Effects of nitrous oxide on dopamine release in the rat nucleus accumbens and expectation of reward

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Abstract

Recently we have shown that nitrous oxide (N_2O) was able to block the expression of morphine-induced conditioned place preference (CPP) in mice. Because dopamine (DA) has also been associated with the positive place conditioning we hypothesis that exposure to N_2O would be significantly associated with a modification of extracellular level of DA. Unbiased place conditioning method was used for mice and rats. Levels of DA, in the nucleus accumbens (Nac), in awake and freely moving rats during positive place conditioning after morphine chronic treatment has been measured by microdialysis. Expression of morphine-induced CPP was totally abolished in mice and rats exposed to N_2O. Results of animals placed in the morphine-paired compartment showed a 75% increase in the extracellular levels of DA, which was blocked by exposure of animals to N_2O. In conclusion we showed the capacity of N_2O to block the expression of morphine-induced CPP in mice and in rats. Then we demonstrated an increase of DA extracellular level in the Nac when animals were placed in the morphine-paired compartment and these increase of DA level was blocked by N_2O.

MESH Keywords Analgesics ; Opioid ; pharmacology ; Animals ; Conditioning (Psychology) ; drug effects ; physiology ; Dopamine ; metabolism ; Drug Interactions ; Male ; Mice ; Mice ; Inbred Strains ; Microdialysis ; Morphine ; pharmacology ; Morphine Dependence ; drug therapy ; metabolism ; Nitrous Oxide ; metabolism ; pharmacology ; Nucleus Accumbens ; drug effects ; metabolism ; Rats ; Rats ; Sprague-Dawley ; Reward ; Spatial Behavior ; drug effects

Introduction

Nitrous oxide (N_2O) is a pharmacologically active gas, with an agonist action on the opioid system (Gillman et al. 1998) and antagonist properties at the NMDA receptors level (Jevtovic-Todorovic et al. 1998). Only few animal studies have been performed to evaluate the mechanisms of action and potential benefits of N_2O on treatment of drugs of abuse. Recently we have shown that N_2O was able to impair the acquisition of morphine-induced conditioned place preference (CPP) and to block the expression of cocaine- and morphine-induced CPP. The effects of the gas were long lasting and persisted at least 4 days following the exposure (Benturquia et al. 2007). Moreover no behavioral modifications in tests usually used to investigate emotional states as compared with control mice were observed in animals exposed to N_2O, ruling out an effect of this gas on attention, anxiety, depression, locomotion and anhedonia (Benturquia et al. 2007). A previous experiment reported that N_2O was able to block amphetamine-induced dopamine release (David et al. 2006), however no experiment investigated the consequences of N_2O exposure in drug free animals exposed to environmental cue previously associated with administration of drugs of abuse.

It is well established that craving is a motivational state associated with a variety of addictive behaviours and a key contributor to relapse. Several neuroimaging studies have shown that cue-elicited craving is linked with endogenous dopamine release in the striatum (Volkow et al. 2008; Wong et al. 2006). Moreover previous studies in monkey showed that dopamine (DA) neurons respond to stimulant environment (Schultz 1986; Ljungberg et al. 1992) and rat studies have shown that with repeated drug exposure, neural stimuli paired with the drug (conditioned stimuli) start to increase DA by themselves (Phillips et al., 2003). Such an effect on DA is believed to underlie the activation of the brain reward system and may play an important role in the reward and/or incentive motivation produced by drugs of abuse (Kelly and Berridge, 2002; Spanagel and Weiss, 1999). However, to our knowledge, the possible changes of extracellular level of DA determined by microdialysis in awake and freely moving rats during positive place conditioning (which may reflect the rewarding properties) after morphine chronic treatment has not been reported. This was performed in this study, in which we investigated also the consequences of N_2O exposure on this increase of extracellular level of DA induced by an environmental cue.

Experimental Procedures

Male CD-1 mice (Iffa Credo, France) weighting 22–24 g at the beginning and male Sprague-Dawley Rats (Janvier, France) weighting 225–250 g at the time of surgery were used. They were housed at temperature (22 ± 1°C) and humidity controlled (50 ± 5%) environment and had access to food and water ad libitum. The animals were treated in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (1985) and in agreement with the local ethical committee. Morphine (Francopia, France) dissolved in saline (0.9% NaCl) was

administered s.c. at 5 (rats) or 10 (mice) mg/kg. N_2O was delivered via specific tubing from bottles containing premixed N_2O/O_2 50/50% (Air Liquide, France). Fresh gases were continuously fed into the hermetic chamber (56 cm long, 56 cm wide, 56 cm high) through an inlet port (4 l/min) and purged by a vacuum set during the time of the test. Oxygen and N_2O concentrations were continuously monitored to confirm premixed gas concentrations (Colin BP 508, France).

