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A study of the role of serotonin in the anxiolytic effect of nitrous oxide in rodents

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Abstract

Rationale: In earlier studies, we have shown that nitrous oxide (N_2O)-induced behavioral effects in rats and mice are mediated by benzodiazepine receptors.
Objectives: This two-part study was conducted in order to investigate the possible role of serotonin (5-HT) in the behavioral effects of N_2O by clarifying its effects on regional brain concentrations of 5-HT and assessing the influence of 5-HT antagonist and reuptake inhibiting drugs on the anxiolytic-like behavioral effect of N_2O.
Methods: In experiment A, male, 150–200 g Sprague–Dawley rats were killed following a 15-min exposure to room air or 70% N_2O. The frontal cortex, hippocampus, corpus striatum and hypothalamus were dissected out and analyzed by HPLC with electrochemical detection for content of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA); dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were also measured. In experiment B, male 18–22 g NIH Swiss mice were pretreated with the 5-HT_2 antagonist cinanserin, the 5-HT_3 antagonist LY-278,584, the 5-HT reuptake inhibitor fluoxetine or saline and tested in the light/dark exploration test under 70% N_2O 30 min after pretreatment.
Results: In experiment A, N_2O produced differential effects on 5-HT neurons in distinct brain areas. There was increased 5-HT turnover in the hypothalamus, decreased turnover in the frontal cortex but no changes in either hippocampus or corpus striatum. By comparison, dopamine turnover in these brain regions was unaltered by N_2O exposure. In experiment B, pretreatment with neither cinanserin, LY-278,584 nor fluoxetine had any appreciable effect on the N_2O-induced increase in time spent in the light compartment. Only cinanserin significantly reduced the N_2O-induced increase in transitions.
Conclusions: While neurochemical results suggest an effect of N_2O on brain 5-HT function, there was no effect of 5-HT_2 or 5-HT_3 antagonists or 5-HT reuptake inhibitor on N_2O-induced anxiolytic-like behavior.

Keywords: Nitrous oxide; Anxiety; 5-Hydroxytryptamine; HPLC quantification of brain monoamines; Light/dark exploration test; Rats; Mice

1. Introduction

Nitrous oxide (N_2O) is the oldest anesthetic gas available and continues to be widely used in combination with other anesthetics for production of surgical anesthesia and, either alone or in combination, for production of conscious sedation in dentistry (Jackson and Johnson, 2002; Paterson and Tahmassebi, 2003). The mechanism of its analgesic effect is thought to involve opioid receptors as the analgesia in human subjects is at least partly reversed by the opioid receptor blocker naloxone (Chapman and Benedetti, 1979; Gillman et al., 1980; Yang et al., 1980). This is consistent with reports that N_2O-induced antinociception in experimental animals is antagonized by opioid receptor blockers (Quock and Vaughn, 1995). The mechanism of its anxiolytic effect in humans is uncertain, although research in animal models of experimental anxiety has implicated benzodiazepine receptors in the reduction in anxiety

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(Quock et al., 1992, 1993; Emmanouil et al., 1994; Li and Quock, 2001).

The current pharmacological management of anxiety focuses on brain mechanisms involving benzodiazepine and 5-hydroxytryptamine (5-HT) receptors. The current research was conducted to ascertain whether 5-HT receptors might also be involved in the anxiolytic effect of N_2O. Towards this end, one study (experiment A) was conducted in rats to determine the influence of N_2O exposure on brain monoamine levels, and another study (experiment B) was conducted in mice to determine the influence of 5-HT receptor antagonists and reuptake inhibitors on N_2O-induced behavioural effects.

2. Materials and methods

2.1. Experiment A

The objective of this experiment was to determine the influence of a 15-min exposure upon 5-HT, dopamine (DA) and their respective metabolites, 5-hydroxyindoleacetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC), in the frontal cortex, hippocampus, corpus striatum and hypothalamus.

2.1.1. Animals

Male Sprague–Dawley rats, weighing 150–200 g, were purchased from Sasco Inc. (Omaha, NE) for this research. After an acclimatization period of 7 days, animals were randomly assigned to one of the following two groups: group I, rats ($n=10$) were exposed to 70% N_2O mixed with 30% oxygen for 15 min; group II, control rats ($n=10$) were exposed to room air for 15 min. 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).

