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Effect of Anesthetics on Calcium Stores and Membrane Order of Brain Microsomes

LAURA C. DANIELL[1] and R. ADRON HARRIS

Department of Pharmacology (L.C.D., R.A.H.) and Veterans Administration Medical Center and Alcohol Research Center (R.A.H.), University of Colorado Health Sciences Center, Denver, Colorado 80262

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SUMMARY

The effects of anesthetic agents from different chemical classes and a nonanesthetic membrane-disordering agent, 2-[2-methoxyethoxy]ethyl-8-[*cis*-2-*n*-octylcyclopropyl]octanoate (A_2C), on calcium stores of whole brain microsomes and on order of microsomal membranes were compared. Calcium release was determined by measurement of the extramicrosomal calcium concentration and membrane order by the fluorescence polarization of diphenylhexatriene (membrane core) and trimethylammonium-diphenylhexatriene (membrane "surface"). *n*-Alkanols (methanol, ethanol, propanol, butanol, pentanol, and hexanol), benzyl alcohol (10–100 mM), and diethyl ether (30–300 mM) released calcium from brain microsomes and decreased the surface and interior membrane order of microsomal membranes. Pentobarbital (0.05–1 mM) did not release calcium from microsomes and did not alter the order of brain microsomal membranes. Halogenated anesthetics (halothane, methoxyflurane, and enflurane), 4-phenyl-1-butanol, and A_2C decreased membrane order but failed to release calcium from brain microsomes. Comparison of the effects of these agents on microsomal calcium release and order of microsomal membranes revealed that decreases in membrane order are unrelated to the calcium-mobilizing actions of anesthetic compounds. In addition, molecular size appeared to limit ability of anesthetic compounds to release calcium from microsomes. For *n*-alkanols, benzyl alcohol, and diethyl ether, the ability to release microsomal calcium was correlated with anesthetic potency. Our results demonstrate, for the first time, direct effects of anesthetic agents on intracellular calcium stores of brain tissue and indicate that these stores may be target sites for anesthetics.

Krnjevic (1) first proposed that general anesthesia is mediated by a mechanism dependent on intracellular calcium. He suggested that, following anesthetic-induced anoxia, mitochondrial calcium is released, resulting in an increase in Ca_i and subsequent calcium-mediated cellular hyperpolarization. More recently, Nicoll and Madison (2) and Carlen *et al.* (3) showed that anesthetics increase a potassium conductance, resulting in cellular hyperpolarization in central mammalian neurons. Because this effect is independent of extracellular calcium, Carlen *et al.* (3) suggested that the ethanol-stimulated calcium-dependent potassium conductance is activated by an increase in Ca_i derived from intracellular sources. Anesthetics also increased calcium-dependent Rb flux in brain synaptosomes and erythrocytes (4, 5). Under resting conditions, ethanol increases neurotransmitter output (6–8), providing suggestive evidence for anesthetic-induced increases in resting Ca_i. The idea that increased Ca_i is important in anesthesia is also supported by previous work that showed that intracerebroventricular injection of calcium increases the duration of ethanol-induced anesthesia (loss of righting reflex) (9).

Our recent work shows that anesthetics from several different chemical classes increase resting Ca_i in brain synaptosomes (10), supporting the idea that anesthetic-induced neuronal hyperpolarization may be due to activation of a calcium-stimulated potassium conductance. We also found that the anesthetic-induced increase in resting Ca_i is correlated with increased synaptosomal membrane fluidity, indicating that brain membranes may be a site of action for anesthetic-induced changes in intracellular calcium homeostasis.

Our previous work with ethanol indicated that the increase in resting Ca_i produced by ethanol in synaptosomes is due to release of calcium from an intracellular site rather than increased permeability of the plasma membrane to calcium (11). The ER or a related structure (12) releases calcium in response

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[1] Present address: Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

ABBREVIATIONS: Ca_i, intracellular ionized calcium concentrations; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; ER, endoplasmic reticulum; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, trimethylammonium-1,6-diphenyl-1,3,5-hexatriene; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; A_2C, 2-[2-methoxyethoxyl]ethyl 8-[*cis*-2-*n*-octylcyclopropy]octanoate.

to several endogenous ligands, notably inositol 1,4,5-trisphosphate (13) and GTP (14), and contains the largest quantity of sequestered calcium in neuronal tissue, compared with other cellular organelles (15). We recently showed that ethanol releases calcium from brain microsomes (a subcellular fraction enriched in vesicles derived from ER) (16), indicating that this could be a site of action for other anesthetic compounds.

