A Pharmacological Analysis of Ganglionic Actions of Some General Anesthetics

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The effects of diethyl ether, halothane, cyclopropane and nitrous oxide on impulse transmission were studied in the stellate ganglion of the spinal dog. The effects of electrical stimulation of pre- and postganglionic sympathetic nerves on heart rate were compared under conditions of normal transmission, with muscarinic transmission (after nicotinic blocking drugs), and with nicotinic transmission (after muscarinic block with atropine).

Diethyl ether had no significant effect on the response to postganglionic stimulation but depressed the response to preganglionic stimulation. The depression of ganglionic transmission by ether was markedly potentiated by small doses of atropine.

Halothane affected ganglionic transmission in a fashion similar to ether but, unlike ether, potentiated the response to postganglionic stimulation.

Cyclopropane produced results similar to halothane whereas nitrous oxide had no effect.

The differential sensitivity of nicotinic and muscarinic ganglionic transmission to general anesthetics may be explained by actions of these agents on either pre- or postsynaptic processes.

THE EFFECTS of general anesthetic agents on synaptic transmission are of interest not only because ganglionic effects of these agents may contribute to their overall influences upon autonomic functions, but also because the effects of anesthetics on the central nervous system probably primarily involve synaptic processes.[1-3] The accessibility and the simpler organization of peripheral ganglia permit the use of experimental approaches which are impossible or at least difficult to apply in the case of central synapses. It is hoped, of course, that synaptic transmission in the peripheral autonomic ganglion reflects the behavior of some central synapses.

Recently, this laboratory described a convenient preparation for the study of the two types of ganglionic transmission found in peripheral ganglia: nicotinic and muscarinic.[4] In the stellate ganglion of the dog, the response to stimulation of preganglionic fibers (as measured by the increase in heart rate produced in an areflexic preparation) cannot be blocked completely by supramaximal doses of hexamethonium and other nicotinic blocking agents. The only effect of these drugs is to shift to the right the curve relating frequency of preganglionic stimulation to increase in heart rate. The maximal heart rate response is not reduced. After hexamethonium, the administration of a small dose of atropine (10 to 30 μg./Kg.), a muscarinic blocking drug, blocks transmission almost completely. Treatment of the animal with procaine or with hemicholinium, agents known to decrease the output of acetylcholine from the presynaptic nerve terminals during stimulation, blocks transmission of both types.[5] We concluded that acetylcholine is the main transmitter agent in the stellate ganglion and that it activates two postganglonic sites which are called, after the terminology of Dale, nicotinic and muscarinic.

These observations are in keeping with results of electrophysiologic studies of other autonomic synapses.[6] The advantage of the dog stellate ganglion preparation lies in the fact that quantitative methods can be used more easily.

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The purpose of the present study was to determine, by the use of atropine and nicotinic blocking agents, the effects of general anesthetic agents on ganglionic transmission. We found that the anesthetics used block ganglionic transmission by a preferential effect on the nicotinic system.

Methods

Adult, mongrel dogs of either sex, weighing between 11 and 17 Kg., were given an intravenous dose of thiopental sodium sufficient to produce surgical anesthesia (300 to 500 mg.). Thiopental was used because in control experiments rapid intravenous injection of 100 mg. had no effect on ganglionic transmission, and it was expected that the blood level of the agent would be quite low by the time the experiment began. (See below.)

The trachea was cannulated and artificial ventilation was instituted with a Starling-type piston pump at a minute volume of 400 ml./Kg. Body temperature was maintained between 37 and 38° C. by infrared heating lamps. Cannulae were inserted into the external jugular vein and into one carotid artery. Both carotid arteries were ligated and the vagi were cut in the midcervical region. The animal was turned to the prone position, the spinal cord was exposed through the atlanto-occipital membrane and transected. The brain was destroyed and the atlanto-occipital foramen plugged with a cork. Destruction of the brain including the medulla obviated the need for further anesthesia and prevented reflex changes in autonomic activity.

In the supine position, the dog's chest was opened in the midline. The stellate ganglion on the right side was identified. The sympathetic trunk below the ganglion was carefully freed of connective tissue so that a preganglionic stimulating electrode could be applied. Usually, the electrode was placed between the rami communicantes from T1 and T2. Postganglionic electrodes were placed either on a major branch emanating from the stellate ganglion or on the vago-sympathetic trunk where it crosses the origin of the right subclavian artery. In the latter case, atropine (1–2 mg./Kg.) had to be administered to prevent the effect of vagal stimulation. The electrodes were covered with pledgets saturated in warm mineral oil. At the end of each experiment complete ganglionic blockade was always produced to verify the placement of the electrodes.