2.1.2. Exposure to nitrous oxide

Rats were exposed in pairs to nitrous oxide inside a medium-size AtmosBag® glove bag (Aldrich, Milwaukee, WI). The sealed glove bag was filled with compressed air or a mixture of N_2O and O_2 (all medical grade, Rockford Industrial Gas, Rockford, IL) via a length of polyethylene tubing using a portable N_2O/O_2 dental sedation system (Porter, Hatfield, PA). The total gas inflow rate was 10 l/min (either 10 l/min compressed air or 7 l/min N_2O+3 l/min O_2). The atmosphere inside the glove bag was confirmed by a POET II® anaesthetic monitoring system (Criticare, Milwaukee, WI).

2.1.3. HPLC quantification of brain monoamines

After 15 min, the glove bag was opened, and the rats were quickly removed and sacrificed by decapitation. The brains were dissected on ice, and the striatum, frontal cortex, hypothalamus and hippocampus were removed. The tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until assayed. 5-HT, 5-HIAA, DA and DOPAC were measured by high performance liquid chromatography (HPLC) with electrochemical detector (ECD), as described by Sharp et al. (1987) with some minor modifications (Papadopoulou-Daifoti et al., 1995). After weighing, the dissected tissues were homogenized and deproteinized in 500 μl of 0.2 N perchloric acid solution containing 7.9 mM $Na_2S_2O_5$ and 1.3 mM Na_2EDTA. The homogenate was centrifuged at 37,000×*g* for 30 min and the supernatant was stored at −80 °C. A reverse-phase ion pair chromatography was used in all analyses. The mobile phase consisted of an acetonitrile −50 mM phosphate buffer (10.5:91.5) pH 3.0, containing 5-octylsulfate sodium salt (300 mg/l) as the ion-pair reagent and (20 mg/l) Na_2EDTA. Reference standards were prepared in 0.2 N perchloric acid solution containing 7.9 mM $Na_2S_2O_5$ and 1.3 mM Na_2EDTA. The sensitivity of the assays was always tested using external standards and an HPLC system BAS-LC4B with an amperometric detector. The working electrode was glassy carbon, the columns were Thermo Hypersil-Keystone™ 150×2.1 mm, 5 μm Hypersil, Elite C18 (Thermo Electron, Cheshire, UK), and the HPLC system was connected to a computer. Samples were quantified by comparison of the areas under peaks with those of reference standards using HPLC software (Chromatography Station for Windows™, Watrex International, Inc., San Francisco, CA). Additionally, the ratios of serotonin (5-HIAA/5-HT) and dopamine (DOPAC/DA) were calculated as indices of the serotonin and dopamine turnover rates which reflect the serotonergic and dopaminergic activity, including release and/or metabolism function (Cransac et al., 1996; Connor et al., 1997).

2.1.4. Statistical analysis of data

Tissue levels of 5-HT, 5-HIAA, DA and DOPAC as well as ratios of 5-HIAA/5-HT and DOPAC/DA in control and N_2O-exposed groups of rats were compared using Student's *t*-test following testing for normality and equal variance.

2.2. Experiment B

The objective of this experiment was to determine the influence of blockade of selected 5-HT receptor subtypes and inhibition of 5-HT reuptake on N_2O-induced anxiolytic-like behavioural response to N_2O in the mouse light/dark exploration test.

2.2.1. Animals

Male NIH Swiss mice, 18–22 g body weight, were purchased from Harlan Laboratories (Indianapolis, IN) and used in these experiments, which were approved by an institutional animal care and use committee. Mice were housed five per cage in the Wegner Hall Vivarium with access to food and water ad libitum. The facility is maintained on a 12-h light/dark cycle (lights on 0700, lights off 1900) under standard conditions (22±1 °C room temperature, 33% humidity). Mice were kept in the holding room for at least 4 days following arrival in the facility. Each animal was used only once and then discarded.

2.2.2. Apparatus

The light/dark exploration box (450 mm length×270 mm width×270 mm height) was constructed of acrylic (Abbott

Plastics, Rockford, IL). An acrylic divider with a 75×75 mm opening at floor level divided the box into a light compartment (three-fifths of the total length) and a dark compartment (two-fifths of the total length). The walls of the light and dark compartments were made of black and white acrylic, respectively. Behavioural observations and assessments were generally performed between 1000 and 1400 h. During all experiments, the light compartment was illuminated by two 40-W white light fluorescent tubes mounted 180 mm directly overhead. In this paradigm, animals were individually placed in the center of the light compartment of the box, facing away from the divider, and 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.

