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Halothane and Isoflurane Increase Spontaneous but Reduce the N-methyl-D-aspartate–evoked Dopamine Release in Rat Striatal Slices

Evidence for Direct Presynaptic Effects

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Background: **Experimental data suggest that volatile anesthetics induce significant changes in extracellular dopamine concentrations in the striatum, a restricted but functionally important brain area. In the present study, the authors used a superfused slice preparation to examine the effects of halothane and isoflurane on both spontaneous and *N*-methyl-D-aspartate (NMDA)-evoked dopamine release in the striatum, and whether these effects involved actions of these anesthetics mediated by γ-aminobutyric acid receptors in this structure.**

Methods: **Radioactivity collected from 5-min fractions was compared in the absence (basal release) or presence (evoked release) of NMDA alone and combined with various pharmacologic or anesthetic agents in slices of the dorsolateral striatum and synaptosomes of the whole striatum preloaded with ^{3}H-dopamine and superfused with artificial cerebrospinal fluid.**

Results: **In tetrodotoxin-treated striatal slices, halothane and isoflurane significantly increased dopamine basal release (EC_{50} = 0.33 mM and 0.41 mM for halothane and isoflurane, respectively). Both agents decreased the NMDA-evoked dopamine release in both the absence (IC_{50} = 0.15 mM and 0.14 mM for halothane and isoflurane, respectively) and presence (IC_{50} = 0.15 mM for both halothane and isoflurane) of tetrodotoxin in slices, and in synaptosomes (IC_{50} = 0.19 mM for both halothane and isoflurane). NMDA-induced dopamine release was significantly enhanced by bicuculline, a γ-aminobutyric acid receptor antagonist. Halothane and isoflurane inhibitory effects on NMDA-evoked dopamine release were significantly reduced in the presence of bicuculline.**

Conclusion: **These results indicate that halothane and isoflurane decrease the NMDA-evoked dopamine release by acting directly at dopamine terminals in striatal slices. They support the involvement of both depression of presynaptic NMDA receptor-mediated responses and enhancement of γ-aminobutyric acid receptor-mediated responses in these effects. (Key words: Excitatory synaptic transmission; inhalational agents; inhibitory synaptic transmission.)**

ALTHOUGH present only in discrete areas of the central nervous system, dopamine (DA) innervation exerts major control on important brain functions such as locomotor activity and cognitive processes.[1] Alteration of the nigrostriatal DA pathway is responsible for the development of Parkinson's disease, whereas dysfunction of the mesocorticolimbic DA neurons has been proposed as a pathogenic hypothesis for some psychotic states.[2–4]

Several lines of evidence suggest that halothane or isoflurane anesthesia is associated with significant changes in extracellular DA concentrations in the striatum *in vivo*. An inverse correlation has been reported between the striatal DA content and halothane anesthetic requirements in mice.[5] Hyperlocomotion during recovery from isoflurane anesthesia is associated with increased DA turnover in the nucleus accumbens and striatum in mice.[6] In addition, the striatal levels of DA or its metabolites measured *in vivo* by microdialysis were increased by clinical halothane and isoflurane concentrations.[7–9] This might be explained in part by blockade of the DA carrier by these agents.[10] Finally, we have previously shown that halothane and isoflurane (1 and 2 minimum alveolar concentration–equivalent

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concentrations) significantly enhanced spontaneous but reduced potassium chloride (KCl)- and *N*-methyl-D-aspartate (NMDA)-evoked DA release from preloaded striatal synaptosomes.[11]

In the rat striatum, glutamate nerve endings originating from the cerebral cortex make synaptic contact with DA terminals arising from the nigrostriatal pathway.[12] Electrical stimulation of the corticostriatal glutamate pathway or local application of glutamate agonists in the vicinity of DA terminals result in a potent depolarization-induced DA release.[13,14] This is mediated in part *via* direct, tetrodotoxin (TTX)-insensitive stimulation of both NMDA and non-NMDA receptors located on DA nerve endings.[14–16] Alternatively, it may be mediated by indirect, TTX-sensitive depolarization of DA terminals resulting from activation of intrastriatal microcircuits.[17,18] DA release is modulated by both excitatory neurotransmitters such as substance P or acetylcholine present in interneurons[18,19] and inhibitory neurotransmitters such as γ-aminobutyric acid (GABA), which is released primarily from recurrent collaterals of medium-sized spiny output neurons extending in their dendritic field, or possibly interneurons.[20,21] GABA exerts a tonic inhibitory control on both spontaneous and NMDA-evoked DA release in the striatum.[21] It is released from striatal slices in response to NMDA stimulation; however, this effect is not observed on a synaptosomal preparation.[22] Therefore, it might be hypothesized that the potent effects of halothane and isoflurane at $GABA_A$ receptors contribute to their depressant effect on NMDA-induced DA release.[11,23] In contrast to synaptosomes, which consist of isolated nerve terminals disconnected from their inputs and outputs, the superfused slice preparation preserves most of the interneurons and recurrent collaterals of striatopallidal or striatonigral efferent cells. Therefore, it represents an appropriate tool to test the role played by the striatal GABAergic systems in the actions of volatile agents.

The aim of the present study was to determine the concentration–response relationship for the effects of halothane and isoflurane on both basal and NMDA-evoked DA release in rat striatal slices and to examine whether these effects involved actions at intrastriatal $GABA_A$ receptors. Additional experiments performed on synaptosomes were included as controls.

Materials and Methods

Handlings procedures according to the *Guide for the Care and Use of Laboratory Animals*[24] were followed throughout. Experiments were performed on male Sprague-Dawley rats (Iffa-Credo, L'Arbresle, France) weighing 200–225 g and housed on a 12:12 light/dark cycle with food and water *ad libitum*. Approval was obtained from the Institutional Animal Care and Use Committee at the University of Paris 7.

Preparation of Striatal Slices and Synaptosomes

Animals were killed by stunning (thoracic shock followed by reflex cardiac arrest) and decapitation. After longitudinal hemisection of the brain, striata were quickly removed and processed to a purified synaptosomal preparation as previously reported in detail.[20,21] Synaptosomes were diluted up to 0.16 mg/ml in an ice-cold Mg^{2+}-free artificial cerebrospinal fluid containing 126.5 mM NaCl, 27.5 mM $NaHCO_3$, 1 mM KCl, 0.5 mM KH_2PO_4, 1.1 mM $CaCl_2$, 0.5 mM Na_2SO_4, 5.9 mM glucose, 10^{-2} mM pargyline, and 1 mM ascorbic acid, adjusted at *p*H 7.3 with 95%/5% [vol/vol] oxygen/carbon dioxide mixture. In Ca^{2+}-free experiments, $CaCl_2$ was omitted from the cerebrospinal fluid.

