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Correlation of inbred mouse sensitivity to nitrous oxide antinociception with brain nitric oxide synthase activity following exposure to nitrous oxide

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Abstract

Inhibition of nitric oxide synthase (NOS) antagonizes nitrous oxide (N_2O)-induced antinociception in mice. This study was conducted to compare brain NOS activity in high responding *C57BL/6* mice, low responding *DBA/2* mice and S_5 mice selectively bred for low responsiveness to N_2O. Exposure to 70% N_2O suppressed acetic acid-induced abdominal constrictions in *C57BL/6* mice but not *DBA/2* or S_5 mice. N_2O exposure also elevated NOS activity in brains of *C57BL/6* mice but not *DBA/2* or S_5 mice. The absence of these effects in *DBA/2* or S_5 mice is further support for the hypothesis that nitric oxide (NO) may play a critical role in N_2O-induced antinociception in mice.

Keywords: Nitrous oxide; Antinociception; Nitric oxide synthase; Short-term selective breeding; Mice

1. Introduction

Current evidence indicates that N_2O-induced antinociception in the mouse abdominal constriction model may be secondary to stimulated neuronal release of dynorphin that then activates κ opioid receptors (Quock and Graczak, 1988; Quock et al., 1990; Quock and Mueller, 1991; Branda et al., 2000; Cahill et al., 2000). The activation of these opioid receptors in the periaqueductal gray matter of the brain (Zuniga et al., 1987; Emmanouil et al., 2004), in turn, inhibits GABAergic neuronal influences upon descending pain modulatory systems that involve the activation of adrenergic receptors in the spinal cord (Sawamura et al., 2000; Fujinaga and Maze, 2002).

Another important component of the antinociceptive effect of N_2O appears to be the gaseous neuromodulator nitric oxide (NO). Experiments previously conducted in this laboratory determined that inhibition of NO production in mice reduced their sensitivity to N_2O-induced antinociception in the abdominal constriction test (McDonald et al., 1994; Ishikawa and Quock, 2003b; Li et al., 2004). The present study employed short-term selective breeding as a means of further implicating NO in the mechanism of N_2O-induced antinociception.

Previous research has reported differences in the responsiveness of inbred mouse strains to N_2O-induced antinociception in the acetic acid abdominal constriction test (Quock et al., 1993). The *C57BL/6* mouse strain demonstrated high responsiveness to N_2O, characterized by a significant decrease in abdominal constrictions under 70% N_2O. The

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DBA/2 mouse strain responded poorly to N_2O, showing very little change in abdominal constrictions. In a recent preliminary report, we found that exposure to 70% N_2O caused a 40% elevation in whole brain NOS activity in *C57BL/6* mice but only a statistically insignificant 10% increase in *DBA/2* mice (Ishikawa and Quock, 2003a). The present research shows that exposure to N_2O increases NOS enzyme activity in whole brain and cerebellum and decreases NOS activity in the corpus striatum of *C576/BL* mice but not DBA or a line of mice that was selectively bred for low responsiveness to N_2O antinociception.

2. Methods

2.1. Animals

Adult male DBA ($n=15$) and C57 male mice ($n=14$) were obtained from the Jackson Laboratory (Bar Harbor, Maine). S_5 mice ($n=14$) with poor responsiveness to N_2O-induced antinociception were the product of a short-term selective breeding program similar to that described by Belknap et al. (Belknap et al., 1997). Breeding began with low-responding male and female mice from the F_2 generation derived from DBA and C57 progenitors (Mueller et al., 2004). Each generation was screened for responsiveness to N_2O-induced antinociception, and the male and female mice with the poorest responsiveness were mated to produce the next generation. Breeding was ended following the F_7 (or S_5) generation.

These experiments were approved by an institutional animal care and use committee and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996). All measures to minimize pain or discomfort were taken by the investigators.

2.2. Antinociceptive testing

Antinociceptive responsiveness to N_2O was assessed by the abdominal constriction test. At 7–8 weeks of age, mice were treated intraperitoneally with 0.6% acetic acid (0.1 ml/10 g body weight); 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 a 6-min period while in a Plexiglas exposure chamber (20 cm W × 35 cm L × 15 cm H) open to room air. One week later, mice were again treated with acetic acid, but this time housed in a closed chamber containing an atmosphere of 70% N_2O in oxygen (O_2). Previous studies showed that mice typically regained their sensitivity to acetic acid-induced abdominal constrictions within this period of time.

2.3. Delivery of nitrous oxide/oxygen

A mixture of 70% N_2O, U.S.P. and 30% O_2, U.S.P. (A&L Welding, Spokane, Washington) was delivered into the chamber at a total inflow rate of 10 l/min using a portable N_2O/O_2 dental sedation system (Porter, Hatfield, Pennsylvania). A POET II anesthetic monitoring system (Criticare, Milwaukee, Wisconsin) was used to verify that desired N_2O/O_2 concentrations had been attained. Exhausted gas was vented to a nearby fume hood.

2.4. NOS assay

Mice were decapitated after 15 min exposure to 70% N_2O or room air (as control). The brains were quickly removed and placed on an ice-cold Petri dish. The brain was cut in half along the midline. One brain half was used to determine NOS activity level per milligram protein. The other brain half was dissected to measure NOS activity in hippocampus, cerebellum, amygdala, midbrain and corpus striatum. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until analyzed.

NOS activity was assayed by measuring the Ca^{2+}-dependent conversion of [^{14}C]L-arginine to [^{14}C]L-citrulline (Huang et al., 1993). On the day of the assay, tissue samples were sonicated in 10 volumes (wt/vol) of 50 mM Tris·HCl (pH 7.4) buffer containing 1.0 mM ethylenediamine tetraacetic acid (EDTA) and 1.0 mM ethyleneglycol tetraacetic acid (EGTA) (homogenization buffer). After centrifugation (12,000 rpm for 20 min at 4 °C), 20 μl supernatant was added to 40 μl 50 mM Tris·HCl (pH 7.4) buffer containing 1.0 mM NADPH, 3.0 μM BH_4, 1.0 μM FAD, 1.0 μM FMN, 1.25 mM $CaCl_2$, and 1.25 μCi/ml [^{14}C]L-arginine (specific activity: 348 mCi/mmol, Amersham Biosciences, Piscataway, New Jersey) and incubated for 30 min at 37 °C. The reaction was terminated by addition of 400 μl stop buffer containing 50 mM HEPES (pH 5.5) and 5.0 mM EDTA. Then the reaction mixture was applied onto a chromatographic column containing 40 mg Dowex AG50WX-8 resin (Bio-Rad, Hercules, California) for separation of [^{14}C]L-citrulline from the unreacted [^{14}C]L-arginine by cation-exchange chromatography and collected into a scintillation vial. Thereafter, the samples were counted for the amount of radioactivity using a model A2500 liquid scintillation counter (Packard Instrument Company, Meriden, Connecticut). The protein content of the supernatant was determined using the bicinchoninic acid (BCA) method and a commercially available assay kit (Pierce Chemical Company, Rockford, Illinois) with bovine albumin as a standard. NOS enzyme activity was expressed in terms of picomole per milligram protein per minute.

2.5. Statistical analysis of data

The responsiveness of each strain to 70% N_2O was determined by Dunnett's *t*-test. Differences in NOS activity levels of N_2O- and room air-exposed animals were analyzed by paired Student's *t*-test.