The mean initial heart rate following cord section and not less than 30 minutes of stabilization was 148 beats/minute in a total of 35 experiments. Arterial pressure immediately following destruction of the brain was high, declined in the subsequent 15 to 20 minutes, and stabilized at a level of 60 to 80 mm. Hg. The administration of anesthetics or ganglionic blocking agents usually reduced the blood pressure further, necessitating the infusion of dextran (6 per cent w./v.). Blood pressure was maintained above 60 mm. Hg at all times. Individual experiments lasted as long as six hours. A normal response to postganglionic stimulation at the end of the experiment demonstrated that the preparations were still in good condition.

Blood pressure was measured in the carotid artery by means of a Statham strain-gauge transducer. The electrocardiogram was recorded from leads placed on the pericardium. The ECG complexes also were used to drive an interval cardiotachograph. The cardiotachogram and the arterial pressure tracing were recorded on a direct-writing oscillograph (Grass, Model 5).

Square wave stimuli were applied to the nerves from an electronic stimulator (Grass, Model S4) connected through a transformer isolation unit. The voltage used was 10 to 20 per cent above that observed to produce a maximal response. Supramaximal voltage was checked at intervals during the experiment. The duration of each stimulus was 1 msec., occasionally 1.5 msec. Stimuli were usually applied for a period of 30 seconds except at the higher frequencies where a period of 15 to 20 seconds was sufficient for a maximal response.

The heart rate was used as indicator of postganglionic nerve activity. The period of stimulation (30 seconds) was sufficient to permit attainment of a steady state of heart rate in most cases. The tachograph tracing gave a

good indication of the time course of the response. The heart rate was counted directly from a high-speed ECG tracing at the peak of the response identified in the tachogram. At the lower frequencies of stimulation (0.1 and 0.3 per sec.), the response of rate to nerve stimulation was not fused. However, since the average response to a single stimulus lasted about 3 to 5 seconds, full fusion occurred at frequencies of 1 per second and above.

The anesthetics were administered for at least 10 minutes prior to the start of stimulation. With ether and halothane, blood samples were taken at the beginning and at the end of a period of observation and analyzed by gas chromatography as described previously.[7] Briefly, the blood samples were extracted with heptane and analyzed with an F & M gas chromatograph (Model 720) equipped with a thermal conductivity detector and a Tide® column. Halothane was administered from a Fluotec® vaporizer, and ether from a Copper Kettle® vaporizer. Flow rates of cyclopropane and nitrous oxide were measured with standard flowmeters. For nitrous oxide, control observations were done with room air. The values obtained were no different from controls obtained with 100 per cent oxygen, which was also used as carrier for the other anesthetics.

The effects of the anesthetics during preganglionic stimulation were tested under the following conditions:

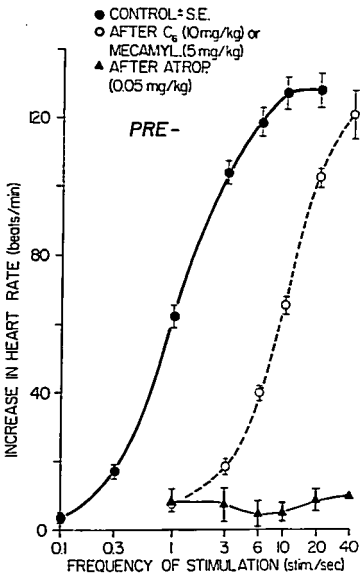

FIG. 1. Increase in heart rate with preganglionic nerve stimulation. Filled circles: control observations (35 experiments). Open circles: after hexamethonium or mecamylamine (13 experiments). Triangles: after hexamethonium or mecamylamine plus atropine (6 experiments).

TABLE 1. Effect of Ether on Postganglionic Stimulation

Freq. of Stim. (Stim./sec.)	Increase in Heart Rate (Beats/min. ± S.E.)					
	0.1	0.3	1	3	6	10
Control	(3) 4.7 ±1.7	(4) 16.3 ± 4.9	(4) 60.0 ±11.3	(4) 107.8 ± 7.7	(4) 122.0 ± 7.8	(4) 120.5 ± 7.5
5% Ether	— — —	(3) 16.7 ± 4.9	(3) 60.6 ± 6.9	(3) 116.3 ± 10.9	(3) 116.7 ± 9.8	(3) 118.0 ± 5.6
10% Ether	— — —	— — —	(3) 51.0 ±16.3	(3) 96.3 ± 11.3	(3) 112.3 ± 8.0	(3) 113.0 ± 8.2

Numbers in parentheses indicate numbers of animals tested.

