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DIFFERENT POPULATIONS OF PROSTAGLANDIN EP3 RECEPTOR-EXPRESSING PREOPTIC NEURONS PROJECT TO TWO FEVER-MEDIATING SYMPATHOEXCITATORY BRAIN REGIONS

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Abstract—The central mechanism of fever induction is triggered by an action of prostaglandin E_2 (PGE_2) on neurons in the preoptic area (POA) through the EP3 subtype of prostaglandin E receptor. EP3 receptor (EP3R)-expressing POA neurons project directly to the dorsomedial hypothalamus (DMH) and to the rostral raphe pallidus nucleus (rRPa), key sites for the control of thermoregulatory effectors. Based on physiological findings, we hypothesize that the febrile responses in brown adipose tissue (BAT) and those in cutaneous vasoconstrictors are controlled independently by separate neuronal pathways: PGE_2 pyrogenic signaling is transmitted from EP3R-expressing POA neurons via a projection to the DMH to activate BAT thermogenesis and via another projection to the rRPa to increase cutaneous vasoconstriction. In this case, DMH-projecting and rRPa-projecting neurons would constitute segregated populations within the EP3R-expressing neuronal group in the POA. Here, we sought direct anatomical evidence to test this hypothesis with a double-tracing experiment in which two types of the retrograde tracer, cholera toxin b-subunit (CTb), conjugated with different fluorophores were injected into the DMH and the rRPa of rats and the resulting retrogradely labeled populations of EP3R-immunoreactive neurons in the POA were identified with confocal microscopy. We found substantial numbers of EP3R-immunoreactive neurons in both the DMH-projecting and the rRPa-projecting populations. However, very few EP3R-immunoreactive POA neurons were labeled with both the CTb from the DMH and that from the rRPa, although a substantial number of neurons that were not immunoreactive for EP3R were double-labeled with both CTbs. The paucity of the EP3R-expressing neurons that send collaterals to both the DMH and the rRPa suggests that pyrogenic signals are sent independently to these caudal brain regions from the POA and that such pyrogenic outputs from the POA reflect different control mechanisms for BAT thermogenesis and for cutaneous vasoconstriction by distinct sets of POA neurons.

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Abbreviations: BAT, brown adipose tissue; CTb, cholera toxin b-subunit; DMH, dorsomedial hypothalamus; DMH-CTb, cholera toxin b-subunit derived from the dorsomedial hypothalamus; EP3R, EP3 receptor; MnPO, median preoptic nucleus; MPO, medial preoptic area; OVLT, organum vasculosum of the lamina terminalis; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline containing Triton X-100; PGE_2, prostaglandin E_2; POA, preoptic area; rRPa, rostral raphe pallidus nucleus; rRPa-CTb, cholera toxin b-subunit derived from the rostral raphe pallidus nucleus.

Key words: GABA, inflammation, rostral medullary raphe, stress response, sympathetic nervous system, thermoregulation.

Fever is a host-defense response that is governed by a brain mechanism triggered by an action of prostaglandin E_2 (PGE_2), which is mostly produced in the brain vasculature in response to immune signals caused by infections (Elmquist et al., 1997; Matsumura et al., 1998; Yamagata et al., 2001). The EP3 subtype of prostaglandin E receptor is abundantly expressed on neurons in specific subregions of the preoptic area (POA): the median preoptic nucleus (MnPO) and medial preoptic area (MPO) (Nakamura et al., 1999, 2000), and most of these neurons contain the inhibitory neurotransmitter, GABA (Nakamura et al., 2002). The action of PGE_2 on these EP3 receptors (EP3Rs), likely an inhibition of EP3R-expressing POA neurons (Narumiya et al., 1999), is responsible for the activation of fever-producing neuronal pathways (Lazarus et al., 2007), although EP3Rs in the POA could also be involved in other physiological functions including thermal hyperalgesia (Hosoi et al., 1997).

