Anesthesiology
69:500–506, 1988

Effects of Halothane on Acetylcholine Release and Sympathetic Ganglionic Transmission

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Depression of synaptic transmission is one of the actions common to many general anesthetics. They appear to act primarily on the chemical transmission process itself without affecting the conduction of impulses in nerve axons. The objective of the present study was to directly examine the effects of halothane on the release of acetylcholine and overall ganglionic transmission. The effects of halothane on ganglionic transmission were studied in the stellate ganglion of the cat *in vitro*. The preganglionic nerves of the stellate ganglion were stimulated electrically to generate threshold and suprathreshold evoked potentials in the postganglionic nerves. The picomole levels of released acetylcholine in the superfusate were determined with a radioenzymatic method using ^{32}P-ATP following a 5-min preganglionic stimulation before and after the addition of 0.28 and 0.59 mM halothane (vaporizer settings of 1% and 2%, respectively). Halothane at both concentrations: 1) depressed sympathetic ganglionic transmission induced by either threshold or suprathreshold stimulation of the preganglionic nerves; and 2) caused a dose-dependent decrease in acetylcholine release during threshold stimulation with no change in neurotransmitter release during suprathreshold stimulation. In summary, the interruption of synaptic transmission by halothane involves at least two mechanisms: 1) decrease in acetylcholine release during low level of synaptic transmission; and 2) postsynaptic decrease in sensitivity to acetylcholine during low and high levels of synaptic transmission. (Key words: Anesthetics, volatile: halothane. Neurotransmitters: acetylcholine. Sympathetic nervous system: evoked potentials; ganglionic transmission; stellate ganglion, synaptic.)

HALOTHANE has been shown to cause significant impairment of cardiovascular stability and reflex regulation.[1–7] Sympathetic efferent nerve activity in the peripheral sympathetic nervous system is attenuated by therapeutic concentrations of halothane, which are below the anesthetic concentrations needed to inhibit axonal transmission.[8–10] Synaptic transmission in the sympathetic ganglia has also been shown to be sensitive to halothane.[9,10] We reported that halothane blocks neuronal transmission across the synapse without disrupting nerve impulse conduction in the afferent or efferent axons.[9] In addition to halothane, a wide range of anesthetics have been found to depress excitatory synaptic transmission in both central and peripheral nervous systems, although there is no general effect common to all synapses and all anesthetics.[6,11,12]

The mechanism by which halothane alters synaptic function is not well understood; however, it may involve: 1) inhibition of neurotransmitter release into the synaptic clef; 2) altered binding of released transmitter to specific receptors on the postsynaptic membrane; and 3) a nonreceptor-mediated inhibition of postsynaptic membrane excitability. Most evidence suggests that the depressant effect of halothane on synaptic transmission does occur at multiple sites including preganglionic release of neurotransmitter as well as postjunctional effects on membranes and receptors.[6,9,13,14] The evidence that halothane inhibits neurotransmitter release in sympathetic ganglia is, however, indirect and must be investigated with more direct studies by measuring acetylcholine (ACh) release simultaneously with measurements of ganglionic transmission.

The purpose of the present study was to directly examine the effects of halothane on the release of ACh and ganglionic transmission. The action of halothane on these processes was investigated in the isolated stellate ganglion of the cat.

Methods

PREPARATION

After approval by the Medical College of Wisconsin Institutional Animal Care and Use Committee, 16 adult cats of either sex weighing 2–4 kg were injected intraperitoneally with atropine (0.3 mg/kg) and 10 minutes later with physostigmine salicylate (1 mg/kg). ACh esterase inhibitor was given to prevent ACh hydrolysis. After 5 min all cats were anesthetized with 5% halothane. Left thoracotomy was performed and the stellate ganglion was quickly dissected and cut along with connecting nerves and placed into a bath containing oxygenated Krebs solution. The organ bath was recirculated with Krebs solution equilibrated with 97% O_2–3% CO_2 and maintained at 37° C and *p*H 7.4. The Krebs solution had the following composition (in mM): Na^+, 137; K^+, 3.0; Ca^{++}, 2.5; Mg^{++},

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Received from the Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin. Accepted for publication April 25, 1988. Supported in part by grants from National Heart, Lung and Blood Institute to Dr. Bosnjak (HL 34708 and HL01901) and the Medical Research Service of the Veterans Administration. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Las Vegas, October 1986.

