The Effects of Halothane on the Responses of Cardiac Sympathetic Ganglia to Various Stimulants

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The effects of halothane on the responses of cardiac sympathetic ganglia to intra-arterial injection of ganglion stimulants were studied in spinal dogs using heart rate as the index of ganglionic activation. Halothane alone, in concentrations of 1 and 1.5 per cent, did not affect the response to injected acetylcholine. Only after blockade of muscarinic receptors in the ganglion did halothane depress the response to acetylcholine. In addition, halothane markedly inhibited the response to DMPP, a nicotinic receptor stimulant, while leaving unchanged the response to McN-A-343, a muscarinic receptor stimulant. We conclude that halothane specifically inhibits the response to nicotinic ganglionic receptors only, and does so through an effect on the postsynaptic neuron. (Key words: Halothane; Sympathetic ganglia; Ganglion stimulants.)

In recent years, it has been firmly established that sympathetic ganglia in some species contain two pharmacologically distinct cholinergic transmission systems.1-4 In addition to the well-known nicotinic system sensitive to block by such drugs as nicotine and hexamethonium, there exists a muscarinic system, whose presence becomes evident only after nicotinic blockade and which, in turn, is blocked by the muscarinic antagonists, atropine and scopolamine. We have demonstrated in the cardiac sympathetic ganglia of the dog a well-developed muscarinic system which, when stimulated, can elicit a maximal response of the heart rate, the indicator of ganglionic activity which we have chosen to study.5,6 Brown,3 recording electrical activity in the postganglionic sympathetic nerve trunks, has shown that the normal synchronous nervous activity in response to preganglionic nerve stimulation is abolished by hexamethonium, leaving a low-amplitude, asynchronous activity, still capable of activating the heart, and abolished by atropine.

With this information as background, the effects on ganglionic transmission of ether, halothane, and cyclopropane were studied.7 All three anesthetics blocked impulse transmission through the cardiac sympathetic ganglia of the dog, with markedly greater effects on transmission via the nicotinic system than via the muscarinic system. Thus, after blockade of the muscarinic system by small doses of atropine, the anesthetics had much greater blocking effects on the ganglia than in the absence of atropine. By contrast, the anesthetics did not greatly change transmission over the muscarinic system after nicotinic block with hexamethonium.

From these experiments with preganglionic nerve stimulation, we were unable to determine whether the general anesthetics affected the presynaptic neuron or the postsynaptic neuron. Anesthetics might reduce the output of the transmitter substance from presy-
naptic nerve endings or, equally possible, they might alter the sensitivity of postjunctional cells to the normally released transmitter, or both actions might prevail.

Recently, we injected ganglion-stimulating drugs into the arterial blood supply of the cardiac sympathetic ganglia of spinal dogs in order to study directly the effects of agents acting on the postganglionic neuron. The current series of experiments was designed to test the effects of halothane on this system. We have found that halothane depresses the response to nicotinic ganglionic stimulants but does not affect the response to drugs activating muscarinic receptors. Moreover, from our experiments, we conclude that the effect of halothane must be primarily on the postjunctional neuron.

### Methods

Mongrel dogs of either sex, weighing between 10 and 18 kg, were anesthetized with sodium pentobarbital, 35 mg/kg, administered intraperitoneally or intravenously. The trachea was cannulated and the lungs ventilated with 100 per cent oxygen by means of a Starling pump. The vagus nerves were then cut in the neck and both carotid arteries ligated.

The spinal cord was exposed through the atlanto-occipital foramen and severed. The brain was pithed with a steel rod. Destruction of the brain and autonomic centers obviated the need for further anesthesia and eliminated major reflex changes in autonomic activity.

The chest was opened in the midline and the right subclavian artery dissected free of connective tissue in the superior mediastinum. A polyethylene catheter was inserted in the right brachial artery and advanced until the tip lay at the junction of the right internal mammary and subclavian arteries. Test injections of 100 µg of acetylcholine (ACh) were made as the catheter’s position was changed until a point was found at which a maximal increase in heart rate was elicited. At that point the catheter was secured in place by a tie around the subclavian artery. Since the response to ganglionic stimulants was found to be temperature-sensitive, the temperature in the area of the ganglia was monitored by a thermistor probe, and maintained between 37 and 38.5 °C. Rectal temperature, monitored with a mercury thermometer, was maintained in the same range by an infrared heat lamp connected to a rheostat. After completion of the surgical procedure, the animals were given 5 mg/kg of heparin intravenously as an anticoagulant.

