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Effects of Volatile Anesthetics, Thiopental, and Ketamine on Spontaneous and Depolarization-evoked Dopamine Release from Striatal Synaptosomes in the Rat

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Background: Recent experimental data indicate that anesthesia is often associated with significant changes in brain concentrations of dopamine (DA), an inhibitory neurotransmitter located in restricted, but functionally important, areas such as the striatum. Whether the presynaptic DA nerve endings represent potential targets for anesthetics remains unknown. Therefore, the current study was designed to investigate the effects of volatile anesthetics, thiopental, and ketamine on both spontaneous and depolarization-evoked DA release from striatal synaptosomes in the rat.

Methods: Purified striatal synaptosomes preloaded with ^3H -DA were superfused with artificial cerebrospinal fluid (1 ml/min). Radioactivity obtained from 1-ml fractions was measured over 15 min; first, in the absence of any treatment (spontaneous release), then in either the absence (time-dependent control) or presence (evoked-release) of anesthetic and pharmacologic agents, and finally, again, without any pharmacologic stimulation. The compounds tested were: potassium chloride (15 and 50 mM), glutamate, N-methyl-D-aspartate (NMDA) and kainate (10^{-4} M and 10^{-3} M), MK-801 (10^{-4} M, an antagonist of NMDA receptors) and 6-cyano-7-nitro-quinoline-2,3-dione (10^{-4} M, an antagonist of D,L- α -amino-3-hydroxy-5-methyl-4-isoxazole propionate [AMPA] receptors), halothane, enflurane, isoflurane (1, 1.5, and 2 minimum alveolar concentrations), ketamine (10^{-5} and 10^{-4} M), and thiopental (10^{-5} and 10^{-4} M).

Results: Volatile anesthetics induced a significant, concentration-related increase in spontaneous ^3H -DA release, but thiopental and ketamine were ineffective. The effect of 2 minimum alveolar concentration enflurane (but not halothane or isoflurane) was significantly enhanced when a Mg^{2+} -free cerebrospinal fluid was used, and was reduced by MK-801 application. Nomifensine (10^{-5} M, a blocker of monoamine transporter) did not affect the ^3H -DA release evoked by volatile anesthetics. Glutamate, kainate, NMDA, and potassium chloride induced a significant, dose-related, Ca^{2+} -dependent ^3H -DA release. Halothane and isoflurane produced a significant and concentration-related decrease in the ^3H -DA peaks evoked by glutamate, kainate, and NMDA; however, enflurane significantly attenuated the glutamate- and kainate-mediated release, but enhanced that evoked by NMDA. Thiopental and ketamine (10^{-4} , but not 10^{-5} M) significantly reduced the glutamate- and NMDA-stimulated release, but only thiopental decreased the kainate-induced effect. Furthermore, the effect of potassium chloride (15 mM) was significantly reduced by all anesthetics examined, whereas that of potassium chloride (50 mM) was unaffected.

Conclusion: The authors conclude that: (1) volatile anesthetics, thiopental, and ketamine exert significant changes in both spontaneous and depolarization-evoked ^3H -DA release in the rat striatum; (2) enflurane uniquely enhances NMDA-receptor mediated dopamine release; and (3) the results obtained from these receptor-mediated effects (AMPA and NMDA) may apply to postsynaptic, as well as presynaptic, glutamate receptors. (Key words: Anesthetics, intravenous: ketamine; thiopental. Anesthetics, volatile: enflurane; halothane; isoflurane. Brain: striatum; synaptosomes. Receptors, glutamate, agonists: kainate; N-methyl-D-aspartate. Receptors, glutamate, antagonists: 6-cyano-7-nitro-quinoline-2,3-dione; MK-801. Sympathetic nervous system, catecholamines: dopamine; nomifensine.)

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SEVERAL lines of evidence indicate that anesthesia may result from interactions between anesthetics and a restricted set of sensitive target sites in the brain that lead to changes in either excitatory or inhibitory neurotransmission in the central nervous system (CNS).^{1,2} In

fact, it is now well documented that the γ -aminobutyric acid (GABA)_A receptor-coupled chloride channel plays a pivotal role in the dominant CNS depressant effects of several chemically distinct classes of anesthetics.⁵ However, data obtained from both clinical and experimental studies indicate that such compounds as α_2 agonists exert, in part, their hypnotic effects by inhibiting the activity of the noradrenergic neurons *via* stimulation of presynaptic α_2 autoreceptors located on the noradrenergic cell bodies.⁴ Thus, CNS neurons containing monoamines (norepinephrine, dopamine, or serotonin) may represent potential targets for anesthetic agents. Surprisingly, however, although many studies have investigated the implications of the noradrenergic neurons in anesthetic mechanisms, little is known about the contribution to anesthesia of the dopaminergic pathways, which represent, as well, potent modulatory systems in the CNS. Indeed, results obtained from electrophysiologic experiments performed both *in vivo* and *in vitro* have established that the effects of DA application on various brain areas are inhibitory, most of these responses being mediated *via* the activation of the DA-D2 receptor subtype.⁵⁻⁷ Also, it is well known that the central dopaminergic pathways participate actively in major brain functions. For instance, loss of the nigrostriatal DA neurons results in the development of Parkinson's disease.⁸ Moreover, the mesocortical and mesolimbic dopaminergic neurons exert a basic control on regulation of cognitive and emotional processes in both animals and humans.^{9,10}

Several lines of evidence indicate that activation of dopaminergic neurons may be involved in some anesthetic mechanisms. Indeed, Johnston *et al.* have shown that the intravenous administration of dihydroxyphenylalanine (L-DOPA) induced a weak, but dose-related, decrease in halothane requirements in dogs.¹¹ In this study, however, it could not be ruled out that some of the findings were explained by an action of L-DOPA mediated either by norepinephrine or epinephrine, or *via* the GABA modulation of the activity of the nigrostriatal dopaminergic neurons. On the other hand, Roizen *et al.* have observed that 1% halothane administered during 90–105 min significantly increased DA levels in the rat nucleus accumbens.¹² Using the 2-deoxy-D-(1-¹⁴C)glucose technique, Savaki *et al.* found that inhalation of halothane (0.5% inspired concentration) produced a dramatic metabolic increase in the substantia nigra, a DA-enriched brain area.¹³ More recently, Segal *et al.* have demonstrated that the halothane anesthetic-sparing effect of L-DOPA

was blocked in mice by systemic administration of a selective antagonist of the D2, but not the D1, receptors.¹⁴ In this study, an inverse correlation was found between halothane anesthetic requirements and DA content of the striatum. Finally, experiments using either the brain microdialysis or voltammetry have contributed to show that striatal extracellular levels of DA or its metabolites were significantly increased during either halothane or isoflurane anesthesia in the rat.¹⁵⁻¹⁸ This indicates that volatile anesthetics may affect DA release and metabolism in the nigrostriatal DA neurons.

