 25, 36). These projections to the LPB include axon collaterals from thalamic projecting neurons in the spinal and trigeminal dorsal horns (57, 75). An anatomical study revealed that axonal swellings from dorsal horn neurons are closely associated with postsynaptic structures of LPB neurons that project to the POA (105), supporting the view that the activation of POA-projecting LPB neurons in response to skin thermal challenges is caused by direct cutaneous thermosensory inputs from dorsal horn neurons (Fig. 1*B*).

In vivo electrophysiological studies examined the functional roles of these cutaneous cool- and warm-sensory pathways through the LPB to the POA in feedforward thermoregulatory responses. Briefly cooling the skin of anesthetized rats elicits increases in sympathetic thermogenesis in BAT, shivering thermogenesis in skeletal muscles, and metabolism (measured with expired CO_2) without substantially affecting body core or brain temperature (Fig. 3*A*) (106, 107). In addition to the thermogenic and metabolic responses, skin cooling also increases heart rate (Fig. 3*A*) (107). This cooling-induced tachycardia would facilitate the distribution of heat produced in thermogenic organs, as well as increase the availability of energy substrates to activated thermogenic organs. Furthermore, increased heart beating would increase the amount of heat production in the heart (cardiac thermogenesis). All of these feedforward cold-defensive responses to skin cooling are immediately eliminated by inhibition of LPBel neurons with bilateral local nanoinjections of muscimol, a $GABA_A$ receptor agonist acting as a neuronal inhibitor (Fig. 3, *A* and *C*), or by blockade of glutamate receptors in the LPBel with bilateral injections of a mixture of AP5 and CNQX, ionotropic glutamate receptor antagonists (105). Therefore, activation of LPBel neurons by glutamatergic inputs, likely from dorsal horn neurons mediating cutaneous cool-sensory signaling, is required to elicit feedforward cold-defensive responses to skin cooling (Fig. 1*B*). Consistent with this notion, rats that have bilateral lesions in the LPB fail to maintain their body temperature in a cool environment (68).

Stimulation of LPBel neurons with NMDA nanoinjection elicits thermogenic, metabolic and tachycardic responses that mimic the cold-defensive responses to skin cooling (105). These responses evoked by LPBel stimulation are blocked by antagonizing glutamate receptors in the MnPO (105), suggesting that LPBel neurons activated by cutaneous cool signals provide glutamatergic inputs to the MnPO (Fig. 1*B*). Further supporting the idea that activation of MnPO neurons by glutamatergic cutaneous cool inputs triggers cold-defensive responses, glutamatergic stimulation of MnPO neurons with NMDA nanoinjection elicits BAT thermogenic, metabolic and tachycardic responses mimicking cold-defensive responses to skin cooling (Fig. 4*D*) (108). Because similar stimulations in other POA subregions do not elicit such responses (108), and LPBel neurons activated in response to skin cooling predominantly project to the MnPO among POA subregions (105), the MnPO appears to be a major POA site that receives cutaneous cool-sensory inputs from the LPBel. Furthermore, inhibition of MnPO neurons blocks BAT thermogenic, shivering, metabolic, and tachycardic responses evoked by skin cooling (106, 108) (Fig. 4, *A* and *B*). These results indicate that the reception of cutaneous cool-sensory signaling by MnPO neurons is an
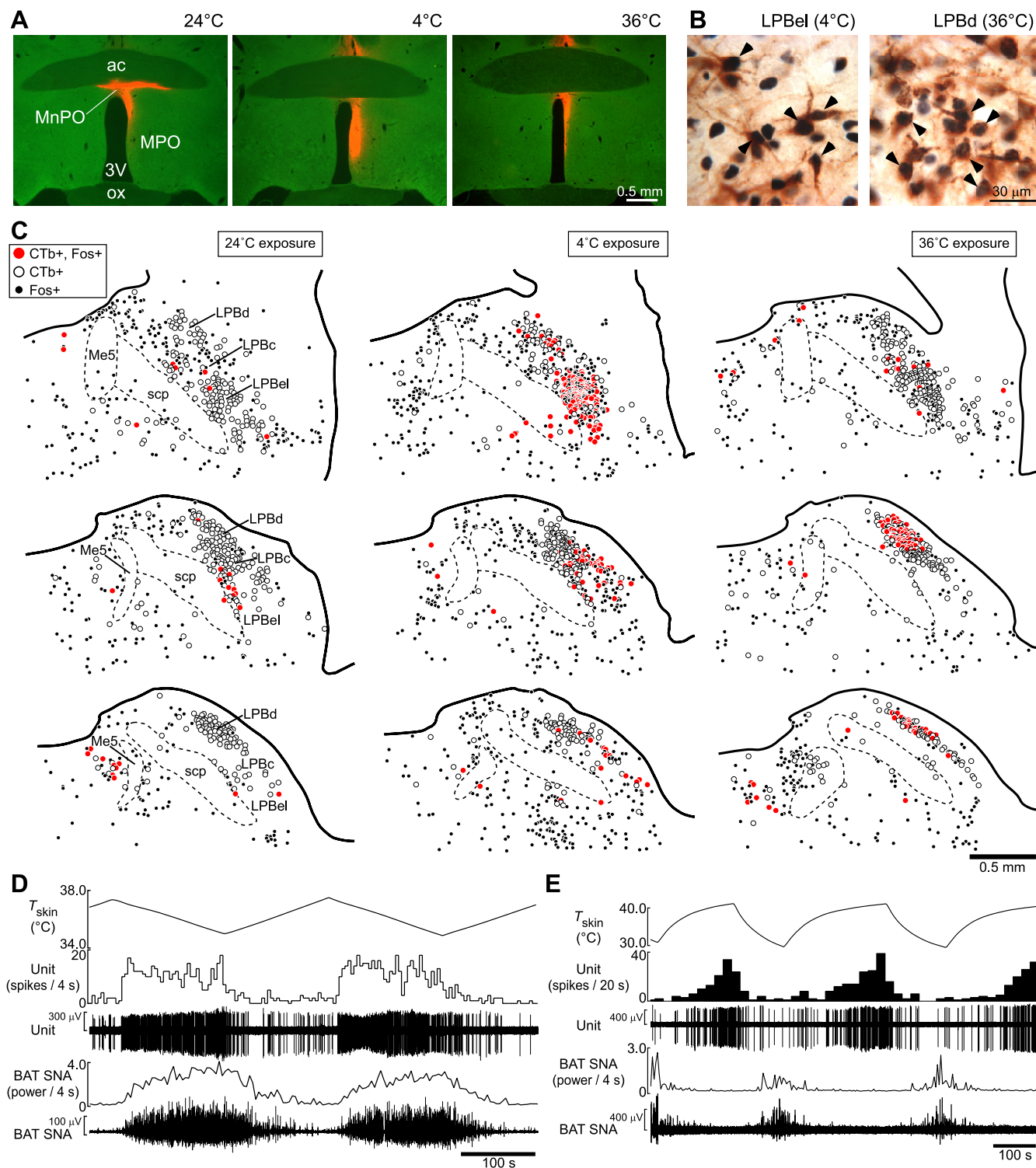
Fig. 2. Preoptic area (POA)-projecting neurons in the lateral parabrachial nucleus (LPB) are activated in response to cutaneous thermosensory inputs. *A-C*: cholera toxin b-subunit (CTb), a retrograde tracer, was injected into the POA (red in *A*) and subsequently, the rats were exposed to a 24°C, 4°C, or 36°C environment. ac, anterior commissure; MnPO, median preoptic nucleus; MPO, medial preoptic area; 3V, third ventricle; ox, optic chiasm. *B*: in the rats exposed to 4°C or 36°C, c-Fos expression (blue-black) was observed in neurons retrogradely labeled with CTb (brown) in the LPB, external lateral subregion (LPBel) or LPB, dorsal subregion (LPBd), respectively. Arrowheads in *B* indicate neurons double-labeled with c-Fos and CTb immunoreactivities. *C*: distribution of c-Fos-immunoreactive cells and CTb-labeled cells in the LPB of the rats shown in *A*. LPBc, central part of the lateral parabrachial nucleus; Me5, mesencephalic trigeminal nucleus; scp, superior cerebellar peduncle. *D* and *E*: in vivo extracellular unit recordings of action potentials (unit) of a skin-cooling-activated LPBel neuron (*D*) and a skin-warming-activated LPBd neuron (*E*) that project to the MnPO. Repeated trunk skin cooling (*D*) or warming (*E*) (T_{skin}) was given. BAT sympathetic nerve activity (SNA) was simultaneously recorded. [From Nakamura and Morrison (104, 105).].