In this study, we examined the effects of anesthetics from different chemical classes on calcium stores of microsomes to determine whether anesthetic-mediated increases in resting Ca_i are due to release of sequestered calcium from ER-derived vesicles. We also examined anesthetic-mediated alterations in membrane order, to determine to what extent alterations in microsomal membrane order are related to the effects of anesthetics on intracellular release of calcium.

Experimental Procedures

Materials. Male ICR mice (Harlan; 60 to 100 days old) were used in all experiments. A_2C (Sigma Chemical Co.) and mitochondrial inhibitors (oligomycin, rotenone, and antimycin) (Sigma) were dissolved in DMSO. Ethanol and pentobarbital were dissolved in H_2O. All other drugs were used without dilution. Indo-1, DPH, and TMA-DPH were obtained from Molecular Probes. Indo-1 was dissolved in H_2O. DPH was dissolved in THF and TMA-DPH in THF/H_2O, 1:1.

Preparation of microsomes. Brain microsomes were prepared from male ICR mice. Whole brains were homogenized in ice-cold sucrose-HEPES (320 mM sucrose, 3 mM HEPES, 0.1 mM EDTA, pH adjusted to 7.4 with Tris base) at 1000 rpm in a Teflon to glass homogenizing tube. The homogenate was centrifuged at 1800 × *g* for 6 min and the supernatant was saved. The pellet was resuspended by hand homogenizing in ice-cold sucrose-HEPES and centrifuged at 1000 × *g* for 6 min. The two supernatants were combined and centrifuged at 17,000 × *g* for 20 min. The microsomal supernatant was centrifuged at 100,000 × *g* for 25 min. The microsomal pellet was resuspended in assay buffer (150 mM KCl, 3 mM $MgCl_2$, 20 mM HEPES, 0.5 mM sodium azide, pH adjusted to 7.0 with Tris base). In most experiments, microsomes were frozen immediately after preparation and thawed immediately before use. Freezing (for up to 4 weeks) and thawing did not alter drug responses. Protein concentrations were determined by the method of Lowry *et al.* (17).

Determination of calcium release from microsomes. Calcium release was quantitated by measurement of the extramicrosomal calcium concentration using indo-1, a fluorescent calcium indicator. An H&L series 300 HH-3 spectrofluorometer with continuous data acquisition was used to monitor emission of indo-1 at 410 and 500 nm (Microcoatings bandpass filters), at an excitation wavelength of 345 nm. The fluorescence of indo-1 was quantitated by measurement of R_{min} and R_{max} (ratio of total fluorescence at 410 nm to that at 500 nm for the calcium-unbound and calcium-bound forms of indo-1, respectively), by determination in the presence of 2 mM EGTA and a free calcium concentration of approximately 2.7 mM, respectively, for each experiment. The extramicrosomal calcium concentration was calculated using a K_D of 250 nM, according to the method of Grynkiewicz *et al.* (18).

Microsomes (0.15 to 0.25 mg of protein/ml final concentration) were suspended in warm (35°) assay buffer with mitochondrial inhibitors [antimycin (0.06 μg/ml), oligomycin (2 μg/ml), and rotenone (1 μM)] and indo-1 (final concentration, 0.45 μM). Mitochondrial inhibitors were included to limit mitochondrial viability but were without effect on responses to ethanol. In addition, marker enzyme activity determinations revealed that mitochondria were not a significant contaminant of the microsomal preparation (16). Each sample was incubated at 32 ± 0.4° for 2.5 min before addition of drugs, except in the case of halothane, methoxyflurane, enflurane, and A_2C. These drugs were incubated with microsomes at 32° for 10 min before measurement of calcium release from microsomes, to increase drug incorporation. Drugs were added as 0.03 to 1.2% of total final sample volume. Volatile anesthetics (halothane, methoxyflurane, enflurane, diethyl ether, and 4-phenyl-1-butanol) were added to microsomes in Inject-a-cell cuvets (Spectrocell, Inc.) to prevent evaporative loss of drug. Calcium release was determined by changes in the extramicrosomal calcium concentration and was expressed as nmol of calcium released/mg of protein.

