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Halothane and Isoflurane Differentially Affect the Regulation of Dopamine and Gamma-aminobutyric Acid Release Mediated by Presynaptic Acetylcholine Receptors in the Rat Striatum

François Salord, M.D., Hawa Keita, M.D.,† Jean-Baptiste Lecharny, M.D.,* Danielle Henzel,‡ Jean-Marie Desmonts, M.D.,§ Jean Mantz, M.D., Ph.D.‖*

Background: **General anesthetics are thought to produce their hypnotic effects mainly by acting at ligand-gated ionic channels in the central nervous system (CNS). Although it is well established that volatile anesthetics significantly modify the activity of the acetylcholine nicotinic receptors of the neuromuscular junction, little is known about their actions on the acetylcholine receptors in the CNS. In this study, the effects of halothane and isoflurane on the regulation of dopamine (DA) (gamma-aminobutyric acid [GABA]) depolarization-evoked release mediated by nicotinic (muscarinic) presynaptic receptors were studied in the rat striatum.**

Methods: **Assay for GABA (dopamine) release consisted of ^{3}H-GABA (^{3}H-DA)-preloaded synaptosomes with artificial cerebrospinal fluid (0.5 ml/min, 37°C) and measuring the radioactivity obtained from 1-min fractions for 18 min, first in the absence of any treatment (spontaneous release, 8 min), then in the presence of depolarizing agents combined with vaporized halothane and isoflurane (0.5–5%, 5 min), and finally with no pharmacologic stimulation (5 min). The depolarizing agents were potassium chloride (KCl; 9 mM) alone or with acetylcholine (10^{-6}–10^{-4} M) and/or atropine (10^{-5} M) for experiments with ^{3}H-GABA, and KCl (15 mM) and nicotine ($10^{-7} - 5 \times 10^{-4}$ M) alone or with mecamylamine (10^{-5} M) for experiments with ^{3}H-DA.**

Results: **Potassium chloride induced a significant, Ca^{2+}-dependent release of both ^{3}H-GABA and ^{3}H-DA. Nicotine produced a concentration-related, mecamylamine-sensitive ^{3}H-DA release that was significantly attenuated by nicotine (10^{-7} M) preincubation. Acetylcholine elicited a dose-dependent, atropine-sensitive reduction of the KCl-evoked ^{3}H-GABA release. Halothane and isoflurane significantly decreased the nicotine-evoked ^{3}H-DA release but had only limited depressant effects on the KCl-stimulated ^{3}H-DA and no action on the KCl-induced ^{3}H-GABA release. The effects of acetylcholine on ^{3}H-GABA release were reversed by halothane but not by isoflurane.**

Conclusion: **Clinically relevant concentrations of halothane and isoflurane significantly, but differentially, alter the presynaptic cholinergic regulation of the release of inhibitory neurotransmitters in the striatum. These results suggest that the cholinergic transmission may represent an important and specific presynaptic target for volatile anesthetics in the CNS. (Key words. Anesthetics, volatile: halothane, isoflurane. Receptors, acetylcholine, nicotinic, agonists: nicotine. Receptors, acetylcholine, nicotinic, antagonists: mecamylamine. Receptors, acetylcholine, muscarinic, antagonists: atropine; receptors. Sympathetic nervous system, catecholamines: dopamine. Neurotransmitters, amino acids: GABA. Brain, striatum: synaptosomes.)**

THE nicotinic acetylcholine receptor is a member of a superfamily of structurally related ligand-gated ionic channels that includes the gamma-aminobutyric acid ($GABA_A$), glycine, and N-methyl-D-aspartate receptors.[1] Elegant experiments recently established that volatile anesthetics used at clinically relevant concentrations exert specific actions at this receptor site. For example, isoflurane causes the nicotinic receptor to flicker.[2] This can be best explained by a kinetic model in which drug molecules have access to a binding (blocking or inhibitory) site when the gate of the channel is either open or closed.[2–5] In addition, isoflurane increases the apparent rates of fast and slow desensitization of the nicotinic cholinergic receptor of the neuromuscular junction.[6]

However, the nicotinic receptor of the neuromus-

* Research Fellow in Neuropharmacology.

† Assistant Professor of Anesthesiology.

‡ Technician.

§ Professor of Anesthesiology and Chairman.

‖ Associate Professor of Anesthesiology.

