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Mechanism of the Differential Effects of Halothane on Nicotinic- and Muscarinic-Receptor-Mediated Responses of the Dog Adrenal Medulla

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The mechanism of the differential effects of halothane on the cholinergic nicotinic and muscarinic responses of adrenal medullary cells was studied using isolated dog adrenals perfused with modified Locke's solution. The concentrations of halothane exhibiting 50% inhibition of catecholamine release induced by nearly equipotent agonists were 0.8% for nicotine, 1.9% for acetylcholine, and 2.8% for muscarine, respectively. Per cent inhibition by halothane (1.5%) of nicotine-induced catecholamine release was 98.5%, and those of veratridine-, acetylcholine-, $CaCl_2$-, Na^+-deprivation- and muscarine-induced catecholamine release were 89.7, 32.5, 21.4, 10.1, and 9.5%, respectively. Halothane showed an inhibitory effect on the agonist-induced catecholamine release in Na^+-free solution to the same extent as in Na^+-containing solution. Tetrodotoxin abolished veratridine-induced catecholamine release completely and decreased nicotine-induced release slightly, whereas it had no effect on either muscarine- or acetylcholine-induced catecholamine release. Verapamil inhibited acetylcholine-induced catecholamine release by 65%, and nicotine- and muscarine-induced release by 79% and 26%, respectively. The results suggest that halothane at clinical concentrations selectively inhibits the nicotinic-receptor-mediated responses of the dog adrenal medulla. The mechanism involved might be the susceptibility to halothane of the Ca^{++} channels that are linked to the respective nicotinic and muscarinic receptors. An inhibition of exocytosis might be also indicated as part of the effect of halothane. (Key words: Anesthetics, volatile: halothane. Ion channels: sodium; calcium. Pharmacology: tetrodotoxin; verapamil. Receptors: nicotinic; muscarinic. Sympathetic nervous system: adrenal medulla; catecholamines.)

GENERAL ANESTHETICS have many effects on synaptic transmission and the physical state of the membranes. They exert various actions at the synapses, affecting both the amount of transmitter released[1,2] and the sensitivity of the postsynaptic membrane to the transmitter.[3,4] Moreover, it is conceivable that different synapses may have varying degrees of stability and susceptibility to anesthetics.[5]

There have been some studies on the interaction of nicotinic and muscarinic acetylcholine receptors with anesthetics in the peripheral organs and the central nervous system. Using cardiac sympathetic ganglia of dogs, Alper *et al.*[6] have demonstrated that halothane alone did not affect the response to injected acetylcholine and that only after blockade of muscarinic receptors in the ganglion, did halothane depress the response to acetylcholine. They concluded that halothane specifically inhibits the response of nicotinic ganglionic receptors alone. Although they have suggested the postsynaptic neuron as the site of action of the anesthetic, the mechanism of the different effects of halothane on these receptors has remained unknown until now. On the other hand, Catchlove *et al.*[7] and Krnjević[8] have suggested that general anesthetics exert their action by blocking muscarinic excitation of the cortical neurons. The mechanism involved was thought to be a diminished respiration of mitochondria followed by an accumulation of internal Ca^{++}. More recent studies by Smaje[4] have failed to confirm these findings. On the contrary, using the olfactory cortex maintained *in vitro*, they found that volatile anesthetics, such as halothane, caused a dose-related augmentation of muscarinic excitation. It is believed that further studies are necessary to clarify the mechanism of action of anesthetics on these acetylcholine-receptor-mediated responses of postsynaptic cells.

The present study was performed in order to obtain detailed information on the mechanism of action of halothane on the cholinergic postsynaptic process. The dog adrenal medulla was used as the experimental model of cholinergic synapse because there has been evidence for nicotinic and muscarinic receptors for acetylcholine in this organ, and both of these are excitatory, having different functions in the selective secretion of norepinephrine and epinephrine.[9]

Materials and Methods

Mongrel dogs of both sexes that weighed 12 to 16 kg were anesthetized with sodium pentobarbital (30 mg/kg iv or ip). Both adrenals were exposed through a midline abdominal incision and isolated outside the bodies together with the adrenolumbar vein. The adrenolumbar vein was cannulated as described by Robinson,[10] and all detectable side branches entering it were tied. The glands were perfused retrogradely at a

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pressure of 60 cmH_2O with a warmed (37° C) modified Locke's solution aerated with 95% O_2 and 5% CO_2, according to the method of Tsujimoto *et al.*[9] The standard solution was composed as follows[11] (in mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, glucose 10 and Tris-HCl buffer 40; *p*H 7.4. In some experiments, Na^+-free Locke's solution, which was of the same composition as described above except that NaCl was replaced by osmotically equivalent concentrations of sucrose, was used. Perfusion was carried out at a constant rate in each experiment, ranging from 0.8 to 1.8 ml/min. About 45 min were allowed before any treatment in order to achieve equilibrium. Drugs dissolved in Locke's solution were administered by continuous infusion by switching a valve on the tubing leading to the glands. The adrenals were stimulated two or three times with various stimulants. The stimulation period was 1 min, followed by 20-min recovery intervals.

