The effect of aging on dopamine release and metabolism during sevoflurane anesthesia in rat striatum: An in vivo microdialysis study

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Abstract

We have previously reported that halothane anesthesia increases extracellular concentrations of dopamine (DA) metabolites in rat striatum using in vivo microdialysis techniques. Aging induces many changes in the brain, including neurotransmission. However, the relationship between aging and changes in neurotransmitter release during inhalational anesthesia has not been fully investigated. The aim of the present investigation was to evaluate the effect of sevoflurane on methamphetamine (MAPT)-induced DA release and metabolism in young and middle-aged rats. Male Sprague-Dawley rats were implanted with a microdialysis probe into the right striatum. The probe was perfused with a modified Ringer’s solution and 40 µL of dialysate was directly injected to an HPLC every 20 min. Rats were administered saline, the same volume of 2 mg kg⁻¹ MAPT intraperitoneally, or 5 µM MAPT locally perfused. After treatments, the rats were anesthetized with 1% or 3% sevoflurane for 1 h. Sevoflurane anesthesia significantly increased the extracellular concentration of DA only in middle-aged rats (52-weeks-old). In young rats (8-weeks-old), sevoflurane significantly enhanced MAPT-induced DA release when administered both intraperitoneally and perfused locally, whereas no significant additive interaction was found in middle-aged rats. These results suggest that aging changes DA release and metabolism in rat brains primarily by decreasing the DA transporter.

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1. Introduction

Aging induces many changes in organisms, including neurotransmission in the brain (Reeves et al., 2002). Parkinson’s disease is one of the most alarming conditions in humans, and aging is a primary risk factor (Collier et al., 2011). Similar cellular mechanisms may be involved in the degeneration of dopaminergic neurons through the course of normal aging and in the aging-related changes that lead to Parkinson’s disease. Midbrain dopamine (DA) neurons are more vulnerable to degeneration than are other neurons (Marras and Lang, 2008). The progressive loss of dopaminergic neurons and the subsequent decrease in DA release in the nigrostriatal pathway results in major disruptions to connections between the thalamus and motor cortex, and leads to Parkinsonian symptoms, such as bradykinesia.

We have previously reported that halothane anesthesia increases extracellular concentrations of DA metabolites in rat striatum, using in vivo microdialysis techniques (Adachi et al., 2005a, 2001c). Inhalational anesthetics likely exert a biphasic effect on DA regulation through potentiation of DA release and the inhibition of DA synthesis (Adachi et al., 2005b). Methamphetamine (MAPT) is a well-known psychotropic stimulant and produces vast changes in DA release in rat striatum. Volatile anesthetics alone mimic the effects on extracellular DA concentration. However, inhalation anesthetics markedly enhance MAPT-induced DA release from axon terminals in rat striatum (Adachi et al., 2005a, 2001c). We hypothesized that volatile anesthetics accelerate DA turnover at a presynaptic site in the brain and that DA metabolites are increased during anesthesia (Adachi et al., 2001c, 2005b).

Oxidative stress followed by inadequate production of reactive oxygen species generates neurodegeneration. DA is usually metabolized, not only by monoamine oxidase-mediated enzymatic oxidation (Adachi et al., 2001a), but also by auto-oxidation to neuromelanin (Fedorow et al., 2005). These metabolic complexes
produce reactive substances; for example, hydrogen peroxide, superoxide anions and hydroxyl radicals. Free radicals react with membrane lipids and produce toxic lipid peroxidation (Ahlskog, 2005). These free radicals are increased in the substantia nigra of patients with Parkinson’s disease (Ahlskog, 2005). Increases in oxidative and metabolic products may indicate an escalation of neurotoxicity and might play an important role in neurodegeneration.

