Smaller effect of propofol than sevoflurane anesthesia on dopamine turnover induced by methamphetamine and nomifensine in the rat striatum: an in vivo microdialysis study

Saori TAHARABARU1), Maiko SATOMOTO2), Takahiro TAMURA1), and Yushi U. ADACHI3)

1)Department of Anesthesia, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya-shi, Aichi 466-8550, Japan
2)Department of Anesthesiology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya-shi, Aichi 466-8550, Japan
3)Department of Surgical Intensive Care Medicine, Nagoya University Hospital, 65 Tsurumai-cho, Showaku, Nagoya-shi, Aichi 466-8550, Japan

Abstract: Volatile anesthetics accelerate dopamine turnover in the brain, especially when used in conjunction with psychotropic agents such as methamphetamine and nomifensine. The effect of intravenous propofol anesthesia on the extracellular dopamine concentrations is unclear. The aim of this study was to compare the effect of two anesthetics on the extracellular concentrations of dopamine and metabolites using an in vivo microdialysis model. Male Sprague Dawley rats were implanted with a microdialysis probe into the right striatum. The probe was perfused with modified Ringer’s solution, and the dialysate was directly injected into a high-performance liquid chromatography system every 20 min. The rats were intraperitoneally administered saline, methamphetamine at 2 mg/kg, or nomifensine at 10 mg/kg. After treatment, the rats were anesthetized with intravenous propofol (20 mg/kg followed by 25 or 50 mg/kg/h) or inhalational sevoflurane (2.5%) for 1 h. Propofol showed no effect on the extracellular concentration of dopamine during anesthesia; however, propofol decreased the dopamine concentration after anesthesia in the high-dose group. Sevoflurane anesthesia increased the concentration of metabolites. Systemic administration of methamphetamine and nomifensine increased the extracellular concentration of dopamine. Sevoflurane anesthesia significantly enhanced the increase in the dopamine concentration induced by both methamphetamine and nomifensine, whereas propofol anesthesia showed no effect on the methamphetamine- and nomifensine-induced dopamine increase during anesthesia. The enhancing effect of psychotropic agent-induced acceleration of dopamine turnover was smaller for propofol anesthesia than for sevoflurane anesthesia.

Key words: dopamine, microdialysis, propofol, sevoflurane, striatum
Introduction

Dopamine (DA) is one of the most important neurotransmitters in a brain. Volatile anesthesia has been shown to markedly enhanced the increase in the extracellular DA concentration induced by methamphetamine and nomifensine administration in the striatum in in vivo microdialysis experiments [2, 4, 15]. Methamphetamine reverses and nomifensine inhibits the DA transporter, and both increase the extracellular concentration of DA in the brain [12]. During general anesthesia using volatile anesthetics, DA turnover is absolutely accelerated [2]. Methamphetamine and nomifensine are well-known psychotropic agents, and activation of the dopaminergic pathway in the limbic system modifies behavioral changes and induces drug abuse [17].

A number of harmful effects of general anesthetics on the central nervous system were recently reported [13]. These effects include damage to the developing brain in neonates [22] and postoperative cognitive dysfunction in geriatric patients [9]. The increase in DA turnover may induce free radicals species in the pathway [6, 8], which can result in neurotoxicity and subsequent oxidative stress. Therefore, anesthesia should be avoided in vulnerable patients [15]. Moreover, patients undergoing treatment with psychotropic drugs are another concern. These patients may have impaired DA homeostasis [17].

Propofol is a popular and widely used intravenous anesthetic. However, the effect of propofol anesthesia on DA regulation in conjunction with psychotic drugs has not been fully investigated [1, 23, 24], and studies comparing volatile anesthetics in terms of their effects on DA release are limited [18]. Propofol anesthesia has a possibility of showing less change in DA turnover and of presenting a smaller toxic effect.

Thus, the current study compared the effects of propofol and sevoflurane anesthesia on the extracellular concentrations of DA and DA metabolites using an in vivo microdialysis study. In addition, the effect of these drugs on DA turnover was examined with or without treatment with methamphetamine and nomifensine.