Blood pressure, measured with a Statham pressure transducer connected to a polyethylene catheter in the left carotid artery, was recorded continuously on a Grass inkwriting polygraph. Blood pressure was maintained between 75 and 100 mm Hg throughout the experiment by the infusion of dextran (6 per cent in saline solution) as required to maintain circulating blood volume. Heart rate was recorded continuously by a cardiograph, as well as counted from high-speed EKG tracings when required.

The ganglion-stimulating drugs (ACh, DMPF, and McN-A-343) were administered via the catheter in the subclavian artery, the injected volume ranging from 0.1 to 0.8 ml, with the same volume always used for a given dose. We previously showed that the response to such injections was abolished by cutting the postganglionic sympathetic fibers.
but not affected by section of the pregangli-
onic fibers; the concomitant injection of India
ink resulted in marked staining of the ganglia,
and the response to the intravenous injection
of ACh resulted only in a heart-rate decrease
after a longer latency period. We infer from
these observations that the cardiac sympa-
thetic ganglia (stellate and inferior cervical)
are the sites of action of the intra-arterially
injected ganglion-stimulating drugs.

At the peak of the heart-rate response to a
given injection, the rate was counted from a
high-speed EKG tracing. The responses so
obtained were plotted against the dose of
agonist, to construct dose-response curves,
and the results were analyzed statistically
using the method of Terry-Hoeffding.

The antagonists, hexamethonium and atro-
pine, were injected intravenously via another
catheter in the left jugular vein. Halothane
was administered to the dog's lungs from a
calibrated Fluotec vaporizer and a non-
rebreathing system using oxygen as the carrier
gas.

The drugs used in this study were: penton-
barbital sodium (Nembutal, Abbott), acetyl-
choline chloride (Merck & Co.), atropine sul-
fate (Merck & Co.), hexamethonium chloride
(Mann Labs), halothane (Ayerst Labs), 1,1-
dimethylphenylpiperazinium iodide or DMPP
(Aldrich Chem.), 4-(m-chlorophenylcarbamoyl-
oxyl)-2-butynyl-trimethyl ammonium chloride
or McNA-A-343 (McNeill Labs), and dextran,
clinical grade (Pharmachem Corp.). All doses of drugs are expressed in terms of their
salts.

Results

Acetylcholine

Injection of ACh into the arterial blood
supply of the cardiac sympathetic ganglia re-
sulted in a dose-dependent increase in heart
rate (fig. 1). The threshold response oc-
curred between 10 and 25 µg and maximal
response at 400–800 µg. The ED₅₀, as deter-
mined from the control dose-response curve,
was 65 µg. When the ACh injections were
repeated following administration of either 1
or 1.5 per cent halothane for 30 to 60 min-
utes, there was no significant difference in the
response to ACh (fig. 1): the ED₅₀ at 1 per

\[ \Delta \text{heart rate} \]

\[ \text{Dose } \text{ACH (mg)} \]

Fig. 2. Average dose-response curves to ACh.
1 = controls, five experiments. 2 = after atropine,
five experiments. 3 = during 1 per cent halothane,
30–60 minutes, three experiments.

\[ \text{cent halothane was 75 µg and at 1.5 per cent} \]
\[ \text{halothane, 85 µg. From these experiments} \]
\[ \text{alone, it appears that halothane had no effect} \]
\[ \text{upon the response of the cardiac sympathetic} \]
\[ \text{ganglia to injected ACh. In addition, these} \]
\[ \text{experiments demonstrated that halothane did} \]
\[ \text{not depress the response of the postganglionic} \]
\[ \text{indicator system.} \]

Effects of Ganglion-Blocking Drugs

on the Response to ACh

Atropine. After administration of atropine
(0.1 to 1.0 mg/kg), the dose–response curve
to ACh was significantly shifted to the right
(fig. 2). The control ED₅₀ in these experi-
ments was 60 µg, compared with the ED₅₀
after atropine of 200 µg, roughly a threefold
change without significant depression of the
maximal response. Statistical analysis shows
that the difference between the two curves is
highly significant (P < 0.005).

