Halothane potentiates the effect of methamphetamine and nomifensine on extracellular dopamine levels in rat striatum: a microdialysis study

Y. U. Adachi¹², K. Watanabe¹, T. Satoh¹ and E. S. Vizi²*

¹Department of Anesthesiology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. ²Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, PO Box 67, H-1450 Budapest, Hungary

*Corresponding author

Brain microdialysis was used to study the in vivo release and metabolism of dopamine (DA) in the rat striatum during halothane anaesthesia. Concentrations were measured in microdialysates collected every 20 min and applied directly to an on-line high-performance liquid chromatograph. Halothane was administered at concentrations of 0.5, 1.0, 1.5 and 2.0%. In another series of experiments, rats were treated intraperitoneally or locally with methamphetamine, a drug of abuse, or with nomifensine, a dopamine uptake blocker and antidepressant, in combination with 0.5 or 1.5% halothane. Halothane anaesthesia did not affect the dialysate (extracellular) concentration of DA at 2.0%. By contrast, the concentrations of DA metabolites [3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] increased during inhaled halothane anaesthesia in a dose-dependent manner and recovered after anaesthesia. Halothane potentiated the ability of methamphetamine to increase the extracellular concentration of DA when administered systemically, whereas only a small increase in DA accumulation was seen when methamphetamine was administered locally via the perfusate. Similarly, the increase in extracellular DA was accentuated by systemic nomifensine during halothane anaesthesia, but no obvious enhancement was observed when it was applied locally. It has been shown that the neurotoxic effect of methamphetamine is mediated by the suboxidation of DA released from the cytoplasm into the extracellular space and transformed into highly reactive free radicals. On the basis of our results, it is suggested that care should be exercised when halothane anaesthesia is used in patients abusing phenylethylamines (amphetamines) or being treated with DA uptake blockers (nomifensine).

Br J Anaesth 2001; 86: 837–45

Keywords: anaesthetics volatile, halothane; brain, dopamine release; brain, striatum; analeptics, methamphetamine; ataractics, nomifensine

Accepted for publication: December 18, 2000

It has been shown¹ that the volatile anaesthetic isoflurane potentiates the ability of nomifensine to increase the extracellular dopamine (DA) concentration in the brain, probably by releasing DA from axon terminals. In addition, halothane has been reported to either increase²⁻⁴ or not affect⁵ the extracellular concentration of DA in the brain during anaesthesia. Miyano et al.⁴ investigated the dose-dependency of the effect of halothane on the extracellular DA concentration, and concluded that the hypoxia induced by halothane may be responsible for the increased extracellular concentration of DA.⁴ In addition, Fink-Jensen et al.⁵ reported that halothane enhanced the ability of both nomifensine and vanoxerine (selective inhibitors of DA reuptake) to increase DA concentration. Moreover, Opacka-Juffry et al.¹ concluded that volatile anaesthetics modulate extracellular DA concentrations by acting on the dopaminergic axon terminals of the nigrostriatal pathway. However, until now no attempt has been made to reinvestigate the effect of halothane anaesthesia on extracellular concentrations of DA and its metabolites. Specifically, no previous studies have included comparison of systemically and locally administered methamphetamine and a DA reuptake inhibitor (nomifensine). These data may yield information regarding the site and mode of action of...
halothane. It is known that methamphetamine, a widely abused psychostimulant, releases DA from the cytoplasm of the axon terminals in the striatum, and nomifensine, a drug used world-wide for depression, prevents DA from being taken up. Therefore, the effect of halothane anaesthesia on the extracellular concentrations of DA and its metabolites [3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] was studied in the presence of methamphetamine or nomifensine.

**Methods**

The experiments were approved by the Committee for Animal Research of our college. Male Sprague–Dawley rats (SLC Laboratory, Tokyo, Japan), weighing 280–320 g, were used. The animals were kept at 20–22°C on a 12 h light–12 h dark cycle (lights on from 07:00 to 19:00 h). All animals had free access to food and drinking water.

**Microdialysis**

Rats were anaesthetized with sevoflurane and ventilated through an orotracheal tube. Surgical procedures were performed after topical application of 1% lidocaine. Using a stereotaxic apparatus, a unilateral guide cannula was implanted just above the striatum (AP +0.6 mm, ML +3.0 mm, DV −3.8 mm) according to the atlas of Paxinos and Watson. The rats were allowed to recover for at least 2 days before experimentation. After each experiment, rats were killed by inhalation of excess isoflurane and intravenous injection of thiopental. Microdialysis probe placement was confirmed by histological examination.