Materials and Methods

The experiments were approved by the institute’s Committee for Animal Research (National Defense Medical College). Male Sprague Dawley rats, 8 weeks old and weighing 280 to 320 g, were used in the experiments (CLEA Japan, Tokyo, Japan). The animals were housed in an animal room kept at 20°C to 22°C and illuminated with a 12-h light/dark cycle (light from 07:00 to 19:00 h). All animals (n=8, each group) had free access to food and drinking water. The number of animals to be analyzed was selected based on the results of our previous study [1–4, 15].

The rats were anesthetized with sevoflurane (Maruishi Pharmaceutical, Osaka, Japan), and surgery was performed with subcutaneous topical application of 1% lidocaine (0.3 ml, AstraZeneca, Tokyo, Japan) for the postoperative analgesia. A unilateral guide cannula was implanted just above the striatum (AP + 0.6 mm, ML + 3.0 mm, DV – 3.8 mm) [20]. The rats were allowed to recover for at least 3 days before beginning the experiment. After each experiment, the rats were killed by excess inhalation of 3% isoflurane (Abbott Japan, Tokyo, Japan), and intravenous injection of 100 mg thiopental (Tanabe Pharma Corporation, Osaka, Japan) through the tail vein using a 24-G metal needle (Terumo, Tokyo, Japan). The brain was removed, and the placement of the microdialysis probe was histologically identified.

On the day of the experiment, the microdialysis probe (o.d. 0.22 mm, membrane length 3 mm, polycarbonate tubing, and cutoff molecular weight 50,000; EICOM, Kyoto, Japan) was carefully inserted into the striatum through a guide cannula, and the rat was immediately placed in a clear open Plexiglas box for recovery. The probe was continuously perfused with modified Ringer’s solution (145.4 mEq/L Na+, 2.8 mEq/L K+, 2.3 mEq/L Ca2+, 150.5 mEq/L Cl−) at a flow rate of 2 µl/min. Samples were collected every 20 min and directly injected into an online analytical system with an autoinjector (EAS-20; EICOM).

The concentrations of DA, 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in each dialysate (40 µl per 20 min) were determined by high-performance liquid chromatography with an electrochemical detector (ECD-300; EICOM). These compounds were separated by reverse-phase ion-pair chromatography with a 5-mm C-18 column (150 mm × 2.1 mm, MAS-ODS, EICOM) using an isocratic mobile phase (0.1 M sodium acetate, 0.1 M citric acid, 1.4 mM sodium 1-octanesulfonate, 5 mM EDTA-Na2 and methanol 13%–14%, pH 3.9) delivered at a flow rate of 230 µl/min by a high-pressure pump (EP-300, EICOM). 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 0.5 pg per sample. DA and its metabolites reached stable baseline concentrations within about 4.5 h after implantation of the probe. Therefore, at least six dialysate samples (40 µl of each collected in 20 min) were collected before starting the pharmacological experiment. The mean value obtained from the last three samples was used as the baseline concentration. The time at which the pharmacological manipulation started is hereafter called “fraction number 1” (Fr. 1, see figures).

All animals were spontaneously breathing without any interventions nor complications throughout the experiments. Rats were administered saline (0.6 ml) as a control and the same volume of methamphetamine (Sumitomo Dainippon Pharma, Osaka, Japan) at 2 mg/kg or the same volume of nomifensine (Sigma-Aldrich, St. Louis, MO, USA) at 10 mg/kg (0.6 ml per 300 g) intraperitoneally with or without 1 h of intravenous propofol or inhaled sevoflurane anesthesia. For the propofol anesthesia groups, each rat was anesthetized by bolus and intravenous administration of 20 mg/kg of propofol (Diprivan, AstraZeneca, Osaka, Japan) followed by continuous infusion at a rate of 25 and 50 mg/kg/h [1] through the tail vein. For the inhaled sevoflurane anesthesia groups, each rat was anesthetized in a semi-closed Plexiglas box, into which 5% sevoflurane was initially introduced at a flow rate of 3 l/min for about 5 min until a steady state was achieved. Subsequently, 2.5% sevoflurane was applied at a rate of 2 l/min, using air (23% oxygen) as the carrier to avoid hypoxia [2, 4, 15]. The rectal temperature of each rat was monitored and maintained at 37°C with an electrical heating pad (Onever, AliExpress, USA) except in the control groups, in which the animals were consistently awake during the experiments. The concentrations of volatile gas and oxygen in the box were monitored using an infrared anesthetic gas analyzer (Capnomac Ultima, Datex, Helsinki, Finland) during each anesthetic procedure. The sampling probe was placed near the rat’s nose, and the respiratory pattern was monitored by capnography. Immediately after the 1 h anesthesia period, the gas in the box was exchanged with room air by forced ventilation.

