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Assessing the role of the medial preoptic area in ethanol-induced hypothermia

Ashley T. Westerman^{a,b}, Peter G. Roma^{b,c,d}, Rebecca C. Price^b, and Juan M. Dominguez^{a,b,e,f,*}

^aDepartment of Psychology, The University of Texas at Austin, Austin, TX USA

^bDepartment of Psychology, American University, Washington, D.C. USA

^cInstitutes for Behavior Resources, Baltimore, MD USA

^dDepartment of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD USA

^eInstitute for Neuroscience, The University of Texas at Austin, Austin, TX USA

^fWaggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX USA

Abstract

Administration of ethanol produces hypothermia. The preoptic area/anterior hypothalamus (POA/AH) contains warm- and cold-sensitive neurons that are important for temperature regulation. The present study evaluated the effect of ethanol on Fos-immunoreactivity (Fos-ir) in the medial preoptic area (MPOA) and the effect of lesions to the MPOA on ethanol-induced hypothermia. Rats receiving 1.5-g/kg ethanol showed an increase in Fos-ir in the MPOA. However, lesions to the MPOA did not affect core body temperature. These findings indicate that ethanol increases neural activity in the MPOA, but this increased activity does not influence ethanol-induced changes in core body temperature.

Keywords

Ethanol; hypothermia; Fos; preoptic area

Administration of ethanol—like other CNS depressants—produces hypothermia. This is evidenced in all studied laboratory species, including cats, dogs, guinea pigs, mice, rabbits, and rats, when animals were kept at normal ambient temperatures [9]. Route of administration or ingestion is not critical with respect to this effect, since ethanol-induced hypothermia is observed following gavage, intraperitoneal injection (i.p.), or inhalation [14].

The preoptic area/anterior hypothalamus (POA/AH) contains warm- and cold-sensitive neurons that are important for temperature regulation [1]. Generally, warming the POA with

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*Corresponding Author: Juan M. Dominguez, Department of Psychology, The University of Texas at Austin, 1 University Station A8000, Austin, TX 78712 Telephone: (512) 471-1157; Facsimile: (512) 471-6175 dominguez@psy.utexas.edu.

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implanted electrodes elicits dissipation of body heat through behavioral and physiological responses, this includes panting, wetting the skin, assuming a stretched-out position, sweating, and increased blood flow to the skin [1]. Alternatively, cooling the POA evokes physiological and behavioral responses that promote preservation of heat and increased heat production, this includes shivering, cutaneous vasoconstriction, moving to a warmer area, or assuming a curled-up position [1]. Studies also show that temperature regulation is impaired following damage to the POA/AH [16], further supporting an important role for this region in thermoregulation.

Here we determined whether the medial preoptic area (MPOA) influences ethanol-induced hypothermia in male rats. The first experiment determined whether ethanol increases neural activity in the MPOA. The second experiment determined whether animals with lesions to their MPOA would experience an altered hypothermic response to ethanol versus sham-lesioned controls.

All animals were individually housed in large polycarbonate bins (26 × 48 × 21 cm) in a climate-controlled colony room (23°C). The room was maintained on a reverse 14/10 hr light/dark cycle, with lights off at 11 A.M. Food and water were freely available at all times throughout the duration of the experiment. Drug administration occurred under red light illumination. All materials and methods were carried out in accordance with NIH guidelines for the treatment of laboratory animals. Additionally, all materials and methods were approved by the Institutional Animal Care and Use Committee (IACUC) at American University, Washington, D.C.