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Address reprint requests to Dr. Mantz: Department of Anesthesiology, Hospital Bichat, 46 rue Henri Huchard, 75018 Paris, FRANCE.

cular junction is not the primary target of general anesthetics and little is known about the sensitivity of the acetylcholine receptors of the central nervous system to these agents. In the striatum (a functionally important supraspinal brain area), cholinergic terminals arise from interneurons that play an important modulatory role on both dopamine (DA) and GABA release.[7] Indeed, acetylcholine stimulates the release of dopamine by activating directly nicotinic receptors located on the striatal dopamine nerve endings.[8-10] Conversely, acetylcholine decreases the potassium chloride (KCl)-evoked GABA release by stimulating muscarinic receptors present on the striatal GABA terminals.[11,12] Gamma-aminobutyric acid is thought to act as an inhibitory neurotransmitter, but this is also probably the case for dopamine. Experiments performed *in vitro* and *in vivo* have helped show that the major electrophysiologic effect of dopamine is hyperpolarization of the postsynaptic membranes, this inhibitory effect being mediated by dopamine D2 receptors.[13-15] Interestingly, the striatal content of dopamine or its metabolites measured *in vivo* by microdialysis in rats was increased by clinical halothane[16-18] and isoflurane[19] concentrations. In addition, Segal *et al.*[20] have shown that the halothane anesthetic-sparing effect of L-dihydroxyphenylalanine was blocked in mice by systemic administration of a selective antagonist of the D2, but not the D1, dopamine receptors. In this study, an inverse correlation was found between halothane anesthetic requirements and dopamine content of the striatum. Together these results suggest that modulating presynaptically not only the GABA but also the dopamine neurotransmission in the striatum might be relevant to the action of volatile anesthetics in the central nervous system.

Whether the modulation of dopamine and GABA release exerted by acetylcholine in the striatum is affected by anesthetics remains to be investigated. Here we used a preparation of synaptosomes (pinched-off nerve endings) originating from the rat striatum to examine the direct effects of inhalational anesthetics on presynaptic GABA and dopamine terminals (fig. 1). Our aim was thus to investigate the effects of halothane and isoflurane (1) on the release of dopamine elicited by activation of presynaptic nicotinic receptors located on dopaminergic terminals, and (2) on the inhibition of the KCl-evoked GABA release mediated by presynaptic muscarinic receptors located on GABA nerve endings.

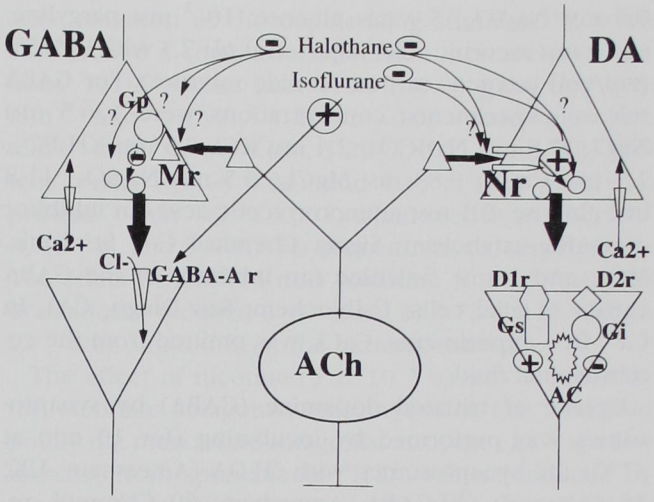

Fig. 1. The connections between acetylcholine interneurons and gamma-aminobutyric(GABA)-ergic and dopaminergic synapses in the striatum. Acetylcholine released from cholinergic interneurons can stimulate either muscarinic receptors located on the GABA terminals or nicotinic receptors present on dopaminergic nerve endings. Activation of the G protein-coupled muscarinic receptors by acetylcholine leads to inhibition of the Ca^{2+}-dependent GABA release, whereas dopamine is released by stimulation of the nicotinic receptors present on the dopaminergic terminals. Putative target sites are suggested for halothane and isoflurane. ACh = acetylcholine; DA = dopamine; Gp = G protein coupled to the muscarinic receptor; Gs = G protein coupled to the dopaminergic D1 receptor (D1r); Gi = G protein coupled to the dopaminergic D2 receptor (D2r); AC = adenylate cyclase; Mr = muscarinic receptor; Nr = nicotinic receptor; GABA-Ar = GABA receptor of the A subtype; + = stimulation; − = inhibition.

Materials and Methods

Handling procedures, as written in the Guide for the Care and Use of Laboratory Animals, were followed throughout. Experiments were performed on male Sprague-Dawley rats (Iffa-Credo, France) weighing 200–225 g and housed on a 12:12 light:dark cycle, with food and water provided *ad libitum*.

The preparation and purification of synaptosomes have been reported in detail elsewhere.[21-23] Briefly, animals were killed by stunning and decapitation. For dopamine release experiments, synaptosomes were diluted up to 0.16 mg/ml in ice-cold artificial cerebrospinal fluid in 126.5 mM NaCl; 27.5 mM $NaHCO_3$; 1 mM KCl; 0.5 mM KH_2PO_4; 1.1 mM $CaCl_2$; 0.83 mM $MgCl_2$;

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). For GABA release experiments, concentrations were 126.5 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 0.5 mM KH_2PO_4, 1.1 mM $CaCl_2$, 0.83 mM $MgCl_2$, 0.5 mM Na_2SO_4, 11.8 mM glucose, 0.1 mM aminooxyacetic acid (an inhibitor of GABA catabolism; Sigma Chemical Co., St. Louis, MO), and 1 mM β-alanine (an inhibitor of the GABA carrier in glial cells; Calbiochem, San Diego, CA). In Ca^{2+}-free experiments, $CaCl_2$ was omitted from the cerebrospinal fluid.