Fig. 3. Inhibition of neuronal activation or antagonizing ionotropic glutamate receptors in the LPB blocks physiological responses to skin cooling or warming in rats. *A*: skin cooling-evoked changes in BAT SNA, BAT temperature (T_{BAT}), expired (Exp.) CO_2, heart rate (HR), arterial pressure (AP), rectal temperature (T_{rec}), and brain temperature (T_{brain}) before and after bilateral nanoinjections (dashed lines) of muscimol into the LPBel. *B*: skin warming-evoked changes in sympathetic cutaneous vasoconstrictor (CVC) activity in the sural nerve (Sural CVC) innervating the hind paw skin, tail skin temperature (T_{tail}), HR, AP, T_{rec}, and T_{brain} before and after bilateral injections (dashed lines) of saline or glutamate receptor antagonists (AP5/CNQX) into the LPBd. *C* and *D*: representative views of injection sites in the LPBel (*C*) and LPBd (*D*), as identified with fluorescent beads (arrows). [From Nakamura and Morrison (104, 105).].
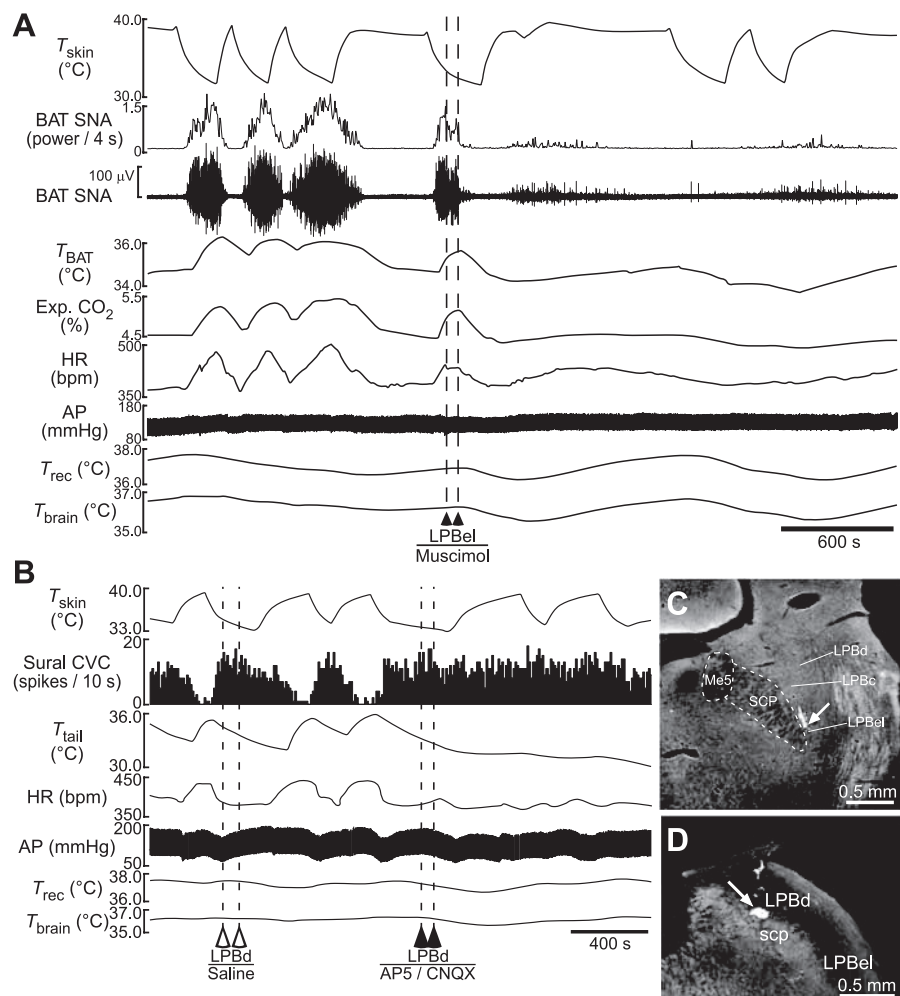
essential step to initiate the feedforward cold-defensive responses to skin cooling (Fig. 1*B*).

Neurons in the LPBd were recently demonstrated to mediate feedforward heat-defensive responses to skin warming (104). Skin warming elicits cutaneous vasodilation, which is a heat-defensive response increasing skin blood flow to facilitate dissipation of body heat from the body surface. This response is mostly elicited through attenuation of cutaneous sympathetic nerve activity, whose tonicity regulates skin blood flow, even under a thermoneutral environment. Skin warming also increases heart rate (104). This warming-induced tachycardia would help the heat-dissipating effect of the simultaneous cutaneous vasodilation by maintaining cardiac output and arterial pressure at a sufficient level to assure optimal increases in cutaneous blood flow. The cutaneous vasodilation and tachycardia evoked by skin warming are eliminated by blockade of glutamate receptors in the LPBd (Fig. 3, *B* and *D*) (104). Furthermore, glutamatergic stimulation of LPBd neurons elicits cutaneous vasodilation and tachycardia associated with rapid drops in body core and brain temperatures, mimicking the heat-defensive responses to skin warming (104). These results indicate that glutamatergic activation of LPBd neurons, likely by inputs from dorsal horn neurons, is required to elicit

heat-defensive responses to skin warming. Stimulation of LPBd neurons also reverses skin cooling-evoked BAT thermogenic, metabolic and tachycardic responses (104), indicating that cutaneous warm-sensory signaling through activation of LPBd neurons not only elicits heat-defensive responses, but also counteracts the cold-defensive actions triggered by cutaneous cool-sensory signaling. The cutaneous vasodilation elicited either by stimulation of LPBd neurons or by skin warming is eliminated by antagonizing glutamate receptors in the MnPO (Fig. 4*C*) (104). Together, with the projection of skin warming-activated LPBd neurons to the MnPO (104), these findings support the notion that LPBd neurons that are activated by cutaneous warm signaling provide direct glutamatergic input to the MnPO and that this LPBd-MnPO pathway is required for eliciting feedforward heat-defensive responses to environmental heat challenges (Fig. 1*B*). Interestingly, antagonizing glutamate receptors in the MnPO does not affect tachycardia evoked by stimulation of LPBd neurons or by skin warming (Fig. 4*C*) (104), suggesting that other projections from LPBd neurons mediate the tachycardic response associated with heat-defensive cutaneous vasodilation. The finding that the MnPO receives both cool- and warm-sensory signals as glutamatergic inputs suggests that cutaneous cool and warm signals from the
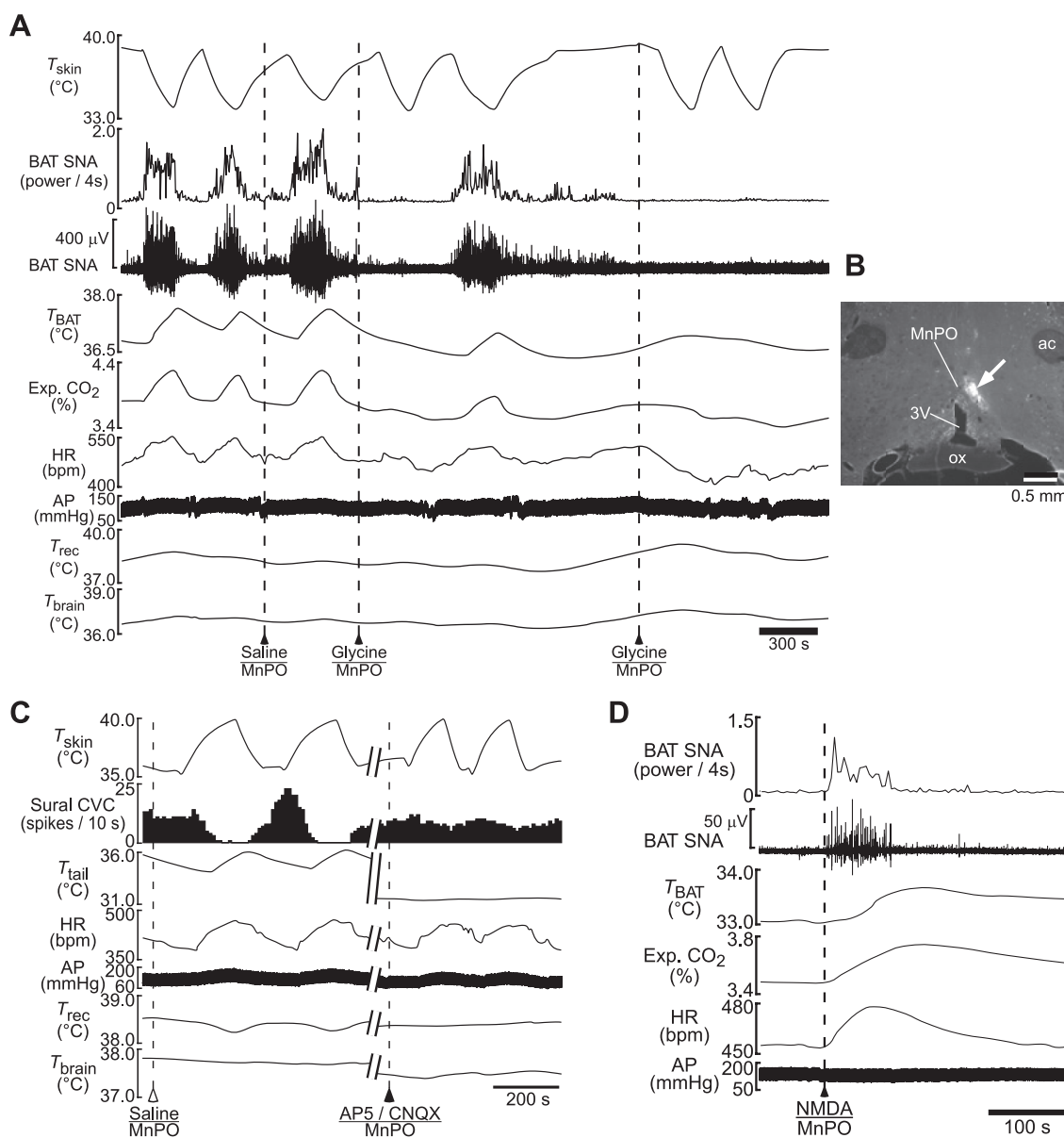
Fig. 4. Neuronal activation in the MnPO by glutamatergic inputs mediates physiological responses to skin cooling and warming. *A*: skin cooling-evoked increases in BAT SNA, T_{BAT}, Exp. CO_2, and HR were consistently blocked by inhibition of MnPO neurons with nanoinjections (dashed lines) of glycine in rats. *B*: an injection site in the MnPO (arrow). *C*: skin warming-evoked inhibition of Sural CVC and increase in T_{tail} were eliminated after an injection of AP5/CNQX into the MnPO (dashed line). Skin warming-evoked increase in HR was not affected by the AP5/CNQX injection. *D*: an injection of NMDA into the MnPO (dashed line) elicits increases in BAT SNA, T_{BAT}, Exp. CO_2, and HR, mimicking skin cooling-evoked responses. [From Nakamura and Morrison (104, 108).].

LPBel and the LPBd, respectively, activate distinct populations of neurons in the MnPO (Fig. 1*B*).

These findings on "thermoregulatory afferent" pathways indicate that the spinal (trigeminal)-LPB-POA pathways mediate cutaneous cool- and warm-sensory signals that are essential for triggering feedforward thermoregulatory responses to defend body core temperature from environmental thermal challenges. In addition to cutaneous thermosensory signals coming through the dorsal horn, the LPB receives massive visceral afferent information related to gastric tension, satiety, taste, thirsty, blood pressure, and temperature through the nucleus of the solitary tract (41, 145). This suggests that the LPB might be a site at which thermal somatosensory signals could be modified by those from the viscera to provide an integrated signal to the POA, a central site controlling a variety of homeostatic functions.