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1.2; Cl^-, 134; HCO_3^-, 15.5; HPO_4^{--}, 1.2; and glucose, 11.5.

With the aid of a dissecting microscope the ganglion was desheathed from the surrounding connective and fibrous tissue. The stellate ganglion (SG) was then pinned to a silastic rubber floor (Sylgard, Dow Corning, Midland, MI) with fine tungsten wire pins. The preganglionic (T_1–T_3 rami communicantes, descending sympathetic chain) and postganglionic (ventral and dorsal ansae subclavia and the stellate cardiac nerve) nerves were placed on bipolar tungsten electrodes for electrical stimulation and extracellular recordings, respectively.

Evoked Potential Measurements

Ganglionic transmission was measured by recording the compound action potential (CAP) extracellularly from the postganglionic nerve (stellate cardiac nerve). The bipolar tungsten electrodes were connected to stimulator isolation units (WP Instruments, New Haven, CT). The preganglionic nerves to the SG were placed on bipolar electrodes and stimulated with square wave monophasic pulses of 1 ms duration at 5 Hz and a constant current of up to 15 mA, using the current necessary to generate a threshold (T) and suprathreshold (2 × T) evoked potential in the postganglionic nerves.

The CAP was displayed and measured on a digital oscilloscope (Nicolet Instruments, Madison, WI) and analyzed for the maximal amplitude in millivolts. The use of the maximal amplitude is valid and accurate only if there is no time dispersion that may decrease the amplitude of the compound action potential without decreasing the number of fibers that are activated. We have previously shown[9] that the depression of the synaptic potentials by halothane was not due to a direct action on the sympathetic nerve fibers. Conduction velocities along the preganglionic and postganglionic nerve fibers were not affected by halothane as indicated by orthodromically and antidromically evoked potentials, respectively. The recording electrodes for the extracellular recordings were connected to a high input impedance preamplifier and an amplifier with high and low pass filters (100–1,600 Hz). Utilizing intracellular recordings from neurons of the stellate ganglia of the guinea pig,[9] we have shown that intracellular responses to supramaximal electrical orthodromic stimulation of the preganglionic nerves was more resistant to even higher concentrations of halothane, as compared to the action potentials evoked during threshold stimulation. We have applied a similar protocol to this study for the measurements of ACh to separate presynaptic and postsynaptic effects. The current used for nerve stimulation was dependent on the size of the nerve trunk and the amount of adherent connective tissue. Current strength was increased gradually until the maximum evoked potential was achieved. We measured the amplitude of the CAP at a fixed latency from the stimulus artifact. The exact latency chosen was dependent on the conduction time of the fibers. Careful checks showed that halothane did not affect the conduction velocity of the fibers, which is in agreement with our previous work utilizing intracellular recordings from the neurons of the stellate ganglion.[9] During the threshold stimulation the height of the evoked CAP was approximately one-half of the maximum evoked potential. Once the current was selected for the appropriate experiment (either threshold or suprathreshold stimulation), it remained constant throughout each experiment.[15] Following the exposure to halothane, postcontrol measurements of the evoked potentials have indicated recovery of its maximal height to be within 5% of the original control.

For some postganglionic nerves CAP is predominantly synaptically induced (stellate cardiac nerve and dorsal ansa subclavia), whereas other nerves (ventral ansa subclavia) carry fibers that do not synapse in the ganglion but traverse the ganglion as preganglionic fibers that synapse in the middle caudal or superior cervical ganglion. Synaptically induced CAP was abolished by hexamethonium chloride (3 mM) at the end of each experiment to verify that the observed changes in the CAP amplitude were related to synaptically mediated events.