Experiment 1 determined whether i.p. injections of ethanol influence neural activity in the MPOA. Twelve adult male Long-Evans/Blue Spruce rats (Harlan Sprague Dawley, Indianapolis, IN) received i.p. injections of either 1.5 g/kg ethanol (15% v/v) (n = 6) or equivolume saline (n = 6). One hour after injection with ethanol or vehicle, animals were sacrificed with an overdose of sodium pentobarbital (120 mg/kg, i.p.). They were then perfused transcardially with 100 mL saline followed by 500 mL 4% paraformaldehyde in 0.1M phosphate buffer (PB). Brains were removed, post-fixed for 1 hr in the perfusion fixative at room temperature, and then stored in a 30% sucrose solution at 4 °C. Coronal sections were cut at 35 µm and stored in cryoprotectant solution. Free floating sections containing the MPOA were immunoprocessed for Fos immunoreactivity. All incubations were performed at room temperature with gentle agitation. Sections were first blocked with 1% H₂O₂ and then soaked in incubation solution (PBS containing 2% normal goat serum and 0.4% Triton X-100). Sections were then incubated overnight in incubation solution and a polyclonal antibody raised in rabbit recognizing Fos (Santa Cruz Biotechnology, Santa Cruz, CA). After sections were incubated with primary antiserum, they were exposed to biotin-conjugated rabbit anti-goat IgG (Vector Laboratories, Burlingame, CA) and then to Avidin Biotin Complex (ABC)–horseradish peroxidase (HRP; ABC Elite in PBS; Vector Laboratories, Burlingame, CA). The peroxidase complex was visualized by exposure to a chromogen solution containing 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) enhanced with nickel sulfate, resulting in a purple–black reaction product. Brain sections including the MPOA were examined and Fos-positive cells were counted in a 400×450 µm area in the medial preoptic nucleus (MPN), a central region in the MPOA, by an experimenter blind to the treatment conditions.

Experiment 2 determined if lesions to the MPOA influence ethanol-induced changes in core body temperature. Thirty-two adult male rats received bilateral radiofrequency lesions to their MPOA (n=16) or sham lesions (n=16). Following a one-week recovery period, 8 rats per group were injected with 1.5 g/kg i.p. ethanol (15% v/v) or equivolume saline. Core body temperatures were digitally measured rectally immediately before injection and 15, 30, 60, and 180 min post-injection. Histo-analysis revealed that all lesions included the MPOA (Figure

2a). All 16 lesions were centered in the MPOA, including the ventromedial and lateromedial preoptic areas. However, 12 of these lesions also extended medially into the periventricular nucleus of the hypothalamus and 5 extended caudally into the anterior and lateroanterior hypothalamic nuclei.

Results of experiment 1 indicate that injections of ethanol increased the number of Fos-positive cells in the MPN (Figures 1a and 1b). An independent samples *t*-test revealed that animals receiving ethanol exhibited a greater number of Fos-labeled cells in their MPN than did vehicle controls ($t_{(11)} = 5.67, p < 0.001$).

Results of experiment two indicate that lesions of the MPOA did not influence ethanol-induced changes in core body temperature (Figure 2b). A $2 \times 2 \times 5$ mixed ANOVA with between groups factors of Lesion (lesion or sham) and Alcohol treatment (1.5 g/kg or vehicle) and a repeated-measures factor of Time since injection (0, 15, 30, 60, and 180 min) was performed on the raw shift in core body temperatures ($^{\circ}\text{C}$). The most complex effect was a significant Alcohol $\times$ Time interaction ($F_{(4,112)} = 11.52, p < 0.001$); follow-up independent samples *t*-tests revealed that the alcohol-treated animals' body temperatures were lower than their vehicle-treated counterparts at all post-injection time points ($t_{(30)}s > 2.6, ps < 0.05$). There were no significant main or interaction effects involving Lesion ($F_s < 1.40, ps > 0.23$). Taken together, these data showed the expected effect of ethanol on core body temperature, but yielded no indication that lesions to the MPOA affect ethanol-induced hypothermia.

These results partially support our initial hypothesis. Results of Experiment 1 indicate that ethanol influences neural activity in the MPOA, as evidenced by an increase in Fos-ir in the MPOA of ethanol-treated rats (Figures 1a and 1b). Chang and colleagues [2] reported Fos-ir in several rat brain regions following i.p. injection of ethanol, including hypothalamic regions. Regions displaying increased Fos-ir following i.p. injections of ethanol included the bed nucleus of the stria terminalis, paraventricular hypothalamic nucleus, the central nucleus of the amygdala, Edinger-Westphal nucleus, locus coeruleus, and parabrachial nucleus [2]. Our findings in the MPOA add to the number of hypothalamic regions that respond to administration of ethanol. However, results of Experiment 2 indicate that radiofrequency lesions to the MPOA did not influence ethanol-induced decreases in core body temperature (Figure 2b).