Secretagogues, such as acetylcholine, nicotine, muscarine, and veratridine, were added to the standard perfusate. $CaCl_2$-induced catecholamine release was achieved as follows. The adrenals were perfused with a modified Locke's solution containing 33 mM of KCl and no $CaCl_2$ and were stimulated with a solution containing 33 mM of KCl and 2.2 mM of $CaCl_2$. In both solutions, the concentration of NaCl was reduced by a corresponding amount. Na^+-deprivation-induced catecholamine release was achieved by perfusion of the glands with Na^+-free Locke's solution containing no Ca^{++} during the stimulation period. The glands were perfused with the standard solution containing no Ca^{++} before and after stimulation.

In controls, the adrenals were stimulated in the absence of any inhibitory drug such as halothane, tetrodotoxin, and verapamil. In the experiments performed to examine the effect of halothane, the adrenals were perfused with the solution containing halothane during a period starting 15 min prior to the second stimulation and lasting until 3 min after the second stimulation. In order to achieve equilibration of halothane in the solution, halothane was added to the aeration mixture through a calibrated vaporizer for 10 min prior to the start of perfusion. The concentration of halothane in the perfusate was measured by a gas chromatographic method.[12] The equilibration of halothane in the solution was achieved within 7 min, and the measured halothane concentrations agreed with predicted values when the solubility coefficient for the solution was taken as 0.75.[12] The third stimulation was done in the absence of halothane in order to examine the reversibility of the effect of halothane.

The inhibitory effects of tetrodotoxin and verapamil on catecholamine release induced by various secretagogues also were examined. These drugs were added to the perfusate during a period starting 10 min prior to the second stimulation and lasting until 3 min after the second stimulation. Other conditions were the same as the controls.

The effluent from the adrenals was collected into glass tubes kept on ice at 1-min intervals starting 1 min prior to the stimulation and lasting for 4 min. Catecholamine content was measured by the trihydroxyindole method of Euler and Floding[13] with modifications using two filter sets[14] without further purification on alumina.[15] In most instances, a 0.2-ml aliquot with 1.0 ml of 1 M acetate buffer (final *p*H 6.3) was used for the assay. Epinephrine and norepinephrine, 0.1 μg, exogenously added to the reaction mixture was measured as 0.098 ± 0.003 μg (mean ± SE, n = 3) and 0.100 ± 0.001 μg (n = 3), respectively. In all cases, it was ascertained that the drugs used did not interfere with the assay. Stimulant-induced catecholamine release was calculated as the difference between spontaneous catecholamine release and release during stimulation, and catecholamine release was expressed by the second stimulation value as per cent of the first stimulation value in order to minimize the individual variations.

Student's *t* test was used for statistical evaluation of the data. *P* values less than 0.05 were considered significant. The drugs used were nicotine (Tokyo Kasei), acetylcholine chloride (Wako Pure Chemical), muscarine chloride (Sigma), veratridine sulfate (Nakarai Chemical), tetrodotoxin (Sankyo), and verapamil (Eiasi).

Results

Spontaneous release of catecholamines during the 1-min period prior to stimulation amounted to 0.9 ± 0.6 μg/min (mean ± SE, n = 8). In controls, acetylcholine-induced catecholamine release during the 3-min collection period on the first stimulation was 20.8 ± 2.3 μg (n = 8); the amounts released on the second and third stimulations were 88.1 ± 2.2% and 79.2 ± 2.9%, respectively, of that released during the initial stimulation. Figure 1 shows the time course of the experiment and the effects of 2% halothane on acetylcholine-induced catecholamine release. Acetylcholine-induced catecholamine release was inhibited by halothane reversibly, since the release on the third stimulation without halothane was restored to the control level.

Figure 2 shows the dose-response curves of the inhibitory effects of halothane on catecholamine release induced by the agonists. Nicotine, 5 μM, and muscarine, 20 μM, were almost comparable to acetylcholine, 20 μM, in terms of total catecholamine release, because the initial release by nicotine and muscarine was 22.8 ± 3.3 μg (n = 4), and 18.4 ± 2.8 μg (n = 7), respectively. The concentrations of halothane exhibiting 50% inhibition

