Ambient Temperature Influences Core Body Temperature Response in Rat Lines Bred for Differences in Sensitivity to 8-Hydroxy-dipropylaminotetralin

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ABSTRACT
Agonist-induced decrease in core body temperature has commonly been used as a measure of serotonin1A (5-HT1A) receptor sensitivity in mood disorder. The thermoregulatory basis for 5-HT1A receptor agonist-induced temperature responses in humans and rats remains unclear. Therefore, the influence of ambient temperature on 5-HT1A receptor-mediated decreases in core body temperature were measured in rat lines bred for high (HDS) or low (LDS) sensitivity to the selective 5-HT1A receptor agonist 8-hydroxy-dipropylaminotetralin (8-OH-DPAT). HDS and LDS rats were injected with either saline, 0.25 or 0.50 mg/kg 8-OH-DPAT at ambient temperatures of 10.5, 24, 30, or 37.5°C, and core temperature was measured by radio-telemetry. For both lines, the thermic response to acute 8-OH-DPAT was greatest at 10.5°C and decreased in magnitude as ambient temperature increased to 30°C, consistent with hypothermia. HDS rats displayed a greater hypothermic response than LDS rats at 10.5, 24, and 30°C. At 37.5°C, LDS rats showed a lethal elevation of temperature in response to 0.50 mg/kg 8-OH-DPAT. All thermic responses to 8-OH-DPAT, including the lethality, were effectively blocked by pretreatment with the 5-HT1A receptor antagonist WAY100635, suggesting line differences in thermoregulatory circuits that are influenced by 5-HT1A receptor activation. Following repeated injection of 8-OH-DPAT, the magnitude of the hypothermic response decreased in both lines at 10.5°C, but increased in HDS rats treated with 0.50 mg/kg 8-OH-DPAT at 30 and 37.5°C. This pattern was reversed in HDS rats following 8-OH-DPAT challenge at 24°C, suggesting that a compensatory thermoregulatory response accounts for changes in the hypothermic response to chronic 8-OH-DPAT.

Serotonergic dysfunction has been implicated in several human disease states including depression and anxiety (Bell et al., 2001). Drugs that modify serotonergic transmission have proven to be efficacious in the treatment of patients with mood disorder (Goodnick and Goldstein, 1998; Gorman and Kent, 1999; Shelton and Brown, 2001). Although there are environmental contributions to these diseases, a subset of the population appears to be biologically or genetically predisposed, possibly through altered sensitivity of serotonin receptor-dependent circuitry (Peroutka, 1998; Roy et al., 1999). Physiological measures that evince differences in the response to serotonergic drugs provide a bridge between animal models of behavior and human disease states, allowing for a better understanding of serotonergic function and mood disorder (Yadid et al., 2000).

Core body temperature is influenced by serotonin 1A (5-HT1A) receptor activation in rats and humans and has been considered a reliable physiological measure of 5-HT1A receptor sensitivity (Lesch et al., 1992; Meltzer and Maes, 1995; Dinan et al., 1997; Yatham et al., 1999). The thermic response to 5-HT1A receptor activation is easily measured and provides an important tool for understanding the mechanisms underlying mood disorder.

It remains unclear whether the 5-HT1A receptor agonist-induced decrease in core body temperature reflects a hypothermic event or a decrease in temperature set point (Oerther, 2000; Zuideveld et al., 2001). Under normal conditions,
Materials and Methods

Animals. Selectively bred male HDS and LDS rats were obtained from the University of North Carolina's Center for Alcohol Studies. The breeding protocol and description of the initial rat stock have been described in detail (Overstreet et al., 1994, 1996; Knapp et al., 2000). Briefly, these lines were established by selectively breeding National Institutes of Health heterogeneous stock rats using a within-family procedure. From the initial population, 10 males and females with the largest hypothermic response to 8-OH-DPAT (0.5 mg/kg s.c.) were randomly mated to establish the HDS group. Similarly, 10 males and females with the smallest response to 8-OH-DPAT were randomly mated to establish the LDS line. The most and least hypothermic male and female from each of the 10 litters were then selected. By the fourth generation, HDS and LDS rats differed significantly in hypothermic response from their parental means. Line differences in hypothermic response to 8-OH-DPAT were stable by generation nine, with HDS rats showing an average decrease of 4.0°C and LDS rats showing a decrease of 0.6°C in response to 0.25 mg/kg 8-OH-DPAT. HDS and LDS rats from generations 15–17 were used for these experiments. Rats were shipped in standard plastic Taconic shipping cartons (Petersburgh, NY), four rats per box, with food, apples, and liquid gel (for fluid). HDS and LDS rats were matched for age and weight. All rats weighed 300 to 450 g and were housed two per cage upon arrival, under standard laboratory conditions (constant temperature of 23 ± 1°C and relative humidity of 40–60%; 12-h light/dark cycle with lights on at 7:00 AM).