Microdialysis probes were obtained from Eicom (Kyoto Japan) (outside diameter 0.22 mm, membrane length 3 mm, polycarbonate tubing, cut-off molecular weight 50 000). On the day of experimentation, at about 07:00 h, the rats were anaesthetized briefly with sevoflurane. The probe was inserted carefully into the striatum through a guide cannula and fixed to the cannula with a screw. This procedure was performed within 5 min of anaesthesia, after which the rat was placed immediately in a clear open Plexiglas box (15 litres in capacity, 27 cm in diameter and 26 cm in height) for recovery. Rats regained consciousness within approximately 3 min. After recovery, the probe was perfused continuously with Ringer solution (in mEq litre⁻¹: 147.0 Na⁺, 4.0 K⁺, 2.4 Ca²⁺, 155.8 Cl⁻) at a flow rate of 2 μl min⁻¹ using a microinfusion pump (ESP-64; Eicom) to determine baseline concentrations of DA and its metabolites. Samples were collected every 20 min and injected directly into an on-line analytical system with the autoinjector (EAS-20; Eicom) as described previously.

The concentrations of DA, DOPAC, 3-MT and HVA in each dialysate sample (40 μl/20 min) were determined by HPLC with an electrochemical detector (ECD-300; Eicom). These compounds were separated by reverse-phase ion-pair chromatography with a 5 μm C-18 column (MA5-ODS, 150×2.1 mm; Eicom) using isocratic mobile phase (0.1 M sodium acetate, 0.1 M citric acid, 1.4 mM sodium 1-octanesulphonate, 5 μM EDTA-Na₂ and methanol 13–14%, pH 3.9), delivered at a flow rate of 230 μl min⁻¹ by a high-pressure pump (EP-300; Eicom). A guard column (MA, 5×4 mm; Eicom) prevented deterioration and plugging of the analytical column. The compounds were quantified by electrochemical detection using a glassy carbon working electrode set at 650 mV against an Ag/AgCl reference electrode. The detection limit for each of the compounds was about 0.1 pg per sample.

DA and its metabolites reached stable baseline concentrations about 4.5 h after microdialysis probe implantation. Thus, at least six dialysate samples (each 40 μl collected in 20 min) were collected before starting an experiment. The mean value obtained from the last three samples was used as baseline release. The time when a pharmacological manipulation started will be hereafter termed ‘Fr. 1’ (fraction 1).

**Experiment 1**

Each rat was anaesthetized in the semi-closed Plexiglas box, into which 3% halothane was initially introduced at a rate of 3 litre min⁻¹ for about 5 min until a steady state was achieved; subsequently 0.5, 1.0, 1.5 or 2.0% halothane was applied at rate of 2 litre min⁻¹, using air (23% oxygen) as a carrier. The control group was administered air through the same line and at the same rate. After a steady state had been achieved, each concentration of halothane was administered for 1 h from Fr. 1 while striatal dialysates were collected. The anaesthetic gas was introduced to the centre of the box and escaped through several small holes used for connecting the rat to the analytical apparatus. Dialysate sampling was continued for 4 h after the conclusion of 1 h of anaesthesia. The rectal temperature of the rat was monitored and maintained at 37°C with an electrical heating pad, except in the control and 0.5% halothane groups, because the animals were consistently awake during inhalation. The concentrations of volatile gas and oxygen in the box were monitored with the infrared anaesthetic gas analyser (Capnomac Ultima; Datex, Helsinki, Finland) during each anaesthetic. Immediately after 1 h of anaesthesia, the gas in the box was exchanged for room air by forced ventilation.

**Experiment 2**

Methamphetamine and nomifensine maleate were tested systemically. Methamphetamine and nomifensine were dissolved in physiological saline (with warming for nomifensine), achieving final concentrations of 5 and 1 mg ml⁻¹ respectively. At Fr. 1, either methamphetamine 2 mg kg⁻¹ (0.6 ml/300 g) or nomifensine 10 mg kg⁻¹ (0.6 ml/300 g) was injected intraperitoneally, with or without subsequent inhalation of 0.5 or 1.5% halothane for 1 h.
Experiment 3

Local application of methamphetamine and nomifensine was tested. Methamphetamine and nomifensine were dissolved in Ringer solution to give a concentration of 5 \( \mu \)mol litre\(^{-1}\). At least six dialysate samples were obtained before starting an experiment and perfusate was changed from standard Ringer solution to solution containing methamphetamine or nomifensine. The time that pretreatment began will be hereafter termed ‘Fr. ±3’ (fraction ±3). Starting 80 min after Fr. 1, the rat received air or 0.5 or 1.5% halothane for 1 h.