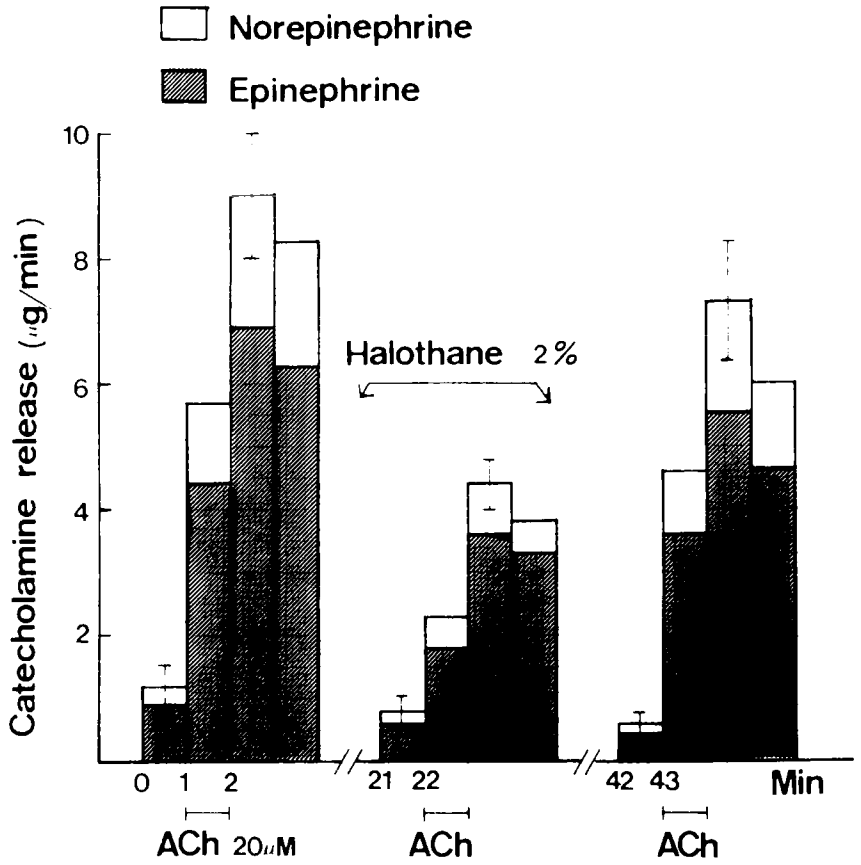

FIG. 1. Time course of the experiments to examine the effect of halothane on acetylcholine-induced catecholamine release from the adrenal medulla. The ordinate represents the rate of catecholamine release during a 1-min period (n = 4, mean ± SE for total catecholamines). Isolated dog adrenals were perfused retrogradely with a modified Locke's solution at 37° C and stimulated three times with acetylcholine. The stimulation period was 1 min, followed by 20-min recovery intervals. Halothane was added to perfusate during the period from 15 min prior to the second stimulation and lasting until 3 min after the second stimulation.

were 0.8% for nicotine, 1.9% for acetylcholine, and 2.8% for muscarine, respectively.

Table 1 shows the release of epinephrine and norepinephrine induced by the agonists. It is characteristic that catecholamine release induced by nicotine or acetylcholine consists of a high proportion of norepinephrine in comparison with that induced by muscarine.[9] Furthermore, the norepinephrine/epinephrine ratio of catecholamines released by nicotine or muscarine remained unchanged when total catecholamine release was decreased to about 50% of control by halothane. On the contrary, the norepinephrine/epinephrine ratio of catecholamines released by acetylcholine was reduced significantly by the anesthetic.

Table 2 shows per cent inhibition by halothane (1.5%) of nicotine-, veratridine-, acetylcholine-, $CaCl_2$-, Na^+-deprivation-, and muscarine-induced catecholamine release from the adrenals. The concentration of the stimulants were nearly equipotent regarding catecholamine release, except for Na^+-deprivation. Catecholamine release induced by each stimulant was inhibited by halothane in a markedly different degree.

Catecholamine releases from adrenals perfused with Na^+-free solution and stimulated by acetylcholine, nic-

TABLE 1. Effects of Halothane on the Norepinephrine (NE)/Epinephrine (E) Ratio of Catecholamines Released by Acetylcholine, Nicotine, and Muscarine

Stimulants	Catecholamine Release on the Second Stimulation (μg/min, Mean ± SE)*					
	Control			Halothane†		
	E	NE	NE/E	E	NE	NE/E
Acetylcholine 20 μM	4.6 ± 0.4	1.5 ± 0.2	0.32 ± 0.02 (n = 8)	2.2 ± 0.2	0.5 ± 0.0	0.23 ± 0.01‡ (n = 4)
Nicotine 5 μM	4.9 ± 0.7	1.9 ± 0.3	0.39 ± 0.03 (n = 4)	2.2 ± 0.3	0.7 ± 0.1	0.34 ± 0.03 (n = 4)
Muscarine 20 μM	4.5 ± 0.5	0.8 ± 0.1	0.18 ± 0.01 (n = 7)	2.0 ± 0.2	0.4 ± 0.1	0.20 ± 0.01 (n = 4)

* The second stimulations were performed in the presence or absence of halothane.

† The concentrations of halothane were chosen to cause about 50% inhibition of catecholamine release induced by each of the agonists, *i.e.*, 2.0% for acetylcholine, 0.8% for nicotine, and 3.0% for muscarine.

‡ Statistically significant change ($P < 0.05$) from the control value.

otine, or muscarine were 17.2 ± 3.1 μg (mean ± SE, n = 3), 15.9 ± 2.8 μg (n = 3), and 17.6 ± 2.3 μg (n = 5), respectively. As shown in figure 3, in the Na^+-free solution also, halothane inhibited nicotine-induced catecholamine release completely, and acetylcholine- and muscarine-induced catecholamine release by 37% and 14%, respectively.