Data were analyzed by two-way analysis of variance with drugs as the between-subjects variable and time as the within-subject variable. For significant (P<0.05) drug or time interactions, a subsequent Newman-Keuls post hoc multiple comparison test was performed (NCSS 2000, Number Cruncher Statistical Systems, Kaysville, UT, USA). The data are presented as the mean ± SEM using percent changes from baseline values.

Results

All animals (n=8, each group) that underwent anesthesia demonstrated no movement with or without mild stimuli, including during insertion of the body temperature monitoring probe into the rectum and application of the heating pad. After termination of anesthesia, all animals showed spontaneous movements within 15 mins.

Propofol had no effect on the extracellular concentration of DA during anesthesia; however, propofol decreased the DA concentration after anesthesia in the high-dose group (Fig. 1). Sevoflurane anesthesia significantly increased the concentrations of metabolites (Fig. 1), whereas only a slight increase in the metabolite concentration (HVA) was observed in the high-dose propofol anesthesia group.

Systemic administration of methamphetamine and nomifensine significantly increased the extracellular concentration of DA (Figs. 2 and 3). Methamphetamine decreased both the DOPAC and HVA concentrations. Propofol anesthesia had no effect on the concentration of DA when used in conjunction with either methamphetamine or nomifensine. The reduction of HVA induced by methamphetamine was significantly enhanced by high-dose propofol anesthesia (Fig. 2). After the administration of nomifensine, propofol anesthesia significantly increased the concentrations of 3-MT and HVA, but not the concentration of DOPAC (Fig. 3).

Sevoflurane anesthesia significantly enhanced the increase in the DA and 3-MT concentrations induced by both methamphetamine and nomifensine (Fig. 4). Sevoflurane profoundly decreased the reduction in the concentrations of DOPAC and HVA induced by the administration of methamphetamine, whereas increases in the concentrations of DOPAC and HVA were induced by nomifensine (Fig. 4).

Discussion

The results of the current study demonstrated that neither anesthetic alone resulted in an increase in the extracellular DA concentration. However, the anesthetics did have significantly different effects on the extracellular concentrations of DA metabolites. Although a no-
Fig. 1. Effect of propofol and sevoflurane anesthesia on the extracellular concentrations of dopamine and metabolites. Control: no anesthesia. Propofol25mg or Propofol50mg: bolus administration of propofol at 20 mg/kg followed by infusion at 25 or 50 mg/kg/h. Sevo2.5%: sevoflurane inhalation at 2.5%. DA: dopamine. DOPAC: 3,4-dihydroxyphenylacetic acid and 3-MT: 3-methoxytyramine. HVA: homovanillic acid. *P<0.05 vs. control group.

Fig. 2. Effect of methamphetamine and propofol anesthesia on the extracellular concentrations of dopamine and metabolites. Control: no anesthesia. Mamp2mg: methamphetamine at 2 mg/kg intraperitoneally. Mamp2mg + P25mg or Mamp2mg + P50mg: intraperitoneal bolus administration of methamphetamine at 2 mg/kg and propofol at 20 mg/kg followed by infusion at 25 or 50 mg/kg/h. DA: dopamine. DOPAC: 3,4-dihydroxyphenylacetic acid. 3-MT: 3-methoxytyramine. HVA: homovanillic acid. *P<0.05 vs. control groups. #P<0.05 vs. Mamp2mg group.
Fig. 3. Effect of nomifensine and propofol anesthesia on the extracellular concentrations of dopamine and metabolites. Control: no anesthesia. Nom10mg: intraperitoneal administration of nomifensine at 10 mg/kg. Nom10mg + P25mg or Nom10mg + P50mg: intraperitoneal bolus administration of nomifensine at 10 mg/kg and propofol at 20 mg/kg followed by infusion at 25 or 50 mg/kg/h. DA: dopamine. DOPAC: 3,4-dihydroxyphenylacetic acid. 3-MT: 3-methoxytyramine. hV a: homovanillic acid. *P<0.05 vs. control groups. #P<0.05 vs. Nom10mg group.

