Biochemical Pharmacology, Vol. 58, pp. 1955–1959, 1999.
© 1999 Elsevier Science Inc. All rights reserved.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00281-6

SHORT COMMUNICATION

Drastic Increase in Nitric Oxide Content in Rat Brain under Halothane Anesthesia Revealed by EPR Method

Nikolajs Sjakste, Larisa Baumane, Dainuvīte Meirena, Lāsma Ļauberte, Maija Dzintare and Ivars Kalviņš

Latvian Institute of Organic Synthesis, Riga LV1006, Latvia

ABSTRACT. A drastic increase in nitric oxide (NO) content was revealed by the EPR method in rat brain cortex and cerebellum under halothane anesthesia. The NO scavenger diethyldithiocarbamate sodium salt (DETC) and ferrous citrate were injected into adult rats 30–60 min before anesthesia. Rats were anesthetized by inhalation of a halothane–oxygen mixture (1%, 1.5%, 2%, or 4%). After different times of anesthesia, rats were decapitated, and brain cortex and cerebellum were dissected, frozen in liquid nitrogen, and subjected to EPR spectroscopy. The concentration of NO was determined from the NO–Fe–DETC radical spectrum. In control animals, NO content in the cerebellum was only 68% of that in the cortex. We observed a time-dependent increase in NO content in the cortex and cerebellum of rats anesthetized with 1.5% halothane. In brain cortex, the NO level increased to six times that of waking animals after 30 min and remained at this level up to 60 min of anesthesia. In cerebellum the changes were less drastic, the NO level showing only a 2-fold increase. The same effect was produced by 1% and 2% halothane. Ketamine, chloral hydrate, and pentobarbital were used as reference drugs. None of these anesthetics produced effects similar to those of halothane. In ketamine-anesthetized rat brain, the NO content slightly decreased. Pentobarbital and chloral hydrate produced an insignificant increase in NO. Data are discussed in the context of possible interference of halothane in the regulation of nitric oxide synthase activity. BIOCHEM PHARMACOL **58**;12:1955–1959, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. spin trapping; nitric oxide; anesthetics; halothane

NO† plays a wide variety of roles in the central nervous system [1]. It was discovered recently that NO is a neurotransmitter (see [2] for review), and it is known to mediate NMDA receptor-associated neurotoxicity (see [3, 4] for review). Very early, it was supposed that halothane, one of the most widely used anesthetics, interferes with the action of NO. Inhibitors of NOS acted synergistically with halothane and decreased the MAC for anesthetics [5]. It was postulated that halothane depresses neurotransmission by L-glutamate and NMDA, thereby inhibiting NO production from the vascular endothelium, and decreases cyclic GMP content in brain. There is evidence of the direct action of halothane on NOS and NO production obtained in simple model systems [6, 7]. More detailed studies revealed that halothane suppressed NMDA-stimulated formation of cyclic GMP [8, 9]. Numerous biochemical and pharmacological studies have attempted to explore the fine mechanisms of inhibition of NO-mediated neurotransmission by halothane [10, 11]. The results of these investigations, however, appear to be extremely contradictory [12, 13]. Study of halothane effects on NOS gene expression revealed an up-regulation, but no down-regulation, as one would expect [14]. The interference of halothane with NOS inhibitor action at the physiological level seems to be evident to some researchers [15], but others consider it extremely complicated and to involve many factors [16]. Halothane by itself produces vasodilatation of brain blood vessels [17, 18] and brain hyperemia [19].

In our opinion, direct measurements of NO in brain of organisms under anesthesia were necessary to clarify the situation. Therefore, we undertook a study of NO production in rat brain during halothane anesthesia. NO production was measured by an EPR method elaborated by Vanin *et al.* and others [20–22]. This method is based on the trapping of NO in a stable complex with iron ion and DETC and detecting its spectrum by means of EPR spectroscopy. The approach has been widely used for NO detection in different systems, including mammal brain tissues [20–25].

* Corresponding author: Prof. Nikolajs Sjakste, Biochemistry Group, Latvian Institute of Organic Synthesis, Aizkraukles Street 21, Riga LV1006, Latvia. FAX 371-7828114; 371-7821038; 371-7553142; E-mail: sjakste@osi.lanet.lv

† *Abbreviations:* NO, nitric oxide; NOS, nitric oxide synthase; DETC, diethyldithiocarbamate sodium salt; NMDA, *N*-methyl-D-aspartate; and MAC, minimal alveolar concentration.

