Role of the Nitric Oxide Pathway in κ-Opioid-Induced Hypothermia in Rats

KHALID BENAMAR, ELLEN B. GELLER, and MARTIN W. ADLER

Center for Substance Abuse Research and Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania

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ABSTRACT

The effect of central and peripheral administration of a nitric oxide synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME), on the hypothermia induced by the selective κ-opioid receptor agonist trans-(±)3,4-dichloro-N-methyl-N-[2-[1-pyrrolidinyl]-cyclohexyl]-benzeneacetamide methane sulfate (U50,488H) was studied in male Sprague-Dawley rats. In the first series of experiments, we examined the effect of subcutaneous (s.c.) administration of L-NAME on the hypothermia induced by s.c. injection of U50,488H. L-NAME, at a dose of 50 mg/kg s.c., had no influence on body temperature (Tb). Coadministration of L-NAME (50 mg/kg, s.c.) with U50,488H (10 mg/kg, s.c.) blocked the hypothermia induced by U50,488H. In the second series of experiments, we investigated the effect of intracerebroventricular (i.c.v.) administration of L-NAME on the hypothermia induced by s.c. injection of U50,488H. L-NAME itself, given i.c.v. at a dose of 1 mg/rat, did not evoke any change in Tb. Administration of L-NAME (1 mg/rat, i.c.v.) caused a significant suppression of U50,488H hypothermia. The results indicate that either central or peripheral nitric oxide synthesis is required for the production of hypothermia induced by U50,488H.

The endogenous opioid system serves several physiological functions, including a role in temperature regulation. Three distinct opioid receptors (μ, κ, and δ) have been identified. Opioid agonists have been investigated in terms of their ability to alter Tb (Clark et al., 1983; Geller et al., 1983, 1986), the response being dependent upon a number of factors including species, strain, dosage, route of administration, ambient temperature, and receptor selectivity (Adler et al., 1988). Previous results from this and other laboratories demonstrated that i.c.v. administration of selective μ-receptor agonists produced hyperthermia (Spencer et al., 1988; Handler et al., 1992; Adler and Geller, 1993), whereas κ-receptor agonists produced hypothermia (Adler et al., 1983, 1986; Spencer et al., 1988).

Nitric oxide (NO), recently recognized as a prominent second messenger (Breden and Saper, 1996), is produced by the enzyme nitric-oxide synthase (NOS) that uses L-arginine to make L-citruline and the radical gas NO. NO has been found in peripheral and central neurons (Breden and Saper, 1996). Three different isoforms of NOS have been described (Lopez-Figueroa et al., 1998). Two are constitutive forms, endothelial and neuronal (Moncada et al., 1991), and the third is inducible (Lowenstein et al., 1992). Within the last few years, a number of studies have been conducted to investigate whether NO plays a role in temperature regulation, fever, and hypothermia. Some authors have suggested that NO has an antipyretic function (Moncada et al., 1991; Gourine, 1995), and some have shown that NO is involved in hypothermia (Branco et al., 1997; Steiner et al., 1998; Almeida and Branco, 2001). However, other articles provide evidence that the formation of NO participates in the development of a febrile response (Lin and Lin, 1996; Scammell et al., 1996; Roth et al., 1998; Benamar et al., 2000). It should be noted that these studies differed in strategies to assess the role of NO, species of experimental animal, pyrogen administered, and route of administration, as well as in inhibitors used.

Although κ-receptor-agonist-induced hypothermia has been intensively investigated, little is known about the mechanisms by which κ-opioids might alter the Tb. The aim of the present study was to investigate the role of NO during the hypothermia induced by U50,488H, a κ-opioid receptor agonist, and to determine whether the effect of L-NAME is the result of its peripheral or its central action.

Materials and Methods

Animals. Male Sprague-Dawley rats (Zivic-Miller Laboratories, Pittsburgh, PA) weighing 250–300 g were used in this study. They were housed two per cage for at least 1 week before surgery and were

ABBREVIATIONS: Tb, body temperature; NO, nitric oxide; NOS, nitric-oxide synthase; U50,488H, trans-(±)3,4-dichloro-N-methyl-N-[2-[1-pyrrolidinyl]-cyclohexyl]-benzeneacetamide methane sulfate; L-NAME, N-nitro-L-arginine methyl ester; POAH, preoptic anterior hypothalamus; CNS, central nervous system.
fed laboratory chow and water ad libitum. The ambient temperature was 22 ± 2°C and a 12-h light/dark cycle was used. All experiments were started between 9:00 and 10:00 AM to minimize the effect of circadian variation in Tb.

**Implantation of Cannula and Transmitter.** Rats were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). Each animal was placed in a stereotaxic instrument. One week before the experiments began, a polyethylene cannula was implanted into the right lateral ventricle and secured to the cranium with dental acrylic according to standard procedures in our laboratory (Adams et al., 1993), and transmitters were implanted i.p. The animals were returned to individual cages in the environmental room.