The POA contains EP3R-expressing neurons that project directly to the dorsomedial hypothalamus (DMH) and to the rostral raphe pallidus nucleus (rRPa) (Nakamura et al., 2002, 2005b), brain regions that provide excitatory drive for the sympathetically-regulated thermal effectors that are essential for eliciting febrile responses, such as brown adipose tissue (BAT) and cutaneous blood vessels (Nakamura, 2004; Morrison et al., 2008). In particular, the rRPa contains sympathetic premotor neurons that are activated in response to central PGE_2 administration and that multi-synaptically control BAT or cutaneous blood vessels such as those in rat tail through their direct innervation of sympathetic preganglionic neurons in the spinal cord (Nakamura et al., 2004, 2005a). Thus, inhibition of neuronal activity in the rRPa blocks heat production in BAT that is evoked by PGE_2 action in the POA (Nakamura et al., 2002; Madden et al., 2003; Rathner et al., 2008) and largely attenuates PGE_2-evoked cutaneous vasoconstriction in rat tail (Rathner et al., 2008), a sympathetic response to restrict body heat loss and increase body temperature. Intriguingly, inhibition of DMH neurons blocks BAT thermogenesis evoked by PGE_2 in the POA (Zaretskaia et al., 2003; Madden et al., 2004; Nakamura et al., 2005b; Rathner et al., 2008), but has no effect on PGE_2-evoked cutaneous vasoconstriction (Rathner et al., 2008).

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These findings led us to hypothesize (Nakamura, 2004; Nakamura et al., 2005b) that pyrogenic signaling in the POA, i.e. PGE_2-mediated inhibition of EP3R-expressing projection POA neurons, increases BAT thermogenesis by removal of direct inhibitory signaling from EP3R-expressing POA neurons to DMH neurons, which would then, in turn, lead to activation of rRPa sympathetic premotor neurons controlling BAT. Similarly, pyrogenic signaling in the POA would stimulate cutaneous vasoconstriction by inhibiting direct inhibitory signaling from EP3R-expressing POA neurons to rRPa sympathetic premotor neurons controlling skin blood vessels (Rathner et al., 2008).

In the present study, we tested the hypothesis that the EP3R-expressing neurons that project to the DMH and to the rRPa constitute segregated populations in POA neurons, potentially mediating differential control of BAT thermogenesis and cutaneous vasoconstriction, respectively, during febrile and cold-defense responses. To this end, we performed a double-tracing experiment in which two types of the retrograde tracer, cholera toxin b-subunit (CTb) conjugated with different fluorophores, were injected into the DMH and the rRPa and the resulting CTb labeling in EP3R-immunoreactive neurons in the POA was analyzed with confocal microscopy.

EXPERIMENTAL PROCEDURES

Animals

All experimental animal protocols were reviewed and approved by Animal Care and Use Committee of Oregon Health and Science University and conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. Male Sprague-Dawley rats (200–250 g, Charles River, Indianapolis, IN, USA) were housed two per cage with *ad libitum* access to feed and water in a room air-conditioned at 24±2 °C with a standard 12-h light/dark cycle.

CTb injection

Rats were deeply anesthetized with chloral hydrate (280 mg/kg i.p.) following introductory gas anesthesia with 3% isoflurane and placed in a stereotaxic apparatus; the incisor bar level was adjusted so that bregma and lambda were at the same dorsal level. Unilateral pressure-injections (Picospritzer II, General Valve, Fairfield, NJ, USA) of Alexa488-conjugated CTb and Alexa594-conjugated CTb (1 mg/ml, 240–280 nl; Molecular Probes, Eugene, OR, USA) were made via glass micropipettes (tip inner diameter: 10–20 μm) stereotaxically positioned in the DMH and in the rRPa, respectively. The coordinates for the DMH injections, which targeted the region combining the dorsal hypothalamic area and the dorsomedial hypothalamic nucleus (see Fig. 1A), were 3.3 mm caudal to bregma, 0.3–0.7 mm lateral to the midline and 8.0–8.5 mm ventral to the brain surface, and those for the injections targeting the rRPa were 11.6 mm caudal to bregma, on the midline and 9.5–9.7 mm ventral to the brain surface. Three to six days later, the animals were re-anesthetized and perfused transcardially with 200 ml of a 0.9% sodium chloride solution followed by 250–300 ml of 4% formaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Subsequently, the brain was post-fixed at 4 °C for 2 h and then cryoprotected with 30% sucrose in 10 mM sodium phosphate buffer (pH 7.4) overnight. The tissues were cut into 25 μm-thick frontal sections on a freezing microtome. The sites of CTb injections were identified with an epifluorescence microscope.

