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Anesthetics Affect the Uptake but Not the Depolarization-evoked Release of GABA in Rat Striatal Synaptosomes

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Background: **Numerous classes of anesthetic agents have been shown to enhance the effects mediated by the postsynaptic γ-aminobutyric acid A ($GABA_A$) receptor-coupled chloride channel in the mammalian central nervous system. However, presynaptic actions of anesthetics potentially relevant to clinical anesthesia remain to be clarified. Therefore, in this study, the effects of intravenous and volatile anesthetics on both the uptake and the depolarization-evoked release of GABA in the rat striatum were investigated.**

Methods: **Assay for specific GABA uptake was performed by measuring the radioactivity incorporated in purified striatal synaptosomes incubated with ^{3}H-GABA (20 nM, 5 min, 37°C) and increasing concentrations of anesthetics in either the presence or the absence of nipecotic acid (1 mM, a specific GABA uptake inhibitor). Assay for GABA release consisted of superfusing ^{3}H-GABA preloaded synaptosomes with artificial cerebrospinal fluid (0.5 ml · min^{-1}, 37°C) and measuring the radioactivity obtained from 0.5 ml fractions over 18 min, first in the absence of any treatment (spontaneous release, 8 min), then in the presence of either KCl alone (9 mM, 15 mM) or with various concentrations of anesthetics (5 min), and finally, with no pharmacologic stimulation (5 min). The following anesthetic agents were tested: propofol, etomidate, thiopental, ketamine, halothane, enflurane, isoflurane, and clonidine.**

Results: **More than 95% of ^{3}H-GABA uptake was blocked by a 10^{-3}-M concentration of nipecotic acid. Propofol, etomidate, thiopental, and ketamine induced a dose-related, reversible, noncompetitive, inhibition of ^{3}H-GABA uptake: IC_{50} = 4.6 ± 0.3 × 10^{-5} M, 5.8 ± 0.3 × 10^{-5} M, 2.1 ± 0.4 × 10^{-3} M, and 4.9 ± 0.5 × 10^{-4} M for propofol, etomidate, thiopental, and ketamine, respectively. Volatile agents and clonidine had no significant effect, even when used at concentrations greater than those used clinically. KCl application induced a significant, calcium-dependent, concentration-related, increase from basal ^{3}H-GABA release, +34 ± 10% ($P < 0.01$) and +61 ± 13% ($P < 0.001$), respectively, for 9 mM and 15 mM KCl. The release of ^{3}H-GABA elicited by KCl was not affected by any of the anesthetic agents tested.**

Conclusions: **These results indicate that most of the intravenous but not the volatile anesthetics inhibit the specific high-affinity ^{3}H-GABA uptake process *in vitro* in striatal nerve terminals. However, this action was observed at clinically relevant concentrations only for propofol and etomidate. In contrast, the depolarization-evoked ^{3}H-GABA release was not affected by anesthetics. Together, these data suggest that inhibition of GABA uptake, which results in synaptic GABA accumulation, might contribute to propofol and etomidate anesthesia. (Key words: Anesthetics, intravenous: etomidate; ketamine; propofol; thiopental. Anesthetics, volatile: enflurane; halothane; isoflurane. Brain: striatum; synaptosomes. Neurotransmitters, amino acids: gamma-aminobutyric acid. Sympathetic nervous system, adrenergic receptors, α_2 agonists: clonidine.)**

AN important target site of general anesthetic action appears to be the modulation of either excitatory or inhibitory synaptic transmission in the central nervous system (CNS).[1] A large body of work has led to propose that actions on the postsynaptic $GABA_A$ receptor complex account for the dominant CNS depressant effects of chemically distinct classes of anesthetics.[2,3] On the other hand, several lines of evidence suggest that some anesthetics also may interfere with presynaptic target sites to modulate either the release or uptake of CNS neurotransmitters.[4] For example, clinically relevant concentrations of halothane exhibit saturable binding

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to rat brain synaptosomes and reduce the depolarization-evoked noradrenergic release from the rat cerebral cortex.[5,6] Also, volatile anesthetics have been shown to inhibit dopamine[7] as well as serotonin[8] uptake by rat brain synaptosomes. We have demonstrated that volatile agents reduce depolarization-evoked dopamine release from striatal synaptosomes in the rat.[9]

Little is known, however, about the presynaptic modulation by anesthetic agents of the inhibitory GABA-mediated neurotransmission. Midazolam and halothane (3%) have been shown to inhibit GABA catabolism in rat brain synaptosomes and/or slices, but these agents failed to affect the high affinity uptake or the spontaneous GABA release in these preparations.[10–12] Similarly, no significant modification was observed in the uptake of GABA into rat brain slices in the presence of barbiturates, ketamine, or urethan.[13–15] On the other hand, results of studies devoted to the involvement of GABA release in the action of anesthetics at the CNS level are conflicting, depending on both the preparation used (*in vivo/in vitro*) and the brain area studied. The potassium-induced GABA release was found to be reduced by barbiturates in whole brain slices,[15] enhanced in the olfactory cortex,[16] and biphasic in the thalamus.[14] In addition, using an *in vivo* microdialysis technique, Osbourne *et al.* have observed that halothane reduces GABA efflux in the dorsolateral rat striatum.[17] However, results obtained from these elegant experiments did not allow to establish whether this was due to an decrease in release or an increase in uptake of GABA.

