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Inhalational Anesthetic Effects on Rat Cerebellar Nitric Oxide and Cyclic Guanosine Monophosphate Production

Appavoo Rengasamy, Ph.D., Thomas N. Pajewski, M.D., Ph.D.,† Roger A. Johns, M.D.‡*

Background: **Inhalational anesthetics interact with the nitric oxide–cyclic guanosine monophosphate (NO-cGMP) pathway in the central nervous system (CNS) and attenuate excitatory neurotransmitter-induced cGMP concentration. The site of anesthetic action on the NO-cGMP pathway in the CNS remains controversial. This study investigated the effect of inhalational anesthetics on N-methyl-D-aspartate (NMDA)-stimulated NO synthase activity and cyclic cGMP production in rat cerebellum slices.**

Methods: **The interaction of inhalational anesthetics with NO synthase activation and cGMP concentration was determined in cerebellum slices of 10-day-old rats. Nitric oxide synthase activity in cerebellum slices was assessed by measuring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline. The cGMP content of cerebellum slices was measured by radioimmunoassay.**

Results: **Isoflurane at 1.5% and 3% enhanced the NMDA-stimulated NO synthase activity by two times while halothane at 1.5% and 3% produced no significant effect. However, the NMDA-stimulated cGMP production was inhibited by both anesthetic agents. The anesthetic inhibition of cGMP accumulation was not significantly altered by a mixture of superoxide dismutase and catalase or by glycine, a coagonist of the NMDA receptor.**

Conclusions: **The enhancement of NMDA-induced NO synthase activity by isoflurane and the inhibition of NMDA-stimulated cGMP production by halothane and isoflurane suggests that inhalational anesthetics interfere with the neuronal NO-cGMP pathway. This inhibitory effect of anesthetics on cGMP accumulation is not due to either their interaction with the glycine binding site of the NMDA receptor or to the action of superoxide anions. (Key words: Anesthetics, inhalational: halothane; isoflurane. Cell signaling: nitric oxide; cyclic guanosine monophosphate. Neurotransmitters: N-methyl-D-aspartate.)**

NITRIC oxide (NO) is a neuronal messenger that mediates glutamate-stimulated changes in cyclic guanosine monophosphate (cGMP) concentration in the central nervous system. Stimulation of the N-methyl-D-aspartate (NMDA) receptor, a major excitatory neurotransmitter subtype, causes an increase in NO and cGMP in the cerebellum.[1,2] Immunohistochemical staining revealed that NO synthase is densely localized in the molecular and granular layer of cerebellum and several other specific regions of the brain.[3] Nitric oxide is implicated in several aspects of neuronal physiology and pathophysiology, including the release of neurotransmitters, long-term potentiation, long-term depression, regulation of cerebral blood flow, and neurotoxicity in the brain.[1,2]

Several studies suggest that general anesthetics may exert their action at least in part through their interaction with the NO-cGMP pathway in the brain. General anesthetics have been shown to depress excitatory synaptic transmission in the central nervous system. For example, halothane attenuates synaptic transmission by glutamate-stimulated cortical neurons[4] and NMDA-stimulated CA-I neurons of the hippocampus.[5,6] Administration of halothane or pentobarbital to rats caused a decrease in cGMP content of the cerebellum and several other regions of the brain.[7,8] Our studies have shown that the minimum alveolar concentration of halothane or isoflurane in rats is decreased by intravenous administration of the NO synthase inhibitors L-N^G-nitro-L-arginine methyl ester or 7-nitroindazole.[9,10] A subsequent study reported that L-N^G-nitro-L-arginine methyl ester did not alter halothane minimum alveolar concentration.[11] Anesthetic interaction with the NO-cGMP pathway in the brain is evident from other studies in which

* Assistant Professor of Research.

† Assistant Professor.

‡ Professor.

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Address reprint requests to Dr. Johns: Department of Anesthesiology, Box 238, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908. Address electronic mail to: raj2d@virginia.edu

NMDA receptor antagonists decreased the minimum alveolar concentration value for anesthetics.[12,13]