In the current study performed on rats, we investigated the effects of volatile anesthetics on DA release evoked from striatal synaptosomes (isolated pinched-off nerve terminals). This preparation allows an assessment of direct effects of anesthetic agents on the presynaptic DA nerve endings located in this structure. For this purpose, the effects of halothane, enflurane, and isoflurane used at clinically relevant concentrations were first analyzed on the basal, unstimulated ³H-DA release. Because depolarization is the normal way that synapses are activated, the release of ³H-DA induced by various depolarizing agents was also examined. For this purpose, potassium chloride, which enhances the activity of voltage-gated Ca²⁺ channels, but also compounds acting at specific presynaptic receptors to stimulate DA release (glutamate, NMDA (N-methyl-D-aspartate), and kainate, the most efficient agonist of the glutamate receptors of the D,L- α -amino-3-hydroxy-5-methyl-4-isoxazole propionate [AMPA] subtype in this model¹⁹), were used. For comparison, the effects on both spontaneous and depolarization-evoked DA release of two intravenous anesthetics, thiopental and ketamine, were also investigated.

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 200–250 g and housed on a 12:12-h light/dark cycle with food and water *ad libitum*.

Synaptosome Preparation and Superfusion

Synaptosomes were prepared from striata and purified on a Percoll gradient according to the procedure described by Dunkley *et al.*²⁰ Rats were killed by stunning and decapitation. Striata were then quickly removed and placed into ice-cold 0.32 M sucrose (containing 1

mm EDTA and 0.25 mm dithiothreitol and adjusted to pH 7.4 with diluted sodium hydroxide). In each experiment, six striata (about 300 mg) were pooled and homogenized in 5 ml of 0.32 M sucrose with ten up-and-down strokes. The homogenate was centrifuged at 2,000 g for 10 min, and the supernatant was layered onto a discontinuous four-step Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) gradient containing 2 ml of each 23, 15, 10, and 3% Percoll (V/V) in the sucrose buffer. After a 5-min centrifugation at 32,500 g, the synaptosomal fractions were collected at the interfaces of layers 10–15 and 15–23%, and diluted up to 0.5 mg/ml of protein with artificial cerebrospinal fluid (CSF; in mM: NaCl, 126.5; NaHCO₃, 27.5; KCl, 1; KH₂PO₄, 0.5; CaCl₂, 1.1; MgCl₂, 0.83; Na₂SO₄, 0.5; glucose, 5.9; pargyline, 10⁻²; ascorbic acid, 1, adjusted at pH 7.3 with 95%/5% [v/v] O₂-CO₂ mixture). In Ca²⁺ (Mg²⁺, respectively)-free experiments, Ca²⁺ (Mg²⁺ respectively) was omitted from the artificial CSF.

Uptake of tritiated dopamine by synaptosomes was performed by incubating the synaptosome preparation with ³H-DA (Amersham, UK; 30 Ci/mmol) during 10 min at 37° C. An aliquot (1 ml) of the synaptosomal suspension was pipetted into each of the 12 superfusion chambers. Synaptosomes were embedded in glass filters (Whatman, GF/F, 0.70- μ m retention capacity) by light suction and 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 superfusion rate has been shown to prevent DA reuptake by the synaptosomal preparation.²¹ After a 30-min washing step (37° C), serial 1-min fractions were collected during three consecutive 5-min periods. The mean value of ³H-DA radioactivity (counts per minute [cpm]) measured over the first 5 min by liquid scintillation spectrometry using Aquasol-2 (New England Nuclear, Boston, MA) was considered the basal (spontaneous) ³H-DA release. During the second 5 min of superfusion, either no treatment (time-dependent control) or various pharmacologic and anesthetic agents (evoked release) were administered to the synaptosome preparation, and radioactivity was again estimated in each 1-min fraction. For the last 5 min, radioactivity was estimated from 1-min fractions in the absence of any anesthetic and pharmacologic agent. The influence of a drug on ³H-DA release was determined by calculating the difference between the maximal peak of radioactivity observed in both the presence and absence (time-dependent control) of the pharmacologic agent used. To allow comparison with pre-

vious studies, the effect of a pharmacologic or anesthetic agent on spontaneous release was expressed as a fraction of basal release, as previously reported.^{19,21–27} On the other hand, the effect of an anesthetic on depolarization-evoked release was expressed as a percentage of either increase or decrease from the control ³H-DA peak elicited by the depolarizing agents.^{19,21,25,27}

Anesthetics and Chemicals

The anesthetic agents tested were: thiopental (10⁻⁵ and 10⁻⁴ M; Nesdonal, Specia, France), ketamine (10⁻⁵ and 10⁻⁴ M; Sigma, La Verpillière, France), halothane (1–2%; Fluothane, Zeneca, Cergy, France), enflurane (2.25–4.5%, Ethrane; Abbott, Rungis, France) and isoflurane (1.5–3%, Forane; Abbott). Volatile anesthetics were delivered through a calibrated vaporizer in an O₂-CO₂ mixture (95/5% v/v, 3 l/min) at gaseous concentrations corresponding approximately to 1, 1.5, and 2 MAC. The 1-MAC value was considered to be 1% for halothane, 1.5% for isoflurane, and 2.25% for enflurane, as previously reported in male rats.²⁸ Volatile anesthetics were equilibrated with the artificial CSF for 60 min at room temperature, and aqueous concentrations in the superfusion chambers were determined by gas phase chromatography according to slight modifications of the method reported by Brachet-Liermain *et al.*²⁹

The pharmacologic agents used to stimulate DA release were potassium chloride (15 and 50 mM), glutamate (10⁻⁴ and 10⁻³ M; Sigma), kainate (an agonist of the AMPA receptors, 10⁻⁴ and 10⁻³ M; Sigma) and NMDA (10⁻⁴ and 10⁻³ M; Sigma). The AMPA/kainate receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) (10⁻⁴ M; Research Biochemical, Illkirch, France) and the NMDA receptor antagonist MK-801 (10⁻⁴ M; Merck-Sharp and Dohme, UK) were used to selectively block the effect of kainate (10⁻³ M; NMDA, 10⁻³ M, respectively) on DA release. The effects of nomifensine hydrochloride (10⁻⁵ M, a nonselective blocker of the DA transporter, Research Biochemical Incorporated) and MK-801 on ³H-DA release elicited by volatile anesthetics were also examined.

