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Halothane Inhibits the Cholinergic-receptor-mediated Influx of Calcium in Primary Culture of Bovine Adrenal Medulla Cells

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Adrenal medulla cells are cholinoceptive cells. Stimulation of the acetylcholine receptor causes the influx of Ca to the cells, and Ca acts as the coupler of the stimulus–secretion coupling. In this study, the authors investigated the effects of halothane on the receptor-mediated influx of ^{45}Ca using cultured bovine adrenal medulla cells. Halothane at clinical concentrations (0.5–2%) inhibited the influx of ^{45}Ca caused by carbachol, with simultaneous inhibition of catecholamine secretion. The influx of ^{45}Ca and the secretion of catecholamines caused by K depolarization were inhibited by a large concentration of Mg, which competes with Ca at Ca channels, but not inhibited by halothane. Inhibition of the ^{45}Ca influx by halothane was not overcome by increase in the carbachol concentration. Inhibition of the ^{45}Ca influx by halothane was examined in comparison with that caused by a large concentration of Mg by the application of Scatchard analysis as the function of the external Ca concentration. Halothane decreased the maximal influx of ^{45}Ca without altering the apparent kinetic constant of Ca to Ca channels. On the contrary, a large concentration of Mg increased the apparent kinetic constant without altering the maximal influx of ^{45}Ca. Based on these findings, the authors suggest that inhibition of the ^{45}Ca influx by halothane was not due to the direct competitive inhibition of Ca channels, nor to the competitive antagonism of agonist–receptor interaction. As a possibility, halothane seems to inhibit the receptor-mediated activation of Ca channels through the interference of coupling between the receptor and Ca channels. (Key words: Anesthetics, volatile: halothane. Ions: calcium. Receptors: nicotinic. Sympathetic nervous system: adrenal medulla; catecholamines.)

THE PRIMARY SITE of action of anesthetics in the central nervous system is believed to be at the synapses. Because of the intrinsic complexity of the central nervous system, however, peripheral synapses have been used as an appropriate model to explore where and how anesthetics act at specific synapses. For this purpose, sympathetic ganglia[1–3] and the adrenal medulla[4–6] have been used as the model of cholinergic synapses.

Halothane has been shown to inhibit the release of norepinephrine from electrically stimulated sympathetic nerves.[7,8] Göthert *et al.*[9,10] have shown that halothane inhibited the secretion of catecholamines induced by acetylcholine from perfused bovine adrenals and suggested that agonist–receptor interaction might be inhibited by halothane in a noncompetitive manner. Recently, Sumikawa *et al.*[5] have examined the inhibitory effects of halothane on the cholinergic postsynaptic process of the canine adrenal medulla and suggested that the most susceptible process might be the Ca influx through nicotinic-receptor-linked channels. Since Ca plays an important role as the coupler in stimulus–secretion coupling,[11] it is interesting to correlate the inhibitory effects of halothane with the inhibition of the Ca influx. However, these previous reports were based on indirect evidence and did not involve the direct measurement of Ca influx.

The present study was undertaken to examine directly the effects of halothane on acetylcholine-receptor-mediated Ca influx and release of catecholamines in cultured bovine adrenal medulla cells, and furthermore, to obtain detailed information on the mode of action of halothane on acetylcholine-receptor-linked Ca channels. Cultured adrenal medullary cells were used for this purpose because these cells have been shown to be a suitable model for analyzing the receptor and ion channel sequences and the mechanism of drug action affecting the stimulus–secretion coupling.[12–17]

Materials and Methods

Adrenal medulla cells were isolated from fresh bovine adrenal medullas by the same methods fundamentally as described by Brooks.[18] Krebs-Ringer phosphate buffer, used throughout the experiments, had the following composition (mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $MgSO_4$ 1.1, NaH_2PO_4 0.85, Na_2HPO_4 2.15, and dextrose 10 with 0.5% bovine serum albumin, *p*H being adjusted to 7.4 with NaOH. In high K (56 mM) medium, NaCl was reduced to maintain the isotonicity of the medium, and other constituents were the same. Adrenal glands were perfused with Ca-free Krebs-Ringer phosphate buffer for 10 min at 37° C to flush out blood materials. The medullary tissues were sliced using a Stadie-Rigg's slicer. The slices were subjected to stepwise digestion with collagenase. The isolated cells were filtered through nylon mesh, collected by centrifugation, and washed three times with Krebs-Ringer phosphate buffer and three times with Eagle's minimum essential medium (MEM). The cells were then plated at a density of 4×10^6 cells/dish (Corning®, 35 mm in diameter) in Eagle MEM containing 10% calf

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serum, aminobenzyl penicillin (60 μg/ml), streptomycin (100 μg/ml) and amphotericin B (0.3 μg/ml). The cells were cultured in 5% CO_2–95% air in culture chamber and used at between 3 to 7 days of culture for experiments of catecholamine secretion and ^{45}Ca influx.