Uptake of tritiated dopamine (GABA) by synaptosomes was performed by incubating (for 10 min at 37°C) the synaptosomes with ^{3}H-DA (Amersham, UK; 30 Ci/mmol) (^{3}H-GABA, Amersham; 60 Ci/mmol, respectively). Aliquots (1 ml) were placed into each of the superfusion chambers using a pipette and embedded in Whatman GF/F glass filters (0.70-μm retention capacity) by light suction, and then superfused at a flow rate of 1 (0.5 ml/min) for experiments containing ^{3}H-DA (^{3}H-GABA) using a superfusion device equipped with an automatic fraction collector (Brandel, Gaithersburg, MD). The 1-ml flow rate was selected because of its ability to prevent reuptake of ^{3}H-DA by the synaptosome preparation.[24] Nipecotic acid (10^{-5} M) was used in the ^{3}H-GABA release experiments as well to eliminate the possibility of reuptake occurring during superfusion of synaptosomes. After a 30-min washing step (at 37°C), serial fractions were collected either every 30 s (experiments on the nicotine-induced ^{3}H-DA release) or every minute (experiments on the KCl-induced ^{3}H-DA or ^{3}H-GABA release) during three consecutive periods. The 30-s period was selected for experiments with nicotine because of the rapid desensitization of the nicotinic response.[25,26] The mean value of the radioactivity (counts per minute [cpm] measured during the first 8 min by liquid scintillation spectrometry using Aquasol-2 [New England Nuclear, Boston, MA]) was considered the basal (spontaneous) ^{3}H-DA or ^{3}H-GABA release. During the next 5 min of superfusion, either no treatment (time-dependent control) or various pharmacologic or anesthetic agents (evoked release) were delivered to the synaptosome preparation, and radioactivity was again estimated in each fraction. For the last 5 min, radioactivity was determined from 1-min fractions in the absence of any treatment. The influence of a drug on the release of either ^{3}H-DA or ^{3}H-GABA was assessed by calculating the difference between the release rate normalized to protein concentration observed in both the presence and the absence (time-dependent control) of the pharmacologic or anesthetic agents used. This unit (pmol · mg protein^{-1} · min^{-1}) allows comparison with data originating from other groups using either the same metric or reporting only the fractional increase from basal release elicited by pharmacologic depolarization.[21-26]

The effects of the following anesthetic agents were studied: halothane (Fluothane; Zeneca, Cergy, France) used at 0.5%, 0.7%, 1%, 1.5%, 2%, 3%, 4%, and 5%; and isoflurane (Forane; Abbott, Rungis, France) used at 0.7%, 1.5%, 2.2%, 3%, 3.7%, 4.2%, and 5%, respectively. Volatile anesthetics were delivered through a calibrated vaporizer in an oxygen-carbon dioxide mixture (95%/5% vol/vol, 3 l/min). The 1 minimum alveolar concentration value was considered to be 1% for halothane and 1.5% for isoflurane, as previously reported in male rats.[27] Volatile anesthetics were equilibrated with the artificial cerebrospinal fluid for 60 min at 37°C, and aqueous concentrations in the superfusion chambers were determined by gas chromatography according to slight modifications of the method reported by Brachet-Liermain *et al.*[28] The pharmacologic agents used in the release experiments were KCl (9 and 15 mM), nicotine (10^{-7}-5×10^{-4} M), acetylcholine (10^{-5}-10^{-4} M), atropine (10^{-5} M), and mecamylamine (10^{-5} M) (Sigma, La Verpillière, France). In the experiments in which preincubation with a low nicotine concentration was performed, synaptosomes were superfused for 20 min with 10^{-7} M nicotine just before the fractions were collected.[26]

Results were considered reliable only if they had been reproduced in at least four independent experiments (each of them run in triplicate). Release data were analyzed by analysis of variance followed by the Student's *t* test corrected for the number of comparisons. A probability value less than 0.05 was considered the threshold for significance. Data are expressed as means ± SD.

Results

Concentrations of Anesthetics Measured in Superfusion Chambers

The aqueous concentrations of volatile anesthetics measured in the chambers after 1 h of equilibration with the superfusion medium at 37°C were 0.17 ± 0.03 mM, 0.23 ± 0.05 mM, 0.31 ± 0.04 mM, 0.43 ± 0.04 mM, 0.55 ± 0.04 mM, 0.96 ± 0.08 mM, 1.43 ± 0.11 mM, and