Statistical analysis

Data were analysed by two-way analysis of variance with drugs as a between-subjects variable and time as a within-subject variable. For each drug with a significant \( (P<0.05) \) drug–time interaction, analysis included one-way analysis of variance followed by Newman–Keuls post hoc comparison (NCSS 2000, Kaysville, UT, USA).

Drugs

Halothane was obtained from Takeda Chemical Industries (Osaka, Japan), Methamphetamine from Dainippon Pharmaceutical (Osaka, Japan) and Nomifensine from ICN Pharmaceuticals (Costa Mesa, CA, USA).

Results

In control experiments, the predrug dialysate concentrations of DA, 3-MT, DOPAC and HVA were 2.25 (SEM 0.26), 3.25 (0.20), 1085 (138) and 393 (23) nM respectively. The concentration of DA increased gradually from 99.1 (1.4) to 108.4 (4.7)% throughout the experiments (from Fr. 0 to Fr. 15), while the concentrations of DA metabolites decreased from 96.1 (1.6) to 78.4 (7.0)% for 3-MT, from 96.7 (1.4) to 79.2 (7.6)% for DOPAC, and from 96.2 (2.0) to 69.9 (8.5)% for HVA. These trends, which have also been observed by others\(^9\) 11–14, are considered to be common in microdialysis experiments on DA dynamics. In the present study, the above changes in concentrations of DA and its metabolites were taken as the baseline with which drug-induced changes were compared.

Effect of halothane anaesthesia

As shown in Fig. 1, the induction of halothane anaesthesia did not change the concentration of DA except in the 2% halothane group. In contrast, all concentrations of halothane studied (0.5, 1.0, 1.5 and 2%) increased the concentrations...
Fig 2 Effects of systemically administered methamphetamine 2 mg kg⁻¹ on the concentrations of extracellular dopamine and its metabolites with or without halothane anaesthesia. Asterisks indicate significant changes ($P<0.05$; Newman–Keuls post hoc comparison) compared with the control value (without anaesthesia) at the corresponding time point.

Fig 3 Effects of locally administered methamphetamine (2 mg kg⁻¹) on the concentrations of extracellular dopamine and its metabolites with and without halothane anaesthesia. Asterisks indicate significant changes ($P<0.05$; Newman–Keuls post hoc comparison) compared with the control value (without anaesthesia) at the corresponding time point.
Fig 4 Effects of systemically administered nomifensine (10 mg kg\(^{-1}\)) on the concentrations of extracellular dopamine and its metabolites with and without halothane anaesthesia. Asterisks indicate significant changes ($P<0.05$; Newman–Keuls post hoc comparison) compared with the control value (without anaesthesia) at the corresponding time point.

Fig 5 Effects of locally administered nomifensine (10 mg kg\(^{-1}\)) on the levels of extracellular dopamine and its metabolites with and without halothane anaesthesia. Asterisks indicate significant changes ($P<0.05$; Newman–Keuls post hoc comparison) compared with the control value (without anaesthesia) at the corresponding time point.
of 3-MT and DOPAC in a dose-dependent manner. Halothane at concentrations less than 1.5% failed to increase HVA. During the 4-h recovery period from anaesthesia, the concentrations of DA metabolites recovered to baseline levels with a delay, but 2% halothane induced a sustained increase in DA concentration that did not recover until the end of the experiment.

**Effect of halothane on rats treated with methamphetamine or nomifensine**

Systemically administered methamphetamine 2 mg kg⁻¹ or nomifensine 10 mg kg⁻¹ markedly increased the concentrations of DA and 3-MT in striatal dialysates, the effect lasting 2.5 h (Figs 2 and 4). After intraperitoneal administration of methamphetamine, the dialysate concentrations of DOPAC and HVA were decreased by 90%. This is consistent with the known ability of methamphetamine to enter the nerve terminals and inhibit monoamine oxidase (MAO). When anaesthesia was with 0.5 or 1.5% halothane, i.e. concentrations that failed to increase the extracellular concentration of DA, the effect of nomifensine was strongly potentiated. Halothane anaesthesia increased DOPAC and HVA in nomifensine-pretreated rats but failed to influence DOPAC and HVA in methamphetamine-pretreated rats.