The influx of ^{45}Ca and secretion of catecholamines were measured as reported previously.[15–17] Cultured cells (4×10^6 cells/dish) were washed twice with 2 ml of ice-cold Krebs-Ringer phosphate buffer and then exposed to various concentrations of halothane for 15 min at 37° C by addition of halothane-saturated Krebs-Ringer phosphate buffer, and the dishes were sealed tightly. The equilibration of halothane in solution was achieved within 15 min. After 15 min of preincubation, the medium was switched to that containing $^{45}CaCl_2$ (1 μCi) and stimulated either by carbachol (3×10^{-4} M), high K (56 mM), nicotine (10^{-4} M) or muscarine (10^{-4} M) for 1 min at 37° C. Hexamethonium, *d*-tubocurarine, Ca channel blockers and large concentrations of Mg (10–20 mM) were also used in some experiments. The reaction was terminated by the addition of diltiazem (10^{-3} M), a Ca channel blocker, and the medium was immediately transferred to test tubes and subjected to catecholamine assay. Cells were washed four times with 2 ml of ice-cold Ca-free Krebs-Ringer phosphate buffer at 15 s intervals. Finally, the cells were solubilized by Triton® X-100 (10%, 1 ml), and the radioactivity was measured by a scintillation counter with an efficiency of 78%. The influx of Ca was expressed as nanomoles of Ca taken up by 4×10^6 cells, calculated from the specific activity of $^{45}CaCl_2$ and the concentration of nonradioactive Ca in the medium. Experiments were done in duplicate, using two dishes for a given concentration of halothane, and the mean averages were subjected to statistical analysis. In some experiments, the effects of halothane on the influx of ^{45}Ca were examined under various concentrations of Ca in the medium, and the results were subjected to kinetic analysis.[19–21] In brief, the influx of ^{45}Ca (I_{Ca}) under various concentrations of external Ca was measured, and the ratio of ^{45}Ca influx to the Ca concentration (S) was plotted against I_{Ca}. Lines were drawn according to least square method. The points where the lines intercept the abscissa show the maximal influx of ^{45}Ca (I_{Ca}max), and the slopes correspond to the apparent kinetic constant (K), which shows the Ca permeability of the cell membranes. The lower the K values are, the more Ca is permeable to the cell membrane.

The halothane concentration in the medium was expressed as the per cent concentration in the gas phase as clinically used. The actual concentration of halothane was checked by gas chromatography, and 1% and 2% halothane were confirmed to correspond to 0.32 mM and 0.63 mM, respectively.

Catecholamines secreted into the medium were extracted with 0.4 M perchloric acid, adsorbed to aluminum hydroxide, and assayed by an improved trihydroxyindole method according to von Euler and Lishajko.[22] The fluorescence was read by a Hitachi fluorescence spectrophotometer with filter sets A (λex = 395 nm, λem = 495) and B (λex = 436, λem = 540). Standards composed of 0.1 μg each of epinephrine and norepinephrine were used. The minimum assay range was 10 ng for both catecholamines, and the recoveries of epinephrine and norepinephrine were 94% and 91%, respectively. Because cultured cells (4×10^6 cells/dish) contained 41.5 ± 5 μg of catecholamines as epinephrine plus norepinephrine, this assay method makes it possible to estimate even 0.02% of total catecholamines in the cells. Halothane and other drugs used in this experiment did not interfere with the catecholamine assay. Thymol, which was added to halothane as the stabilizer, had no effect on the secretion of catecholamines or the influx of ^{45}Ca.

$^{45}CaCl_2$ (0.5–2 Ci/mmol) was obtained from Amersham. Collagenase (Type 1), soybean trypsin inhibitor, carbachol, and muscarine were from Sigma. Eagle MEM was from Nissui. Halothane was from Hoechst; diltiazem and verapamil were gifts from Tanabe and Eisai. Hexamethonium and *d*-tubocurarine were from Tokyo Kasei, and nifedipine was from Bayer. All other chemicals were analytical grade from Nakarai Chemicals.

Statistical Analysis

The data were expressed as mean ± SE. The results of repeated measurements and multiple groups were analyzed by one-way analysis of variance. Multiple pairwise comparisons between groups were assessed by a Bonferroni *t* test. A P value of < 0.05 was considered significant.

