British Journal of Pharmacology (1999) 127, 679–684
http://www.stockton-press.co.uk/bjp

Halothane enhances exocytosis of [^{3}H]-acetylcholine without increasing calcium influx in rat brain cortical slices

*,[1]R. S. Gomez, [2]M. A. M. Prado, [3]F. Carazza & [2]M. V. Gomez

[1]Departamento de Cirurgia, Sala 4000 Faculdade de Medicina da UFMG, Avenida Alfredo Balena, 190 CEP: 30130-100, Belo Horizonte-Minas Gerais-Brasil; [2]Departamento de Farmacologia, ICB-UFMG, Avenida Alfredo Balena, 190 CEP: 30130-100, Belo Horizonte-Minas Gerais-Brasil and [3]Departamento de Química, ICEX-UFMG, Avenida Alfredo Balena, 190 CEP: 30130-100, Belo Horizonte-Minas Gerais-Brasil

1 The effect of halothane on the release of [^{3}H]-acetylcholine ([^{3}H]-ACh) in rat brain cortical slices was investigated.

2 Halothane (0.018 mM) did not significantly affect the basal and the electrical field stimulation induced release of [^{3}H]-ACh. However, halothane (0.063 mM) significantly increased the basal release of [^{3}H]-ACh and this effect was additive with the electrical field stimulation induced release of [^{3}H]-ACh.

3 The release of [^{3}H]-ACh induced by 0.063 mM halothane was independent of the extracellular sodium and calcium ion concentration and was decreased by tetracaine, an inhibitor of Ca^{2+}-release from intracellular stores or dantrolene, an inhibitor of Ca^{2+}-release from ryanodine-sensitive stores.

4 Using 2-(4-phenylpiperidino)-cyclohexanol (vesamicol), a drug that blocks the storage of ACh in synaptic vesicles, we investigated whether exocytosis of this neurotransmitter is involved in the effect of halothane. Vesamicol significantly decreased the release of [^{3}H]-ACh evoked by halothane.

5 It is suggested that halothane may cause a Ca^{2+} release from intracellular stores that increases [^{3}H]-ACh exocytosis in rat brain cortical slices.

Keywords: Acetylcholine; transmitter release; anaesthetics, volatile; halothane; rat brain cortical slices; ions; intracellular Ca^{2+} release; exocytosis

Abbreviations: ACh, acetylcholine; EGTA, ethyleneglycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid; paraoxon, diethyl ρ-nitrophenyl phosphate; vesamicol, 2-(4-phenylpiperidino)-cyclohexanol

Introduction

There have been extensive efforts to characterize the mechanism of action of volatile anaesthetics, but their molecular and cellular actions are still a matter of debate. A possible site of action for general anaesthetics could be the presynaptic terminal as indicated by results showing that synaptic transmission is more sensitive to the effects of general anaesthetics than axonal conduction (Larrabee *et al*., 1952; Richards, 1983; Griffiths & Norman, 1993). Thus, the investigation of the effects of anaesthetics on the release of neurotransmitters may provide information on the mechanisms that contribute to the general effect of these substances during anaesthesia.

Acetylcholine (ACh) is a transmitter implicated in memory, learning, cognitive behaviour and it is also of importance during sleep (Mitchell, 1963; Kanai & Szerb, 1965; Collier & Mitchell, 1967; Griffiths & Norman, 1993; Keifer *et al*., 1994; Winkler *et al*., 1995). Although there has been some reports of the action of volatile anaesthetics on ACh release, it is not clear how these drugs alter the release of this transmitter. Some authors reported significant inhibition of potassium-stimulated release of ACh in the presence of halothane (Johnson & Hartzell, 1985; Griffiths *et al*., 1995) while others observed no effect (Bazil & Minneman, 1989a,b). However, to our knowledge all studies examined the release of ACh from the central nervous system (CNS) using high potassium stimulation, and little effort has been made to follow the effects of anaesthetics on ACh release induced by electrical stimulation.

In the course of an investigation in brain cortical slices, we observed that halothane increased the basal release of ACh ([^{3}H]-ACh). When associated with electrical stimulation, the release of this transmitter was additive. Therefore, the aim of the present work was to further investigate the mechanisms involved on halothane-induced release of [^{3}H]-ACh in rat brain cortical slices.

Methods

Release of [^{3}H]-ACh from rat brain cortical slices

All procedures were approved by the local ethics committee. Adult Wistar rats (200–250 g) of either sex were decapitated and had their brains removed. Slices of cerebral cortex (0.5 mm) were obtained using a McIlwain Tissue Slicer (Brinkman Instruments Inc., U.K.). The brain cortical slices were weighed (40 mg) and then placed into the incubating medium.