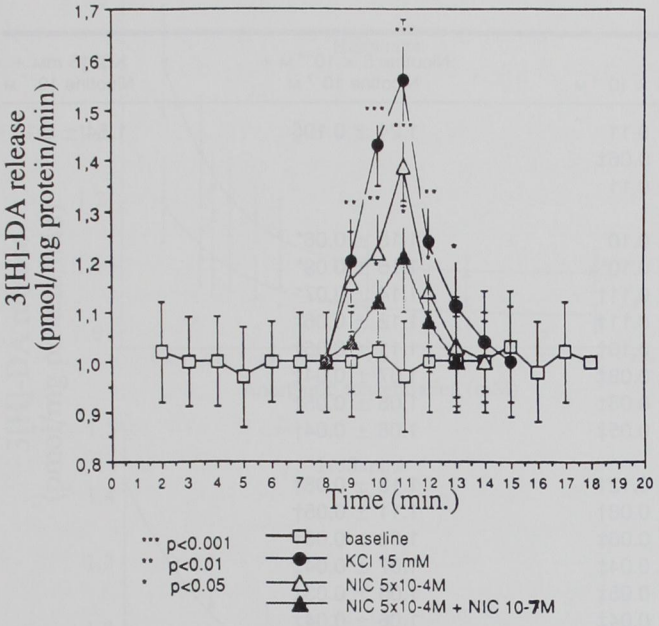

Fig. 2. Time course of the release rate of ^{3}H-DA elicited by various depolarizing stimuli from preloaded striatal synaptosomes. Data (means ± SD) are expressed as pmol · mg protein^{-1} · min^{-1}. The peaks of radioactivity corresponding to the pooling of two consecutive 30-s periods of sampling are displayed for every minute. Depolarizing agents were applied between minutes 8 and 13. Preincubation with nicotine (10^{-7} M) was performed for 20 min just before sampling. KCl = potassium chloride; NIC = nicotine.

2.03 ± 0.14 mM for halothane used at 0.5%, 0.7%, 1%, 1.5%, 2%, 3%, 4%, and 5%, respectively; 0.20 ± 0.03 mM, 0.33 ± 0.03 mM, 0.47 ± 0.05 mM, 0.61 ± 0.05 mM, 0.77 ± 0.08 mM, 0.94 ± 0.09 mM, and 1.12 ± 0.12 mM for isoflurane used at 0.7%, 1.5%, 2.2%, 3%, 3.7%, 4.2%, and 5%, respectively.

Effects of Volatile Anesthetics on the Stimulation of Dopamine Release Mediated by Presynaptic Nicotinic Receptors

The ^{3}H-DA concentration estimated in the incubation medium during the uptake phase was 250 ± 15 pmol/mg protein, and the initial basal release rate measured during a 5-min period was approximately 1.02 ± 0.09 pmol · mg protein^{-1} · min^{-1}. The basal release rate was not affected by removing Ca^{2+} from the superfusion medium. Potassium chloride (15 mM) elicited a significant increase from the spontaneous ^{3}H-DA release rate: (1.59 ± 0.12 pmol · mg protein^{-1} · min^{-1}; $P < 0.001$; fig. 2). It was followed by a rapid return to baseline after cessation of the depolarizing stimulus. Nicotine also evoked a concentration-related increase from the basal ^{3}H-DA release rate: 1.10 ± 0.03 pmol · mg protein^{-1} · min^{-1} (NS), 1.21 ± 0.05 pmol · mg protein^{-1} · min^{-1} ($P < 0.05$), 1.29 ± 0.07 pmol · mg protein^{-1} · min^{-1} ($P < 0.01$), and 1.43 ± 0.11 pmol · mg protein^{-1} · min^{-1} ($P < 0.001$) when used at 10^{-6} M, 10^{-5} M, 10^{-4} M, and 5×10^{-4} M concentrations, respectively. The effect of KCl (15 mM) (nicotine 5×10^{-4} M) was dramatically reduced (−87 ± 8% or −78 ± 12%) when Ca^{2+} was omitted from the external medium.

The effect of nicotine (5×10^{-4} M) was blocked by the nicotinic antagonist mecamylamine (10^{-5} M), but not by the muscarinic antagonist atropine (10^{-5} M). The increase from spontaneous ^{3}H-DA release induced by nicotine (5×10^{-4} M) was significantly attenuated by preincubation with a 10^{-7} M nicotine concentration applied during 20 min before nicotine (5×10^{-4} M; fig. 2 and table 1). This low nicotine concentration (10^{-7} M) failed to induce a dopamine release by itself. In contrast, the KCl-evoked ^{3}H-DA release was unaffected by nicotine (10^{-7} M) preincubation.

The effects of halothane and isoflurane on the depolarization-evoked ^{3}H-DA release are displayed in table 1 and figure 3. Both halothane and isoflurane produced a significant reduction of the nicotine-induced peaks of radioactivity (median inhibitory concentration = 0.24 ± 0.04 mM and 0.22 ± 0.05 mM for halothane and isoflurane, respectively). At all anesthetic concentrations, desensitization due to nicotine 10^{-7} M preincubation was not altered by anesthetics, because the magnitude of reduction of the nicotine-induced ^{3}H-DA release was not significantly different in either the presence or absence of preincubation of synaptosomes with a 10^{-7} M concentration of nicotine (fig. 3). On the other hand, halothane had only a limited and barely dose-dependent action on the KCl-induced release, but isoflurane had a more clearly concentration-related effect (table 1).