Results

Secretion of Catecholamines and the Influx of ^{45}Ca Evoked by Acetylcholine Receptor Stimulation in Cultured Bovine Adrenal Medulla Cells

The adrenal medulla cells used in this experiment contained 41.5 ± 5.0 μg (mean ± SEM, n = 10) of catecholamines per dish (4×10^6 cells). The spontaneous release of catecholamines from the cells during 1 min of incubation was less than 1% of the total catecholamines in the cells. Stimulation by carbachol (3×10^{-4} M) caused a rapid secretion of catecholamines, which was transient and tapered off within 1 min. The secretion of catecholamines in the presence of carbachol amounted to 12.04 ± 0.24% (n = 10).

The influx of ^{45}Ca to the nonstimulated cells was rather slow. Stimulation by carbachol caused an instantaneous influx of ^{45}Ca, which reached a plateau within 1 min. The

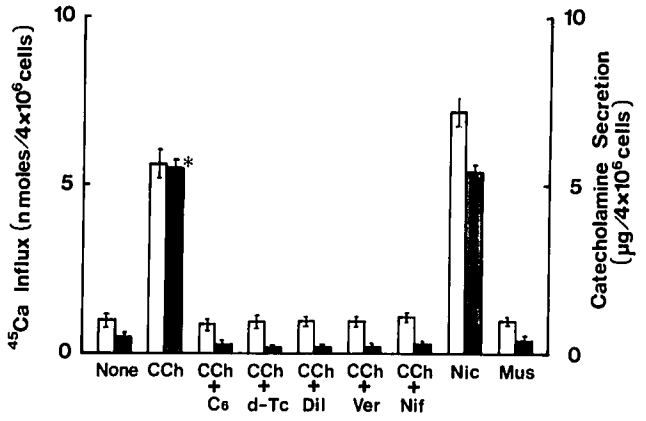

FIG. 1. Secretion of catecholamines and the influx of ^{45}Ca in cultured bovine adrenal medulla cells. Adrenal medulla cells (4×10^6 cells/dish) were incubated in Krebs-Ringer phosphate buffer with 1 μCi of $^{45}CaCl_2$ in the presence of carbachol (CCh, 3×10^{-4} M), nicotine (Nic, 10^{-4} M) or muscarine (Mus, 10^{-4} M) for 1 min at 37° C. In some dishes, hexamethonium (C_6, 10^{-3} M), *d*-tubocurarine (*d*-Tc, 10^{-3} M), diltiazem (Dil, 10^{-3} M), verapamil (Ver, 10^{-3} M) or nifedipine (Nif, 10^{-3} M) were simultaneously added along with carbachol. Catecholamines secreted into the medium (*shaded columns*) and ^{45}Ca taken up by the cells (*open columns*) were measured. Values are the means from six to 10 (*) separate experiments and SEMs are expressed by the vertical bars.

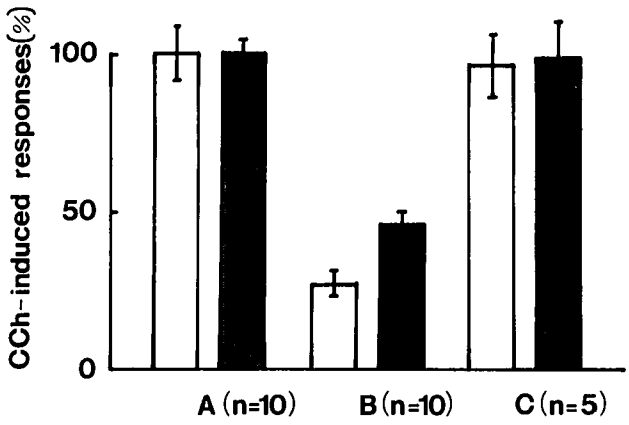

FIG. 3. Reversibility of the effects of halothane on the secretion of catecholamines and the influx of ^{45}Ca in cultured bovine adrenal medulla cells. Secretion of catecholamines (*shaded columns*) and the influx of ^{45}Ca (*open columns*) were measured in control cells (A), halothane 2%-equilibrated cells (B), and halothane preexposed cells (C). Cells used in experiment C were equilibrated with 2% halothane, as were cells B, and then washed out by the control medium for 15 min. Cells were stimulated by carbachol (3×10^{-4} M) for 1 min at 37° C. Secretion of catecholamines and the influx of ^{45}Ca caused by carbachol in control cells (A) were expressed as 100% responses. Values are the mean average, and the SEMs are expressed by the vertical bars. The number of experiments is shown in parentheses.

influx of ^{45}Ca in the presence of carbachol (3×10^{-4} M) was 5.58 ± 0.23 nmol · 4×10^6 cells^{-1} · min^{-1} (n = 10).