The release of [^{3}H]-ACh into the incubating fluid was studied after labelling the tissue ACh with [methyl-^{3}H]-choline chloride (78 Ci mmol^{-1}; Amersham Searle), as previously described by Gomez *et al*. (1996) and Casali *et al*. (1997). Briefly, endogenous ACh stores were first depleted by incubation at 37°C for 15 min in high-K^+ (50 mM) salt medium (3.0 ml) and the slices were separated from the incubating medium by centrifugation (5000 × *g* for 10 min). To

*Author for correspondence; E-mail: gomez@mono.icb.ufmg.br

label the endogenous pools of ACh, the slices were incubated in salt medium for 30 min (37°C) with 0.11 μCi ml^{-1} of [methyl-^{3}H]-choline (free of choline carrier). The slices were then separated from the incubating fluid by centrifugation (5000 × *g* for 10 min) followed by two washes with a medium containing 1.0 μM choline.

Experimental protocol

Stock solutions of saturated halothane were prepared prior to each experimental session. 10 ml of incubating medium was equilibrated with liquid anaesthetic (100 or 400 μl) at 37°C in Teflon-capped glass vials for 30 min. Subsequently, 500 μl of stock, anaesthetic-saturated solution was added to 1.0 ml of the incubation medium with a glass syringe. The vial was immediately capped and mixed. In some experiments, slices were stimulated 5 min thereafter by electrical field stimulation with a platinum electrode (10 Hz, 0.5 ms, 10 V) for a further 5 min. In order to investigate the effect of halothane on the basal release of [^{3}H]-ACh, we also used halothane in the absence of electrical field stimulation.

To clarify the mechanism(s) by which halothane changes release of [^{3}H]-ACh, the slices were previously incubated for 15 min in the absence or presence of 0.5 μM tetrodotoxin, 2.0 mM EGTA, 100 μM Cd^{2+}, tetracaine (50 or 500 μM) or dantrolene (0.1 – 100 μM). Thereafter, the slices were incubated for 5 min in the absence or presence of halothane (0.063 mM). At this concentration and time the release of [^{3}H]-ACh was linear (data not shown). When the action of 2-(4-phenylpiperidino)-cyclohexanol (vesamicol) was examined, the drug was added to slices 10 min before incubation with [methyl-^{3}H]-choline. In these conditions, a concentration of vesamicol ten times larger than that used in the present study does not alter the incorporation of ^{3}H label (Leão *et al.*, 1995). The incubation medium used contained (in mM): NaCl 136, KCl 2.7, $CaCl_2$ 1.8, glucose 5.5, Tris base 10, and 20 μM diethyl *p*-nitrophenyl phosphate (paraoxon) to prevent hydrolysis of [^{3}H]-ACh. The final pH was adjusted to 7.4. The Ca^{2+} free solution was prepared by removing $CaCl_2$ and adding 2.0 mM ethyleneglycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA).

The saturation of the stock solution and the aqueous concentration of halothane in the incubating medium after a 5 min incubation was confirmed by n-heptane extraction and measurement by gas chromatography using a Hewlett Packard Series II-5890 gas chromatograph (Rutledge *et al.*, 1963).

Measurement of [^{3}H]-ACh release

Aliquots (300 μl) of the samples were counted for radioactivity by liquid scintillation spectrophotometry using a Packard spectrophotometer. In each group of experiments, [^{3}H]-ACh and [^{3}H]-choline were separated from the supernatants by the choline kinase method (Goldberg & McCamman, 1973; Prado *et al.*, 1993) and [^{3}H]-ACh represented about 65% of the total radioactivity released. Confirming this data, the same magnitude of ^{3}H efflux induced by halothane was also obtained when paraoxan was replaced by 10 μM hemicholinium-3 (HC-3), suggesting that accumulation of ACh due to cholinesterase inhibition was not altering the release of transmitter (data not shown). In addition, there was little release of ^{3}H during halothane stimulation in the absence of paraoxan and HC-3 (145 ± 5% of basal release, mean ± s.e.mean for three experiments in the absence of paraoxan or HC-3 compared with 269 ± 4% in the presence of paraoxan), indicating that most [^{3}H]-ACh released can be hydrolized and taken back by the slices in a HC-3 sensitive way.

Chemicals

Halothane was a generous gift from Halocarbon (River Edge, New Jersey, U.S.A.). Paraoxon, tetracaine, cadmium and dantrolene were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The effective (−)-enantiomer of vesamicol was a gift of Professor S. M. Parsons (University of California at Santa Barbara CA, U.S.A.), and in this article the term vesamicol refers to the (−)-enantiomer. The n-heptane chromatography grade was obtained from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical grade and were obtained from the usual commercial sources.

Statistical analysis

Results are presented as mean ± s.e.mean values. Difference between means were determined by analysis of variance (ANOVA) and multiple comparison tests.

Results

Effects of halothane on the resting and stimulated release of [^{3}H]-ACh

Table 1 shows that 0.018 mM halothane did not affect the basal or the electrical field induced release of [^{3}H]-ACh ($P > 0.05$). However, 0.063 mM halothane increased by 2.7 times the release of [^{3}H]-ACh ($P < 0.05$). This effect remained at higher concentrations of halothane (0.117 and 0.235 mM) and the release of [^{3}H]-ACh was linear up to 0.063 mM halothane and 5 min of incubation (data not shown). Moreover, the release of [^{3}H]-ACh in the presence of 0.063 mM halothane and electrical field stimulation was additive ($P < 0.05$).