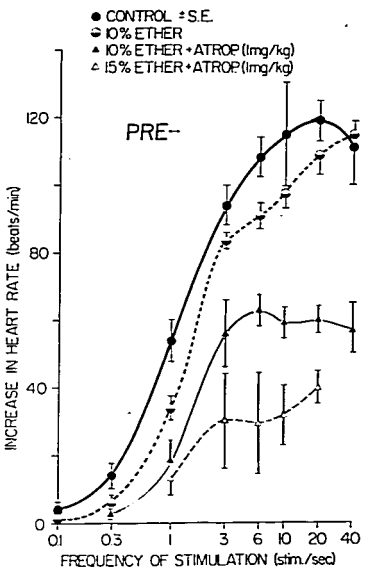

FIG. 2. Effect of ether on response of heart rate to preganglionic nerve stimulation. Filled circles: control observations. Half-circles: responses to 10 per cent ether (blood concentration 85–100 mg./100 ml.). Solid triangles: responses to 10 per cent ether plus atropine, 1 mg./Kg. Open triangles: responses to 15 per cent ether (blood concentrations 140–150 mg./100 ml.) plus atropine, 1 mg./Kg. Further details in Table 2a, b.

a) with normal transmission, *i.e.*, in the presence of intact nicotinic and muscarinic pathways;

b) after administration of atropine, *i.e.*, with only nicotinic transmission intact;

c) after administration of hexamethonium (C_6) or mecamylamine, *i.e.*, with only muscarinic pathways intact;

d) after pretreatment with hemicholinium, when transmission was depressed as the result of decreased transmitter output.

In addition, the effects of the anesthetics on the response to postganglionic nerve stimulation were tested.

The following drugs were used: hexamethonium bromide (Burroughs Wellcome), mecamylamine hydrochloride (Merck, Sharp and Dohme), atropine sulfate (Merck, Sharp and Dohme), hemicholinium-3 (Aldrich Chemical Company), physostigmine sulfate (Merck, Sharp and Dohme). The doses given refer to the salts.

The diethyl ether used was Ether Merck, U.S.P. Halothane was as Fluothane® (Ayerst), and thiopental was as Pentothal Sodium® (Abbott Laboratories). Cyclopropane and nitrous oxide were obtained from Medical-Technical Corporation.

Curves relating frequency of nerve stimulation to the observed increase in heart rate were constructed. In cases where the response was not sustained, the maximal rate attained during stimulation was used. Standard errors were calculated for the mean values of the various groups of observations. The statistical significance of differences between the mean values of groups of observations was tested by an overall analysis of variance at selected frequencies. It is to be noted, in the section of results, that for all P values reported, F values (analysis of variance) were significant below the 0.10 level. The specific differences between group means were tested by the Duncan Multiple Range Test, as adapted by Duncan for comparison of unequal means.[8] In his article, Duncan states that the nature of the test itself is conservative, and suggests, therefore, that one regard a P value of 0.10 or less as significant. This practice was adhered to in the analysis of our data.

Results

Control observations. Stimulation of preganglionic nerve fibers caused an increase in heart rate and blood pressure. Only the change in heart rate was evaluated. The response to a single stimulus lasted between three and five seconds and a smooth heart-rate curve resulted from frequencies of 1/sec. and higher. The response was well sustained for the duration of stimulation and subsided slowly following cessation of stimulation. When the maximal heart rates attained at each frequency were plotted against frequency of stimulation on semilogarithmic paper, a curve of sigmoid shape resulted (fig. 1). The maximal increase occurred at a stimulation frequency of about 10/sec., and higher frequencies up to