ACh Determination

The amount of ACh released from the isolated stellate ganglion into the 1.0 ml superfusate bath was determined by modification of the radioenzymatic method of McCaman and Stetzler.[16] Because we determined the amount of ACh released into the Krebs superfusate and not in ganglionic tissue, the organic extraction procedure of that method was omitted. A 0.1-ml aliquot of the superfusate around the isolated ganglion was lyophilized and incubated in 10 μl of a solution containing choline kinase and ATP to convert released choline to choline phosphate. To hydrolyze the released ACh and convert the ACh-derived choline to ^{32}P-choline phosphate, we then added ACh esterase and ^{32}P-ATP. The ^{32}P-choline phosphate was separated from unreacted ^{32}P-ATP by ion exchange chromatography and used as an estimate of the amount of ACh released from the isolated ganglion into the 1-ml superfusate bath. To prevent ACh hydrolysis by ganglionic AChE, physostigmine salicylate was included in the superfusate. Because of the presence of physostigmine salicylate, we found it necessary to use eel AChE in the assay at a concentration 30 times greater than that originally used by McCaman and Stetzler.[16] Optimization of the physostigmine concentration to maximize released ganglionic ACh and minimize inhibition of the radioenzymatic assay is described in "Results."

TABLE 1. Effect of Physostigmine Salicilate on ACh Release

Physostigmine salicilate (μg/ml)	0	1.5	3.0	6.0
ACh release (pmol/0.1 ml)				
0 Hz	0.3 ± 0.1	1.0 ± 0.1*	2.6 ± 0.2*	2.7 ± 0.4*
5 Hz	0.6 ± 0.1	4.5 ± 0.4*†	12.5 ± 0.7*†	13.6 ± 2.3*†

* $P < 0.05$ *versus* 0 μg/ml. † $P < 0.05$ *versus* 0 Hz.

For analysis of released ACh, duplicate 100 μl aliquots of superfusate were removed from the 1-ml ganglion bath just before (pre) and just after (post) 5-min periods using the following stimulation frequency: 0 Hz, 5 Hz, 5 Hz, 0 Hz, and 5 Hz. Between these 5-min periods the ganglion was washed for 5 min by changing the 1.0-ml bath superfusate each minute. This schedule was repeated in the presence of halothane at concentration of 0.28 or 0.59 mM in random order (vaporizer settings of 1 and 2%, respectively). Relatively low bath concentrations of halothane for these settings were due to a very low recirculating bath volume. ACh release was averaged for control and postanesthetic stimulation periods. The 100-μl aliquots were placed in a 0.3 ml conical polypropylene microtube and immediately frozen on dry ice and then lyophilized. The dried residue was redisolved in 10 μl of mixture containing 0.1 M sodium phosphate buffer (*p*H 8.0), 180 mM ATP, 3 mM $MgCl_2$, and 0.75 mg of choline kinase (Sigma Chemical Co., St. Louis, MO). We then vortexed these tubes thoroughly to ensure that all the dried residue was dissolved and then incubated them at 37° C. After 15 min we placed the tubes on ice and added 5 μl of a mixture consisting of 0.1 M sodium phosphate buffer (*p*H 8.0), 0.3 units of AChE (eel, Type V, Sigma Chemical Co.), and 2.0 μCi of gamma ^{32}P-ATP (7000 Ci/mmol, New England Nuclear, Boston, MA). The tubes were incubated at 37° C, and at the end of 15 min we added 20 μl of 0.3 M barium acetate. Each tube was then mixed by vortexing and centrifuged at 100 × *g* for 5 min.

The ^{32}P-labeled choline phosphate derived from ACh released from the ganglion was separated from unreacted ^{32}P-ATP by anion exchange chromatography on Ag1-X8 resin (200–400 mesh, BioRad, Richmond, CA) as described by McCaman and Stetzler.[16] For our assay 30 μl of the final assay mixture was applied to the column, and the column was washed with 1.5 ml of freshly prepared 0.05 M NaOH. The NaOH wash from the column was collected in 5-ml plastic scintillation vials. We placed the vials in the scintillation spectrometer (Packard Model 4530, Downers Grove, IL) and estimated the amount of ^{32}P from the beta radiation. Counting efficiency was about 30%.