Thus, a better understanding of the role played by presynaptic GABA mechanisms on the action of anesthetic agents requires further investigation. Here, we used a preparation of synaptosomes (pinched-off nerve endings) originating from the rat striatum (a GABA-enriched brain structure) to assess direct effects of anesthetic agents on the presynaptic GABA terminals located in this subcortical area. The aim of the current study was to examine separately the influence of anesthetics on the specific GABA uptake process and the release of GABA elicited by KCl depolarization.

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 *ad libitum*.

Anesthetics

The effects of the following anesthetic and/or pharmacologic agents were studied on ^{3}H-GABA uptake and release: nipecotic acid (a specific inhibitor of GABA uptake, 10^{-6} to 10^{-3} M; Sigma, La Verpillière, France), thiopental (10^{-5} to 10^{-2} M; Nesdonal, Specia, France), ketamine (10^{-5} to 10^{-3} M; Sigma), propofol (10^{-5} to 10^{-3} M), etomidate (10^{-5} to 10^{-3} M), clonidine (Sigma; 10^{-9} to 10^{-6} M), halothane (Fluothane, Zeneca Pharmaceuticals, Cergy, France), enflurane (Ethrane), and isoflurane (10^{-5} to 10^{-2} M; Forane, Abbott, Rungis, France). Volatile anesthetics were first prepared as a 10^{-1}-M stock solution in dimethyl sulfoxide (DMSO, Merck Sharp and Dohme, Darmstadt, Germany). Dilutions of the volatile anesthetics were obtained by using glass syringes (Hamilton, Reno, NV) of appropriate volumes of the 10^{-1}-M solution into 5 ml CSF. The final aqueous concentrations of halogenated agents in the tubes (5 ml capacity) after dilution and equivalent periods were determined in a parallel experimental set up by chromatography.[18] Precautions were taken to minimize evaporation of volatile anesthetics leaving the tubes covered with Teflon foils during the serial dilutions and with Parafilm (Greenwich, CT) for the final tubes placed into the superfusion device. The vehicles tested were DMSO (used at a 0.1–10% concentration range for preparation of halogenated agents, propofol, and etomidate) and 0.01% thymol (Merck, Sharp and Dohme, used for stabilizing halothane).

GABA Uptake Assay

Preparation of synaptosomes has been reported in detail elsewhere.[8] Synaptosomes were diluted up to 0.16 mg/ml in an ice-cold artificial cerebrospinal fluid (CSF; in mM: NaCl 126.5, $NaHCO_3$ 27.5, KCl 2.4, KH_2PO_4 0.5, $CaCl_2$ 1.1, $MgCl_2$ 0.83, Na_2SO_4 0.5, glucose 11.8, aminooxyacetic acid (Sigma, an inhibitor of GABA catabolism) 0.1, and beta-alanine (an inhibitor of the GABA carrier in glial cells; Calbiochem, San Diego, CA) 1, adjusted to *p*H 7.3 with 95%/5% (v/v) oxygen/carbon dioxide mixture. In Ca^{2+}-free experiments, $CaCl_2$ was omitted from the artificial CSF.

Uptake of [2,3]^{3}H-GABA (Amersham, UK; 60 Ci/mmol) was performed by incubating 1-ml aliquots of synaptosomes (5 min, 37°C) with 20 nM ^{3}H-GABA in either the absence (control) or presence of anesthetic and/or pharmacologic agents. This duration of incu-

bation was determined according to preliminary experiments indicating that ^{3}H-GABA uptake increased linearly and reproducibly with time between 3 and 10 min of incubation at 37°C in this preparation. The reaction was stopped by vacuum-filtration through Whatman GF/F filters (0.70 μm retention capacity) and washing with ice-cold CSF (30 ml), and the radioactivity retained on the filters (counts per minute) measured by liquid scintillation spectrometry using Aquasol-2 (New England Nuclear, Boston, MA). Specific ^{3}H-GABA uptake was considered the difference between the radioactivity measured in both the presence and absence of 10^{-3} M nipecotic acid.

To determine whether any observed affects of anesthetics were not due to irreversible synaptosomal damage, ^{3}H-GABA uptake was examined after removal of the anesthetic from the buffer/synaptosomal mixture by vacuum and stirring rapidly for 60 min, as previously reported in dopamine uptake studies.[7] Aliquots were removed at 30 and 60 min from an uptake mixture initially containing a concentration of anesthetic corresponding to that one inhibiting 50% of the specific high affinity GABA uptake (IC_{50}), and ^{3}H-GABA uptake was determined as a function of the time that the mixture had been exposed to vacuum and stirring. Control experiments also were run in parallel to allow comparison with anesthetic-free mixtures exposed to the same conditions of vacuum and stirring.