Microdiscs of striatal tissue were prepared according to a slightly modified version of the procedure described by Galli *et al.*[22] Brains were quickly removed from the skull and placed for 10 min in the same cooled cerebrospinal fluid. Microdiscs (0.9-mm diameter, 0.35 mg protein per disc) of tissue were punched out with a stainless steel tube from 300-μm sagittal rat brain slices prepared with a vibratome (Campden Instruments, London, United Kingdom). Microdiscs of tissue from the dorsolateral striatal matrix-enriched area were obtained from three consecutive slices in each hemisphere (laterality 4.80–3.75 mm according to the atlas of Paxinos and Watson[23]), with one microdisc punched out in each slice.

Superfusion and Collection of Samples

Uptake of DA by synaptosomes and tissue microdiscs was performed by incubating (15 min at 37°C) the preparations (final volume, 12 ml for synaptosomes and 5 ml for microdiscs) with [2,5,6]^{3}H-DA (Amersham, Little Chalfont, United Kingdom; 25 μl, 44 nM, 24 Ci/mM, 1 mCi/ml). Aliquots of the synaptosomal preparation (1 ml) were pipetted into each of the superfusion chambers (15-mm diameter, 2-mm height) and embedded in Whatman (Whatman International Ltd., Maidstone, England) GF/F glass filters (0.70-μm retention capacity) by light suction. Each microdisc of the striatum was introduced individually in a microsuperfusion chamber (6.35-mm diameter, 9.5-mm height). Synaptosomes or microdiscs were then superfused at a 1-ml/min flow rate using a

superfusion device equipped with an automatic fraction collector (Brandel, Gaithersburg, MD). The 1-ml/min flow rate was selected because of its ability to prevent reuptake of ^{3}H-DA by the preparations.[11,25] After a 30-min washing step (37°C), serial fractions were collected every minute during three consecutive periods; the mean value of the radioactivity (counts/min) measured over the first 5 min by liquid scintillation spectrometry using Aquasol-2 (New England Nuclear, Boston, MA) was considered the basal (spontaneous) ^{3}H-DA release. During the next 5 min, either no treatment (time-dependent control) or various pharmacologic or anesthetic agents were delivered to the preparations, and radioactivity was again estimated in each fraction. Because of a 3-ml dead volume occupied by the inside of the connect tubes, pharmacologic agents delivered at a 1-ml/min flow rate reached striatal tissue or synaptosomes only after a delay averaging 180 s. For the final 9 min, radioactivity was determined from 1-min fractions in the absence of any treatment.

Anesthetics and Chemicals

The effects of the following pharmacologic agents were studied: nomifensine (a blocker of the high affinity DA uptake, 10^{-5} M; Sigma, L'Isle d'Abeau, France), NMDA (5×10^{-7}–10^{-3} M; Sigma), MK 801 (an NMDA receptor antagonist, 10^{-8}–10^{-5} M; Merck-Sharp and Dohme, Darmstadt, Germany), glycine (10^{-6} M; Sigma), 7-chloro-kynurenate (an antagonist of the glycine site at the NMDA receptor, 10^{-4} M; Research Biochemical Incorporated, Illkirch, France), TTX (a blocker of the voltage-operated sodium channels that suppresses action potentials, 10^{-10}–10^{-7} M; Sigma), and bicuculline (an antagonist of the $GABA_A$ receptors, 10^{-5} M; Sigma). Halothane (Fluothane; Zeneca, Cergy, France) and isoflurane (Forane; Abbott, Rungis, France) were delivered through a calibrated vaporizer in an oxygen/carbon dioxide mixture (95%/5% vol/vol, 3 l/min) at various concentrations ranging from 0.25% to 5%. The minimum alveolar concentration value was considered to be 1% for halothane and 1.5% for isoflurane, as previously reported for male rats.[26] Volatile anesthetics were equilibrated with the reservoir of artificial cerebrospinal fluid for 60 min at 37°C, and aqueous concentrations in the superfusion chambers were determined by gas phase chromatography according to a slightly modified version of the method of Brachet-Liermain *et al.*[27]

Expression of Results and Statistical Analysis

Results were considered reliable only if they had been reproduced in four independent experiments (each of them performed in triplicate). The influence of a drug on ^{3}H-DA release was assessed by calculating the difference between the release observed in the presence and absence (time-dependent control) of the pharmacologic agent applied over the 5-min period. This was expressed as a fractional increase from spontaneous release. Only minor, nonsignificant differences in ^{3}H-DA release evoked by NMDA were observed from one microdisc of tissue to the other. Therefore, results obtained with microdiscs of tissues punched out on three successive sagittal slices on each side of the brain were combined for statistical analysis.

Concentration–response curves, IC_{50} values, and Hill coefficients were computer-generated using GraphPAD software (Intuitive Software for Science, San Diego, CA). The following four-parameter logistic equation was used to fit the curves to the data:

$$Y = A + (B - A)/[1 + (10^C/10^X)^D]$$

where X represents the logarithm of drug concentration, and A and B represent the minimum (bottom) and maximum (top) of X values, respectively. C is the logarithm of the IC_{50} value, and D is the Hill coefficient or slope factor that is positive for curves in which the Y value increases with increasing X and is negative for curves in which the Y value decreases with increasing X.

Normality of distributions was first assessed by the Fisher test for equality of variances. Statistical analysis was then performed by analysis of variance with Scheffé's *post hoc* correction for multiple comparisons. A P value < 0.05 was considered the threshold for significance. Data are expressed as mean ± SD.

Results

The ^{3}H-DA concentration estimated in the incubation medium during the uptake phase was 250 ± 29 pmol/mg protein, and the initial basal release rate measured over 5 min averaged 1.08 ± 0.10 and 2.9 ± 0.32 pmol · mg protein^{-1} · min^{-1} for synaptosomes and microdiscs, respectively. Omitting Ca^{2+} from the superfusion medium did not affect basal release rate. The aqueous concentrations of volatile anesthetics measured in the chambers after 1 h of equilibration with the superfusion medium at 37°C are given in table 1.

