Increased noradrenaline release from rat preoptic area during and after sevoflurane and isoflurane anesthesia

Noriyuki Anzawa MD,
Tetsuya Kushikata MD,
Hirobumi Ohkawa MD,
Hitoshi Yoshida MD,
Takeshi Kubota MD,
Akitomo Matsuki MD

Purpose: To study the effects of sevoflurane and isoflurane on noradrenaline release from the rat preoptic area (POA).

Method: Sixteen male Wistar rats were studied. A microdialysis probe with a 2 mm long semipermeable membrane was implanted in the POA. Dialysates were collected at intervals of ten minutes. After obtaining five control samples for 50 min, 30 min inhalation of 3% sevoflurane or 1.8% isoflurane was performed. After cessation of the inhalation, five more samples were obtained for 50 min as recovery phase. Noradrenaline (NA) concentration in the dialysates was measured by high pressure liquid chromatography with an electrochemical detector.

Results: Both sevoflurane and isoflurane caused marked increases in NA release from the rat POA (sevoflurane 233% at 20 min, isoflurane 357% at ten minutes after the start of inhalation). The marked NA releases were also observed during the emergence from sevoflurane and isoflurane anesthesia (sevoflurane 269% at 20 min, isoflurane 368% at ten minutes in the recovery phase).

Conclusion: This study suggests that enhanced release of NA in the POA during sevoflurane and isoflurane may explain the excitatory phase observed during the peri-anesthetic period with these agents.

Objectif : *Étudier les effets du sévoflurane et de l'isoflurane sur la libération de noradrénaline en provenance de l'aire préoptique du rat (APO).*

Méthode : *L'étude a porté sur 16 rats mâles Wistar. Une sonde pour microdialyse munie d'une membrane semiperméable de 2 mm de longueur a été implantée dans l'APO. Les dialysats ont été recueillis à intervalles de dix minutes. Après avoir obtenu cinq échantillons témoins de 50 min, on a administré du sévoflurane à 3 % ou de l'isoflurane à 1,8 % pendant 30 min. Une fois l'inhalation stoppée, on a recueilli cinq autres échantillons pendant 50 min considérées comme une récupération. La concentration de noradrénaline (NA) des dialysats a été mesurée par chromatographie liquide haute performance avec un détecteur électrochimique.*

Résultats : *Le sévoflurane et l'isoflurane ont fait beaucoup augmenter la libération de NA de l'APO de rat (le sévoflurane, de 233 % à 20 min, l'isoflurane, de 357 % à dix minutes après le début de l'inhalation). La libération marquée de NA a été aussi observée pendant le réveil (269 % à 20 min avec le sévoflurane et 368 % à dix minutes avec l'isoflurane).*

Conclusion : *Cette étude indique qu'en présence de sévoflurane et d'isoflurane, la libération accrue de NA dans l'APO peut expliquer la phase excitatrice périanesthésique.*

NORADRENERGICneurons in the central nervous system are involved in the regulation of various physiological events including sleep-awake cycle, stress responses, emotion, attention and learning. Much evidence has indicated an association of brain noradrenergic activity with effects of general anesthetics. Ablation of noradrenergic neurons in the brain stem reduces minimum alveolar concentrations of halothane and cyclopropane[1] and our previous studies showed that NA release in the posterior hypothalamus of rats increased during emergence from inhaled anesthesia.[2–4]

The preoptic area (POA) of the anterior hypothalamus is also deeply involved in the regulation of sleep, body temperature and stress responses.[5–11] This region has been suggested as an important site of hypnotic action of general anesthesia, because lesioning and microinjection of clonidine into the POA induce insomnia and arousal,[12–14] while microinjection of a benzodiazepine receptor agonist or pentobarbitone into the POA also induces a hypnotic state in rats.[15,16]

From the Department of Anesthesiology, University of Hirosaki School of Medicine, Hirosaki 036-8562, Japan.
Address correspondence to: Dr. Noriyuki Anzawa, 5 Zaifu-cho, Hirosaki, Aomori-Ken, 036-8562, Japan. Phone: 81-172-39-5111; Fax: 81-172-39-5112.
Accepted for publication January 16, 2001.