Received 24 November 1998; accepted 22 June 1999.

MATERIALS AND METHODS

Chemicals

DETC, ferrous sulfate, and sodium citrate were from Sigma. Halothane was purchased from Laboratories Belamont. Ketamine was from Alfasan. Xylazine was from Dopharma. Sodium pentobarbital was from Veterinaria AG. Chloral hydrate was from Fluka.

Animal Treatment and Anesthesia

Animals were purchased from Grindex. All manipulations with animals were performed according to Latvian legislation. Male Wistar rats weighting 200–300 g were used in experiments. They were anesthetized either by inhalation of halothane in oxygen (1; 1.5; 2; 4%), using the Cyprane Fluotec rodent ventilator (Cyprane Ltd.), or by intraperitoneal injections of ketamine (100 mg/kg) with myorelaxant xylazine (5 mg/kg), pentobarbital (60 mg/kg), or chloral hydrate (300 mg/kg). In order to keep body temperature constant during anesthesia, the animals were placed on a heated support. The body temperature during narcosis was constantly monitored with a rectal thermometer. The gas flux was kept constant throughout the administration of halothane.

Administration of Spin Trap Agents

To determine NO content in brain tissue, we used the protocol originally elaborated by Vanin *et al.* [20] and later modified for peculiarities of brain tissue [23]. Rats were injected intraperitoneally with 400 mg/kg of the NO scavenger DETC and ferrous citrate (40 mg/kg ferrous sulfate + 200 mg/kg sodium citrate subcutaneously) 30–60 min before decapitation.

Sacrifice, Brain Dissection, and Sample Preparation

After the animals had remained under anesthesia for the required time, they were decapitated. Brains were removed and cooled at −20° for 3 min. Brain cortex and cerebellum were dissected. Brain tissue was extruded through a 3-mL plastic syringe to a glass tube (2.5 mm in diameter, 30 mm in height), frozen in liquid nitrogen, and subjected to EPR spectroscopy. We used standard tubes with fixed volume that were always completely filled with tissue slurry, which enabled us to standardize the amount of tissue used in our experiments. Samples were prepared rapidly without any delay, which also enabled us to standardize the time taken to prepare samples, which was equal for all samples (10 min).

EPR Measurements

Tubes with samples were placed in EPR spectrometer quartz duar. Relative NO content in the samples was evaluated by comparing the intensity of the NO signal with a standard signal intrinsic to the duar. EPR spectra were recorded in liquid nitrogen using the EPR spectrometer Radiopan SE/X2544. Conditions of EPR measurements were: 2.5 mW microwave power, 9.24 GHz microwave frequency, 100 kHz modulation frequency, 0.4 mT modulation amplitude, and 2.5×10^5 receiver gain.

Statistical Analysis

Significance of differences between the groups (waking animals and animals under anesthesia, or groups differing in concentration of anesthetic or time under anesthesia) was evaluated according to Student's *t*-test ($P < 0.05$).

RESULTS AND DISCUSSION

Figure 1 presents the spectra of NO in rat brain tissues. The spectra all have a typical shape of the Cu–DETC spectrum with a superposed NO peak. The intensity of the Cu–DETC spectrum indicates the bioavailability of the tissue under study to DETC. We regarded the height between the maximum of the peak at g = 2.047 and the trough at g = 2.025 as the size of the NO–Fe–DETC signal (I_0) [25]. Our spectra reproduce very well previously published spectra of NO–Fe–DETC in brain tissues [20–25]. To determine the background NO level in rat brain, rats were injected with the NO scavenger DETC and ferrous citrate and sacrificed 30 min later. The average signal intensity in the cortex of these animals will be referred to as 100% for further determinations (100 ± 37; N = 15). NO content in cerebella of animals from the same group was lower, 68 ± 19; N = 15. In measurements made after longer time intervals following scavenger administration (45 or 60 min), the intensity of the NO signal remained within the same range after 45 and 60 min (100 ± 31; N = 6 and 146 ± 80; N = 6, respectively). After a shorter time (15 min) the signal was very weak albeit detectable. These differences must have been due to DETC pharmacokinetics and, in order to avoid their influence in our experiments, we determined NO content in brain within a time range of 30 and 60 min after DETC administration.