The efficacy and toxicity of psychotropic drugs almost certainly change with age since body size and organ maturity are both important factors in determining drug effects (Wynne and Blagburn, 2010). Recently, the potential toxicity of volatile anesthetics on the developing brain has been an area of focus in anesthesiology (Jevtovic-Todorovic et al., 2003; Satomoto et al., 2009). Just like in the neonatal central nervous system, geriatric neurons might be more susceptible to psychotropic drugs, including anesthetics and hallucinogens (Wynne and Blagburn, 2010). Geriatric patients have many opportunities to receive general anesthesia, followed by psychotropic medications. However, the effects of aging and general anesthesia on the release of neurotransmitters, including DA, have rarely been investigated due to the difficulty and technical limitations involved in conducting in vivo experiments in aged animals.

The aim of the present investigation was to evaluate the effect of inhaled sevoflurane anesthesia and MAPT on extracellular DA concentrations and DA metabolites in young and middle-aged rat striatum using an in vitro microdialysis technique. We examined whether aging discernibly affects extracellular DA homeostasis during general anesthesia.

2. Materials and methods

2.1. Materials

Male Sprague–Dawley rats, 8 weeks old and weighing 280–320 g as young rats and 52–54 weeks old and weighing 560–780 g as middle-aged rats, were used in the experiments (CLEA Japan, Tokyo, Japan). The animals were gently housed in an animal room at 20–22 °C and illuminated with a 12-h light/dark cycle (light from 07:00 to 19:00 h). All animals had free access to food and drinking water. The experiments were approved by the Committee for Animal Research in the institute.

2.2. Microdialysis

Rats were anesthetized with sevoflurane and surgery was performed with the 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) following the atlas of Paxinos and Watson (1998). The rats were allowed to recover for at least 3 days before the experiment began. After each experiment, the rats were killed by excess inhalation of isoflurane and intravenous injection of thiopental. The brain was removed, and the placement of the microdialysis probe was identified histologically. Microdialysis probes were obtained from EICOM (Kyoto, Japan) (o.d. 0.22 mm, membrane length 3 mm, polycarbonate tubing, cut-off mol. wt. 50,000). At about 07:00 a.m. on the day of experiment, the probe was inserted carefully into the striatum through a guide cannula and fixed to the cannula with a screw, 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 mmol/L NaCl, 2.8 mmol/L KCl, 2.3 mmol/L CaCl₂, 150.5 mmol/L L-1 Cl⁻) at a flow rate of 2 ml/min using a micro-infusion pump (ESP-64, EICOM, Kyoto, Japan) to determine the baseline concentrations of DA and its metabolites. Samples were collected every 20 min and directly injected into an online analytical system with an auto-injector (EAS-20, EICOM), as described elsewhere (Adachi et al., 2005, 2001c).

The concentrations of DA, 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in each dialysate (40 ml/20 min) were determined by HPLC with an electrochemical detector (EC-D, EICOM). These compounds were separated by reverse-phase ion-pair chromatography with a 5-mm C18 column (MAB-ODS, 150 mm × 2.1 mm, EICOM) using an isotonic 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 2.30 ml/min by a high-pressure pump (EP-300, EICOM). A guard column (MA, 5 mm × 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 a Ag/AgCl reference electrode. The detection limit for each of the compounds was roughly 0.5 pg per sample. DA and its metabolites reached stable baseline concentrations within about 4.5 h after implantation of the microdialysis probe. Therefore, at least six dialysate samples (each 40 ml 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).

2.3. Experiments

Rats were given saline (0.6 ml) or the same volume of MAPT 2 mg·kg⁻¹ (0.6 ml/300 g) intraperitoneally with or without 1-h sevoflurane anesthesia (1.0 or 3.0%). In other groups, MAPT was dissolved in Ringer’s solution to give a concentration of 5 μM. Each rat was anesthetized in a semi-closed Plexiglas box, into which 3% sevoflurane was initially introduced at a flow rate of 31 min⁻¹ for about 5 min until a steady state was achieved. Subsequently, 1.0% or 3.0% sevoflurane was applied at a rate of 21 min⁻¹, using air (23% oxygen) as the carrier for avoiding hypoxia (Orset et al., 2005). The rectal temperature of the rat was monitored and maintained at 37 °C with an electrical heating pad, except in the control groups, because the animals were consistently awake during 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 anesthesia. The sampling probe was placed near to rats nose and respiratory pattern was monitored by capnomgram. Immediately after the 1-h anesthesia, the gas in the box was exchanged with room air by forced ventilation.