Determination of the fluorescence polarization of DPH and TMA-DPH. Fluorescence polarization of DPH and TMA-DPH was measured using an HH-3 T-format spectrofluorometer with KV389 emission filters (Schott Optical, Duryea, PA) and a 03FCG001 excitation filter (Melles Griot, Irvine, CA), at excitation wavelengths of 361 and 364 nm for DPH and TMA-DPH, respectively. The probes were incorporated into microsomal membranes by addition of 0.25 mg of the probe/ml of microsomal suspension (0.15 to 0.25 mg of protein/ml) with vortexing and incubation in a shaking water bath at 35° for 15 min. After baseline polarization was measured, drugs were added in sequential increments and readings were taken immediately (<45 sec) after addition. All readings were made with sample temperature at 32 ± 0.4°. Drug-induced changes in fluorescence polarization were calculated by subtraction of values measured in the presence of drug from values measured in the absence of added drug. Previous work has shown that ethanol and barbiturates do not alter the fluorescent lifetime of DPH, indicating that drug-induced changes in the polarization of the probe are due to changes in probe mobility (19–21).

Determination of behavioral effect of 4-phenyl-1-butanol. Mice were injected intraperitoneally with DMSO (controls) or 4-phenyl-1-butanol dissolved in DMSO. The mice were observed for 20 min for qualitative determination of effects of 4-phenyl-1-butanol, including ataxia and anesthesia (defined as loss of righting reflex).

Results

The effects of addition of a 100 mM concentration of ethanol, 1-propanol, and 1-butanol on microsomal calcium stores are shown in Fig. 1. Microsomal calcium release induced by these agents was maximal within 10 sec after addition (first measurement interval) and did not increase thereafter. Other anesthetic agents (discussed below) that released calcium from microsomes also produced maximal effects at the first measurement interval (10 or 30 sec) (data not shown).

n-Alkanols (methanol, ethanol, propanol, butanol, pentanol, and hexanol) released calcium from brain microsomes in a concentration-dependent manner (Fig. 2A). The order of po-

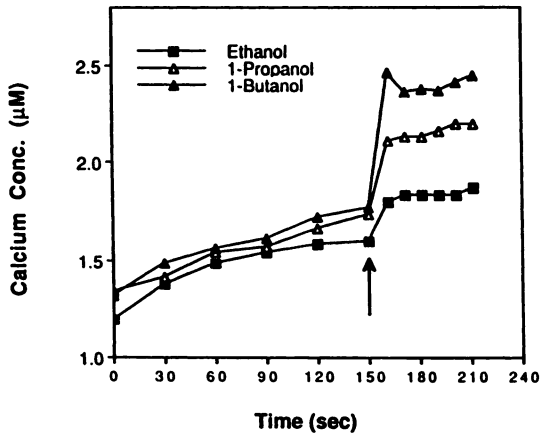

Fig. 1. Effect of ethanol, 1-propanol, and 1-butanol on calcium stores of brain microsomes. The extramicrosomal calcium concentration was measured using indo-1 at 30-sec intervals before and 10-sec intervals after addition of a 100 mM concentration of each alcohol. Data are representative of two experiments.