Although inhalational anesthetics have been shown to interact with the NO-cGMP pathway, the specific site(s) of anesthetic action remain controversial. In our preliminary studies, inhalational anesthetics inhibited the glutamate-stimulated accumulation of cGMP in rat cerebellum slices, indicating that anesthetics may interact with NO production, its action, or both.[14] Tobin *et al.*[15] reported that both halothane and isoflurane attenuated the activity of isolated brain NO synthase.[15] Contrary to this, studies from our laboratory[16] and others[17,18] have shown that inhalational anesthetics do not alter isolated NO synthase activity. Recently, inhibition of NO synthase isolated from polymorphonuclear leukocytes exposed to halothane was reported.[19] Terasako *et al.*[20] reported that anesthetics inhibit cGMP accumulation in rat cerebellum slices in response to excitatory amino acids but not to sodium nitroprusside. This suggests that anesthetics may act on the production of NO but not on its action. Similarly, our recent studies in endothelial cell-vascular smooth muscle coculture have shown that anesthetics inhibit the NO-cGMP pathway distal to receptor activation in the endothelial cells and proximal to NO activation of soluble guanylyl cyclase and do not directly interact with NO.[21] In contrast, Blaise *et al.*[22] reported that halothane has no effect on endothelial NO release but interferes with the half-life or redox form of NO. Further studies showed that inhalational anesthetics do not interfere with the stimulation of isolated soluble guanylyl cyclase.[23] The mechanism and site of anesthetic action on the NO-cGMP pathway remain controversial.[24]

The current studies were designed to elucidate the effect and mechanism of inhalational anesthetic action on the NO-cGMP pathway in the central nervous system. The effect of anesthetics on NMDA-stimulated NO synthase activity and cGMP accumulation was investigated using rat cerebellum slices.

Materials and Methods

Materials

Superoxide dismutase and catalase were obtained from Sigma Chemical Company (St. Louis, MO). L-[^{3}H]arginine (55 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Dowex AG50W-X8 cation exchange resin and Bio-Rad protein assay reagent were obtained from Bio-Rad Laboratories (Richmond, CA).

Determination of Nitric Oxide Synthase Activity and Cyclic Guanosine Monophosphate Accumulation in Cerebellar Slices

Sprague-Dawley rats were killed by cervical disarticulation and their cerebella were rapidly removed. The methods and procedures of this study were approved by the animal research committee of the University of Virginia. Nitric oxide synthase activity and cGMP accumulation in rat cerebellum slices were determined as described previously.[25] Briefly, thin slices (0.4 mm) were cut from the cerebella of 10-day-old rats in both sagittal and coronal planes using a McIlwain tissue chopper. The slices were incubated for 1 h in Krebs-Henseleit buffer containing 118 mM NaCl, 4.7 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11 mM glucose (Buffer A) continuously supplied with 95% oxygen/5% carbon dioxide at 37°C. Nitric oxide synthase activity was determined by measuring the conversion of L-[^{3}H]cbarginine to L-[^{3}H]citrulline in a 4-ml gas-tight vial capped with a Teflon septum. Supplying the vial with a specific gas mixture was achieved by temporarily inserting a 22-gauge needle for inlet and a similar needle for outlet at will. Buffer A (530 μl) equilibrated with 95% oxygen/5% carbon dioxide was injected into the vial and supplied with 95% oxygen/5% carbon dioxide for 2 min. A small volume (40 μl) of the gravity-packed cerebellum slices was injected into the vial, and a humidified 95% oxygen/5% carbon dioxide mixture with or without the anesthetic agent was passed through the vial for 4 min. After incubating the reaction mixture with or without the anesthetic agent for 15 min at 37°C, a 30-μl solution containing L-[^{3}H]arginine (3 μCi) with buffer A or NMDA was injected. The amount of cerebellum slices and L-[^{3}H]arginine used in these experiments ranged from 30-40 μl and 1.5-3 μCi, respectively. The reaction was stopped after 15 min by adding 400 μl ice-cold buffer A containing 10 mM arginine and 10 mM ethylene diaminetetraacetic acid. The mixture was transferred to an Eppendorf tube and centrifuged at 10,000*g* at 4°C. The supernatant was removed and 1 ml of 1 M trichloroacetic acid was added to the pellet. The sample was sonicated for 3 × 15 s and 50 μl aliquots were saved for protein determination. The sample was centrifuged and 0.85 ml supernatant was transferred to a 5-ml glass vial. Trichloroacetic acid in the supernatant was extracted three times with 2 ml diethyl ether. The sample was freeze-dried and then reconstituted with 1 ml of 20 mM HEPES, *p*H 5.5, containing 2 mM ethylene diaminetetraacetic acid. The recovery of L-[^{3}H]-citrulline