In order to clarify the role of ion channels in the nicotinic and muscarinic responses, the effects of tetrodotoxin and verapamil on the catecholamine release were examined. As shown in figure 4, 0.6 μM of tetrodotoxin abolished veratridine-induced catecholamine release almost completely, and decreased nicotine-induced catecholamine release slightly, whereas it had no effect on either muscarine- or acetylcholine-induced catecholamine release. On the other hand, 10 μM of verapamil showed an inhibitory effect on the catecholamine release induced by these agonists, *i.e.*, the response to acetylcholine was inhibited by 65%, that to nicotine by 79%, and that to muscarine by 26%, respectively (fig. 5).

Discussion

The results have shown that halothane at clinical concentrations selectively inhibits catecholamine release induced by stimulation of the nicotinic receptors of the dog adrenal medulla, whereas muscarinic-receptor-mediated catecholamine release is inhibited by halothane only at high concentrations. In the dog adrenal medulla, the end results of stimulation of the medullary cells via both the nicotinic and muscarinic receptors are functionally the same, *i.e.*, evoking catecholamine release. However, catecholamine release via the muscarinic response lasts longer than that via the nicotinic response.[9] This phenomenon is in accordance with the proposition by Purves[16] that the physiologically important difference between the two types of receptors is their speed of response.

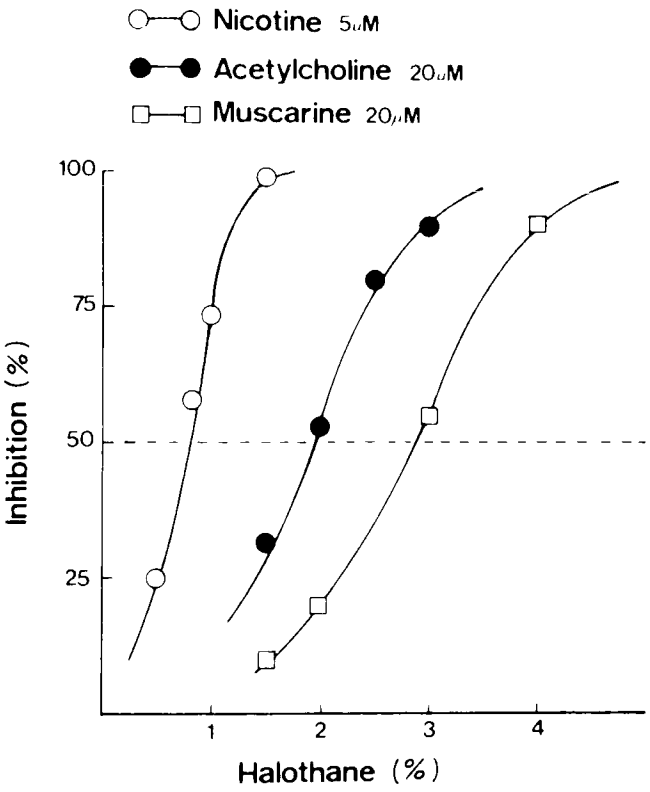

FIG. 2. The dose-response curves with respect to the inhibitory effects of halothane on the agonist-induced catecholamine release from the adrenal medulla. The second stimulations were performed in the presence or absence of halothane and per cent inhibition by halothane was calculated by comparison with the control condition. Each point represents the value determined by at least three experiments.

The mechanisms involved in the inhibitory effect of halothane on the catecholamine release and in the differential effects of halothane on the nicotinic and muscarinic receptors might be advanced as follows.

1) Halothane might inhibit the intracellular process of exocytosis. This mechanism is probably involved at

TABLE 2. Effects of Halothane 1.5% on Catecholamine Release from the Adrenal Medulla Induced by Various Stimulants

Stimulants		Catecholamine Release: First Stimulation (μg/min, Mean ± SE)	Catecholamine Release: Second Stimulation (Per Cent of First Stimulation Value, Mean ± SE) Control	Catecholamine Release: Second Stimulation (Per Cent of First Stimulation Value, Mean ± SE) Halothane 1.5%	Inhibition by Halothane* (Per Cent)
Muscarine	20 μM	6.1 ± 0.9 (n = 7)	86.3 ± 2.1 (n = 7)	78.1 ± 2.2† (n = 5)	9.5
Na^+-deprivation		3.2 ± 0.5 (n = 5)	78.5 ± 3.0 (n = 5)	67.9 ± 2.7† (n = 5)	10.1
$CaCl_2$	2.2 mM	6.3 ± 1.0 (n = 6)	83.7 ± 4.0 (n = 3)	65.8 ± 3.3† (n = 3)	21.4
Acetylcholine	20 μM	6.9 ± 0.7 (n = 8)	88.1 ± 2.2 (n = 8)	59.5 ± 2.4‡ (n = 4)	32.5
Veratridine	100 μM	5.8 ± 0.8 (n = 6)	72.9 ± 6.5 (n = 3)	7.5 ± 1.5‡ (n = 3)	89.7
Nicotine	5 μM	7.6 ± 1.1 (n = 4)	89.6 ± 3.5 (n = 4)	1.4 ± 0.6‡ (n = 3)	98.5

* The second stimulation was performed in the presence or absence of halothane, and per cent inhibition by halothane was calculated in comparison with the control condition.

