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Short communication

Effects of a nitric oxide donor on behavior and interaction with nitrous oxide in the mouse light/dark exploration test

Shuang Li, Raymond M. Quock*

Department of Pharmaceutical Sciences, College of Pharmacy and Graduate Program in Pharmacology and Toxicology, Washington State University, P.O. Box 646534, Pullman, WA 99164-6534, USA

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Abstract

There is controversy whether endogenous nitric oxide (NO) is involved in anxiogenesis or anxiolysis. This study was conducted to determine the influence of the NO donor, 3-morpholinosyndnonimine (SIN-1), on resting and nitrous oxide (N_2O)-induced behaviors in the mouse light/dark exploration test. I.c.v. doses of 0.3 and 1.0 μg SIN-1 both increased the time spent in the light compartment. When pretreated with 0.1 μg SIN-1, mice responded to N_2O with an apparent additive increase in the time spent in the light compartment. These findings further support a functional role of NO in regulation of anxiety and mediation of N_2O-induced behavior.

Keywords: Nitric oxide (NO); Nitric oxide (NO) donor; Nitrous oxide (N_2O); Light/dark exploration test; Anxiety

1. Introduction

Nitric oxide (NO) is a neuromodulator and intercellular retrograde messenger that mediates several important functions in the central nervous system (Prast and Philippu, 2001; Ohkuma and Katsura, 2001). Among these functions is anxiety, and currently there is controversy as to the role played by NO in anxiety.

Earlier experiments from our laboratory have demonstrated an essential role of NO in mediating the anxiolytic effects of nitrous oxide (N_2O) and benzodiazepines (Quock and Nguyen, 1992; Caton et al., 1994; Li and Quock, 2001), implicating a functional role of NO in relief of anxiety. Consistent with this hypothesis, other groups have reported that acute treatment of rats with non-specific inhibitors of nitric oxide synthase (NOS) reduced open-arm activity in the elevated plus-maze, which is suggestive of increased anxiety (De Oliveira et al., 1997; Vale et al., 1998; Monzon et al., 2001). However, other investigators implicate NO in anxiogenesis since NOS inhibitors appear to suppress indices of anxiety in some experimental models (Volke et al., 1997; Dunn et al., 1998; Yildiz et al., 2000).

Because the actual role of NO in anxiety is still unclear, this research utilized i.c.v. administration of an NO donor to determine its behavioral effects in the light/dark exploration test. Further, we investigated the interaction between the NO donor and N_2O, which has been shown to exhibit apparent anxiolytic properties (Quock and Nguyen, 1992; Caton et al., 1994; Li and Quock, 2001).

2. Materials and methods

2.1. Animals

Male NIH Swiss mice, 18–22 g body weight, were purchased from Harlan Sprague–Dawley Laboratories (Indianapolis, IN) and maintained in the Vivarium under standard conditions (12-h light:dark cycle, 22 ± 1 °C room temperature, 33% humidity). Mice were kept in the holding room for at least 4 days following arrival in the facility. All experiments were approved by an institutional animal care and use committee. Each animal was used only once, then discarded.

2.2. Apparatus

Gas exposures and experiments were conducted inside a light/dark exploration box (as described in Li and Quock,

* Corresponding author. Tel.: +1-509-345-5956; fax: +1-509-335-5902.
E-mail address: quockr@wsu.edu (R.M. Quock).

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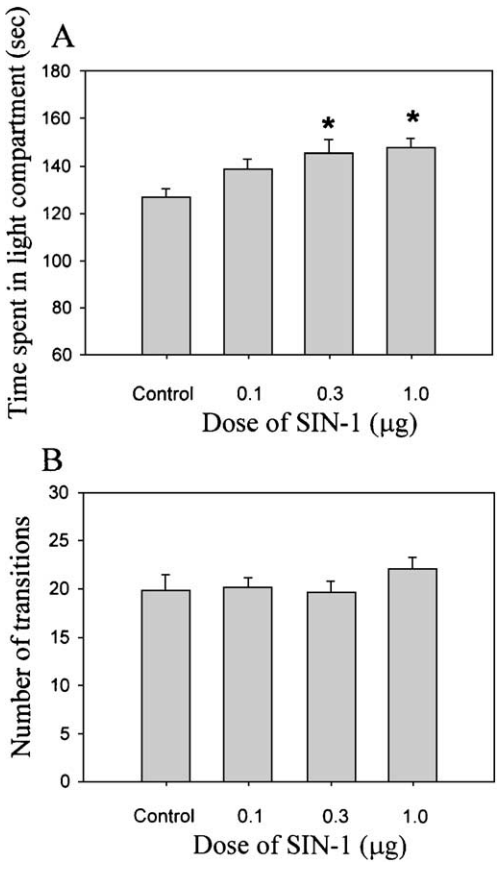

Fig. 1. Anxiolytic effects of NO-donor in the light/dark exploration test, (A) time spent in the light compartment and (B) number of transitions. The data are expressed as mean ± S.E.M. of 10 mice per group. Significance of difference: $*P<0.05$, compared with VEH control (one-way ANOVA followed by a post-hoc Bonferroni test versus control).