Behavioural studies were performed both in mice and rats. Place preference paradigm was based on that previously described (Benturquia et al. 2007). The place preference apparatus consisted of two conditioning compartments separated by a neutral compartment. The movement and location of animals were recorded by computerized monitoring software (Videotrack, Viewpoint, France). Briefly, the protocol consisted of three phases: 1) Preconditioning phase (one day): drug-naive animals had free access to both compartments for 20 min, and the time spent in each compartment was recorded. 2) Conditioning phase: This phase consisted of 6 days in which each conditioning chamber was closed. On the first conditioning day, animals were treated with morphine and placed in one of the conditioning environments individually for 20 min. The following conditioning day, animals were given saline in the opposite compartment and this sequence alternated during the next 4 days. Control animals received saline every day and were submitted to an alterned sequence between the two compartments. 3) Postconditioning phase (one day): This phase took place 24 h after the final conditioning session and was carried out exactly as the preconditioning phase. Then, animals were exposed to N_2O before or during the 20 min test sessions. Results are expressed in scores (mean±S.E.M.) calculated as the difference between postconditioning and preconditioning time spent in the drug-paired compartment.

In the neurochemical study in rats, the experiment was conducted as described above.

For surgery and brain dialysis procedure, rats were anesthetized by an intraperitoneal injection of a mixture of ketamine/xylazine (80/10 mg/kg) and placed in a stereotaxic apparatus (Unimécanique). The skull was exposed and a guide canula (CMA 12, Phymep, France) was stereotaxically implanted in the Nac. The coordinates, taken from the atlas of Paxinos and Watson (1986) were +1.2 mm anterior to the interaural, ±0.8 mm lateral to the midline, and −6.2 mm under the skull surface. Animals were used for experiments after a recovery period of 5 –7 days. Three hours before the start of microdialysis, the rats were gently restrained, the stylus was removed from the guide cannula and the probe (CMA 12, Phymep, France) was implanted and perfused at 2.0 µl/min for routine sample analysis. The perfusate consisted of artificial cerebrospinal fluid containing (in mM) 140 NaCl, 4 KCl, 1 $MgCl_2$, 1.9 NaH_2 PO_4, 1.9 Na2HPO4, 1.2 $CaCl_2$ (pH 7.4). Two hours after the beginning of the perfusion, two samples were collected to determine the basal efflux of DA, before introducing the animals in the drug-paired compartment and exposed them to N_2O, during the collect of two new samples. Samples were collected every 30 min in tubes containing 5µl $HClO_4$ 0.4 M in order to prevent DA oxidation and maintained in dry ice and conserved at −80°C until the quantification. HPLC apparatus coupled to electrochemical detector (Coulochem II, ESA Inc., US) as described previously (Malagie et al. 2001) was used to quantify DA levels. Data were analyzed by a one-way (behavioral studies) or two-way (microdialysis) ANOVA, followed by a Newman-Keuls test (Statview, SAS institute Inc, USA). Significance was accepted with $P< 0.05$.

Results

As illustrated in Figure 1a mice which received morphine at 10 mg/kg (s.c.) developed a significant preference for the drug-paired compartment. Expression of morphine-induced CPP was totally abolished in animals exposed to N_2O during the test (morphine + N_2O in) as well as before the test (morphine + N_2O), $F(3,55) = 4.143$, $P = 0.0102$.

These results confirmed the capacity of N_2O to block the expression of morphine-induced CPP previously observed in mice, while extending it to the fact that this result can be obtained when animals were exposed to N_2O for 20 min before the test.

As illustrated in Figure 1b rats which received morphine at 5 mg/kg (s.c.) developed a significant preference for the drug-paired compartment. Expression of morphine-induced CPP was totally abolished by exposure to N_2O before the test, $F(3,43) = 2.895$, $P = 0.0460$.

As shown in Figure 2, there was no significant change in the extracellular levels of DA in control rats during the experiments. The basal level of DA in the Nac of rats conditioned with morphine was not significantly different from that the control rats. When the animals were placed in the morphine-paired compartment, the analysis of the microdialysis samples showed a large increase (75%) in the extracellular levels of DA ($F(1,49) = 4.401$, $P = 0.041$). Moreover, this increase observed in morphine treated rats was blocked by exposure of animals to N_2O.

Discussion

All these results confirmed those previously obtained in mice (Benturquia et al., 2007) and extended them to rats and were also consistent with results obtained with amphetamine (David et al. 2006). N_2O was able to block the expression of morphine-induced CPP when animals were exposed to the gas during the test, and more interestingly before the test. This suggests that N_2O may block the motivational value of morphine.