Extracellular Na^+ and Ca^{2+} independence of halothane induced release of [^{3}H]-ACh

Halothane could affect the release of [^{3}H]-ACh through an alteration in Ca^{2+} or Na^+ influx, causing an increase in

Table 1 Effect of halothane on the release of acetylcholine induced by electrical field stimulation in rat brain cortical slices

	[^{3}H]-ACh (d.p.m. mg^{-1} of tissue)	
Volatile anaesthetic	*Control*	*Electrical field stimulation* (10 V, 0.5 ms, 10 Hz)
None	35.3 ± 2.9	87.6 ± 7.2*
Halothane (0.018 mM)	45.3 ± 5.9	89.1 ± 6.1*
Halothane (0.063 mM)	94.9 ± 5.2**	189.2 ± 15.6*†
Halothane (0.117 mM)	105.8 ± 4.8**	193.2 ± 13.2*†
Halothane (0.235 mM)	104.7 ± 6.2**	196.3 ± 16.5*†

*Statistically different from the control value, $P < 0.05$. **Statistically different from the value without volatile anaesthetic, $P < 0.05$. †Statistically different from the electrical field stimulation value, $P < 0.05$. Brain cortical slices (40 mg) loaded with [^{3}H]-choline were preincubated for 5 min in salt medium in the absence (control) or in the presence of halothane (0.018 or 0.063 mM) and then stimulated for 5 min with electrical field stimulation (10 V, 0.5 ms, 10 Hz). Data are mean ± s.e.mean values from at least three different experiments performed in duplicate. For other details, see text.

transmitter output. Table 2 shows that tetrodotoxin, a known Na^+ channel blocker (Narahashi *et al*., 1964), failed to affect the release of [^{3}H]-ACh induced by halothane. Moreover, these data also show that the release of [^{3}H]-ACh evoked by halothane was not affected by 2.0 mM EGTA (no calcium added to the medium) or 100 μM Cd^{2+}, a nonspecific blocker of calcium channels (Fox *et al*., 1987). The lack of effect of external calcium on the action of halothane was also confirmed when $^{45}Ca^{2+}$ uptake in rat brain cortical synaptosomes was measured as previously described (Miranda *et al*., 1998). In these experiments, the influx of $^{45}Ca^{2+}$ in synaptosomes after 30 s of exposure to 0.063 mM halothane (at 35°C) was not changed (mean $\pm$ s.e.mean of three experiments performed in duplicate; control: 2760 ± 260 d.p.m. mg^{-1} protein; halothane: 2830 ± 218 d.p.m. mg^{-1} protein).

Table 2 Effect of tetrodotoxin, EGTA or Cd^{2+} on the release of acetylcholine induced by halothane in rat brain cortical slices

	[^{3}H]-ACh (d.p.m.$^{-1}$ of tissue)	
	Control	*Halothane* (0.063 mM)
Control	37.8 ± 2.4	95.1 ± 5.9*
Tetrodotoxin (0.5 μM)	32.5 ± 3.4	91.5 ± 4.8*
EGTA (2.0 mM)	38.8 ± 2.9	90.1 ± 6.8*
Cd^{2+} (100 μM)	39.1 ± 3.8	101.7 ± 8.1*

*Statistically different from the control value, $P<0.05$. Brain cortical slices (40 mg) loaded with [^{3}H]-choline were preincubated for 15 min in salt medium in the absence (control) or in the presence of tetrodotoxin (0.5 μM), EGTA (2.0 mM) or Cd^{2+} (100 μM) and then incubated for 5 min in the absence (control) or presence of halothane (0.063 mM). The experiments with EGTA were performed in a calcium free medium. Data are mean $\pm$ s.e.mean values from at least three different experiments performed in duplicate. For other details, see text.

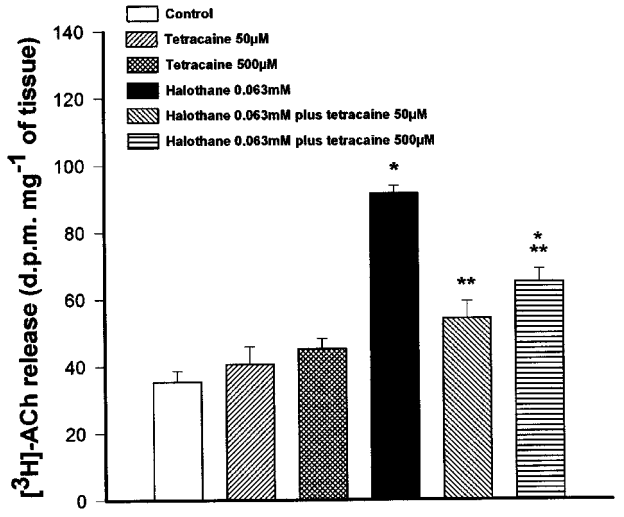

Figure 1 The effect of tetracaine on the release of [^{3}H]-ACh induced by halothane from rat brain cortical slices. Brain cortical slices (40 mg) loaded with [^{3}H]-choline were preincubated for 15 min in salt medium in the absence or in the presence of tetracaine (50 or 500 μM). They were then incubated for 5 min in the absence or presence of halothane (0.063 mM). Data are mean $\pm$ s.e.mean (bars) values from at least three different experiments performed in duplicate. For other details, see text. *Statistically different from the control value, $P<0.05$. **Statistically different from the halothane value, $P<0.05$.