Central Thermosensation for Body Temperature Regulation

Neurons whose activity is affected by local brain temperature are abundantly localized in the POA (112, 113). Most of these thermosensitive neurons in the POA are neurons activated by local warm temperatures (warm-sensitive neurons) (14) and warm-sensitive POA neurons exhibit tonic discharge at thermoneutral temperatures (112, 113). The tonic discharge of some warm-sensitive POA neurons is reduced by skin cooling, as well as by local preoptic cooling, and their thermosensitivity to preoptic local temperature is affected by the

level of skin temperature (15), suggesting an integration of central and cutaneous thermosensory signals in the POA. Local cooling of the POA evokes BAT thermogenesis, as well as shivering thermogenesis (47, 59), and local warming of the POA evokes cutaneous vasodilation, as well as salivary secretion (evaporative heat loss response in rats and mice) (65, 66). These characteristics of warm-sensitive neurons support a model in which they integrate cutaneous and local thermal information and function as inhibitory projection neurons in the medial preoptic area (MPO; see below) that are tonically active at thermoneutral temperatures to control the tone of the efferent pathways driving thermoregulatory effector responses (Fig. 1*B*). Projection of warm-sensitive neurons outside of the POA remains to be investigated. Suggesting a role of warm-sensitive POA neurons in metabolic regulation, a recent study reported that a direct action of insulin on warm-sensitive POA neurons reduces their firing rates, and injection of insulin into the POA elicits a rise in body core temperature involving BAT thermogenesis (144).

Several ion channels that are localized in the cell bodies of POA neurons, such as hyperpolarization-activated cyclic nucleotide-gated channels and background potassium leak channels, have been proposed to contribute to the thermosensitivity of warm-sensitive neurons (180). However, studies on the molecular and neurophysiological mechanisms underlying the thermosensitivity of warm-sensitive neurons have not reached a conclusion (13, 69). Identification of specific histochemical markers for thermosensitive neurons would facilitate the characterization of these neurons, as well as the local circuitry in the POA, although several attempts have been made to find such markers (33).

In support of a role of temperature sensation in the spinal cord in thermoregulation, temperature changes in the spinal cord affect the activity of thermoregulatory neurons in the POA (44). This finding implies the existence of thermosensitive neurons in the spinal cord that can sense changes in local temperatures. However, changes in spinal temperatures could also be sensed by TRP channels located in the central endings of primary somatosensory fibers in the dorsal horn (7, 173), and if this is the case, such spinal thermal information could be integrated with cutaneous thermal signals at the level of dorsal horn somatosensory neurons.

These thermosensory mechanisms in the central nervous system (CNS) are considered to monitor the changes in temperature of blood circulating from peripheral tissues, including thermoregulatory effectors, because tissue temperatures in the CNS, especially the ventral portion of the brain, including the POA, which receives abundant blood flow from body core sites, are strongly affected by changes in blood temperature. This thermal feedback from periphery through the central monitoring of blood temperature is an important component in the thermoregulatory system (Fig. 1*A*). However, the regulation through the feedback mechanism involves fluctuation of CNS temperatures to trigger thermoregulatory responses. Such fluctuation in the central tissue temperatures could affect neuronal functions in the CNS and thereby, compromise the performance of any neurally controlled physiological functions. In contrast, feedforward regulation triggered by cutaneous thermosensation allows the body to maintain temperatures in the core sites, including the CNS, even under environmental thermal challenges, if they are not extreme. Actually, temper-

atures in deep body core structures, including the brain, do not change in a cold environment as much as skin temperatures (17, 38, 51, 78). Therefore, the central thermosensation would be expected to play a role *1*) in setting the basal tone of thermoregulatory efferent pathways, *2*) in enhancing thermoregulatory responses in situations of extreme thermal environments when the feedforward thermoregulatory responses have proven inadequate to prevent changes in brain or body core temperature and *3*) in responding to challenges to thermal homeostasis involving changes in temperature within the body, such as exercise, intake of hot fluids, or hemorrhage.

Central Reception of Pyrogenic Signals from the Immune System

The POA also receives pyrogenic signaling from the immune system (Fig. 1*A*). The immune-brain signaling mechanism for triggering fever during infection has been a focus of research. In laboratories, systemic injection of LPS, an endotoxin, into rodents has been used as an experimental model to mimic acute inflammatory symptoms, including fever. Injection of LPS (5–100 µg/kg) causes febrile elevation of body temperature with two or three peaks over several hours (34, 137). Although the development of the multiphasic fever is dependent on actions of prostaglandin PGE_2, a pyrogenic mediator (119), early (peaking ~1 h after LPS injection) and late (peaking 1–6 h) phases are likely mediated by PGE_2 from different sources.

The late phases, which embody a major part of LPS-induced fever (34, 137), are mediated by PGE_2 produced in brain vasculature. Inflammatory cytokines that are released from immune cells in response to infection or endotoxins from bacterium act on endothelial cells of blood vessels in the brain and thereby, induce expression of enzymes for biosynthesis of PGE_2 in these cells (87, 185). The enzymes for PGE_2 biosynthesis include secretory phospholipase A_2 IIA, cyclooxygenase-2, and microsomal PGE synthase (61, 87, 185). There are also reports that such expression of cyclooxygenase-2 is induced in perivascular glial cells (35). Large attenuation of LPS-induced fever by microinjection of a cyclooxygenase inhibitor into the POA (148) suggests that PGE_2 that is produced in vasculature around the POA during infection diffuses into the parenchyma to act on the fever-triggering neural mechanism in the POA (see below). A recent study reported that the receptor-activator of NF-κB ligand, RANKL and its receptor, RANK, mediate febrile signaling from inflammatory cytokines to activate the PGE_2-mediated pyrogenic mechanism and also that children with RANK mutations exhibit impaired fever during pneumonia (48). However, it remains to be determined how RANK existing in the parenchyma can be involved in the cytokine-triggered mechanism of PGE_2 production within brain vasculature.

The early phase of LPS-induced fever is likely mediated by PGE_2 produced in peripheral organs. Within 40 min after intravenous LPS injection, expression of cyclooxygenase-2 and microsomal PGE synthase is induced in the lung and liver, but not yet in the hypothalamus, and the plasma PGE_2 level is elevated (61, 156). The hepatic and pulmonary cells that express cyclooxygenase-2 were found to be macrophages (156). Neutralization of blood PGE_2 by intravenous injection of an anti-PGE_2 antibody reduces the early phase of LPS-
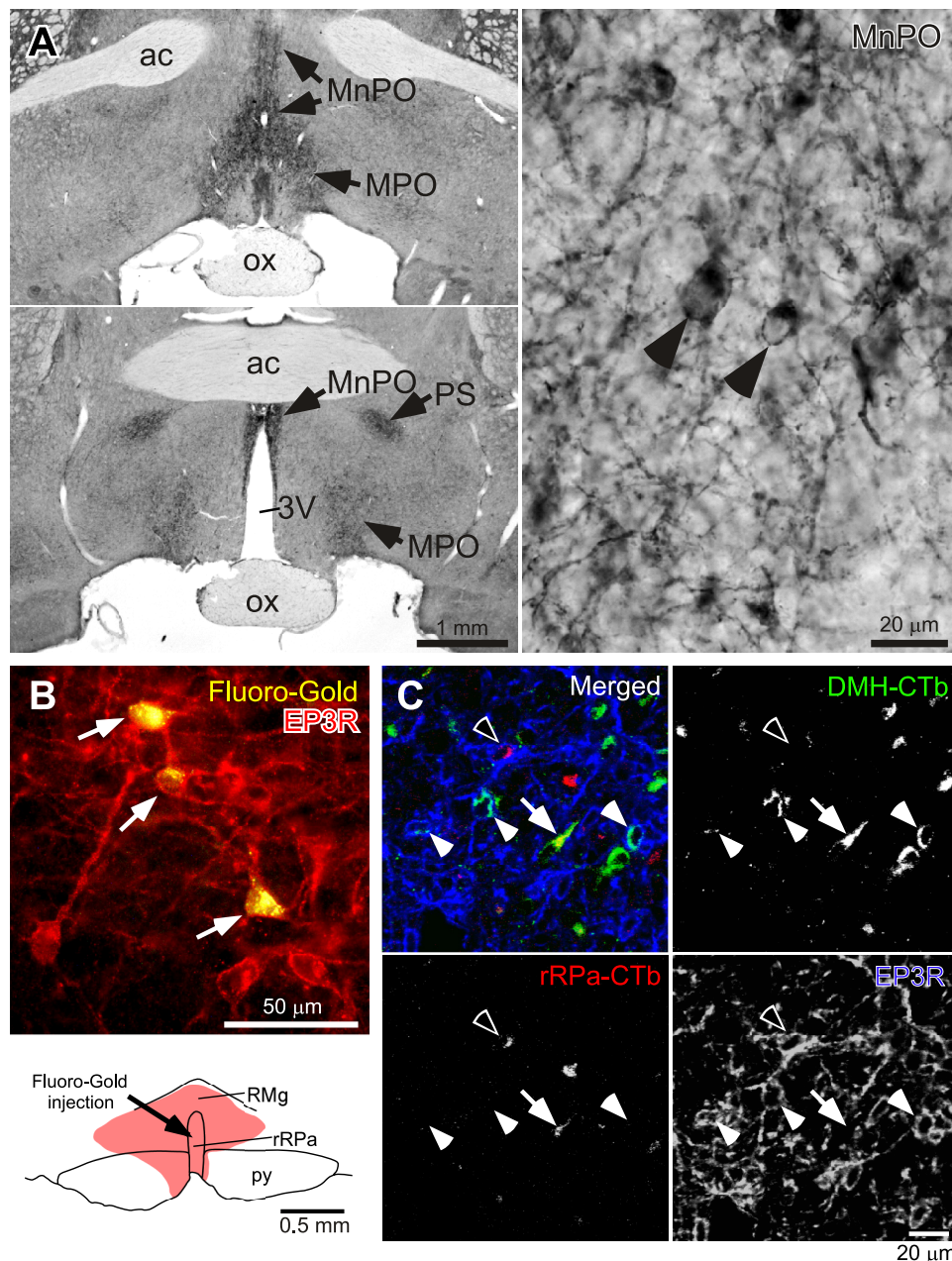
Fig. 5. Projections of EP3 receptor (EP3R)-expressing POA neurons to the DMH and to the rostral medullary raphe (rMR). *A*: EP3R immunohistochemistry in the rat POA. Intense immunoreactivity is distributed in the MnPO, MPO, and parastrial nucleus (PS) (*left*). In the MnPO, immunoreactivity is localized on dendritic processes and plasma membranes of neuronal cell bodies (*right; see arrowheads*). *B*: EP3R-immunoreactive POA neurons retrogradely labeled (arrows) with the tracer, Fluoro-Gold, injected into the rMR (*bottom; see pink area*). py, pyramidal tract. *C*: confocal images of EP3R-immunoreactive POA neurons retrogradely labeled from the DMH and from the rMR. Fluorescence signals of Alexa Fluor-488-conjugated CTb (transported from the DMH), Alexa Fluor-594-conjugated CTb (transported from the rRPa) and EP3R immunoreactivity are pseudocolored. EP3R-immunoreactive neurons labeled with DMH-CTb (solid arrowheads), EP3R-immunoreactive neurons labeled with rRPa-CTb (open arrowheads) and neurons labeled with both DMH-CTb and rRPa-CTb but lacking EP3R immunoreactivity (arrows) were densely distributed in the MnPO and MPO. However, neurons triple-labeled with EP3R-immunoreactivity, DMH-CTb, and rRPa-CTb were hardly found. [From Nakamura et al. (99, 102, 111).].

induced fever and exogenous intravenous injection of PGE_2 induces a rise in body temperature that is similar to the early phase of LPS-induced fever (156). These findings suggest that the early phase of fever is triggered by PGE_2 that is produced by immune system-activated hepatic and pulmonary macrophages and that is delivered to the POA through blood circulation.