It is well established that cue-elicited craving is linked with endogenous DA release (Volkow et al. 2008; Wong et al. 2006). In the present study we observed a transient increase in DA efflux when the animals were placed in the drug-paired compartment during the microdialysis experiment. This increase may reflect an anticipation of the rewarding effect, associated with the memory of the reinforcing effects obtained with morphine in this compartment during the conditioning phase. It is interesting to note that this increase also occurs in response to the drug itself (Di Chiara and Imperato, 1985; 1988). This regulation of extracellular DA efflux may be important in the expression of morphine-induced positive place conditioning, because opioid reward measured by the CPP paradigm depends on midbrain DA related mechanisms (Bozarth, 1987; Bals-Kubik et al., 1993). This hypothesis is in agreement with the observed lack of the increase of DA extracellular concentration following N_2O exposure in morphine-treated rats, parallel to the blockade of the CPP expression. However, this observation could directly be dependent on the NMDA antagonist properties of the gas (Jevtovic-Todorovic et al. 1998; Balon et al. 2003), as several recent papers have suggested that cue-induced reinstatement of drug seeking is sensitive to NMDA receptor antagonism (Backstrom and Hyytia, 2007). In agreement with this hypothesis it has been shown that N_2O and memantine, which both possess low-affinity antagonistic properties at the NMDA receptor (David et al. 2006) were able to block DA release induced by drugs of abuse. However surprisingly this was not the case with the prototypical NMDA receptor antagonist MK-801 (David et al., 2006). Due to differences in affinity for NMDA receptor subtypes, it was suggested that nitrous oxide, like memantine, might act through NMDA receptors that express the NR2D subunit (David et al., 2006), which are mainly located on interneurons in the striatum-accumbens complex (Standaert et al., 1996).

In summary, we showed the capacity of N_2O to block the expression of morphine-induced CPP in mice and in rats. Then we demonstrated an increase of DA extracellular level in the Nac when animals were placed in the morphine-paired compartment and these increase of DA level was blocked by N_2O. It is also interesting to observe that N_2O by itself did not modify DA efflux in the Nac in our experimental conditions. This is not in agreement with the results reported by Sakamoto et al. (2006) after 60% N_2O exposure. This higher percentage may explain the discrepancies between both studies. The absence of DA increase in the present study might be related with our previous results, showing the lack of rewarding effects of N_2O (Benturquia et al. 2007).

In conclusion, we confirmed the previous behavioral results obtained in rodents and clinical reports, which proposed that N_2O could be effective in the treatment of drug withdrawal syndrome (Gillman and Lichtigfeld 1998; 2000). However, these findings have not been confirmed by a study comparing the effects of N2O versus placebo (Alho et al., 2003). Nevertheless, the present data suggest that N_2O might have possible utility in treating opioid addiction. This is supported not only by the inhibition of CPP induced by drug associated cues but also by the blockade of DA efflux in the Nac after N_2O exposure. However, there is ample evidence that recreational N_2O inhalation is an increasing phenomenon. Nevertheless, there exists across a number of studies a great variability in the degree to which the gas is perceived by volunteers as having pleasant effect or is liked (see Cho et al. 1997). The other treatments already available (methadone, buprenorphine) may also have abuse potentials (Boothby and Doering, 2007), that may be highly reduced under control medical deliverance.

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Footnotes:

Section Editors: Behavioural Neuroscience

Abbreviations

Nac: nucleus accumbens
DA: dopamine
N_2O: nitrous oxide
CPP: conditioned place preference

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Figure 1

a- Influence of N_2O upon the expression of conditioned place preference induced by morphine (10 mg/kg, s.c.). Mice were exposed to N_2O during the test (morphine + N_2O in) or for 20 minutes before the test (morphine + N_2O). b- Influence of N_2O upon the expression of conditioned place preference induced by morphine (5 mg/kg, s.c.). Rats were exposed to N_2O for 20 minutes before the test. Each column represents the mean score ± S.E.M. (n = 11–13 per group). * P< 0.05 compared with the three other groups, (Newman-Keuls test).

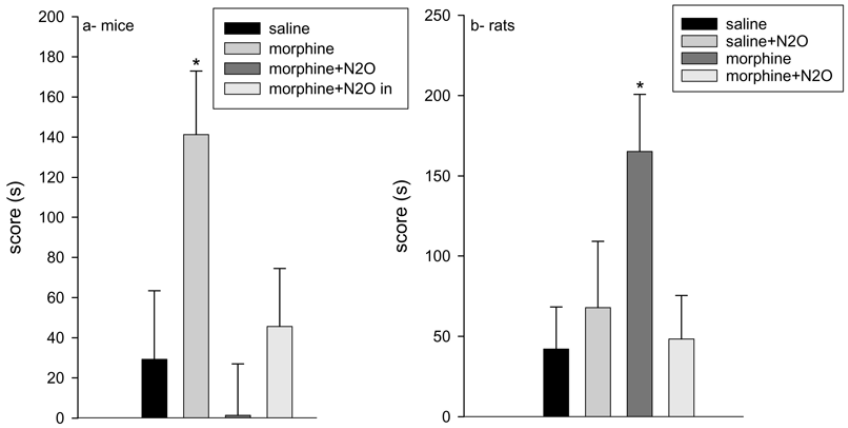

Figure 2

determination of the extracellular levels of dopamine in the nucleus accumbens of rats chronically treated with saline or morphine to induce a positive place conditioning. The dialysis samples were collected every 30 minutes. The basal concentrations of DA, in the NAc microdialysates were 20.78 ± 1.02, 19.41 ± 1.83, 22.34 ± 1.65 and 20.55 ± 1.23 pg/sample for the saline, saline + N_2O, morphine, morphine + N_2O groups, respectively. Results are expressed as percent (mean ± S.E.M.) of baseline value obtained before introduction of the animals in the drug-paired compartment (n = 5–7). * P< 0.05 versus the saline group, (ANOVA test).