Nicotine (10^{-4} M) substituted for carbachol in evoking the secretion of catecholamines and the influx of ^{45}Ca. Muscarine (10^{-4} M) did not substitute for carbachol either in the secretion of catecholamines or the influx of ^{45}Ca, although muscarine has been demonstrated to cause the secretion of catecholamines from the perfused dog adrenals.[5,23] Secretion of catecholamines and the influx of ^{45}Ca were both inhibited by hexamethonium (10^{-3} M), *d*-tubocurarine (10^{-3} M), or Ca channel blockers such as diltiazem (10^{-3} M), verapamil (10^{-3} M), and nifedipine (10^{-3} M) (fig. 1).

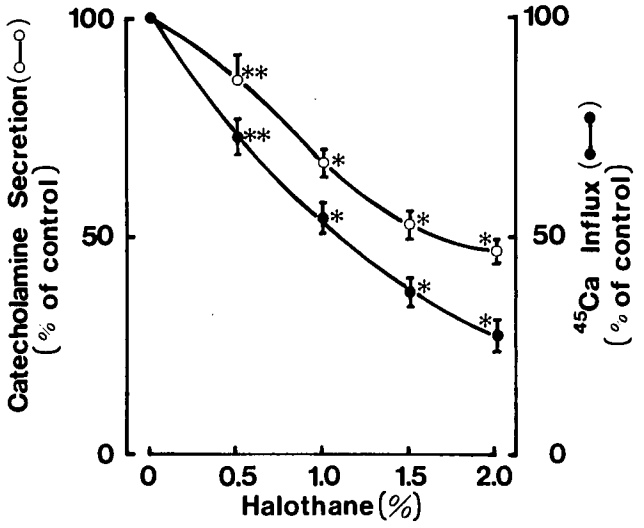

FIG. 2. Effects of halothane on the secretion of catecholamines and the influx of ^{45}Ca evoked by carbachol in cultured bovine adrenal medulla cells. Adrenal medulla cells (4×10^6 cells/dish) were equilibrated with 0.5–2% halothane and then stimulated with carbachol (3×10^{-4} M) in the presence of $^{45}CaCl_2$ for 1 min at 37° C. Catecholamines secreted into the medium and ^{45}Ca taken up by the cells were measured. Values are the means from ten separate experiments, expressed by the percentage to carbachol-evoked responses in the absence of halothane. SEMs are expressed by the vertical bars. $^{**}P < 0.05$; $^{*}P < 0.01$.

EFFECTS OF HALOTHANE ON THE SECRETION OF CATECHOLAMINES AND THE INFLUX OF ^{45}CA IN CULTURED BOVINE ADRENAL MEDULLA CELLS

Halothane, at the concentration equilibria of 0.5% to 2%, had no effects on the spontaneous release of catecholamines, but it did inhibit the secretion of catecholamines evoked by carbachol in a dose-dependent manner (fig. 2). Halothane at the same concentration also inhibited the influx of ^{45}Ca evoked by carbachol, while it had no effects on the influx of ^{45}Ca in nonstimulated cells.

The inhibitory effects of halothane on the secretion of catecholamines and the influx of ^{45}Ca were reversible, with cells restoring their responses to carbachol after a washout of halothane (fig. 3).

In cultured bovine adrenal medulla cells, high K solution causes the depolarization of cell membranes and induces the secretion of catecholamines and the influx of

^{45}Ca. Halothane (0.5% to 2%) did not inhibit the secretion of catecholamines or the influx of ^{45}Ca evoked by high K (fig. 4).

Effects of a Large Concentration of Mg on the Secretion of Catecholamines and the Influx of ^{45}Ca

Divalent cations, such as Mg and Mn, have been known to inhibit the secretion of catecholamines by their competition with Ca at Ca channels.[24] In cultured bovine adrenal medulla cells, large concentrations of Mg (10–20 mM) inhibited the secretion of catecholamines and the influx of ^{45}Ca that had been evoked by carbachol and high K. The secretion of catecholamines caused by high K was inhibited to a greater extent than that caused by carbachol ($P < 0.01$). Similarly, the influx of ^{45}Ca caused by high K was inhibited to a greater extent than that caused by carbachol ($P < 0.01$, table 1).

Mode of Inhibition of the ^{45}Ca Influx Evoked by Carbachol by Halothane and Mg

In order to ascertain the mode by which halothane inhibits the influx of ^{45}Ca, we compared the inhibition of the ^{45}Ca influx by halothane (fig. 5*A*) and by a large concentration of Mg (fig. 6*A*) under various concentrations of Ca in the medium. Inhibition of the ^{45}Ca influx caused by halothane was observed to a similar extent, irrespective of the Ca concentration in the medium, while inhibition by Mg was reduced by the increase of the Ca concentration (fig. 7).