TABLE 2a. Effect of Ether on Preganglionic Stimulation

Freq. of Stim. (Stim./sec.)	Increase in Heart Rate (Beats/min ± S.E.)							
	0.1	0.3	1	3	6	10	20	40
Control	(6) 4.0 ±1.3	(10) 13.5 ± 4.5	(10) 53.6 ± 6.5	(10) 93.6 ± 5.8	(10) 108.0 ± 5.5	(9) 115.6 ± 15.7	(6) 119.3 ± 6.3	(4) 111.0 ± 10.5
10% ether no atropine	(2) 1.5 ±1.1	(5) 6.6 ± 1.6	(7) 33.6 ± 3.0	(7) 83.6 ± 2.7	(7) 91.3 ± 3.8	(7) 97.3 ± 4.0	(7) 109.0 ±5.6	(5) 115.0 ± 4.3
10% ether with atropine	—	(3) 3.3 ± 0.2	(5) 18.6 ± 5.9	(5) 55.8 ±10.1	(5) 63.2 ± 4.1	(5) 59.2 ± 4.5	(4) 61.3 ± 4.0	(3) 57.3 ± 7.5
15% ether with atropine	—	—	(2) 13.0 ± 5.0	(2) 30.0 ±14.0	(2) 29.0 ± 15.0	(2) 32.5 ± 9.5	(2) 40.5 ± 5.5	—

Numbers in parenthese indicate numbers of animals tested.

TABLE 2b. Results of Duncan Test on Means of Preganglionic Responses during Various Concentrations of Ether

	Freq. of Stim. (Stim./sec.)	Control	10%	10% + Atrp.	15% + Atrp.
Control	20	—	N.S.*	$P<0.01$	$P<0.01$
	6	—	$P<0.05$	$P<0.01$	$P<0.01$
	1	—	$P<0.05$	$P<0.01$	$P<0.01$
10%	20	N.S.	—	$P<0.01$	$P<0.01$
	6	$P<0.05$	—	$P<0.01$	$P<0.01$
	1	$P<0.05$	—	N.S.	N.S.
10% + Atrp.	20	$P<0.01$	$P<0.01$	—	$P<0.10$
	6	$P<0.01$	$P<0.01$	—	$P<0.05$
	1	$P<0.01$	N.S.	—	N.S.

Each mean heart rate response at the specified frequency is compared with all other means at that frequency and probabilities of differences are as noted.

* N.S. = No significant.

40/sec. did not produce a significantly greater response. Fifty per cent of maximal increase occurred at a frequency of about 1/sec. The maximal increase averaged 128 beats/min., about the same as that observed during infusion of norepinephrine in the isolated, denervated heart.[9]

Administration of hexamethonium (10 mg./Kg.) or mecamylamine (5 mg./Kg.) did not abolish impulse transmission but shifted the frequency-response curve to the right (fig. 1). The 50 per cent increase in rate now occurred at about 10 stimuli/sec. and approximated the same maximum as in the controls. Previous experiments [5] have shown that additional doses of the blocking agents do not cause any further shift of the curve, indicating that the antagonism is indeed maximal. However, a dose of 0.05 mg./Kg. of atropine nearly completely blocked transmission (fig. 1). Atropine alone without prior administration of a nicotinic blocking agent does not affect the frequency-response curve even in doses as high as 1 mg./Kg.[4]

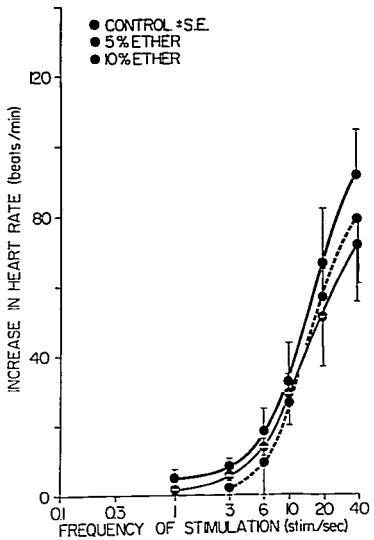

FIG. 3. Effect of ether on response of heart rate in the presence of muscarinic ganglionic transmission. Filled circles with solid line: control observations. Barred circles with solid line: responses to 5 per cent ether (blood concentration 40–60 mg./100 ml.). Filled circles, dashed line: responses to 10 per cent ether (blood concentrations 85–110 mg./100 ml.). Further details in Table 3.

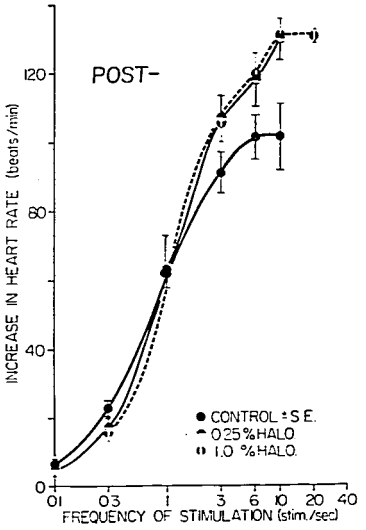

FIG. 4. Effect of halothane on response of heart rate during postganglionic stimulation. Filled circles: control observations. Half-circles: 0.25% halothane (blood concentrations 4–8 mg./100 ml.). Barred circles: responses to 1.0% halothane (blood concentrations 10–18 mg./100 ml.). Further details in Tables 4a, b.