Effects of Volatile Anesthetics on the Reduction of Gamma-aminobutyric Acid Release Mediated by Presynaptic Muscarinic Receptors

The initial ^{3}H-GABA basal release rate measured during a 5-min period was approximately 0.90 ± 0.07 pmol · mg protein^{-1} · min^{-1}. The time course of the depolarization-induced GABA release is displayed in figure 3. Potassium chloride (9 mM) elicited a significant increase from the spontaneous ^{3}H-GABA release rate (1.30 ± 0.10 pmol · mg protein^{-1} · min^{-1}; $P < 0.001$) fol-

Table 1. Effects of Halothane and Isoflurane on ^{3}H-DA Release

	KCl 15 mM	Nicotine 5×10^{-4} M	Nicotine 5×10^{-4} M + Nicotine 10^{-7} M	KCl 15 mM + Nicotine 10^{-7} M
Control	1.59 ± 0.12	1.43 ± 0.11	1.24 ± 0.10§	1.54 ± 0.10
Mecamylamine (10^{-5} M)		1.09 ± 0.06‡		
Atropine (10^{-5} M)		1.36 ± 0.11		
Halothane (mM)				
0.17	1.57 ± 0.12	1.29 ± 0.10*	1.15 ± 0.08*	
0.23		1.25 ± 0.10*	1.15 ± 0.09*	
0.31	1.48 ± 0.13	1.23 ± 0.11†	1.14 ± 0.07*	
0.43		1.21 ± 0.11†	1.12 ± 0.06*	
0.55	1.43 ± 0.09*	1.18 ± 0.10†	1.11 ± 0.06†	
0.96		1.14 ± 0.08‡	1.07 ± 0.04†	
1.43		1.12 ± 0.06‡	1.08 ± 0.05†	
2.03		1.13 ± 0.05‡	1.06 ± 0.04†	
Isoflurane (mM)				
0.20	1.55 ± 0.11	1.26 ± 0.12†	1.13 ± 0.08†	
0.33	1.40 ± 0.08*	1.21 ± 0.08†	1.11 ± 0.05†	
0.47		1.10 ± 0.06‡	1.08 ± 0.06†	
0.61	1.25 ± 0.12†	1.07 ± 0.04‡	1.07 ± 0.04‡	
0.77		1.09 ± 0.05‡	1.05 ± 0.03‡	
0.94		1.11 ± 0.04‡	1.06 ± 0.04‡	
1.12		1.07 ± 0.04	1.05 ± 0.03‡	

Data (mean ± SD) are expressed in pmol · mg protein^{-1} · min^{-1}.

* $P < 0.05$ *versus* control.

† $P < 0.01$ *versus* control.

‡ $P < 0.001$ *versus* control.

§ $P < 0.01$ *versus* nicotine (5×10^{-4} M).

lowed by a prompt return to baseline after cessation of the depolarization. This effect was significantly attenuated by acetylcholine (10^{-4} M): 1.15 ± 0.09 pmol · mg protein^{-1} · min^{-1} *versus* 1.30 ± 0.10 pmol · mg protein^{-1} · min^{-1} ($P < 0.01$; table 2, fig. 4). The inhibitory action of acetylcholine was reversed in the presence of atropine (10^{-5} M), but not mecamylamine (10^{-5} M) (table 2). Halothane and isoflurane did not significantly affect the release of ^{3}H-GABA produced by KCl depolarization. In contrast, halothane was found to antagonize the inhibitory action of acetylcholine on GABA release (median effective concentration = 0.20 ± 0.03 mM; fig. 5). This effect was not significantly different from that of atropine, alone or in association with halothane. This property was not shared by isoflurane, because that agent failed to alter the inhibitory influence of acetylcholine on the KCl-evoked ^{3}H-GABA release (table 2).

Discussion

The primary original finding of this study is that volatile anesthetics used at clinically relevant concentrations can interfere with responses mediated by central, presynaptic acetylcholine receptors. More precisely, equipotent concentrations of halothane and isoflurane exert significant, but nonidentical, actions on the responses involved in GABA and dopamine release in the striatum and mediated by presynaptic acetylcholine receptors. Both agents significantly decreased the release of dopamine mediated by nicotinic receptors, whereas they produced only modest effects on that elicited by KCl depolarization. Halothane was also found to antagonize the inhibition of KCl-evoked GABA release mediated by presynaptic muscarinic receptors. This effect, however, was not shared by isoflurane.