To differentiate the mode of inhibition by halothane of the receptor-mediated influx of ^{45}Ca, *i.e.*, whether it is competitive or noncompetitive, the Scatchard analysis of the influx of ^{45}Ca was used in a way similar to that used by Stjärne,[20,21] who analyzed the kinetics of secretion of sympathetic transmitter as a function of external Ca by Michaelis-Menten kinetics. The ratio of the I_{Ca} to the Ca concentration (S) was plotted against I_{Ca} to determine the I_{Ca}max and apparent kinetic constant (K) of Ca to Ca channels. In control cells stimulated by carbachol, the I_{Ca}max value was 12.6 ± 1.3 nmol/4 × 10^6 cells (n = 10), and the K value was 3.8 ± 0.5 mM (n = 10). Halothane 1% reduced the I_{Ca}max value to 7.6 ± 0.6 nmol/4 × 10^6 cells (n = 6, $P < 0.01$), but did not alter the K value (3.6 ± 0.5 mM, n = 6, fig. 5*B*). On the contrary, a large concentration of Mg (10 mM) increased the K value to 7.7 ± 0.8 mM (n = 6, $P < 0.01$) without affecting the I_{Ca}max value (12.3 ± 1.6 nmol/4 × 10^6 cells, n = 6, fig. 6*B*).

Inhibition of the ^{45}Ca influx caused by halothane was not overcome by the increase in the carbachol concentration used to stimulate the acetylcholine receptor (data not shown), while inhibition by *d*-tubocurarine was overcome as reported previously.[14]

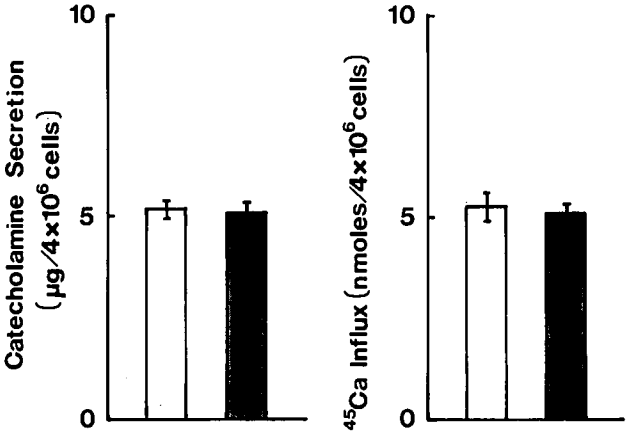

FIG. 4. Effects of halothane on the secretion of catecholamines and the influx of ^{45}Ca evoked by high K. Adrenal medulla cells (4 × 10^6 cells/dish) were stimulated by high K (56 mM) for 1 min at 37° C. Catecholamines secreted into the medium and ^{45}Ca taken up by the cells were measured in control cells (*open columns*) and halothane 2%-equilibrated cells (*shaded columns*). Secretion of catecholamines and the influx of ^{45}Ca in nonstimulated cells were subtracted. Values are the means from six separate experiments; SEMs are expressed by the vertical bars.

Discussion

The results have shown that halothane at clinical concentrations inhibited the secretion of catecholamines and the influx of ^{45}Ca caused by carbachol in cultured bovine adrenal medulla cells. The inhibitory effect of halothane on the secretion of catecholamines is in accordance with previous reports.[5,9,10] Because of the parallelism between the diminution of the ^{45}Ca influx and the secretion of catecholamines, the major contributing mechanism for the inhibition of catecholamine secretion would be the

TABLE 1. Effects of a Large Concentration of Mg on Secretion of Catecholamines and Influx of ^{45}Ca

	Catecholamine Secretion (μg/4 × 10^6 cells)	^{45}Ca Influx (nmol/4 × 10^6 cells)
Carbachol (3×10^{-4} M)	5.31 ± 0.17 (n = 6)	4.58 ± 0.23 (n = 10)
Carbachol + Mg (20 mM)	3.77 ± 0.17* (n = 6)	2.19 ± 0.18* (n = 6)
High K (56 mM)	5.13 ± 0.19 (n = 6)	5.30 ± 0.23 (n = 6)
High K + Mg (20 mM)	1.60 ± 0.10† (n = 6)	1.20 ± 0.10† (n = 6)

* $P < 0.01$, compared with control.
† $P < 0.01$, compared with effects on carbachol evoked responses.
Adrenal medulla cells (4 × 10^6 cells/dish) were stimulated by carbachol (3×10^{-4} M) or high K (56 mM) for 1 min at 37°C, in the presence and absence of a large concentration of Mg (20 mM). Catecholamine secretion and the influx of ^{45}Ca in nonstimulated cells were subtracted. Number of experiments is shown in parentheses.