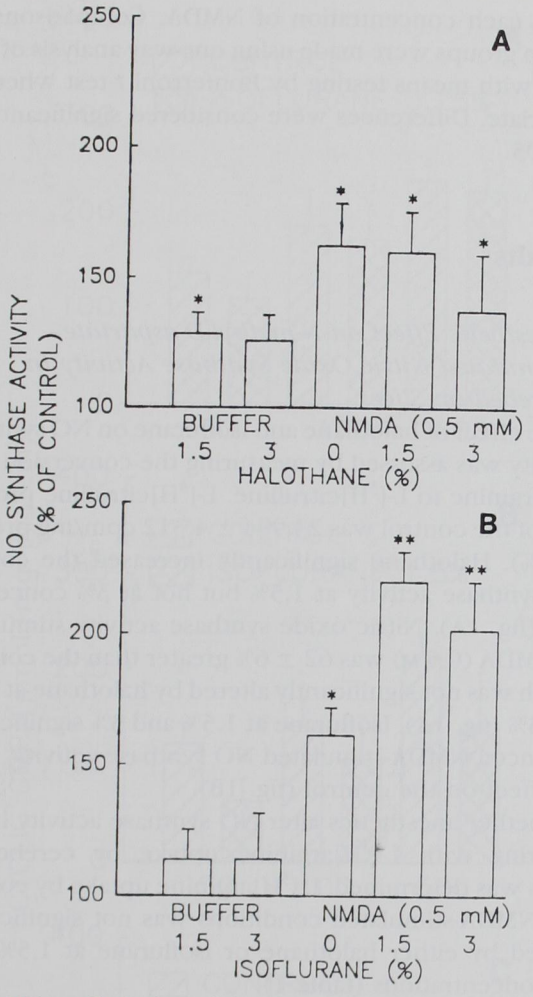

Fig. 1. Effect of halothane (*A*) and isoflurane (*B*) on rat cerebellum nitric oxide (NO) synthase activity. Control (buffer) and N-methyl-D-aspartate (NMDA)-stimulated NO synthase activity in cerebellum slices was assessed by measuring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline at different concentrations of anesthetic agent, as described in Materials and Methods. L-[^{3}H]citrulline production by the control in the absence of anesthetics was 23,994 ± 4,312 cpm/mg protein (100%). The data are means ± SEM of four experiments. *Significantly ($P < 0.05$) different from control in the absence of anesthetics; **significantly ($P < 0.05$) different from both control and NMDA in the absence of anesthetics.

in this above procedure was 89%. The sample was applied to a 1-ml Dowex AG50WX-8 (Na^+ form) column and L-[^{3}H]-citrulline eluted with 1.5 ml water. The radioactivity of the eluate was determined by liquid scintillation counting at 49% efficiency.

To measure cGMP, cerebellum slices equilibrated with a 95% oxygen/5% carbon dioxide mixture were supplied with gas with or without the anesthetic agent for 4 min. After 15 min, cerebellum slices were stimulated with buffer or NMDA at different concentrations. After 3 min, the reaction was stopped by adding 0.4 ml of 0.25 M HCl. The sample was sonicated for 3 × 15 s and 100 μl samples were separated in aliquots for protein determination. The rest of the volume in each sample was centrifuged and cGMP in the supernatant was analyzed by radioimmunoassay.[26]

Effect of Anesthetics on L-[^{3}H]arginine Uptake by Cerebellum Slices

Whether anesthetics alter NO synthase activity by interfering with L-[^{3}H]arginine uptake was determined because L-[^{3}H]arginine uptake was the first step in measuring NO synthase activity. L-[^{3}H]arginine uptake was performed as described for NO synthase activity measurement. The uptake of L-[^{3}H]arginine by cerebellum slices was stopped by adding 0.4 ml ice-cold buffer A containing 10 mM arginine and 10 mM ethylene diaminetetraacetic acid. The sample was centrifuged and the supernatant was discarded. To the pellet, 1 ml distilled water was added and sonicated for 3 × 15 s. Samples were analyzed for radioactivity and protein content.

For protein determination, the sonicate was hydrolyzed in 0.5 M NaOH at 60°C for 30 min and then reacted with the Bio-Rad protein reagent. Bovine serum albumin was used as the standard. Anesthetic concentration in the reaction sample buffer was determined as described previously.[16]

Data Analysis

Data were expressed as the means ± SEM. Paired *t* tests were used to compare control values with anesthe-

Table 1. Effect of Halothane and Isoflurane on L-[^{3}H]arginine Uptake under Control and NMDA-stimulated Conditions

	L-[^{3}H]arginine Uptake (% control)			
	Halothane		Isoflurane	
Assay Condition	1.5%	3%	1.5%	3%
Control	108 ± 10	100 ± 4	100 ± 7	96 ± 2
NMDA (100 μM)	95 ± 8	93 ± 5	97 ± 7	87 ± 8

NMDA = *N*-methyl-D-aspartate.

Rat cerebellum slices were incubated with or without anesthetics in buffer containing L-[^{3}H]arginine and immediately stimulated with buffer (control) or 100 μM NMDA as described in "Materials and Methods." L-[^{3}H]arginine uptake was expressed as percentage of the control in the absence of anesthetics. Data are mean ± SEM of three experiments.