Temperature Measurement. Radio transmitters (resolution of 0.01°C; MiniMitter, Sun River, OR) were implanted into the peritoneal cavity of anesthetized rats (ketamine 1 ml/kg and xylazine 0.33 ml/kg) (Balcells-Olivero et al., 1998). Following surgery, all rats were housed singly. After a 7-day recovery period, the core temperatures of HDS (n = 48) and LDS (n = 48) rats were measured noninvasively by radiotelemetry. Temperature box chambers that allowed ambient temperature to be controlled to within ± 0.1°C were used to examine the effects of environmental temperature on drug response (Malberg and Seiden, 1998). On the day of experimentation, rats were placed into temperature chambers maintained at 10.5 (cool), 24 (neutral), 30 (warm), or 37.5°C (hot). After a 25-min acclimation period, individual rats were briefly removed, injected, and returned to their temperature boxes. Rat core body temperatures were continuously recorded, before and after injection, and averaged each minute for a 1.5- to 5-h period. All rats received 0.9% saline on the 1st day. On the subsequent 14 days, rats received 0.5 mg/kg or 0.25 mg/kg s.c. 8-OH-DPAT (Research Biochemicals International, Natick, MA). On days 7 and 14 of 8-OH-DPAT treatment, all groups were injected with 8-OH-DPAT at a neutral ambient temperature. The 0.25 (low) and 0.5 (high) mg/kg doses of 8-OH-DPAT were chosen for these studies because they cause significant decreases in core temperature compared with saline for both HDS and LDS rat lines at room temperature (Overstreet et al., 1994, 1996). On day 15, rats were pretreated with 0.1 mg/kg s.c. of the selective 5-HT1A antagonist WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(Wyeth-Ayerst, Princeton, NJ) 25 to 27 min before treatment with 8-OH-DPAT at cool, neutral, warm, or hot ambient temperatures. Due to unexpected lethality in response to 8-OH-DPAT, chronic studies for low- and high-dose 8-OH-DPAT were not run for LDS rats at a hot ambient temperature. Therefore, a group of naive LDS rats, was pretreated with WAY100635 (0.1 mg/kg s.c.) 25 to 27 min before receiving an acute injection of 8-OH-DPAT (0.5 mg/kg s.c.) at a hot ambient temperature.

Data Analysis. For all experiments, the 1st minute core temperature, preinjection, and postinjection change in core body temperature were determined. For each rat, the preinjection change in core body temperature was defined as the difference between the 1st minute temperature and the temperature taken 1 min prior to injection. For each rat, the postinjection change in core body temper-
Influence of Ambient Temperature on the Core Body Temperature following Saline Injection. HDS and LDS rats were treated with acute saline at cool (10.5°C), neutral (23°C), warm (30°C), or hot (37.5°C) ambient temperatures, and core body temperature responses were recorded by radiotelemetry (Fig. 1). No line differences were observed in the 25- to 27-min preinjection time period. There was no significant difference between the change in core body temperature measured during the preinjection period for HDS (Fig. 1B) or LDS (Fig. 1A) rats at a cool, neutral, or warm ambient temperature. At a hot ambient temperature, a significant increase in preinjection core body temperature was observed in both lines. The mean change in core body temperature was 1.3 ± 0.2°C for HDS and 1.3 ± 0.1°C for LDS rats.

Following saline injection, HDS and LDS rats maintained their core body temperature at all ambient temperatures to within 1°C of their core body temperature at the time of injection. No line differences in core body temperature were observed following saline injection at any ambient temperature.