To study the influence of halothane anesthesia on NO content in rat brain, rats were anesthetized with 1.5% halothane. Animals were sacrificed at different time intervals after starting narcosis. The NO scavenger was injected 60 min before decapitation. After 5 min of narcosis, the NO content in brain cortex had already doubled and by 30 min it had increased six-fold, remaining at this level for up to 60 min of anesthesia. The difference in the EPR spectra of waking and halothane-anesthetized animals is clearly seen in Fig. 1 (compare spectra *a* and *e*, *a′* and *e′*): the height between the maximum peak at g = 2.047 and the trough at g = 2.025 as the size of (I_0) is six times more intensive in the cortex preparation of halothane-anesthetized rats (*e*) than that of waking rats (*a*). In Fig. 2, data are presented in quantitative form.

The dependence of NO content in brain cortex on

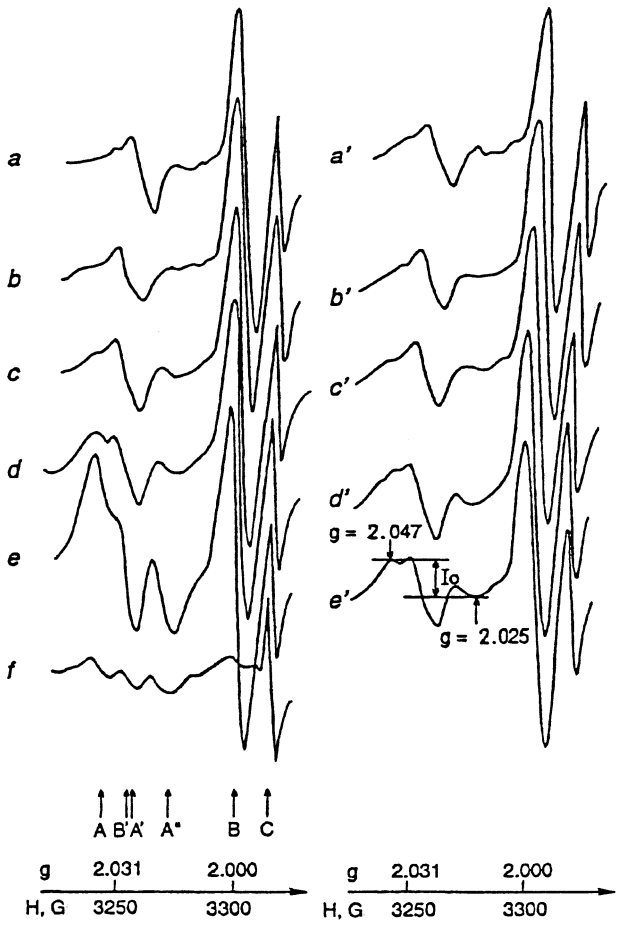

FIG. 1. EPR signals obtained from the brain cortex *(a–e)* and cerebellum *(a′–e′)* during various anesthetic regimens: *a, a′*-waking animals; *b, b′*-ketamine (100 mg/kg, 30 min); *c, c′*-chloral hydrate (300 mg/kg, 30 min); *d, d′*-pentobarbital (60 mg/kg, 30 min); *e, e′*-halothane (1.5%, 30 min), *f*-NO–Fe–DETC signal without Cu–DETC signal. A, A′, A″: signals from the NO–Fe–DETC complex; *B, B′*: signals from Cu–DETC complex; C: standard signal. Conditions of EPR spectroscopy: 2.5 mW microwave power, 0.4 mT modulation amplitude, 9.24 GHz microwave frequency, and 100 kHz modulation frequency, 2.5×10^5 receiver gain. All rats were administered with DETC (400 mg/kg) ferrous citrate (40 mg/kg Fe_2SO_4 + 200 mg/kg sodium citrate subcutaneously) 60 min before decapitation. g = g factor and H = tension of magnetic field.