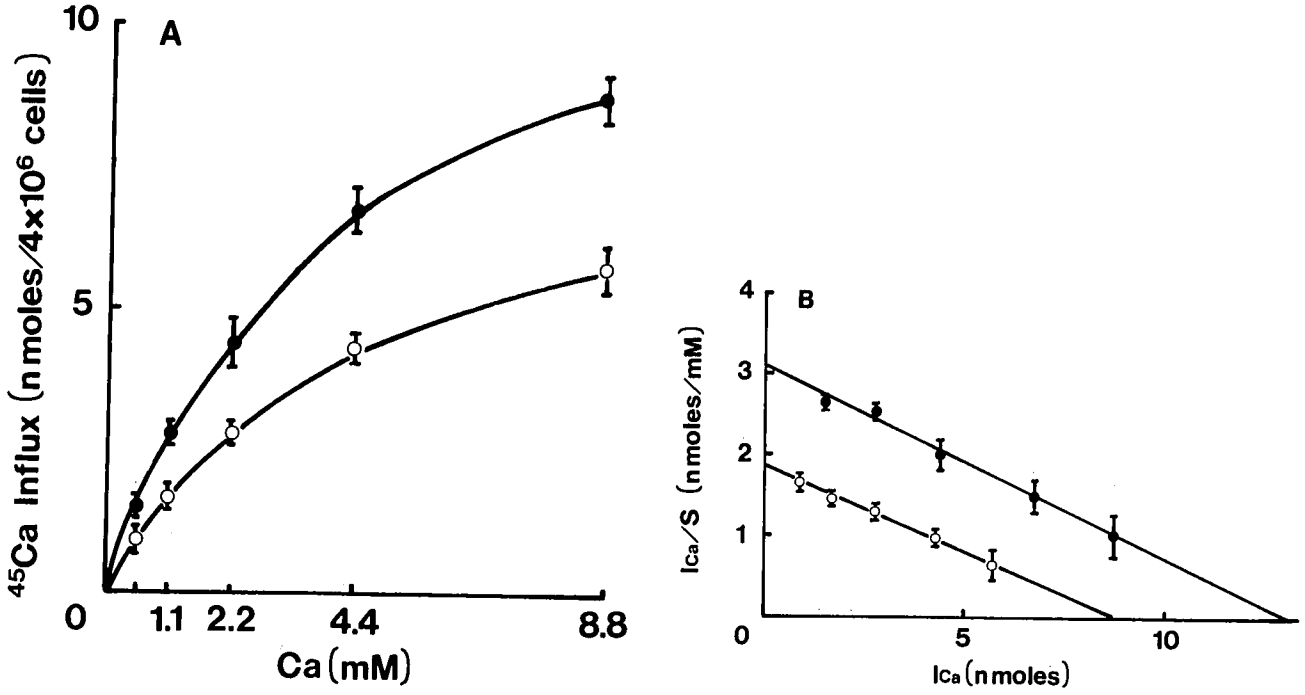

FIG. 5. Inhibition by halothane of the carbachol-evoked influx of ^{45}Ca under various concentrations of Ca in the medium. Influx of ^{45}Ca caused by carbachol (3×10^{-4} M) was measured in control (●) and halothane 1%-equilibrated cells (○) under various concentrations of Ca in the medium. Cells were stimulated with carbachol for 1 min at 37° C. The influx of ^{45}Ca in nonstimulated cells was subtracted. Values are the means from six to ten separate experiments, and SEMs are expressed by the vertical bars (*A*). *B* shows the Scatchard plots from *A*.

diminution of the Ca influx. This confirms the assumption by Sumikawa *et al.*[5] that the process most susceptible to the anesthetic would be the nicotinic-receptor-mediated influx of Ca. As opposed to the canine adrenal medulla in which both nicotinic and muscarinic receptors are involved in catecholamine secretion,[5,23] the acetylcholine