Standard curves containing 0.5 to 10 pmol/0.1 ml of ACh were prepared in the superfusate used for the isolated ganglion preparation. Counts per minute for each concentration of ACh, less the counts per minute obtained from blanks containing no ACh, were plotted against the concentration of ACh in the standards. The standard curve was linear throughout the concentration range studied with a lower limit of detectability of 0.5 pmol/0.1 ml and a coefficient of variation for the assay of 10%. The amount of ACh released from the ganglion during the 5-min test period was determined from the difference in CPM obtained in the pre and post samples. We found this radioenzymatic assay to be sensitive to many drugs besides physostigmine. Therefore, it is essential that the artificial superfusate used to superfuse the ganglion in each experiment be used to construct the standard curve. In addition to ACh, if other neurotransmitters are coreleased, it is presumed that they will be released during the control stimulation and exert their effect, if any, upon the assay and serve as its own control.

STATISTICAL ANALYSIS

All data are presented as the mean ± SE. Differences between means were determined by analysis of variance (ANOVA) and multiple comparisons tests. The ANOVA was computed for data expressed as percent of control and for transformed percentages. Angular or inverse sine transformation of percentages was performed according to Steel and Torrie.[17] Probability of less than 0.05 was used for statistical significance.

Results

The effect of different concentrations of physostigmine on ACh release is shown in table 1. Because AChE readily hydrolyzes released ACh in the synaptic cleft, only small amounts of ACh can diffuse into the superfusate without the presence of physostigmine. With physostigmine present (1.5, 3, and 6 μg/ml), there is a dose-dependent increase in ACh release during identical suprathreshold stimulation at 5 Hz. Complete inhibition of AChE occurs when physostigmine concentration reaches 3 μg/ml with no further increase in ACh release seen with 6 μg/ml. Higher doses of physostigmine (3 and 6 μg/ml) coupled with high-frequency preganglionic stimulation lead to high ACh output, which saturates the synaptic cleft and postganglionic neurons with free ACh. Under these conditions synaptic transmission is partly or wholly blocked, although the ganglion cells may be firing rapidly and asynchronously. Because progressively higher doses of

TABLE 2. Effects of Halothane on Compound Evoked Potentials and ACh Release

	Evoked Potentials (mV)		ACh Release (pmol/0.1 ml)	
	Threshold Stimulation (8)	Suprathreshold Stimulation (8)	Threshold Stimulation (8)	Suprathreshold Stimulation (8)
Control				
0 Hz	—	—	0.9 ± 0.3	2.0 ± 0.6
5 Hz	770 ± 150	1,350 ± 340	4.5 ± 0.5‡	7.8 ± 1.1‡
0.28 mM halothane				
0 Hz	—	—	0.6 ± 0.2	1.1 ± 0.3
5 Hz	250 ± 40*	860 ± 210*	3.0 ± 0.5*‡	7.8 ± 0.8‡
0.59 mM halothane				
0 Hz	—	—	0.9 ± 0.2	1.3 ± 0.3
5 Hz	230 ± 80*	470 ± 100*†	2.6 ± 0.3*‡	8.0 ± 0.8‡

* $P < 0.05$ *versus* control.
† $P < 0.05$ *versus* 0.28 mM halothane.
‡ $P < 0.05$ *versus* 0 Hz.

physostigmine also cause a dose-dependent decrease in the slope of the ACh standard curve, we chose a lower dose of physostigmine (1.5 μg/ml) for all experiments.

Evoked Potential Responses

During the threshold stimulation of the preganglionic nerves, it appears that the number of preganglionic fibers that is stimulated is lower as compared to suprathreshold stimulation, and therefore, the summation of acetylcholine release upon the postganglionic neurons is decreased during the threshold stimulation. Therefore, the number of preganglionic fibers recruited during threshold stimulation is lower than the number of fibers that are stimulated during the suprathreshold stimulation. The number of stimulated fibers remains the same throughout the experiment as evident from the height of evoked potentials. The failure in the synaptic transmission during the threshold stimulation occurs not because of the change in the total number of recruited fibers, but because of the difference in ACh release and sensitivity of postganglionic neurons to released ACh.