Statistical Analysis

Data are expressed as mean \pm SEM. Results were considered reliable only if they had been reproduced in four independent experiments at least (each of them run in triplicate). Data were analyzed by ANOVA followed by Student's *t* test, a *P* value less than 0.05 being considered the threshold for significance.

Results

Pharmacologic Characterization of Spontaneous and Depolarization-Evoked ^3H -DA Release from Striatal Synaptosomes

The ^3H -DA concentration estimated in the incubation medium during the uptake phase was 250 ± 25 pmol/mg protein. A typical example of the time course of spontaneous ^3H -DA release is displayed in figure 1. A slight, nonsignificant decrease with time was observed in basal release measured in the absence of any anesthetic and pharmacologic agent. As shown on this figure, only small, nonsignificant changes in baseline occurred when either Ca^{2+} or Mg^{2+} were omitted from the superfusion medium. More precisely, Mg^{2+} depletion resulted in a slight increase (maximum: $+11 \pm 5\%$) in ^3H -DA release in comparison with time-dependent controls. Similarly, the decrease in release observed after depletion of external Ca^{2+} was $-14 \pm 7\%$.

In contrast, the depolarizing agent potassium chloride induced a dose-related, Ca^{2+} -dependent ^3H -DA release from the synaptosome preparation (fig. 2A). Potassium chloride, 15 mM (50 mM, respectively), induced a $51 \pm 6\%$ ($P < 0.01$; $104 \pm 12\%$, $P < 0.001$, respectively) increase from basal release. This ^3H -DA peak occurred rapidly after potassium chloride application and was followed by a rapid decrease in radioactivity that occurred before the stimulus was discontinued (fig. 2A). Glutamate, NMDA, and kainate produced a concentration-related stimulation of ^3H -DA release (fig. 2B–D). More precisely, glutamate 10^{-4} M (10^{-3} M) elicited a $29 \pm 3\%$ ($P < 0.05$; $49 \pm 9\%$, $P < 0.01$, respectively) increase from basal release. It is noteworthy that glutamate effects were not significantly different in either the presence or absence of physiologic levels of Mg^{2+} (0.83 mM; fig. 2B). The ^3H -DA peak elicited by glutamate was observed until the application of this pharmacologic agent was stopped. It was followed by progressive restoration of basal release. The AMPA receptor agonist kainate induced a $24 \pm 6\%$ ($P < 0.05$; $36 \pm 7\%$, $P < 0.01$) increase in ^3H -DA release when used at a 10^{-4} M (10^{-3} M, respectively) concentration (fig. 2C). However, a stimulatory effect of NMDA was observed only when Mg^{2+} was omitted from the medium (fig. 2D). Under these conditions, the increase in ^3H -DA release evoked by a 10^{-4} M (10^{-3} M) NMDA concentration was $28 \pm 7\%$ ($P < 0.05$; $42 \pm 6\%$, respectively, $P < 0.01$; fig. 2D). The stimulation of ^3H -DA release elicited by glutamate, kainate, and NMDA was dependent on the presence of external Ca^{2+} . Indeed, when Ca^{2+}

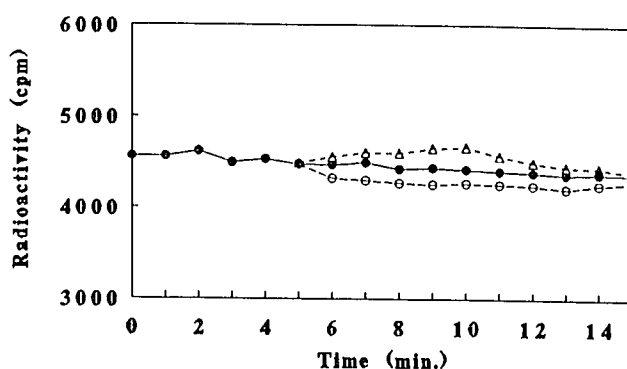


Fig. 1. Typical example of the time-course of spontaneous ^3H -DA release from preloaded synaptosomes and the influence of Ca^{2+} or Mg^{2+} depletion on this release. Normal superfusion medium (filled circles) was replaced by either Ca^{2+} -free (open circles) or Mg^{2+} -free (open triangles) CSF between minutes 5 and 10. Data represent raw ^3H -DA release (cpm) obtained from one representative experiment.

was omitted from the medium, the peaks induced by a 10^{-3} M concentration of these agents was reduced by 85 ± 7 , 78 ± 9 , and $84 \pm 10\%$ for glutamate, kainate, and NMDA, respectively. However, the effect of NMDA (10^{-3} M) was completely blocked by application of the selective NMDA receptor antagonist MK-801 (10^{-4} M; fig. 3). In contrast, it was not significantly reduced by administration of the AMPA receptor antagonist CNQX (10^{-4} M). Conversely, the effect of kainate (10^{-3} M) was blocked by CNQX (10^{-4} M) but not MK-801 (10^{-4} M). Spontaneous release was not affected by application of either MK-801 (10^{-4} M) or CNQX (10^{-4} M; fig. 3).

Effects of Anesthetic Agents on Spontaneous ^3H -DA Release from Striatal Synaptosomes

The aqueous concentrations of volatile anesthetics measured in the chambers after 1 h of equilibration with the superfusion medium were 0.36 ± 0.04 , 0.47 ± 0.05 , and 0.64 ± 0.04 mM for halothane used at 1, 1.5, and 2%, respectively; 0.52 ± 0.05 , 0.67 ± 0.03 , and 0.84 ± 0.06 mM for enflurane used at 2.25, 3.4, and 4.5%, respectively; and 0.35 ± 0.05 , 0.49 ± 0.04 , and 0.69 ± 0.06 mM for isoflurane used at 1.5, 2.25, and 3%, respectively. As shown in figure 4, volatile agents induced a concentration-related increase in ^3H -DA release. In fact, halothane elicited a 22 ± 7 ($P < 0.05$), 30 ± 6 ($P < 0.01$), and $39 \pm 5\%$ ($P < 0.01$) increase from basal ^3H -DA release at 1, 1.5, and 2 MAC concentrations, respectively. The effect of halothane on spontaneous ^3H -DA release was reversible, because