2.4. Statistics

Data were analyzed by two-way analysis of variance with drugs as a between-subjects variable and time as a 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 were presented as mean ± SEM using percent changes from baseline values.

2.5. Drugs

Sevoflurane was obtained from Maruishi (Maruishi Pharmaceutical Col, Ltd., Osaka, Japan). Methamphetamine was purchased from Daipun Pharmaceutical (Osaka, Japan).

3. Results

Sevoflurane anesthesia significantly increased the extracellular concentration of DA metabolites, 3-MT, DOPAC and HVA in both 8-week old young rats (Fig. 1) and 52-week old middle-aged rats (Fig. 2) in a dose-dependent manner. After inhalation, the concentration of metabolites returned to pre-anesthetic levels. DA concentration was not significantly changed in young rats following anesthetic inhalation. However, in middle-aged animals, inhalation of high concentrations of sevoflurane increased DA levels in a manner consistent with anesthesia duration.

Intraparenchymal administration of MAPT markedly increased the extracellular concentration of DA and 3-MT and decreased that of DOPAC and HVA in all animals (Figs. 3 and 4). Sevoflurane anesthesia enhanced DA and 3-MT concentrations in young rats administered MAPT. In middle-aged animals, there were no significant differences in DA and 3-MT concentrations between the non-anesthetized and anesthetized groups. Sevoflurane counteracted the MAPT-induced reduction of HVA in both young and middle-aged animals; however, the small but significant increase in DOPAC was reduced by MAPT administration only in middle-aged rats.

Perfusion of MAPT also significantly increased extracellular DA and 3-MT concentrations (Figs. 5 and 6). This effect was significant in young animals but not in middle-aged rats. However, there was no apparent change in DOPAC and HVA in either age group.

4. Discussion

In this study, we demonstrate that sevoflurane anesthesia has a tendency to slightly increase extracellular DA in young rats; however, in middle-aged rats, a much larger increase in DA was
**Fig. 1.** In young rats, effect of sevoflurane anesthesia on the extracellular concentrations of dopamine (DA, upper left) and its metabolites, 3-methoxytyramine (3-MT, upper right), 3,4-dihydroxyphenylacetic acid (DOPAC, lower left) and homovanillic acid (HVA, lower right). In this and the following figures, the ordinate of each graph shows the concentration of DA or a metabolite expressed as the percentage of the baseline concentration, which is the mean of three consecutive values immediately before pharmacological manipulations. *P<0.05 compared with control value at each fraction. Each point is the mean (SEM) (n=5–7 per group). Dialysate fractions were obtained every 20 min. Sevoflurane anesthesia did not change the extracellular concentration of DA, whereas sevoflurane significantly increased those of metabolites (3-MT, DOPAC, HVA) in a concentration-dependent manner compared with control in young rats.

**Fig. 2.** In middle-aged rats, effect of sevoflurane anesthesia on the extracellular concentrations of dopamine and its metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid and homovanillic acid. *P<0.05 compared with control value at each fraction. Each point is the mean (SEM) (n=5–7 per group). Sevoflurane anesthesia increased the extracellular concentration of DA in middle-aged rats as same as those of other DA metabolites in a concentration-dependent manner compared with control group.
Fig. 3. In young rats, effect of sevoflurane anesthesia and intraoperative administration of methamphetamine on the extracellular concentrations of dopamine and its metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid and homovanillic acid. *P<0.05 compared with control value at each fraction, # P<0.05 compared with values in the methamphetamine treatment group at each fraction. Each point is the mean (SEM) (n=5–7 per group). Sevoflurane anesthesia increased the extracellular concentration of DA in middle-aged rats as same as those of other DA metabolites in a concentration-dependent manner compared with control group.