To distinguish between competitive and noncompetitive mechanisms of any observed inhibition of ^{3}H-GABA uptake by anesthetics, kinetics of anesthetic-induced inhibition were investigated by incubating synaptosomes (37°C, 5 min) with increasing concentrations of ^{3}H-GABA (20–200 nM). Reaction was stopped by the addition of ice-cold CSF followed by vacuum filtration, and the radioactivity retained on the filters was measured. The kinetic parameters of the reaction (V_{max}, Km) were determined from double reciprocal plots of experiments performed in the absence (control) and presence of a fixed concentration of anesthetic corresponding to the IC_{50} value.

GABA Release Assay

Release experiments were performed as previously reported by Galli *et al.*[19] The synaptosome suspension was first incubated with ^{3}H-GABA (10 min, 37°C). Aliquots (1 ml) were pipetted into each of the 12 superfusion chambers and embedded in Whatman GF/F glass filters by light suction, then superfused at a 0.5 ml/min flow rate with artificial CSF using a superfusion device equipped with an automatic fraction collector (Brandel, Gaithersburg, MD). After a 30-min washing step (37°C), serial 0.5 ml fractions were collected during three consecutive periods. The mean value of ^{3}H-GABA radioactivity measured over the 8 first min was considered the basal (spontaneous) ^{3}H-GABA release. During the next 5 min of superfusion, either no treatment (time-dependent control) or various pharmacologic and/or anesthetic agents (evoked release) were delivered to the synaptosome preparation, and radioactivity was again estimated in each 0.5-ml fraction. The potassium chloride concentrations used were low (9 and 15 mM) to mimic physiologic depolarizations. For the last 5 min, radioactivity was determined from 1-min fractions in the absence of any treatment. The influence of a drug on ^{3}H-GABA release was assessed by calculating the difference between the maximal peak of radioactivity observed in both the presence and the absence (time-dependent control) of the pharmacologic or anesthetic agents used. To allow comparison with previous studies, the effect of an anesthetic agent on spontaneous release was expressed as a percentage of either increase or decrease from the time-dependent control basal release. The action of an anesthetic on depolarization-evoked release was displayed as a fraction of the control ^{3}H-GABA peak elicited by KCl. This presentation of data facilitated comparison with results obtained from previous studies.[19–21] Nipecotic acid was used in the release experiments as well to eliminate the possibility of reuptake occurring during superfusion of the synaptosomes. Therefore, the values taken into account were those obtained by subtracting results done in the absence nipecotic acid from those in the presence of nipecotic acid (1 mM) only.

Statistical Analysis

In uptake experiments, concentration-response curves were generated directly from computer and IC_{50} values for inhibition of ^{3}H-GABA uptake were calculated using the GraphPAD Software (San Diego, CA). The functions used to fit the curves to the data was the following four-parameter logistic equation:

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

where X represents the logarithm of anesthetic concentration, A and B, respectively, the minimum (bottom) and maximum (top) of X values. C is the logarithm of the IC_{50} value, and D 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. Results were considered reliable only if they had been reproduced in four independent experiments at least (each of them run in triplicate). Uptake and release data were analyzed by ANOVA followed by Student's *t* test corrected for the number of comparisons. The measured concentrations of volatile anesthetics were compared to the theoretical ones using the Student's *t* test. A *P* value less than 0.05 was considered the threshold for significance. Unless specified, data are expressed as mean ± SD.

Results

Concentrations of Volatile Anesthetics

The concentrations of volatile anesthetics were measured after dilution and equivalent periods of time in three independent experiments (each of them run in triplicate). The actual concentrations measured in the final samples and corresponding to the anticipated 100, 10, 2, 1, 0.5, and 0.1 mM, respectively, were 93 ± 11, 9.4 ± 0.4, 1.7 ± 0.3, 0.96 ± 0.06, 0.43 ± 0.05, and 0.07 ± 0.01 mM, respectively, for halothane; 95 ± 10, 9.2 ± 0.5, 1.8 ± 0.2, 0.91 ± 0.07, 0.41 ± 0.06, and 0.07 ± 0.01 mM, respectively, for isoflurane; and 91 ± 9, 9.6 ± 0.4, 1.8 ± 0.3, 0.93 ± 0.06, 0.44 ± 0.05, and 0.08 ± 0.01 mM, respectively, for enflurane. As was observed in a previous study in which a similar methodology was used for dilution of halogenated agents from a concentrated DMSO solution,[22] these values were nonsignificantly different from those anticipated theoretically.