In striatal slices, no significant variation in basal (spon-

Table 1. Aqueous Concentrations of Halothane and Isoflurane Measured in the Superfusion Chambers at 37°C after 1 h Equilibration

Vaporized (%)	Aqueous (mM)
Halothane	
0.25	0.09 ± 0.04
0.5	0.16 ± 0.05
0.7	0.24 ± 0.07
1	0.32 ± 0.09
1.5	0.42 ± 0.07
2	0.57 ± 0.09
3	0.94 ± 0.12
4	1.40 ± 0.15
5	2.06 ± 0.21
Isoflurane	
0.35	0.10 ± 0.05
0.7	0.20 ± 0.08
1	0.27 ± 0.08
1.5	0.34 ± 0.08
2.2	0.45 ± 0.11
3	0.60 ± 0.09
3.7	0.80 ± 0.13
4.2	0.95 ± 0.15
5	1.15 ± 0.19

Data are expressed as the mean ± SD.

taneous) release was observed with time. NMDA application induced a concentration-related increase in ^{3}H-DA release (fig. 1A). The 10^{-4}-M concentration of NMDA produced a 1.62 (in fractional units, $P < 0.05$) increase from spontaneous release and was used as the reference depolarizing stimulus to elicit ^{3}H-DA release in microdiscs of striatal tissue. This release was markedly decreased by MK 801 in a concentration-related fashion (fig. 1B). It was significantly enhanced by a 10^{-6}-M glycine concentration (in fractional units: 1.84 ± 0.13; $P < 0.01$), but coapplication of 7 chloro-kynurenate (10^{-4} M) with glycine suppressed the glycine-induced potentiation of NMDA on ^{3}H-DA release (fig. 1C).[28] MK 801, glycine, and kynurenate had no effect *per se* on spontaneous release. As shown in figure 1D, TTX induced a significant, concentration-related decrease in the NMDA-evoked ^{3}H-DA release from striatal slices. A ceiling effect was noted in TTX action. The TTX concentration of 5×10^{-8} M was used for testing the effects of anesthetics, because this concentration attenuated ≈90% of the TTX-sensitive NMDA-evoked release in the slices. Consistent with previous reports,[28] we found that this TTX concentration decreased basal DA release by 40% in striatal slices. Nomifensine (10^{-5} M) failed to affect basal or NMDA-evoked release under these experimental conditions.

Halothane and isoflurane produced a significant increase in spontaneous ^{3}H-DA release in TTX-treated slices (5×10^{-8} M; EC_{50} = 0.33 mM and 0.41 mM for halothane and isoflurane, respectively; fig. 2). Both agents induced a significant, concentration-related decrease of ^{3}H-DA release evoked by NMDA in slices (fig. 3). The IC_{50} values obtained for these inhibitory effects of halothane and isoflurane were 0.15 mM and 0.14 mM, respectively. The best fit of the curves to the data was always obtained when the Hill coefficient was forced to 1. The attenuation of the NMDA-evoked release by volatile anesthetics yielded a ceiling effect. The IC_{50} values for the inhibitory effects of halothane and isoflurane in microdiscs of tissue were not significantly different in either the presence or absence of TTX (5×10^{-8} M) in the medium (IC_{50} values: 0.15 mM and 0.15 mM for halothane and isoflurane, respectively; fig. 3).

Muscimol (10^{-5} M) did not affect basal release. In contrast, it induced a mild but significant decrease in NMDA-evoked 3H-DA release in slices (fig. 4). In contrast, bicuculline application (10^{-5} M) produced a marked increase in NMDA-evoked 3H-DA release. In the presence of bicuculline, the inhibitory effect of halothane and isoflurane was significantly attenuated (fig. 4).

In the synaptosomal preparation, the ^{3}H-DA release elicited by NMDA (10^{-5}-M, 10^{-4}-M, 5×10^{-4}-M, and 10^{-3}-M concentrations) was (in fractional units): 1.10 ± 0.12 (not significant), 1.24 ± 0.13 ($P < 0.05$), 1.30 ± 0.15 ($P < 0.05$), and 1.37 ± 0.20 ($P < 0.01$), respectively. For comparative purposes with previous studies, the 10^{-3}-M NMDA concentration was selected.[11] The effect of NMDA (10^{-3} M) was completely blocked by MK 801 (10^{-5} M; 97% ± 13%) but was not significantly affected by TTX application (10^{-7} M). Halothane and isoflurane produced a concentration-related decrease in the NMDA-evoked ^{3}H-DA release (fig. 5). The IC_{50} values for these inhibitory effects were 0.19 mM for both anesthetics.

Discussion

The present study indicates that halothane and isoflurane exert complex effects on DA release in striatal slices: they enhance the spontaneous release of striatal DA but decrease the NMDA-evoked in a concentration-related fashion. The latter effects are observed at particularly low concentrations of anesthetics whether the activity of the local interneurons and afferent fibers involved in DA release is depressed or not. These findings