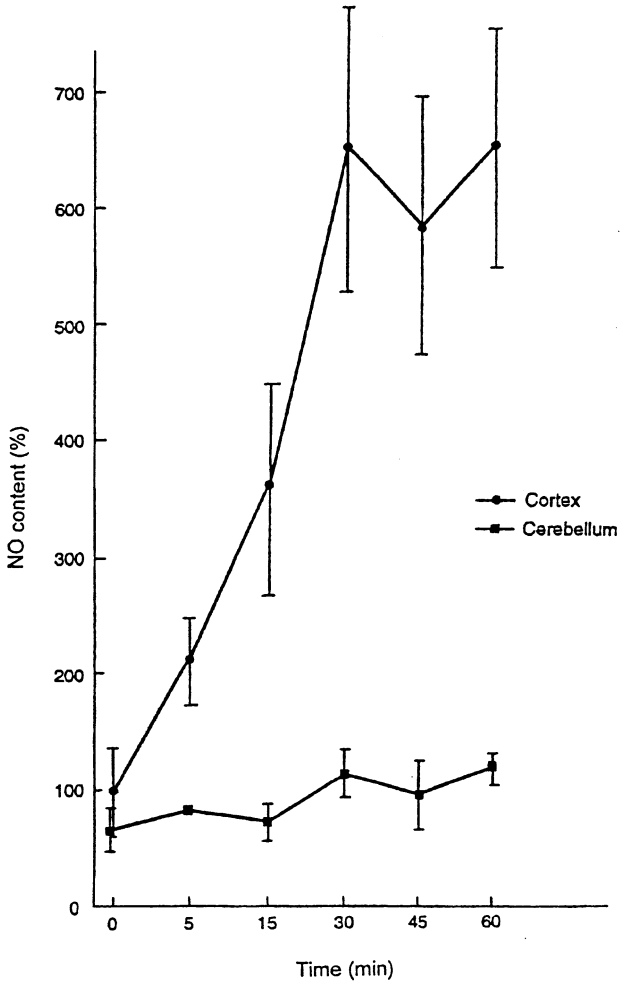

FIG. 2. Nitric oxide content in rat brain cortex and cerebellum during development of halothane anesthesia (1.5%). Data shown are the means ± SD, N = 4–15. Abscissa: time, minutes; ordinate: NO content, percentage of the mean value in the cortex of waking animals.

halothane concentration was evaluated in animals subjected for 45 min to different concentrations of anesthetic. Anesthesia with 1% halothane increased NO content up to 669 ± 166; N = 10, and 1.5% and 2% halothane raised it to 585 ± 105; N = 11 and 546 ± 51; N = 6, respectively. Apparently, in this range of concentrations, NO content in brain cortex does not depend on halothane concentration, as differences in NO content are not statistically significant. We were not interested in lower concentrations, as the lowest of the studied concentrations was close to MAC for halothane (0.7%) and a decrease in the anesthetic concentration made it difficult to achieve narcosis.

Time was necessary to obtain a significant increase in NO content. After 1 min of anesthesia, there was only a tendency for NO increase in the cortex (131 ± 44; N = 14), although a high concentration of halothane (4%) was applied to achieve anesthesia in this short time interval. In cerebellum, halothane anesthesia produced less pronounced changes in NO content. After 1-min anesthesia produced by 4% halothane, the increase in the NO content was not yet significant, 76 ± 16%; N = 5 compared to 68 ± 19; N = 15. During anesthesia with 1.5% halothane, a 1.5-fold increase in NO content was achieved within 30–60 min (Fig. 2). The effect was more pronounced with higher concentrations of anesthetic: after 45 min with 2% halothane, NO in cerebellum rose to 271 ± 32; N = 3. Thus, halothane anesthesia produces a drastic increase in NO content in brain cortex and a similar, although less pronounced, effect in cerebellum. In order to elucidate whether this effect was due to the drug action or the result of the anesthesia itself, we compared the action of halothane with that of other anesthetics with different chemical structures and mechanisms of action.

CHLORAL HYDRATE. Under chloral hydrate action, NO increased slightly in brain cortex 30 min after administration (178 ± 49; N = 4). No effect was detected in cerebellum (55 ± 8; N = 4).

KETAMINE. After 30 min, ketamine action did not produce any changes in NO content in the cortex (102 ± 8; N = 4) and manifested a tendency to decrease 30 min later (51 ± 22; N = 4). In cerebellum, NO content remained unchanged 30 min after ketamine administration (70 ± 26; N = 4), decreasing 30 min later (45 ± 5; N = 4).

PENTOBARBITAL. Anesthesia with this barbiturate slightly increased the NO content in brain cortex (137 ± 12; N = 3) and cerebellum (84 ± 15; N = 3).