Effects of Anesthetics on ^{3}H-GABA Uptake

The ^{3}H-GABA concentration estimated in the incubation medium during the uptake phase was 125 ± 30 pM. Synaptosomal ^{3}H-GABA uptake after the 5-min incubation averaged 0.87 ± 0.08 pM/mg protein. The specific uptake inhibitor nipecotic acid produced a dose-related inhibition of ^{3}H-GABA transport into synaptosomes (IC_{50} = 3.5 ± 0.3 × 10^{-6} M, fig. 1). A 10^{-3}-M concentration of nipecotic acid was found to inhibit more than 95% of ^{3}H-GABA uptake. Of the intravenous anesthetics tested, propofol, etomidate, thiopental, and ketamine induced an inhibitory effect on ^{3}H-GABA transport (IC_{50} values (mean ± SEM) = 4.6 ± 0.3 × 10^{-5} M, 5.8 ± 0.3 × 10^{-5} M, 2.1 ± 0.4 × 10^{-3} M, and 4.9 ± 0.5 × 10^{-4} M for propofol, etomidate, thiopental, and ketamine, respectively (fig. 1). In contrast, neither clonidine nor volatile anesthetics were found to significantly affect ^{3}H-GABA uptake by the synaptosomal preparation (fig. 1). In agreement with previous studies, DMSO and thymol, applied at the vehicle concentrations used in the current study, failed to affect GABA uptake.[23]

Reversibility of the effects of propofol, etomidate, thiopental, and ketamine on the uptake of ^{3}H-GABA was obtained in all cases. As shown on figure 2, uptake activities of the anesthetic-exposed solutions were not significantly different from time-dependent control uptake activities measured at either 30 or 60 min after washout.

The kinetic parameters of ^{3}H-GABA uptake were determined in either the absence (control) or presence of the concentrations of intravenous anesthetics inhibiting 50% of the transport of GABA into synaptosomes (IC_{50}) reported above. Changes in Km values due to anesthetic exposure were nonsignificant in comparison with control. In contrast, all anesthetic agents tested produced a significant reduction in V_{max} (fig. 3 and table 1). These data suggest that inhibition of ^{3}H-GABA uptake by these anesthetics is mediated by noncompetitive mechanisms.

Effects of Anesthetics on KCl-evoked ^{3}H-GABA Release

Results obtained from the release assay are illustrated in figure 3. No significant variation was observed in the time course of spontaneous ^{3}H-GABA release. No significant modification in baseline occurred when Ca^{2+} was omitted from the superfusion medium. The effect of nipecotic acid (10^{-3} M) on spontaneous release failed to achieve statistical significance. In contrast, KCl (9 and 15 mM) application induced a significant and dose-related increase from baseline. KCl, 9 and 15 mM, respectively, elicited a 34 ± 10% ($P < 0.01$) and 61 ± 13% ($P < 0.001$), respectively, increase from basal release (fig. 4). This ^{3}H-GABA peak occurred rapidly after KCl application and was followed by a rapid return of radioactivity to baseline, which was observed before the stimulus was discontinued. The KCl-evoked ^{3}H-GABA release was completely blocked when external Ca^{2+} was omitted from the medium (fig. 4). All anesthetic agents tested failed to affect significantly both the spontaneous and KCl-evoked release of ^{3}H-GABA (fig. 5 and table 2).

Discussion

The current study demonstrates that some intravenous but not volatile anesthetics exert significant actions on