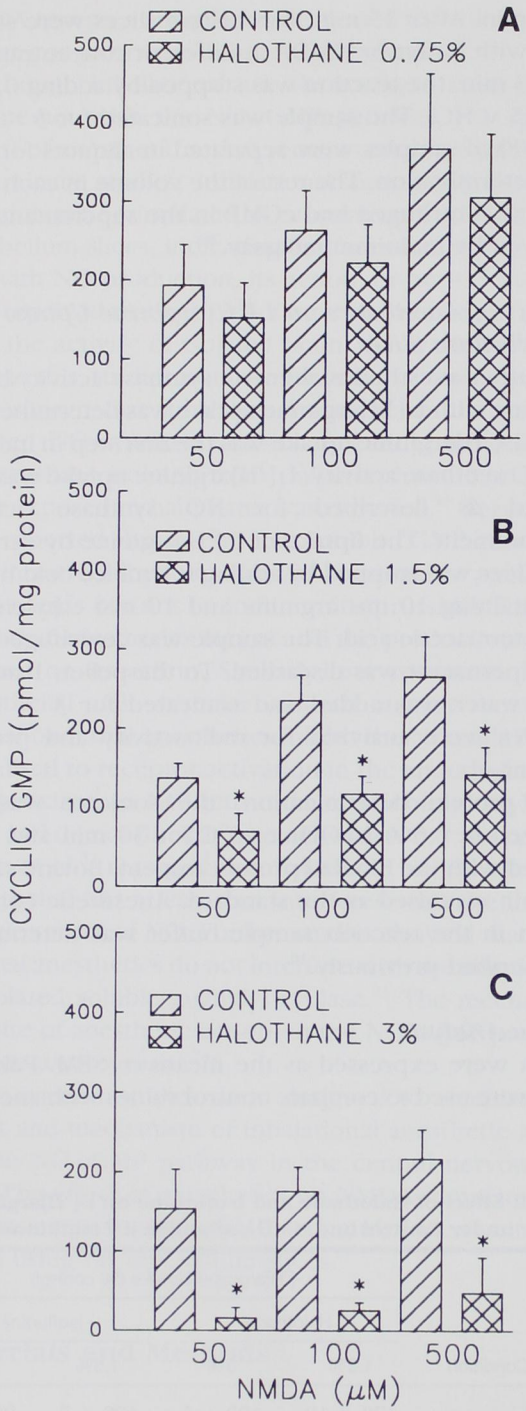

Fig. 2. Effect of halothane on N-methyl-D-aspartate (NMDA)-stimulated cyclic guanosine monophosphate (cGMP) accumulation in rat cerebellum slices. Cerebellum slices were exposed to halothane (*A*, 0.75%; *B*, 1.5%; *C*, 3%) and then stimulated with varying concentrations of NMDA. Cyclic GMP content was measured as described in Materials and Methods. The data are means ± SEM of three to seven experiments. *Significantly ($P < 0.05$) different from the respective control.

tic at each concentration of NMDA. Comparisons between groups were made using one-way analysis of variance with means testing by Bonferroni *t* test when appropriate. Differences were considered significant at $P < 0.05$.

Results

Anesthetic Effect on N-methyl-D-aspartate-stimulated Nitric Oxide Synthase Activity in Cerebellum Slices

The effect of halothane and isoflurane on NO synthase activity was assessed by measuring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline. L-[^{3}H]citrulline production of the control was 23,994 ± 4,312 cpm/mg protein (100%). Halothane significantly increased the control NO synthase activity at 1.5% but not at 3% concentration (fig. 1A). Nitric oxide synthase activity stimulated by NMDA (0.5 mM) was 62 ± 6% greater than the control, which was not significantly altered by halothane at 1.5% and 3% (fig. 1A). Isoflurane at 1.5% and 3% significantly enhanced NMDA-stimulated NO synthase activity, with no effect on the control (fig. 1B).

Whether anesthetics alter NO synthase activity by interfering with L-[^{3}H]arginine uptake by cerebellum slices was determined. L-[^{3}H]arginine uptake by control and NMDA-stimulated conditions was not significantly altered by either halothane or isoflurane at 1.5% and 3% concentrations (table 1).