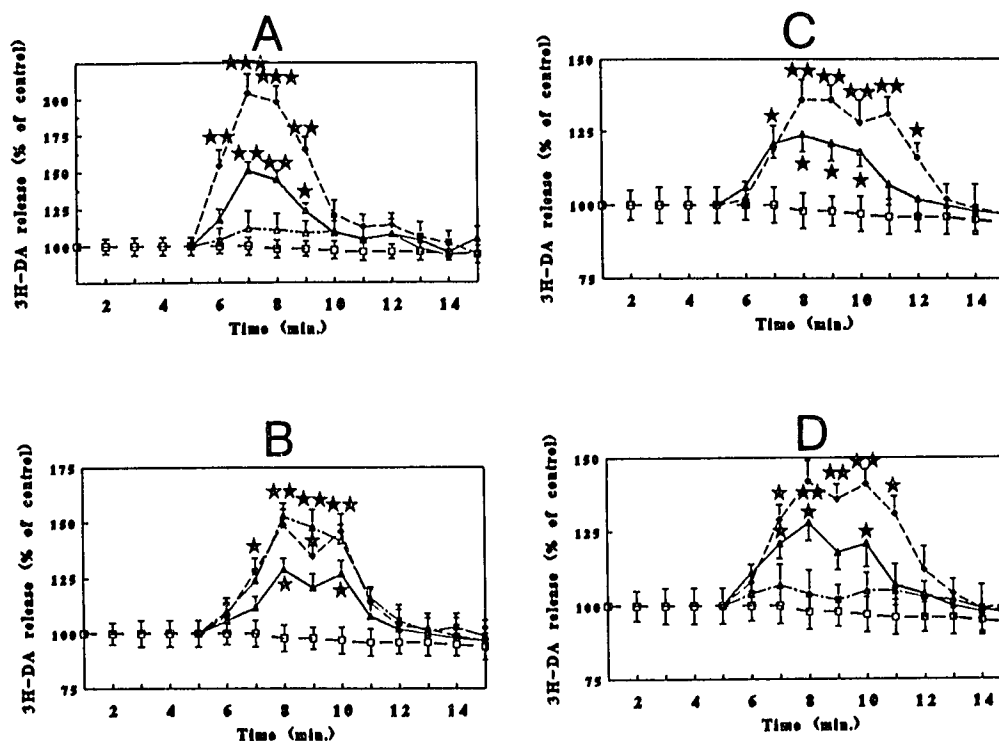


Fig. 2. Time-course of the release of ^3H -DA elicited by various depolarizing agents from preloaded striatal synaptosomes. (A) Effects of potassium chloride, 15 mM (filled triangles); 50 mM (filled squares); and 15 mM without Ca^{2+} (open triangles). (B) Effects of glutamate applied either in normal CSF at a 10^{-4} M (filled triangles) or 10^{-3} M (filled squares) concentration, or in Mg^{2+} -free CSF (10^{-3} M, open triangles). (C) Effects of kainate (10^{-4} M, filled triangles; and 10^{-3} M, filled squares) applied in normal CSF. (D) Effects of NMDA (10^{-4} M, filled triangles; and 10^{-3} M, filled squares) in the absence of Mg^{2+} , and of NMDA (10^{-3} M) in the presence of Mg^{2+} (0.83 mM, open triangles). Data (mean \pm SEM) are expressed as a fraction of time-dependent controls (basal release, open squares). All depolarizing agents were applied between minutes 5 and 10. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$.

the time course of radioactivity paralleled that of the time-dependent control after cessation of anesthetic delivery (fig. 5). Also, the same profile was observed for either isoflurane or enflurane (data not shown). It is noteworthy that these agents were less potent than halothane, because enflurane caused a 5 ± 8 (not significant), 11 ± 4 (not significant), and $25 \pm 6\%$ ($P < 0.05$), and isoflurane a 12 ± 6 (not significant), 25 ± 5 ($P < 0.05$), and $33 \pm 6\%$ ($P < 0.01$) increase in ^3H -DA release when used at 1, 1.5, and 2 MAC concentrations, respectively (fig. 4). The maximal increase in release elicited by volatile anesthetics was significantly reduced when Ca^{2+} was omitted from the medium ($-58 \pm 10\%$, $P < 0.01$ for enflurane; $-52 \pm 8\%$, $P < 0.01$ for halothane; and $-48 \pm 7\%$, $P < 0.01$ for isoflurane; fig. 6). No significant difference in halothane- and isoflurane-induced ^3H -DA release was observed whether or not physiologic levels of Mg^{2+} (0.83 mM) were present in the medium, although the effect of

enflurane (2 MAC) was significantly increased in the absence of Mg^{2+} ($34 \pm 7\%$, $P < 0.05$; fig. 6).

To examine whether the presynaptic NMDA receptors located on DA nerve terminals were involved in the action of volatile anesthetics reported above, the effect of the NMDA antagonist MK-801 (10^{-4} M, delivered in a Mg^{2+} -free artificial CSF) on spontaneous, NMDA-, halothane-, enflurane-, and isoflurane-induced ^3H -DA release was analyzed. No significant difference was observed in halothane or isoflurane effects (2 MAC) when experiments were performed in either the presence or absence of MK-801. In contrast, the effect of a 2-MAC enflurane concentration was significantly reduced when MK-801 was present in the Mg^{2+} -depleted solution ($-65 \pm 10\%$, $P < 0.01$; fig. 6). In contrast, MK-801 (10^{-4} M) failed to block the enflurane-induced ^3H -DA peak in the presence of physiologic concentrations of Mg^{2+} (fig. 6).