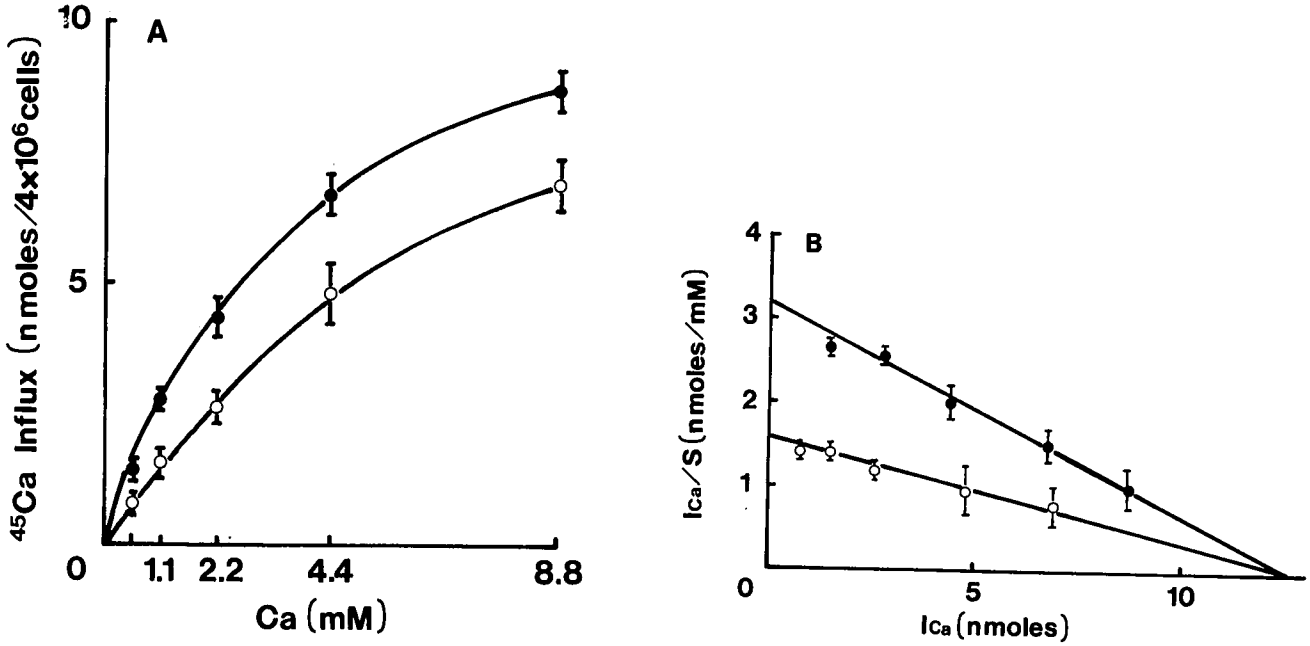

FIG. 6. Inhibition by a large concentration of Mg of the carbachol-evoked influx of ^{45}Ca under various concentrations of Ca in the medium. Influx of ^{45}Ca caused by carbachol (3×10^{-4} M) was measured in the presence (○) and absence (●) of 10 mM Mg, under various concentrations of Ca in the medium. Cells were stimulated with carbachol for 1 min at 37° C. The influx of ^{45}Ca in nonstimulated cells was subtracted. Values are the means from six separate experiments, and the SEMs are expressed by the vertical bars (*A*). *B* shows the Scatchard plots from *A*.

receptors in the bovine adrenal medulla that contribute to secretion of catecholamines are entirely nicotinic in nature because nicotine, but not muscarine, can substitute for carbachol, and the secretion was blocked by hexamethonium and *d*-tubocurarine.

Influx of Ca to the cells is the most critical step in triggering the secretion of catecholamines,[11] and stimulation of acetylcholine receptor has been shown to cause the rise in cytoplasmic-free Ca concentration measured by fluorescence probe.[25] The influx of Ca should be diminished either by: 1) the interference of receptor–agonist interaction; 2) direct blockade of Ca channels; or 3) the disruption of coupling between receptor and Ca channels. In perfused bovine adrenal glands, Göthert *et al.*[10] have shown that halothane inhibits the secretion of catecholamines evoked by acetylcholine in a noncompetitive manner to acetylcholine. They suggested that halothane does not impair the permeability of cell membranes to Ca, while it may cause a conformational change of membrane proteins, particularly of the nicotinic receptor. However, in the mammalian neuromuscular junction,[26] halothane has been shown not to interfere with the binding of cholinergic ligand to nicotinic receptor, because the dissociation constant of *d*-tubocurarine to nicotinic receptor was not altered by halothane. In our experiment, inhibition of the ^{45}Ca influx by halothane was not restored by the increase in carbachol concentration, showing that the inhibition was not due to the competition at the agonist binding site.

High K depolarization causes the influx of Ca through the voltage-dependent Ca channels and causes the secretion of catecholamines. Halothane did not inhibit the influx of ^{45}Ca or the secretion of catecholamines by high K. Thus, it seems that halothane has no direct action on Ca channels. The third possibility, that halothane may disrupt the coupling between receptor and Ca channels, would be the mechanism that contributes the most to the inhibitory action of halothane.