2001). Behavioral observations and assessments were generally performed each day between 1000 and 1400 h.

In this paradigm, animals were individually placed in the center of the light compartment of the box, facing away from the divider, then observed for 5 min. The time spent in the light chamber of the box as well as the number of transitions between the light and dark compartments were recorded for each mouse. A mouse was considered to have entered the new area when all four legs crossed the threshold into the compartment.

2.3. Drugs

The following drugs were used in this research: N_2O, USP, O_2, USP and compressed air, USP (all from A&L Welding, Spokane, WA); 3-morpholinosydnonimine (SIN-1; Sigma, St. Louis, MO). The gases N_2O and O_2 were delivered into the light/dark box via a length of polyethylene tubing via a portable N_2O/O_2 dental sedation system (Porter, Hatfield, PA). A POET II anesthetic monitoring system (Criticare, Milwaukee, WI) was used to ascertain that the desired N_2O-O_2 atmospheres had been attained within the filling time. SIN-1 was prepared in 0.9% physiological saline and administered i.c.v. 30 min prior to testing, as described by Xu et al. (1995). The dose range of SIN-1 was 0.1–1.0 μg/mouse in a volume of 4.0 μl. Control animals were treated with vehicle (VEH) and/or exposed to compressed air.

2.4. Statistical analysis of the data

All data were analyzed by one- or two-way analyses of variance (ANOVAs). When a significant F value was found, post-hoc analysis was performed by Bonferroni test.

3. Results

3.1. Anxiolytic effects of NO-donor in the light/dark exploration test

As shown in Fig. 1A and B, SIN-1 affected the time spent in the light compartment but not the number of transitions. A dose of 0.1 μg caused a slight—though statistically insignificant—increase, but 0.3 and 1.0 μg both

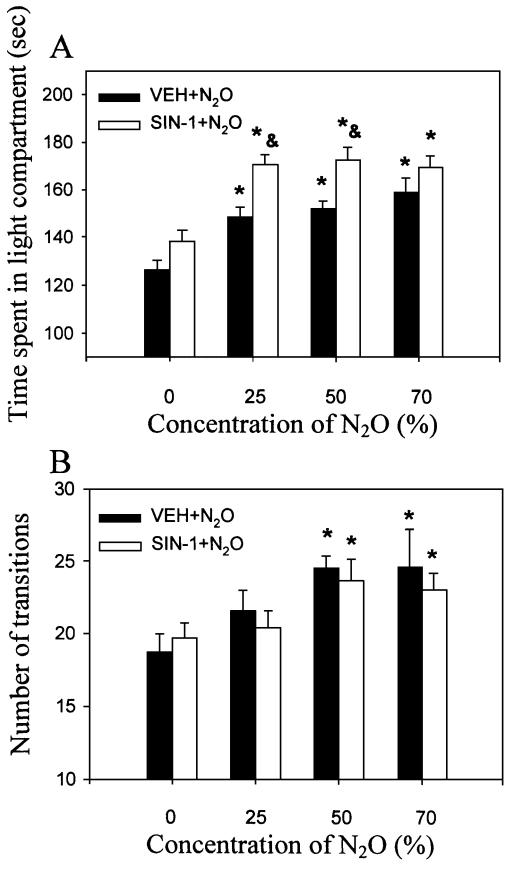

Fig. 2. Influence of NO-donor on N_2O-induced changes in (A) time spent in the light compartment and (B) number of transitions. The data are expressed as mean±S.E.M. of 10 mice per group. Significance of difference: $*P<0.05$, compared with its corresponding control (compressed air-exposed) group; and, $\&P<0.05$, compared with the VEH+N_2O control (two-way ANOVA followed by a post-hoc Bonferroni test).

caused a significant increase in the amount of time spent in the light compartment [one-way ANOVA, $F(3,36)=3.9$, $P<0.05$].

3.2. Influence of NO-donor on behavioral effects of N_2O

Fig. 2A and B shows that mice exposed to N_2O exhibited increases in both time spent in the light compartment as well as number of transitions between compartments. These increases were all statistically significant from the baseline behavior exhibited by control (compressed air-exposed) animals [time: F_{N_2O} $(3,72)=20.84$, $P<0.001$; transitions: F_{N_2O} $(3,72)=5.37$, $P<0.01$].