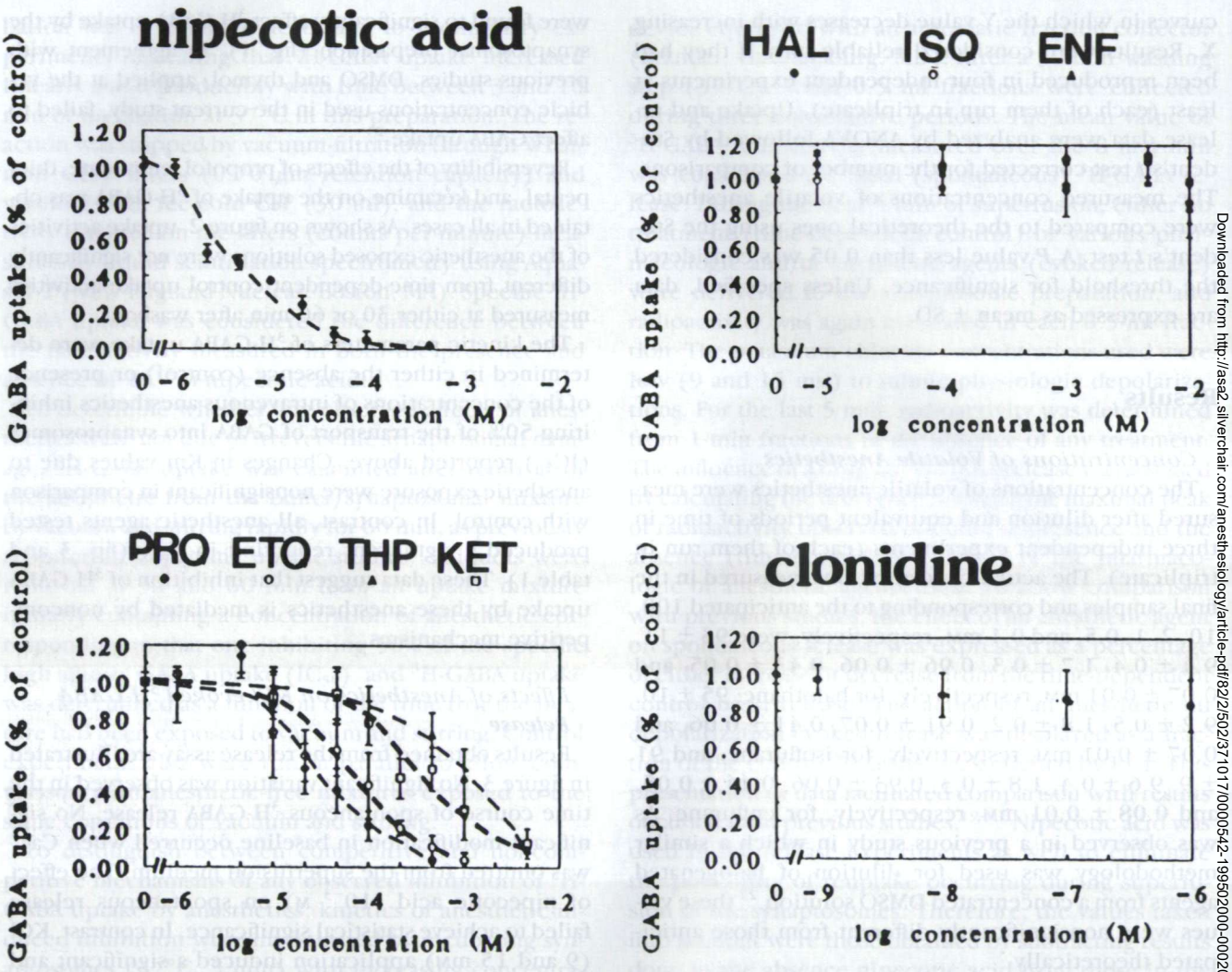

Fig. 1. Concentration-response curves of the effects of anesthetic agents on the specific high-affinity uptake of ^{3}H-GABA by synaptosomes obtained from the rat striatum. Data are expressed as mean ± SEM and result from at least four independent experiments run in triplicate. For nipecotic acid, propofol, etomidate, thiopental, and ketamine, IC_{50} values could be derived from a nonlinear computerized fitting of the data (see Methods).

the presynaptic GABAergic nerve terminals located in the rat striatum *in vitro*. Propofol, etomidate, thiopental, and ketamine were found to inhibit the specific high-affinity GABA uptake in this brain area. In contrast, the release of ^{3}H-GABA evoked by KCl depolarization was not affected by the anesthetics studied.

Methodologic Considerations and Limitations

In the current study, the effects of anesthetics on the uptake and on the depolarization-evoked release of ^{3}H-GABA were examined on synaptosomes prepared from the rat striatum. Advantages and drawbacks of working on this preparation have been addressed in a study originating from our laboratory.[4] Synaptosomes provide a reliable and reproducible model for analyzing, *in vitro*, the direct presynaptic effects of anesthetics on neurotransmitter release or uptake.[4–9,19] However, unlike striatal slices, they preclude looking at indirect, tetrodotoxin-sensitive mechanisms or to address the role played by diffusible mediators involved in neurotransmitter release, such as nitric oxide. Therefore, the results obtained here apply only to uptake and release in synaptosomes, whereas nerve terminals *in situ* subjected to impulse

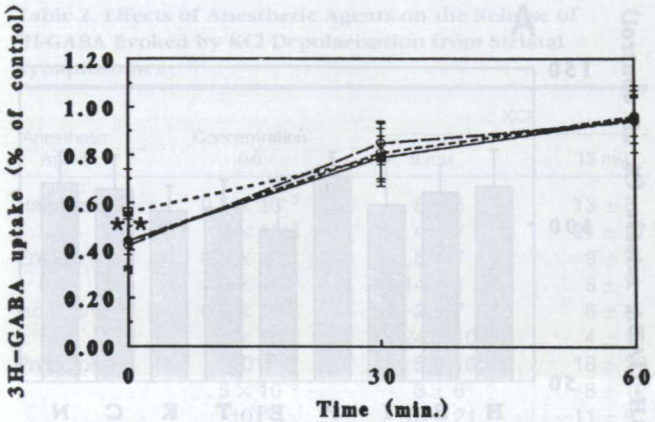

Fig. 2. Reversibility of the effects of propofol (●), etomidate (○), thiopental (— ○ —), and ketamine (□) on the uptake of ^{3}H-GABA by striatal synaptosomes. Anesthetics used at concentrations corresponding to their IC_{50} values determined in the uptake assay were removed from the synaptosome suspension at t = 0. Uptake was measured at 30 and 60 min after washout of the anesthetics. Data are expressed as mean ± SD. *$P < 0.05$ and **$P < 0.01$ *versus* vehicle control.

activity and a transmitter-enriched environment may exhibit a different behavior.