2.2.3. Drugs

The following drugs were used in this experiment: N_2O and O_2 (both medical grade, A&L Welding, Spokane, WA), LY-278,584 maleate (1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-1*H*-indazole-3-carboxamide maleate, Research Biochemicals International, Inc., Natick, MA), and cinanserin hydrochloride and fluoxetine hydrochloride (Tocris Cookson, Inc., Ellisville, MO).

N_2O and O_2 were delivered into the light/dark box via a length of polyethylene tubing using a portable N_2O/O_2 dental sedation system (Porter, Hatfield, PA). The gases were delivered in a 7:3 proportion in a total inflow rate of 10 l/min (i.e., 7.0 l/min N_2O+3.0 l/min O_2=70% N_2O in O_2). A POET II® anaesthetic monitoring system was used to ascertain that the desired atmosphere of N_2O and O_2 were attained within the filling time.

LY-278,584, cinanserin and fluoxetine were prepared in 0.9% physiological saline and administered intraperitoneally at doses of 1.0, 2.5 and 10 mg/kg, respectively, in a volume of 0.1 ml/10 g body weight. Pretreatment drug doses were determined in preliminary experiments. N_2O and O_2 control groups received the same volume of vehicle (0.9% physiological saline). LY-278,584, cinanserin and fluoxetine pretreatment times were all 30 min prior to testing.

2.2.4. Statistical analysis of data

The mean behavioral endpoints for N_2O in groups of mice in the absence and presence of LY-278,584 and fluoxetine were analyzed by two-way analysis of variance (ANOVA) and post hoc Bonferroni test. Due to a non-normal distribution, the influence of cinanserin pretreatment on N_2O effects was analyzed by Kruskal–Wallis non-parametric ANOVA.

3. Results

3.1. Experiment A

The neurochemical results show that a 15-min exposure to 70% N_2O caused differential changes in 5-HT and 5-HIAA concentrations in different brain regions (Fig. 1). In the hypothalamus, there was a 23% increase in levels of 5-HIAA but not 5-HT, increasing the ratio of 5-HIAA/5-HT from 1.53 to 1.83 ($p<0.05$). In the frontal cortex, there was a 26% increase in amounts of 5-HT but not 5-HIAA, decreasing the ratio of 5-HIAA/5-HT from 0.92 to 0.83 ($p>0.05$). In the hippocampus, there were inappreciable effects of N_2O on 5-HT and 5-HIAA

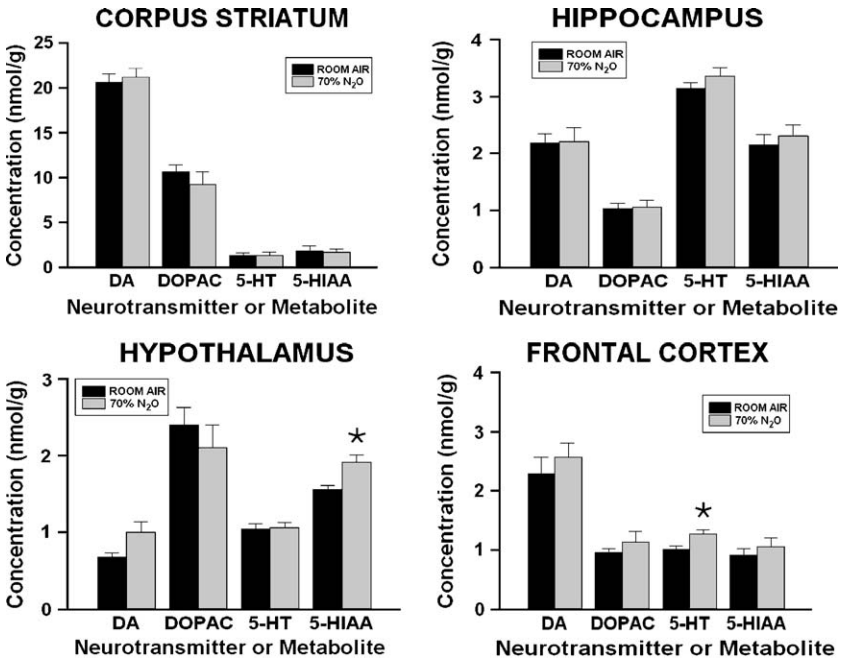

Fig. 1. Comparison of DA, DOPAC, 5HT and 5HIAAA concentrations in the hypothalamus (upper left panel), frontal cortex (upper right panel), hippocampus (lower left panel) and frontal cortex (lower right panel) of compressed air (solid bars)- and N_2O (shaded bars)-exposed rats. Each bar represents the mean and vertical lines the S.E.M. of 10 rats per group. Significance of difference: *$p<0.05$ compared to room air exposure.