Effect of intracellular calcium stores blockers on the release of [^{3}H]-ACh evoked by halothane

It has been demonstrated that volatile anaesthetics, including halothane, stimulate Ca^{2+} release or leakage from the intracellular stores of cardiac (Wheeler *et al*., 1988; Connelly & Coronado, 1994), skeletal (Palade, 1987; Nelson & Sweo, 1988) and vascular smooth muscle cells (Tsuchida *et al*., 1993) as well in synaptosomes (Daniell & Harris, 1988) and hippocampal slices (Mody *et al*., 1991). Figure 1 illustrates the effect of 50 and 500 μM tetracaine on [^{3}H]-ACh release induced by halothane. At these concentrations, it has been demonstrated that tetracaine blocks calcium release from intracellular stores (Ohnishi *et al*., 1979; Pike *et al*., 1989; Györke *et al*., 1997), and in our experiments decreased by 75.7 ± 4.0 and 64.4 ± 3.5%, respectively, the release of [^{3}H]-ACh evoked by halothane ($P<0.05$). There was also a trend for an increase in the rate of basal [^{3}H]-ACh release in the presence of tetracaine but it did not reach statistical significance.

We also investigated the effect of dantrolene (0.1 – 100 μM), a blocker of Ca^{2+} release from ryanodine-sensitive stores (Van Winkle, 1976; Morgan & Bryant, 1977; Ohta *et al*., 1990), on the release of [^{3}H]-ACh stimulated by halothane. Dantrolene did not affect the basal release of [^{3}H]-ACh (data not shown) but caused a dose dependent reduction in volatile anaesthetic evoked ACh release (Figure 2, $P<0.05$). The IC_{50} for dantrolene to inhibit [^{3}H]-ACh evoked release by halothane was close to 4.0 μM (Figure 2).

[^{3}H]-ACh exocytosis induced by halothane

To investigate the possibility that halothane increases the exocytotic release of [^{3}H]-ACh, vesamicol, a drug that blocks the storage of ACh in synaptic vesicles by inhibiting the vesicular ACh transporter (Parsons *et al*., 1993), was used. As

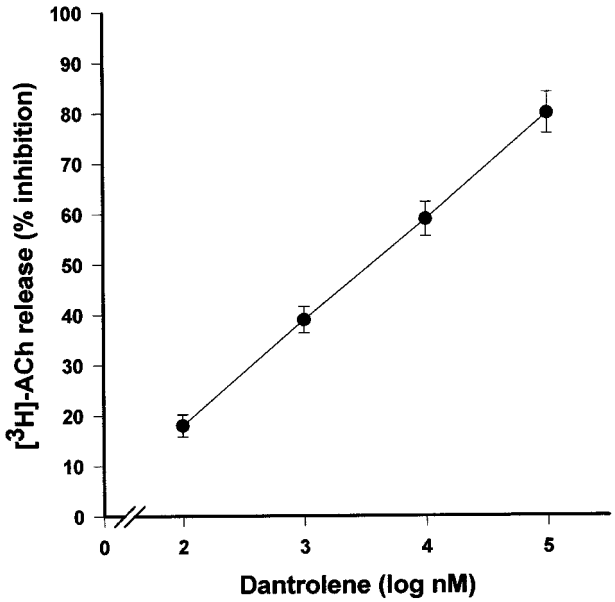

Figure 2 The effect of dantrolene concentration on the release of [^{3}H]-ACh induced by halothane from rat brain cortical slices. Brain cortical slices (40 mg) loaded with [^{3}H]-choline were preincubated for 15 min in salt medium in the presence of dantrolene at the indicated concentrations in abscissa. They were then incubated for 5 min in the presence of halothane (0.063 mM). Data are mean $\pm$ s.e.mean (bars) values from at least three different experiments performed in duplicate. For other details, see text.