† $P < 0.05$.

‡ $P < 0.01$, compared with control.

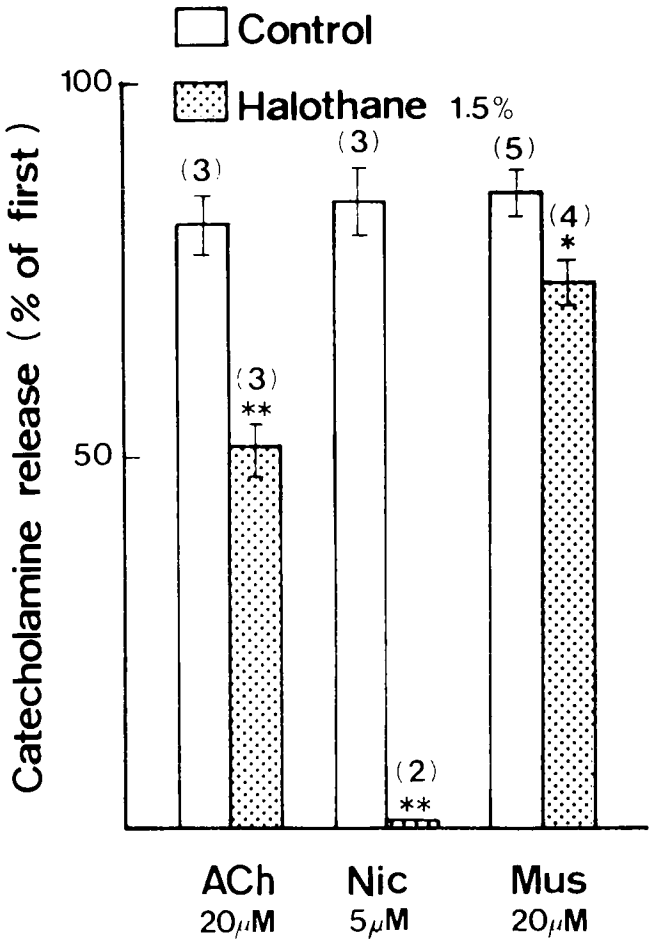

FIG. 3. Effects of halothane on catecholamine release induced by acetylcholine (ACh), nicotine (Nic), and muscarine (Mus) in the Na^+-free perfusate (mean ± SE). NaCl in the perfusate was replaced by osmotically equivalent concentrations of sucrose. The ordinate represents the second stimulation value compared with the first stimulation value. The number of experiments is indicated by the figure in parentheses. $*P < 0.05$; $**P < 0.01$, compared with control.

least in part because Na^+-deprivation-induced catecholamine release is inhibited slightly by halothane. It has been demonstrated that catecholamines could be released by exocytosis during Na^+ deprivation in spite of the absence of extracellular Ca^{++}.[17] The result would indicate that the exocytosis might be inhibited about 10% in the presence of 1.5% halothane. As exocytosis is considered to be a common process of catecholamine release, the following interpretation seems possible. At the concentration of 1.5%, halothane shows 33% inhibition of acetylcholine-induced catecholamine release; the inhibition of the exocytosis seems to contribute about 10%. It appears likely that the inhibition of the exocytosis might be partly due to the inhibition of the catecholamine storage mechanism of the chromaffin granules, followed by a decrease in the catecholamine content of the granules.[18,19]

Concerning the inhibition of exocytosis, Muldoon *et al.*[20] and Roizen *et al.*[21] stimulated postganglionic sympathetic neurons electrically and found that 1% halothane inhibited the catecholamine release by 27 and 57%, respectively. The difference in the per cent inhibition of exocytosis between our results and their results might be attributed to the differences of species and organs, or to the possibility that the electrically stimulated release of catecholamines might involve the activation of ionic channels in addition to the intracellular process of exocytosis.