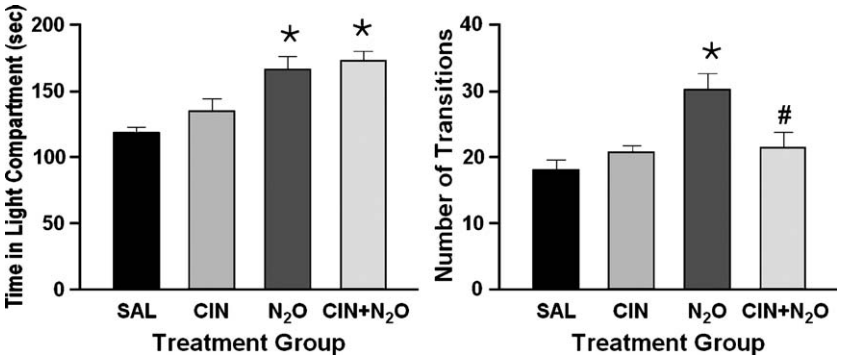

Fig. 2. The influence of pretreatment with cinanserin (CIN) on N_2O-induced anxiolytic-like behavior in the light/dark exploration test: time in light compartment (left) and number of transitions (right). Each bar represents the mean and vertical lines the S.E.M. of 10–20 mice per group. Significance of difference: $^{*}p<0.05$ compared to saline (SAL) control group; $^{\#}p<0.05$ compared to N_2O group.

concentrations, and the ratio of 5-HIAA/5-HT changed from 0.68 to 0.69 ($p>0.05$). In the corpus striatum, there were also inconsequential changes in levels of 5-HT and 5-HIAA, and the ratio of 5-HIAA/5-HT was decreased slightly from 1.33 to 1.28 ($p>0.05$).

The HPLC results also showed that exposure to 70% N_2O had no significant effect on either DA or DOPAC in the hypothalamus, frontal cortex, corpus striatum or hippocampus.

3.2. Experiment B

In the behavioural experiments, exposure to N_2O caused significant increases in both time spent in the light compartment ($p<0.05$) and the number of transitions ($p<0.05$). Pretreatment with cinanserin had no influence on the N_2O-induced increase in time ($p>0.05$) but did significantly attenuate the N_2O-induced increase in transitions ($p<0.05$) (Fig. 2).

Pretreatment with LY-278,584 did not alter the effects of N_2O on increasing the time spent in the light compartment and the number of transitions in the light/dark exploration test (Fig. 3). A two-way ANOVA (factor N_2O × factor LY) was used to analyze the data [time spent in the light compartment: $F_{N2O}(1,62)=9.98$, $p<0.005$; $F_{LY}(1,62)=0.09$, $p>0.05$; $F_{N2O\times LY}(1,62)=0.28$, $p>0.05$; number of transitions: $F_{N2O}(1,62)=28.78$, $p<0.0001$; $F_{LY}(1,62)=0.51$, $p>0.05$; $F_{N2O\times LY}(1,62)=0.27$, $p>0.05$].

Pretreatment with FLX had no influence on the effects of N_2O on increasing the time spent in the light compartment and the number of transitions in the light/dark exploration test (Fig. 4). A two-way ANOVA (factor N_2O × factor FLX) was used to analyze the data [time spent in the light compartment: $F_{N2O}(1,66)=8.71$, $p<0.01$; $F_{FLX}(1,66)=0.29$, $p>0.05$; $F_{N2O\times FLX}(1,66)=1.33$, $p>0.05$; number of transitions: $F_{N2O}(1,66)=28.82$, $p<0.0001$; $F_{FLX}(1,66)=0.54$, $p>0.05$; $F_{N2O\times FLX}(1,66)=0.03$, $p>0.05$].

4. Discussion

Our laboratory was the first to report striking similarities in behavioural response and pharmacological interactions between N_2O and benzodiazepines in animal models of experimental anxiety (Quock et al., 1987, 1992, 1993; Czech and Quock, 1993; Emmanouil et al., 1994; Li and Quock, 2001). These findings support the idea that N_2O can mimick in some way the action of benzodiazepines at binding sites that are coupled to $GABA_A$ receptors forming the chloride ion channel (Zorumski and Isenberg, 1991).