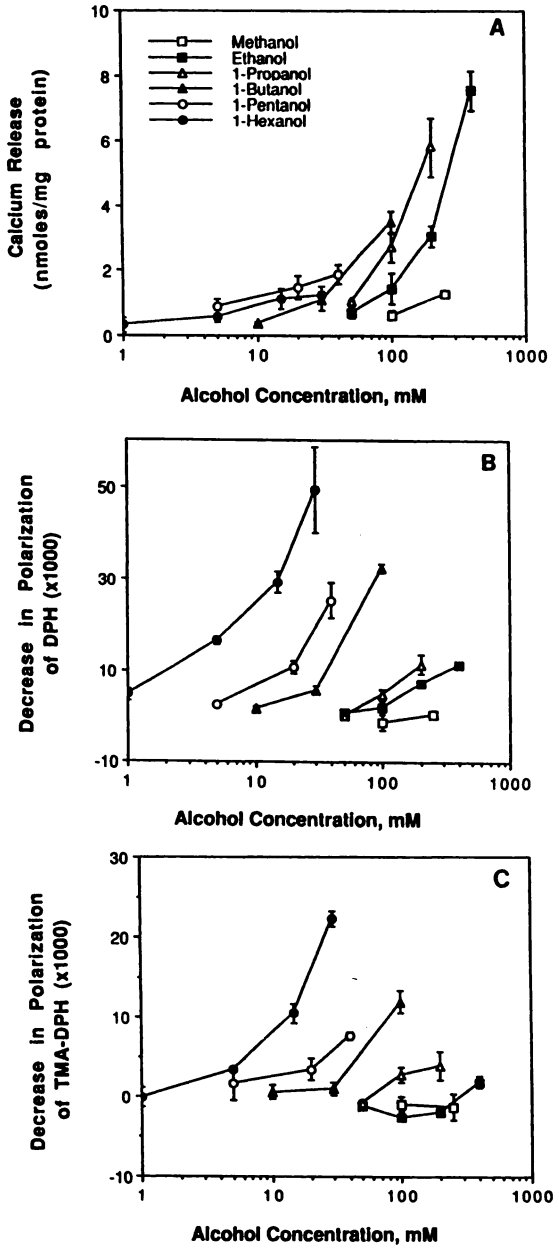

Fig. 2. Effect of *n*-alkanols on calcium stores and membrane order of brain microsomes. Calcium release and fluorescence polarization of DPH or TMA-DPH were measured 30 sec after addition of various concentrations of each alcohol. Data are the means ± standard errors of three to six experiments.

tency for release of microsomal calcium increased as follows: methanol < ethanol < propanol < butanol < pentanol = hexanol. Anesthetic concentrations of 1-alkanols are: methanol, 360 mM;[2] ethanol, 120 mM; 1-propanol, 54 mM; 1-butanol, 12 mM; 1-pentanol, 4 mM;[3] and 1-hexanol, 0.7 mM (22). [All anesthetic concentrations cited are those required to produce nonresponsiveness (lack of muscular movement in response to environmental stimulation) in 100% of subjects and reflect blood concentrations or concentrations applied to the bath for tadpole data.] Each of the *n*-alkanols released calcium from brain microsomes at concentrations within (methanol, ethanol, propanol, and butanol) or slightly higher (pentanol and hexanol) than the anesthetic concentration. The concentration dependence for microsomal calcium release produced by methanol, ethanol, *n*-propanol, and *n*-butanol appeared to be linear (Fig. 2A).

DPH is a lipophilic probe that is incorporated into the interior of membranes. Depolarization of DPH fluorescence is due to a decrease in order of the membrane interior (23). TMA-DPH is a charged derivative of DPH and is not membrane permeable. Decreases in the fluorescence polarization of TMA-DPH are due to effects on the upper region of the membrane and primarily reflect decreased order in the glycerol and upper acyl regions (23). The ability of *n*-alkanols to decrease polarization of DPH increased in the order methanol < ethanol < propanol < butanol < pentanol < hexanol (Fig. 2B). The *n*-alkanols decreased the polarization of TMA-DPH to a lesser extent than DPH but had a qualitatively similar effect, with approximately the same order of potency (Fig. 2C).

The anesthetic concentration of benzyl alcohol is approximately 2 mM (24). A supraanesthetic concentration of benzyl alcohol (10 mM) released a small amount of calcium but larger concentrations (30, 60, and 100 mM) released a larger amount of calcium than similar concentrations of all of the *n*-alkanols tested (Table 1). The largest concentration tested (100 mM) released 12.58 ± 1.89 nmol of calcium/mg of protein (mean ± SE, three experiments). The polarization of DPH was reduced by benzyl alcohol to a greater extent than by similar concentrations of *n*-alkanols, except for *n*-hexanol (Table 1, Fig. 2B). Benzyl alcohol also reduced the polarization of TMA-DPH (Table 1).