When the ACh injections were repeated
after 30 to 60 minutes of administration of 1
per cent halothane, the dose–response curve
shifted even further to the right, with an ED₅₀
in three experiments of 650 µg, representing
an additional post-atropine shift by a factor
of three and a total shift from the control
curve by a factor of 11. Again, the depression
by halothane was highly significant (P
Fig. 3. Average dose-response curves to ACh. 1 = controls, three experiments. 2 = during 1.5 per cent halothane, 30–60 minutes, three experiments. 3 = after atropine, 0.001–0.01 mg/kg, three experiments.

<0.005) when compared with both of the earlier curves.

Figure 3 shows the results of three experiments in which the order of administration of the drugs was reversed and much smaller doses of atropine were administered. During the administration of halothane alone, there was no significant depression of the response to injected ACh. Following the administration of atropine in very small doses (1 μg/kg in one experiment and 10 μg/kg in two), the curve shifted significantly to the right; the ED_{90} of the control curve was 65 μg; after halothane, 85 μg; and with halothane and atropine, 700 μg. The combination of halothane and atropine again resulted in a total shift to the right of the dose-response curve, roughly by a factor of 11, to a position identical to that depicted in figure 2 where atropine preceded halothane.

Several important points are brought out by these results. First, halothane alone does not change the response to injected ACh. Second, after muscarinic receptor blockade by atropine, there is a significant shift to the right of the ACh dose-response curve. Finally, after both drugs, regardless of the order of administration, the curve is displaced further to the right. Thus, muscarinic receptor blockade by atropine unMASKS the true magnitude of the depressant effect of halothane on the response to ACh of the nicotinic system within the ganglion.

Hexamethonium. The interrelationships among ACh, atropine, and hexamethonium (C6) in this preparation have been worked out by Fleisch et al. Unlike atropine alone, C6 alone did not displace the ACh dose-response curve significantly. If anything, there was a slight shift to the left. However, after a loading dose of atropine (0.1–1.0 mg/kg), C6 caused a progressive shift of the curve to the right in a dose-dependent fashion.

In three experiments, when halothane was administered to the dog following C6, in the absence of atropine, there was either no change or a small shift to the left of the ACh dose-response curve. There was no evidence of a atropine-like depressant action of halothane on the muscarinic system.

Figure 4 shows an experiment in which C6 was injected after atropine and halothane. There was a progressive dose-related shift to the right of the ACh dose-response curve without depression of the maximal response. The 1 mg/kg dose of C6, which, after atropine alone, would have been expected to shift the dose-response curve roughly twofold, now caused a tenfold shift to the right of the curve. This result would have been expected from a
10 mg/kg dose of C6 by itself, suggesting that halothane and C6 are behaving in an additive fashion.

Finally, in two experiments, one of which is illustrated in figure 5, the administration of halothane after large doses of atropine and C6 had no additional effect on the position of the curve or the maximal response.

From these experiments, it appears that halothane does not have atropine-like muscarinic blocking properties, but rather behaves like hexamethonium. Moreover, the combination of hexamethonium and halothane resulted in effects identical to those following a bigger dose of C6 alone.

**THE EFFECTS OF SPECIFIC GANGLIONIC STIMULANTS**

To substantiate the observations with ACh, specific cholinergic ganglionic stimulants were used. In each of three experiments, the effect of halothane on the response to a nicotinic receptor stimulant, DMPP, was observed (fig. 6). In the presence of 1 per cent halothane, the dose–response curve to DMPP was markedly depressed both in slope and in maximal response. The differences were significant (*P < 0.05*) at the 80-, 200-, and 400-μg doses. In two further experiments, 1.5 per cent halothane had an even greater depressant effect on the action of DMPP.