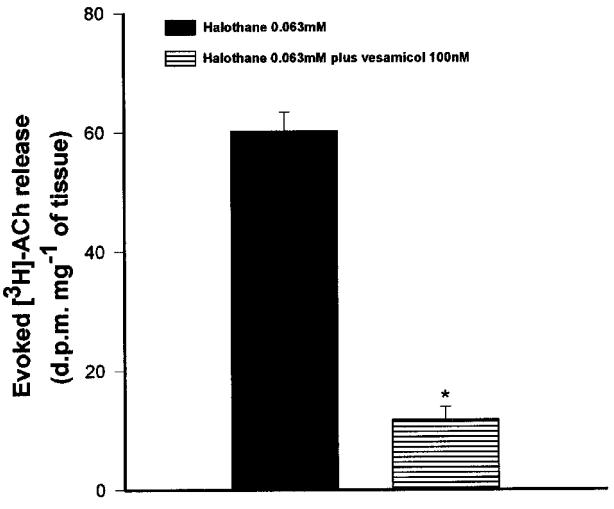

Figure 3 The effect of vesamicol on halothane-evoked [^{3}H]-ACh release from rat brain cortical slices. Brain cortical slices (40 mg) were first incubated for 10 min in the absence or presence of vesamicol (100 nM). They were then loaded with [^{3}H]-choline and after that incubated for 5 min in the absence or presence of halothane (0.063 mM). Data are shown as release of [^{3}H]-ACh evoked by halothane (total release minus basal in the absence or in the presence of vesamicol). Data are mean$\pm$s.e.mean (bars) values from at least three different experiments performed in duplicate. For other details, see text. *Statistically different from the halothane value, $P<0.05$.

a consequence of vesamicol action, the vesicular release of ACh is suppressed. Vesamicol decreases basal release of ACh (Ricny & Collier, 1986), and in our experiments we observed a similar effect of 50% inhibition of basal [^{3}H]-ACh release. Thus the data in Figure 3 is shown as total release of [^{3}H]-ACh induced by halothane in the absence (filled bar) or in the presence of vesamicol (hatched bar) minus the value of basal release obtained in the absence or in the presence of the vesicular blocker, respectively. Figure 3 shows that vesamicol (100 nM) decreased (80.4$\pm$4.9%) the evoked [^{3}H]-ACh release induced by halothane. Greater concentrations of vesamicol (up to 1.0 μM) did not produce larger inhibition of the release of [^{3}H]-ACh evoked by the anaesthetic (data not shown).

Discussion

It is not completely clear how volatile anaesthetics cause anaesthesia, but one possible consequence of their action is to alter presynaptic activity and the release of neurotransmitters. Indeed, halothane has been shown to inhibit choline uptake into rat brain synaptosomes (Johnson & Hartzell, 1985; Griffiths & Norman, 1993; Griffiths *et al*., 1994). We have studied here the effect of halothane on the release of [^{3}H]-ACh as an experimental model to determine how this volatile anaesthetic could affect neuronal secretion. To avoid any effect of halothane on choline uptake, the terminals were first loaded with radiolabelled choline in the absence of halothane and the release of [^{3}H]-ACh in the presence of this agent was determined.

There are few studies of the effect of volatile general anaesthetics on the release of ACh. In some of these studies, halothane was shown to decrease potassium-evoked ACh release from rat cortical synaptosomes (Johnson & Hartzell, 1985) and rat cortical slices (Griffiths *et al*., 1995). In contrast, no effect on potassium-stimulated ACh release was observed in rat cerebral cortex in the presence of 1.25% halothane, 3% enflurane or 0.2% methoxyflurane (Bazil & Minneman, 1989a, b). To our knowledge, all published studies investigated the effect of halothane in the CNS using only KCl depolarization, but did not investigate this effect following a more physiological and relevant stimulation, such as electrical field stimulation.

We observed that 0.018 mM halothane had no effect on the basal release of [^{3}H]-ACh (Table 1). At this concentration, the evoked release of [^{3}H]-ACh by electrical field stimulation was also not affected. In contrast, 0.063 mM halothane significantly increased the basal release of [^{3}H]-ACh. Interestingly, the association of field stimulation and 0.063 mM halothane caused additive release of [^{3}H]-ACh which result was not seen with 0.018 mM halothane. Both effects remained when higher concentrations of halothane (0.117 and 0.235 mM, Table 1) were used. In the cat sympathetic ganglion, Bosnjak *et al*. (1988) showed a decreased ACh release induced by electrical stimulation using higher concentration of halothane than those used in our experiments (0.28 and 0.59 mM). Although this apparent discrepancy could be explained by the halothane dose used, tissue and methodological differences should also be taken into account. Moreover, it is worth noting that, as in the present study, Griffiths *et al*. (1995) reported a trend to an increase in the release of ACh in brain cortical slices from unstimulated samples at all concentrations of halothane tested (0.037–1.19 mM).

The release of ACh evoked by electrical field stimulation is Na^+ and Ca^{2+} dependent and is inhibited by tetrodotoxin and the absence of calcium (Nakashima *et al*., 1990; Yokoyama *et al*., 1990). In our experiments, we demonstrated that EGTA, Cd^{2+} or tetrodotoxin did not significantly affect basal or halothane-induced ACh release. Moreover, halothane did not increase $^{45}Ca^{2+}$ influx in synaptosomes of rat brain cortical slices. Thus, the release of [^{3}H]-ACh in brain cortical slices induced by halothane is independent of the presence of extracellular Na^+ or Ca^{2+} and did not involve the entry of Ca^{2+} by calcium channels. Altogether, these results suggest the existence of different mechanisms for the release of [^{3}H]-ACh induced by halothane and electrical field stimulation from rat brain cortical slices.