**i.c.v. Injection and Body Temperature.** One week after surgery, the rats were tested in an environmental room (21 ± 0.3°C ambient temperature and 52 ± 2% relative humidity). Tb was measured by a biotelemetry system (Mini-Mitter, Sunriver, OR) using calibrated transmitters. Signals from the transmitter were delivered through a computer-linked receiver. This method minimizes stress to animals during the Tb reading. Thus, the Tb could be monitored continuously and recorded without restraint or any disturbance to the animal.

Either saline or drug was injected i.c.v. in a volume of 5 μl. With aseptic procedures, drugs were administered by inserting the needle tip of a 10-μl syringe (Fisher Hamilton Scientific, Malvern, PA) into the polyethylene cannula. All injections and Tb measurements have performed in the environmental room with animals in individual cages.

**Drugs.** U50, 488H (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile pyrogen-free saline and injected s.c. at doses of 2.5, 5, and 10 mg/kg. N-Nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich) was also dissolved in saline. Doses were 50 mg/kg for s.c. injection and 1 mg/rat for i.c.v. injection. The doses of L-NAME used were based on those used in previous studies (Benamar et al., 2001).

**Statistical Analysis and Histology Analysis.** All results were expressed as Tb changes (ΔTb; mean ± S.E.M.) from baseline. Statistical analysis was carried out with the use of the student’s t test. A value of P < 0.05 was considered statistically significant. Cannula placement was confirmed by checking the location of the tip by 1% Evan’s blue after the experiment, according to standard procedures in our laboratory (Xin et al., 1997).

**Results**

**Effect of s.c. Administration of U50,488H on Body Temperature.** As shown in Table 1, administration of U50–488H in a dose of 2.5 to 10 mg/kg s.c. produced hypothermia in a dose-dependent manner. Tb was measured every 5 min. The lowest dose did not alter Tb significantly compared with vehicle (P > 0.05). However, 5 mg of U50,488H caused a small decrease in Tb, which reached a peak of −0.70 ± 0.14°C at 30 min (P < 0.05) and returned to baseline levels about 240 min postinjection. With 10 mg/kg, the peak of hypothermia was −1.49 ± 0.24°C at 60 min postinjection. The Tb returned to baseline levels about 540 min. Accordingly, a dose of 10 mg/kg was chosen for subsequent experiments.

**Effect of s.c. or i.c.v. Injection of l-NAME on Tb.** During the 240-min recording period, no significant change in Tb was observed after s.c. injection of 50 mg/kg l-NAME compared with the effect of injection of an equivalent volume of vehicle (saline) (Fig. 1; P > 0.05). Mean Tb before injection was 37.35 ± 0.24°C for the saline group and 37.37 ± 0.21°C for the l-NAME group. Injection of 1 mg/rat of l-NAME i.c.v. did not alter Tb significantly compared with saline (3 μl) given i.c.v. (Fig. 2; P > 0.05). Mean Tb before injection was 37.54 ± 0.20°C for the saline group and 37.52 ± 0.23°C for the l-NAME group.

**Effect of s.c. Injection of l-NAME on Hypothermia Induced by 10 mg/kg U50,488H.** In vehicle-treated animals, s.c. injection of 10 mg/kg U50,488H produced a decrease in Tb that peaked at 60 min (−1.47 ± 0.22°C). After 2 h postinjection, Tb started to increase slowly (Fig. 1). Treatment with l-NAME (50 mg/kg) inhibited the hypothermic response induced by U50,488H (Fig. 1; P < 0.05). Mean Tb before injection was 37.55 ± 0.24°C for the saline/U50,488H group and 37.41 ± 0.19°C for the l-NAME/U50,488H group.

**Effect of i.c.v. Injection of l-NAME on Hypothermia Induced by 10 mg/kg U50,488H.** Coadministration of l-NAME i.c.v. at dose of 1 mg/rat with U50,488H s.c. at dose of 10 mg/kg blocked the hyperthermic response during the 240-min recording period (Fig. 2; P > 0.05). Mean Tb before injection was 37.60 ± 0.17°C for the saline/U50,488H group and 37.61 ± 0.20°C for the l-NAME/U50,488H group.

**Discussion**

A reduction of Tb is part of the response to a number of different stimuli. Hypothermia in some situations can be a beneficial response because it reduces oxygen consumption, promotes a leftward shift of the oxygen-hemoglobin dissociation curve, and may promote survival during periods of hypoxia. However, hypothermia may also be detrimental in certain situations, such as in patients with infections or traumatic injuries, where it can delay the host’s response to the infection or injury. Further studies are needed to better understand the mechanisms underlying the effects of l-NAME on the hypothermic response induced by U50,488H.
Using selective opioid agonists and antagonists, we deter-
doses caused a profound decrease in Tb (Geller et al., 1983).
ies in our laboratory showed that 4 to 15 mg/kg morphine
1988; Nemmani et al., 2001). Although there is evidence of
so-called opioid mediation of hypothermia. Furthermore, opioid drugs
are mediated by \( \mu \), \( \delta \), and \( \kappa \)-opioid receptors. Previous studies in our laboratory showed that 4 to 15 mg/kg morphine (given s.c.) markedly increased Tb in the rat, but higher doses caused a profound decrease in Tb (Geller et al., 1983).