Fig. 4. Effect of sevoflurane anesthesia on the extracellular concentrations of dopamine and metabolites after treatment with methamphetamine or nomifensine. Control: no anesthesia. Mampt2mg: intraperitoneal administration of methamphetamine at 2 mg/kg. Nom10mg: intraperitoneal administration of nomifensine at 10 mg/kg. Sevo: sevoflurane inhalation at 2.5%. DA: dopamine. DOPAC: 3,4-dihydroxyphenylacetic acid. 3-MT: 3-methoxytyramine. hV a: homovanillic acid. *P<0.05 vs. control groups. #P<0.05 vs. Mampt2mg or Nom10mg group.
towardly dose-dependent defect of propofol on the DA concentration was not found, propofol was found to have had a smaller effect on DA turnover than did sevoflurane in animals with or without administration of dopaminergic psychotropic agents. Previous studies regarding the extracellular concentrations of DA and metabolites during anesthesia with continuous infusion of propofol are limited [24], and we are the first to report results using continuous infusion techniques.

In our previous study [2, 4, 15], volatile anesthetics including halothane, isoflurane, and sevoflurane did not affect the extracellular concentration of DA during anesthesia, despite inducing significant increases in the DA metabolites 3-MT, DOPAC, and HVA. We speculated that volatile anesthetics might accelerate DA turnover in the presynaptic site of neurons [2]. The acceleration of DA release would be compensated for by regulation of the DA transporter, and the concentration of extracellular DA did not change during anesthesia [5]. Therefore, under the condition of DA transporter insufficiency induced by the administration of psychotropic agents, drastic changes may occur during anesthesia [8].

Although the extracellular concentration of DA was maintained, the increase of metabolites suggested the acceleration of oxidation of DA and the increase of production of toxic free radical species [6, 8]. The oxidative stress might be harmful to living things and especially harmful to the central nervous system [9, 22]. Parkinson’s disease is one of the most well-known degenerative diseases of the central nervous system, and the key mechanism of neuronal damage induced by oxidative stress is a focus of studies [7]. Eliminating excess reactive oxygen species and regulating oxidative reactions might be a therapeutic option [9]. General anesthesia for living things susceptible to oxidative stress should be applied carefully and anesthetics showing a smaller effect of acceleration of DA metabolism would be appropriate [22].

Several studies have shown opposing results for the extracellular concentration of DA in the brain following anesthesia [19, 23, 24]. The release of DA from striatal neurons may be mediated by the action of GABAergic [11, 16] and cholinergic interneurons [3]. The highly complex networks of neurons and interneurons make it difficult to explain the change in DA release by simple anesthetic action. For example, propofol anesthesia is known to have antiemetic effects compared with volatile anesthetics [26]. DA antagonists, including droperidol and metoclopramide, are popular antiemetics that are used for general anesthesia in operating rooms [21] and increased DA release [4]. Regarding DA regulation, the interaction between dopaminergic activity and propofol anesthesia could explain the findings in this study.

The mechanism of general anesthesia, especially for achieving hypnosis, has mainly focused on GABAergic inhibitory neuronal circuits [10]. Solt et al. [14, 25] recently demonstrated that dopaminergic stimulation induced an apparent arousal response during general anesthesia. The dextroamphetamine-induced change in electroencephalography was prevented by pretreatment with SCH-23390, a D1 DA receptor antagonist. Acceleration of the dopaminergic pathway may modify and reverse anesthesia by both intravenous anesthetics such as propofol and volatile anesthetics such as sevoflurane [14].