Basically, the striatal synaptosomes consist of different subpopulations of presynaptic nerve endings originating from a restricted but functionally important brain area. The specific GABA carrier is present on synaptosome membranes of the GABA terminals located in this structure.[19] GABA released from striatal nerve endings is likely to originate from either striatal GABA interneurons or from recurrent collaterals arising from striatopallidal or striatonigral output neurons.[24,25] Interestingly, convincing evidence has been provided that the striatum is anatomically and functionally heterogeneous. Subcompartmentalization of this brain area into matrix and striosomes correlates with major biochemical and pharmacologic differences, such as the control of presynaptic dopamine release in the cat.[24,26] To date, whether these differences also apply to GABA release in the rat striatum remains unknown. However,

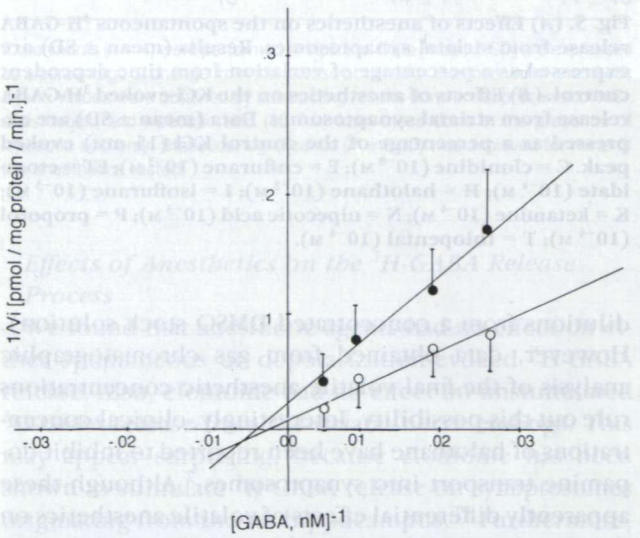

Fig. 3. Example of a double reciprocal plot allowing determination of the kinetic parameters of the inhibition of ^{3}H-GABA uptake by thiopental. In these experiments (n = 4), the concentration of substrate (^{3}H-GABA) was increased from 20 to 200 nM in the presence (●) or absence (○) of a fixed thiopental concentration corresponding to the IC_{50} value. Data are mean ± SD.

Table 1. Kinetic Parameters for the Uptake of ^{3}H-GABA by Striatal Synaptosomes

	K_m (μM)	V_{max} (pmol/mg off protein per minute)	n
Control	6.3 ± 0.8	10.80 ± 1.2	4
Propofol	5.5 ± 0.9	7.96 ± 0.96*	4
Etomidate	5.8 ± 0.7	7.85 ± 1.10*	4
Ketamine	6.2 ± 1.1	4.46 ± 0.89†	4
Thiopental	5.4 ± 0.9	3.57 ± 0.81†	4

Values are mean ± SD; n refers to the number of independent experiments.
* $P < 0.05$, † $P < 0.01$ (ANOVA and Student's *t* test).

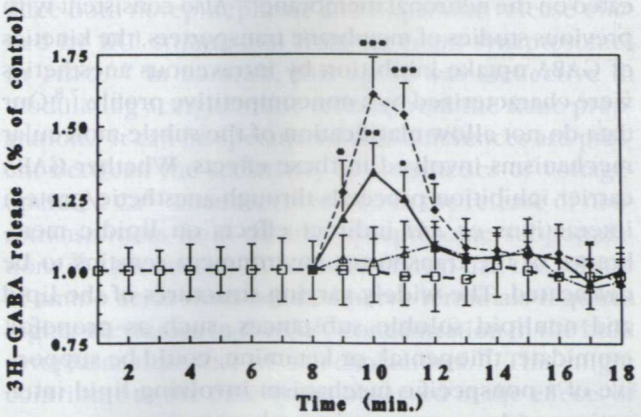

Fig. 4. Time-course of the release of ^{3}H-GABA elicited by various depolarizing agents from preloaded striatal synaptosomes: baseline (□), potassium chloride 9 mM (▲), potassium chloride 15 mM (♦), and potassium chloride (9 mM) in a Ca^{2+}-free medium (△). Depolarizing stimuli were applied between minutes 8 and 13. Experiments were performed in the presence of nipecotic acid (10^{-3} M). Data are mean ± SD. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

it cannot be excluded that differences might exist in the actions of anesthetics on GABA uptake or release between matrix- and striosome-enriched areas of the rat striatum.

Effects of Anesthetics on the ^{3}H-GABA Uptake Process

The originality of the current study is that we separately investigated the uptake and release processes. The specific GABA uptake inhibitor nipecotic acid was found to potently block the incorporation of ^{3}H-GABA into synaptosomes. This indicates that the uptake process studied here was the specific one for GABA.[27] Of the different anesthetics examined, only propofol, etomidate, ketamine, and thiopental were found to significantly inhibit ^{3}H-GABA uptake. The effects of propofol and etomidate were observed at concentrations that were clinically relevant, because these corresponded to the range of plasma concentrations that might be observed in the anesthetized patient. Reversibility of anesthetic effects on GABA uptake further suggests that these actions might occur *in vivo,* because they were not associated with irreversible damage of synaptosome membranes. However, the IC_{50} values reported for thiopental and ketamine suggest that the effects of these compounds on GABA uptake were obtained for anesthetic concentrations surpassing clinical relevance.