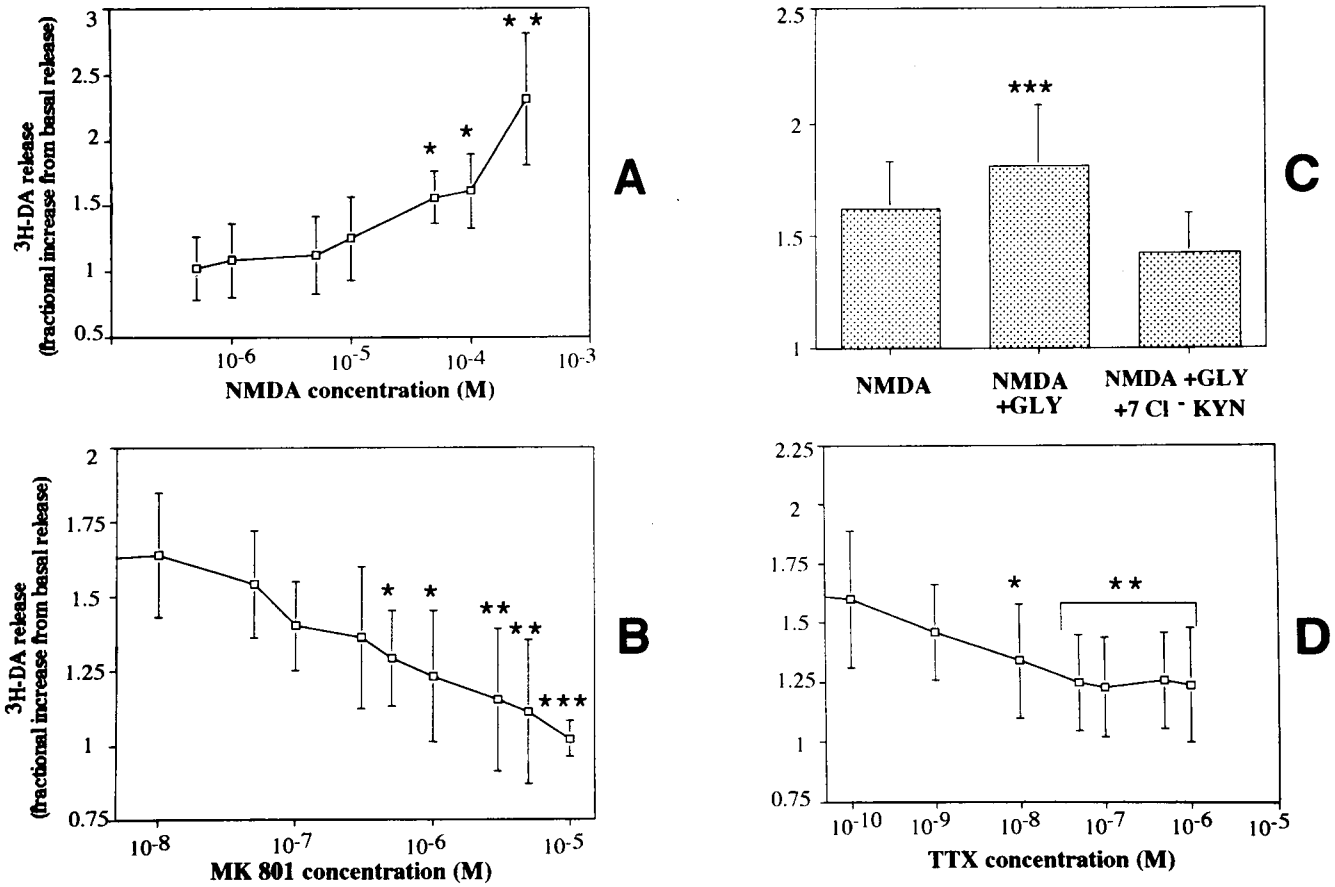

Fig. 1. Pharmacologic characterization of *N*-methyl-D-aspartate (NMDA)-induced dopamine (DA) release in striatal slices. (*A*) Concentration–response curve for NMDA. (*B*) Concentration–response curve for MK 801 using a fixed NMDA concentration (10^{-4} M). (*C*) Effect of glycine (10^{-6} M) and 7-chloro-kynurenate (10^{-4} M) on NMDA (10^{-4} M)-evoked DA release. (*D*) Concentration–response curve for TTX using a 10^{-4}M NMDA concentration. Data (mean ± SD) are expressed as a fractional increase from basal release. *P < 0.05, **P < 0.01, ***P < 0.001 *vs.* basal release (*A*) and *vs.* NMDA-evoked release (*B–D*). TTX = tetrodotoxin; GLY = glycine; 7 Cl-KYN = 7 chloro-kynurenate.

show that volatile agents modulate transmitter release mainly *via* direct presynaptic effects at DA terminals in the striatum and the involvement of presynaptic $GABA_A$ receptors in at least part of their inhibitory effects on NMDA-evoked release.

Methodologic Considerations and Limitations

The synaptosomal preparation provides a useful tool to examine direct presynaptic effects of anesthetics on transmitter release.[10,11,14,16] However, it precludes examination of indirect, TTX-sensitive mechanisms or the role of diffusible mediators, such as nitric oxide, involved in NMDA-stimulated DA release.[29] The use of striatal slices is particularly interesting because it allows separate examination of the effects of anesthetics on the direct, TTX-insensitive and indirect, TTX-sensitive DA release elicited by NMDA application. However, the fact that drug-induced neurotransmitter release is sensitive to TTX application does not rule out the possibility of direct presynaptic action at DA terminals. Indeed, several nonexclusive mechanisms could contribute to the NMDA-evoked GABAergic inhibitory control of ^{3}H-DA release resistant to TTX.[21] These include local modifications of the GABA neuronal uptake process,[30] release of GABA either from collaterals of medium-sized spiny neurons or GABAergic interneurons through processes involving TTX-insensitive sodium channels,[31] or dendritic release of GABA as already reported in the rat olfactory bulb.[28] In the present study, the NMDA-evoked ^{3}H-DA release was insensitive to TTX in the synaptosomal preparation.

We used ^{3}H-DA to preload DA nerve endings in the

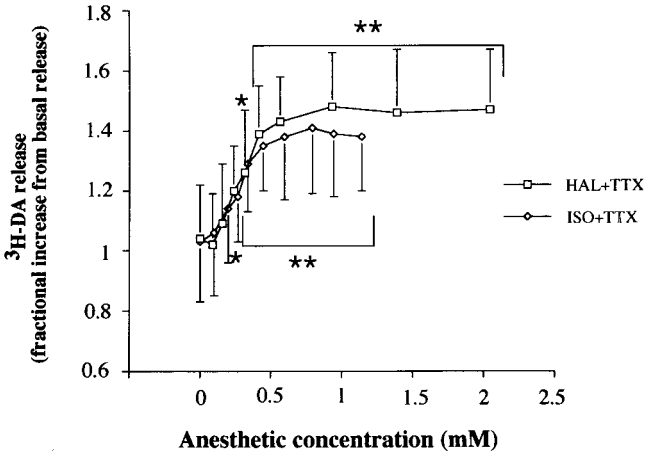

Fig. 2. Effects of halothane and isoflurane on spontaneous ^{3}H-DA release rate in striatal slices. Experiments were performed in the presence of a tetrodotoxin (5×10^{-8} M). Data (mean ± SD) are expressed as a fractional increase from basal release. *$P < 0.05$, **$P < 0.01$ *vs.* basal release.

present study. This technique allows a specific prelabeling of DA terminals in the striatum, because only DA nerve endings possess the high affinity carrier for this neurotransmitter among the various neuronal subpopulations present in this brain area. Release of the newly synthesized DA from ^{3}H-tyrosine could also have been used.[15] The newly synthesized DA is preferentially released from nerve endings by depolarization, and this phenomenon is also calcium-dependent.[32] However, this technique does not separate effects of anesthetics on release and synthesis of DA.

Effects of Halothane and Isoflurane on Basal ^{3}H-DA Release in the Striatum

Halothane and isoflurane were found to significantly enhance spontaneous ^{3}H-DA release in a concentration-related fashion in TTX-treated slices. These findings are consistent with the effects of a restricted number of concentrations of volatile anesthetics on ^{3}H-DA release reported in striatal synaptosomes.[11] This effect may have been caused by the blockade of the DA carrier, because halothane and isoflurane show significant effects on this transporter *in vitro*[10]; however, this was likely not to be the case for two reasons: (1) the 1-ml/min flow prevents from DA reuptake subjected to both KCl- and NMDA-induced release from synaptosomes[11,25]; and (2) nomifensine, an inhibitor of DA uptake, failed to affect either spontaneous or NMDA-elicited release in the present study.

