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Research report

Brain delta$_2$ opioid receptors mediate SNC-80-evoked hypothermia in rats

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Abstract

Despite insights into an increasingly significant role for delta opioid receptors in thermoregulation, it is unclear whether delta receptors located in the brain or periphery play the more critical role in body temperature regulation. Moreover, it is not entirely clear which delta receptor phenotype, delta$_1$ or delta$_2$, mediates the hypothermic actions of delta agonists. Because SNC-80 distributes into central and peripheral compartments and produces rapid hypothermia following systemic injection, the nonpeptide delta agonist is particularly useful in discriminating the site of action of delta receptor-mediated hypothermia. To determine the locus and phenotype of delta receptor which mediates SNC-80-induced hypothermia, we injected SNC-80 and phenotype selective delta antagonists to male Sprague–Dawley rats. SNC-80 (10–50 mg/kg, im) evoked hypothermia that peaked 30 min post-injection. Naltrexone (5 mg/kg, sc), an opioid antagonist, or naltrindole (5 mg/kg, sc), a delta antagonist, blocked the hypothermic response to SNC-80 (35 mg/kg, im). The hypothermia caused by SNC-80 (35 mg/kg, im) was blocked by a delta$_2$ antagonist, naltriben (2.5 mg/kg, sc), but was not affected by BNTX (5 and 10 mg/kg, sc), a delta$_1$ antagonist. The administration of naltriben (10 μg/rat, icv) 30 min before SNC-80 (35 mg/kg, im) prevented SNC-80-evoked hypothermia. In contrast, methylnaltrexone (5 mg/kg, sc), a peripherally restricted opioid antagonist, did not affect the hypothermia caused by SNC-80. The present data demonstrate that selective activation of brain delta$_2$ receptors is a major mechanism of SNC-80-evoked hypothermia in rats.

Theme: Neurotransmitters
Topic: Opioids: anatomy, physiology, and behavior

Keywords: SNC-80; Delta; Opioid; Hypothermia; Naltriben; Naltrindole

1. Introduction

Delta, mu, and kappa opioid receptors mediate the pharmacological actions of opioids. Similar to cannabinoids, vanilloids, benzodiazepines, neuroleptics, and other drugs, opioids cause marked changes in body temperature [1,14,17,26,30,35,37,47]. Therefore, change in body temperature is one biological effect that provides a quantitative measure of opioid activity. Along with analgesia, gastrointestinal transit, learning, respiratory depression, and motor activity, opioid-induced thermoregulatory effects have been used to evaluate activity in opioid analogues and endogenous opioids [11,22–24]. It is well established that kappa receptor activation produces hypothermia, whereas mu receptor activation causes hyperthermia in unrestrained rats [1,17,42,46].

The relevance of delta receptors in thermoregulation is less clear. Early studies suggested a minor involvement for delta receptors [9,20,21], but a more significant role for these receptors in thermoregulation has been demonstrated recently in rats [32,39]. Low doses of deltorphin-II, a delta$_2$ agonist, injected centrally produce hypothermia [39]. Higher doses of deltorphin-II evoke hypothermia followed by hyperthermia. A delta antagonist, naltrindole, antagonizes the deltorphin-II-evoked hypothermia, indicat-

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ing that the decline in body temperature is mediated by delta receptors [39]. However, it is not clear which delta receptor phenotype mediates the hypothermia [5].

There is interest in the commercial development of delta agonists because of the anticipated low side-effect profile, including reduced effects on respiration and gastrointestinal transit. The diphenylmethylpiperazine derivative, SNC-80, is one such agonist. SNC-80 is highly selective for delta receptors over mu and kappa receptors, and several observations suggest that the pharmacological profile of SNC-80 differs from that of peptide agonists, such as DPDPE ($delta_1$), DADLE (mu/delta), and deltorphin-II ($delta_2$) [6,8,11,13,25,44]. SNC-80 possesses antinociceptive properties in rhesus monkeys [31] and in the warm-water tail flick test in mice [8]. Although SNC-80 is 1000-fold less potent than deltorphin-II in stimulating locomotor activity in rats, the lower potency does not correlate with differences in brain penetration for SNC-80 and deltorphin-II or with the superior binding affinity and efficacy of SNC-80 at delta opioid receptors in rat brain homogenates [16]. The high delta selectivity of SNC-80 is useful in discriminating the functional role of delta receptors in convulsions, depression, antinociception, locomotion, and thermoregulation.