Thus it seems relevant to investigate the changes in noradrenergic activity in the POA during and after inhaled anesthesia. However, there is no study concerning the effects of inhaled anesthetics on noradrenaline (NA) release in the POA. We herein report the pattern of NA release in the rat POA during induction and emergence from sevoflurane and isoflurane anesthesia, as these agents have been most widely employed in the clinical situation, using a microdialysis technique.

Method and subjects

The study was approved by the animal experiment committee of our institution. Sixteen male Wistar rats (Japan Clea, Kyoto, Japan) weighing 280–320 g were used for the study. They were housed for at least a week before the experiment. They were kept in a 12 hr light-dark cycle environment (lights on 8:00 a.m. to 8:00 p.m.) at a temperature of 22–24°C and with a humidity level of 40%. They could access food and water freely. All experiments were conducted from 10:00 a.m. to 3:00 p.m because of the circadian rhythm of NA.

Rats were mounted on a stereotaxic frame under pentobarbitone anesthesia (50 $mg \cdot kg^{-1}$ *ip*). A stainless guide cannula was stereotaxically implanted unilaterally into the POA with the following coordinates (A: -0.92, L:2.5 at an angle of 11, V: 7.0 mm) in relation to the bregma, according to the atlas by Paxinos and Watson.[17] The cannula was fixed to the skull with dental resin and stainless steel screws. After each experiment, location of the probe was verified by histologic examination.

Forty-eight hours were allowed for rats to recover from the influence of the guide cannula implantation and a probe (A-I-12-2, Eicom, Kyoto, Japan) with a 2-mm long semipermeable membrane tip was inserted through the guide cannula. The dialysis probe was perfused at a rate of 2 $\mu L \cdot min^{-1}$ with an artificial cerebrospinal fluid solution (NaCl 128 mM; KCl 2.6 mM; $CaCl_2$ 1.3 mM; $MgCl_2$ 0.9 mM; $NaHCO_3$ 20 mM; Na_2HPO_4 1.3 mM) containing 1 mM pargyline. The latter was included to prevent degradation of NA. Dialysates were collected every ten minutes during the experiment.

Each rat was introduced into a plastic housing 40 cm in diameter. After obtaining five control samples during 50 min, 3% sevoflurane or 1.8% isoflurane in air was administered in the housing at a flow rate of 4 $L \cdot min^{-1}$ for 30 min. Three percent sevoflurane and 1.8% isoflurane correspond with approximate 1.3 minimum alveolar concentration, respectively.[18,19] During this period a heating pad was applied to prevent hypothermia. After the end of the inhalation period, five more samples were taken for 50 min of the recovery period. Calibrated Forawick vaporizers (Muraco medical Co., Tokyo, Japan) were used to vaporize sevoflurane and isoflurane, and a Capnomac™ (Datex, IMI, Koshigaya Japan) was used to monitor concentrations of inhalational anesthetics, oxygen and carbon dioxide in the housing throughout the experimental procedure.

NA was measured by a high-performance liquid chromatograph equipped with an electrochemical detector. The samples (18 μL^{-1}) were injected manually into ODS-C18 reverse-phase column (2.1 x 150 mm CA-5ODS: Eicom, Kyoto, Japan) maintained at 25C. The mobile phase was made from 0.1 M phosphate buffer (pH 6) containing EDTA 200 $mg \cdot L^{-1}$, 1-octanesulfonate 400 $mg \cdot L^{-1}$ and 5% methanol. The flow rate of the buffer was 220 $\mu L \cdot min^{-1}$ and the oxidation potential of the graphite electrode was set at +400 mV against an Ag/AgCl reference electrode (ECD-300, Eicom, Kyoto, Japan). The detection limit of the assay was 300 $fg \cdot 18\ \mu L^{-1}$. The detector response was linear beyond the range of our measurements.