Thus, during anesthesia produced by three different reference drugs, we did not observe any drastic increase in NO–Fe–DETC formation during halothane action. We conclude that halothane greatly increases NO content in brain. This effect is apparently due to an increase in NO production rather than to an accumulation of the NO–Fe–DETC complexes over time. In models similar to ours, a rapid decline in the signal intensity has been observed in the course of brain ischemia [22]. This would not be possible if the complexes were accumulated in the brain. It should be pointed out that the observed increase in intensity of the NO–Fe–DETC complex EPR spectrum indeed reflects changes in NO concentration in rat brain. Although some reports indicate that the Fe–DETC complex can reduce nitrite to nitric oxide [26], it is unlikely that the Fe–DETC complex can interact with charged nitrite in living tissue, as the complex is highly hydrophobic and compartmentalized in biomembrane fraction. Special studies [27] confirm this point of view. The increase in the EPR signal in rat brain under halothane anesthesia might also be due to an increase of Fe–DETC accessibility to NO under action of the drug. Although difficult to test experimentally, this hypothesis seems to be unlikely. We suggest that either halothane acts as a catalyst of the complex formation—and this hypothesis has no chemical background—or that it facilitates DETC transport to the brain. In the latter case, we should have observed a stronger signal in cerebellum, since it contains more neuronal NOS [2]. Moreover, it was shown that under experimental conditions analogous to ours, the Fe–DETC trap was nearly equally distributed throughout the body and was not a limiting factor for the trapping of NO under either basal or stimulated conditions [28]. DETC concentration in tissues is 20- to 200-fold higher than NO concentration [29]. Cu–DETC spectra in our experiments also indicated that we had a large excess of Fe–DETC complex over NO. Possible modification of blood–brain barrier permeability during narcosis also cannot change much Fe–DETC concentration in brain, as even osmotic disruption of the barrier did not influence it [30].

We believe that together with indirect data indicating involvement of NO production in halothane action [17, 19, 31–33] our results indicate increased production of NO under halothane anesthesia. The mechanism of the observed phenomenon is still to be elucidated. Halothane might activate NOS directly, as has been observed in some studies [9]. The observed increase in NO concentration in brain tissue might also be due to activation of endothelial NOS, as halothane possesses a vasodilatating effect that is mediated by endothelial NOS [17, 19, 32, 34, 35]. Disturbance of energy metabolism in brain under halothane action [36–39] can provoke ischemization of brain tissues and trigger NO production [22–25]. Our data comparing the action of several anesthetics are consistent with previous reports of an attenuation of the increase in arterial pressure provoked by NOS inhibitors under halothane narcosis, a phenomenon that is not observed under the action of other anesthetic agents (ketamine, barbiturates, isoflurane, and others) [40, 41]. In this case, increased NO content under halothane anesthesia should have equilibrated the decrease in NO production due to action of the NOS inhibitor, as other anesthetics could not produce this effect.

This work was supported by a grant from the Latvian Council of Science. We thank Dr. R. Johns (University of Virginia, Charlottesville), Prof. A.F. Vanin (Institute of Chemical Physics, Moscow), and Dr. M. Vēveris for reading the manuscript and helpful comments, and Dr. A. Kleschyov (INSERM U392, Université Louis Pasteur, Faculté de Pharmacie, Strasbourg) for his advice. We thank Mrs. A. Kropfinger for editing the manuscript.

References

1. Knowles RG and Moncada S, Nitric oxide synthases in mammals. *Biochem J* **298:** 249–258, 1994.
2. Garthwaite J and Boulton CL, Nitric oxide signaling in the central nervous system. *Annu Rev Physiol* **57:** 683–706, 1995.
3. Dawson VL and Dawson TM, Nitric oxide neurotoxicity. *J Chem Neuroanat* **10:** 179–190, 1996.
4. Moncada S, Palmer RMJ and Higgs EA, Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol Rev* **43:** 109–142, 1991.
5. Johns RA, Moscicki JC and DiFazio CA, Nitric oxide synthase inhibitor dose-dependently and reversibly reduces the threshold for halothane anesthesia. A role for nitric oxide in mediating consciousness? *Anesthesiology* **77:** 779–784, 1992.
6. Galley HF, Nelson LR and Webster NR, Anesthetic agents decrease the activity of nitric oxide synthase from human polymorphonuclear leucocytes. *Br J Anaesth* **75:** 326–329, 1995.
7. Tobin JR, Martin LD, Breslow MJ and Traystman RJ, Selective anesthetic inhibition of brain nitric oxide synthase. *Anesthesiology* **81:** 1264–1269, 1994.
8. Terasako K, Nakamura K, Miyawaki I, Toda H, Kakuyama M and Mori K, Inhibitory effects of anesthetics on cyclic guanosine monophosphate (cGMP) accumulation in rat cerebellar slices. *Anesth Analg* **79:** 921–926, 1994.
9. Rengasamy A, Pajewski TN and Johns RA, Inhalational anesthetic effects on rat cerebellar nitric oxide and cyclic guanosine monophosphate production. *Anesthesiology* **86:** 689–698, 1997.
10. Zuo Z, Tichotsky A and Johns RA, Halothane and isoflurane