A structural analog of benzyl alcohol with three additional methylene groups, 4-phenyl-1-butanol, failed to release calcium from brain microsomes (Table 1). 4-Phenyl-1-butanol produced a concentration-dependent reduction in the polarization of DPH and TMA-DPH (Table 1). A 3 mM concentration of 4-phenyl-1-butanol produced substantial decreases in the fluorescence polarization of DPH and TMA-DPH, approximately equivalent to the decrease in polarization of DPH and TMA-DPH produced by 100 mM *n*-butanol (Table 1, Fig. 2, B and C). The effect of 4-phenyl-1-butanol *in vivo* was determined in ICR mice. The threshold anesthetic dose was approximately 1 mmol/kg (0.15 g/kg) intraperitoneally and the lethal dose was approximately 3 mmol/kg (0.45 g/kg) intraperitoneally ($n = 5$) (data not shown). Blood and tissue concentrations of 4-phenyl-1-butanol were not determined. The anesthetic effects of 4-phenyl-1-butanol were short-lasting (diminishing within 5 to 15 min after intraperitoneal administration), possibly due to rapid redistribution of the drug (data not shown).

Anesthetic concentrations of diethyl ether range from 10 to 50 mM (25). Diethyl ether released calcium from brain microsomes with a threshold of approximately 30 mM (Table 1). The concentration response for diethyl ether-induced calcium release was approximately linear and nonsaturable up to 300 mM. Diethyl ether decreased the polarization of DPH and TMA-DPH in a concentration-dependent manner (Table 1).

Anesthetic concentrations of pentobarbital range from 0.05 to 0.20 mM (26). Pentobarbital did not release calcium from brain microsomes at any of the concentrations tested (0.05 to 1.0 mM) (Table 1). Pentobarbital also failed to alter the fluo-

[2] Estimated as three times the anesthetic concentration of ethanol given by Pringle *et al.* (22).

[3] Estimated as one third of the anesthetic concentration of *n*-butanol given by Pringle *et al.* (22).

TABLE 1

Comparison of effects of anesthetic and nonanesthetic agents on microsomal calcium stores and microsomal membrane order

Effects of agents were measured 30 sec (diethyl ether, benzyl alcohol, 4-phenyl-1-butanol, and pentobarbital) or 10 min (all others) after drug addition. Values are the means ± standard errors of three to five experiments.

Agent	Concentration	Calcium release	Decrease in Polarization (×1000)	
			DPH	TMA-DPH
	mM	nmol/mg of protein		
Benzyl alcohol	10	0.28 ± 0.01	8.8 ± 2.9	2.9 ± 0.9
	30	3.62 ± 0.56	24.5 ± 3.6	9.6 ± 1.7
	60	5.53 ± 0.45	46.8 ± 1.8	16.6 ± 1.4
	100	12.58 ± 1.89	73.3 ± 1.4	30.8 ± 2.5
Diethyl ether	10	0.08 ± 0.33	10.4 ± 2.3	0.7 ± 2.6
	30	0.43 ± 0.25	25.0 ± 4.4	3.6 ± 0.2
	100	1.73 ± 0.49	50.8 ± 5.1	12.9 ± 1.8
	300	6.77 ± 0.88	102.0 ± 22.1	46.1 ± 5.2
4-Phenyl-1-butanol	1	−0.16 ± 0.38	6.7 ± 1.3	5.0 ± 1.4
	3	−0.35 ± 1.17	32.3 ± 6.1	10.2 ± 3.7
	10	ND[a]	86.7 ± 12.9	18.4 ± 1.4
	30	ND	159.6 ± 9.6	55.8 ± 1.8
Pentobarbital	0.05	−0.17 ± 0.18	−1.3 ± 1.4	−2.3 ± 2.0
	0.25	−0.34 ± 0.18	−1.1 ± 1.3	−2.6 ± 1.6
	0.50	−0.61 ± 0.58	−0.2 ± 1.0	−1.7 ± 1.9
	1.00	0.29 ± 1.70	1.9 ± 1.0	1.9 ± 1.8
Halothane	10	−0.83 ± 0.34	ND	ND
Methoxyflurane	10	0.04 ± 1.07	31.7 ± 7.2	12.3 ± 1.0
Enflurane	10	0.20 ± 0.82	26.6 ± 5.7	11.5 ± 1.0
A_2C	1 μmol/mg of protein	−0.03 ± 0.29	52.7 ± 8.8	7.7 ± 0.9

[a] ND, not determined.

rescence polarization of DPH or TMA-DPH in microsomal membranes at these concentrations (Table 1).