Central Processing of Pyrogenic Signals for Fever Development

Action of the pyrogenic mediator PGE_2 on neuronal cells triggers the central circuitry mechanisms for fever development. From extensive injection mapping with PGE_1 throughout subcortical brain sites, the POA was identified as the sole region in which the E-series of PGs (PGEs) can act to produce fever (158, 183). Fever evoked by injection of PGEs into the POA is associated with involuntary somatic and sympathetic effector responses, such as shivering, BAT thermogenesis, and cutaneous vasoconstriction (3, 106, 171). PGE_1 injection into the POA also induces warm-seeking behavior (30), which is a voluntary behavior during fever that facilitates elevation of body temperature through selection of a warmer environment. However, lack of deficiency in LPS-induced warm-seeking behavior following lesion of the POA (1) suggests that warm-seeking behavior during fever is mediated by redundant mechanisms, some of which do not involve the POA.

Within the POA, the MPO and MnPO were found highly responsive to PGE_2 for its pyrogenic action (147). Similar POA subregions harbor many neurons that express the EP3 subtype of PGE receptor with the subcellular distribution in their somata and

dendritic fibers (Fig. 5A) (99, 100). PGE receptors have four subtypes, EP1, EP2, EP3, and EP4 (114), and mRNA expression for the EP1 and EP4 receptors, in addition to the EP3 receptor, is detected in the POA (120). Among mice lacking each of these PGE receptor subtypes, EP3 receptor-deficient mice completely fail to show a febrile response to any of PGE_2, interleukin-1β, and LPS (176), and EP1 receptor-deficient mice exhibit partially attenuated fever in response to LPS injection (119). Furthermore, spatially specific, genetic deletion of the EP3 receptor exclusively in MnPO and MPO neurons resulted in a large attenuation of fever in response to PGE_2 or LPS (72). These findings indicate that PGE_2 triggers the neuronal processes for fever induction by acting principally on the EP3 receptors located on POA neurons.

Findings from in vitro experiments suggest that activation of EP3 receptors mostly inhibit neuronal functions through their negative coupling to adenylate cyclase via G_i proteins (114). Although no study has directly recorded the activity of EP3 receptor-expressing neurons in the POA, the tonic activity of most warm-sensitive neurons in the POA is inhibited by the E-series of PGs (133, 149). Furthermore, intracerebroventricular application of PGE_2 reduces cAMP level in the POA and intracerebroventricular administration of an inhibitor of phosphodiesterase, an intracellular enzyme degrading cAMP, blunts fever evoked by intra-POA PGE_2 injection (154). Therefore, it is likely that binding of PGE_2 to EP3 receptors on POA neurons inhibits the activity of the neurons through reducing their intracellular cAMP levels, and this action triggers the efferent febrile neural signaling (Fig. 1B). The possibility that EP3 receptor-expressing POA neurons exhibit tonic firing activity whose attenuation triggers fever is consistent with a finding that inhibition of MPO neurons with muscimol injection elicits hyperthermic, shivering, metabolic, tachycardic, and neuroendocrine responses similar to fever (127, 192). This POA mechanism of triggering fever is similar to the notion that reduction of tonic activity of warm-sensitive neurons in the POA leads to cold-defensive responses (Fig. 1B). However, it has not been examined whether EP3 receptor-expressing POA neurons are identical to warm-sensitive neurons.

The EP3 receptor-expressing population of POA neurons directly innervates the dorsomedial hypothalamus (DMH) and the rostral medullary raphe region (rMR), including the rostral raphe pallidus nucleus (rRPa) and the adjacent raphe magnus nucleus (RMg) (Fig. 5, B and C) (102, 110, 111). A double-retrograde tracing study, in which tracers conjugated with different fluorophores were injected into the DMH and the RMg, revealed that separate populations of EP3 receptor-expressing POA neurons project to these brain sites (Fig. 5C) (111). This observation indicates independent control of DMH and RMg neurons from the POA by these separate neuronal populations. The DMH and rMR contain neurons whose activation leads to thermogenesis, cutaneous vasoconstriction, and tachycardia (see below). In support of the idea that the efferent neural signaling from EP3 receptor-expressing POA neurons finally controls thermoregulatory effector organs, trans-synaptic retrograde viral tracing from BAT resulted in labeling of EP3 receptor-expressing POA neurons (187). Taken together with expression of a marker for GABAergic neurons in most EP3 receptor-expressing POA neurons (102), these findings raise the notion that the neuronal population expressing EP3 receptors in the POA tonically inhibits neurons in the DMH and rRPa through their direct GABAergic projections under

normal conditions (Fig. 1B, left) and inhibition of the tonic activity of EP3 receptor-expressing POA neurons by an action of PGE_2 during infection leads to disinhibition of the neurons in the DMH and rRPa, which then drive febrile effector responses as described below (Fig. 1B, right).

The POA local mechanism for triggering fever can be modulated by norepinephrine. The level of norepinephrine in the POA is increased when body temperature declines following peaks of multiphasic fever evoked by LPS and application of norepinephrine into the POA attenuates LPS-induced fever (152). However, how norepinephrine acts on the febrile local mechanism in the POA is unknown. Although adrenoreceptor agonists and antagonists have been applied into the POA to examine their effects on LPS- or PGE_2-induced fever, inconsistent results have been obtained (37, 128). Noradrenergic inputs to the POA are likely provided by A1, A2, and locus coeruleus neurons in the pons and the medulla oblongata (184). These noradrenergic neuronal populations express c-Fos in response to LPS injection (49). Lesion of the locus coeruleus attenuates fever induced by LPS or intracerebroventricular PGE_2 in rats under a subneutral environmental temperature (23°C), but not under a neutral temperature (28°C) (2). These results suggest a modulatory role of locus coeruleus noradrenergic neurons in the central mechanism of fever, potentially through their projections to the POA, although the POA receives fewer noradrenergic projections from the locus coeruleus than those from A1 or A2 neurons (49).

Local Mechanism in the POA for Thermoregulatory Responses to Cutaneous Thermosensory Signals

How do cutaneous thermosensory signals that are received by MnPO neurons trigger the efferent signaling mechanisms to drive thermoregulatory responses? Stimulation of MnPO neurons with NMDA or disinhibition of MnPO neurons with bicuculline, a $GABA_A$ receptor antagonist, elicits BAT thermogenic, shivering, metabolic and tachycardic responses, mimicking cold-defensive responses to skin cooling (Fig. 4D) (106, 108, 129). All of these responses evoked by activation of MnPO neurons are reversed by bilateral blockade of $GABA_A$ receptors in the MPO (106, 108). Antagonizing $GABA_A$ receptors in the MPO also reverses BAT thermogenic, shivering, metabolic and tachycardic responses evoked by skin cooling (107, 127). These results indicate that the skin cooling-evoked physiological responses are dependent on GABAergic inhibition of MPO neurons, which is potentially provided by the MnPO neurons activated by a cutaneous cool-sensory input from the LPBel (Fig. 1B). The idea that MnPO neurons provide a local GABAergic connection to the MPO is also supported by previous anatomical observations that some MnPO neurons innervate the MPO (175) and that the MnPO contains many GABAergic neurons (42, 102). Furthermore, many neurons in the MnPO, rather than other POA subregions, are activated (express c-Fos) in response to lowered environmental temperature (16) and the extracellular level of GABA in the POA is elevated during cold exposure and reduced during heat exposure in free-moving rats (60).

In addition to these findings, several other physiological observations support the idea that neurons in the MPO provide tonic descending inhibition to caudal brain sites, including the DMH and rMR, and inhibition of the tonic activity of the MPO

neurons by cutaneous cool-sensory signals disinhibits the neurons in the caudal sites, which then drive thermogenic, metabolic and tachycardic responses (Fig. 1*B*). For example, a coronal transection just caudal to the POA evokes BAT thermogenesis (26) and lesion of the MPO, but not that of the ventral lateral preoptic area (LPO), and evokes hyperthermia through increasing metabolism and eliciting shivering thermogenesis (164). Furthermore, GABAergic inhibition of MPO neurons, but not those in the MnPO or LPO, increases body core temperature, EMG activity (shivering), metabolism, and heart rate (127, 192). This model of cool input-triggered POA mechanism is similar to the "disinhibition mechanism" for triggering fever that involves inhibition of EP3 receptor-expressing POA neurons by PGE_2. Whether the MPO neurons regulating cold-defensive responses are identical to EP3 receptor-expressing neurons and/or warm-sensitive neurons is an important question for future studies.

The POA local mechanism proposed here can also explain the regulation of cutaneous vasomotion, which works for both cold defense and heat defense: vasoconstriction through activation of cutaneous vasoconstrictor (CVC) sympathetic nerves reduces skin blood flow and attenuates heat dissipation from the body surface, and vasodilation, mostly through inhibition of CVC sympathetic nerves, increases skin blood flow and facilitates heat dissipation. Transection of descending outputs from the POA or GABA inhibition of MPO neurons enhances skin vasoconstriction through increasing CVC sympathetic nerve activity (134, 170). Furthermore, disinhibition of MnPO neurons increases CVC sympathetic nerve activity (169). Therefore, similar to the regulation of thermogenesis, metabolism and heart rate from the POA, CVC sympathetic nerve activity appears to be also regulated by descending tonic inhibition from MPO neurons, which are postulated to receive cutaneous thermosensory inputs from cooling-activated GABAergic inhibitory neurons and warming-activated excitatory neurons in the MnPO (Fig. 1*B*). However, as a caveat, unilateral blockade of $GABA_A$ receptors in the MPO was shown to have no effect on cooling-evoked activation of CVC sympathetic nerve (169). Although bilateral blockade might have inhibited the increase in CVC sympathetic nerve activity similarly to BAT sympathetic activity (107), it is also possible that descending inhibitory MPO neurons controlling CVC sympathetic nerve activity are regulated by non-GABAergic inputs.