Fig. 4. In middle-aged rats, effect of sevoflurane anesthesia and intraoperative administration on the extracellular concentrations of dopamine and its metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid and homovanillic acid. *P<0.05 compared with control value at each fraction, # P<0.05 compared with values in the methamphetamine treatment group at each fraction. Each point is the mean (SEM) (n=5–7 per group). Sevoflurane anesthesia increased the extracellular concentration of DA in middle-aged rats as same as those of other DA metabolites in a concentration-dependent manner compared with control group.
Fig. 5. In young rats, effect of sevoflurane anesthesia and local administration of methamphetamine as perfusate on the extracellular concentrations of dopamine and its metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid and homovanillic acid. *P<0.05 compared with control value at each fraction. #P<0.05 compared with values in the methamphetamine treatment group at each fraction. Each point is the mean (SEM) (n=5–7 per group). Sevoflurane anesthesia increased the extracellular concentration of DA in middle-aged rats as same as those of other DA metabolites in a concentration-dependent manner compared with control group.

Fig. 6. In middle-aged rats, effect of sevoflurane anesthesia and local administration of methamphetamine as perfusate on the extracellular concentrations of dopamine and its metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid and homovanillic acid. *P<0.05 compared with control value at each fraction. #P<0.05 compared with values in the methamphetamine treatment group at each fraction. Each point is the mean (SEM) (n=5–7 per group). Sevoflurane anesthesia increased the extracellular concentration of DA in middle-aged rats as same as those of other DA metabolites in a concentration-dependent manner compared with control group.
measured. These changes cannot be attributed to an increased DA metabolism with age since the increases in DA metabolites were similar in both young and middle-aged rats. The results in young rats were consistent with previous experiments using halothane (Adachi et al., 2005a, 2001c), but not isoflurane (Adachi et al., 2008, 2005b). Isoflurane significantly increased extracellular DA concentration after high-dose inhalation, as did sevoflurane anesthesia in middle-aged rats in the current investigation. We cannot explain the differing results with distinct anesthetics. However, the potentiation of DA release and the acceleration of DA metabolism could certainly be a common basal effect of volatile anesthetics on DA regulation (Fink-Jensen et al., 1994; Opaka-Juffry et al., 1991) and on DA homeostasis modulated by aging.

Profound and excess anesthesia can induce ischemia and hypoxia as a result of cardiovascular insufficiency in experimental animals. Anesthetics cause circulatory (Raner et al., 1994) and respiratory depression (van den Elsen et al., 1998). Subsequent ischemic and hypoxic changes might induce neuronal damage in the brain, which impairs the regulation of release and reuptake of neurotransmitters such as DA (Toner et al., 2001). In the current investigation, we administered sevoflurane to experimental animals with 23% oxygen to prevent hypoxia-induced neuronal damage (Toner et al., 2001), and to avoid the acceleration of DA metabolism by high concentrations of oxygen (Adachi et al., 2001a).

To our knowledge, there is no evidence that the anesthetic protocol used here leads to the development of severe ischemic or hypoxic brain injury in vivo (Orset et al., 2005). However, changes in blood pressure, heart rate and partial pressure of arterial carbon dioxide were not investigated during anesthesia. All rats showed no apparent behavioral changes after anesthesia recovery, and fatal cell damage in the central nervous system could not be induced in the whole experiments.