According to the dose–response curve shown in figure 1, the TTX concentration used was expected to block action potential propagation along nerve membranes ≈90% of the striatal interneurons or fibers. Consistent with previous reports, we found that TTX alone reduced spontaneous ^{3}H-DA release by 40% from slices.[19] This might be a result of the inhibition of striatal interneurons such those containing acetylcholine, substance P, or nitric oxide, all of which exert presynaptic facilitative control on DA release.[17–19] Indeed, recent electrophysiologic and biochemical experiments indicate that presynaptic voltage-operated sodium channels show some sensitivity to volatile agents.[33,34] In addition, halothane blocks synaptic excitation of local interneurons in some brain areas, such as the hippocampus.[35] However, this hypothesis is not consistent with the lack of effect of the anesthetics on the TTX-sensitive portion of ^{3}H-DA release observed in the present study. Therefore, the concentration-related enhancement by halothane and isoflurane of the spontaneous ^{3}H-DA release in TTX-treated slices together with our previous data[11] support that this effect is mainly caused by direct stimulatory action of volatile anesthetics at DA terminals. Membrane fluidization—which correlates with the ability of volatile agents to increase resting calcium levels,[36] increase by volatile anesthetics of Ca^{2+} permeability of synaptosomal membranes,[37] or release of intraneuronally stored neurotransmitter, as was demonstrated for GABA[38,39]—may contribute to the stimulatory effect of inhalational anesthetics on spontaneous ^{3}H-DA release.

Effects of Halothane and Isoflurane on NMDA-evoked ^{3}H-DA Release in the Striatum

A major finding made in the present study is that halothane and isoflurane used at particularly low concentrations markedly decreased the NMDA-evoked DA release in striatal slices in a concentration-related fashion. With respect to the IC_{50} values reported for each anesthetic, this observed effect was with the same potency in the presence or absence of activity of the striatal interneurons. In addition, it was present in synaptosomes in a dose-related fashion. Taken together, these data confirm and extend our previous observations[11] and show that volatile anesthetics decrease the NMDA receptor-mediated DA release from striatal slices by acting directly at presynaptic DA terminals.

NMDA application evoked a potent, concentration-related stimulation of ^{3}H-DA release in striatal slices, which is consistent with previous observations.[21] The stimulatory effect of NMDA on DA release was enhanced by glycine, a positive allosteric effector at the NMDA receptor, and blocked by MK 801, an antagonist of the

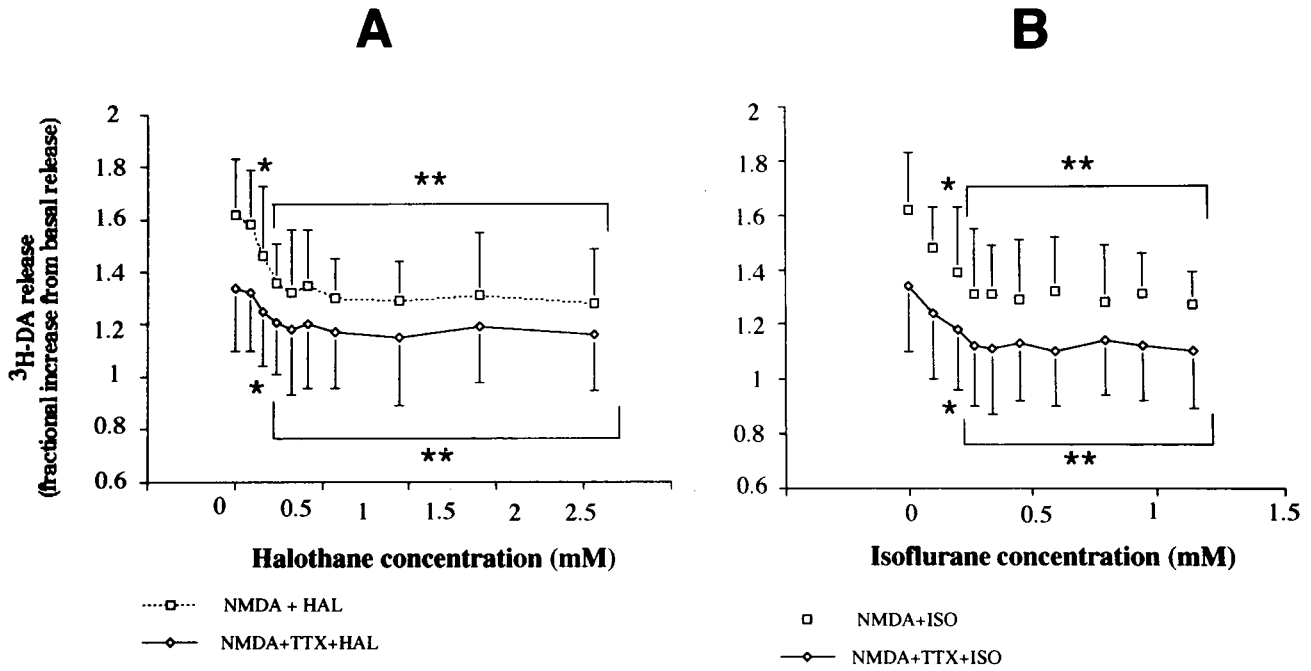

Fig. 3. Effects of halothane (*A*) and isoflurane (*B*) on ^{3}H-dopamine (DA) release rate stimulated by *N*-methyl-D-aspartate (NMDA; 10^{-4} M) in striatal slices. Experiments were performed in either the presence (*white triangles*) or absence (*white squares*) of tetrodotoxin (TTX; 5×10^{-8} M). Data (mean ± SD) are expressed as a fractional increase from basal release. HAL = halothane; ISO = isoflurane. **P* < 0.05, ***P* < 0.01 *vs.* NMDA-induced release.

NMDA receptor. The potentiation by glycine of NMDA-evoked DA release was no more observed in the presence of 7-chloro-kynurenate, an antagonist of the glycine site at the NMDA receptor. These findings show that DA release observed in the present study was actually caused by stimulation of striatal NMDA receptors. The release of ^{3}H-DA evoked by NMDA in the presence of TTX was markedly reduced in comparison with that elicited without TTX in the superfusion medium. In contrast, NMDA-evoked ^{3}H-DA release was insensitive to TTX in synaptosomes. This further shows that, in slices, NMDA stimulates DA release both directly and indirectly.[14] The higher NMDA concentrations (10^{-3} M *vs.* 10^{-4} M) required to significantly stimulate ^{3}H-DA release in synaptosomes compared with slices could be a result of a lower extracellular concentration of endogenous glycine in the synaptosomal preparation.