Baker and Meert used delta antagonists to characterize the hypothermic effects of (+)-4-[(*aR*)-*a*-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80), a nonpeptide delta agonist, in mice [3]. That study demonstrated that SNC-80 induces dose-dependent hypothermia that is blocked by naltrindole and decreased by low doses of naltriben, a $delta_2$ antagonist. On the other hand, BNTX, a $delta_1$ antagonist, did not alter the SNC-80-evoked hypothermia. The close association of delta receptors with the preoptic anterior hypothalamus (POAH), considered to be a primary site of thermoregulation in the brain, emphasizes the potential importance of these receptors in the regulation of body temperature [2,15,27,28].

Despite the fact that recent studies have established a significant role for delta opioid receptors in the regulation of body temperature in rats and mice [3,32,39], the delta receptor phenotype that mediates the hypothermia and the location of that phenotype is unclear [40]. To determine the delta receptor phenotype that mediates hypothermia in rats and to elucidate a central or peripheral locus, we administered SNC-80 and phenotype selective delta antagonists to rats. Our data reveal that the central administration of a $delta_2$ antagonist blocks the hypothermic effects of SNC-80. In contrast, a peripherally restricted opioid antagonist, methylnaltrexone, did not significantly alter SNC-80-evoked hypothermia. Our results indicate that the activation of $delta_2$ opioid receptors in the brain is a major mechanism by which SNC-80 causes hypothermia in rats. Yet, unlike in mice, our data do not support a significant role for peripheral opioid receptors in the hypothermic effect of SNC-80 [3].

2. Materials and methods

2.1. Animals

All animal use procedures were conducted in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Temple University Animal Care and Use Committee. Male Sprague–Dawley rats (Zivic-Miller, Pittsburgh, PA, USA) weighing 100–125 g were housed 1 per cage for a minimum of 5 days before experimental use. Rats were maintained on a 12-h light/dark cycle and fed rat chow and water ad libitum.

2.2. Drugs

SNC-80 was purchased from Tocris–Cookson (St. Louis, MO, USA) and obtained from the National Institutes on Drug Abuse [13]. Methylnaltrexone was obtained from the National Institutes on Drug Abuse [12]. Naltrexone hydrochloride, a general opioid antagonist; naltrindole HCl, a delta opioid antagonists; naltriben mesylate, a selective $delta_2$ antagonist; and 7-benzylidenenaltrexone maleate (BNTX), a selective $delta_1$ antagonist, were provided by NIDA [7,33,34,40]. SNC-80 was dissolved in 3% lactic acid to a final concentration of 50 mg/ml, and dilutions were made with sterile water. Naltriben mesylate was dissolved in 10% DMSO/sterile water to a final concentration of 2.5 mg/ml, and dilutions were made with sterile water. All other drugs were dissolved in sterile water. SNC-80 was administered intramuscularly (im) into the right thigh. The im route was chosen for SNC-80 to minimize the possibility that SNC-80 absorption would be hindered by the absorption of opioid antagonists. In our laboratory, the im administration route is used commonly for cannabinoid studies [35,37]. All other drugs were administered subcutaneously (sc), except for naltriben, which was given sc and intracerebroventricularly (icv). Doses were based on the salt forms of the drugs described above and chosen according to previous body temperature studies.

2.3. Cannula implantation

Rats were anesthetized with an intraperitoneal (ip) injection of a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). A polyethylene cannula was implanted stereotaxically into the right lateral ventricle according to procedures in our laboratory [36]. Dental acrylic was used to secure cannula to the cranium.

2.4. Experimental protocol

Between 8 and 9 AM on the morning of the experiment, rats were removed from the Animal Facility and placed one per cage into an environmental room, which was maintained