Cellular activity is dependent on cytosolic free calcium concentration and intracellular Ca^{2+} stores have an important role in this process. The effect of tetracaine on the release of [^{3}H]-ACh evoked by halothane is consistent with the concept of calcium release from internal stores induced by halothane in cholinergic neurons, as previously described in synaptosomes (Daniell & Harris, 1988), hippocampal slices (Mody *et al*., 1991) and in clonal pituitary cells (GH_3) (Hossain & Evers, 1994). The greater inhibition of tetracaine at 50 μM compared with 500 μM on halothane evoked [^{3}H]-ACh release could be explained by a dual effect of this drug on the calcium intracellular stores. Therefore, higher concentrations of tetracaine (above 1.0 mM) caused a gradual increase in intracellular store calcium load and subsequent activation of the Ca^{2+}-release channels by Ca^{2+} inside the internal stores (Pike *et al*., 1989; Györke *et al*., 1997). Indeed, we observed that tetracaine (1.0 and 2.0 mM) did not significantly affect the [^{3}H]-ACh release evoked by halothane (data not shown).

There is evidence indicating the presence of intracellular calcium stores in neurons that are sensitive to ryanodine receptor ligands such as caffeine and ryanodine (Miller, 1991; McPherson & Campbell, 1993). Indeed, our results showing a significant inhibition of halothane induced release of [^{3}H]-ACh by dantrolene suggests the involvement of ryanodine-sensitive stores in cholinergic neurons that are able to supply calcium for transmitter release.

It has long been known that the release of neurotransmitters requires the presence of extracellular Ca^{2+}. According to the calcium hypothesis, the synaptic release of neurotransmitters occurs through an exocytotic process triggered by Ca^{2+} inflow associated with presynaptic depolarization (Katz & Miledi, 1967). However, in recent years, evidence demonstrating transmitter release in the absence of external Ca^{2+} has been reported (reviewed by Adam-Vizi, 1992). Despite the large amount of contradictory data on this issue, one possibility is that neurotransmitter release under certain circumstances might be entirely independent of external Ca^{2+}, but triggered by the efflux of Ca^{2+} from internal stores (mitochondria, endoplasmic reticulum, etc.). Our results with EGTA, Cd^{2+}, tetracaine and dantrolene support this possibility. With respect to release in Ca^{2+}-free medium, most of the data in the literature suggests that transmitter release under these conditions occurs following efflux of transmitters from cytoplasmic sources. However, our experimental data with vesamicol, a compound known to inhibit the ACh transporter of cholinergic vesicles, suggests that most of the release of [^{3}H]-ACh induced by halothane from brain cortical slices occurs from an occluded tissue compartment, that uses the vesicular ACh transporter to accumulate ACh. The present results are thus distinct from the observation that extracellular Ca^{2+}-independent release does not require the vesicular ACh pool (Adam-Vizi *et al*., 1991; Adam-Vizi, 1992).

In conclusion, this study indicates that halothane may alter intracellular calcium homeostasis in cholinergic neurons, which effect could account for an extracellular Ca^{2+}-independent exocytotic release of ACh from rat brain cortical slices. Further investigations are necessary to identify the target by which halothane induces the release of ACh from cholinergic neurons.

We thank D.M. Miranda for her help with $^{45}Ca^{2+}$ influx experiments. We also thank Adriane A. Pereira, Andreia A. Guimarães and Antonio C.S. Gomes for technical assistance and Dr L.A. de Marco for reading and suggestions in this manuscript. Supported by FAPEMIG, CNPq, PADCT, FINEP, PRPq-UFMG and PRONEX.

References

ADAM-VIZI, V. (1992). External Ca^{2+}-independent release of neurotransmitters. *J. Neurochem.,* **58,** 395–405.

ADAM-VIZI, V., DERI, Z., VIZI, E.S., SERSHEN, H. & LAJTHA, A. (1991). Ca^{2+}-independent veratridine-evoked acetylcholine release from striatal slices is not inhibited by vesamicol (AH5183): mobilization of distinct transmitter pools. *J. Neurochem.,* **56,** 52–58.

BAZIL, C.W. & MINNEMAN, K.P. (1989a). Effects of clinically effective concentrations of halothane on adrenergic and cholinergic synapses in rat brain in vitro. *J. Pharmacol. Exp. Ther.,* **248,** 143–148.

BAZIL, C.W. & MINNEMAN, K.P. (1989b). Clinical concentrations of volatile anesthetics reduce depolarization-evoked release of [^{3}H]norepinephrine, but not [^{3}H]acetylcholine, from rat cerebral cortex. *J. Neurochem.,* **53,** 962–965.