All values obtained were expressed as mean ± SD. Repeated measured ANOVA and Scheffe F test were used for analysis of NA values. A value of $P < 0.05$ was considered significant.

Results

The NA concentrations during the control period were similar in both groups. In the sevoflurane group, NA concentrations increased during anesthesia, as

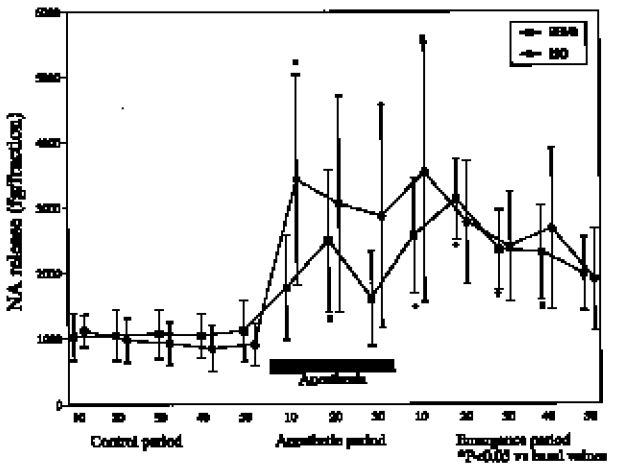

FIGURE Noradrenaline (NA) release during anesthesia with sevoflurane and isoflurane. Each value indicates the mean ± SD for eight rats. * indicates statistical difference from basaline values.

shown in the Figure. The value at 20 min after the start of inhalation was significantly increased (P <0.01) compared to baseline. NA concentrations started to decrease 30 min after the beginning of the anesthetic. NA concentrations were again increased at ten and 20 min in the emergence period (P <0.01 for ten and 20 min values). Thereafter they were followed by a gradual decrease, even though they remained high at 30 and 40 min (P <0.05).

In the isoflurane group, NA concentrations increased immediately after inhalation of isoflurane. The value at ten minutes after the start of inhalation was significantly increased (P <0.05). NA concentrations started to decrease 20 min after the beginning of inhalation. NA concentrations increased again ten minutes (P <0.05) during the emergence period.

Discussion

The main finding of the present study is that both isoflurane and sevoflurane significantly increased NA release from the POA during induction and emergence from anesthesia. Our previous studies demonstrated that NA release in the posterior hypothalamus increased during the emergence from halothane, isoflurane and sevoflurane anesthesia.[2–4] In contrast, such an increase in NA is not observed during the recovery from *iv* anesthetics as demonstrated by Mizuno *et al.* who reported that NA release in the POA decreased by 40–50% of basal release after 35 $mg \cdot kg^{-1}$ pentobarbitone *ip.*[20] A slight decrease in NA release in the medial prefrontal cortex was reported during propofol and midazolam anesthesia by Kubota *et al.*; however, the reduction did not return to the preanesthetic level even in the recovery phase of anesthesia.[21] Ketamine has unique effects on NA release in the brain. We have already reported that ketamine appreciably increased NA release from the medial prefrontal cortex during the anesthetic and recovery phases in rats.[22] The NA release following emergence from ketamine anesthesia was much higher than that observed during anesthesia itself. The peak effect was observed 40–50 min after the recovery from anesthesia, and this phase coincided with the excitatory phase elicited by ketamine. Consequently, the increase in NA release may contribute to the appearance of excitation after ketamine anesthesia.

NA release in the POA is enhanced during excitation induced by various stresses such as heat stress.[8] Clinically, inhalational anesthetics often induce excitation during induction and emergence from anesthesia. However, *iv* anesthetics such as propofol and midazolam, which do not enhance NA release in the brain, are reported to cause no excitement. As a high incidence of agitation is observed during sevoflurane induction in children,[23] the marked increase in NA release in this study could provide a possible explanation for the excitation induced by inhalational anesthesia, inasmuch as our results may be extrapolated to humans.