According to the model presented here (Fig. 1*B*), inhibition of MnPO neurons would block cutaneous thermosensory inputs required to trigger feedforward thermoregulatory responses. Consistently, such inhibition blocks skin cooling-evoked increases in thermogenic, metabolic, and cardiac parameters (106, 108). Inhibition of MnPO neurons by itself does not induce BAT thermogenesis, shivering, or tachycardia (106, 108). However, inhibition of MnPO neurons with injection of GABA elicits skin vasoconstriction through activation of CVC sympathetic nerves (170). These contrasting effects of inhibition of MnPO neurons on the thermoregulatory effector activities might be explained by different contributions of warm-sensitive POA neurons to these thermoregulatory effector responses. In the study of CVC sympathetic nerve recording (170), the trunk skin of rats was warmed up to 39.5°C before GABA injection to reduce the baseline CVC sympathetic nerve activity. In this condition, cutaneous warm inputs to the MnPO

would be more intense than cutaneous cool inputs and thereby, the activity of MPO neurons that provide descending inhibition would be increased, leading to attenuation of CVC sympathetic nerve activity. Then, injection into the MnPO with an agent that inhibits neuronal activation, such as GABA, blocks the reception of both cutaneous warm and cool signals from the LPB (104, 105, 108). Under this condition, the POA local mechanism that regulates descending inhibitory signaling from the MPO would be devoid of thermosensory afferent inputs and likely dependent on local temperature sensation by warm-sensitive neurons, which are tonically active under a thermoneutral range of local brain temperature (113). The tonic descending inhibition from warm-sensitive neurons by itself might be insufficient to keep halting the caudal neuronal activity that drives CVC sympathetic outflow and, therefore, GABA injection into the MnPO elevated CVC sympathetic nerve activity (170). On the other hand, this level of tonic activity of warm-sensitive neurons is likely sufficient to keep suppressing the caudal mechanisms driving thermogenic, metabolic, and cardiac responses.

Efferent Pathways Mediating Command Signaling from the POA to Premotor Neurons in the rMR and to the Hypothalamic-Pituitary-Adrenal Axis

The descending inhibition from the MPO is considered to tonically control the activities of neurons in caudal sympatho-excitatory sites, among which the most investigated are the DMH and rMR. The rMR is a ventromedial medullary region immediately rostral to the rostral pole of the inferior olivary complex and consists of the rRPa, the adjacent RMg and the laterally extending parapyramidal nucleus (Fig. 7*J*) (101, 102). As described below, the rMR is known as a medullary region that contains sympathetic premotor neurons that control BAT thermogenesis, cutaneous vasomotion and heart rate for thermoregulation and fever (101, 103). Recent findings suggest that the rMR also contains somatic premotor neurons that drive shivering (106). Experimental findings in the last decade support the idea that regulation of these premotor neurons by descending signaling pathways from the POA is a fundamental component in the efferent control of thermoregulatory and febrile responses.

Cold exposure or injection of PGE_2 into the POA activates many neurons in the rMR, as evidenced by their c-Fos expression (12, 19, 97, 102). Inhibition of neurons in the rMR with drug injections blocks physiological responses, including BAT thermogenesis, shivering, cutaneous vasoconstriction, and tachycardia, which are evoked by skin cooling or injection of PGEs into the POA, cerebroventricle or vein (Fig. 7*A*-*C*, *E* and *F*) (70, 81, 93, 102, 106, 107, 124, 125, 134). These findings indicate that activation of rMR neurons is a required process in the efferent mechanisms that drive these cold-defensive and febrile responses.

Disinhibition of rMR neurons by antagonizing local $GABA_A$ receptors with bicuculline injection elicits BAT thermogenesis, shivering, cutaneous vasoconstriction and tachycardia (10, 21, 94, 97, 115). This physiological evidence suggests that thermoregulatory premotor neurons in the rMR are controlled by tonic GABAergic inputs. As described above, EP3 receptor-expressing neurons in the POA likely provide such GABAergic inputs through their direct projections to the rMR (Fig. 1*B*)

(102, 111), although there might also be other sources of such GABAergic inputs somewhere else. Antagonizing ionotropic glutamate receptors in the rMR also blocks BAT thermogenesis and tachycardia evoked by skin cooling or PGE$_2$ into the POA (81, 107) or evoked by stimulation of DMH neurons (22). Also, blockade of excitatory amino acid receptors in the rMR reduces skin cooling-evoked increase in CVC sympathetic nerve activity (169). Stimulation of DMH neurons induces c-Fos expression in rMR neurons (190). Therefore, glutamatergic inputs are required for the activation of premotor neurons in the rMR that mediate cold-defensive or febrile efferent signaling from the POA and one of the potential sources of such glutamatergic inputs is the DMH. Recently, a subset of MnPO neurons projecting to the rMR was found to increase their firing activity in response to skin cooling (169). Although the role of these neurons in thermoregulation is unknown, they might also contribute to the excitatory input to the rMR to drive cold-defensive responses.

The DMH consists of the dorsomedial hypothalamic nucleus and dorsal hypothalamic area. DMH neurons are activated (express c-Fos) in response to cold exposure, systemic LPS injection, or stress (16, 19, 146). Inhibition of neurons in the DMH with muscimol injections immediately reverses BAT thermogenesis, shivering, and tachycardia evoked by skin cooling or PGE$_2$ injection into the POA (Fig. 6, A–F) (82, 106, 107, 110, 172, 189), indicating that these cold-defensive and febrile responses require activation of DMH neurons. Furthermore, antagonizing GABA$_A$ receptors in the DMH elicits BAT thermogenesis, cutaneous vasoconstriction and tachycardia (20, 134, 191). EP3 receptor-expressing POA neurons innervate DMH neurons (Fig. 5C) (110, 111). These findings are consistent with the model in which tonic GABAergic inhibitory neurotransmission from the POA controls thermoregulatory neurons in the DMH, and this tonic inhibition is reduced by skin cooling or by PGE$_2$ action on EP3 receptors in the POA to disinhibit the DMH neurons (Fig. 1B). In addition to the attenuated GABAergic input from the POA, activation of thermoregulatory neurons in the DMH appears to require glutamatergic excitatory inputs, because blockade of ionotropic glutamate receptors within the DMH blocks BAT thermogenesis evoked by PGE$_2$ injection into the POA (82). However, the source of such glutamatergic inputs has yet to be determined.

BAT thermogenesis and tachycardia evoked by stimulation of DMH neurons are eliminated by inhibition of neurons in the rMR (20, 143) or by blockade of glutamate receptors in the rMR (22), suggesting that glutamatergic signals driving thermogenesis and tachycardia are transmitted from the DMH to the rMR to activate premotor neurons. Although the pathways responsible for the efferent signaling from the DMH to the rMR is still uncertain, several anatomical observations support the possibility that a direct monosynaptic pathway from the DMH to the rMR transmits the thermogenic and cardiac drives. Retrograde tracing studies have shown that the DMH provides numerous projections to the rMR and that such rMR-projecting neurons cluster in the region consisting of the dorsal hypothalamic area and the dorsal tip of the dorsomedial hypothalamic nucleus (Fig. 6G) (55, 110, 142), which is the site responsive to stimulation for eliciting tachycardia and BAT thermogenesis (20, 142). Similar distribution of DMH neurons is observed following trans-synaptic retrograde labeling from BAT (20).

Furthermore, rMR-projecting neurons in the DMH express c-Fos in response to cold exposure, stress or systemic LPS injection (146, 186). A double neural tracing study, in which anterograde and retrograde tracers were injected into the POA and rMR, respectively, revealed that GABAergic terminals that were anterogradely labeled from the POA formed close appositions with some DMH neurons that were labeled retrogradely from the rMR (i.e., rMR-projecting neurons) (Fig. 6H) (110). This observation of the putative synapses supports the notion that the activity of rMR-projecting neurons in the DMH is controlled by GABAergic inputs from the POA (Fig. 1B). Taken together, these lines of evidence support the mechanistic model for activation of rMR premotor neurons, in which cutaneous cool inputs to the POA or PGE$_2$ action on EP3 receptor-expressing POA neurons stimulates excitatory signaling from the DMH to the rMR through disinhibition of rMR-projecting DMH neurons and resultant activation of premotor neurons in the rMR leads to drives of BAT thermogenesis, shivering, and tachycardia through activation of bulbospinal signaling (Fig. 1B).

In contrast to thermogenesis and tachycardia, cutaneous vasoconstriction evoked by cooling or PGE$_2$ injection into the POA is not attenuated by inhibition of DMH neurons (134). This result excludes the role of DMH neurons in the efferent signaling for cold-defensive and febrile cutaneous vasoconstriction. However, the DMH might be involved in cutaneous vasoconstriction evoked by other stimuli, such as stress, because stimulation of DMH neurons can elicit cutaneous vasoconstriction (134). The efferent signaling mechanism of cold-defensive and febrile cutaneous vasoconstriction that bypasses the DMH raises a potential role of a direct projection from the POA to the rMR in this physiological response, which contrasts to the POA-DMH-rMR pathway for driving thermogenesis and tachycardia (Fig. 1B). The anatomical observation that separate populations of EP3 receptor-expressing POA neurons project to the DMH and to the rMR (111) further supports the idea that descending tonic GABAergic signals from POA neurons independently regulate thermogenesis/tachycardia and cutaneous vasoconstriction through their projections to the DMH and to the rMR, respectively (Fig. 1B). The possibility of separate regulations of thermogenesis and cutaneous vasomotion is consistent with a physiological observation that during cooling, the sympathetic outflow to BAT and that to the tail skin vasculature in rats are activated at different threshold temperatures (126). Even within the DMH, separate neuronal populations appear to control different autonomic effectors. Small injections of D,L-homocysteic acid to stimulate local neurons were made in the DMH of anesthetized rats to examine their effects on phrenic activity, CVC sympathetic nerve activity and heart rate (168). This functional mapping study revealed slightly different topographies of the DMH subregions that are responsive for activation of these physiological parameters (168).