To determine whether ^3H -DA release elicited by ha-

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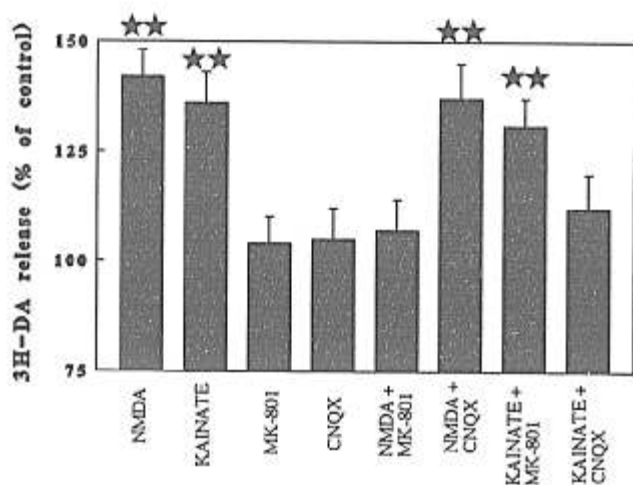


Fig. 3. Pharmacologic characterization of the ³H-DA release evoked by the glutamate agonists NMDA and AMPA from preloaded striatal synaptosomes. Data are expressed as a fraction of control (basal) release. Agonists (NMDA and kainate) and antagonists (MK-801 and CNQX) were used at 10^{-3} M (10^{-4} M, respectively) concentrations. ** $P < 0.01$.

logenated anesthetics could be mediated by reversal of the membrane DA transporter, the effects of halothane, enflurane, and isoflurane were compared in the absence and presence of nomifensine (10^{-5} M), a blocker of the monoamine transporters.²¹ Nomifensine alone did not significantly modify the basal release of ³H-DA. No significant difference could be observed between ³H-DA release evoked by volatile anesthetics in either the presence or absence of nomifensine (fig. 6). However, neither ketamine nor thiopental (10^{-5} and 10^{-4} M) had any effect on basal release (fig. 7).

Effects of Anesthetic Agents on Depolarization-Evoked ³H-DA Release from Striatal Synaptosomes

The effects of anesthetic agents on depolarization-evoked ³H-DA release are reported in table 1. The volatile agents halothane and isoflurane induced a significant, concentration-related decrease in the release evoked by glutamate (10^{-3} M), kainate (10^{-3} M), or NMDA (10^{-3} M). Enflurane was also found to significantly reduce glutamate and kainate peaks, but not the effect of NMDA. It is interesting, however, that enflurane used at both 1 and 2 MAC concentrations significantly enhanced the release of DA evoked by NMDA. On the other hand, thiopental (10^{-4} M, but not 10^{-5} M) significantly reduced the effect of glutamate (10^{-3} M), NMDA (10^{-3} M), and kainate (10^{-3} M) on release, with a predominant action on the NMDA-evoked peak. Ketamine (10^{-4} M, but not 10^{-5} M)

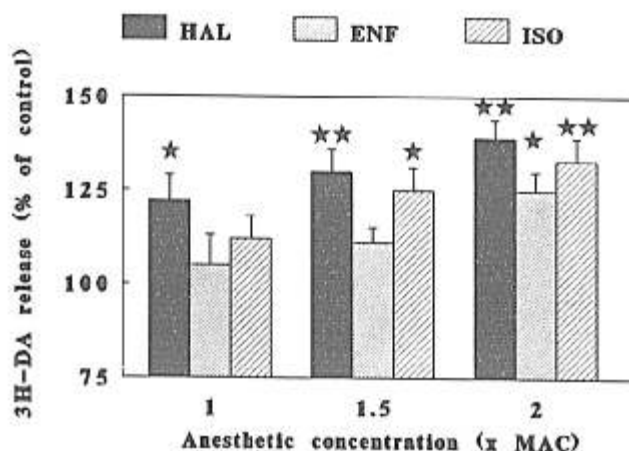


Fig. 4. Effects of halothane (HAL), enflurane (ENF), and isoflurane (ISO) on the spontaneous ³H-DA release evoked from preloaded striatal synaptosomes. Data are expressed as a fraction of control (basal) release. Gaseous concentrations corresponding to 1 MAC were 1, 2.25, and 1.5% for halothane, enflurane, and isoflurane, respectively. * $P < 0.05$; ** $P < 0.01$.

significantly attenuated the release elicited by NMDA (10^{-3} M) and glutamate (10^{-3} M). However, ketamine had no effect on the kainate-mediated ³H-DA release. The release of DA induced by potassium chloride (15 mM) was significantly reduced by all anesthetic agents examined in this study. In contrast, the potassium chloride (50 mM)-evoked peak was unaffected by any of the anesthetics tested.

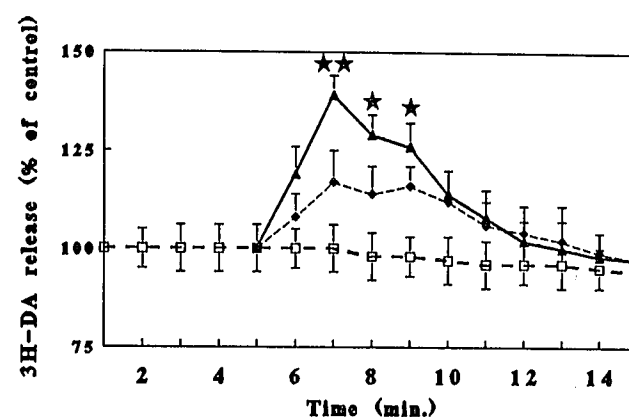


Fig. 5. Time course of the effect of halothane application (2 MAC) on the spontaneous ³H-DA release from preloaded striatal synaptosomes observed in either the presence (filled triangles) or absence (filled squares) of external Ca^{2+} . Halothane was applied between minutes 5 and 10 at a 2% gaseous concentration. Data (mean \pm SEM) are expressed as a percentage of time-dependent control (basal, open squares) release. * $P < 0.05$; ** $P < 0.01$.

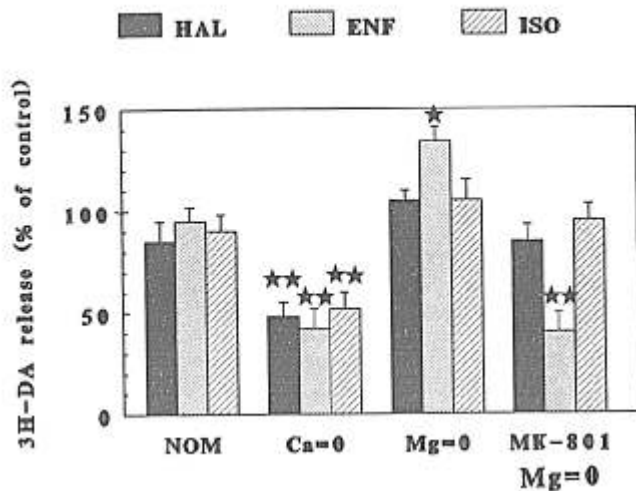


Fig. 6. Effects of Ca^{2+} - and Mg^{2+} -free media, the NMDA antagonist MK-801 (10^{-4} M), and the DA uptake inhibitor nomifensine (NOM, 10^{-5} M) on the release of ^3H -DA from preloaded striatal synaptosomes elicited by a 2-MAC concentration of inhalational anesthetics. Note that nomifensine alone had no significant action on spontaneous release. Data (mean \pm SEM) are expressed as a fraction of control release (effect of 2 MAC obtained in the presence of Mg^{2+}). Gaseous concentrations corresponding to 1 MAC were 1, 2.25, and 1.5% for halothane, enflurane, and isoflurane, respectively. * $P < 0.05$; ** $P < 0.01$.