TABLE 3. Effect of Ether on Muscarinic Transmission

Freq. of Stim. (Stim./sec.)	Increase in Heart Rate (Beats/min. ± S.E.)					
	1	3	6	10	20	40
Control	(3) 5.0 ±2.6	(3) 8.3 ±1.8	(3) 18.3 ± 7.4	(3) 32.3 ±11.9	(3) 66.6 ±16.9	(3) 90.7 ±14.1
5% Ether	(2) 2.5 ±0.4	(3) 6.3 ±1.9	(3) 13.7 ± 4.1	(3) 29.0 ± 6.8	(3) 51.7 ±14.5	(3) 72.7 ±15.9
10% Ether	(2) 0	(2) 2.5 ±2.5	(2) 9.0 ± 9.9	(4) 27.0 ± 5.7	(4) 57.3 ± 9.6	(3) 79.0 ±18.5

Numbers in parentheses indicate numbers of animals tested.

TABLE 4a. Effect of Halothane on Postganglionic Stimulation

Freq. of Stim. (Stim./sec.)	Increase in Heart Rate (Beats/min. ± S.E.)						
	0.1	0.3	1	3	6	10	20
Control	(5) 6.0 ±1.4	(5) 22.4 ± 2.8	(5) 61.8 ± 4.8	(5) 90.8 ± 5.6	(5) 101.4 ± 8.4	(5) 101.6 ± 9.6	— — —
0.25%	(3) 4.7 ±1.6	(4) 17.0 ± 6.0	(4) 60.5 ±10.5	(4) 107.3 ± 6.7	(4) 117.3 ± 8.1	(3) 129.3 ± 6.2	— — —
1.0%	— — —	(3) 15.7 ± 2.3	(3) 60.7 ±10.7	(3) 106.0 ± 6.0	(4) 118.5 ± 6.5	(4) 130.0 ± 5.6	(3) 130.3 ± 1.8

Numbers in parentheses indicate numbers of animals tested.

Stimulation of the postganglionic nerve at the time of complete block of preganglionic stimulation elicited a normal response. In two experiments, pre- and postganglionic nerves were stimulated alternately. The effect of postganglionic stimulation remained practically unchanged, whereas the above-described results of preganglionic stimulation took place with administration of the cholinolytic drugs.

Diethyl Ether. When diethyl ether was administered after a reproducible control curve had been established, the response to postganglionic stimulation was not significantly altered with inspired concentrations of 5 per cent and 10 per cent ether (table 1). Five per cent ether was not administered during preganglionic stimulation because the effect of 10 per cent ether on normal transmission was relatively small. However, 5 per cent ether was given during postganglionic stimulation. We wished to see whether the low concentration would have a discernible effect, since 0.25 per cent halothane was found to potentiate responses to postganglionic stimulation (see below).

The curve describing the responses to preganglionic stimulation was shifted to the right (fig. 2). With 10 per cent ether in the inspired gas (blood concentration between 85–100 mg./100 ml.) the effect was statistically significant at some frequencies (table 2b). However, greater weight derives from the fact that the changes were always in the same direction, that their magnitude was related to the concentration of the anesthetic, and that they were reversible when the anesthetic was discontinued. When atropine (0.1–1.0 mg./Kg.) was added, the frequency-response curve was shifted further and a marked depression of the maximal response was apparent at frequencies between 6 and 40/second (fig. 2, table 2a). When ether was administered to a preparation pretreated with atropine, its effect was much greater than in a nonatropinized animal. Thus, the sequence of the administration of ether and atropine was unimportant. Upon discontinuation of ether, the changes

TABLE 4b. Results of Duncan Test on Means of Postganglionic Responses during Various Concentrations of Halothane

	Freq. of Stim. (Stim./sec.)	Control	0.25%	1.0%
Control	10	—	$P<0.10$	$P<0.05$
	3	—	$P<0.10$	$P<0.10$
0.25%	10	$P<0.10$	—	N.S.*
	3	$P<0.10$	—	N.S.
1%	10	$P<0.05$	N.S.	—
	3	$P<0.10$	N.S.	—

Each mean heart rate response at the specified frequency is compared with all other means at that frequency, and probabilities of differences are as noted.