Several factors influence ethanol-induced hypothermia. One such factor is ambient temperature, as a dose of ethanol that produces hypothermia in animals kept at 20–23 $^{\circ}\text{C}$ fails to induce hypothermia when ambient temperature is increased to 30–38 $^{\circ}\text{C}$ [9]. However, even at a comfortable ambient temperature, changing intracranial temperature in the POA/AH evokes behavioral and physiological responses that allow for thermoregulation, without impacting rectal temperature [11], which was measured in the present experiments. Thus, it is possible that the MPOA plays a more important role in peripheral thermoregulation than in central (or core) thermoregulation. Indeed, although large lesions to the POA/AH disrupt thermoregulation, such lesions do not thwart rats from maintaining their body temperature at levels approaching normal via behavioral responses (e.g., sweating, panting, shivering, etc) [15]. Evidence of this nature confirms that the MPOA and other hypothalamic areas are not the only brain regions involved in thermoregulation [15], as other regions such as the medulla, midbrain, and spinal cord are also involved in the regulation of body temperature [12]. As such, future experiments should assess the role of the MPOA in peripheral thermoregulation per se by directly assessing peripheral body temperature as opposed to core body temperature. Notwithstanding, here we elucidate on the role of the MPOA specifically on core body temperature and its response to ethanol.

Ethanol's effects on thermoregulation may also be important in altering ethanol's motivational effects [3,6,4,5,8]. In support of this conclusion, preference for, or aversion to, ethanol or ethanol-paired stimuli was found to be related to the degree of hypothermia induced by administration of ethanol. Specifically, the greater the hypothermia experienced by animals the more aversive the motivational effects of ethanol. In one such study, rats drank more ethanol in a self-administration paradigm when its effects were experienced at high ambient temperatures, which reduced ethanol-induced hypothermia [5]. When ethanol was consumed at normal ambient temperatures, the animals consumed less of the drug [5]. These findings are similar to other studies showing that ethanol-induced conditioned taste aversion in rats is attenuated when ethanol-induced hypothermia is blocked by exposure to high ambient temperatures [4]. Conversely, ethanol-induced conditioned taste aversion is enhanced when ethanol-induced hypothermia was increased by exposure to low ambient temperatures [7].

In summary, although the hypothalamus is crucial for thermoregulatory processes, temperature regulation is a complex phenomenon involving a variety of peripheral and central mechanisms. While ethanol increases neural activity in the MPOA, the present study suggests that this increase in neural activity is not responsible for ethanol-induced changes in core body temperature in male rats. The fact that we found no lesion effect certainly does not invalidate the role of the hypothalamus in thermoregulation, but as discussed, it does suggest the primacy of peripheral mechanisms in hypothermia induced by IP ethanol.

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Figure 1.

(a) Photomicrographs illustrating Fos-labeled cells in the medial preoptic nucleus (MPN), a central nucleus in the medial preoptic area (MPOA), after i.p. injections of vehicle (left) or ethanol (right) (bar, 60 μ m). (b) The MPN of animals receiving ethanol contained more Fos-labeled cells than in those receiving vehicle. Values are expressed as mean $\pm$ SEM (* $p < 0.001$).

Figure 2.

(a) Representative perimeters of smallest lesion (black) and largest lesion (dash) for animals with radio-frequency lesions of the MPOA. Coordinates for this figure are (top to bottom) from bregma -0.30 , -0.40 , -0.80 , -0.92 , and -1.30 mm, drawn from Paxinos and Watson [13]. (b) Treatment with ethanol significantly decreased body temperature at all post-injection time points. However, there were no significant main or interaction effects indicating an influence of MPOA lesions. Values are expressed as mean $\pm$ SEM (* $p < 0.05$).