Kinetics of ^{3}H-GABA uptake were in agreement with the characteristics of the high-affinity GABA carrier located on the neuronal membrane.[28] Also consistent with previous studies of membrane transporters, the kinetics of GABA uptake inhibition by intravenous anesthetics were characterized by a noncompetitive profile.[7,8] Our data do not allow clarification of the subtle molecular mechanisms involved in these effects. Whether GABA carrier inhibition proceeds through anesthetic/protein interactions or *via* indirect effects on lipidic membranes of the transporter environment remains to be delineated. The widely varying structures of the lipid and nonlipid soluble substances, such as propofol, etomidate, thiopental, or ketamine, could be supportive of a nonspecific mechanism involving lipid interactions and synaptosomal membranes.

On the other hand, clonidine and the volatile anesthetics failed to interfere with GABA transport. It might have been argued that the lack of effect of halogenated agents on GABA uptake was explained by much lower final concentrations than those expected theoretically with respect to the dilution procedure used here (serial dilutions from a concentrated DMSO stock solution). However, data obtained from gas chromatographic analysis of the final volatile anesthetic concentrations rule out this possibility. Interestingly, clinical concentrations of halothane have been reported to inhibit dopamine transport into synaptosomes.[7] Although these apparently differential effects of volatile anesthetics on dopamine *versus* GABA uptake could be explained in part by differences in the experimental conditions achieved in the two studies, it can be suggested that the specific GABA and dopamine carriers exhibit distinct sensitivities to inhalational anesthetics in the rat brain.

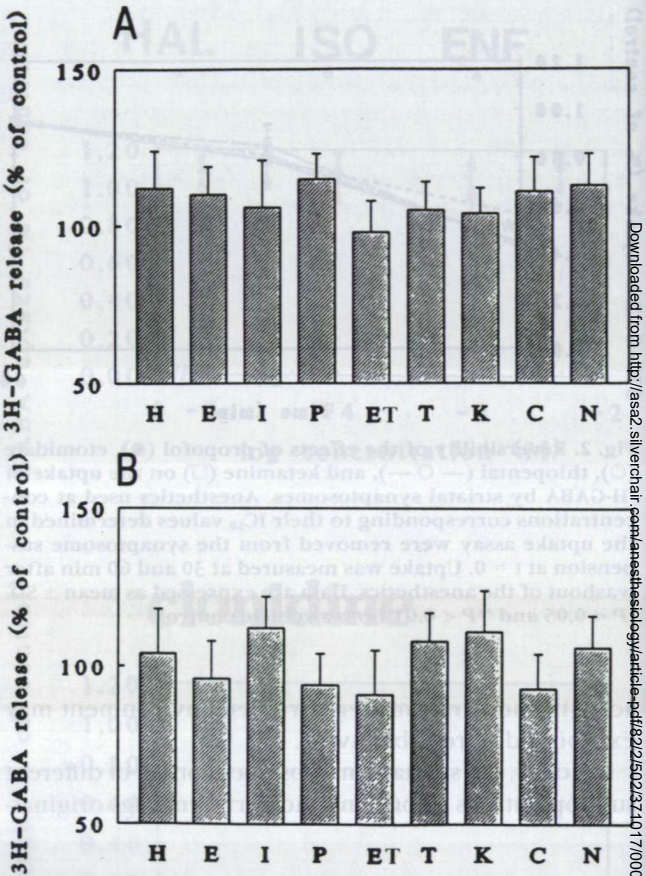

Fig. 5. (*A*) Effects of anesthetics on the spontaneous ^{3}H-GABA release from striatal synaptosomes. Results (mean ± SD) are expressed as a percentage of variation from time dependent control. (*B*) Effects of anesthetics on the KCl-evoked ^{3}H-GABA release from striatal synaptosomes. Data (mean ± SD) are expressed as a percentage of the control KCl-(15 mM) evoked peak. C = clonidine (10^{-8} M); E = enflurane (10^{-3} M); ET = etomidate (10^{-4} M); H = halothane (10^{-3} M); I = isoflurane (10^{-3} M); K = ketamine (10^{-4} M); N = nipecotic acid (10^{-3} M); P = propofol (10^{-4} M); T = thiopental (10^{-4} M).

Table 2. Effects of Anesthetic Agents on the Release of ^{3}H-GABA Evoked by KCl Depolarization from Striatal Synaptosomes

Anesthetic Agent	Concentration (M)	KCl 9 mM	KCl 15 mM
Halothane	0.5×10^{-3}	−8 ± 6	13 ± 8
	2×10^{-3}	4 ± 7	21 ± 17
Enflurane	0.5×10^{-3}	8 ± 7	−9 ± 7
	2×10^{-3}	14 ± 1	8 ± 7
Isoflurane	0.5×10^{-3}	−2 ± 7	6 ± 8
	2×10^{-3}	24 ± 10	4 ± 11
Propofol	10^{-5}	5 ± 10	18 ± 20
	5×10^{-5}	3 ± 6	−8 ± 12
	10^{-4}	18 ± 21	−11 ± 9
	5×10^{-4}	23 ± 10	−18 ± 15
Etomidate	10^{-5}	5 ± 7	6 ± 5
	5×10^{-5}	16 ± 9	14 ± 11
	10^{-4}	14 ± 13	−7 ± 6
	5×10^{-4}	6 ± 5	−11 ± 9
Thiopental	10^{-5}	−7 ± 8	11 ± 12
	5×10^{-5}	12 ± 9	−14 ± 7
	10^{-4}	−13 ± 8	12 ± 9
	5×10^{-4}	−21 ± 17	−19 ± 14
Ketamine	10^{-5}	11 ± 9	21 ± 18
	5×10^{-5}	5 ± 7	−13 ± 11
	10^{-4}	8 ± 5	−23 ± 18
	5×10^{-4}	−13 ± 10	−14 ± 11
Clonidine	10^{-9}	15 ± 12	16 ± 9
	10^{-8}	−4 ± 7	−8 ± 7
	10^{-7}	−9 ± 8	12 ± 9
	10^{-6}	−14 ± 11	−11 ± 10