In the striatum, basal DA levels are reported to be lower in aged rats than in young animals (Gerhardt and Maloney, 1999). However, stimulus-evoked overflow of DA is not diminished in aged rats, and dopaminergic neurons may compensate for the progressive changes that occur with aging (Gerhardt and Maloney, 1999; Stanford et al., 2001). Volatile anesthetics exert small changes in impulse-dependent DA release and induce significant changes in cytoplasmic and non-vesicular DA release (Adachi et al., 2001b; Diniz et al., 2007). Sevoflurane might potentiate cytoplasmic DA release from axon terminals. Significant and consistent increases in DA metabolites, particularly the concentration of 3-MT, support this hypothesis (Adachi et al., 2005a,b, 2001c). The anesthesia-induced modification of DA homeostasis could lead to unexpected symptoms. Iselin-Chaves et al. (2009) reported a case of naloxone-responsive acute dystonia and Parkinsonism immediately after general anesthesia. In a clinical setting, anesthesia is associated with the administration of multiple drugs, including opiates, which cause instability in neurotransmission (Walsh et al., 2010). Even in young animals, stressful events alter dopaminergic function (Novick et al., 2011). Cruz-Muros et al. (2009) reported decreased expression of the DA transporter in striatal dopaminergic-cells of aged rats. Striatal DA transporter function has also been reported to decline with aging, leading to reduced DA reuptake in the brain of rats (Salvatore et al., 2003) and humans (Erixon-Lindroth et al., 2005). We hypothesize that dopaminergic neurons activate compensatory mechanisms directed at maintaining DA transporter function and that these mechanisms may be associated with preserved motor function in normal geriatrics. However, sevoflurane anesthesia might enhance DA release and inhibit the reactive increase of DA reuptake by the transporter, which declines with aging. This is supported by the results of this study; i.e., the increased extracellular DA concentration in middle-aged, but not young, rat brains during anesthesia.

General anesthesia with isoflurane (Jansson et al., 2004) or propofol (Wang et al., 2000) has been shown to decrease acetylcholine (ACh) release in the brain of aged rats. These changes have been touted as a mechanism of promotion of peri-operative impairment of brain function in geriatric patients (Avidan and Evers, 2011). Since ACh release from striatal cholinergic interneurons is inhibited by DA release from nigrostriatal axon terminals (Vizi and Labos, 1991), anesthesia-induced increases in DA release into the extracellular space might be followed by decreases in ACh (Adachi et al., 2002). Recently, Whittington and Virág (2010) reported that when an age-adjusted equipotent dose of inhaled anesthetic similarly decreased ACH levels in young and aged rats. This suggests that the relative anesthetic potency could be adjusted for aging by altering the minimum alveolar concentration (MAC) (Gregory et al., 1969). However, numerous variables, including effects on the inter-neuronal system, cannot be fully explained by the MAC (Tanifuji, et al.). The nigrostriatal dopaminergic system plays an important role in motor activity and cognitive function during anesthesia (Erixon-Lindroth et al., 2005; Solt et al., 2011), which affects the MAC. In general, anesthesia increases DA release in rat brains and these changes are followed by a decrease in ACH release.

Nomifensine, a DA reuptake inhibitor (Eshleman et al., 1994), has been reported to enhance DA outflow in young rats (Opaka-Juffry et al., 1991; Stanford et al., 2001). Methamphetamine releases DA from the intracellular pools by reversing the DA transporter (Eshleman et al., 1994; Fink-Jensen et al., 1994). Volatile anesthetics reportedly enhance the effect of MAO on DA release (Adachi et al., 2005a, 2001c; Fink-Jensen et al., 1994; Opaka-Juffry et al., 1991). In the current investigation, the enhancement of MAO-induced DA release with sevoflurane anesthesia was observed only in young rats. We hypothesized that anesthesia-driven DA release might become apparent only when the DA transporter was reversely activated by MAO. The feedback regulation of DA by reuptake through the DA transporter was more dramatic in young rats than in aged rats (Cruz-Muros et al., 2009; Erixon-Lindroth et al., 2005; Salvatore et al., 2003). Thus, the change in DA would be more robust in young animals during anesthesia by reuptake inhibition. Another possible explanation is that sevoflurane might accelerate MAO-induced reversal of DA release through the DA transporter (Diniz et al., 2007), and the enhancement would be negligible due to the DA transporter decline in the aged rat brain (Cruz-Muros et al., 2009; Salvatore et al., 2003). However, the latter hypothesis does not support the increased DA concentration during anesthesia that occurs without MAO in middle-aged rats.