Methodologic Considerations and Limitations

In the current study, the effects of volatile anesthetics on the release of dopamine and GABA were examined on synaptosomes prepared from the rat striatum. This allows comparison with previous data on the modulation by anesthetics of the striatal release of dopamine and GABA.[21–26] The depolarizing agents were applied briefly (5 min). As was observed here, this experimental

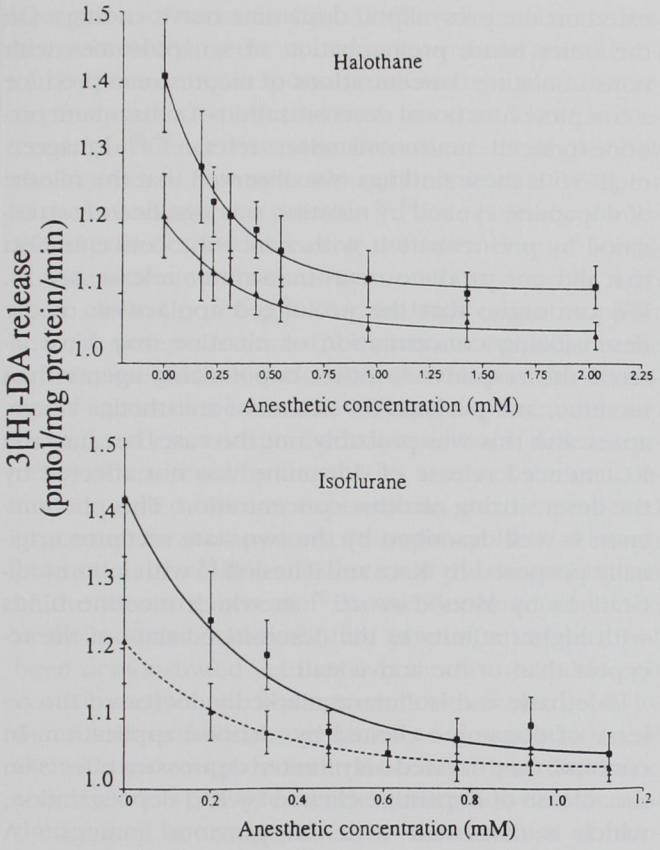

Fig. 3. Effects of halothane (*upper*) and isoflurane (*lower*) on ^{3}H-DA release rate stimulated by nicotine (5×10^{-4} M from striatal synaptosomes. Experiments were performed in either the presence (filled triangles) or absence (filled squares) of a desensitizing nicotine concentration (10^{-7} M). Data (means ± SD) are expressed in pmol · mg protein^{-1} · min^{-1}.

paradigm allows us to study phenomena that depend on the presence of external Ca^{2+}. However, the interpretation of the data must account for the fact that physiologic depolarization, which occurs during a period of milliseconds, may exhibit different sensitivity to anesthetics compared with pharmacologic depolarization applied during several minutes to the synaptosomal preparation.

Synaptosomes provide a reliable and reproducible model for analyzing, *in vitro*, the direct presynaptic effects of anesthetics on neurotransmitter release, uptake, or metabolism of either subcortical or cortical[29,30] areas. However, it must be emphasized that the use of synaptosomes precludes examining indirect, tetrodotoxin-sensitive mechanisms mediated by local interneurons. In addition, we cannot address the role of endogenous diffusible mediators such as nitric oxide, which is involved in the striatal dopamine release triggered by N-methyl-D-aspartate and putatively implicated in the cholinergic regulation of dopamine release *via* N-methyl-D-aspartate/acetylcholine/dopamine interactions.[7,31] Therefore, we cannot preclude that nerve terminals *in situ* subjected to impulse activity and a transmitter-enriched environment may behave differently.

The synaptosomes were obtained from the corpus striatum, which represents a functionally important brain area particularly implicated in motor activity control.[32] Results obtained from striatal preparations may not be extended to other brain areas. In addition, the subcompartmentalization of the striatum into matrix and striosomes is highly relevant to the regulation of neurotransmitter release by acetylcholine, because the choline acetyltransferase content represents one of the major criteria that substantiate this separation.[32] Important differences have been observed in the presynaptic control of dopamine release between striosomal and matrix-enriched striatal areas in the cat.[33] Thus we cannot rule out that anesthetics may differentially affect

Table 2. Effects of Halothane and Isoflurane on ^{3}H-γ-Aminobutyric Acid Release

	KCl 9 mM	KCl 9 mM + ACH 10^{-4} M
Control	1.30 ± 0.10	1.15 ± 0.09*
Mecamylamine (10^{-5} M)		1.17 ± 0.08*
Atropine (10^{-5} M)		1.34 ± 0.09†
Halothane (mM)		
0.17	1.29 ± 0.11	1.22 ± 0.08
0.23		1.25 ± 0.08‡
0.31	1.27 ± 0.12	1.29 ± 0.08†
0.43		1.34 ± 0.08†
0.55	1.24 ± 0.12	1.35 ± 0.07†
0.96		1.33 ± 0.08†
1.43		1.35 ± 0.09†
2.03		1.35 ± 0.11†
Halothane (0.55 mM) + atropine (10^{-5} M)		1.35 ± 0.12†
Isoflurane (mM)		
0.20	1.26 ± 0.12	1.20 ± 0.09
0.33	1.26 ± 0.11	1.18 ± 0.10
0.61	1.25 ± 0.10	1.12 ± 0.10

Data (mean ± SD) are expressed in pmol · mg protein^{-1} · min^{-1}.