Experiments (n = 4) were performed in triplicate. Data (mean ± SD) are expressed as a percentage of change from the release obtained in the absence of anesthetic agent. Values were obtained by subtracting results done in the presence of KCl, anesthetics, and nipecotic acid (10^{-3} M) from those done in the presence of nipecotic acid only. Statistical significance ($P < 0.05$) was achieved for none of the anesthetics tested.

Effects of Anesthetics on the ^{3}H-GABA Release Process

We found that anesthetic agents had no effect on either spontaneous or depolarization-evoked ^{3}H-GABA release. Also, clonidine had no effect on unstimulated ^{3}H-GABA release from the striatal nerve endings. This may appear surprising, because clonidine has been shown to stimulate ^{3}H-GABA release on synaptosomes originating from the rat hippocampus.[29] Furthermore, elegant studies performed on the rat hippocampal slice preparation have shown that α-adrenergic agonists increase GABAergic inhibition by presynaptically enhancing endogenous GABA release.[30] These differences likely can be attributed to the lack of noradrenergic innervation of the striatum, while the hippocampus receives massive noradrenergic inputs from the locus ceruleus.[24,31]

It can be speculated that the effects of anesthetics on GABA release evoked by depolarization may be more relevant to the *in vivo* situation than those observed on basal, unstimulated release, because depolarization is the normal way synapses are activated. The release of GABA from synaptosomes is classically mediated by both exocytotic mechanisms, which depend on depolarization and calcium entry, and reversal of the GABA carrier, which is independent from the presence of external Ca^{2+} and is driven by the sodium gradient.[32–34] KCl depolarization is able to trigger both mechanisms on the synaptosome preparation. However, it has been shown that the exocytotic release of ^{3}H-GABA is more pronounced than the carrier-mediated process in synaptosomes from the adult rat brain.[35] In agreement with these findings, we found that the KCl-evoked release of ^{3}H-GABA was completely blocked in the absence of external calcium. Second, the magnitude of the KCl-evoked ^{3}H-GABA peaks reported here are in good agreement with previous studies.[19] Third, anesthetics failed to affect depolarization-evoked GABA release, regardless whether the specific high-affinity uptake process was blocked by nipecotic acid. These results strongly support that the release process investigated there was primarily the exocytotic one.[19] Therefore, anesthetics seem not to interfere with exocytotic release of GABA from preloaded striatal synaptosomes *in vitro*. Halothane has been shown to reduce both norepinephrine and dopamine release elicited by KCl stimulation from rat brain synaptosomes *in vitro*.[6,9] In contrast, this agent was ineffective in modulating acetylcholine release from the same preparation.[6] It can be speculated that differences are present between the sensitivity to anesthetics of voltage-operated Ca^{2+} channels involved in the release of neurotransmitters from distinct synaptosome subpopulations.[36] Alternatively, it has been shown that the release of amino acids from isolated nerve terminals requires higher elevations in the Ca^{2+} concentration in the bulk cytoplasm than that of catecholamines.[37] This might contribute to the differences observed in the effects of halothane, enflurane, and isoflurane on GABA *versus* dopamine release in the rat striatum. More generally, it can be suggested that volatile anesthetics will interfere selectively with stimulus-secretion coupling in specific neuronal phenotypes.

In summary, we have shown that intravenous but not volatile agents interfere with the specific high-affinity

GABA uptake process in a synaptosome preparation *in vitro*. However, the similarities between the concentrations required to modulate ^{3}H-GABA uptake *in vitro* and those associated with propofol and etomidate anesthesia *in vivo* suggest that synaptic GABA accumulation may occur during anesthesia induced by both agents. Whether these findings are of potential clinical relevance cannot be extrapolated from our experimental data. It has been demonstrated that only events that are elicited by large stimuli or corresponding to paroxysmal activity are enhanced by blocking GABA uptake.[38,39] Whether these patterns of activities occur during anesthesia remains speculative. However, it can be suggested that a major consequence of inhibiting GABA uptake is to increase the background levels of GABA-activating extrasynaptic $GABA_A$ or even $GABA_B$-mediated synaptic transmission.[38] Thus, some actions at selective presynaptic CNS target sites might contribute to the mechanisms by which propofol or etomidate anesthesia is produced.

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