* N.S. = Not significant.

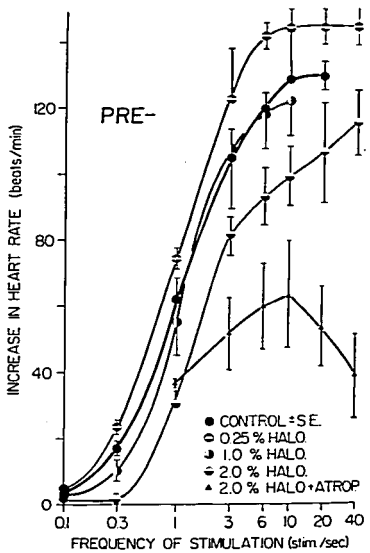

FIG. 5. Effect of halothane on response of heart rate to preganglionic nerve stimulation. Filled circles: control observations. Barred circles: responses to 0.25 per cent halothane (blood concentrations 4–8 mg./100 ml.). Vertical half-circles: responses to 1.0 per cent halothane (blood concentrations 10–18 mg./100 ml.). Horizontal half-circles: responses to 2.0 per cent halothane (blood concentrations 20–35 mg./100 ml.). Triangles: responses to 2.0 per cent halothane plus atropine, 0.05 mg./Kg. Further details in Tables 5a, b.

were largely reversible although full reversal was not obtained when ether had been present for prolonged periods. The most marked recovery was seen at the higher frequencies of stimulation where the depression had been most pronounced.

In view of the fact that atropine in the absence of anesthetics had no effect on the frequency-response curve,[4] it must be concluded that ether exerts a depressant effect on nicotinic transmission which is partly masked by muscarinic transmission. Blocking the latter by atropine unmasks the full nicotinic depression produced by ether.

There was considerable variation in the magnitude of the depressant effect produced by a given concentration of ether, especially following administration of atropine (fig. 2, table 2a). However, since the depression varied with the concentration of the anesthetic, closely similar curves could have been produced by varying the concentration of ether.

With the grouping of experiments by concentration of inspired ether (table 2b), the effect of 10 per cent ether alone was on the borderline of statistical significance. The addition of atropine caused further displacement of the frequency-response curve, which was significant at the 1 per cent level at the higher frequencies of stimulation. At low frequencies, only the effect of the combination of ether plus atropine was statistically significant.

These results indicate that the muscarinic system is more resistant to ether than the nicotinic system. They do not reveal whether or not there is any effect on muscarinic transmission itself. In order to answer this question, ether was given to animals pretreated with full doses of hexamethonium or mecamylamine (see control observations). We then observed that ether did indeed depress muscarinic transmission (fig. 3, table 3). Again, in a given experiment, the magnitude of the effect increased with the concentration of the anesthetic and was easily reversible although, with the number of experiments done, an inspired concentration of 10 per cent did yield a statistically significant result (table 3). The depression observed at an inspired concentration of 10 per cent ether was about the same as that seen in the untreated preparation (no C_6 or atropine) but much less than that after atropine had been given.

Halothane: Halothane, in concentrations between 0.25 and 1.0 volumes per cent in the inspired gas, potentiated the response of the heart rate to postganglionic stimulation (fig. 4, table 4a). As with norepinephrine infusion in the isolated heart[9] the potentiation was restricted to high levels of adrenergic activity. In four experiments, the administration of 2.0 per cent halothane did not alter the response to postganglionic stimulation in a consistent fashion. There was some depression of the response to low-frequency and either no effect or a potentiation of the response to high-frequency stimulation (above 6 per second).

TABLE 5a. Effect of Halothane on Preganglionic Stimulation

Freq. of Stim. (Stim./sec.)	Increase in Heart Rate (Beats/min. ± S.E.)							
	0.1	0.3	1	3	6	10	20	40
Control	(17) 5.4 ±0.9	(19) 16.8 ± 1.8	(19) 62.2 ± 3.7	(19) 104.5 ± 15.6	(19) 119.0 ± 4.1	(18) 127.5 ± 19.2	(7) 128.6 ± 4.5	— — —
0.25%	(4) 4.8 ±0.8	(4) 23.3 ± 1.6	(4) 74.8 ± 1.6	(4) 122.8 ± 3.8	(4) 141.5 ± 8.8	(4) 147.2 ± 6.7	(4) 149.5 + 5.7	(3) 149.0 ± 7.1
1.0%	(5) 4.2 ±1.3	(5) 10.2 ± 2.7	(5) 55.6 ± 9.6	(5) 107.8 ± 6.8	(5) 118.0 ± 9.5	(5) 122.2 ± 9.2	— — —	— — —
2.0% no atropine	(2) 2.0 ±0.0	(3) 2.3 ± 1.3	(7) 30.6 ± 3.2	(7) 82.4 ± 6.8	(7) 93.6 ± 8.3	(7) 98.7 ± 9.7	(5) 106.8 ± 15.0	(4) 115.8 ± 10.6
2.0% + atropine	— — —	— —	(5) 36.0 ± 1.9	(5) 51.0 ± 11.3	(5) 59.2 ± 13.2	(5) 62.8 ± 15.5	(5) 53.2 ± 12.0	(5) 28.2 ± 13.0