Application of PGE$_2$ into the POA or inhibition of MPO neurons with muscimol injection also elevates plasma ACTH levels through activation of the hypothalamic-pituitary-adrenal axis (98, 192). Similar neuroendocrine responses are also observed during LPS-induced fever and during cold exposure (32, 174). ACTH stimulates release of glucocorticoid, which would then increase glucose availability and facilitate lipolysis to provide more fuel for thermogenesis. Injection of PGE$_2$ into
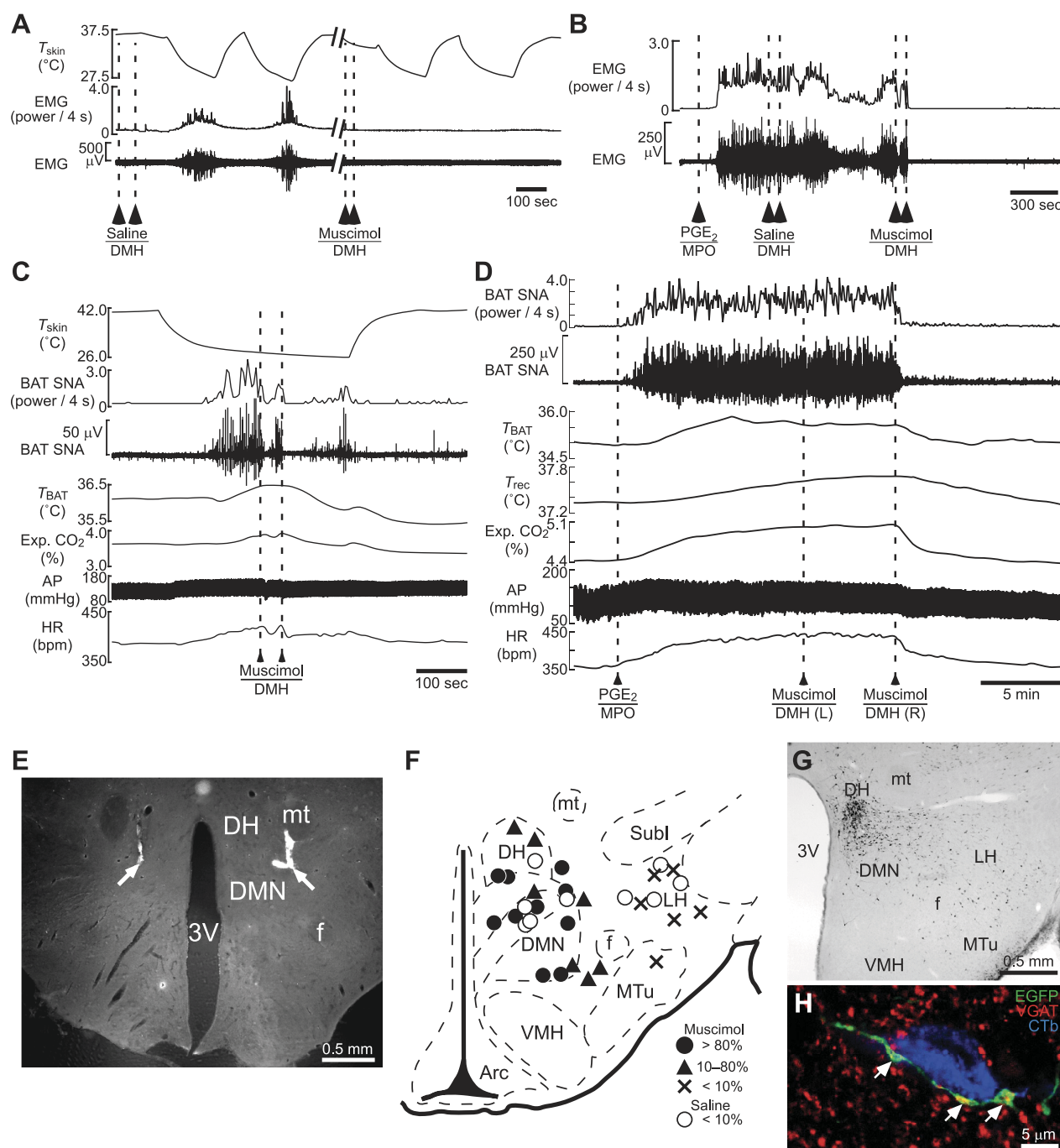
Fig. 6. Neurons in the DMH mediate shivering, BAT thermogenic, metabolic, and cardiovascular responses to skin cooling or PGE$_2$ injection into the MPO. *A* and *B*: increases in electromyogram (EMG; shivering) that were evoked by skin cooling (*A*) or by PGE$_2$ injection into the MPO (*B*) in rats were eliminated following bilateral nanoinjections (dashed lines) of muscimol into the DMH, which consists of the dorsomedial hypothalamic nucleus (DMN) and dorsal hypothalamic area (DH) (see *F*). *C* and *D*: skin cooling- or PGE$_2$-evoked increases in BAT SNA, T_{BAT}, Exp. CO$_2$ and HR were immediately reversed by bilateral muscimol injections (dashed lines) into the DMH. The muscimol injections also reversed PGE$_2$-evoked increases in T_{rec} and AP. *E*: representative view of bilateral injections into the DMH (arrows). f, fornix; mt, mammillothalamic tract. *F*: composite drawing of sites of saline and muscimol injections with their inhibitory effects on the increase in BAT SNA evoked by PGE$_2$ into the POA (see *D*). The inhibitory effect was expressed as inhibition percent of PGE$_2$-evoked increase in BAT SNA and graded. Arc, arcuate nucleus; LH, lateral hypothalamic area; MTu, medial tuberal nucleus; SubI, subincertal nucleus; VMH, ventromedial hypothalamic nucleus. *G*: cluster of DMH neurons retrogradely labeled with Fluoro-Gold injected into the rMR. *H*: confocal image showing that axon swellings in the DMH labeled both with a viral anterograde tracer, enhanced green fluorescent protein (EGFP), from the POA and with vesicular GABA transporter (VGAT), a marker of GABAergic terminals, were closely associated (arrows) with a DMH neuron retrogradely labeled with CTb injected into the rMR. [From Nakamura and Morrison (106, 107) and Nakamura et al. (110).].

the POA or inhibition of MPO neurons increases c-Fos expression in the paraventricular hypothalamic nucleus (PVH) (56, 147), the hypothalamic output center for the hypothalamic-pituitary-adrenal axis. The increase in plasma ACTH that is evoked by muscimol injection into the MPO is suppressed by neuronal inhibition in the PVH (56). These results confirm that the neuroendocrine response from the POA is mediated by PVH neurons. Intriguingly, both the increase in plasma ACTH and the c-Fos expression in the PVH that are evoked by muscimol injection into the MPO are eliminated by inhibition of DMH neurons (56). This finding suggests that the DMH mediates the signaling from the POA to the PVH to stimulate the hypothalamic-pituitary-adrenal axis. In contrast to the neuroendocrine response, stimulation of the PVH exerts an inhibitory effect on BAT sympathetic nerve activity and thermogenesis (83). This sympathoinhibition by PVH stimulation is effective on BAT sympathetic nerve activation evoked by body cooling, PGE_2 injection into the POA, or stimulation of DMH neurons, but not that evoked by disinhibition of rRPa neurons with antagonizing local $GABA_A$ receptors (83). Therefore, the inhibition of BAT thermogenesis by PVH stimulation is likely mediated through a GABAergic input to the rRPa. However, a direct GABAergic pathway from the PVH to the rRPa is unlikely to exist, because PVH neurons are non-GABAergic (159). The inhibitory signaling mechanism from the PVH to the rRPa remains to be determined.

Bulbospinal Premotor Signaling from rMR Neurons

Neurons in the rMR are consistently labeled following inoculation of pseudorabies virus into thermoregulatory and cardiac effectors, such as BAT, cutaneous blood vessels and stellate ganglion (controlling the heart), subsequently to its infection of sympathetic preganglionic neurons (5, 19, 63, 121, 153, 187). The results from the trans-synaptic retrograde viral tracing raise the possibility that the rMR contains sympathetic premotor neurons that control these effectors through their direct excitatory projections to sympathetic preganglionic neurons in the spinal intermediolateral cell column (IML).

The rMR contains many neurons projecting to the IML (77). Although the rMR harbors abundant serotonergic neurons, a dominant population of neurons in the rMR that are activated by febrile signaling from the POA or psychological stress is nonserotonergic (76, 102). Anatomical studies revealed that cold exposure, injection of PGE_2 into the POA, or psychological stress induces c-Fos expression in many rMR neurons that express vesicular glutamate transporter 3 (VGLUT3) (Fig. 7, *I* and *J*) (76, 101). Because VGLUT3 transports the neurotransmitter glutamate into vesicles (167), VGLUT3-expressing neurons in the rMR potentially release glutamate from their axon terminals. Anterograde tracing revealed that VGLUT3-expressing rMR neurons directly innervate sympathetic preganglionic neurons (Fig. 7*H*) and trans-synaptic viral retrograde tracing from BAT or skin blood vessels labeled many VGLUT3-expressing rMR neurons (101). These lines of evidence indicate that VGLUT3-expressing rMR neurons serve as sympathetic premotor neurons that function for thermoregulation and fever by mediating efferent signaling to BAT and skin blood vessels through their glutamatergic innervation of sympathetic preganglionic neurons. The glutamatergic bulbospinal premotor signaling is further supported by physiological observations that BAT thermogenesis evoked by stimulation of rMR neurons is blocked by antagonizing glutamate receptors in the upper thoracic IML (Fig. 7*G*) (101) and that nanoinjection of glutamate or NMDA into similar spinal sites elicits BAT thermogenesis (84, 101). Furthermore, cutaneous sympathetic nerve discharge evoked by stimulation of the rMR was eliminated by spinal application of an antagonist for excitatory amino acid receptors (123). These findings indicate that the glutamatergic pathway from the rMR to the IML is an essential component of the premotor signaling that controls BAT thermogenesis and cutaneous vasoconstriction (Fig. 1*B*). The anatomical observation that cell bodies of VGLUT3-expressing rMR neurons are surrounded by numerous GABAergic terminals (103) supports the idea that sympathetic premotor neurons in the rMR are controlled by GABAergic inputs. Although VGLUT3-expressing rMR neurons might also mediate shivering response, whether these neurons innervate somatomotor neurons in the spinal ventral horn remains to be examined.