The fractional equivalent reduction by volatile agents of TTX-sensitive and -insensitive responses to NMDA stimulation in slices suggests direct presynaptic actions of volatile agents at DA terminals. Interestingly, the inhibitory effect of an IC_{50} concentration of halothane and isoflurane was significantly, but not totally, reversed by

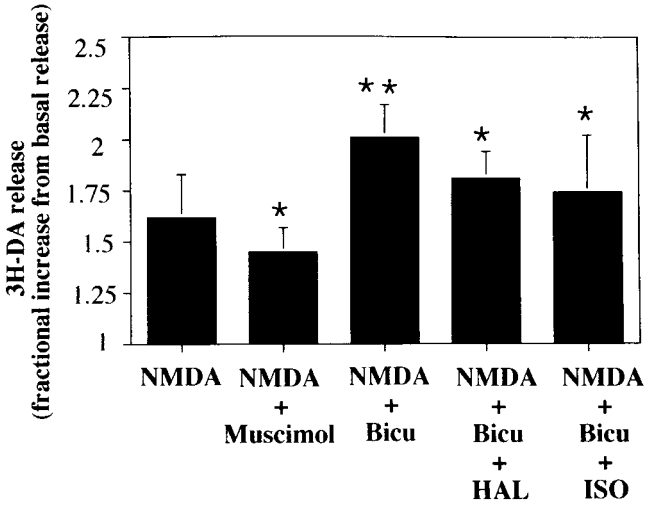

Fig. 4. Effects of muscimol (10^{-5} M), bicuculline (10^{-5} M), and combinations of halothane (0.15 mM) and isoflurane (0.14 mM) with bicuculline (10^{-5} M) on the *N*-methyl-D-aspartate (NMDA)-evoked ^{3}H-dopamine release in striatal slices. Halothane and isoflurane were used at their IC_{50} concentrations. Data (mean ±SD) are expressed as fractional increase from basal release. HAL = halothane; ISO = isoflurane; Bicu = bicuculline. **P* < 0.05, ***P* < 0.01 *vs.* NMDA-induced release.

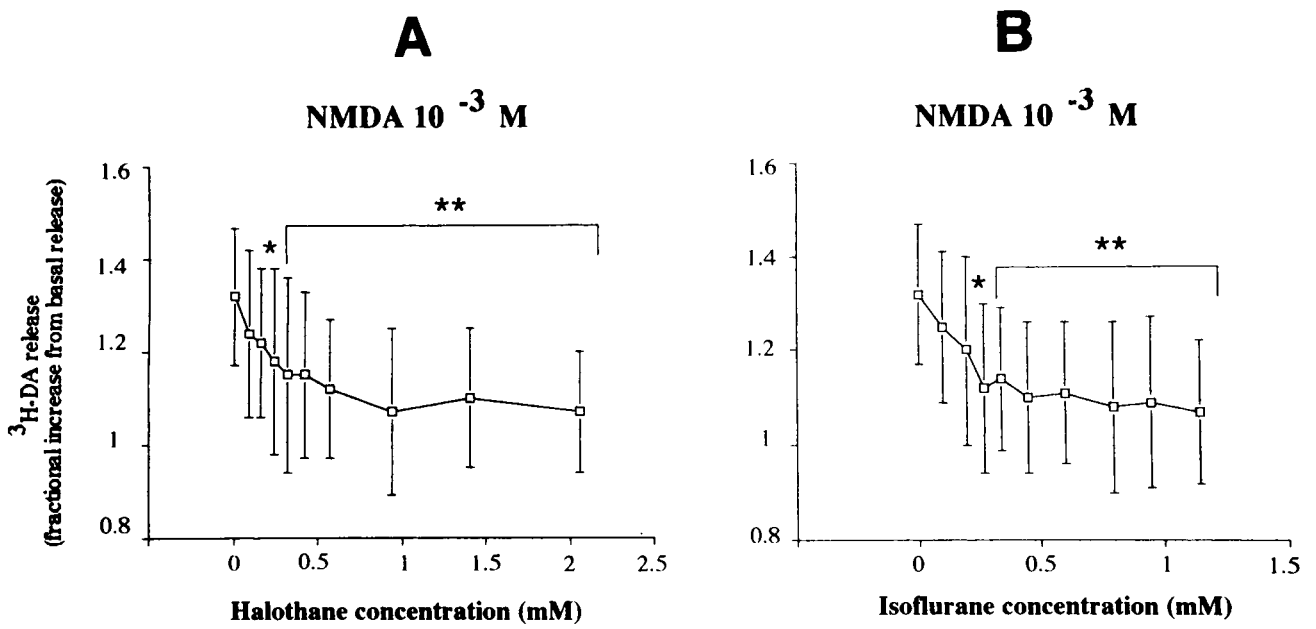

Fig. 5. Effects of halothane (*A*) and isoflurane (*B*) on ^{3}H-dopamine (DA) release rate stimulated by *N*-methyl-D-aspartate (NMDA; 10^{-3} M) in striatal synaptosomes. Data (mean ± SD) are expressed as a fractional increase from basal release. *$P < 0.05$, **$P < 0.01$ *vs.* NMDA-induced release.

bicuculline used at a concentration that blocked the tonic GABAergic inhibitory control of NMDA-evoked DA release.[20,21] Consistent with this finding, we observed a slight but significant decrease in NMDA-induced ^{3}H-DA release by muscimol, an agonist of $GABA_A$ receptors. The presence of these receptors on striatal DA terminals is strongly supported by previous pharmacologic experiments.[21,40] On the other hand, volatile anesthetics have been shown to potently enhance the activity of the $GABA_A$ receptor-coupled-chloride current.[41] Whether muscimol and volatile anesthetics share common target sites at $GABA_A$ receptors cannot be determined from our data. It can be proposed that that the inhibitory effects of inhalational anesthetics on NMDA-evoked ^{3}H-DA release are mediated in part *via* enhancement of the action of GABA released from recurrent collaterals or dendrites of striatal output neurons on the $GABA_A$ receptors present on DA terminals. The potency of volatile anesthetics to block NMDA-induced ^{3}H-DA release was greater in slices than in synaptosomes. The potentiation of GABA action on its receptors in slices may account for this difference, because no NMDA-evoked GABA release occurs in the synaptosomal preparation.[22]