The limitations of the study should be addressed. The depth of anesthesia was not evaluated in the current investigation. It was possible that the anesthetic depth was different between propofol and sevoflurane anesthesia. Behavioral changes were not evaluated in the current investigation. However, all anesthetic regimens in the study achieved a stable anesthetic state including hypnosis, adequate respiration, and immobility during anesthesia. Both doses of propofol showed different profiles of changes in DA and metabolites compared with sevoflurane.

Conclusions

Propofol anesthesia had a smaller effect on DA release and metabolism than sevoflurane in animals treated with psychotropic agents. Propofol anesthesia may have a lower effect on the dopaminergic nervous system than do other anesthetic drugs.

Conflict of Interest

There are no conflicts of interest with respect to the current investigation.

Acknowledgments

The current study was funded by a grant-in-Aid for Scientific Research (19591787) from the Japan Society for the Promotion of Science.
References

1. Adachi, Y., Wada, H., Watanabe, K., Uchihashi, Y., Higuchi, H., and Satoh, T. 2000. The effect of propofol anesthesia on striatal dopamine of awake freely moving and anesthetized rats examined by in vivo microdialysis study. *Masui* 49: 1076–1081 (in Japanese). [Medline] [CrossRef]

2. Adachi, Y.U., Satomoto, M., Higuchi, H., Watanabe, K., Yamada, S., and Kazama, T. 2005. Halothane enhances dopamine metabolism at presynaptic sites in a calcium-independent manner in rat striatum. *Br. J. Anaesth.* 95: 485–494. [Medline] [CrossRef]

3. Adachi, Y.U., Watanabe, K., Higuchi, H., Satoh, T., and Zsilave, G. 2002. Halothane enhances acetylcholine release by decreasing dopaminergic activity in rat striatal slices. *Neurochem. Int.* 40: 189–193. [Medline] [CrossRef]

4. Adachi, Y.U., Yamada, S., Satomoto, M., Higuchi, H., Watanabe, K., Kazama, T., Mimuro, S., and Sato, S. 2008. Isoflurane anesthesia inhibits clozapine- and risperidone-induced dopamine release and anesthesia-induced changes in dopamine metabolism was modified by fluoxetine in the rat striatum: an in vivo microdialysis study. *Neurochem. Int.* 52: 384–391. [Medline] [CrossRef]

5. Brodnik, Z.D. and España, R.A. 2015. Dopamine uptake dynamics are preserved under isoflurane anesthesia. *Neurosci. Lett.* 606: 129–134. [Medline] [CrossRef]

6. Camarero, J., Sanchez, V., O’Shea, E., Green, A.R., and Colado, M.I. 2002. Studies, using in vivo microdialysis, on the effect of the dopamine uptake inhibitor GBR 12909 on 3,4-methylenedioxymethamphetamine (‘ecstasy’)—induced dopamine release and free radical formation in the mouse striatum. *J. Neurochem.* 81: 961–972. [Medline] [CrossRef]

7. Dias, V., Junn, E., and Mouradian, M.M. 2013. The role of oxidative stress in Parkinson’s disease. *J. Parkinins Dis.* 3: 461–491. [Medline] [CrossRef]

8. Eisenhofer, G., Kopin, I.J., and Goldstein, D.S. 2004. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol. Rev.* 56: 331–349. [Medline] [CrossRef]

9. Evered, L., Silbert, B., Scott, D.A., Ames, D., Maruff, P., and Blennow, K. 2016. Cerebrospinal Fluid Biomarker for Alzheimer Disease Predicts Postoperative Cognitive Dysfunction. *Anesthesiology* 124: 353–361. [Medline] [CrossRef]

10. Franks, N.P. and Zecharia, A.Y. 2011. Sleep and general anesthesia. *Can. J. Anaesth.* 58: 139–148. [Medline] [CrossRef]

11. Grasshoff, C., Herrera-Marschitz, M., Goiny, M., and Kretschmer, B.D. 2005. Modulation of ventral pallidal dopamine and glutamate release by the intravenous anesthetic propofol studied by in vivo microdialysis. *Amino Acids* 28: 145–148. [Medline] [CrossRef]