ACH = acetylcholine.

* $P < 0.01$ *versus* KCl 9 mM.

† $P < 0.01$ *versus* control.

‡ $P < 0.05$ *versus* control.

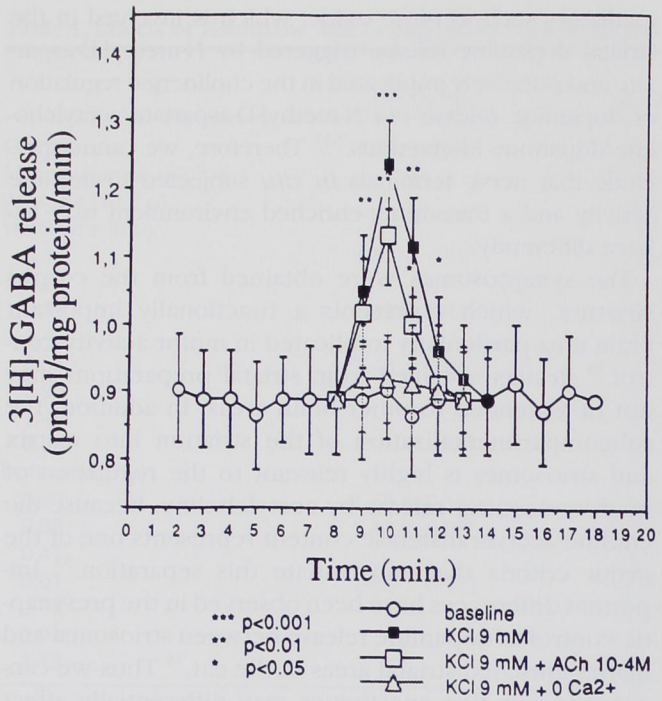

Fig. 4. Time course of the release rate of ^{3}H-GABA elicited by KCl and inhibitory effects of acetylcholine. Data (means ± SD) are expressed in pmol · mg protein^{-1} · min^{-1}. Experiments were performed in the presence of nipecotic acid (10^{-5} M). Radioactivity was collected every minute. Potassium chloride and acetylcholine were applied between minute 8 and 13. KCl = potassium chloride; ACh = acetylcholine

the control of dopamine or GABA release mediated by presynaptic acetylcholine receptors in matrix- and striosome-enriched areas of the striatum.

Effects of Halothane and Isoflurane on the Release of Dopamine Mediated by Activation of Presynaptic Nicotinic Receptors

The release of dopamine evoked by nicotine probably was mediated by acetylcholine receptors of the nicotinic subtype, because it was blocked by the nicotinic antagonist mecamylamine but not by the muscarinic antagonist atropine. These findings correspond with previous results showing that the nicotinic receptors involved in the control of dopamine release in the striatum are sensitive to mecamylamine.[10] The lack of effect of tetrodotoxin in blocking the release of dopamine elicited by nicotinic agonists on striatal slices[10] and the present results obtained on synaptosomes support that these effects were mediated by nicotinic receptors located on the presynaptic dopamine nerve endings. On the other hand, preincubation of synaptosomes with nonstimulating concentrations of nicotine can produce a complete functional desensitization of subsequent nicotine-induced neurotransmitter release.[25,26] In agreement with these findings, we observed that the release of dopamine evoked by nicotine was significantly attenuated by preincubation with a nicotine concentration that did not produce neurotransmitter release *per se*. We can argue that the prolonged application of the desensitizing concentration of nicotine may have altered the response to other depolarizing agents than nicotine, and perhaps the action of anesthetics at synapses. But this was probably not the case, because the KCl-induced release of dopamine was not affected by the desensitizing nicotine concentration. This phenomenon is well described by the two-state receptor originally proposed by Katz and Thesleff,[34] with later modifications by Monod *et al.*,[35] in which nicotine binds with higher affinity to the desensitized state of the receptor than to the active state.

Halothane and isoflurane markedly decreased the release of dopamine elicited by nicotine application. In contrast, they exerted only limited depressant effects on the release of dopamine elicited by KCl depolarization, which is consistent with our previous findings.[21] A marked reduction of the nicotine-evoked dopamine release was observed at concentrations of volatile anesthetics clearly within the clinical range (median inhibitory concentration = 0.24 ± 0.04 mM and 0.22 ± 0.05 mM for halothane and isoflurane, respectively). These values were the same in the presence of a desensitizing con-