As summarized in table 2, halothane at both concentrations caused a decrease in ganglionic transmission during threshold and suprathreshold stimulation. We recorded a greater decrease in the compound evoked action potential amplitude during the threshold stimulation (68%) with a low level of halothane (0.28 mM) than in the suprathreshold stimulation (36%) at the same level of halothane. This finding is not unexpected because the ganglionic transmission is more labile during the threshold stimulation. It appears that the number of preganglionic fibers is lower, and therefore, the summation of ACh release upon the postganglionic neurons is decreased during the threshold stimulation. No further drop in the transmission during threshold stimulation is seen at the higher level of halothane (0.59 mM), indicating that a critical number of synaptic failures has already occurred. At the higher dose of halothane the overall magnitude of the transmission is lower during the threshold stimulation than during the suprathreshold stimulation (470 *vs.* 230 mV). The compound evoked potentials recorded in this study from the postganglionic nerves were completely abolished by hexamethonium chloride (3 mM) administration at the end of each experiment.

ACh Release

The rate at which a superfused ganglion discharges ACh at rest is small compared with the rate at which it can discharge ACh when its preganglionic fibers are excited. Release of ganglionic ACh during the 5 min rest period (0 Hz) amounted to approximately 20% of that released during the 5 min, 5 Hz preganglionic stimulation. This continuous resting release of ACh is due to the slow diffusion of previously released ACh. It also represents a spontaneous quantal release of the transmitter, analogous to that occurring at the neuromuscular junction. The spontaneous ACh release (0 Hz, no evoked potentials) and stimulated release (spontaneous plus evoked release at 5 Hz) from the stellate ganglion is shown during the threshold and suprathreshold stimulation in table 2. Here again, during the threshold stimulation halothane caused a dose-dependent decrease in net ACh release, which along with postsynaptic effects, results in a precipitous drop in ganglionic transmission. During the suprathreshold stimulation, despite the dose-dependent fall in ganglionic transmission caused by halothane, release of the preganglionic ACh did not change significantly.

Figure 1 compares the effects of halothane concentration on the evoked potential response and ACh release during the suprathreshold stimulation and expresses them as a percentage of the control values. The spontaneous release of ACh (0 Hz) was subtracted from the evoked release (5 Hz stimulation), and expressed as percent of control (no halothane). Because the amount of ACh available for the postganglionic stimulation did not change as a function of halothane concentration, failure in ganglionic transmission must have occurred at the postjunctional

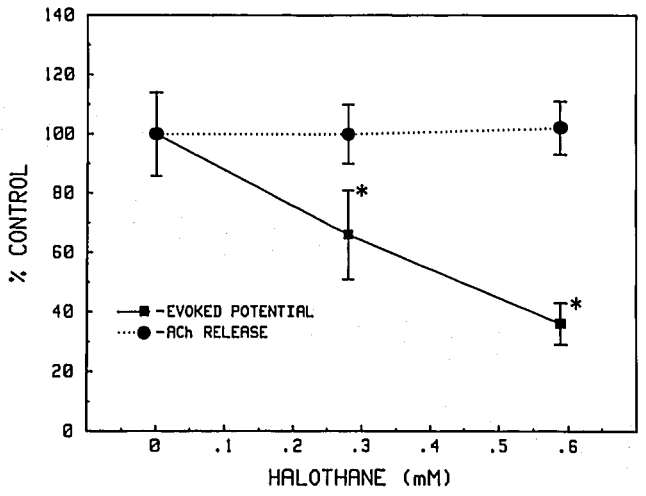

FIG. 1. Effects of halothane on ACh release and ganglionic transmission of the stellate ganglion during suprathreshold electrical stimulation of the preganglionic nerves. *$P < 0.05$ *versus* 0 mM halothane.

receptor sites. During the threshold stimulation (fig. 2) it is likely that both preganglionic decrease in ACh release and postganglionic sites are affected by halothane, contributing to a greater fall in ganglionic transmission (68% *vs.* 36%).