Discussion

Numerous studies have shown that general anesthetics enhance inhibitory neurotransmission by increasing the activity of receptor-operated ionic channels at the postsynaptic level.¹⁻³ In contrast, whether clinically relevant concentrations of anesthetic agents may also interfere with presynaptic target sites to either stimulate release or depress uptake of inhibitory neurotransmitters remains to be clearly established. In the current study, we provide evidence that anesthetics, particularly volatile agents, directly act at presynaptic target sites to modulate both spontaneous and depolarization-evoked release of DA. More precisely, we have shown that: (1) volatile anesthetics, thiopental, and ketamine exert complex, even opposite, effects on the spontaneous, unstimulated *versus* depolarization-evoked ^3H -DA release from striatal synaptosomes in the rat; (2) the action of enflurane differs from that of all other anesthetics tested insofar as this agent enhances the NMDA receptor-mediated mechanisms involved in both the spontaneous and depolarization-evoked release; and (3) the involvement the DA transporter in the effects of volatile anesthetics on spontaneous release is not supported by our findings.

Methodologic Considerations

In the current study, the effects of anesthetics on spontaneous and depolarization-evoked DA release were examined on synaptosomes obtained from the rat striatum. Synaptosomes are considered the simplest preparation on which direct effects of pharmacologic agents on calcium-dependent neurotransmitter release can be studied.³⁰ It should be noted that volatile anesthetics do not necessarily modulate the release of neurotransmitters from nerve terminals containing different mediators in a similar fashion. For example, halothane reduces the depolarization-evoked noradrenergic release, but it has no influence on the release of acetylcholine from the rat cerebral cortex.²⁵ The preparation used in our study consists of different subpopulations of nerve endings originating from a unique, DA-enriched, and functionally important brain area, the striatum. Of these various subpopulations of nerve endings, only the DA terminals are considered to be able to take up tritiated DA *via* activation of the specific DA carrier, and, consequently, to release this transmitter. In fact, the rat caudate nucleus receives no noradrenergic innervation, and only very few serotonergic inputs from the midbrain,³¹ two neuronal subpopulations that are also theoretically able to take up and release DA. This strongly supports the fact that the main subpopulation of synaptosomes used in our study was, indeed, the DA one.

Previous experiments performed *in vivo* have shown that the release of DA can be stimulated in the striatum primarily by activation of the glutamatergic

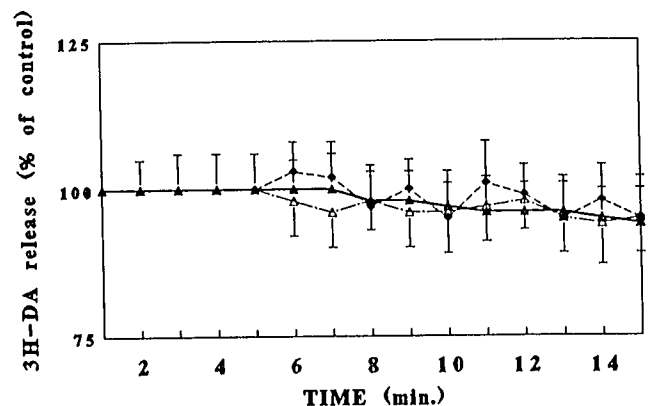


Fig. 7. Time course of the effects of thiopental (10^{-4} M) and ketamine (10^{-4} M) on the spontaneous ^3H -DA release from preloaded striatal synaptosomes. Drugs were applied between minutes 5 and 10. Data (mean \pm SEM) are expressed as a fraction of control (basal, filled triangles) release.

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Table 1. Effects of Anesthetic Agents on the Release of ³H-DA Evoked by Depolarizing Agents from Striatal Synaptosomes

Anesthetic Agent		KCl		Glutamate 10 ⁻³ M	NMDA 10 ⁻³ M	Kainate 10 ⁻³ M
		15 mM	50 mM			
Halothane	1 MAC	-41 ± 6*	+11 ± 6	-32 ± 9*	-34 ± 9*	-32 ± 6*
	2 MAC	-54 ± 4*	-9 ± 4	-56 ± 8*	-52 ± 10*	-55 ± 7*
Isoflurane	1 MAC	-34 ± 8*	-4 ± 8	-41 ± 7*	-37 ± 10*	-35 ± 9*
	2 MAC	-49 ± 7*	-9 ± 13	-62 ± 10*	-57 ± 6*	-52 ± 11*
Enflurane	1 MAC	-26 ± 7†	-5 ± 8	-18 ± 6	+21 ± 9†	-31 ± 9*
	2 MAC	-43 ± 9*	-7 ± 9	-28 ± 7†	+35 ± 11*	-54 ± 11*
Ketamine	10 ⁻⁵ M	-6 ± 9	+7 ± 10	-7 ± 11	-14 ± 11	+6 ± 7
	10 ⁻⁴ M	-21 ± 6†	-11 ± 12	-22 ± 6†	-65 ± 9*	-4 ± 10
Thiopental	10 ⁻⁵ M	-15 ± 5	+6 ± 10	-18 ± 6	-14 ± 7	-11 ± 6
	10 ⁻⁴ M	-38 ± 8*	-9 ± 9	-40 ± 11*	-51 ± 12*	-26 ± 9†

Data (mean ± SEM) are expressed as a percentage of either increase or decrease from the release obtained in the absence of anesthetic agents (control). Gaseous concentrations corresponding to 1 MAC were 1, 1.5, and 2.25% for halothane, isoflurane, and enflurane, respectively.

* $P < 0.01$.