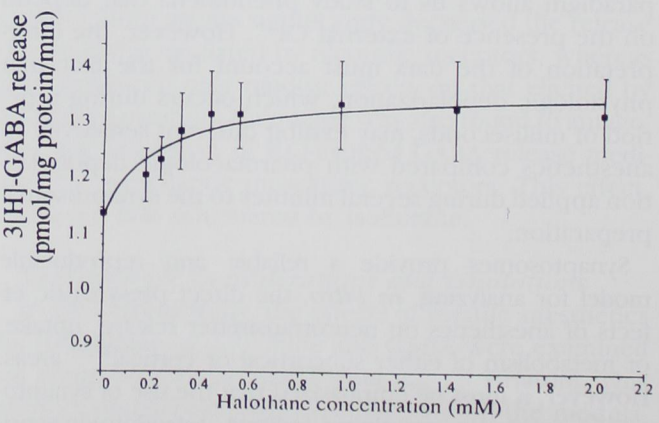

Fig. 5. Reversal by halothane of the reduction by acetylcholine of the ^{3}H-GABA release elicited by KCl. Data (means ± SD) are expressed in pmol · mg protein^{-1} · min^{-1}.

centration of nicotine (fig. 3). The molecular mechanisms involved in the decrease by volatile anesthetics of the stimulating response mediated *via* nicotinic receptors cannot be inferred from our data. There is converging evidence that inhalational anesthetics modify the kinetics of desensitization of the nicotinic receptor at the neuromuscular junction.[6-10] The potency of volatile anesthetics in decreasing the open time of acetylcholine-activated channels was found to correlate with their clinical potency.[36] Our findings cannot support that the desensitization of the nicotinic response is the primary mechanism underlying the decrease by inhalational agents of the nicotine-induced dopamine release. Indeed, under our experimental conditions, desensitization was unaltered by anesthetics at any of the concentrations tested. Several explanations can be proposed to account for this apparent discrepancy. First, although promotion by volatile anesthetics of the desensitization of the nicotinic receptor is well documented at the neuromuscular junction, this phenomenon has not been demonstrated yet in the brain. Second, although not supportive, our findings cannot exclude that halothane and isoflurane may promote desensitization of the nicotinic receptors located on the striatal dopamine terminals. It is possible that desensitization due to anesthetics may be compensated by other actions of halothane and isoflurane at these synapses, such as facilitation of dopamine release. Indeed, we showed previously that the spontaneous release of dopamine from the striatum was enhanced by these agents.[21]

Effects of Halothane and Isoflurane on the Inhibition of the Potassium Chloride-evoked GABA Release Mediated by Activation of Presynaptic Muscarinic Receptors

Corresponding with our previous findings, we observed that volatile anesthetics failed to affect the KCl-induced release of GABA from striatal synaptosomes.[22,23] The inability of anesthetics to alter the KCl-evoked GABA release suggest that the Ca^{2+} channels and downstreams signaling events are not highly sensitive to volatile anesthetics in this subpopulation of striatal nerve endings. This observation is consistent with the finding that synaptosomal glutamate release, which is also closely coupled to specific Ca^{2+} channel activation, is not highly sensitive to volatile anesthetics.[37]

The inhibition by acetylcholine of the KCl-evoked GABA release probably was due to stimulation of presynaptic muscarinic receptors, because it was reversed by atropine, and was unaffected by mecamylamine. The pharmacologic characteristics of these presynaptic muscarinic receptors (insensitivity to pirenzepine, sensitivity to atropine) are close to those of muscarinic autoreceptors located on cholinergic nerve endings and negatively regulating acetylcholine release.[11,12] This receptor has been classified as an M3 muscarinic receptor.[38] The halothane concentrations used to reverse the effects of acetylcholine on the KCl-evoked GABA release were also clinically relevant (median effective concentration = 0.20 ± 0.03 mM). The effect of halothane was not significantly different in either the presence or absence of atropine. It was reported previously that halothane decreased acetylcholine release in the medial pontine reticular formation, suggesting disruption by the anesthetic of cholinergic signaling.[39] Halothane depressed Ca^{2+}-activated currents in response to stimulation by methylcholine of M1 muscarinic receptors expressed in *Xenopus* oocytes.[40] In addition, halothane has been proposed to alter the affinity of the G protein-coupled muscarinic receptors from the rat brain stem.[41] The exact molecular location of halothane on the muscarinic receptor/second messenger complex is still unclear.[42] Although halothane may bind directly to this presynaptic muscarinic receptor, it is more likely that it could also disrupt cholinergic signaling by acting at the muscarinic receptor-coupled-G-protein or even distal to this step, at the effector (kinases, phosphatases, or both).

Isoflurane used at equipotent concentrations did not antagonize the reduction by acetylcholine of the KCl-evoked GABA release. Several lines of evidence suggest that the effects of these inhalational anesthetics at the cellular and molecular level in the central nervous system are not identical.[43-45] Our results are consistent with the lack of depression of muscarinic signaling previously reported for isoflurane.[46] Because the prominent role of the septohippocampal cholinergic innervation in learning and memory has been emphasized,[42] it remains to be determined whether muscarinic inhibition is more relevant to side effects or to anesthetic action (amnesia) of halothane.

In conclusion, we showed that halothane and isoflurane exert significant, by distinct, effects on the responses mediated by central, presynaptic, acetylcholine receptors. Depression of the nicotinic responses helps reduce the excitatory neurotransmission in the central nervous system and may help produce unconsciousness by these agents. The clinical significance of the effects of volatile agents on muscarinic signaling remains to be elucidated.

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