inhibit vasodilatation due to constitutive but not inducible nitric oxide synthase. Implications for the site of anesthetic inhibition of the nitric oxide/guanylyl cyclase signaling pathway. *Anesthesiology* **84:** 1156–1165, 1996.
11. Iranami H, Hatano Y, Tsukiyama Y, Maeda H and Mizumoto K, A beta-adrenoreceptor agonist evokes a nitric oxide–cGMP relaxation mechanism modulated by adenylyl cyclase in rat aorta. Halothane does not inhibit this mechanism. *Anesthesiology* **85:** 1129–1138, 1996.
12. Rengasamy A, Ravichandran LV, Reikersdorfer CG and Johns RA, Inhalational anesthetics do not alter nitric oxide synthase activity. *J Pharmacol Exp Ther* **273:** 599–604, 1995.
13. Tagliente TM, Royal BC, Beasley J and Maayani S, A modified citrulline assay of NOS activity in rat brain homogenates does not detect direct effects of halothane on the kinetics of NOS activity. *Brain Res* **767:** 393–396, 1997.
14. Zuo Z and Johns RA, Inhalational anesthetics up-regulate constitutive and lipopolysaccharide-induced inducible nitric oxide synthase expression and activity. *Mol Pharmacol* **52:** 606–612, 1997.
15. Sigmon DH, Florentino-Pineda I, van Dyke RA and Beierwaltes WH, Halothane impairs the hemodynamic influence of endothelium-derived nitric oxide. *Anesthesiology* **82:** 135–143, 1995.
16. Chelly JE, Doursout MF, Lechevalier T, Liang YY, Chelly F, Hartley C and Kilbourn RG, Cardiac and regional hemodynamic interactions between halothane and nitric oxide synthase activity in dogs. *Anesthesiology* **85:** 142–149, 1996.
17. Harkin CP, Hudetz AG, Schmeling WT, Kampine JP and Farber NE, Halothane-induced dilatation of intraparenchymal arterioles in rat brain slices: A comparison to sodium nitroprusside. *Anesthesiology* **86:** 885–894, 1997.
18. Ogawa K, Yamamoto M, Mizumoto K and Hatano Y, Volatile anesthetics attenuate hypocapnia-induced constriction in isolated dog cerebral arteries. *Can J Anaesth* **44:** 426–432, 1997.
19. Smith JJ, Hudetz AG, Bosnjak ZJ and Kampine JP, The role of nitric oxide in cerebrocortical laser Doppler flow response to halothane in the rat. *J Neurosurg Anesthesiol* **7:** 187–195, 1995.
20. Mikoian VD, Kubrina LN and Vanin AF, Detection of the generation of nitric oxide from L-arginine in the murine brain *in vivo* using EPR. *Biofizika* **39:** 915–918, 1994.
21. Henry Y, Lepoivre M, Drapier JC, Ducrocq C, Boucher JL and Guissani A, EPR characterization of molecular targets for NO in mammalian cells and organelles. *FASEB J* **7:** 1124–1134, 1993.
22. Sato S, Tominaga T, Ohnishi T and Ohnishi T, Electron paramagnetic resonance study on nitric oxide production during brain focal ischemia and reperfusion in rat. *Brain Res* **647:** 91–96, 1994.
23. Shutenko Z, Henry Y, Pinard E, Seylaz J, Potier P, Berthet F, Girard P and Sercombe R, Influence of the antioxidant quercetin *in vivo* on the level of nitric oxide determined by EPR in rat brain during global ischemia and reperfusion. *Biochem Pharmacol* **57:** 199–208, 1999.
24. Kuppusamy P, Ohnishi ST, Numagami Y, Ohnishi T and Zweier JL, Three-dimensional imaging of nitric oxide production in rat brain subjected to ischemia-hypoxia. *J Cereb Blood Flow Metab* **15:** 899–903, 1995.
