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Nitrous oxide-antinociception is mediated by opioid receptors and nitric oxide in the periaqueductal gray region of the midbrain

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Abstract

Previous studies have shown that nitrous oxide (N_2O)-induced antinociception is sensitive to antagonism by blockade of opioid receptors and also by inhibition of nitric oxide (NO) production. The present study was conducted to determine whether these occur within the same brain site. Mice were stereotaxically implanted with microinjection cannulae in the periaqueductal gray (PAG) area of the midbrain. In saline-pretreated mice, exposure to 70% N_2O resulted in a concentration-dependent antinociceptive effect in the mouse abdominal constriction test. Pretreatment with an opioid antagonist in the PAG significantly antagonized the antinociceptive effect. Pretreatment with an inhibitor of NO production in the PAG also significantly antagonized the antinociceptive effect. These findings suggest that N_2O acts in the PAG *via* an NO-dependent, opioid receptor-mediated mechanism to induce antinociception.

1. Introduction

Nitrous oxide, also known as "laughing gas" or simply N_2O, has gained clinical acceptance because of its anesthetic, analgesic and anxiolytic properties, which have been utilized in medicine and dentistry. How such a simple inorganic chemical structure can evoke this wide range of pharmacological effects is intriguing.

Exposure of test animals to N_2O results in an antinociceptive effect that is sensitive to antagonism by naloxone and other opioid receptor blockers (Berkowitz et al., 1976; Quock and Graczak, 1988), indicating a key role for opioid receptors in the drug effect. We have previously reported that the antinociceptive effect of N_2O is also antagonized by inhibition of nitric oxide (NO) production (McDonald et al., 1994; Ishikawa and Quock, 2003a; Li et al., 2004), implicating an involvement of

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NO in the drug effect as well. The present study was conducted to ascertain whether the opioid receptors and NO function that are involved in N_2O-induced antinociception are co-localized in the periaqueductal gray (PAG) of the midbrain, which has been shown to be involved in N_2O-induced antinociception (Zuniga et al., 1987; Hodges et al., 1994; Fang et al., 1997).

2. Experimental procedures

2.1. Subjects

A total of 265 male NIH Swiss mice, 18–22 g body weight, were obtained from Harlan Laboratories (Indianapolis, IN) and were housed five per cage in an AAALAC-accredited animal facility. Food and water were available *ad libitum*. The facility was maintained on a 12-h light/dark cycle (lights on 0700–1900) under standard conditions (22 ± 1 °C room temperature, 33% humidity). Mice were kept in the holding room for at least four days following arrival in the facility prior to use. The animals were tested in control and experimental groups of 8–12 mice per group.

The experimental protocol was approved by the Institutional Review Committee for the Use and Care of Animal Subjects of Washington State University and is in compliance with *The Guide for the Care and Use of Laboratory Animals* (US National Research Council, 1996). All care was taken to minimize pain and discomfort in the experimental animals.

2.2. Surgery

Under isoflurane anesthesia, mice were mounted in a digital stereotaxic system (Cartesian Research, Inc., Sandy, OR). Using aseptic technique, 26-G stainless steel external guide cannulae (Plastics One, Roanoke, VA) were stereotaxically directed at the PAG of the midbrain at coordinates −3.0 mm AP, ±0.2 mm ML, and −3.0 mm DV (Franklin and Paxinos, 2001). The tips of the external guide cannulae were positioned 1.0 mm dorsal to the target sites. Cannulae were secured to the calvarium using stainless steel screws and dental cement. Each cannula was plugged with a solid 33-gauge dummy cannula. After surgery, mice were allowed a minimum of five days recovery time before testing.

2.3. Histological verification of microinjection site

At the end of the experiments, animals were anesthetized with 2.5% isoflurane, and 0.5 μl 10% India ink dye was microinjected into the PAG over 15 min to mark the microinjection site. Thirty minutes after dye injections, mice were perfused with cold phosphate–buffered saline followed by 4% formaldehyde. The brains were dissected out and cryoprotected in 25% sucrose. Forty-μm coronal sections were cut on a cryostat and processed for staining with 0.5% cresyl violet. The sections were later observed under a stereomicroscope and photographed (Fig. 1).