The above conclusion not withstanding, it has also long been recognized that 5-HT plays a crucial role in the regulation of anxiety (Gorman et al., 2002). It is plausible that N_2O may exert a direct or indirect action upon 5-HT neurotransmission in producing its anxiolytic effect. Previously, it was demonstrated

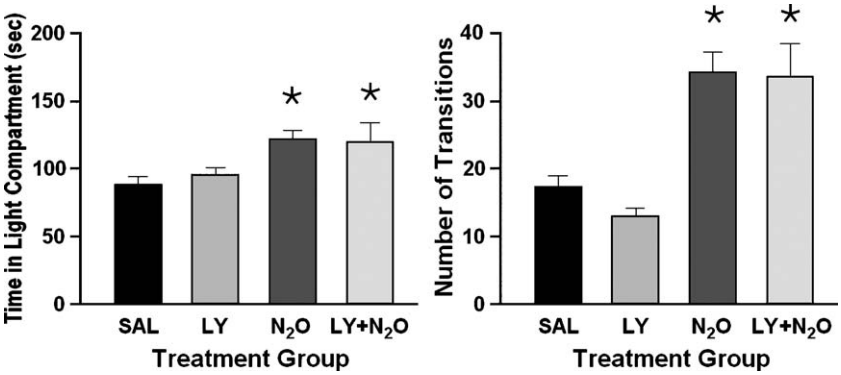

Fig. 3. The influence of pretreatment with LY-278,584 (LY) on N_2O-induced anxiolytic-like behavior in the light/dark exploration test: time in light compartment (left) and number of transitions (right). Each bar represents the mean and vertical lines the S.E.M. of 10–20 mice per group. Significance of difference: $^{*}p<0.05$ compared to saline (SAL) control group.

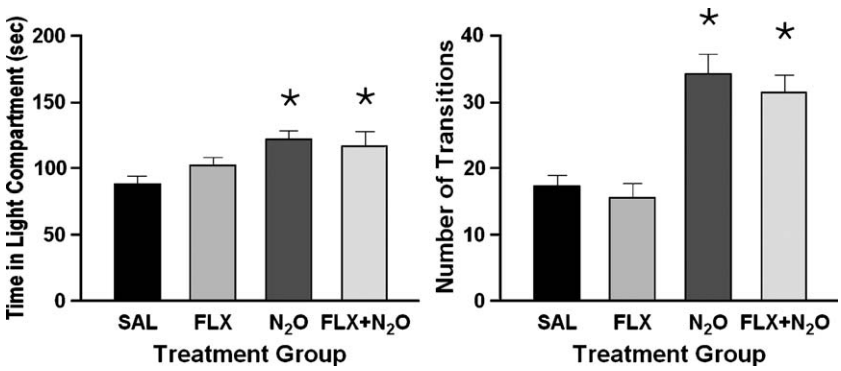

Fig. 4. The influence of pretreatment with fluoxetine (FLX) on N_2O-induced anxiolytic-like behavior in the light/dark exploration test: time in light compartment (left) and number of transitions (right). Each bar represents the mean and vertical lines the S.E.M. of 10–20 mice per group. Significance of difference: $^*p < 0.05$ compared to saline (SAL) control group.

that N_2O-induced antinociception was enhanced by blockade of 5-HT_{1C} and/or 5-HT_2 receptors and antagonized by blockade of 5-HT_3 receptors (Mueller and Quock, 1992).

4.1. Experiment A

While there appear to be many investigations of the influence of N_2O on regional brain levels of the catecholamines norepinephrine and dopamine (Abdul-Kareem et al., 1991; Murakawa et al., 1994a; Kofke et al., 1995; Karuri et al., 1998; Turle et al., 1998), there have been few studies of the effect of N_2O on brain 5-HT.