A high concentration (10 mM) of each of the halogenated anesthetics, halothane, methoxyflurane, and enflurane, did not release calcium from brain microsomes, even after a 10-min incubation (Table 1). This concentration is 10 to 300 times higher than the anesthetic concentrations of these agents (0.03–1 mM) (27–29). Methoxyflurane and enflurane reduced the polarization of DPH and TMA-DPH (Table 1). Halothane could not be tested because it quenched the fluorescence of DPH and TMA-DPH. A_2C, a membrane-disordering agent, also failed to release calcium from brain microsomes but reduced the polarization of DPH and slightly reduced the polarization of TMA-DPH (Table 1).

To assess the possible relationship between anesthetic-induced decreases in membrane order and anesthetic-induced calcium release from microsomes, the two quantities were compared for all drugs studied, except halothane, by least squares linear regression analysis. For comparison between drug-induced calcium release and depolarization of DPH and TMA-DPH, the r^2 values were 0.32 and 0.33 (n = 36), respectively. However, two outlier values contributed by 300 mM diethyl ether and 100 mM benzyl alcohol excessively influenced the computation (Table 1). After exclusion of these two values, the r^2 values were 0.03 and 0.03 (n = 34) for decreases in polarization of DPH and TMA-DPH, respectively. The comparison between reduction of the polarization of DPH and TMA-DPH and ability to release microsomal calcium was also examined after exclusion of compounds that were ineffective in releasing microsomal calcium (4-phenyl-1-butanol, halothane, methoxyflurane, enflurane, and A_2C). The r^2 values were 0.40 and 0.42 (n = 27). After exclusion of the two outlier values noted above, the r^2 values were 0.07 and 0.07 (n= 25) for comparison of effects of these compounds on polarization of DPH and TMA-DPH with microsomal calcium release, respectively. These results indicate that increased membrane fluidity cannot account for the ability of these compounds to release microsomal calcium.

The anesthetic concentration of all compounds tested that released microsomal calcium (n-alkanols, benzyl alcohol, and diethyl ether) was correlated with the concentration of each agent required to release 2 nmol of calcium/mg of protein (Fig. 3). This value of calcium release was chosen because it reflected the approximate amount of calcium released by an anesthetic concentration of ethanol. For the least potent calcium-mobilizing anesthetics tested (methanol, ethanol, and diethyl ether), there was approximately a one-to-one correspondence between

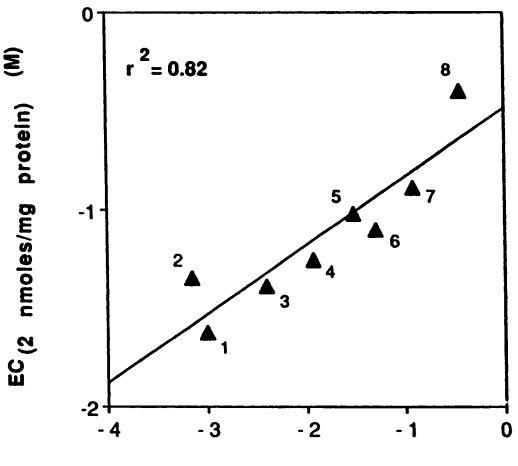

Fig. 3. Comparison of anesthetic potency with effective concentration of anesthetic required to release 2 nmol of calcium/mg of protein from brain microsomes. Inactive compounds (4-phenyl-1-butanol, halothane, methoxyflurane, enflurane, and A_2C) were excluded. Calcium release was measured 30 sec after drug addition. The data shown reflect comparisons for benzyl alcohol (1), n-hexanol (2), n-pentanol (3), n-butanol (4), diethyl ether (5), n-propanol (6), ethanol (7), and methanol (8) (n = 8).

the concentration required to release 2 nmol of calcium/mg of protein and the anesthetic concentration (Fig. 3). The drug concentration required for release of 2 nmol of calcium/mg of protein was 5 to 10 times larger than the anesthetic concentration for the most potent calcium-mobilizing anesthetics (benzyl alcohol, *n*-pentanol, and *n*-hexanol) (Fig. 3).