Anesthetic Effect on N-methyl-D-aspartate-stimulated Cyclic Guanosine Monophosphate Accumulation in Cerebellum Slices

The effect of different concentrations of halothane and isoflurane on cGMP accumulation in cerebellum slices was determined in the absence or presence of various concentrations of NMDA. Basal cGMP concentration was not significantly altered by either halothane or isoflurane at 0.75%, 1.5%, or 3% concentration (data not shown). However, cGMP production stimulated by NMDA (50 μM, 100 μM, and 500 μM) was significantly attenuated by halothane at 1.5% and 3% (figs. 2B and C) but not at 0.75% (fig. 2A). Isoflurane at 0.75% did not produce any significant effect on cGMP production at different NMDA concentrations (fig. 3A). A significant inhibition of cGMP production was observed at 1.5% isoflurane when stimulated with 50 μM but not with 100 μM or 500 μM NMDA (fig. 3B). Isoflurane at 3%

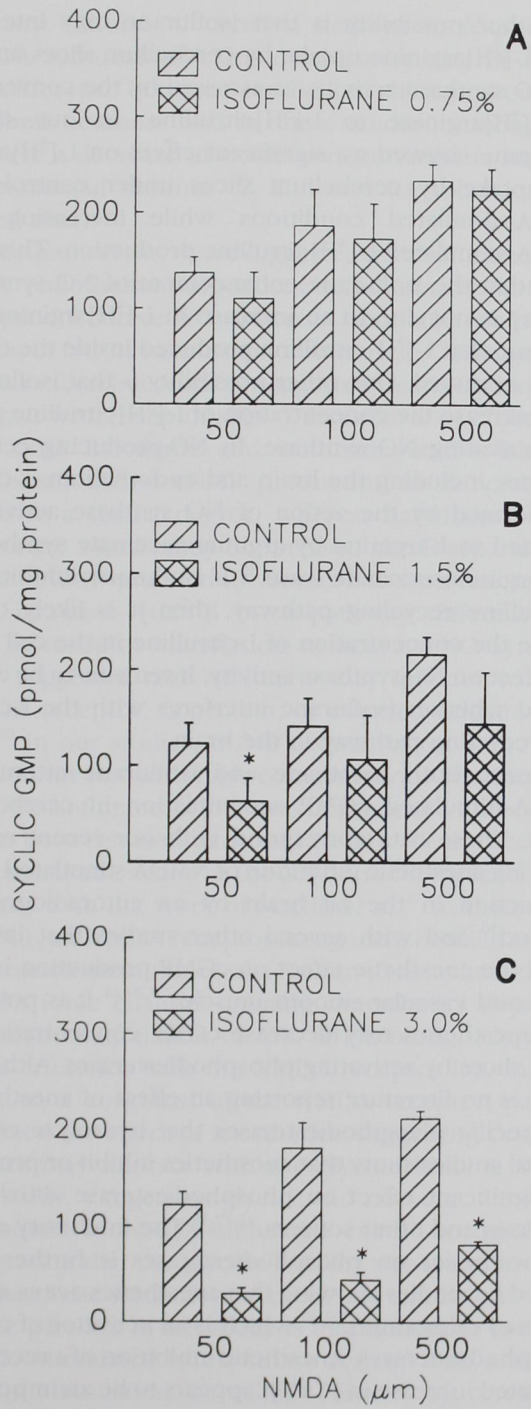

Fig. 3. Effect of isoflurane on N-methyl-D-aspartate (NMDA)-stimulated cyclic guanosine monophosphate (cGMP) accumulation in rat cerebellum slices. After exposure to halothane (*A*, 0.75%; *B*, 1.5%; *C*, 3%), cerebellum slices were stimulated with varying concentrations of NMDA and cGMP content was measured as described in Materials and Methods. The data are means ± SEM of three to seven experiments. *Significantly ($P < 0.05$) different from the respective control.

Table 2. Effect of SOD and Catalase on Anesthetic Inhibition of NMDA-induced cGMP Concentration

	cGMP (pmol/mg)		
Assay Condition	Control	Halothane (3%)	Isoflurane (3%)
NMDA (100 μM)	287 ± 21	39 ± 7	72 ± 8
NMDA (100 μM) + SOD and catalase (each 100 units)	299 ± 27	38 ± 5	89 ± 12

SOD = superoxide dismutase; NMDA = *N*-methyl-D-aspartate; cGMP = cyclic guanosine monophosphate.

Rat cerebellum slices were treated with or without a mixture of SOD and catalase (each 100 units) and exposed to halothane or isoflurane at 3%. After stimulation with 100 μM NMDA, cGMP content was measured as described in "Materials and Methods." Data are mean ± SEM of four experiments.

produced a significant attenuation of cGMP accumulation at all concentrations of NMDA tested (fig. 3C).

To determine whether superoxide anion is involved in the observed anesthetic responses, the effect of a mixture of superoxide dismutase and catalase on cGMP accumulation was examined. A mixture of superoxide dismutase and catalase (100 units each per milliliter) caused no significant effect on anesthetic inhibition of NMDA-stimulated cGMP concentration (table 2). Studies by Martin *et al.*[27] have suggested that glycine, a coagonist of the NMDA receptor, attenuated the inhibitory effect of anesthetics on NMDA-induced ion channel opening. Glycine (100 μM), when tested on the anesthetic inhibition of NMDA-induced cGMP accumulation but did not produce any significant effect, although cGMP accumulation by NMDA stimulation was significantly increased (table 3).

