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Research report

Increasing the availability of L-arginine and nitric oxide increases sensitivity of nitrous oxide (N_2O)-insensitive inbred mice to N_2O-induced antinociception

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ABSTRACT

Nitrous oxide (N_2O)-induced antinociception in mice is dependent on the neuromodulator nitric oxide (NO). In contrast to C57BL/6J (B6) mice, DBA/2J (D2) mice fail to respond to N_2O with a robust antinociceptive response or with an increase in brain nitric oxide synthase (NOS) enzyme activity, suggesting that failure of D2 mice to respond to N_2O might result from a deficit of NO function. Therefore, it was of interest to determine whether increasing the availability of NO might increase sensitivity of D2 mice to N_2O. Male D2 mice were pretreated with sub-antinociceptive intracerebroventricular doses of the NO donor 3-morpholinosydnoimine or the NO precursor L-arginine then assessed for responsiveness to N_2O-induced antinociception using the acetic acid abdominal constriction test. Both pretreatments increased the antinociceptive responsiveness of D2 mice to N_2O. These results indicate that the NOS enzyme in D2 mice is functional and that the deficit in NO function that obstructs sensitivity to N_2O-induced antinociception may lie in availability or utilization of L-arginine.

1. Introduction

The anesthetic gas nitrous oxide (N_2O) evokes a robust antinociceptive response in experimental animals that is sensitive to antagonism by inhibition of nitric oxide synthase (NOS) and interference with nitric oxide (NO) production in the brain (McDonald et al., 1994; Ishikawa and Quock, 2003a; Cope et al., 2010). In contrast to C57BL/6J (B6) mice, DBA/2J (D2) mice demonstrate a weak antinociceptive response to N_2O (Quock et al., 1993) and failure to show an increase in brain nitric oxide synthase (NOS) enzyme activity (Ishikawa and Quock, 2003b), initially suggesting that failure of D2 mice to respond to N_2O might result from a deficit of NO function. The correlation between poor antinociceptive responsiveness and weak NOS stimulation was confirmed by an S_5 generation selectively bred for low responsiveness to N_2O (Henry et al., 2005). The present study was carried out to further explore the unique resistance of the D2 strain to respond to N_2O with stimulated NOS activity and antinociception.

2. Materials and methods

2.1. Animals

Male NIH Swiss mice (18–23 g) were obtained from the Harlan Laboratories (Indianapolis, IN) and male C57BL/6 and DBA/2 mice (18–23 g) were obtained from The Jackson Laboratories (Bar Harbor, Maine). These experiments were approved by the Washington State University Institutional Animal Care and Use Committee (IACUC) with post-approval review and carried out in accordance with The Guide for the Care and Use of Laboratory Animals, 8th Edition (National Academies Press, Washington, DC, 2010).

Mice were housed five per cage in the Animal Resource Unit at Washington State University with access to food and water ad libitum. The facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), was maintained on a regular 12-h light:dark cycle (lights on 0700–1900 h) under standard conditions of temperature (22 ± 1 °C) and humidity (33%). Mice were kept in the holding room for at least four days following arrival in the facility and prior to experimentation.

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2.2. Antinociceptive testing

Antinociceptive responsiveness was assessed using the abdominal constriction test (Siegmund et al., 1957). This nociceptive model was selected in lieu of tests employing a thermal noxious because this model is significantly more sensitive to detection of κ–opioid antinociceptive activity than are thermal tests (Tyers, 1980) and because our previous studies have implicated κ–opioid mechanisms in mediation of N_2O-induced antinociception (Quock et al., 1990; Quock and Mueller, 1991).

Mice were treated i.p. with 0.1 mL per 10 g body weight of 0.6% acetic acid. Exactly 5 min later, the number of abdominal constrictions – lengthwise stretches of the torso with concave arching of the back – in each animal was counted for 6 min. The degree of antinociception (inhibition of abdominal constrictions) produced in various treatment groups of mice was calculated by the following formula:

$$\%\text{antinociception} = 100 \times \frac{\#\text{constrictions in control mice} - \#\text{constrictions in treated mice}}{\#\text{constrictions in control mice}}$$

2.3. Exposure to N_2O

Nitrous Oxide, U.S.P. and Oxygen, U.S.P. (A-L Compressed Gases Inc., Spokane, WA) were mixed and delivered using a dental-sedation system (Porter, Hatfield, PA) at a total flow rate of 10 L/min. Mice were individually exposed in a clear Plexiglas® exposure chamber (35 cm L × 20 cm W × 15 cm H) with gas inlet and outlet ports. The concentrations of N_2O and O_2 delivered into the box were monitored using a POET II® anesthetic monitoring system (Criticare, Waukesha, WI). Exhausted gases were routed by polyethylene tubing to a nearby fume hood.