Discussion

Recent studies indicate that the reflex attenuation produced by halothane and other inhalational anesthetics (isoflurane and enflurane) is the result of actions at both peripheral and central sites.[7,9,12,18-20] They have shown that preganglionic sympathetic nerve activity is modestly inhibited in the presence of halothane, enflurane, or isoflurane in the intact and decerebrate cat.[19,21-23] The authors concluded that central inhibitory actions of these anesthetics could account for some of their depressant effects on the cardiovascular system but that a major part of their depressant effects on the cardiovascular system occur peripherally. They found that baseline levels and reflex-induced changes in preganglionic sympathetic nerve activity are decreased by 2 MAC halothane,[7,18] suggesting depression of central nervous system centers. In addition, a significantly greater depression of postganglionic sympathetic efferent activity versus preganglionic activity is found at 2 MAC halothane and isoflurane, indicating a ganglionic-blocking effect of both anesthetics.[7,18,24]

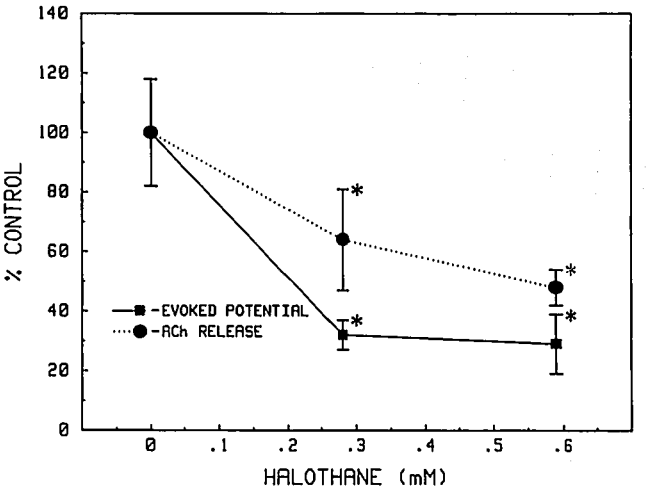

FIG. 2. Effects of halothane on ACh release and ganglionic transmission of the stellate ganglion during threshold electrical stimulation of the preganglionic nerves. *$P < 0.05$ *versus* 0 mM halothane.

These studies suggest that an inhibitory action of these general anesthetics on ganglionic transmission in peripheral sympathetic ganglia may play an important role in causing peripheral depressant effects. In a study that examined synaptic transmission in an isolated guinea pig stellate ganglion, halothane reduced synaptic transmission at concentrations that did not alter axonal conduction or the intracellular stimulation of the excitable neurons.[9] At clinically relevant concentrations halothane depressed excitatory postsynaptic potentials produced by preganglionic nerve stimulation and ultimately abolished generation of postganglionic action potentials.

Most of the experimental evidence available suggests that there is no general effect of inhalational anesthetics on synaptic transmission common to all synapses and all anesthetics. It is probable that the pharmacologic actions at peripheral ganglia induced by halothane are the results of its action at several possible sites. Halothane might inhibit sympathetic ganglionic transmission by producing inhibitory effects on axons innervating ganglion cells, inhibition of neurotransmitter synthesis, storage, release, or reuptake, inhibitory actions of postjunctional receptors, and nonreceptor-linked inhibitory actions on postjunctional membranes.

Evidence has been accumulated from studies in the cat, dog, guinea pig, rat, rabbit, and monkey[6,9,10,11,25-32] that general anesthetics have selectivity with regard to inhibition of sympathetic ganglionic transmission. At clinically useful concentrations they appear to inhibit synaptic transmission without affecting axonal transmission of impulses.[9] It was postulated that the ganglionic-blocking effects of halothane are the result of a depressant effect on responses mediated mainly through nicotinic postganglionic receptors.[11,28] It has been suggested that inhalational anesthetics may produce synaptic failure by changing the kinetics of postsynaptic conductance.[33] In addition, a decrease in neurotransmitter release has been suggested as well.[9] Because the release of neurotransmitter is calcium-dependent, it is possible that halothane may exert its ganglionic-blocking effects by altering this Ca^{++} related

process in a manner similar to its action in cardiac muscle.[34,35]