By contrast, when a muscarinic receptor stimulant, McN-A-343, was administered intra-arterially, there was no significant difference between the dose–response curves before and during administration of halothane (fig. 7). The control ED₅₀ was 130 μg, that during 1 per cent halothane, 150 μg. Thus, in these experiments there was a striking difference between the effect of halothane on the response to nicotinic receptor stimulation and its effect on the response to muscarinic receptor stimulation.

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**Fig. 5.** Effects of atropine, halothane, and C6 on heart-rate response to ACh. Female dog, weight 13.0 kg. For further explanation, see text. 1 = control. 2 = after atropine, 1.0 mg/kg. 3 = C6, 15 mg/kg. 4 = halothane, 1 per cent, 60 minutes. 5 = C6, 30 mg/kg.

**Fig. 6.** Average dose–response curves to DMPP. 1 = control, five experiments. 2 = during 1 per cent halothane, 30 minutes, three experiments.

**Fig. 7.** Average dose–response curves to McN-A-343. 1 = control, four experiments. 2 = during 1 per cent halothane, 30 minutes, four experiments.
Discussion

Studies of the effects of anesthetics on impulse transmission through autonomic ganglia have recently been reviewed. The results with direct electrical recording of nerve activity and with the use of heart-rate or blood-pressure response as an index of ganglionic transmission have indicated that anesthetics inhibit ganglionic transmission. Only one group of investigators has concluded that halothane does not affect ganglionic transmission in sympathetic ganglia.

The increasing knowledge of synaptic and ganglionic physiology and pharmacology has made it possible to analyze in more detail the effects of anesthetics. In an earlier study with preganglionic sympathetic nerve stimulation, we showed that ether, halothane, and cyclopropane significantly depressed transmission through the nicotinic, hexamethonium-sensitive pathway, while having little effect on impulse transmission over the muscarinic, atropine-sensitive pathway in the cardiac sympathetic ganglia of the dog. We used as an index of inhibition of ganglionic transmission the difference in the heart-rate response to preganglionic as compared with postganglionic sympathetic nerve stimulation. The response of the indicator system (preganglionic adrenergic nerve-cardiac pacemaker) was not affected by ether and was actually somewhat potentiated by both cyclopropane and halothane. We concluded, therefore, that the site of action of the anesthetics was within the ganglion, but left open the question whether the effect was pre- or postsynaptic.

The current series of experiments presents evidence that halothane antagonizes the ganglionic response to direct injection of the transmitter, ACh, after muscarinic blockade with atropine, as well as the response to the selective nicotinic stimulant, DMPP, in the absence of atropine. The site of action of the anesthetics within the ganglia remains unidentified.

Agonists may stimulate autonomic ganglia either by direct activation of postganglionic receptors or indirectly, by stimulating the release of ACh from presynaptic nerve endings, as suggested by Volle and Koelle. The preponderance of evidence favors the postsynaptic neuron as the site of action of injected ganglionic stimulants. First, it has been demonstrated repeatedly that ACh is still able to stimulate chronically denervated ganglia, i.e., after degeneration of presynaptic nerve endings. This does not eliminate the possibility that, in intact innervated ganglia, ACh, in addition to its direct stimulatory action on the postsynaptic neuron, may provoke the release of ACh from presynaptic nerve endings. Evidence against the latter hypothesis has recently been presented by Collier et al., who failed to demonstrate release of radioactively-labelled ACh from the ganglion following arterial injection of ACh when labelled ACh was readily released by preganglionic nerve stimulation.

In addition, we have investigated the response to nerve stimulation and intra-arterial injection of ACh in animals treated with hemicholinium-3, a drug which depletes presynaptic stores of ACh. At a time when the response to preganglionic nerve stimulation was almost totally abolished, the response to the direct ganglionic injection of ACh was actually potentiated. Vickerson and Varma have recently shown an increase in response to the muscarinic agonist, McN-A-343, in denervated ganglia, evidence for a postsynaptic site of action for this agonist as well.