2.4. Drugs

The following drugs were used in this research: L-arginine (L-ARG); and 3-morpholinosydnoimine (SIN-1) (both from Sigma Chemical Co., St. Louis, MO). L-ARG and SIN-1 were dissolved in 0.9% physiological saline and microinjected by the intracerebroventricular (i.c.v.) route according to the method of Haley and McCormick (1957). Briefly, mice were anesthetized with IsoFlo® (isoflurane, U.S.P., Abbott Laboratories, North Chicago, IL). A short incision was made along the midline of the scalp using a scalpel, and the skin was pulled back to expose the calvarium. The i.c.v. microinjection was made, using a 10-μL microsyringe (Hamilton, Reno, NV) with a 26-gauge cemented needle. The microsyringe was held vertically by hand at a point on the calvarium 2.0 mm lateral and 1.0 mm caudal from bregma to a depth of −2.0 mm from the skull surface. Penetration was controlled by a large-bore needle through which the microsyringe needle was inserted; the larger hypodermic needle served as a collar to limit penetration of the microsyringe needle to 2.0 mm. A volume of 4.0 μL of drug solution or vehicle was delivered directly into the lateral cerebral ventricle over 30 s. The i.c.v. doses of L-ARG and SIN-1 were 0.1 and 0.01 μg/mouse, respectively. These were sub-threshold doses of L-ARG and SIN-1 that alone failed to elicit an appreciable antinociceptive effect.

2.5. Statistical analysis of data

A two-way ANOVA with a *post-hoc* Dunnett's multiple comparison test was used to compare the antinociceptive responsiveness of different treatment groups of the three mouse strains to N_2O-induced antinociception.

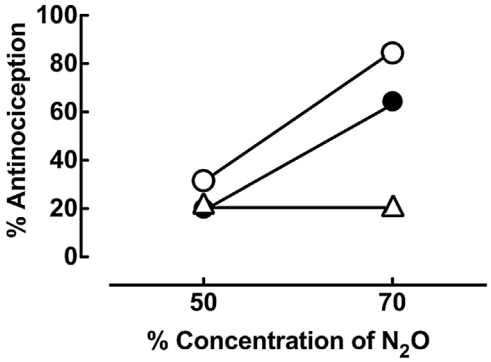

Fig. 1. N_2O dose-response curves in NIH Swiss, B6 and D2 mouse strains: NIH Swiss AD_{50} = 55.7% (48.2–64.4%); B6 AD_{50} = 59.3% (54.0–65.1%); and D2 $AD_{50} = \infty$. Each symbol represents the mean ± SEM of the response of 10–12 mice per group.

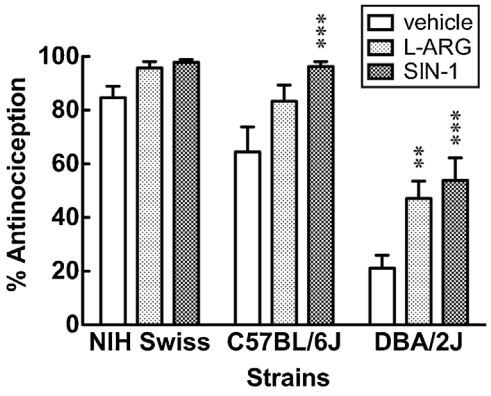

Fig. 2. Antinociceptive effects of N_2O in NIH Swiss, B6 and D2 mouse strains pretreated i.c.v. with vehicle, L-ARG and SIN-1. Each column represents the mean antinociceptive response ± SEM of at least 10–12 mice per group. Significance of differences: strain: $F(2,67) = 16.21$, $p < 0.0001$; drug: $F(2,67) = 67.96$, $p < 0.0001$; interaction: $F(4,67) = 1.031$, $p = 0.3976$ (two-way ANOVA). Symbols: **, $p < 0.01$, ***, $p < 0.001$, compared to the vehicle + N_2O groups (Dunnett's multiple comparison test).

3. Results

N_2O exposure induced a robust antinociceptive effect in NIH Swiss and B6 mice but not in D2 mice. This is evidenced by a comparison of the dose-response curves for N_2O-induced antinociceptive in the three mouse strains (Fig. 1). The relative sensitivity of the strains to N_2O-induced antinociception is NIH Swiss = B6 » D2. The analgesic dose 50% values were 55.74% (48.22–64.42%, 95% confidence limits) for NIH Swiss mice, 59.28% (53.99–65.09%) for B6 mice, and ∞ for D2 mice.

The three strains were exposed to 70% N_2O following i.c.v. pretreatments that would indirectly or directly increase brain levels of L-arginine or NO. Neither L-ARG nor SIN-1 significantly enhanced the antinociceptive effect of N_2O in NIH Swiss mice; however, the N_2O response was already near maximal. However, both L-ARG and SIN-1 significantly increased the antinociceptive responsiveness of both B6 and D2 mice to N_2O (Fig. 2).

4. Discussion

The antinociceptive effect of N_2O has long been investigated (Berkowitz et al., 1976; Zuniga et al., 1987). Recent preclinical investigations have demonstrated that, in addition to providing relief from acute pain, N_2O is also capable of suppressing chronic pain in animal models of neuropathic pain for weeks following a single exposure (Bessière et al., 2010; Ben Boujema et al., 2015).