Locally administered methamphetamine increased the extracellular concentrations of DA and 3-MT and decreased those of DOPAC and HVA. Locally administered nomifensine caused a gradual increase in DA and 3-MT concentrations (Fig. 5). Halothane anaesthesia failed to affect this change, whereas 1.5% halothane increased DOPAC and HVA concentrations. Halothane 1.5% significantly increased the extracellular concentrations of DA metabolites, including 3-MT, DOPAC and HVA when methamphetamine or nomifensine was administered locally (Figs 3 and 5).

**Discussion**

The aim of the present study was to elucidate in detail the site and the mode of action of halothane anaesthesia on the concentrations of extracellular DA and its metabolites in the striatum of the rat using in vivo microdialysis techniques under different experimental conditions. To this end, methamphetamine, a widely abused drug, and nomifensine, a selective DA uptake blocker, were administered systematically or locally, thereby increasing the extracellular concentration of DA in two ways: methamphetamine, like amphetamine, releases DA from the cytoplasm into the extracellular space and nomifensine prevents the reuptake of DA already present in the extracellular space. DA concentrations in in vivo microdialysis experiments are known to be affected by many factors, including the period of time after probe implantation, the type of perfusion medium and animal stress. Although the effects of halothane anaesthesia on the concentrations of extracellular DA and its metabolites and on acetylcholine release and transmitter metabolism have been studied by several authors, no consensus about the mechanism of action of halothane anaesthesia has been reached. Osborne et al. and Stahle et al. have reported increases in the extracellular concentration of DA in the rat striatum during halothane anaesthesia, whereas Fink-Jensen et al. have found no change in DA. Miyano et al. have investigated the dose-dependency of halothane on the concentrations of DA and its metabolites, and showed that anaesthesia increases DA and DOPAC concentrations during inhalation of 2% halothane. In our experiments, the extracellular concentration of DA was unchanged during halothane anaesthesia except when it was used at an extremely high concentration (2%). The DA metabolites in the dialysate, including 3-MT, DOPAC and HVA, were increased by halothane inhalation. Our results and those of other investigators are consistent: halothane <2.0% does not change the concentration of extracellular DA; however, at concentrations of ≥2.0%, halothane increases extracellular DA.

Miyano et al. and Fink-Jensen et al. have suggested that halothane may cause respiratory depression and subsequent brain hypoxia, thereby increasing extracellular DA. In the central nervous system, severe hypoxia followed by ischaemia increases the extracellular concentrations of different transmitters, including DA, by leakage from damaged axons. Therefore, our experiments suggest that halothane at high concentrations might cause secondary damage to neurones as a result of either hypoxia induced by respiratory depression or ischaemia induced by circulatory depression. During hypoglycaemia, the drop in intracellular ATP might cause inhibition of the sodium pump and the accumulation of [Na⁺], resulting in the reverse operation of DA transporters, leading to DA release. This release is [Ca²⁺]₀-independent and not affected by neuronal firing. In our previous investigation, the observation that halothane anaesthesia induced hypercapnia suggested a state of respiratory depression.

In another series of experiments, we investigated the effect of halothane on methamphetamine- and nomifensine-induced changes in the extracellular DA concentration. Nomifensine is a DA reuptake inhibitor and has been reported to increase the extracellular DA concentration, concentrations of DOPAC and HVA remaining unchanged. Nomifensine prevents the uptake of DA released primarily from non-synaptic varicosites in response to axonal activity, thereby increasing DA concentration in the extracellular space. In the present study, halothane anaesthesia potentiated the increase in extracellular DA induced by systemically administered nomifensine, whereas it failed to enhance the increase of DA when nomifensine was administered locally in the perfusate. Augmentation of anaesthesia-induced DA release by DA reuptake inhibitors had been reported by Opacka-
Juffry et al. for isoflurane and nomifensine and by Fink-Jensen et al. for halothane and vanoxerine. They have suggested that volatile anaesthetics might increase the rate of release of DA, but is then compensated for by increased reuptake activity at axon terminals. However, the results of the present investigation reveal that DA release from axon terminals in the striatum of rats is not activated by halothane when the reuptake process is inhibited by local administration of nomifensine. Therefore, it should be concluded that it is the enhanced neural activity induced by halothane that potentiates the effect of the reuptake inhibitor on DA release rather than some additional effect of volatile anaesthetics at nerve endings.