† $P < 0.05$.

corticostriatal fibers.³² The presynaptic regulation of DA release by glutamate has now been extensively studied and characterized in the rat striatum.^{19,22-24,33-35} More precisely, data obtained from both *in vivo* and *in vitro* experiments clearly indicate that glutamate stimulates DA release in this structure *via* both indirect mechanisms depending on action potential propagation^{33,34} and direct, tetrodotoxin-insensitive ones.²³ Moreover, glutamate has been found to directly stimulate DA release from striatal synaptosomes by acting on both presynaptic NMDA and AMPA receptors located on the DA terminals.^{19,22-24} This probably explains why glutamate was found to be more effective in releasing ³H-DA than either NMDA or kainate in the current study. As previously suggested, glutamate acts first on AMPA receptors to eliminate the Mg²⁺ block, which allows activation of the NMDA receptors.²² Thus, the synaptosome preparation used here provides a reliable and reproducible model for analyzing, *in vitro*, the direct presynaptic effects of anesthetics on spontaneous and depolarization-evoked DA release in the striatum. However, working with synaptosomes also has some limitations. Unlike striatal slices, synaptosomes do not preserve the local microcircuits involved in DA release, and, thus, preclude looking at indirect, tetrodotoxin-sensitive mechanisms. Furthermore, the role played by diffusible mediators, such as nitric oxide (which has been shown to stimulate DA release in the striatum³⁵), cannot be addressed using synaptosomes, because the striatal DA terminals do not possess the nitric oxide synthase.³⁶

Effects of Volatile Anesthetics on ³H-DA Release from Striatal Synaptosomes

We found that volatile anesthetics used at clinically relevant concentrations exert differential effects on spontaneous *versus* depolarization-evoked ³H-DA release from preloaded striatal synaptosomes. Indeed, these agents applied alone on the synaptosome preparation triggered an ³H-DA efflux, one half of which depends on the presence of external Ca²⁺. In contrast, they reduced the release of ³H-DA evoked by depolarizing pharmacologic agents, such as potassium chloride (15 mM, but not 50 mM) or most of the glutamate agonists.

A slight, nonsignificant decrease in spontaneous ³H-DA release with time when a classic CSF containing physiologic concentrations of Mg²⁺ was used. A small, nonsignificant increase in baseline was noted when Mg²⁺ was omitted from the superfusion medium. Altogether, these findings indicate that using an Mg²⁺-free CSF had only little influence on synaptosome excitability. Similarly, a slight, nonsignificant decrease in spontaneous release was noted when Ca²⁺ was omitted from the medium. This indicates that, unlike the Ca²⁺ release of ³H-DA evoked by either volatile anesthetics or depolarizing pharmacologic agents, the basal release corresponds primarily to an external Ca²⁺-independent phenomenon. The lack of effect of the NMDA antagonists MK-801 and ketamine on spontaneous release are consistent with this finding, because no tonic activation of the NMDA receptors is expected to occur with this synaptosome preparation. Consequently, this rules out the possibility that tonically activated NMDA receptors

may contribute to the spontaneous, unstimulated ^3H -DA release in our conditions.

Our results seem to differ from those obtained recently *in vitro* by El-Maghrabi and Eckenhoff. These authors observed that volatile anesthetics did not stimulate ^3H -DA release from preloaded synaptosomes.³⁷ Several explanations can be proposed to reconcile these apparently contradictory results. First, these discrepancies may be caused by the use of different experimental approaches to measure DA release from preloaded synaptosomes in the two studies. Alternatively, they may be caused by the heterogeneity of the preparation used (rat whole brain synaptosomes) in El-Maghrabi and Eckenhoff's study. Indeed, this model probably contains various subpopulations of presynaptic terminals, including those originating from other monoamine-containing fibers (*i.e.*, the serotonergic and noradrenergic neurons). This statement is further supported by the efficiency of imipramine, a potent blocker of the monoamine uptake systems located on serotonergic fibers,³⁸ to block ^3H -DA transport in their study.³⁷ Thus, the sensitivity to volatile anesthetics of these fibers may be different from that of the DA ones. El-Maghrabi and Eckenhoff also made the interesting finding that volatile anesthetics block ^3H -DA uptake by nerve terminals.³⁷ However, the increase in ^3H -DA release elicited by these agents in our study is probably not accounted for by inhibition of DA reuptake by synaptosomes for at least two reasons: first, the superfusion rate used (1 ml/min) has been previously shown to prevent DA reuptake under similar experimental conditions, and,²¹ second, nomifensine, as well as ketamine, which also blocks reuptake of monoamines in the CNS,³⁹ failed to affect spontaneous release in our study.

Our results indicate that halothane, enflurane, and isoflurane stimulate the spontaneous release of ^3H -DA by mechanisms that are, in part, dependent on the presence of external Ca^{2+} , but remain to be delineated. It can be suggested that the Ca^{2+} -dependent release elicited by volatile anesthetics may result from lipophilic interactions with the synaptosome membranes. Indeed, it has been shown that halothane increases Ca^{2+} concentrations in whole brain synaptosomes. The ability of this agent to increase resting Ca^{2+} levels was correlated with its membrane fluidizing action.⁴⁰ However, halothane has also been shown to trigger the release of intraneuronally stored Ca^{2+} .⁴¹ The existence of rough endoplasmic reticulum-like Ca^{2+} stores in nerve terminals and a ryanodine receptor in brain indicates that

the mobilization of Ca^{2+} from endogenous stores may be important in neurotransmitter release. Also, it cannot be ruled out that volatile agents may also enhance the so-called external Ca^{2+} -independent neurotransmitter release,⁴² because about one half of the increase in ^3H -DA release was still observed in the absence of external Ca^{2+} in our study. It is possible that these agents enhance DA release by reversal of the DA transporter. This mechanism has been previously suggested to occur with compounds such as serotonin or D-amphetamine in the striatum.⁴³ If this had been the case, however, halothane and isoflurane effects would have been expected to be decreased by the DA transport inhibitor, nomifensine, which was not supported by our data. In any case, our results support that, in this preparation, the machinery for transmitter release *per se* is enhanced, rather than inhibited, by volatile anesthetics.