Although full dose–response curves have not been generated with bicuculline, we observed that the reversal of the inhibitory effect of inhalational agents by bicuculline was only partial. This may suggest that volatile agents also act directly at either NMDA receptors or their environment to reduce ^{3}H-DA release. Our findings may suggest that downstream sites common to both KCl- and NMDA-elicited challenges are involved in halothane and isoflurane effects. However, some data show that the NMDA receptor-mediated response is particularly sensitive to isoflurane. Volatile anesthetics have been shown to depress NMDA-induced Ca^{2+} entry in cultured hippocampal neurons.[42] In addition, isoflurane is particularly effective at blocking glutamate transmission in numerous models.[43,44] This is in agreement with the greater sensitivity to isoflurane of the NMDA-evoked ^{3}H-DA release reported in our study. In addition, recent patch-clamp experiments indicate that isoflurane action at the NMDA receptor may show a high degree of specificity in some models.[45] Conversely, the KCl-evoked release of ^{3}H-GABA was not affected by these inhalational anesthetics in similar experimental conditions,[46] whereas synaptosomal glutamate release, which is also closely coupled to specific Ca^{2+} channel activation, is not highly sensitive to volatile anesthetics.[47] However, the N- type channels, which play a key role in presynaptic stimulus-secretion coupling in the brain,[48] are depressed by clinical concentrations of halothane or isoflurane.[37] Therefore, it cannot be excluded that a decrease in the activity of these voltage-

gated Ca^{2+} channels may contribute to the anesthetic-induced decrease in depolarization-evoked DA release in striatal slices.

Physiologic Implications

A potent inhibitory action of volatile anesthetics was observed on the NMDA-induced ^{3}H-DA release from striatal terminals. In contrast, a stimulatory effect of these agents on spontaneous release was observed in either the slice preparation and synaptosomes *in vitro*, although at higher concentrations than the effect on NMDA-evoked release, or in *in vivo* studies. Several explanations can be proposed to account for this apparent discrepancy. Isoflurane may both stimulate spontaneous DA release from striatal terminals and reduce glutamate release from striatal nerve endings, as was reported in some brain areas.[44,47] Alternatively, isoflurane depresses neuronal excitability by hyperpolarizing the cell membrane of all neocortical neurons in humans.[49] This results in reduction of the activity of the corticostriatal glutamate pathway and subsequent decrease in glutamate-stimulated DA release. Thus, the stimulatory effect of inhalational anesthetics on spontaneous DA release would be more perceptible than the inhibitory one on depolarization-evoked DA release, resulting in an increase in DA extracellular concentrations in the striatum. The lack of effect of anesthetics on the local circuits involved in the regulation of DA release is consistent with this hypothesis.

In conclusion, we provide evidence that clinical concentrations of halothane and isoflurane enhance basal but decrease NMDA-evoked DA release in the rat striatum *via*, at least in part, actions at $GABA_A$ receptors. It can be speculated that the inhibitory effects of volatile agents on glutamate transmission might contribute to some of their neuroprotective effects against striatal ischemic injury.[50]

References

1. Brozoski TJ, Brown BM, Rosvold HE, Goldman-Rakic PS: Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of Rhesus monkey. Science 1979; 205:929–32

2. Da Prada M, Keller HK, Pieri L, Kettler R, Haefely WE: The pharmacology of Parkinson's disease: Basic aspects and recent advances. Experientia 1984; 40:1165–304

3. Le Moal M, Simon H: Mesocorticolimbic dopaminergic network: Functional and regulatory roles. Physiol Rev 1991; 71:155–234

4. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG: Dopamine receptors: From structure to function. Physiol Rev 1998; 78:189–225

5. Segal IS, Walton JK, Irwin I, DeLanney LE, Ricaurte GA, Langston JW, Maze M: Modulating role of dopamine on anesthetic requirements. Eur J Pharmacol 1990; 186:9–15

6. Irifune M, Sato T, Nishikawa T, Masuyama T, Nomoto M, Fukuda T, Kawahara M: Hyperlocomotion during recovery from isoflurane anesthesia is associated with increased dopamine turnover in the nucleus accumbens and striatum in mice. ANESTHESIOLOGY 1997; 86: 464–75

7. Stahle L, Collin AK, Ungerstedt U: Effects of halothane anesthesia on extracellular levels of dopamine, dihydroxy phenylacetic acid, homovanillic acid and 5-hydroxyindolacetic acid in rat striatum: A microdialysis study. Arch Pharmacol 1990; 342:136–40

8. Miyano K, Tanifuji Y, Eger EI: The effect of halothane dose on striatal dopamine: An in vivo microdialysis study. Brain Res 1993; 605:342–4

9. Opacka-Juffry J, Ahier RG, Cremer JE: Nomifensine-induced increase in extracellular striatal dopamine is enhanced by isoflurane anaesthesia. Synapse 1991; 7:169–71

10. El-Maghrabi EA, Eckenhoff RG: Inhibition of dopamine transport in rat brain by volatile anesthetics. ANESTHESIOLOGY 1993; 78:750–6

11. Mantz J, Varlet C, Lecharny JB, Henzel D, Lenot P, Desmonts JM: Effects of volatile anesthetics, thiopental, and ketamine on spontaneous and depolarization-evoked dopamine release from striatal synaptosomes in the rat. ANESTHESIOLOGY 1994; 80:352–63

12. Graybiel AM: Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 1990; 13:244–54

13. Nieoullon A, Chéramy A, Glowinski J: Release of dopamine evoked by electrical stimulation of the motor and visual areas of the cerebral cortex in both caudate nuclei and in the substantia nigra in the cat. Brain Res 1978; 145:69–83

14. Krebs MO, Desce JM, Kemel ML, Gauchy C, Godeheu G, Chéramy A, Glowinski J: Glutamatergic control of dopamine release in the rat striatum: Evidence for presynaptic N-methyl D-aspartate receptors on dopaminergic nerve terminals. J Neurochem 1991; 56:81–5

15. Krebs MO, Trovero F, Desban M, Gauchy C, Glowinski J, Kemel ML: Distinct presynaptic regulation of dopamine release through NMDA receptors in striosome- and matrix-enriched areas of the rat striatum. J Neurosci 1991; 11:1256–62

16. Wang JTK: Presynaptic glutamate receptors modulate dopamine release from striatal synaptosomes. J Neurochem 1991; 57:819–22

17. Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC: Striatal interneurones: Chemical, physiological and morphological characterization. Trends Neurosci 1995; 18:527–35

18. Reid MS, Herrera-Marschitz M, Hökfelt T, Linderfors N, Persson H, Ungerstedt U: Striatonigral GABA, dynorphin, substance P and neurokinin A modulation of nigrostriatal dopamine release: Evidence for direct regulatory mechanisms. Exp Brainm Res 1990; 82:293–303