2) Halothane might block Na^+ channels, resulting in abolishing acetylcholine-induced depolarization of chromaffin cells. It has been shown that acetylcholine produces a membrane depolarization of chromaffin cells[22,23] and that this depolarization was due to inward currents of Na^+ and Ca^{++}, with Na^+ contributing more.[22] Although it was suggested that halothane might block Na^+ channels,[24] this mechanism is unlikely in the

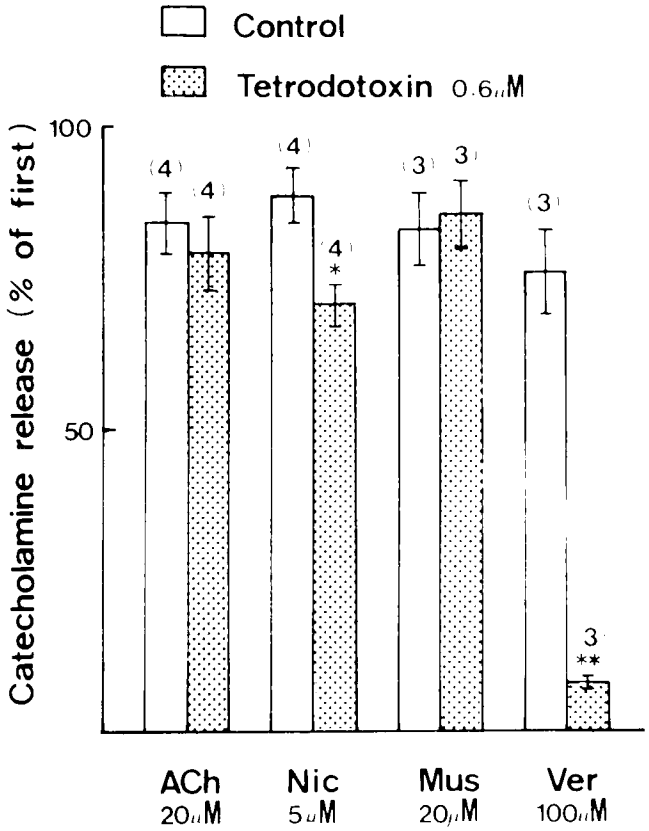

FIG. 4. Effects of tetrodotoxin on catecholamine release from the adrenal medulla induced by acetylcholine (ACh), nicotine (Nic), muscarine (Mus), and veratridine (Ver) (mean ± SE). The second stimulations were performed in the presence or absence of tetrodotoxin. The ordinate represents the second stimulation value compared with the first stimulation value. The number of experiments is indicated by the figure in parentheses. $*P < 0.05$; $**P < 0.01$, compared with control.

chromaffin cells for the following reasons. Although tetrodotoxin abolished veratridine-induced catecholamine release completely, it decreased nicotine-induced catecholamine release only slightly, whereas it had no effect on either muscarine- or acetylcholine-induced catecholamine release. Furthermore, release of catecholamines by acetylcholine was obtained in Na^+-free media, and was inhibited by halothane to the same extent as in Na^+-containing media. These results suggest that veratridine and at least the nicotinic receptors seem to activate fast Na^+ channels in chromaffin cells comparable to those of neuronal axons. However, they do not appear to play an essential role in acetylcholine-induced catecholamine release. This finding is in agreement with Kirpekar and Prat[25] and Ritchie.[26] Accordingly, the site of action of halothane seems not to be the Na^+ channels of chromaffin cells.

3) Halothane might block Ca^{++} channels in the membranes of chromaffin cells. It has been well-established that acetylcholine-induced catecholamine release is dependent on extracellular Ca^{++}, and that the entry of Ca^{++} into the cell triggers exocytotic secretion of catecholamines. Recently, it has been demonstrated that there are two pathways for Ca^{++} entry during stimulus-secretion coupling, *i.e.*, a voltage-dependent Ca^{++} channel and the acetylcholine-receptor-linked channel.[26,27] The results show that the voltage-dependent Ca^{++} channel is resistant to the action of halothane because $CaCl_2$-induced catecholamine release in high KCl solution[27] was not inhibited markedly by the anesthetic. On the other hand, the agonist-induced catecholamine releases in the Na^+-free media were inhibited by the anesthetic to almost the same extent as those in the Na^+-containing media. In Na^+-free media, nicotine and muscarine probably stimulate catecholamine release by Ca^{++} influx through the receptor-linked channel.[27] Therefore, it is believed probable that there are two types of acetylcholine-receptor-linked Ca^{++} channels, and that halothane blocks the nicotinic-receptor-linked Ca^{++} channel, but has no effect on the muscarinic-receptor-linked Ca^{++} channel. The hypothesis that there are two types of acetylcholine-receptor-linked Ca^{++} channels is supported by the fact that there is a difference in the sensitivity to the Ca^{++} channel blocker,[28] verapamil, between nicotine- and muscarine-induced catecholamine release.

4) Agonist-receptor interaction might be influenced by halothane, *i.e.*, acetylcholine-nicotinic receptor binding is blocked by halothane, whereas acetylcholine-muscarinic receptor binding is unaffected. Göthert *et al.*[29] suggested that this might be the mechanism involved in the inhibitory effect of halothane on catecholamine release from the bovine adrenal medulla. However, this mechanism is unlikely because of the following reasons.