The noradrenergic neurons in the POA are involved in the regulation of the autonomic nervous system and it is possible that an increased NA release in the POA is also associated with activation of sympathetic activity. In support of this hypothesis, Poole reported that the microinjection of NA into POA in rats increases arterial pressure.[24]

The POA receives major noradrenergic innervation from the locus coeruleus (LC) and the other cell groups in the medula oblongata and pons. Noradrenergic neurons in the LC are strongly activated during recovery from halothane anesthesia.[25] This response is due to an increase in excitatory amino acid input into the LC. Further, a similar response occurs during the excitatory phase following opioid withdrawal.[26] Consequently, we could postulate that sevoflurane and isoflurane stimulate noradrenergic neurons in the LC leading to activation of noradrenergic neurons in the POA to induce the excitation observed with these agents.

Another possibility for volatile anesthetics to cause enhanced NA release is due to direct effect on the neuronal terminal. A neural cell culture study demonstrated that clinical concentrations of volatile anesthetics including halothane, enflurane, isoflurane and methoxyflurane stimulate phorbol ester evoked NA release by an increase in cytoplasmic Ca^{++} concentrations.[27] This study suggests that volatile anesthetics can directly affect the neuronal cell to induce neurotransmitter release. It is possible that our findings reflect not only indirect effect on POA function via LC but also a direct pharmacological action of inhaled anesthetics on the POA neurons. However, we need a more detailed study to clarify this point.

In conclusion, sevoflurane and isoflurane induce enhanced NA release from the POA during induction and recovery from anesthesia in rats. This NA release may help explain the excitatory phase observed during the peri-anesthetic period with these agents.

References

1 *Roizen MF, White PF, Eger EI II, Brownstein M.* Effects of ablation of serotonin or norepinephrine brain- stem areas on halothane and cyclopropane MACs in rats. Anesthesiology 1978; 49: 252–5.

2 *Chave S, Kushikata T, Ohkawa H, Ishiara H, Grimaud D, Matsuki A.* Effects of two volatile anesthetics (sevoflurane and halothane) on the hypothalamic noradrenaline release in rat brain. Brain Res 1996; 706: 293–6.