25. Olesen SP, Müller A, Mordvintcev PI, Busse R and Mülsch A, Regional measurements of NO formed *in vivo* during brain ischemia. *Acta Neurol Scand* **95:** 219–224, 1997.
26. Hiramoto K, Tomiyama S and Kikugawa K, Appearance of electron spin resonance signals in the interaction of dithiocarbamate–Fe(II) with nitrogen dioxide and nitrite. *Free Radic Res* **27:** 505–509, 1997.
27. Mülsch A, Vanin A, Mordvintcev P, Hauschild S and Busse R, NO accounts completely for the oxygenated nitrogen species generated by enzymic L-arginine oxygenation. *Biochem J* **288:** 597–603, 1992.
28. Vanin AF and Kleschyov AL, EPR detection and biological implications of nitrosyl non-heme iron complex In: *Nitric Oxide in Allograft Rejection and Anti-Tumour Responses* (Eds. Lukiewicz SJ and Zweier JL), pp. 49–82. Kluwer Academic Publishers, Norwell, MA, 1998.
29. Mülsch A, Mordvintcev P, Bassenge E, Jung F, Clement B and Busse R, *In vivo* spin trapping of glyceryl trinitrate-derived nitric oxide in rabbit blood vessels and organs. *Circulation* **92:** 1876–1882, 1995.
30. Hooper DC, Ohnishi ST, Kean R, Numagami Y, Dietzschold B and Koprowski H, Local nitric oxide production in viral and autoimmune diseases of the central nervous system. *Proc Natl Acad Sci USA* **92:** 5312–5316, 1995.
31. Koenig HM, Pelligrino DA and Albrecht RF, Halothane vasodilatation and nitric oxide in rat pial vessels. *J Neurosurg Anesthesiol* **5:** 264–27, 1993.
32. McPherson RW, Kirsch JR, Moore LE and Traystman RJ, N omega-nitro-L-arginine methyl ester prevents cerebral hyperemia by inhaled anesthetics in dogs. *Anesth Analg* **77:** 891–897, 1993.
33. McCandless DW and Wiggins RC, Cerebral energy metabolism during the onset and recovery from halothane anesthesia. *Neurochem Res* **6:** 1319–1326, 1981.
34. Manohar M and Parks C, Porcine regional brain and myocardial blood flows during halothane-O_2 and halothane-nitrous oxide anesthesia: Comparisons with equipotent isoflurane anesthesia. *Am J Vet Res* **45:** 465–473, 1984.
35. Hansen TD, Warner DS, Todd MM, Vust LJ and Trawick DC, Distribution of cerebral blood flow during halothane versus isoflurane anesthesia in rats. *Anesthesiology* **69:** 332–337, 1988.
36. Savaki HE, Desban M, Glowinski J and Besson MJ, Local cerebral glucose consumption in the rat. I. Effects of halothane anesthesia. *J Comp Neurol* **213:** 36–45, 1983.
37. Shapiro HM, Greenberg JH, Reivich M, Ashmead G and Sokoloff L, Local cerebral glucose uptake in awake and halothane-anesthetised primates. *Anesthesiology* **48:** 97–103, 1978.
38. Johnson GV and Hartzell CR, Halothane-induced alterations of glucose and pyruvate metabolism in rat cerebra synaptosomes. *J Neurochem* **44:** 1838–1844, 1985.
39. Fujibayashi T, Sugiura Y, Yanagimoto M, Harada J and Goto Y, Brain energy metabolism and blood flow during sevoflutane and halothane anesthesia: Effects of hypocapnia and blood pressure fluctuations. *Acta Anaesthesiol Scand* **38:** 413–418, 1994.
40. Wang Y-X, Zhou T, Chua TC and Pang CCY, Effects of inhalation and intravenous anesthetic agents on pressor response to NG-nitro-L-arginine. *Eur J Pharmacol* **198:** 183–188, 1991.
41. Greenblatt EP, Loeb AL and Longnecker DE, Endothelium-dependent circulatory control - a mechanism for the differing peripheral vascular effects of isoflurane versus halothane. *Anesthesiology* **77:** 1178–1185, 1992.