BOSNJAK, Z.J., DUJIC, Z., ROERIG, D.L. & KAMPINE, J.P. (1988). Effects of halothane on acetylcholine release and sympathetic ganglionic transmission. *Anesthesiology,* **69,** 500–506.

CASALI, T.A.A., GOMEZ, R.S., MORAES-SANTOS, T., ROMANO-SILVA, M.A., PRADO, M.A.M. & GOMEZ, M.V. (1997). Different effects of reducing agents on ω-conotoxin GVIA inhibition of [^{3}H]-acetylcholine release from cortical slices and guinea-pig myenteric plexus. *Br. J. Pharmacol.,* **120,** 88–92.

COLLIER, B. & MITCHELL, J.F. (1967). Release of ACh during consciousness and after brain lesions. *J. Physiol. (Lond),* **188,** 83–88.

CONNELLY, T.J. & CORONADO, R. (1994). Activation of the Ca^{2+} release channel of cardiac sarcoplasmic reticulum by volatile anesthetics. *Anesthesiology,* **81,** 459–469.

DANIELL, L.C. & HARRIS, R.A. (1988). Neuronal intracellular calcium concentrations are altered by anesthetics: Relationship to membrane fluidization. *J. Pharmacol. Exp. Ther.,* **245,** 1–7.

FOX, A.P., NOWYCKY, M.C. & TSIEN, R.W. (1987). Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurons. *J. Physiol. (Lond),* **394,** 149–172.

GOLDBERG, A.M. & MCCAMAN, R.E. (1973). The determination of picomole amounts of acetylcholine in mammalian brain. *J. Neurochem.,* **20,** 1–8.

GOMEZ, R.S., GOMEZ, M.V. & PRADO, M.A.M. (1996). Inhibition of Na^+,K^+-ATPase by ouabain opens calcium channels coupled to acetylcholine release in guinea pig myenteric plexus. *J. Neurochem.,* **66,** 1440–1447.

GRIFFITHS, R., GREIFF, J.M.C., BOYLE, E., ROWBOTHAM, D.J. & NORMAN, R.I. (1994). Volatile anesthetics inhibit choline uptake into rat synaptosomes. *Anesthesiology,* **81,** 953–958.

GRIFFITHS, R., GREIFF, J.M.C., HAYCOCK, J., ELTON, C.D., ROWBOTHAM, D.J. & NORMAN, R.I. (1995). Inhibition by halothane of potassium-stimulated acetylcholine release from rat cortical slices. *Br. J. Pharmacol.,* **116,** 2310–2314.

GRIFFITHS, R. & NORMAN, R.I. (1993). Effects of anaesthetics on uptake, synthesis and release of transmitters. *Br. J. Anaesth.,* **71,** 96–107.

GYÖRKE, S., LUKYANENKO, V. & GYÖRKE, I. (1997). Dual effects of tetracaine on spontaneous calcium release in rat ventricular myocytes. *J. Physiol. (Lond),* **500,** 297–309.

HOSSAIN, M.D. & EVERS, A.S. (1994). Volatile anesthetic-induced efflux of calcium from IP_3-gated stores in clonal (GH_3) pituitary cells. *Anesthesiology,* **80,** 1379–1389.

JOHNSON, G.V.W. & HARTZELL, C.R. (1985). Choline uptake, acetylcholine synthesis and release, and halothane effects in synaptosomes. *Anesth. Analg.,* **64,** 395–399.

KANAI, T. & SZERB, J.C. (1965). Mesencephalic reticular activating system and cortical acetylcholine output. *Nature,* **205,** 80–82.

KATZ, B. & MILEDI, R. (1967). The timing of calcium action during neuromuscular transmission. *J. Physiol. (Lond).,* **189,** 533–544.

KEIFER, J.C., BAGHDOYAN, H.A., BECKER, I. & LYDIC, R. (1994). Halothane anaesthesia causes decreased acetylcholine release in the pontine reticular formation and increased EEG spindles. *Neuroreport,* **5,** 577–580.

LARRABEE, M.G. & POSTERNAK, J.M. (1952). Selective action of anesthetic on synapses and axons in mammalian sympathetic ganglia. *J. Neurophysiol.,* **15,** 92–114.

LEÃO, R.M., GOMEZ, M.V., COLLIER, B. & PRADO, M.A.M. (1995). Inhibition of potassium-stimulated acetylcholine release from rat brain cortical sliced by two high-affinity analogues of vesamicol. *Brain Res.,* **703,** 86–92.

MCPHERSON, P.S. & CAMPBELL, K.P. (1993). The Ryanodine Receptor/Ca^{2+} release channel. *J. Biol. Chem.,* **268,** 13765–13768.

MILLER, R.J. (1991). The control of neuronal Ca^{2+} homeostasis. *Prog. Neurobiol.,* **37,** 255–285.