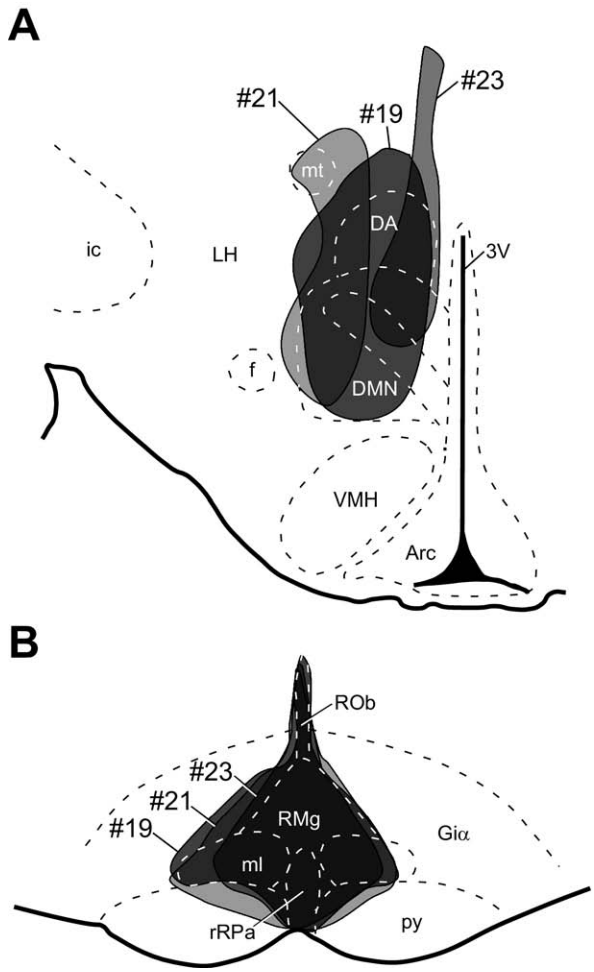

Fig. 1. Sites of CTb injections. (A) Alexa488-conjugated CTb and Alexa594-conjugated CTb were injected into the DMH (A; bregma, −3.30 mm) and into the rRPa (B; bregma, −11.80 mm), respectively. Areas where injected CTb spread are shown on the brain maps adopted from an atlas of Paxinos and Watson (1998). Injections in the three animals (#19, #21 and #23) that were used for histochemical analyses are shown. 3V, Third ventricle; Arc, arcuate nucleus; DA, dorsal hypothalamic area; DMN, dorsomedial hypothalamic nucleus; f, fornix; Giα, alpha part of the gigantocellular reticular nucleus; ic, internal capsule; LH, lateral hypothalamic area; ml, medial lemniscus; mt, mammillothalamic tract; py, pyramidal tract; ROb, raphe obscurus nucleus; RMg, raphe magnus nucleus; VMH, ventromedial hypothalamic nucleus.

The post-injection survival time of longer than 3 days was adopted from previous retrograde tracing with CTb in rats (Li et al., 1995; Nakamura et al., 2005b; Nakamura and Morrison, 2008a) and we found no obvious difference in the numbers of CTb-labeled neurons in the POA between the survival times of 3 and 6 days. Out of 23 rats that received the double CTb injections in the present study, three rats had CTb injections centered at both the DMH and the rRPa sites that have been reported to mediate febrile signals from the POA: inactivation of neurons in these regions blocks febrile thermogenic responses to PGE_2 into the POA (Nakamura et al., 2002, 2005b). Brain sections of these three rats were subjected to subsequent histological analyses. Prior to confocal microscopy, the other 20 animals were excluded from the present immunohistochemical analyses, since their CTb injections missed or were not centered on either or both of the DMH and rRPa sites.