A large concentration of Mg has been shown to inhibit the voltage-dependent Ca channels selectively.[27] In our experiment, large concentrations of Mg inhibited the secretion of catecholamines and the influx of ^{45}Ca induced by high K to greater extents than those induced by carbachol. This finding may imply that influx of ^{45}Ca evoked by carbachol occurred also through the receptor-associated Ca channels in addition to the voltage-dependent Ca channels, as reported previously.[28,29] There was considerably more inhibition of the ^{45}Ca influx than of catecholamine secretion with a large concentration of Mg (table 1) and to a lesser extent with halothane (fig. 2). As an explanation for this, the exogenous Ca, intruded into the cells by receptor stimulation, might have mobilized the endogenous Ca from Ca pools, and this Ca was altogether used for the secretion of catecholamines.

In the present study, we applied kinetic methods to characterize the mode of action of halothane on the receptor-mediated influx of Ca in comparison with the effect of a large concentration of Mg. A large concentration of Mg increased the apparent kinetic constant of Ca to Ca channels without altering the maximal influx of ^{45}Ca, showing that Mg caused the direct competition at Ca channels and decreased the Ca permeability of the cells. On the contrary, halothane decreased the maximal influx of ^{45}Ca without altering the apparent kinetic constant of Ca to Ca channels, showing that Ca permeability itself was not affected by halothane.

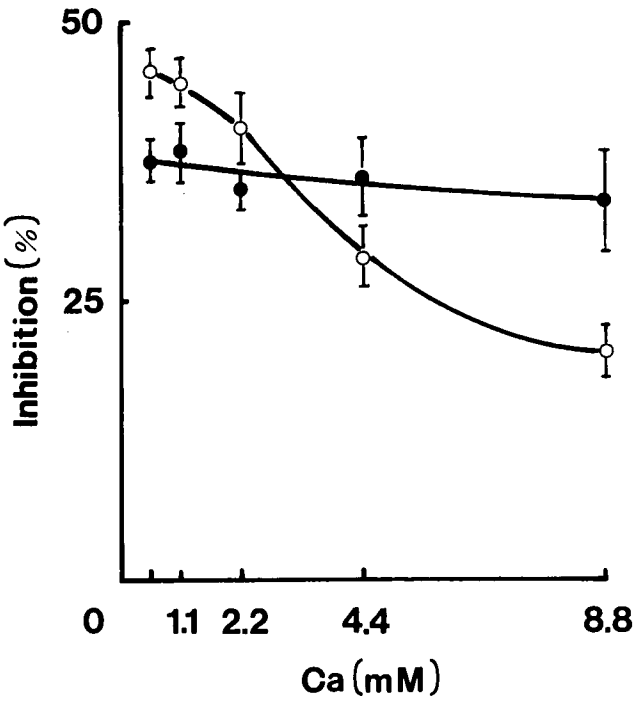

FIG. 7. Per cent inhibition of the carbachol-evoked influx of ^{45}Ca by halothane and large Mg under various concentrations of Ca in the medium. Inhibition of ^{45}Ca influx by halothane (●) and large Mg (○), which was presented in Fig. 5*A* and 6*A*, is expressed by the per cent to each respective control values as the function of Ca concentration in the medium.

Adrenal medulla cells are of neural origin and share many common properties with sympathetic ganglia. Therefore, the effects of halothane on adrenal medulla cells and those on sympathetic ganglia could be considered analogously. In isolated hamster stellate ganglion, Christ[1] reported that halothane decreased the potentials evoked by preganglionic stimuli as well as the discharges elicited by nicotinic agonist. He hypothesized that halothane depresses the postsynaptic response to nicotinic stimulation and also, probably, depresses transmitter release from the presynaptic terminals. Bosnjak *et al.*[3] investigated the inhibitory effects of halothane on ganglionic transmission in guinea pig stellate ganglion and showed that the depression of transmission was most likely due to a decrease in transmitter release, although alterations in post-

synaptic receptor properties could have been involved as well. Seagard *et al.*[2] reported that, although halothane modifies each site of the baroreceptor reflex arch, the depression in postsynaptic activity was greater than that in preganglionic activity. These findings show that halothane modifies the ganglionic transmission by acting at multiple sites, at least at presynaptic and postsynaptic sites. In our experiment, we used cultured bovine adrenal medulla cells, which are cholinoceptive in nature, but the presynaptic innervation is absent. Thus, the effects of halothane reported in this experiment are purely confined to the postsynaptic action of this anesthetic.

In conclusion, in cultured bovine adrenal medulla cells, halothane inhibits acetylcholine receptor-mediated influx of Ca in a noncompetitive manner, as is evidenced by Scatchard analysis. Because halothane seems not to inhibit Ca channels directly and not to affect agonist–receptor interaction, the inhibition may be due to the interference of coupling between the acetylcholine receptor and Ca channels, *i.e.*, to the decrease in the number of Ca channels that respond to receptor stimulation.

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