A 15-min exposure to 70% N_2O induced a significant increase in the 5-HIAA/5-HT ratio in hypothalamus which reflects an increased serotonergic activity. In particular, this increase was due to increased 5-HIAA levels. An increase in 5-HIAA and/or 5-HIAA/5-HT ratio suggests increased 5-HT activity, because the increase in metabolite tissue levels ex vivo and/or the increase in metabolite/neurotransmitter ratio reflect increased neurotransmitter activity in vivo (Shannon et al., 1986; Thorre et al., 1997). On the other hand, a 15-min exposure to 70% N_2O induced a significant decrease of 5-HIAA/5-HT ratio in the cerebral cortex, which was attributed to increased 5-HT tissue content without any effect on the 5-HIAA content. The differential effects of N_2O indicate that serotonergic neurons are differentially affected by exposure to N_2O in distinct brain areas. It has been suggested that central 5-HT plays a key role in the etiology of anxiety (Handley, 1995; Pellow and File, 1986). In the neurochemical studies, it was reported that changes in the content of 5-HT and its turnover rate in the brain are associated with anxiogenesis (Iversen, 1984; Chaouloff et al., 1998).

Serotonergic synapses are most densely concentrated in limbic regions including the amygdala and bed nucleus of the stria terminalis (BNST), thought to play a seminal role in anxiety (Davis, 1998), as well as the ventral striatum and hypothalamus. There are much less dense but not insignificant concentrations in cortical regions as measured by serotonin transporter binding in humans and non-human primates (Smith et al., 1999).

The limbic system contains dense serotonergic projections. Studies of fear conditioning have shown that 5-HT inhibits cortical and thalamic excitatory drive into lateral nucleus of the amygdala that is critical in fear conditioning (LeDoux, 1998). These authors conclude that increased 5-HT may decrease the sensitivity of the amygdala to activating (particularly aversive) stimuli (Stutzmann et al., 1998). Others have shown in an in vitro amygdala slice preparation that 5-HT mediates this inhibition primarily via activation of 5-HT_2 receptors on inhibitory interneurons within basolateral amygdala (Rainnie, 1999).

Serotonin has many electrophysiologic functions in its target areas (Aghajanian, 1995), and the combination of excitatory, inhibitory and modulatory roles lead to a complex electrophysiology that can be summed up as potentiating gating. Cortical modulation by 5-HT is mediated by multiple excitatory and inhibitory receptors. There seems to be a complicated dose–response relationship in cortical circuitry modulated by 5-HT. In some cases, moderate levels of 5-HT are needed to potentiate glutamatergic action, but higher 5-HT levels lead to inhibition (Aghajanian, 1995). Although its role in cortical processing is complex (Buhot, 1997), its importance is clearly implicated by the serotonergic modulation by hallucinogens (e.g., LSD and mescaline) and atypical antipsychotics (e.g., clozapine, quetiapine).

The rise in 5-HT content in cerebral cortex, seen after exposure to 70% N_2O, suggests that the predominant effect of N_2O under our experimental conditions was to modify the neuronal activity possibly by inhibiting its own release at the level of presynaptic 5-HTI_A autoreceptors (Pineyro and Blier, 1999). However, the cortical effects of N_2O did not seem to be generalized to hypothalamus, thus showing a regional specificity of N_2O effects on serotonergic neurons. Hypothalamic modulation by 5-HT may be involved in appetite control, modulation of the HPA stress response and sexual behavior (Rainnie, 1999). More detailed neurochemical studies are needed to elucidate the mechanism by which N_2O exerts its anxiolytic activity through the central serotonergic neuron.

No significant differences in the levels of dopamine were reported. A previous report indicated alterations in steady-state levels of DA but not of NE in the brains of rats exposed to 75% N_2O for 4 h. However, a 2 h exposure did not alter either DA or NE levels (Karuri et al., 1998). Variations in methodology, including the method of sacrifice (microwave killing vs.

decapitation), may have contributed to these differences; it is, therefore, difficult to compare the results of our study with that report.

4.2. Experiment B

Following the identification of 5-HT receptor subunits (Glennon et al., 1995), there has been a major research endeavour to determine the roles of these in mediating anxiety and anxiolytic drug effect. We decided to directly test for 5-HT involvement by assessing the interaction between N_2O and 5-HT receptor blocking and reuptake inhibitor drugs.

There are earlier reports that 5-HT_2 receptors in the periaqueductal gray matter subserve negative reinforcement and that 5-HT_2 antagonists can suppress this central aversive system (Jenck et al., 1989). 5-HT_2 antagonists can relieve anxiety (Deakin, 1988; Raheja et al., 1995). The 5-HT_{1C}/5-HT_2 antagonist mianserin was reported to be effective in significantly reducing the anxiogenic-like behaviour observed in mice following withdrawal from ethanol (Lal et al., 1993). However, the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) was also reported to elicit a strong anxiolytic effect comparable to that of benzodiazepines (Nic Dhonnchadha et al., 2003; Ripoll et al., 2005).