The transmission of nerve impulses in sympathetic ganglia is achieved primarily by a nicotinic action of presynaptically released ACh, which induces the f-EPSP in the adrenergic neurons of the ganglia. Besides these fast responses that directly convey individual signals, the slow synaptic potentials are also present in mammalian sympathetic ganglia.[36,37] The cellular mechanisms of the muscarinic slow responses are not fully clarified but appear to involve the contribution of cyclic nucleotides. In addition, it has been demonstrated that a number of peptides are present in the peripheral sympathetic ganglia and that they might serve as transmitters serving as modulators in regulating the membrane excitability and transmitter release. The physiologic significance and the mode of action of the slow potentials has not been fully characterized and, for some peptides, even studied. For instance, very little is known about the synthesis, storage, release, and inactivation of peptides in sympathetic ganglia. In addition, the innervation of the stellate ganglion by neuropeptide neurons is small compared to the prevertebral ganglia.[38] In summary, nicotinic cholinergic receptors are responsible for the initiation of the postsynaptic action potential. Activation of other postsynaptic receptors is followed by slow changes in the membrane potential that do not trigger spike initiation but play a modulatory role.

A preganglionic stimulus triggers release of ACh, which is due to calcium entry into the preganglionic terminal.[39] During repetitive stimulation of preganglionic fibers ACh output is greater compared with that produced by a single stimulus. This facilitation appears to be due to the activation of residual calcium remaining in the terminal from previous impulses.[40] In addition, it has been shown that presynaptic receptors are involved in the regulation of transmitter release. The ability of atropine to enhance ACh release from brain slices,[41] myenteric plexus,[42] bullfrog sympathetic ganglia,[43] and superior cervical ganglion[44] suggested the existence of a muscarinic feedback mechanism capable of regulating the neurotransmitter output. The released ACh could act through muscarinic receptors and inhibit further transmitter release. When atropine is present this inhibition is prevented and enhanced ACh release occurs.

The presynaptic sodium–potassium pump's function in the regulation of transmitter release has also been investigated.[45] Following the pump inhibition with ouabain there is an increase in the spontaneous release of ACh. The mechanism of increased transmitter release caused by pump inhibition is presumed to involve sodium loading in the presynaptic terminal.[46] The high intracellular sodium concentration would enhance sodium–calcium exchange and increase intracellular calcium and therefore stimulate ACh release. The increase in intracellular sodium concentration would be exaggerated in the presence of preganglionic stimulation.

It is most likely that halothane decreases the release of acetylcholine at threshold and suprathreshold stimulation of preganglionic nerves. However, during suprathreshold stimulation halothane might also interfere with binding of acetylcholine to the muscarinic presynaptic receptors and therefore effectively increase the acetylcholine release *via* the inhibition of a negative feedback to the presynaptic receptors. This hypothesis should be tested using presynaptic blockers.

The results of this study show that interruption of synaptic transmission by halothane could involve several sites: 1) presynaptic depression of ACh release that is most evident during the threshold electrical stimulation (low sympathetic efferent nerve activity); 2) presynaptic interference with negative feedback mechanism during high ACh release (suprathreshold stimulation); and 3) postsynaptic decrease in sensitivity to the endogenously released neurotransmitter. This postsynaptic effect is most likely present during the threshold and suprathreshold stimulation (low and high level of sympathetic efferent nerve activity) when the release of ACh is either decreased or unaffected but accompanied by a decrease in ganglionic transmission.

These results directly confirm our previous findings on the effects of halothane at the cellular level, which were analyzed by means of intracellular recordings.[9] Therefore, it is possible that halothane decreases ACh release during low level synaptic transmission along with alteration of postsynaptic receptors or membrane properties. The mechanisms involved in these inhibitory actions of halothane are unclear at the present time and are worthy of further investigation.

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