Table 3. Effect of Glycine on Anesthetic Inhibition of NMDA-induced cGMP Concentration

	cGMP (pmol/mg)		
Assay Condition	Control	Halothane (3%)	Isoflurane (3%)
NMDA (100 μM)	213 ± 9	35 ± 3	46 ± 9
NMDA (100 μM) + glycine (100 μM)	275 ± 23*	31 ± 9	63 ± 13

NMDA = *N*-methyl-D-aspartate; cGMP = cyclic guanosine monophosphate.

Rat cerebellum slices were exposed to halothane or isoflurane at 3% and stimulated with NMDA (100 μM) in the presence and absence of glycine (100 μM). cGMP content was measured as described in "Materials and Methods." Data are mean ± SEM of three experiments.

* Significantly ($P < 0.05$) different from NMDA in the absence of anesthetics.

Discussion

In this study, we investigated the effects of inhalational anesthetics on NMDA-stimulated NO and cGMP production in rat cerebellum slices. Halothane and isoflurane inhibited NMDA-induced cGMP content of cerebellum slices. Unlike previous studies showing a lack of effect of halothane and isoflurane on neuronal NO synthase, the current studies investigated the action using isolated brain slices with intact cellular milieu. Despite their inhibitory action on NMDA-stimulated cGMP accumulation in cerebellum slices, isoflurane significantly enhanced NMDA-stimulated NO synthase activity, whereas halothane produced no significant effect. This study presents new insights into the mechanism of inhalational anesthetic action on NMDA-stimulated NO synthase activity in the rat cerebellum.

First, the mechanism of inhalational anesthetic action on NO synthase activity in different tissues is controversial.[15,16,19,23,24] Contrary to our expectation, in this study, isoflurane enhanced the activity of NMDA-stimulated NO synthase in cerebellum slices, whereas halothane produced no significant effect. The effect of isoflurane on NO synthase activity might be due to a direct action on NO synthase, as reported previously.[15] However, recent studies show that isoflurane or other inhalational anesthetics do not alter isolated NO synthase activity, excluding a direct interaction of anesthetics with NO synthase.[16–18] Then how can isoflurane enhance the activity of NO synthase in cerebellum slices? Isoflurane may increase the cytosolic free calcium ($[Ca^{2+}]i$) and enhance NMDA-stimulated NO synthase activity in the intact cerebellum. Anesthetic agents have been shown to increase the $[Ca^{2+}]i$ in unstimulated cells[28,29] and to inhibit receptor activated increase in $[Ca^{2+}]i$.[30,31] Isoflurane has been shown to reduce glutamate and NMDA-mediated calcium fluxes in brain slices by approximately 60% and thereby delay cellular injury induced by simulated ischemia.[32] Recently Miao *et al.*[33] observed that both halothane and isoflurane decreased the intrasynaptosomal calcium level, presumably by inhibiting calcium entry through specific neuronal calcium channels. Isoflurane may interact with other cofactors of NO synthase and enhance NMDA-stimulated NO synthase activity. The isoflurane enhancement of NMDA-stimulated NO synthase activity is supported by a recent report in which isoflurane increased the cGMP content of primary cultures of cerebral neurons stimulated with NMDA, presumably through increased production of NO.[34]

Another possibility is that isoflurane may interfere with L-[^{3}H]arginine uptake by cerebellum slices and alter NO synthase activity, as assessed by the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline. In our study, isoflurane showed no significant effect on L-[^{3}H]arginine uptake by cerebellum slices under control and NMDA-stimulated conditions while increasing the NMDA stimulated L-[^{3}H]citrulline production. This suggests that the isoflurane enhancement of NO synthase activity is not due to an increase in L-[^{3}H]arginine uptake and that L-[^{3}H]citrulline produced inside the tissue is not extruded. One other possibility is that isoflurane may increase the concentration of L-[^{3}H]citrulline without activating NO synthase. In NO-producing cellular systems, including the brain and endothelium, L-citrulline formed by the action of NO synthase activity is recycled to L-arginine by argininosuccinate synthetase and argininosuccinate lyase.[35] If isoflurane inhibits the L-citrulline recycling pathway, then it is likely to increase the concentration of L-citrulline in the cell with no effect on NO synthase activity. It remains to be determined whether isoflurane interferes with the L-citrulline recycling pathway in the brain.