We found no additive effect of sevoflurane anesthesia on MAO-induced changes in middle-aged rats. Due to maturity and the marked difference in body weight, MAO could possibly be administered at a larger dose in aged rats when given intraperitoneally, if corrected for actual body weight. The effect of MAO on dopaminergic neurons might be modified by pharmacokinetic and pharmacodynamic parameters, which consist of many variables, including aging, body weight, cardiac function, circulatory volume and clearance capacity. Moreover, the effects of MAO and nomifensine on DA release are dependent on the method of administration (Adachi et al., 2001c). Thus, we applied MAO to rats with both an intraperitoneal bolus injection and continuous microdialysis perfusion, the latter of which was independent of animal size. Despite these differences in drug administration, we observed consistent changes in DA and 3-MT during anesthesia, which support our results regarding the effect of MAO.

Oxidative stress is responsible for the major changes that occur in aging (Barja, 2004). Excess release of neurotransmitter is followed by increases in free radicals through sequential oxidative metabolism (Ahlskog, 2005). One of the supplemental drugs for Parkinson’s disease is selegiline, a monoamine oxidase inhibitor, and entacapone, a catechol-o-methyltransferase inhibitor. These
inhibitors suppress metabolism of DA administered as l-Dopa (Adachi et al., 2001a). Although no curative effect in Parkinson’s patients has been demonstrated, these inhibitors do reduce the formation of reactive substances. A reduction in hydrogen peroxide, superoxide anions and hydroxyl radicals could prevent neuronal damage. In our study, neither peri-anesthetic behavioral nor cognitive dysfunction was determined. However, the increase in DA metabolites was consistent in both young and middle-aged rats. We would expect that if neuronal cells were vulnerable and beginning to degenerate with age, additional oxidative stress would be more harmful in aged animals than in young animals.

The in vivo microdialysis experiments used in this study have a series of technical limitations (Adachi et al., 2005a, 2001c; Fink-Jensen et al., 1994; Opica-Juffry et al., 1991), which differ from many in vitro investigations (Adachi et al., 2001b, 2002). The actual concentration or amount of DA and its metabolites in the brain could not be measured, because the recovery rate of neurotransmitters in the perfuse from the extracellular space into the dialyzing probe through the membrane was not predetermined. However, any alterations in DA recovery or release that might have occurred in the current study were consistent in all experiments and with previous reports (Adachi et al., 2005a,b, 2001c). Moreover, non-synaptic communication and chemical messaging through the extracellular space are the primary controls of brain function (Vizi et al., 2010). Neurotransmission could be considered a long pathway in the brain; e.g., inter-hemispheric connections (Lieu and Subramanian, 2012) and direct intranuclear absorption (Chao et al., 2012). We could not separate the effect of sevoflurane anesthesia on central nervous system function from that on altered consciousness, the subsequent analgesia or the nature of sleep during anesthesia. Thus, further investigations are required.

5. Conclusion

Our findings suggest that aging reduces the sensitivity of the DA transporter to sevoflurane anesthesia and increases the extracellular DA concentration. The risk of anesthesia-induced apoptotic neurodegeneration in the developing brain has been of interest recently (Jevtovic-Todorovic et al., 2003). Thus, the increased extracellular DA concentration during anesthesia may damage neurons in aged animals in a manner similar to that seen in neonates (Satomo et al., 2009).

Conflict of interest

None declared.

References