3 *Ohkawa H, Kushikata T, Satoh T, Hirota K, Ishihara H, Matsuki A.* Posterior hypothalamic noradrenaline release during emergence from sevoflurane anesthesia in rats. Anesth Analg 1995; 81: 1289–91.
4 *Kushikata T, Anzawa N, Yoshida H, Hirota K.* Isoflurane anesthesia influences norepinephrinergic neuronal activity in the rat anterior and posterior hypothalamus. JSPA 1997; 10: 3–8.
5 *Koyama Y, Hayaishi O.* Firing of neurons in the preoptic/anterior hypothalamic areas in rat: its possible involvement in slow wave sleep and paradoxical sleep. Neurosci Res 1994; 19: 31–8.
6 *Kumar VM, Sharma R, Wadhwa S, Manchanda SK.* Sleep-inducing function of noradrenergic fibers in the medial preoptic area. Brain Res Bull 1993; 32: 153–8.
7 *Szymusiak R, McGinty D.* Sleep-waking discharge of basal forebrain projection neurons in cats. Brain Res Bull 1989; 22: 423–30.
8 *Kendrick KM, De La Riva C, Hinton M, Baldwin A* Microdialysis measurement of monoamine and amino acid release from the medial preoptic region of the sheep in response to heat exposure. Brain Res Bull 1989; 22: 541–4.
9 *Pacak K, Palkovits M, Kvetnansky R, Kopin IJ, Goldstein DS.* Stress-induced norepinephrine release in the paraventricular nucleus of rats with brainstem hemisections: a microdialysis study. Neuroendocrinology 1993; 58: 196–201.
10 *Yokoo H, Tanaka M, Tanaka T, Tsuda A* Stress-induced increase in noradrenaline release in the rat hypothalamus assessed by intracranial microdialysis. Experientia 1990; 46: 290–2.
11 *Yokoo H, Tanaka M, Yoshida M, Tsuda A, Tanaka T, Mizoguchi K.* Direct evidence of conditioned fear-elicited enhancement of noradrenaline release in the rat hypothalamus assessed by intracranial microdialysis. Brain Res 1990; 536: 305–8.
12 *John J, Kumar VM, Gopinath G, Ramesh V, Mallick H.* Changes in sleep- wakefulness after kainic acid lesion of the preoptic area in rats. Japanese Journal of Physiology 1994; 44: 231–42.
13 *John J, Kumar VM.* Effect of NMDA lesion of the medial preoptic neurons on sleep and other functions. Sleep 1998; 21: 587–98.
14 *Ramesh V, Kumar VM, John J, Mallick H.* Medial preoptic alpha-2 adrenoceptors in the regulation of sleep-wakefulness. Physiol Behav 1995; 57: 171–5.
15 *Mendelson WB.* Sleep induction by microinjection of pentobarbital into the medial preoptic area in rats. Life Sci 1996; 59: 1821–8.
16 *Mendelson WB, Martin JV.* Characterization of the hypnotic effects of triazolam microinjections into the medial preoptic area. Life Sci 1992; 50: 1117–28.
17 *Paxinos G, Watson C.* The Rat Brain in Stereotaxic Coordinates, 2nd ed. San Diego: Academic Press, 1986.
18 *Eger II EI, Johnson BH.* Rates of awakening from anesthesia with I-653, halothane, isoflurane, and sevoflurane: a test of the effect of anesthetic concentration and duration in rats. Anesth Analg 1987; 66: 977–82.
19 *Crawford MW, Lerman J, Saldivia V, Carmichael FK.* Hemodynamic and organ blood flow responses to halothane and sevoflurane anesthesia during spontaneous ventilation. Anesth Analg 1992; 75: 1000–6.
20 *Mizuno T, Ito E, Kimura F.* Pentobarbital sodium inhibits the release of noradrenaline in the medial preoptic area in the rat. Neurosci Lett 1994; 170: 111–3.
21 *Kubota T, Hirota K, Yoshida H, et al.* Effects of sedatives on noradrenaline release from the medial prefrontal cortex in rats. Psychopharmacology 1999; 146: 335–8.
22 *Kubota T, Hirota K, Anzawa N, Yoshida H, Kushikata T, Matsuki A.* Physostigmine antagonizes ketamine-induced noradrenaline release from the medial prefrontal cortex in rats. Brain Res 1999; 840:175–8.
23 *Constant I, Dubois M-C, Piat V, Moutard M-L, McCue M, Murat I.* Changes in electroencephalogram and autonomic cardiovascular activity during induction of anesthesia with sevoflurane compared with halothane in children. Anesthesiology 1999; 91: 1604–15.
24 *Poole* S. Cardiovascular responses of rats to intrahyothalamic injection of carbachol and noradrenaline. Br J Pharmacol 1983; 79: 693–700.
25 *Saunier CF, Akaoka H, de La Chapelle B, et al.* Activation of brain noradrenergic neurons during recovery from halothane anesthesia. Anesthesiology 1993; 79: 1072–82.
26 *Airio J, Atee L.* The involvement of noradrenergic transmission in the morphine-induced locomotor hyperactivity in mice withdrawn from repeated morphine treatment. Br J Pharmacol 1999; 126: 1609–19.
27 *Tas PWL, Koschel K.* Volatile anesthetics stimulate the porbol ester evoked neurotransmitter release from PC12 cell through an increase of the cytoplasmic Ca^{2+} ion concentration. Biochim Biophys Acta 1991; 1091: 401–4.