Methamphetamine is a widely abused drug that, like amphetamine, is able to release DA. Amphetamine has been reported to increase the concentration of extracellular DA and 3-MT but to decrease the concentrations of DOPAC and HVA. Methamphetamine releases DA from a cytoplasmic pool, which is the major source of substrate for the metabolizing enzyme, monoamine oxidase, MAO. Halothane anaesthesia at concentrations (0.5 and 1.5%) at which the extracellular concentration of DA is not increased, potentiated the ability of methamphetamine to induce DA release [without induction of anaesthesia there was a 3-fold increase, but with halothane there was a 13-fold increase (Fig. 2)]. Methamphetamine reduced the concentrations of DOPAC and HVA when administered either systemically or locally, whereas increases in DOPAC and HVA in response to halothane anaesthesia were found only when methamphetamine was administered locally. However, the results of the present investigation reveal that methamphetamine might reduce DA not only at an axon terminal but also at other neuronal pathways, because locally administered methamphetamine did not prevent the increase in DOPAC induced by halothane, suggesting that halothane modifies DA metabolism in axon terminals.

Our findings argue against the possibility that halothane acts directly on the DA transporters, at least at concentrations less than 2%. At concentrations higher than 1.5% effects of halothane on the transporter cannot be excluded. Previous in vitro studies with rat brain synaptosomes support this conclusion. In these experiments, halothane inhibited DA uptake, and either increased or did not alter the release of DA from synaptosomes. If halothane were indeed a DA reuptake inhibitor, as indicated by in vitro studies, the effects of nomifensine in our experiments should have been further increased. However, except at the highest concentration (2%), this was not the case.

With systemic administration of methamphetamine or nomifensine, we demonstrated that 1.5% halothane potentiated the methamphetamine-induced increase in DA concentration. In addition, the increase in 3-MT induced by methamphetamine, but not nomifensine, was enhanced by halothane anaesthesia. The observation that the 3-MT concentration was increased in methamphetamine-pretreated rats suggests that halothane also alters DA metabolism at postsynaptic sites, because 3-MT is a catabolic product of the postsynaptic enzyme, catechol-o-methyltransferase. Differences observed between the effects of methamphetamine and nomifensine on DA release and metabolism support the conclusion that halothane modifies the metabolism of DA in neural transmission pathways.

In conclusion, the present study clearly shows that halothane anaesthesia increases the concentrations of extracellular DA metabolites in the rat striatum, whereas the concentration of DA remains changed. Halothane augmented methamphetamine- and nomifensine-induced increases in extracellular DA concentration when the drugs were administered systemically. These findings suggest that potentiation of DA release by halothane anaesthesia results from increased neural activity in the nigrostriatal pathway rather than from modulation of DA release or DA reuptake at axon terminals. Excessive release of DA may produce striatal neurotoxicity. This suggests that halothane potentially affects neuronal firing in the dopaminergic pathway of the rat striatum, at least in the context of excessive release, reuptake and metabolism of DA. Because the striatum is part of the limbic system, it seems likely that halothane anaesthesia has similar effects in the rest of the limbic system, e.g. the nucleus accumbens. Therefore, the use of this type of anaesthesia in patients who are drug abusers may be problematical.

Acknowledgements
We would like to express our sincere thanks to Dr K. Okamoto and Professor J. Okamoto for reviewing and commenting on this manuscript.

References
1 Opacka-Juffry J, Ahier RG, Cremer JE. Nomifensine-induced increase in extracellular striatal dopamine is enhanced by isoflurane anaesthesia. Synapse 1991; 7: 169–71
3 Stahle L, Collin AK, Ungerstedt U. Effects of halothane anaesthesia on extracellular levels of dopamine, dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindolacetic acid in rat striatum: a microdialysis study. Naunyn-Schmiedebergs Arch Pharmacol 1990; 342: 136–40
5 Fink-Jensen A, Ingwersen SH, Nielsen PG. Halothane anesthesia
enhances the effect of dopamine uptake inhibition on interstitial levels of striatal dopamine. Naunyn-Schmiedebergs Arch Pharmacol 1994; 349: 229–44.


40. Parker EM, Cubeddu LX. Effects of d-amphetamine and dopamine synthesis inhibitors on dopamine and acetylcholine neurotransmission in the striatum. I. Release in the absence of...
41 Parker EM, Cubeddu LX. Effects of d-amphetamine and dopamine synthesis inhibitors on dopamine and acetylcholine neurotransmission in the striatum. II. Release in the presence of vesicular transmitter stores. J Pharmacol Exp Ther 1986; 237: 192–203
46 Saller CF, Salama AI. 3-Methoxytyramine accumulation: effects of typical neuroleptics and various atypical compounds. Naunyn-Schmiedebergs Arch Pharmacol 1986; 334: 125–32