Second, both halothane and isoflurane attenuated NMDA-stimulated cGMP accumulation in cerebellum slices. These data correspond with our recent report showing anesthetic inhibition of NMDA-stimulated GMP production in the rat brain by an autoradiographic method[36] and with several other studies that investigated the anesthetic effect on cGMP production in the brain and vascular smooth muscle.[7,8,20,24] It is possible that anesthetics may decrease cGMP concentration in brain slices by activating phosphodiesterases. Although there is no literature reporting an effect of anesthetics on specific phosphodiesterases that hydrolyze cGMP, several studies show that anesthetics inhibit or produce no significant effect on phosphodiesterase activity of the brain and other sources.[21,37,38] The inhibitory effect of anesthetics on phosphodiesterases is further supported by studies showing that anesthetics act as antagonists of calmodulin,[39,40] which is an activator of cGMP phosphodiesterases. Anesthetic inhibition of a receptor-mediated increase in $[Ca^{2+}]i$ appears to be an important mechanism for the decrease in cGMP production. Puil *et al.*[30] have reported that isoflurane and halothane attenuated a glutamate-stimulated increase in $[Ca^{2+}]i$ in cultured hippocampal neurons. Similarly, volatile anesthetics have been shown to depress evoked transmitter release by reducing calcium entry.[31] A recent report shows that isoflurane increases the cGMP content in

primary cultures of cerebral neurons in response to NMDA stimulation.[34] This discrepancy may be due to the difference between cerebellum slices and cultured cerebral neurons used in these studies, although we observe a decrease in cGMP concentration in cultured hippocampal neurons (Z Zuo and RA Johns, unpublished data).

Nakamura *et al.*[41] have suggested that isoflurane attenuates endothelium-dependent relaxation by inhibiting the production of NO, whereas halothane inhibits the action of NO in vascular smooth muscle.[41] Further studies in rat cerebellar slices suggested that isoflurane may interact with receptor sites.[20] Terasako *et al.*[20] observed that both halothane and isoflurane at 2% inhibited NMDA-stimulated cGMP production in rat cerebellar slices to a similar degree, whereas halothane but not isoflurane significantly suppressed the D-aspartate–induced cGMP production. Because D-aspartate stimulation increases $[Ca^{2+}]i$ by a pathway different from NMDA receptor stimulation to activate NO synthase, they suggested that isoflurane may interact with the NMDA receptor, G protein, or coupled calcium channel.[20] In our studies, cGMP accumulation with 50 μM NMDA but not with high concentrations was inhibited by 1.5% isoflurane, which was consistent with a competition between isoflurane and NMDA for the receptor. The anesthetic action at the NMDA receptor level corresponds with the work of Martin *et al.*[27] In their study, MK801, an NMDA receptor antagonist, binding to cerebral cortex membranes was measured as an index of the NMDA ionic channel opening. Anesthetics depressed glutamate stimulation of MK 801 binding, suggesting an interference with the NMDA receptor ion channel. Glycine, a coagonist of the NMDA receptor, partially reversed the effect of anesthetics, suggesting that anesthetics may interact with the glycine binding site. In our study, exogenous glycine did not alter anesthetic inhibition of NMDA-stimulated cGMP accumulation, suggesting that the glycine binding site is not involved in anesthetic inhibition of cGMP accumulation.

The paradox in this study is that isoflurane enhances NMDA-stimulated NO synthase activity with a simultaneous attenuation of cGMP content of cerebellum slices. An increase in NO synthase activity is expected to increase cGMP concentration in the tissue. On the contrary, there are conditions under which an increased production of NO may adversely affect the accumulation of cGMP content. In a previous study, we observed an increase in NO production as measured by the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline under hyperoxic conditions.[42] However, when the NO-producing enzyme system was coupled to a bioassay system consisting of vascular smooth muscle cells, the cGMP accumulation in vascular smooth muscle cells was drastically reduced under hyperoxic conditions. This suggested an involvement of superoxide in inactivating NO and subsequently decreasing the concentration of cGMP in the tissue. Whether superoxide is involved in the inhibition of cGMP accumulation in cerebellum slices is not known. Shayevitz *et al.*[43] reported that halothane and isoflurane increase pulmonary artery endothelial cell sensitivity to oxidant stress. They have suggested that anesthetics may produce oxidant load by enhancing the conversion of xanthine dehydrogenase to xanthine oxidase or by attenuating antioxidant systems, including glutathione reductase. This is supported by the studies of Yoshida and Okabe[44] that showed attenuation of acetylcholine-induced relaxation of canine mesenteric arteries by sevoflurane by a superoxide-mediated mechanism. In that study, the attenuation of relaxation was partially reversed by superoxide dismutase or deferoxamine, indicating that superoxide anion or hydroxyl anion was in part responsible for the effect of sevoflurane. This indicates that superoxide may be responsible for the anesthetic-induced decrease in cGMP content in brain slices. However, in our study, a mixture of superoxide dismutase and catalase (100 units each per milliliter) produced no significant effect on the anesthetic inhibition of NMDA-stimulated cGMP production, suggesting that superoxide generation may not be involved in anesthetic inhibition of NMDA-stimulated cGMP production.