12. Heal, D.J., Gosden, J., and Smith, S.L. 2014. Dopamine reuptake transporter (DAT) “inverse agonism” – a novel hypothesis to explain the enigmatic pharmacology of cocaine. *Neuropharmacology* 87: 19–40. [Medline] [CrossRef]

13. Hudson, A.E. and Hemnings, H.C. Jr. 2011. Are anaesthesiologists toxic to the brain? *Br. J. Anaesth.* 107: 30–37. [Medline] [CrossRef]

14. Kenny, J.D., Taylor, N.E., Brown, E.N., and Solt, K. 2015. Dextroamphetamine (but Not Atomoxetine) Induces Reanimation from General Anesthesia: Implications for the Roles of Dopamine and Norepinephrine in Active Emergence. *PloS One* 10: e0131914. [Medline] [CrossRef]

15. Kimura-Kuroiwa, K., Adachi, Y.U., Mimuro, S., Obata, Y., Kawamata, M., Sato, S., and Matsuda, N. 2012. The effect of aging on dopamine release and metabolism during sevoflurane anesthesia in rat striatum: an in vivo microdialysis study. *Brain Res. Bull.* 89: 223–230. [Medline] [CrossRef]

16. Krasowski, M.D., Koltehine, V.V., Rick, C.E., Ye, Q., Finn, S.E., and Harrison, N.L. 1998. Propofol and other intravenous anesthetics have sites of action on the gamma-aminobutyric acid type A receptor distinct from that for isoflurane. *Mol. Pharmacol.* 53: 530–538. [Medline] [CrossRef]

17. McMahon, L.R. and Cunningham, K.A. 2003. Discriminative stimulus effects of (-)-ephedrine in rats: analysis with catecholamine transporter and receptor ligands. *Drug Alcohol Depend.* 70: 255–264. [Medline] [CrossRef]

18. Müller, C.P., Pum, M.E., Amato, D., Schüttrich, J., Huston, J.P., and Silva, M.A. 2011. The in vivo neurochemistry of the brain during general anesthesia. *J. Neurochem.* 119: 419–446. [Medline] [CrossRef]

19. Pain, L., Gobaille, S., Schlee, C., Aunis, D., and Oberling, P. 2002. In vivo dopamine measurements in the nucleus accumbens after nonanesthetic and anesthetic doses of propofol in rats. *Anesth. Analg.* 95: 915–919. [Medline] [CrossRef]

20. Paxinos, G. and Watson, C. 1988. The Brain in Stereotaxic Coordinates, 4th ed. Adacemic Press, San diego, CA.

21. Pendeville, P.E., Veyckemans, F., Van Boven, M.J., and Steiner, J.R. 1993. Open placebo controlled comparison of the antiemetic effect of droperidol, metoclopramide or a combination of both in pediatric strabismus surgery. *Acta Anesthesiol. Belg.* 44: 3–10. [Medline] [CrossRef]

22. Satomoto, M., Satoh, Y., Terui, K., Miyao, H., Takishima, K., Ito, M., and Imaki, J. 2009. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. *Anesthesiology* 110: 628–637. [Medline] [CrossRef]

23. Semba, K., Adachi, N., and Araï, T. 2005. Facilitation of serotonergic activity and amnesia in rats caused by intravenous anesthetics. *Anesthesiology* 102: 616–623. [Medline] [CrossRef]

24. Shirasaka, T., Yonaha, T., Onizuka, S., and Tsuneyoshi, I. 2011. Effects of orexin-A on propofol anesthesia in rats. *J. Anesth.* 25: 65–71. [Medline] [CrossRef]

25. Solt, K., Cotten, J.F., Cimenser, A., Wong, K.F., Chemali, J.J., and Brown, E.N. 2011. Methylphenidate actively induces emergence from general anesthesia. *Anesthesiology* 115: 791–803. [Medline] [CrossRef]

26. Soppitt, A.J., Glass, P.S., Howell, S., Weatherwax, K., and Gan, T.J. 2000. The use of propofol for its antiemetic effect: a survey of clinical practice in the united States. *Anesth. Analg.* 90: 1433–1436. [Medline] [CrossRef]