Effects of Acute 8-OH-DPAT. Following acute saline experiments, HDS and LDS rats were treated with low- or high-dose 8-OH-DPAT at cool, neutral, warm, or hot ambient temperatures (Fig. 1). The change in core body temperature was defined as the difference between the highest and lowest temperature recorded following injection of 8-OH-DPAT (Fig. 2). There was an overall significant effect of line (P < 0.001), ambient temperature (P < 0.001), and dose (P < 0.05). On day 1 of treatment, HDS rats (Fig. 2, C and D) showed a significantly larger core body temperature response than LDS rats (Fig. 2, A and B) in all conditions (P < 0.001), except at a hot ambient temperature in response to low-dose 8-OH-DPAT.

The change in core temperature for HDS rats on day 1 was greatest at a cool ambient temperature and decreased in magnitude at warmer ambient temperatures (Fig. 2C,D). The change in core temperature for HDS rats treated at a neutral ambient temperature was significantly smaller than that of HDS rats treated at a cool ambient temperature (P < 0.001) and significantly larger than that of HDS rats treated at warm (P < 0.001) or hot (P < 0.001) ambient temperatures on day 1 in response to both low- and high-dose 8-OH-DPAT. Thus, the magnitude of the thermic response in HDS rats is highly dependent on ambient temperature and does not follow the expected pattern for a change in hypothalamic set point, but rather appears to be a hypothermic event.

LDS rats did not show a significant difference in the magnitude of the 8-OH-DPAT-induced decrease in core temperature for rats treated at cool, neutral, and warm ambient temperatures (Fig. 2A,B). Following both low- and high-dose 8-OH-PAT, a significant effect was observed for LDS rats treated at a hot ambient temperature compared with cool, neutral, and warm ambient temperatures (P < 0.001). Following high-dose 8-OH-DPAT at a hot ambient temperature, LDS rats showed an unexpected, immediate, and lethal increase in core body temperature (Fig. 2B). Three of four rats perished within 15 min of injection with core body temperatures of 41, 42, and 44°C. Consequently, a second group of four LDS rats were injected with high-dose 8-OH-DPAT and run in hot temperature boxes set to automatically cool when core body temperatures rose to 41°C. This rescue attempt was ineffectual and within 20 min of injection, one rat from this study perished with a core body temperature of 42°C despite an ambient temperature below neutral. The rats showed excessive salivation and urination, suggesting that they were actively trying to cool themselves. The remaining LDS rats were immediately removed from the temperature boxes and sacrificed. LDS rats treated with low-dose 8-OH-DPAT at a hot ambient temperature did not differ in their core temperature response from saline-treated rats or low-dose-treated HDS rats. HDS and LDS rats showed a similar gradual increase in core temperature following recovery from 8-OH-DPAT injection between the 4th and 5th hour of exper-
ambient temperature. Unexpectedly, none of the LDS rats treated with low-dose 8-OH-DPAT survived the full 5-h experiment at a hot ambient temperature and were found dead in the temperature chambers. In contrast, HDS rats that were run at the same time appeared normal. Lethality at a hot ambient temperature was not observed for low- or high-dose 8-OH-treated HDS rats or saline-treated HDS and LDS rats (Fig. 2). The lethality observed in LDS rats suggests: 1) line differences in compensatory thermoregulatory responses following administration of 8-OH-DPAT in a hot environment and 2) some life-sustaining process is more sensitive to the elevation of core temperature in LDS than HDS rats.

**Effects of Chronic 8-OH-DPAT.** HDS and LDS rats were treated with chronic low- or high-dose 8-OH-DPAT at cool, neutral, warm, or hot ambient temperatures for 13 days. An unexpected influence of ambient temperature on the thermic response was observed in HDS (P < 0.001) and LDS (P < 0.001) rats following repeated treatment with 8-OH-DPAT (Fig. 2). A significant decrease in the magnitude of the HDS thermic response to low- and high-dose 8-OH-DPAT was observed on day 6 (P < 0.001) and day 14 (P < 0.001) compared with day 1 of repeated injection at a cool ambient temperature (Fig. 2, C and D). At a neutral ambient temperature, a decrease in the magnitude of the thermic response was observed on treatment days 6 (P < 0.01) and 13 (P < 0.05) compared with day 1 in HDS rats treated with low-dose 8-OH-DPAT (P < 0.01). No significant change in response was observed for HDS rats treated at warm or hot ambient temperatures. Unexpectedly, an increase in the magnitude of the HDS thermic response to repeated injection of high-dose 8-OH-DPAT was observed on treatment days 6 (P < 0.001) and 13 (P < 0.01) compared with day 1 for HDS rats treated at a warm ambient temperature, as well as on treatment days 6 (P < 0.001) and 13 (P < 0.05) compared with day 1 for HDS rats treated at a hot ambient temperature (Fig. 2D).