Using selective opioid agonists and antagonists, we determined that these effects were due to the actions of morphine on \( \mu \) - and \( \kappa \)-receptors, respectively (Geller et al., 1982). Thus, \( \mu \)-receptor agonists caused hyperthermia (Spencer et al., 1988; Handler et al., 1992; Adler and Geller, 1993), and \( \kappa \)-receptor agonists produced hypothermia in rats (Adler et al., 1983, 1986; Spencer et al., 1988; Handler et al., 1992; Chen et al., 1996). In the present study, the selective \( \kappa \)-agonist U50,488H produced a dose-dependent hypothermic response, with a maximum decrease in Tb induced by 10 mg/kg. The data are in line with previous studies showing that the \( \kappa \)-opioid receptor is involved in hypothermia (Adler et al., 1988; Nemmani et al., 2001). Although there is evidence of \( \kappa \)-involvement, little is known about its mechanism of action.

Since the hypothermic effect of U50,488H, a \( \kappa \)-opioid agonist, was blocked by nor-BNI, a selective \( \kappa \)-opioid antagonist (Xin et al., 1997), the effect of U50,488H on body temperature must be by a \( \kappa \)-mediated mechanism. It was demonstrated recently that the hypothermia induced by U50,488H is po-
tentiated by a selective 5-HT inhibitor, suggesting a role of 5-HT in the mediation of hypothermia (Nemmani et al., 2001). Also, U50,488H-induced hypothermia was potentiated by pretreatment with chlorpromazine, indicating that biogenic amines can interact with the \( \kappa \)-opioid-receptor-mediated hypothermia (Adler et al., 1986). Similarly, U50,488H in combination with neurotensin produced a dose-dependent additive effect (Handler et al., 1995).

The preoptic anterior hypothalamus (POAH) is generally considered to be the primary site for central control of Tb because a large population of thermosensitive neurons is located there (Boulant, 1980) and its destruction or inactivation disrupts thermoregulation. It receives and integrates the Tb information from both central and peripheral sensors and sends the modulating signal to direct Tb regulatory effectors in maintaining Tb around a given temperature. It has been demonstrated that the POAH is a primary func-
tional area in Tb responses to opioids (Xin et al., 1997). At least three types of opioid receptors, \( \mu \), \( \kappa \), and \( \delta \), have been discovered in the CNS, including the POAH (Mansour et al., 1987). NOS is widely distributed in the CNS. Dense NOS staining occurs in hypothalamus (Bredt and Snyder, 1992).

The importance of NO can be demonstrated by inhibition of its effects (Rees et al., 1990) by the use of \( L \)-arginine analogs such as \( L \)-NAME. In the present study, \( L \)-NAME was chosen because it is a nonselective inhibitor of NOS that acts on both the constitutive and inducible isoforms of the enzyme.

The present results showed that i.c.v. administration of \( L \)-NAME significantly reduced the U50,488H-induced hypothermia, suggesting that NO production in the CNS plays an important role in the development of the hypothermic response. The central involvement of NO in the hypothermic process has been demonstrated previously. Thus, \( L \)-NAME abolished the hypothermia caused by insulin infusion, providing evidence that the NO pathway in the CNS plays a role in insulin-induced hypothermia (Almeida and Branco, 2001). Also, central administration of \( L \)-NAME attenuated the hypothermia induced by arginine vasopressin (Steiner et al., 1998). Moreover, rats treated with a high dose of lipopolysaccharide showed a strong presence of NOS in various areas in the brain, including the POAH (Gath et al., 1999).

The involvement of opioid receptors in the modulation of NO has been supported by various recent studies. For example, it has been demonstrated that lipopolysaccharide-induced expres-
sion of inducible NOS by splenocytes is modulated through the activation of endogenous opioid in the CNS (Lysele and How, 1999), and \( L \)-NAME (given i.c.v.) alters the hyperthermia induced by morphine (15 mg/kg, i.p.) (Denamar et al., 2001).

The present experiments also demonstrate that blocking the endogenous NO production by systemic injection of \( L \)-NAME suppresses the hypothermia induced by U50,488H. Our findings indicate that the hypothermia induced by U50,488H requires the production of NO. Since the sites of action of \( L \)-NAME seem to be distributed throughout the body, including brown adipose tissue, where they are responsible for heat production, and vascular smooth muscle, where they are responsible for heat conservation (Taylor and Bishop, 1993; Nagashima et al., 1994), the hypothesis that U50,488H-induced hypothermia can act at peripheral sites via NO cannot be excluded.

In conclusion, the present studies have demonstrated that...
the selective κ-opioid receptor agonist U50,488H, injected s.c. at dose of 10 mg/kg in rats, produced hypothermia, and the effect could be blocked by central or peripheral pretreatment with the NOS inhibitor l-NAME. These findings clearly indicate the involvement of NO in the generation of hypothermia induced by U50, 488H.

References