Consistent with the notion that VGLUT3-expressing rMR neurons release glutamate in the IML, VGLUT3-immunoreactive axon terminals in the IML were found to form asymmetric synapses (109, 160), which are characteristic of excitatory synapses. However, VGLUT3 is also expressed in GABAergic raphe-spinal neurons providing symmetric synapses in the IML (160). These observations suggest that the VGLUT3-expressing population of rMR neurons could provide both excitatory and inhibitory signals to the IML, although the physiological role of such inhibitory bulbospinal signaling is unknown.

Double-immunohistochemical labeling for VGLUT3 and serotonin revealed that 10–15% of VGLUT3-expressing rMR neurons contain serotonin (101) and that in the IML, 6–22% (depending on thoracic levels) of VGLUT3-containing axon terminals also contain serotonin (109). These observations suggest that some VGLUT3-expressing rMR neurons, al-

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Fig. 7. Neurons in the rMR mediate cold-defensive and febrile shivering, BAT thermogenic, metabolic, and tachycardic responses, likely through VGLUT3-expressing glutamatergic raphe-spinal premotor pathways. *A* and *B*: increases in EMG (shivering) evoked by skin cooling (*A*) or by a PGE_2 injection into the MPO (*B*) in rats were eliminated following an injection (dashed lines) of muscimol into the rRPa. *C* and *D*: an injection of glycine (*C*) or 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; *D*) into the rRPa (dashed lines) immediately reversed skin cooling-evoked increases in BAT SNA, T_{BAT}, Exp. CO_2, and HR. *E*: an injection site into the rRPa (arrow). *F*: BAT thermogenesis (rise in T_{BAT}) evoked by a PGE_2 injection into the MPO was blocked by a prior injection of muscimol into the rRPa. *G*: BAT thermogenesis triggered by bicuculline-induced disinhibition of rMR neurons was blocked by prior injections of AP5/CNQX into the IML. *H*: confocal image in the IML showing that axon swellings double-labeled with a viral anterograde tracer (EGFP) from the rMR and with VGLUT3 immunoreactivity were associated (arrowheads) with a dendrite immunoreactive for choline acetyltransferase (ChAT), a marker for sympathetic preganglionic neurons. *I*: double immunostaining for VGLUT3 (blue-black) and c-Fos (brown) in the rMR following an intracerebroventricular injection of saline or PGE_2. Open and solid arrowheads indicate VGLUT3-immunoreactive neurons that are negative and positive for c-Fos immunoreactivity, respectively. *J*: distribution of neurons immunoreactive for VGLUT3 and c-Fos in the ventral medulla. cRPa, caudal raphe pallidus nucleus; DAO, dorsal accessory olivary nucleus; Giv, ventral part of the gigantocellular reticular nucleus; MAO, medial accessory olivary nucleus; PIO, principal inferior olivary nucleus; PPy, parapyramidal nucleus; ROb, raphe obscurus nucleus. [From Nakamura et al. (101, 102) and Nakamura and Morrison (106, 107).].
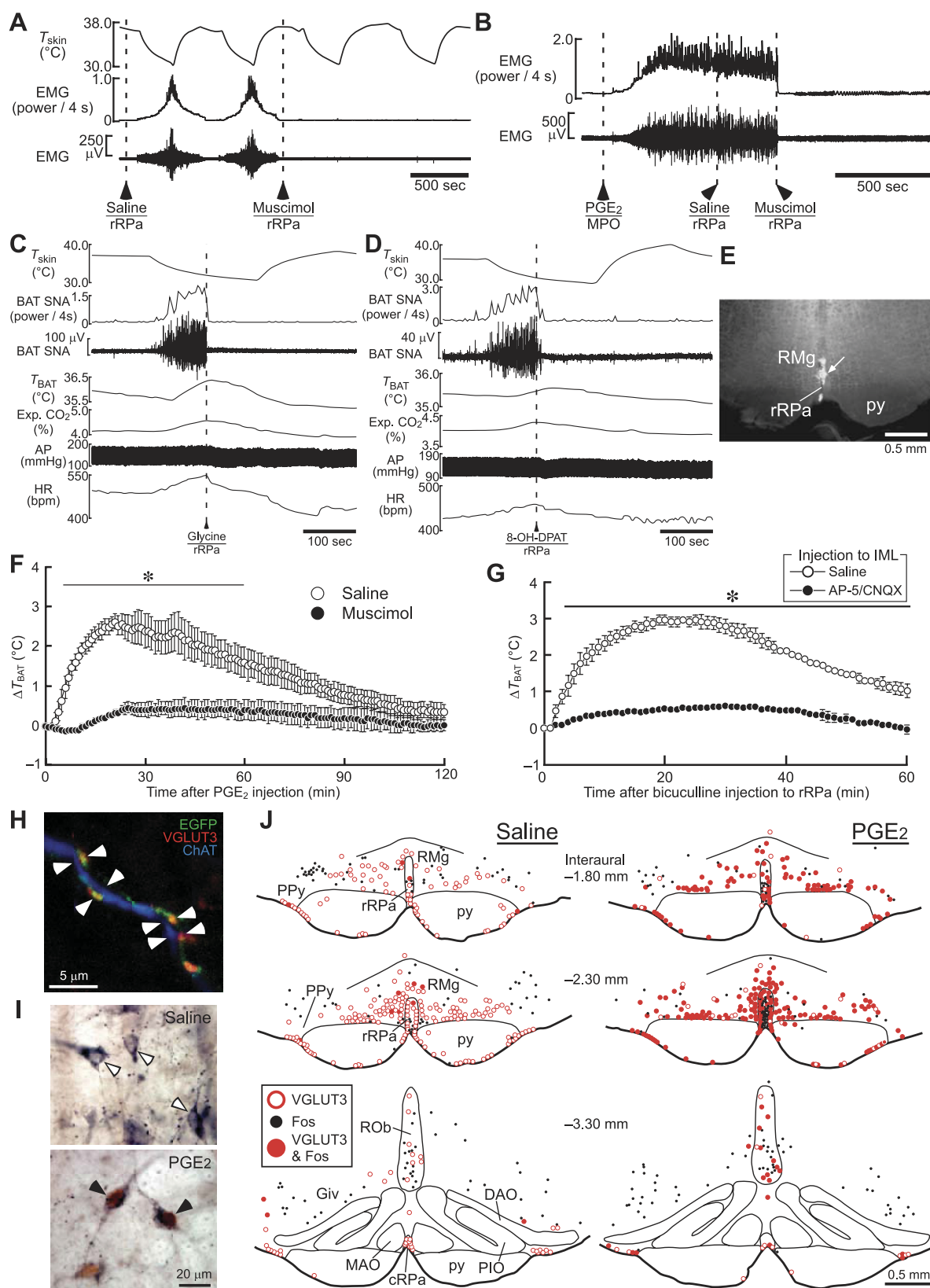
though not predominant, corelease glutamate and serotonin in the IML. Because injection of serotonin into the thoracic IML does not evoke rapid BAT thermogenesis (84), action of serotonin by itself seems insufficient to elicit depolarization of sympathetic preganglionic neurons controlling BAT. However, serotonin released in the IML can play a modulatory role by potentiating the excitatory effect of glutamatergic neurotransmission onto sympathetic preganglionic neurons controlling BAT (84). Consistent with this idea, blockade of serotonin receptors in the IML attenuates cooling-evoked BAT thermogenesis, and this attenuation is ascribable to blockade of 5-HT_{1A} and 5-HT_7 receptors in the IML (80). Raphe-spinal serotonergic signaling is also involved in the regulation of cutaneous vasomotion through 5-HT_{2A} receptors in the spinal cord, as evidenced by a physiological study in which CVC sympathetic nerve discharge evoked by stimulation of the rMR was reduced by spinal application of a 5-HT_{2A} receptor antagonist (123). In further support of the significant role of serotonergic neurons in cold defense, mice whose central serotonergic neurons are almost all eliminated show blunted BAT thermogenic and shivering responses to intense cold exposure (53) and acute inhibition of central serotonergic neurons transiently lowers body temperature under room temperature (135).

Serotonin modulates not only spinal neurotrasmission, but also the activity of rMR neurons. Injection of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, into the rMR blocks BAT thermogenic, metabolic, and tachycardic responses to skin cooling (Fig. 7D) or intravenous injection of leptin (92, 107), as well as shivering evoked by cooling or PGE_2 injection into the POA (18, 106). These sympathoinhibitory effects of 5-HT_{1A} receptor agonists through their action in the rMR might be a major pharmacological cause of severe hypothermia observed following systemic administration of such drugs in rats (52), mice (43), and humans (11). Activation of 5-HT_{1A} receptors is considered to inhibit neuronal functions through their negative coupling to adenylate cyclase via G_i proteins (6), and these receptors are located in spinal projecting neurons in the medullary raphe region (50). These findings suggest that serotonergic inputs to the rMR attenuate the activity of thermoregulatory sympathetic and somatic premotor neurons in the rMR through 5-HT_{1A} receptors potentially located on their somatodendritic portions (Fig. 1B). However, the sources of such serotonergic inputs are unknown.