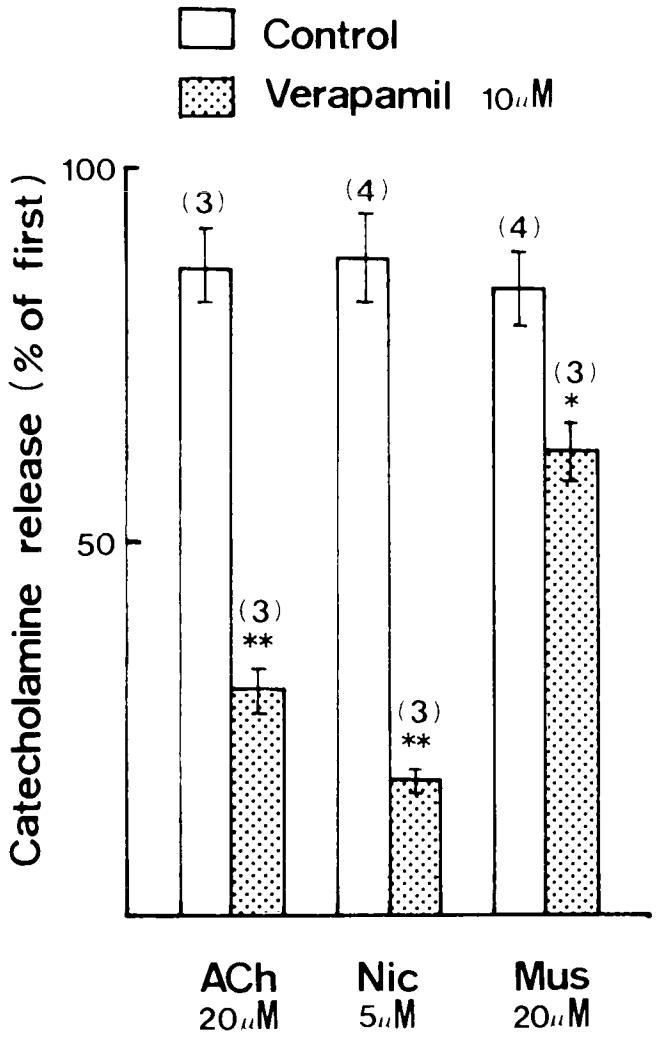

FIG. 5. Effects of verapamil on catecholamine release from the adrenal medulla induced by acetylcholine (ACh), nicotine (Nic), and muscarine (Mus) (mean ± SE). The second stimulation was performed in the presence or absence of verapamil. The ordinate represents the second stimulation value compared with the first stimulation value. The number of experiments is indicated by the figure in parentheses. $**P < 0.05$; $*P < 0.01$, compared with control.

1) The inhibitory effects of halothane are not a receptor protein-specific phenomenon. Catecholamine release induced by veratridine, which is known as Na^+ ionophore,[25] is also blocked markedly. 2) There is no evidence for the inhibition by halothane of ligand-nicotinic receptor binding; on the contrary, general anesthetics do not affect the binding of *d*-tubocurarine to the functional and nondesensitized receptors at the isolated guinea pig lumbrical muscle.[30] More recently, Young *et al.*[31] have demonstrated that volatile anesthetics facilitate a structural transition of membrane-bound acetylcholine receptor protein induced by the agonist carbamoylcholine, but do not inhibit the binding of the ligand to the nicotinic receptor. 3) Recent studies

have confirmed the entirely nicotinic nature of the acetylcholine receptors in the bovine adrenal medulla.[15,32] Therefore, bovine adrenals are not considered a suitable model for examining the cellular responses mediated by nicotinic and muscarinic receptors.

In the present experiment, equivalence of various stimulants were not determined by taking detailed dose-response curves. Therefore, it is considered possible that the dose *vs.* release curves might not be exactly parallel among the stimulants, and accordingly, further investigations might be necessary to get the final answers.

In conclusion, halothane at clinical concentrations selectively inhibits catecholamine release induced by stimulation of the nicotinic receptors of the dog adrenal medulla, whereas muscarinic-receptor-mediated catecholamine release is inhibited by halothane only at high concentrations. The mechanism involved in the differential effects of halothane on the responses mediated by these receptors in the adrenal medulla might be the susceptibility to halothane of the Ca^{++} channels that are linked to each receptor. An inhibition of exocytosis might be also indicated as part of the effect of the anesthetic.