Discussion

Our results show that certain anesthetics, *n*-alkanols, benzyl alcohol, and diethyl ether, release calcium from brain microsomes. These results extend our previous findings, which showed that *n*-alkanols and diethyl ether increase resting Ca_i in whole brain synaptosomes (10). Release of calcium from intracellular stores may be responsible for increases in resting Ca_i and consequent stimulation of calcium-activated potassium conductances produced by these anesthetics in brain tissue (2, 3). We found that halogenated anesthetics (halothane, methoxyflurane, and enflurane) and pentobarbital did not release microsomal calcium even at supraanesthetic concentrations. The increase in resting Ca_i in synaptosomes that is produced by halothane, which we demonstrated previously (10), may be due to other mechanisms. Indeed, previous work showed that halothane reduced sequestration of calcium by brain mitochondria (30), inhibited the Ca-ATPase that mediates calcium sequestration by sarcoplasmic reticulum (31), and inhibited Na/Ca exchange in cardiac sarcolemma (32). One or several of these actions may be responsible for halothane-induced increases in resting Ca_i. The lack of effect of pentobarbital on microsomal calcium stores agrees with our previous work, which showed that pentobarbital does not alter resting Ca_i (10). Neuronal hyperpolarization produced by pentobarbital is probably due to another mechanism. Previous work showed that pentobarbital stimulates γ-aminobutyric acid receptor-mediated activation of a chloride conductance (33), indicating that pentobarbital produces hyperpolarization by specific action at a γ-aminobutyric-acid receptor.

The concentrations of *n*-alkanols and diethyl ether required to release microsomal calcium were within or slightly higher than the anesthetic concentrations of these agents. However, the concentrations required to demonstrate effects of *n*-alkanols and diethyl ether on resting synaptosomal Ca_i (10) were larger than concentrations required to demonstrate release of calcium from whole brain microsomes. These differences are probably due to the lack of sensitivity inherent in the fura-2 technique for measurement of Ca_i of synaptosomes, because the requirement for large loading concentrations of fura-2 results in buffering of drug-induced changes in Ca_i (34).

Our previous work demonstrated a correlation between anesthetic-induced increases in resting Ca_i and decreases in synaptosomal membrane order (10). However, we found that the ability of anesthetic compounds to decrease the order of the interior and surface of microsomal membranes was not strongly correlated with anesthetic-induced calcium release from microsomes. In fact, A_2C, a nonanesthetic membrane-disordering agent (35) which produced large decreases in order of the interior of microsomal membranes, did not release calcium from microsomes. These results indicate that anesthetic-induced decreases in membrane order are probably unrelated to release of microsomal calcium produced by these compounds. The idea that increased membrane order is related to anesthesia has been recently questioned, in view of exceptions such as A_2C (35). Unlike the other anesthetics tested, pentobarbital did not decrease the polarization of DPH even at a high concentration (1 mM). Although 1 mM pentobarbital disorders synaptic plasma membranes, it does not disorder myelin membranes or lipids extracted from synaptic plasma membranes (19). Thus, effects of pentobarbital on membrane physical properties are dependent on the type of membrane tested and microsomal membranes are apparently resistant to these effects.

Although we found that a nonspecific disordering action of anesthetics on microsomal membranes was unrelated to their ability to release microsomal calcium, compounds active in releasing microsomal calcium appeared to be related by molecular size and chemical structure. The ability of anesthetic and nonanesthetic agents tested in this study to increase synaptosomal Ca_i and release microsomal calcium is compared with molecular size in Table 2. Agents that are active in releasing microsomal calcium exhibit molecular volumes ranging from 41 ml/mol (methanol) to 125 ml/mol (1-hexanol). The most potent compounds were methanol, ethanol, and 1-propanol, with molecular volumes of 41, 58, and 75 ml/mol, respectively. 4-Phenyl-1-butanol was anesthetic in mice and produced a large decrease in the polarization of DPH (indicating that it was present at high concentrations in microsomal membranes) but did not release microsomal calcium. This compound is similar to benzyl alcohol but is larger in size by the addition of three methylene groups (Table 2). A_2C, a nonanesthetic fatty acid derivative presumably with the largest molecular size of the compounds tested in this study, also produced large decreases in polarization of DPH and TMA-DPH but failed to release microsomal calcium. On the other hand, the halogenated anesthetics, halothane, methoxyflurane, and enflurane, which are also inactive at microsomal calcium stores, are similar in size to their nonhalogenated active counterparts (Table 2). These compounds decreased the order of microsomal membranes, indicating that they were not excluded from microsomal membranes due to solubility limitations. Molecular size alone, therefore, does not account for the inability of these agents to release