Numbers in parentheses indicate numbers of animals tested.

TABLE 5b. Results of Duncan Test on Means of Preganglionic Responses during various Concentrations of Halothane

	Freq. of Stim. (Stim./sec.)	0.25%	Control	1.0%	2.0%	2.0% + Atropine
0.25%	40	—	—	—	$P<0.10$	$P<0.005$
	20	—	N.S.*	—	$P<0.05$	$P<0.005$
	6	—	$P<0.10$	N.S.	$P<0.005$	$P<0.005$
	3	—	$P<0.10$	$P<0.10$	$P<0.005$	$P<0.005$
	1	—	$P<0.10$	$P<0.05$	$P<0.005$	$P<0.005$
Control	40	—	—	—	—	—
	20	N.S.	—	—	$P<0.10$	$P<0.005$
	6	$P<0.10$	—	N.S.	$P<0.05$	$P<0.005$
	3	$P<0.10$	—	N.S.	$P<0.01$	$P<0.005$
	1	$P<0.10$	—	N.S.	$P<0.005$	$P<0.005$
1%	40	—	—	—	—	—
	20	—	—	—	—	—
	6	N.S.	N.S.	—	$P<0.10$	$P<0.005$
	3	$P<0.10$	N.S.	—	$P<0.05$	$P<0.005$
	1	$P<0.05$	N.S.	—	$P<0.005$	$P<0.005$
2%	40	$P<0.10$	—	—	—	$P<0.005$
	20	$P<0.05$	$P<0.10$	—	—	$P<0.005$
	6	$P<0.005$	$P<0.05$	$P<0.10$	—	$P<0.01$
	3	$P<0.005$	$P<0.01$	$P<0.05$	—	$P<0.005$
	1	$P<0.005$	$P<0.005$	$P<0.005$	—	$P<0.10$

Each mean heart rate response at the specified frequency is compared with all other means at that frequency, and probabilities of differences are noted.

* N.S. = Not significant.

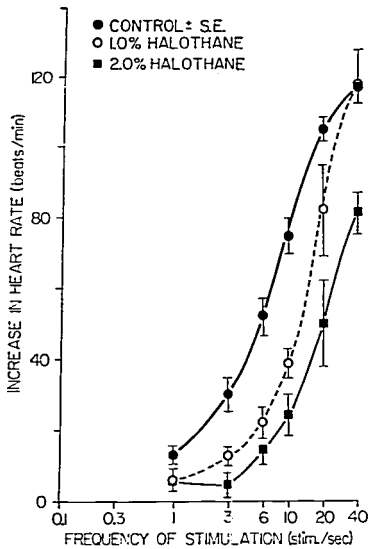

FIG. 6. Effect of halothane on response of heart rate in the presence of muscarinic ganglionic transmission. Filled circles: control observations. Open circles: 1.0% halothane (blood concentration 10–18 mg./100 ml.). Squares: 2.0% halothane (blood concentrations 20 to 35 mg./100 ml.). Further details in Tables 6a, b.

When the preganglionic nerve was stimulated, a potentiation was apparent at the lowest concentration of halothane (0.25 per cent —blood level 4–8 mg./100 ml.) (fig. 5, table 5), probably reflecting the potentiation of postganglionic nervous activity. With a concentration of 1 per cent halothane (blood level 10–18 mg./100 ml.) the curve coincided with the control. However, since there was marked postganglionic potentiation at this concentration (fig. 4), the downward shift of the preganglionic curve as compared with the potentiated postganglionic curve represents a real depression of ganglionic transmission, a depression still more pronounced with higher concentrations of the anesthetic. As with ether, administration of a small dose of atropine (0.05 mg./Kg.), markedly increased the depression by the anesthetic (fig. 5, table 5, fig. 7). When halothane was given after prior administration of a nicotinic blocking agent, *i.e.*, during muscarinic transmission, a moderate depressant effect of the anesthetic was observed (fig. 6, tables 6a, b). In all cases considerable reversal of the depression was obtained when the anesthetic was discontinued (fig. 7).