Fig. 2A also shows the effects of SIN-1 pretreatment on the time spent in the light compartment during exposure to N_2O [F_{N_2O} $(3,72)=20.84$, $P<0.001$; $F_{SIN\text{-}1}$ $(1,72)=24.84$, $P<0.001$; interaction, $F_{N_2O} \times$ SIN-1 $(3,72)=0.83$, not significant]. But post-hoc test showed a significant difference between groups exposed to 25% and 50% ($P<0.05$, Bonferroni test) but not 70% N_2O. Fig. 2B shows the effects of SIN-1 pretreatment on the number of transitions during exposure to N_2O [F_{N_2O} $(3,72)=5.37$, $P<0.01$; $F_{SIN\text{-}1}$ $(1,72)=0.43$, not significant ($P<0.05$); $F_{N_2O} \times$ SIN-1 $(3,72)=0.82$, not significant].

4. Discussion

SIN-1 spontaneously releases NO as follows: SIN-1+$OH^- \Rightarrow$ SIN-1A + $O_2 \Rightarrow$ SIN-1C + O_2^- + NO (Freelisch et al., 1989). As an NO donor, SIN-1 has been utilized to generate exogenous NO in the brain and determine the direct effects of NO on physiological or behavioral function. In earlier studies, SIN-1 has been administered to experimental animals and found to evoke flight reaction (De Oliveira et al., 2000), non-REM sleep (Kapas and Krueger, 1996) and hyperalgesia (Machelska et al., 1998). Further, SIN-1 has been reported to potentiate β-endorphin-induced antinociception (Xu et al., 1995) and reverse serotonin-induced head twitching (Kim et al., 1999).

The present results show that higher i.c.v. doses of SIN-1 (0.3 and 1.0 μg) significantly increased the amount of time spent by animals in the light compartment but not the number of transitions between the compartments. The light/dark exploration test is based on the natural tendency of rodents to initially avoid brightly illuminated spacious areas (Hascoët et al., 2001). In this paradigm, anxiolytic agents increase the amount of time spent in the light compartment. The number of transitions serves as an index of locomotor activity and the effect of the agent on locomotor behavior. It is generally acknowledged that the time spent in the light compartment is a more sensitive index of the anxiolytic action of drugs than the number of transitions (Hascoët and Bourin, 1998).

While SIN-1 increased only the time spent in the light compartment, benzodiazepines increase *both* time and number of transitions. The latter represents the disinhibiting effect of benzodiazepines that subsides at higher doses due to their sedative action (Li and Quock, 2001). N_2O also produces concentration-related increases in both time and number of transitions; however, in the case of N_2O, the increase in transitions is apparently due to an opioid receptor-mediated locomotor stimulatory effect of the drug (Hynes and Berkowitz, 1982).

These results are in accord with previous reports from our laboratory that implicate a role for NO in mediating the anxiolytic effects of N_2O and benzodiazepines (Quock and Nguyen, 1992; Caton et al., 1994; Li and Quock, 2001). These results are also consistent with other reports that NOS-inhibitors tend to increase anxiety in the elevated plus-maze (De Oliveira et al., 1997; Vale et al., 1998; Monzon et al., 2001).

To further explore this apparent role of NO in suppressing anxiety, we pretreated mice with 0.1 μg SIN-1, i.c.v. to determine its influence of the known effects of N_2O in the light/dark exploration test (Li and Quock, 2001). The amount of time spent in the light compartment by SIN-1 + N_2O mice was greater than that spent by N_2O control mice at 25% and 50% but not 70% N_2O. The curves of the control (VEH + N_2O) and test (SIN-1 + N_2O) are generally parallel to one another, and there is no statistically significant interaction between SIN-1 and N_2O. Consequently, the difference between the two groups is attributed to an additive effect of the slight increase observed with SIN-1 (Fig. 1A) and the effect of N_2O (Fig. 2A). The lack of a difference at 70% is probably the result of the near-maximal responses already attained at the lower concentrations of N_2O (25% and 50%).

While the findings of this study support our hypothesis of a functional role of NO in regulation of anxiety and mediation of N_2O-induced behavior, it must be acknowledged that there remains some controversy of the precise role of NO in anxiogenesis and anxiolysis. Some researchers report that NOS-inhibitors are capable of suppressing anxiety in animal models of experimental anxiety (Volke et al., 1997; Dunn et al., 1998). Monzon et al. (2001) have attributed these apparent contradictions to different experimental procedures and routes of administration. They suggest that the levels of NO in amygdala and hippocampus influence the expression of anxiety; however, systemic administration of NOS-inhibitors might affect the synthesis of NO throughout the body and cause peripheral side effects (e.g., hypotension) that might influence behavioral function. On the other hand, NO may have biphasic influences (i.e., both excitatory and inhibitory) based on a complex interaction among glutamatergic, GABAergic and other (e.g., opioid) neuronal systems, all of which are modulated by NO (Lovick and Key 1996; Wang et al., 1997; Hall and Behbehani 1998; Lin et al., 2000). While these advances have help clarify some facets of the role of NO in the brain, further research is needed to gain an understanding of the precise role of NO in anxiety.

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