In sum, then, the evidence strongly supports the hypothesis that stimulants injected directly into sympathetic ganglia act postsynaptically. We are led, therefore, to the conclusion that halothane, which antagonizes the responses of the ganglia to ACh and DMPP, must do so primarily by an action on the postsynaptic neuron. Studies of the effects of halothane at another cholinergic synapse, the neuromuscular junction, also support the view that the anesthetic alters the response of the postsynaptic membrane to the transmitter.

This conclusion raises a second question of considerable interest, namely, how to explain the specificity of the depressant effect of halothane. In our experiments with both preganglionic sympathetic nerve stimulation and direct injection of ganglion stimulants, halo-
halothane depressed the response of the nicotinic system without affecting the response of the muscarinic system. This is even more interesting in view of the recent demonstration of the existence of both types of receptors in the same cell of the cervical sympathetic ganglion of the rabbit. The anesthetics are generally thought of as agents which do not interact with specific receptor sites but rather alter the responses of biological systems in a different, less specific fashion. Therefore, the specific depression of the nicotinic system by halothane probably represents a change in some property of the postsynaptic neuron, which alters the response to activation of one type of receptor but not another. In view of our ignorance of the precise molecular steps which follow drug–receptor combinations, the mechanism by which halothane acts remains undefined. It is of interest to note the recent demonstration by Kobayashi and Libet that the intracellular events following activation of nicotinic receptors differ from those following activation of muscarinic receptors in ganglion cells of the rabbit and the frog. In addition, the two types of postsynaptic potentials varied in their sensitivity to inhibition by both metabolic inhibitors and hypoxia. Thus, even though the end results of impulse transmission over both nicotinic and muscarinic pathways may be functionally the same, i.e., activation of postganglionic adrenergic nerves, the intracellular events and consequent vulnerability to depression may be quite different.

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References


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**Drugs**

**PSEUDOCHOLINESTERASE** Ten patients, ranging in age from 16 to 61 years, were given cyclophosphamide (Cytoxan) at a dose of 25 mg per kg, intravenously. One day after the infusion of the drug the pseudocholinesterase level of the serum was reduced to an average of 33 per cent of normal. By the third day the enzyme level was still reduced by 50 per cent. Eight days after the infusion the enzyme level had reached about 75 per cent of normal. The period of apnea following injection of succinylcholine, 1 mg per kg, was markedly prolonged in these patients as long as the enzyme levels were depressed. Conceivably, true cholinergic reactions can also occur, resulting in excessive formation of secretions, bradycardia and cardiac arrest. Patients on medication with cytotoxic drugs should undergo at least a gross test for pseudocholinesterase level before surgery. The mode of action of cyclophosphamide is apparently analogous to the action of other alkylphosphates. (Priesching, A., Seidl, H., and Steinbrecithiner, K.: Cyclophosphamide (Endoxan) and Pseudocholinesterase—Clinical—Experimental Studies, Wien. Klin. Wschr. 79: 238 (March) 1967.)

**VITAMIN K₁** Vitamin K₁ was given to six volunteers 24 hours before, 48 hours before, 48 hours after, and simultaneously with, 40 mg of warfarin. Coagulation was restored most uniformly, or least disturbed, when vitamin K₁ preceded warfarin, and the greatest variation in response occurred when it was given 48 hours after warfarin. (Zieve, P. D., and Solomon, H. M.: Variation in the Response of Human Beings to Vitamin K₁, J. Lab. Clin. Med. 73: 103 (Jan.) 1969.)

**STEROIDS AND ASTHMA** Although the eosinopenic potency of glucocorticoids is well correlated with antiasthmatic and anti-inflammatory effectiveness, the eosinopenic response to 40 mg of cortisol given intravenously was depressed in steroid-resistant asthmatics. In addition, the cortisol turnover was rapid in these patients. Asthma requiring unusually large doses of steroid for control may be associated with a decreased eosinopenic response to cortisol and an accelerated plasma cortisol clearance. (Schweitz, H. J., Lowell, F. C., and Melby, J. C.: Steroid Resistance in Bronchial Asthma, Ann. Int. Med. 69: 493 (Sept.) 1968.)