19. Giorguieff MF, Le Floc'h ML, Glowinski J, Besson MJ: Involvement of presynaptic receptors of nicotinic and muscarinic types in the control of the spontaneous release of dopamine from striatal dopaminergic terminals in the rat. J Pharmacol Exp Ther 1977; 200:535–44

20. Krebs MO, Gauchy C, Desban M, Glowinski J, Kemel ML: Role of dynorphin and GABA in the inhibitory regulation of NMDA-induced dopamine release in striosome- and matrix-enriched areas of the rat striatum. J Neurosci 1994; 14:2435–43

21. Krebs MO, Kemel ML, Gauchy C, Desban M, Glowinski J: Local GABAergic regulation of the N-methyl-D-aspartate-evoked release of dopamine is more prominent in striosomes than in matrix of the rat striatum. Neuroscience 1993; 57:249–60

22. Galli T, Desce JM, Artaud F, Kemel ML, Chöramy A, Glowinski J:

Modulation of GABA release by α-amino-3-hydroxy-5-methylisoxazole-4-propionate and N-methyl-D-aspartate receptors in matrix-enriched areas of the rat striatum. Neuroscience 1992; 50:769–80

23. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates. London, Academic Press, 1986

24. National Institutes of Health: Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23. Bethesda, MD, National Institutes of Health, 1996, pp 58–9

25. Bowyer JF, Masserano JM, Weiner N: Inhibitory effects of amphetamine on potassium-stimulated release of ^{3}H-dopamine from striatal slices and synaptosomes. J Pharmacol Exp Ther 1987; 240:177–86

26. Mazze RI, Rice SA, Baden JM: Halothane, isoflurane and enflurane MAC in pregnant and nonpregnant female and male mice and rats. ANESTHESIOLOGY 1985; 62:99–105

27. Brachet-Liermain E, Ferrus L, Caroff J: Electron capture detection and measurement of fluothane (halothane) in blood. J Chromatogr Sci 1971; 9:49–53

28. Jaffé EH, Cuello AC: Release of 3H-γ-aminobutyric acid from the external plexiform layer of the rat olfactory bulb: Possible dendritic involvement. Neuroscience 1980; 5:1859–69

29. Hanbauer I, Wink D, Osawa Y, Edelman GM, Gally JA: Role of nitric oxide in NMDA-evoked release of 3H-dopamine from striatal slices. NeuroReport 1992; 3:409–12

30. Carvalho CM, Bandeira-Duarte C, Ferreira IL, Carvalho AP: Regulation of carrier-mediated excitotoxic release of ^{3}H-GABA in rat brain synaptosomes. Neurochem Res 1991; 16:763–73

31. Fukuda J, Kameyama M: Tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in tissue-cultured spinal ganglion neurons from adult mammals. Brain Res 1980; 182:191–7

32. McMillen BA, German DC, Shore PA: Functional and pharmacological significance of brain dopamine and norepinephrine storage pools. Biochem Pharmacol 1980; 29:3045–50

33. Rehberg B, Xiao YH, Duch DS: Central nervous system sodium channels are significantly suppressed at clinical concentrations of volatile anesthetics. ANESTHESIOLOGY 1996; 84:1223–33

34. Ratnakumari L, Hemmings HC Jr: Inhibition of presynaptic sodium channels by halothane. ANESTHESIOLOGY 1998; 88:1043–54

35. Perouansky M, Kirson ED, Yaari Y: Halothane blocks synaptic excitation of inhibitory interneurons. ANESTHESIOLOGY 1996; 85:1431–8

36. Daniell LC, Harris RA: Neuronal intracellular calcium concentrations are altered by anesthetics: Relationship to membrane fluidization. J Pharmacol Exp Ther 1988; 245:1–7

37. Xu F, Sarti P, Zhang J, Blanck TJJ: Halothane and isoflurane alter calcium dynamics in rat cerebrocortical synaptosomes. Anesth Analg 1998; 87:701–10

38. Mody I, Tanelian DL, MacIver MB: Halothane enhances tonic neuronal inhibition by elevating intracellular calcium. Brain Res 1991; 538:319–23

39. Doze VA, McIver MB: Halothane acts on ryanodine sensitive calcium release channels to enhance presynaptic GABA release (abstract). ANESTHESIOLOGY 1998; 89:A722

40. Chesselet MF: Presynaptic regulation of neurotransmitter release in the brain: Facts and hypothesis. Neuroscience 1984; 12:347–75

41. Tanelian DL, Kosek P, Mody I, MacIver MB: The role of the GABAA receptor/chloride channel complex in anesthesia. ANESTHESIOLOGY 1993; 78:757–76

42. Puil EH, El-Beheity H, Baimbridge KG: Anaesthetic effects on glutamate-stimulated increase in intraneuronal calcium. J Pharmacol Exp Ther 1990; 255:955–61

43. Bickler PE, Buck LT, Hansen BM: Effects of isoflurane and hypothermia on glutamate receptor-mediated calcium influx in brain slices. ANESTHESIOLOGY 1994; 81:1461–9

44. MacIver MB, Mikulec AA, Amagasu SM, Monroe FA: Volatile anesthetics depress glutamate transmission via presynaptic actions. ANESTHESIOLOGY 1996; 85:823–34

45. Ming Z, McCown TJ, Criswell E, Breese GR, Mueller RA: NMDAR1 splicing alters the effects of isoflurane on NMDA receptors: The role of the exon 5 insert (abstract). ANESTHESIOLOGY 1998 89:A701

46. Salord F, Keita H, Lecharny JB, Henzel D, Desmonts JM, Mantz J: Halothane and isoflurane differentially affect the regulation of dopamine and gamma-aminobutyric acid release mediated by presynaptic acetylcholine receptors in the rat striatum. ANESTHESIOLOGY 1997; 86:632–41

47. Schlame M, Hemmings HC Jr: Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. ANESTHESIOLOGY 1995; 82:1406–16

48. Lynch C, Pancrazio JJ: Snails, spiders, and stereospecificity: Is there a role for calcium channels in anesthetic mechanisms? ANESTHESIOLOGY 1994; 81:1–5

49. Berg-Johnsen J, Langmoen IA: Mechanisms concerned in the direct effect of isoflurane on rat hippocampal and human neocortical neurons. Brain Res 1990; 507:28–34

50. Koorn R, Kahn RA, Brannan TS, Martineztica J, Weinberger J, Reich DL: Effect of isoflurane and halothane on in vivo ischemia-induced dopamine release in the corpus striatum of the rat: A study using cerebral microdialysis. ANESTHESIOLOGY 1993; 79:827–35