MIRANDA, D.M., ROMANO-SILVA, M.A., KALAPOTHAKIS, E., DINIZ, C.R., CORDEIRO, M.N., MORAES-SANTOS, T., PRADO, M.A.M. & GOMEZ, M.V. (1998). *Phoneutria nigriventer* toxins block tityustoxin -induced calcium influx in synaptosomes. *Neuroreport,* **9,** 1371–1373.

MITCHELL, J.F. (1963). The spontaneous and evoked release of acetylcholine from the cerebral cortex. *J. Physiol. (Lond),* **165,** 98–116.

MODY, I., TANELIAN, D.L. & MACIVER, M.B. (1991). Halothane enhances tonic neuronal inhibition by elevating intracellular calcium. *Brain Res.,* **538,** 319–323.

MORGAN, K.C. & BRYANT, S.H. (1977). The mechanism of action of dantrolene sodium. *J. Pharmacol. Exp. Ther.,* **201,** 138–147.

NAKASHIMA, Y., SUGIYAMA, S., SHINDOH, J., TAKI, F., TAKAGI, K., SATAKE, T. & OZAWA, T. (1990). Effects of sodium channel blockers on electrical field stimulation-induced guinea-pig tracheal smooth muscle contraction. *Arch. Int. Pharmacodyn. Ther.,* **306,** 130–138.

NARAHASHI, T., MOORE, J. & SCOTT, W.R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axon. *J. Gen. Physiol.,* **147,** 965 – 974.

NELSON, T.E. & SWEO, T. (1988). Ca^{2+} uptake and Ca^{2+} release by skeletal muscle sarcoplasmic reticulum. *Anesthesiology,* **69,** 571 – 577.

OHNISHI, S.T. (1979). Calcium-induced calcium release from fragmented sarcoplasmic reticulum. *J. Biochem.,* **86,** 1147 – 1150.

OHTA, T., ITO, S. & OHGA, A. (1990). Inhibitory action of dantrolene on Ca-induced Ca^{2+} release from sarcoplasmic reticulum in guinea pig skeletal muscle. *Eur. J. Pharmacol.,* **178,** 11 – 19.

PALADE, P. (1987). Drug-induced Ca^{2+} release from isolated sarcoplasmic reticulum. II. Releases involving a Ca^{2+}-induced Ca^{2+} release channel. *J. Biol. Chem.,* **262,** 6141 – 6148.

PARSONS, S.M., PRIOR, C. & MARSHALL, I.G. (1993). Acetylcholine transport, storage, and release. *Int. Rev. Neurobiol.,* **35,** 279 – 390.

PIKE, G.K., ABRAMSON, J.J. & SALAMA, G. (1989). Effects of tetracaine and procaine on skinned muscle fibres depend on free calcium. *J. Musc. Res. Cell. Motil.,* **10,** 337 – 349.

PRADO, M.A.M., GOMEZ, M.V. & COLLIER, B. (1993). Mobilization of a vesamicol-insensitive pool of acetylcholine from a sympathetic ganglion by ouabain. *J. Neurochem.,* **61,** 45 – 56.

RICHARDS, C.D. (1983). Actions of general anaesthetics on synaptic transmission in the CNS. *Br. J. Anaesth.,* **55,** 201 – 207.

RICNY, J. & COLLIER, B. (1986). Effect of 2-(4-phenylpiperidino)cyclohexanol on acetylcholine release and subcellular distribution in rat striatal slices. *J. Neurochem.,* **47,** 1627 – 1633.

RUTLEDGE, C.O., SEIFEN, E., ALPER, M.H. & FLACKE, W. (1963). Analysis of halothane in gas and blood by gas chromatography. *Anesthesiology,* **24,** 862 – 867.

TSUCHIDA, H., NAMBA, H., YAMAKAGE, M., FUJITA, S., NOTSUKI, E. & NAMIKI, A. (1993). Effects of halothane and isoflurane on cytosolic calcium ion concentrations and contraction in the vascular smooth muscle of the rat aorta. *Anesthesiology,* **78,** 531 – 540.

VAN WINKLE, W.B. (1976). Calcium release from skeletal muscle sarcoplasmic reticulum; Site of action of dantrolene sodium? *Science,* **193,** 1130 – 1131.

WHEELER, D.M., RICE, R.T., HANSFORD, R.G. & LAKATTA, E.G. (1988). The effect of halothane on the free intracellular calcium concentration of isolated rat heart cells. *Anesthesiology,* **69,** 578 – 583.

WINKLER, J., SUHR, S.T., GAGE, F.H., THAL, L.J. & FISHER, L.J. (1995). Essential role of neocortical acetylcholine in spatial memory. *Nature,* **375,** 484 – 487.

YOKOYAMA, K. & YAGASAKI, O. (1990). Effects of Ca^{2+} blockers on the various types of stimuli-induced acetylcholine release from guinea-pig ileum myenteric plexus. *Jpn. J. Pharmacol.,* **52,** 109 – 114.

(Received October 10, 1998
Revised March 8, 1999
Accepted March 17, 1999)