LDS rats treated with low-dose 8-OH-DPAT at a cool ambient temperature showed a decrease in the magnitude of their thermic response on treatment days 6 (P < 0.001) and 13 (P < 0.001) compared with day 1 (Fig. 2A). A similar decrease in the magnitude of the thermic response on treatment days 6 (P < 0.01) and 13 (P < 0.05) compared with day 1 was observed for LDS rats treated with high-dose 8-OH-DPAT at a cool ambient temperature (Fig. 2B). No significant difference in thermic response to repeated injection of high- or low-dose 8-OH-DPAT was observed for LDS rats treated at neutral or warm ambient temperatures.

**Influence of Pretreatment Ambient Temperature on Change in Core Body Temperature following Challenge with 8-OH-DPAT at Neutral Ambient Temperature.** HDS and LDS rats treated with chronic high-dose 8-OH-DPAT at cool, warm, or hot ambient temperatures were challenged with 8-OH-DPAT at a neutral ambient temperature on treatment days 7 and 14. The change in core body temperature for these rats was then compared with rats treated solely at neutral ambient temperature to further characterize the influence of pretreatment temperature on the thermic response to 8-OH-DPAT.

A clear effect of pretreatment ambient temperature (P = 0.02) was observed on the HDS responses to 8-OH-DPAT challenge at a neutral ambient temperature (Fig. 3B). On day 14, HDS rats treated at cool (P < 0.01) or neutral (P < 0.05) ambient temperatures showed a significantly smaller thermic response to 8-OH-DPAT than rats treated at a hot ambient temperature. This reversal pattern suggests that changes in the magnitude of thermic response observed at different pretreatment ambient temperatures represent a
learned thermoregulatory compensatory change evoked by repeated exposure to cool, warm, and hot environments.

**Effect of WAY100635.** The hypothermic response to high-dose 8-OH-DPAT was blocked by the 5-HT$_{1A}$ antagonist WAY100635 in HDS and LDS rats at all ambient temperatures on the day following chronic treatment (Fig. 4). Since chronic studies could not be completed for LDS rats at high ambient temperatures, the thermic response to acute 8-OH-DPAT is shown following pretreatment with WAY100635 (Fig. 4A). Following pretreatment with WAY100635, HDS and LDS rats injected with high-dose 8-OH-DPAT did not differ significantly from saline-treated rats in preinjection or postinjection maximal change in core body temperature. WAY100635 effectively blocked the lethal response to acute 8-OH-DPAT observed in LDS rats at a hot ambient temperature (Fig. 4). All LDS rats pretreated with WAY100635 survived the hot ambient environment and did not differ in appearance or behavior from HDS rats. This finding supports the idea that the lethal increase in core body temperature observed in LDS rats is caused by the action of 8-OH-DPAT on 5-HT$_{1A}$ receptors.

**Discussion**

These findings suggest that the 8-OH-DPAT-induced decrease in core body temperature is highly sensitive to ambient temperature and represents a hypothermic event that does not involve a change in temperature set point. The HDS and LDS rat lines differ in the magnitude of their hypothermic responses to 8-OH-DPAT at several ambient temperatures. Administration of 8-OH-DPAT in a hot environment causes a lethal hyperthermia in the LDS, but not HDS, rat line. Ambient temperature influences the magnitude of the thermic response following repeated injection of 8-OH-DPAT. The changes in the hypothermic response to 8-OH-DPAT in the HDS line, evoked by repeated injection, may reflect a compensatory thermoregulatory response that is independent of a change in 5-HT$_{1A}$ receptor sensitivity.