TABLE 2

Comparison of effects of anesthetic and non-anesthetic agents on calcium homeostasis with molecular size

Results of effects of anesthetics on intrasynaptosomal calcium concentrations are derived from Daniell and Harris (10). Microsomal calcium release was graded as amount of calcium released by a concentration of the agent equivalent to five times the anesthetic concentration. Molecular size values were estimated from molecular weights and densities determined at 20 or 25°.

Agent	Anesthetic	Increases Ca_i	Releases microsomal calcium	Molecular size
				ml/mol
Methanol	Yes	Yes	++++	41
Ethanol	Yes	Yes	+++++	58
1-Propanol	Yes	Yes	++++	75
1-Butanol	Yes	Yes	+	92
1-Pentanol	Yes	Yes	+	108
1-Hexanol	Yes	Yes	+	125
Diethyl ether	Yes	Yes	++	104
Benzyl alcohol	Yes	ND[a]	+	103
Halothane	Yes	Yes	−	106
Methoxyflurane	Yes	ND	−	116
Enflurane	Yes	ND	−	142
Pentobarbital	Yes	No	−	NA
4-Phenyl-1-butanol	Yes	ND	−	154
A_2C	No	ND	−	NA

[a] ND, not determined.
[b] NA, not available.

microsomal calcium. On the other hand, all agents that are active in releasing microsomal calcium exhibited a similar chemical structure, that is, one electronegative atom (oxygen) at one end of the molecule and a hydrophobic region at the other. For these compounds, potency appears to increase with increasing molecular size and peak at values of 40 to 80 ml/mol, suggesting a role for molecular size in effects of these compounds on microsomal calcium stores. Inactive compounds were either larger in size (4-phenyl-1-butanol and A_2C) or possess electronegative substituents at various positions (pentobarbital and the halogenated anesthetics).

Taken together, our findings indicate that anesthetic-induced microsomal calcium release may be dependent on both molecular size and structure and unrelated to anesthetic-induced disordering of the interior or surface of microsomal membranes and they indicate that anesthetics release microsomal calcium by action at a protein site within microsomal membranes. The existence of convulsant nonanesthetic halogenated compounds such as 2-bromo-1,1,1-trifluoroethane (36), which is chemically similar to halothane except for the absence of a chloride substitution on C-2, suggests that anesthetics may bind to a specific saturable site in neuronal membranes. This idea is supported by the recent observation of saturable binding sites for halothane in brain tissue (37). Our results indicate that the site mediating calcium release from brain microsomes probably best accomodates anesthetic compounds the size of ethanol and may require partial polar characteristics such as a hydroxyl group. This site could be a channel or transporter. Our previous work showed that ethanol-induced calcium release is highly temperature dependent (16). We also found that anesthetic-induced calcium release from microsomes is maximal within 10–30 sec. The rapid effect of these compounds is suggestive of activation of a channel in microsomal membranes. Further work using stop-flow techniques to measure initial rates of anesthetic-induced calcium release will be required to determine the characteristics of the microsomal membrane site that is responsive to anesthetics.

In summary, we found that anesthetics of several chemical classes release calcium from brain microsomes. Anesthetic-induced release appears to be unrelated to disordering of microsomal membranes and may be determined, at least in part, by molecular size and polarity. The anesthetics studied in this report are all capable of causing cellular hyperpolarization of central neurons. However, the lack of similarity of effect of anesthetics from various chemical classes on microsomal calcium stores and on resting Ca_i indicates that a single mechanism of calcium-mediated hyperpolarization produced by anesthetics does not exist.

Acknowledgments

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Send reprint requests to: Laura C. Daniell, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.