Consistent with earlier results (Emmanouil et al., 1994), exposure to 70% N_2O significantly increased the amount of time spent by mice in the light compartment of the light/dark box and the number of transitions made by mice between the light and dark compartments of the box. The increase in time spent in the light compartment reflects an anxiolytic-like behavioural response to N_2O. The increase in transitions is related to a locomotor stimulatory effect of N_2O in response to indirect activation of opioid receptors (Hynes and Berkowitz, 1979). There is an apparent involvement of brain dopamine in the locomotor response to N_2O (Dorris and Truong, 1993; Hynes and Berkowitz, 1983).

In the present study, selective blockade of 5-HT_2 receptors with cinanserin had no effect on the N_2O-induced increase in time spent in the light compartment but did significantly attenuate the N_2O-induced increase in number of intercompartmental transitions. As the time spent in the light compartment is a more critical index of anxiolytic-like activity, these findings would dissociate 5-HT_2 receptors in the behavioural response to N_2O.

Other research has implicated 5-HT_3 receptors in anxiety, although this remains controversial. N_2O has been reported to modulate 5-HT_3 receptor activity (Yamakura and Harris, 2000; Suzuki et al., 2002), and 5-HT_3 antagonists have been reported to produce anxiolytic-like behavioural responses in a wide range of animal tests (Tyers et al., 1987; Jones et al., 1988; Costall and Naylor, 1992, 2004; Costall et al., 1993). However, other laboratories were unable to replicate these findings (Johnston and File, 1988; File and Johnston, 1989). A number of clinical studies also reported that 5-HT_3 antagonists are generally ineffective in reducing anxiety (Schweizer and Rickels, 1991; Wilde and Markham, 1996; Olivier et al., 2000).

In our experiments, the blockade of 5-HT_3 receptors with LY-278,584 (Fludzinski et al., 1987) failed to influence the anxiolytic-like behavioral response of N_2O in the light/dark exploration test, suggesting that 5-HT_3 receptors are perhaps not involved in the behavioural response to N_2O. This is consistent with an earlier report that blockade of 5-HT_3 receptors failed to reduce PAG stimulation-induced aversive behaviour (Jenck et al., 1989).

LY-278,584 also failed to reduce the number of intercompartmental transitions. This is in agreement with earlier reports that 5-HT_3 receptors do not play a role in regulating spontaneous locomotor activity (Kelley et al., 2003; Hodge et al., 2004) and 5-HT_3 antagonists have no appreciable effect on locomotor activity (Jones et al., 1988).

An additional experiment was conducted using the selective 5-HT reuptake inhibitor fluoxetine. If N_2O promotes the release of 5-HT to activate 5-HT_2 receptors, inhibiting the reuptake of 5-HT would be expected to enhance the anxiolytic-like response to N_2O. However, the behavioural effect of N_2O was unaffected by fluoxetine in doses previously shown to inhibit 5-HT reuptake. It is not known whether inhibition of 5-HT in specific brain regions rather than globally might influence the behavioural response to N_2O.

Brain 5-HT is widely distributed, is seemingly involved in numerous physiological regulatory mechanisms and may potentially participate in counteracting mechanisms. Part of the diversity of functions of 5-HT is likely due to the fact that effects of 5-HT are mediated by as many as 13 distinct seven-transmembrane-spanning, G-protein-coupled receptors (GPCRs) and at least one ligand-gated ion channel (Hoyer et al., 2002). The multiplicity of 5-HT receptors and their unique distribution in the limbic system suggest that more brain region- or pathway-specific analysis of 5-HT function may be required for a more complete answer to the question of whether 5-HT mechanisms are involved in the anxiolytic effect of N_2O.

In summary, acute exposure of rats to 70% N_2O significantly elevated 5-HT turnover in the hypothalamus, decreased turnover in the frontal cortex but no changes in either hippocampus or corpus striatum. While cognizant of potential species differences in drug effect, we found that the N_2O-induced increase in time spent in the light compartment was unaltered by 5-HT_2 or 5-HT_3 receptor blockade or inhibition of 5-HT reuptake and the N_2O-induced in transitions was sensitive to antagonism by 5-HT_2 receptor blockade but not 5-HT_3 receptor blockade or inhibition of 5-HT reuptake.

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