Anesthetic interaction with soluble guanylyl cyclase may decrease cGMP accumulation. Studies by Terasako *et al.*[20] showed no significant effect of halothane and isoflurane on sodium nitroprusside-induced cGMP production in rat cerebellum. This suggested that halothane and isoflurane do not interact with soluble guanylyl cyclase. However, studies by Hart *et al.*[45] showed that halothane inhibited NO-induced cGMP production in denuded rat aortic rings. Thus it appears that inhalational anesthetics may interfere with NO activation of soluble guanylyl cyclase. Hart *et al.*[45] have suggested that inhalational anesthetics may compete with NO for binding to the heme group of soluble guanylyl cyclase. If this is true, anesthetics are expected to inhibit NO or sodium nitroprusside activation of isolated soluble guanylyl cyclase. However, we found that there was no significant effect of inhalational anesthetics on NO or sodium nitroprusside activation of isolated soluble gua-

nylyl cyclase.[23] In addition, NO, sodium nitroprusside, and nitroglycerin-stimulated cGMP accumulation in cultured vascular smooth muscle cell or endothelial cell-vascular smooth muscle cell cocultures was not significantly altered by 0-5% halothane and isoflurane.[21] These studies suggest that the anesthetic inhibition of NO-induced cGMP accumulation may not be due to a direct competition between NO and inhalational anesthetics for binding to soluble guanylyl cyclase in the brain.

The mechanism of anesthetic inhibition of NMDA-stimulated cGMP accumulation may be due to the ability of anesthetics to interact with multiple neuronal pathways. For example anesthetics have been shown to increase the concentration of neurotransmitters, including gamma-aminobutyric acid[46] and serotonin,[47] which may feed back on the NO-cGMP pathway and alter NMDA-induced cGMP production in the cerebellum. Serotonin at low nanomolar concentrations has been shown to inhibit NMDA-stimulated cGMP production in rat cerebellum.[48] The inhibitory serotonin receptors appear to interact with NMDA receptors at both postsynaptic sites and presynaptic terminals. This suggests that inhalational anesthetics interact with other neurotransmitter pathways that modulate NMDA-induced cGMP accumulation in the cerebellum.

Although our interest in investigating the mechanism of anesthetic inhibition of the NO pathway was stimulated by our observations that inhibition of neuronal NO synthase decreases minimum alveolar concentration for anesthetics,[9,10] the possibility that inhibition of the NO pathway could account for other actions of anesthetics on neuronal physiology should be considered. Several studies suggest that NO plays a role in regulating cerebral blood flow (CBF), electroencephalogram (EEG) activity, and cerebral oxygen consumption. Administration of L-N^G-nitro-L-arginine methyl ester, an inhibitor of NO synthase, in different animal models has been shown to reduce CBF under various conditions, suggesting that NO contributes to CBF regulation.[49-51] 3-morpholinosydnonimine, an NO donor, administered after 3 min of middle cerebral artery occlusion in rats, enhanced the recovery of CBF and EEG amplitude and reduced the size of the infarct.[52] Nitric oxide also appears to regulate EEG activity[52] and cerebral oxygen consumption.[53,54] If anesthetics inhibit the NO pathway, we would expect a decrease in CBF and EEG. However, inhalational anesthetics have been shown to increase CBF and decrease cerebral oxygen consumption and EEG activity.[55-57] Several studies have shown that anesthetic-induced increases in CBF are prevented by inhibitors of NO synthase, suggesting that anesthetics may actually stimulate the NO pathway.[51,58] Alternatively, these results could be explained by inhibition of basal NO unrelated to the anesthetic action. Indeed, Todd *et al.*[59] have shown that NO is not a primary mediator of anesthetic CBF effects.[59] Overall, these studies suggest that the anesthetic inhibition of the NO signaling pathway is not related to anesthetic effects on CBF, EEG activity, and cerebral oxygen consumption, which must be mediated by other mechanisms.

In conclusion, we have shown that isoflurane significantly enhanced NMDA-stimulated NO synthase activity, with a simultaneous decrease in cGMP accumulation in cerebellum slices. Halothane produced no significant effect on NMDA-stimulated NO synthase activity but attenuated NMDA-stimulated cGMP accumulation.

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