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References

1. Weakly JN: Effect of barbiturates on "quantal" synaptic transmission in spinal motoneurons. J Physiol (Lond) 204:63–77, 1969
2. Proctor WR, Weakly JN: A comparison of the presynaptic and postsynaptic actions of pentobarbitone and phenobarbitone in the neuromuscular junction of the frog. J Physiol (Lond) 258:257–268, 1976
3. Barker JL: Selective depression of postsynaptic excitation by general anesthetics. Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia. Edited by Fink BR. New York, Raven Press, 1975, pp 135–153
4. Smaje JC: General anaesthetics and the acetylcholine sensitivity of cortical neurones. Br J Pharmacol 58:359–366, 1976
5. Wall PD: The mechanisms of general anesthesia. ANESTHESIOLOGY 28:46–52, 1967
6. Alper MH, Freish JH, Flacke W: The effects of halothane on the responses of cardiac sympathetic ganglia to various stimulants. ANESTHESIOLOGY 31:429–436, 1969
7. Catchlove RFH, Krnjević K, Maretić H: Similarity between effects of general anesthetics and dinitrophenol on cortical neurones. Can J Physiol Pharmacol 50:1111–1114, 1972
8. Krnjević K: Is general anesthesia induced by neuronal asphyxia? Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia, Edited by Fink BR. New York, Raven Press, 1975, pp 93–98
9. Tsujimoto A, Nishikawa T: Further evidence for nicotinic and muscarinic receptors and their interaction in dog adrenal medulla. Eur J Pharmacol 34:337–344, 1975
10. Robinson RL: Stimulation of the release of catecholamines from isolated adrenal glands by tyramine. J Pharmacol Exp Ther 151:55–58, 1966
11. Sumikawa K, Amakata Y, Kashimoto T, Izumi F: Effects of cyclopropane on catecholamine release from bovine adrenal medulla. ANESTHESIOLOGY 53:49–52, 1980
12. Renzi F, Waud BE: Partition coefficients of volatile anesthetics in Krebs' solution. ANESTHESIOLOGY 47:62–63, 1977
13. Euler US von, Floding I: A fluorimetric method for differential estimation of adrenaline and noradrenaline. Acta Physiol Scand 33 (Suppl 118):45–56, 1955
14. Euler US von, Lishajko F: Improved technique for the fluorimetric estimation of catecholamines. Acta Physiol Scand 51:348–356, 1961
15. Schneider AS, Herz R, Rosenheck K: Stimulus-secretion coupling in chromaffin cells isolated from bovine adrenal medulla. Proc Natl Acad Sci USA 74:5036–5040, 1977
16. Purves RD: Function of muscarinic and nicotinic acetylcholine receptors. Nature 261:149–151, 1976
17. Lastowecka A, Trifaró JM: The effect of sodium and calcium ions on the release of catecholamines from the adrenal medulla: Sodium deprivation induces release by exocytosis in the absence of extracellular calcium. J Physiol (Lond) 236:681–705, 1974
18. Bangham AD, Mason WT: Anesthetics may act by collapsing *p*H gradients. ANESTHESIOLOGY 53:135–141, 1980
19. Sumikawa K, Amakata Y, Yoshikawa K, Kashimoto T, Izumi F: Catecholamine uptake and release in isolated chromaffin granules exposed to halothane. ANESTHESIOLOGY 53:385–389, 1980
20. Muldoon SM, Vanhoutte PM, Lorenz RR, Van Dyke RA: Venomotor changes caused by halothane acting on the sympathetic nerves. ANESTHESIOLOGY 43:41–48, 1975
21. Roizen MF, Thoa NB, Moss J, Kopin IJ: Inhibition by halothane of release of norepinephrine, but not of dopamine-β-hydroxylase, from guinea-pig vas deferens. Eur J Pharmacol 31:313–318, 1975
22. Douglas WW, Kanno T, Sampson SR: Influence of the ionic environment on the membrane potential of adrenal chromaffin cells and on the depolarizing effect of acetylcholine. J Physiol (Lond) 191:107–121, 1967
23. Brandt BL, Hagiwara S, Kidokoro Y, Miyazaki S: Action potentials in the rat chromaffin cell and effects of acetylcholine. J Physiol (Lond) 263:417–439, 1976
24. Trudell JR: A unitary theory of anesthesia based on lateral phase separations in nerve membranes. ANESTHESIOLOGY 46:5–10, 1977
25. Kirpekar SM, Prat JC: Release of catecholamines from perfused cat adrenal gland by veratridine. Proc Natl Acad Sci USA 76:2081–2083, 1979
26. Ritchie AK: Catecholamine secretion in a rat pheochromocytoma cell line: Two pathways for calcium entry. J Physiol (Lond) 286:541–561, 1979
27. Stallcup WB: Sodium and calcium fluxes in a clonal nerve cell line. J Physiol (Lond) 286:525–540, 1979
28. Arqueros I., Daniels AJ: Analysis of the inhibitory effect of verapamil on adrenal medullary secretion. Life Sci 23:2415–2422, 1978
29. Göthert M, Dorn W, Loewenstein I: Inhibition of catecholamine release from the adrenal medulla by halothane. Site and mechanism of action. Naunyn Schmiedebergs Arch Pharmacol 294:239–249, 1976
30. Waud BE, Waud DR: The effects of diethyl ether, enflurane, and isoflurane at the neuromuscular junction. ANESTHESIOLOGY 42:275–280 1975
31. Young AP, Brown FF, Halsey MJ, Sigman DS: Volatile anesthetic facilitation of *in vitro* desensitization of membrane-bound acetylcholine receptor from Torpedo California. Proc Natl Acad Sci USA 75:4563–4567, 1978
32. Sumikawa K, Kashimoto T, Izumi F, Yoshikawa K, Amakata Y: Effects of muscle relaxants on catecholamine release from adrenal medulla. Res Commun Chem Pathol Pharmacol 25:205–214, 1979