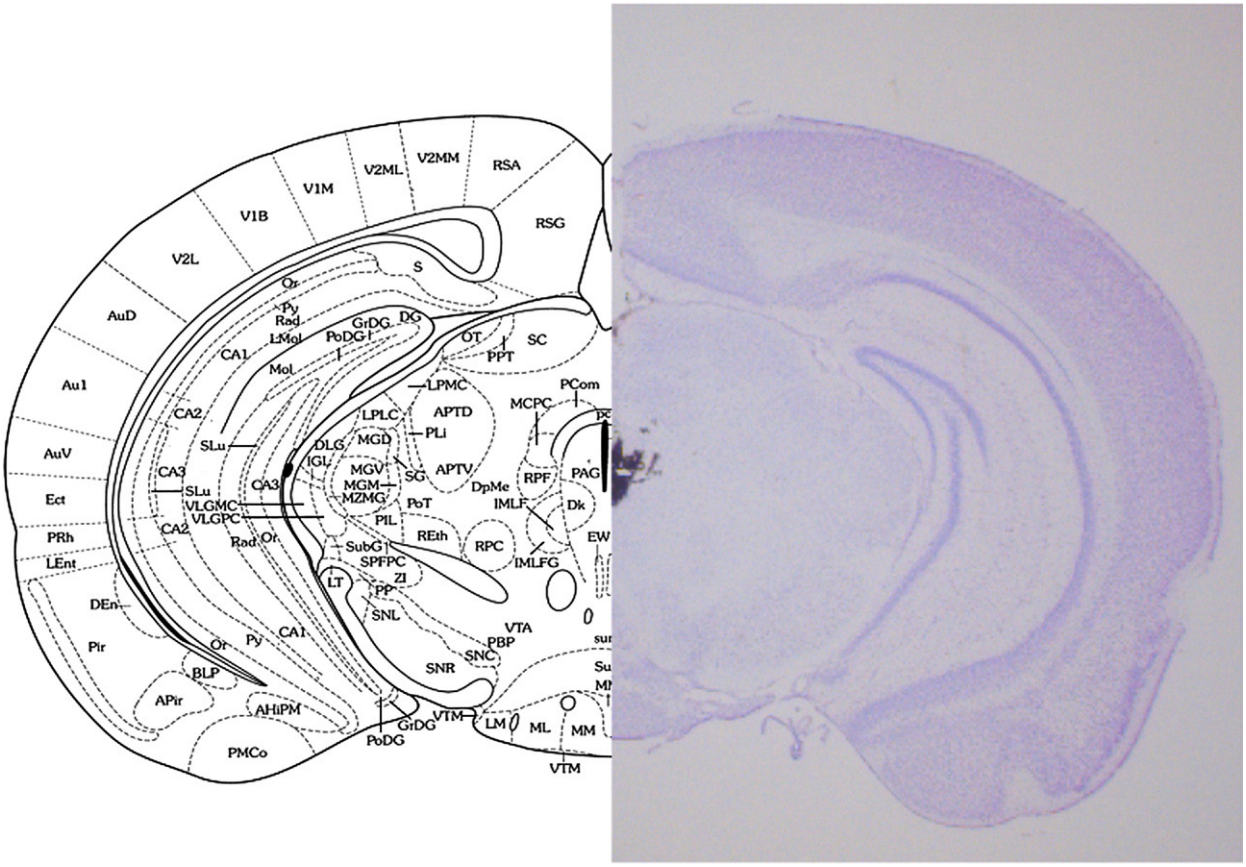

Figure 1 Photomicrograph of cresyl violet-stained coronal mouse brain section and corresponding atlas plate (Franklin and Paxinos, 2001) indicating the typical site and spread of microinjection as assessed by stain injections. All doses of β-CNA and TRIM were injected at a volume of 0.5 ml into the PAG.