We also observed that potassium chloride, glutamate, NMDA, and kainate induce a dose-related ^3H -DA release from striatal synaptosomes. These effects were clearly dependent on the presence of external Ca^{2+} , which is in agreement with previous reports.^{19,21-25,33,42} Indeed, depolarization evoked by either potassium chloride or glutamate agonists leads to a rapid influx of Ca^{2+} into the nerve endings, and triggers the release of neurotransmitter.³⁰ We observed that clinical concentrations of both halothane and isoflurane decreased the depolarization-evoked ^3H -DA release from preloaded striatal synaptosomes. Interestingly, depression of either potassium chloride-, glutamate-, or NMDA-induced Ca^{2+} entry by halothane and isoflurane in cultured hippocampal neurons has been already observed.⁴⁴ Our findings are also consistent with previous observations made on cortical slices, in which volatile anesthetics reduced the potassium chloride-evoked release of norepinephrine, but not acetylcholine.²⁵ Accordingly, we observed that inhibition of potassium chloride (15 mM)-induced ^3H -DA release by volatile anesthetics could be surmounted by increasing the strength of depolarization until reaching "supraphysiologic" potassium concentrations (50 mM).^{25,45} This indicates that these agents may specifically affect the stimulus secretion process, possibly by altering the activity of the voltage-operated Ca^{2+} channels located on the DA terminals. Indeed, volatile anesthetics have been previously shown to inhibit dihydropyridine binding to brain membranes in the rat.⁴⁶ Furthermore, patch-clamp studies have demonstrated that clinically relevant concentrations of halothane and isoflurane reduce both the low- and high-voltage activated Ca^{2+} currents located

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on sensory neurons.⁴⁷ Our results also indicate that halothane and isoflurane decrease the release of DA evoked by glutamate, AMPA, and NMDA. Halothane and isoflurane applications to cultured hippocampal neurons have been shown to prevent the increases in intracellular Ca^{2+} produced by the interactions of glutamate with receptors of either the kainate or NMDA subtype.⁴⁴ Thus, our findings confirm and extend previous results by showing that these agents decrease neurotransmitter release triggered by direct stimulation of the presynaptic glutamate receptors (NMDA and AMPA) present on DA terminals in the striatum.

Enhancement by Enflurane of the NMDA-Mediated 3H -DA Release

Interestingly, although the action of enflurane on spontaneous DA release seemed to be close to that of the other volatile agents, it exhibited some particularities that were not shared by the other vapors. Indeed, the enflurane (but neither halothane nor isoflurane)-induced DA release was enhanced by almost 40% when the synaptosome preparation was superfused with magnesium-free CSF. It is well known that depolarization suppresses the blockade of the NMDA receptor-coupled channel because of the presence of Mg^{2+} ions.⁴⁸ Although few changes occurred in baseline in the absence of Mg^{2+} , it cannot be definitely ruled out that enflurane only increases the depolarization of the synaptosome, and, therefore, has a secondary effect on the NMDA receptor. However, administration of the NMDA antagonist MK-801 significantly reduced the effects of enflurane (but not halothane or isoflurane) on 3H -DA release in the absence, but not in the presence, of Mg^{2+} . Moreover, unlike the other vapors, enflurane was found here to reduce the glutamate-evoked DA release by primarily decreasing the kainate-mediated effect, although it enhanced the NMDA-evoked release. MacIver and Kendig have previously shown that the enflurane-induced seizure-like burst discharges from CA1 neurons observed on hippocampal slices in the rat were completely suppressed by 5-APV, an NMDA receptor antagonist.⁴⁹ They suggested that enflurane may enhance either the tonic release of glutamate from synaptic terminals or the NMDA receptor/ionophore activity. Enflurane has recently been shown to increase the Ca^{2+} -dependent release of endogenous glutamate from the mouse cerebral cortex.⁵⁰ Our data indicate that about one half of enflurane action on spontaneous DA release proceeds *via* direct or indirect activation of the presynaptic NMDA receptors, and the rest very

likely represents a mechanism shared with halothane and isoflurane. That enflurane may increase the activity of the NMDA receptors is consistent with the presence of epileptiform discharges during anesthesia performed with this agent.⁵¹ Therefore, activation of NMDA receptors by enflurane may also occur at postsynaptic target sites. More generally, it can be suggested that, with respect to the receptor-dependent effects, the results presented here may apply to postsynaptic, as well as presynaptic, AMPA and NMDA receptors.

Influence of Thiopental and Ketamine on 3H -DA Release from Striatal Synaptosomes

Unlike volatile agents, ketamine and thiopental used at clinically relevant concentrations were ineffective in stimulating 3H -DA release from striatal nerve terminals. The lack of effect of ketamine on basal release is not surprising, because DA release is stimulated by glutamate agonists in the rat striatum. Ketamine has been shown to induce anesthesia, at least in part, by blocking glutamate receptors of the NMDA subtype.⁵² Accordingly, we found that clinically relevant concentrations of ketamine significantly attenuated the glutamate- and NMDA-, but not kainate-, mediated 3H -DA release. On the other hand, thiopental is known to enhance the efficacy of the GABA-receptor-coupled chloride channel, which, in turn, leads to neuronal hyperpolarization.³ To date, however, there is no evidence for a regulation of DA release involving presynaptic GABA receptors located on DA terminals. It is interesting that, unlike halothane, pentobarbital application failed to alter Ca^{2+} levels in either synaptosomes⁴⁰ or cultured hippocampal neurons.⁴¹ In the current study, thiopental inhibited the 3H -DA release evoked by potassium chloride, as well as that evoked by excitatory amino acids. Barbiturates have been reported to non-competitively inhibit postsynaptic responses elicited by kainate and quisqualate more potently than those evoked by NMDA.⁵² Our findings are consistent with these results; however, we did not observe a significant difference in the magnitude of decrease of kainate- and NMDA-evoked effects by thiopental. This may be related to slight differences in some pharmacologic properties of pre- *versus* postsynaptic AMPA and NMDA receptors.

Possible Physiologic Relevance of the Study

It is always difficult to extrapolate *in vitro* results to processes occurring *in vivo*. However, the similarities between the concentrations required to modulate 3H -DA release and those associated with anesthesia *in vivo*

indicate that this effect may contribute to production of unconsciousness. It can be speculated that the effects of anesthetics on DA 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 that synapses are activated. Nevertheless, it should be taken into account that at least three neurotransmitter systems (glutamatergic, GABAergic, and cholinergic) participate in the control of presynaptic DA release in the striatum *in vivo*^{19,53-56}. Therefore, the results obtained here apply only to spontaneous and evoked release from synaptosomes, whereas nerve terminals *in situ* subjected to impulse activity and a transmitter-enriched environment may behave quite differently.

In summary, we have shown that anesthetic agents exert a complex influence on the release of DA, an inhibitory neurotransmitter, from the rat striatum. Some of these findings may be relevant to the changes in the striatal DA content associated with general anesthesia. Our results emphasize that the presynaptic nerve endings located in a restricted, but functionally important, brain area, the striatum, may represent potential targets for general anesthetics. Thus, it would be interesting to investigate the effects of anesthetics on the release of other inhibitory neurotransmitters present in this structure, such as GABA, which has potential importance with regard to anesthetic mechanisms.

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