**Baseline Thermoregulatory Function.** Naïve HDS and LDS rats did not differ in their thermoregulatory ability to maintain core body temperature at different ambient temperatures. Saline-treated HDS and LDS rats displayed splayed body posture and salivation at a hot ambient temperature, behaviors associated with heat loss (Gordon, 1993). At a cool ambient temperature, both lines displayed piloerection and huddled body posture, behaviors associated with heat conservation (Gordon, 1993). HDS and LDS rats maintained their core body temperature at cool, neutral, and warm ambient temperatures. At a hot ambient temperature,
a significant increase in core body temperature that reached a plateau within 30 min of exposure, was observed in saline-treated HDS and LDS rats. This response was previously observed in regular rats in a hot environment, suggesting that hot ambient temperature challenges the ability of both HDS and LDS rats to maintain core body temperature in a way that is similar to normal rats (Gordon, 1993).

**8-OH-DPAT-Evoked Hypothermia.** A significant effect of ambient temperature on 8-OH-DPAT-induced decrease in core body temperature, consistent with a hypothermic event, was observed in HDS and LDS rat lines. For both HDS and LDS rats, a cool ambient temperature enhanced the decrease in core body temperature elicited by 8-OH-DPAT injection. As ambient temperatures increased, the magnitude of the thermic response to 8-OH-DPAT decreased. This was true with the exception of an unexpected and lethal increase in core body temperature in response to high-dose 8-OH-DPAT observed in LDS rats at a hot ambient temperature. Previous studies have shown that 8-OH-DPAT causes heat loss through cutaneous vasodilation and decreased metabolism, effects that interfere with the normal thermoregulatory responses activated by environmental temperature (Lin et al., 1998). Cutaneous vasodilation would have a greater cooling effect in a cold environment relative to a neutral environment. In a hot environment, it is difficult to lose heat via cutaneous vasodilation compared with a neutral environment. Therefore, we would expect a cold environment to facilitate and a hot environment to attenuate 8-OH-DPAT-induced heat loss. This explains the observed effect of ambient temperature on 8-OH-DPAT-mediated heat loss in HDS rats and is consistent with a hypothermic event.

The line differences observed for the thermic response to 8-OH-DPAT could be attributable to a stronger compensatory mechanism or thermal sensitivity to environmental temperature in the LDS compared with the HDS rat. The blunted hypothermic response to 8-OH-DPAT and decreased influence of cool, neutral, and warm ambient temperature observed for LDS rats compared with HDS rats suggests that compensatory responses to skin temperature are increased in the LDS rat. In a cool environment, blood flow to the skin is decreased in an attempt to conserve heat (Gordon, 1993; Nagashima et al., 2000). Cutaneous vasoconstriction in response to a cool environment may counteract the cutaneous vasodilation induced by 8-OH-DPAT. Therefore, if LDS rats have a greater compensatory response to skin temperature than HDS rats, we would expect increased cutaneous vasoconstriction in LDS rats exposed to a cool environment, resulting in decreased 8-OH-DPAT-induced cutaneous vasodilation and a subsequent blunted hypothermic response. The magnitude of the line difference in the hypothermic response to 8-OH-DPAT should therefore decrease as ambient temperature increases, which is what we have observed.

It is well established that acute treatment with 8-OH-DPAT decreases core body temperature (Hjorth, 1985; Hutton et al., 1987; Larsson et al., 1990; Uphouse et al., 1991; Millan et al., 1993). Therefore, it was surprising to find that acute systemic treatment with high-dose 8-OH-DPAT at a hot ambient temperature causes a lethal increase in core body temperature in the LDS rat line. This response is effectively blocked by the 5-HT1A receptor antagonist WAY100635, showing that the lethality is caused by the 5-HT1A receptor agonist properties of 8-OH-DPAT. It is possible that the hyperthermia observed in LDS rats represents an extreme example of a rat gaining heat from the environment. At hot ambient temperatures, cutaneous blood flow increases in an attempt to cool body temperature. However, if ambient temperatures are extremely hot, this increase in cutaneous blood flow is reversed to prevent a situation in which more heat is picked up from the environment than lost to it (Gordon, 1993). The increase in cutaneous blood flow in response to treatment with 8-OH-DPAT would cause an initial rise in skin temperature that, for the LDS rat in a hot environment, may result in a reversal of cutaneous blood flow and subsequent acute lethal hyperthermia. Cutaneous vasodilation in response to low-dose 8-OH-DPAT is not enough to cause immediate lethal hyperthermia in the LDS line. The delayed lethality observed following low-dose 8-OH-DPAT may occur as a result of physiological line differences in compensatory response to heat exposure elicited by 5-HT1A receptor activation.