Psychological Stress-Induced Hyperthermia

Psychological stress strongly affects the central thermoregulatory system and induces a rise in body core temperature. Psychological stress-induced hyperthermia is a fundamental physiological response broadly observed in mammals and exposure to intense and repeated stress often results in chronic hyperthermia, a symptom often called "psychogenic fever" (118). Patients with psychogenic fever exhibit a rise in body temperature above normal range as long as psychologically stressful situations last (117). Hyperthermia induced by social defeat stress, a psychological stress model, in rats is reduced by systemic injection of an antagonist of the β_3-adrenoreceptor (Fig. 8A) (76), the adrenoreceptor subtype abundant in BAT and responsible for BAT thermogenesis (196). Furthermore, BAT temperature is increased in response to immobilization
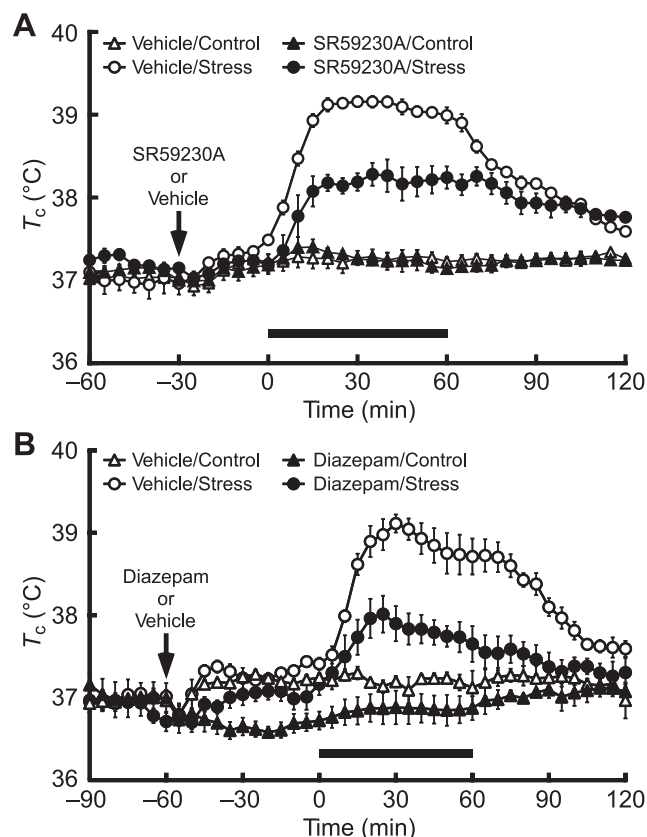

Fig. 8. Effects of the β_3-adrenoblocker SR59230A (A) or the anxiolytic drug diazepam (B) on social defeat stress-induced increase in body core (abdominal) temperature (T_c). Rats ($n = 5$ per group) received an intraperitoneal injection of the drug or their respective vehicles (arrows) and were subsequently exposed to social defeat stress (Stress) or left undisturbed (Control) during the period indicated by the horizontal bars. [From Lkhagvasuren et al. (76).].

stress (151). These results suggest that similar to infection-induced fever, psychological stress-induced hyperthermia also involves sympathetic thermoregulatory responses, including BAT thermogenesis.

However, stress-induced hyperthermia and infection-induced fever show different sensitivities to some pharmacological agents. For example, stress-induced hyperthermia, but not infection-induced fever, is attenuated by anxiolytic drugs, such as diazepam (Fig. 8B) (76, 122, 178, 193), whereas cyclooxygenase inhibitors block infection-induced fever, but not stress-induced hyperthermia (76, 155, 178, 182, 188). Furthermore, EP3 receptor-deficient mice can exhibit intact stress-induced hyperthermia, but fail to exhibit LPS-induced fever (119). These findings indicate that psychological stress triggers hyperthermic responses in a manner independent of the PGE_2-EP3 receptor mechanism and suggest that anxiolytic drugs exert their inhibitory effect on stress-induced hyperthermia not by acting on the thermoregulatory efferent pathways from the POA, but potentially by acting on stress-processing structures, such as the limbic system.

Nevertheless, several studies suggest that the DMH and rMR are involved in stress-evoked autonomic responses. Neuronal inhibition in the DMH with muscimol injections eliminates tachycardic and pressor responses to air jet stress (161), although its effect on stress-induced hyperthermia remains to be

tested. DMH neurons projecting to the rMR express c-Fos in response to air jet or restraint stress (146). Social defeat stress induces c-Fos expression in VGLUT3-expressing neurons in the rMR and systemic injection of diazepam inhibits the social defeat stress-induced c-Fos expression, as well as hyperthermia (Fig. 8*B*) (76). These findings raise the possibility that a direct pathway from the DMH to the rMR delivers stress-driven sympathoexcitatory signals to activate sympathetic premotor neurons controlling thermal effectors. However, this mechanism might not be applicable to hyperthermia observed in some other stress models. Conditioned fear stress increases core body temperature without involving evident BAT thermogenesis (86) and induces c-Fos expression in spinally projecting neurons in the perifornical area and paraventricular nucleus of the hypothalamus and in the A5 noradrenergic area of the pons, but not in the rMR (23). Ablation of orexin-containing neurons, which are mostly distributed in the perifornical area, markedly attenuates hyperthermia evoked by repetitive insertion of a temperature probe into the rectum (handling stress) (195). Further studies are awaited to determine how a variety of psychological stresses stimulate sympathoexcitatory efferent mechanisms to elicit hyperthermia.

Summary and Perspectives

Thermoregulation and fever involve a variety of effector responses. The major focus of this review was on the central circuitry mechanisms that underlie nonshivering thermogenesis in BAT, shivering thermogenesis in skeletal muscles, and cutaneous vasomotion that are involuntarily evoked (or inhibited) in response to environmental thermal challenges or pyrogenic stimuli. As illustrating in Fig. 1*B*, I have summarized the schematic model of the central circuitries based on research findings. In warm environments, sensory signals from cutaneous warm receptors ascend through the dorsal horn to the LPBd as a glutamatergic input. Activated LPBd neurons then provide another glutamatergic input to the MnPO, where local neurons are postulated to be activated and provide excitatory signals to descending projection neurons in the MPO. The activated MPO neurons, which are potentially warm-sensitive neurons, send GABAergic inhibitory signals to neurons in the DMH or the rMR to suppress sympathetic drives to BAT and CVCs (leading to cutaneous vasodilation), as well as shivering somatic motor outputs. In cold environments, sensory signals from cutaneous cool receptors ascend through the dorsal horn to the LPBel as a glutamatergic input. Activated LPBel neurons then provide a glutamatergic input to the MnPO, where GABAergic neurons are activated and inhibit the descending projection neurons in the MPO. In case of infection, the activity of the descending projection neurons in the MPO is attenuated by an action of the febrile mediator, PGE$_2$ on EP3 receptors potentially located on these neurons. The cool input- or PGE$_2$-induced attenuation of the GABAergic descending inhibition from the MPO to the DMH and rMR leads to disinhibition of neurons in the DMH and rMR. For driving BAT thermogenesis and shivering, activated DMH neurons provide an excitatory input to premotor neurons in the rMR. Activated sympathetic premotor neurons in the rMR provide excitatory inputs to sympathetic preganglionic neurons in the IML to drive BAT thermogenesis and cutaneous vasoconstriction. Excitatory signaling from rMR neurons to somatomotor

neurons in the spinal ventral horn drives shivering, although the spinal circuitry mechanism that produces the tremor-like muscle movements of shivering is unknown.

Although most of the findings presented in this review were obtained from experiments using rodents, fMRI studies in humans have reported that body cooling activates the parabrachial nucleus and rMR (88, 150), supporting the relevance of the present central circuitry model to the human thermoregulatory system. A recent big breakthrough in research on human thermoregulation and metabolism is the discovery of BAT in adult humans (31, 141, 177, 179). Until recently, the presence of BAT was thought to be relevant only in small mammals and human infants, with negligible physiological relevance in adult humans (116). PET/CT scanning in adult humans has detected cooling-activated uptake of radiolabeled fluorodeoxyglucose (FDG) (indicative of glucose utilization) into fat deposits in the supraclavicular and paraspinal regions, in which adipocytes expressing uncoupling protein 1, a marker of brown adipocytes, were found (Fig. 9, *A* and *B*) (31, 141, 177, 179). Intriguingly, the cooling-activated FDG uptake into BAT was
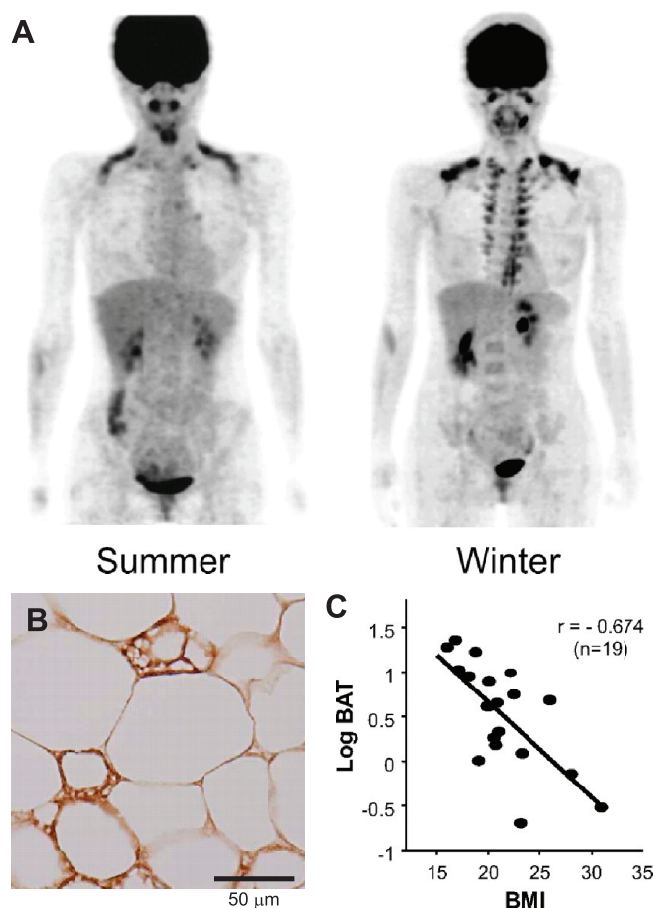
Summer   Winter

Fig. 9. BAT exists in adult humans and is remarkably activated by cooling in winter and the intensity of BAT activation is inversely correlated to body mass index (BMI) of subjects. *A*: PET/CT scanning visualized cooling-activated uptake of FDG into supraclavicular and paraspinal BAT of an adult female human subject. FDG uptake into BAT was more intense in summer than in winter. *B*: Uncoupling protein 1-positive brown adipocytes in fat depots obtained from the supraclavicular region of an adult human. *C*: cooling-activated FDG uptake into BAT was inversely correlated to BMI of the subjects. [From Saito et al. (141).].

increased in winter compared with summer and inversely correlated to body mass index, as well as fat mass of the subjects (Fig. 9, *A* and *C*) (31, 141, 177). These findings indicate important roles of BAT in the regulations of body temperature and metabolism in adult humans, and the central circuitry regulating BAT thermogenesis could be a target for obesity treatment.

In addition to metabolic control, the central thermoregulatory system is also closely linked to systems controlling other homeostatic functions, such as osmoregulation, biological rhythm generation (e.g., ultradian, circadian, and seasonal rhythms), respiration, appetite, and sleep. However, the central mechanisms underlying these functional relationships are poorly understood. Orchestrated CNS regulations of peripheral organs, including thermoregulatory effectors are essential to maintain homeostasis. The pivotal functions of the POA that it integrates afferent signals and provides efferent command signals suggest the POA as a potential brain region that coordinates the orchestrated effector regulations. Although, as described in this review, efferent outflow pathways to a variety of effector organs are likely controlled by separate sets of projection neurons in the POA, how the POA local circuitry accomplishes the coordination of these projection neuronal activities is still a big open question in the field.

DISCLOSURES

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

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