**Functional Implications.** As described above, HDS rats appear to make less compensatory thermoregulatory adjustments than LDS rats following acute treatment with 8-OH-DPAT, perhaps because they are less sensitive to changes in skin temperature. With repeated exposure to hot and cool ambient temperature over days, a secondary compensatory response to environment appears to develop in HDS rats. This is shown as a decrease in the magnitude of 8-OH-DPAT induced hypothermia at a cold ambient temperature and increase in the magnitude of 8-OH-DPAT-induced hypothermia at a hot ambient temperature following chronic daily treatment. This effect of pretreatment at different ambient temperatures is readily observed following 8-OH-DPAT challenge at a neutral ambient temperature. HDS rats pretreated in a cool environment show a smaller hypothermic response to 8-OH-DPAT challenge at a neutral ambient temperature compared with HDS rats pretreated in warm and hot environments. These results are strong evidence that the developed compensatory response is not solely dependent on thermal information from cutaneous receptors but reflects a central change in the sensitivity and/or function of thermoregulatory neurons. Furthermore, the HDS rats do not show tolerance or desensitization following 14 days of treatment with high-dose 8-OH-DPAT at a neutral ambient temperature, suggesting that the changes in response to repeated injection at cool and hot ambient temperatures do not reflect a change in 5-HT1A receptor sensitivity to 8-OH-DPAT.

If the observed line differences in the thermic response to 8-OH-DPAT represent differences in compensatory thermoregulatory mechanisms, then there may not be line differences in 5-HT1A receptor function. Similarly, the blunted thermic response to 5-HT1A receptor agonist buspirone observed in patients with depression and panic disorder may also represent a difference in thermoregulatory sensitivity (Lesch et al., 1990a, 1991).

Because the partial 5-HT1A agonist buspirone has proven to be an effective anxiolytic medication, drug company research has focused on the development of specific full 5-HT1A receptor agonists to further improve the treatment of anxiety and as an adjunct treatment for depression. Increasingly, emergency medical facilities are faced with cases of hyperthermia associated with serotonin syndrome caused by the interaction of medications prescribed for mood disorder (McGugan, 2001). Interestingly, lethal hyperthermia is
known to be a leading cause of death in patients taking monoamine oxidase inhibitors in response to the accumulation of high levels of serotonin in the synapse (Sporer, 1995). The decrease in core body temperature evoked by ipsapirone, a 5-HT1A receptor antagonist, is significantly blunted in older subjects, as is the 8-OH-DPAT-induced hypothermia observed in LDS rats (Gelfin et al., 1995). The lethal hyperthermic response of LDS rats to 5-HT1A receptor stimulation serves to caution the use of specific full 5-HT1A receptor antagonists for the treatment of anxiety and depression, especially for patients whose thermoregulatory responses to environmental temperature are compromised by age or disease (Epstein et al., 1997).

Pindolol, a 5-HT1A receptor antagonist, has been effectively used to enhance the antidepressant response to selective serotonin reuptake inhibitors (Blier, 2001). The ability of WAY100635 to block the lethal hyperthermic response to 8-OH-DPAT in the LDS line suggests that the combined administration of 5-HT1A receptor antagonists with antidepressant medications that enhance serotonergic transmission may provide, in addition to rapid onset of efficacy, protection against drug-induced hyperthermia.

It is clear that drugs influencing serotonergic transmission can perturb normal thermoregulatory responses to environmental temperature (Wappler et al., 2001; Hojer et al., 2002; Parrott, 2002). However, the mechanisms by which this occurs are not well understood. Further research regarding the influence of serotonin on thermoregulatory processes is necessary to ensure that the growing number of patients identified with mood disorder receive safe treatment.

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References


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