Figure 7 illustrates another feature of the actions of both halothane and ether. While the response to preganglionic nerve stimulation was normally well sustained during the period of stimulation (panel 1) this was not so when a higher concentration of anesthetic was given. A marked "fading" of the response occurred (panels 2, 3, and 4). In all curves presented, this fading was not taken into account and the maximal rate attained was plotted. This tended to produce underestimation of the effects of the anesthetics.

Figure 7 illustrates the rapidity of reversal of the ganglionic depression when the anesthetic was withdrawn (panels 5 and 6). Often, marked reversal was seen as early as two minutes following discontinuation.

Cyclopropane and Nitrous Oxide: No effect upon the response to either pre- or postganglionic stimulation was seen with 80 volumes per cent of nitrous oxide in three experiments with or without atropine. The effects of cyclopropane (three experiments) were qualitatively similar to those of halothane, including the potentiation of the postganglionic response. However, concentrations higher than the clinical anesthetic concentration of 20 volumes per cent were usually required. With 40 per cent, a marked effect was observed, but even a concentration of 60 volumes per cent did not cause full block, resembling the effect of 2 per cent halothane or 15 per cent diethyl ether. As with halothane and ether, the addition of atropine accentuated the blocking effect of cyclopropane.

Hemicholinium: Hemicholinium, which blocks synthesis of acetylcholine, depresses ganglionic transmission when the stores of acetylcholine in the preganglionic nerve terminals are exhausted by prolonged stimulation.[10] In four experiments a depression of the control curve to about 50 per cent of normal was produced in the presence of the drug

TABLE 6a. Effect of Halothane on Muscarinic Transmission

Freq. of Stim. (Stim./sec.)	Increase in Heart Rate (Beats/min. ± S.E.)					
	1	3	6	10	20	40
Control	(9) 13.1 ± 2.4	(9) 30.3 ± 4.5	(9) 52.3 ± 4.7	(9) 74.6 ± 4.5	(9) 104.8 ± 3.1	(9) 116.9 ± 4.0
1.0%	(3) 6.3 ± 3.6	(4) 12.5 ± 2.4	(5) 21.8 ± 4.0	(5) 38.4 ± 3.7	(5) 81.4 ± 13.4	(5) 117.0 ± 10.9
2.0%	(3) 5.0 ± 2.8	(3) 4.7 ± 2.6	(3) 14.7 ± 4.8	(4) 24.3 ± 5.8	(4) 49.8 ± 13.4	(4) 81.0 ± 7.3

Numbers in parentheses indicate numbers of animals tested.

(3 mg./Kg.). We reasoned that the depression of transmission caused by a decreased release of transmitter might render the preparation more sensitive to a possible additional depression of release of transmitter by the anesthetics. Further depression by halothane and ether did occur but no strikingly greater sensitivity to the anesthetics was seen.

Physostigmine: Physostigmine given to a preparation with intact nicotinic transmission has no potentiating action.[11] After partial or full blocking doses of a nicotinic agent, physostigmine does not reverse the depression of nicotinic receptors. In contrast, physostigmine consistently potentiates muscarinic ganglionic transmission and reverses a muscarinic block brought about by atropine.[11]

In five experiments we found that physostigmine (0.1 mg./Kg.) did not overcome the depression of nicotinic transmission brought about by halothane or ether. In three experiments, muscarinic ganglionic transmission which was partially depressed by either halothane or ether was restored or potentiated by physostigmine.

TABLE 6b. Results of Duncan Test on Means of Responses during Muscarinic Transmission with Various Concentrations of Halothane

	Freq. of Stim. (Stim./sec.)	Control	1.0%	2.0%
Control	40	—	N.S.*	$P<0.01$
	20	—	$P<0.10$	$P<0.01$
	10	—	$P<0.01$	$P<0.01$
	6	—	$P<0.01$	$P<0.01$
	1	—	N.S.	$P<0.10$
1%	40	N.S.	—	$P<0.01$
	20	$P<0.10$	—	$P<0.05$
	10	$P<0.01$	—	N.S.
	6	$P<0.01$	—	N.S.
	1	N.S.	—	N.S.
2%	40	$P<0.01$	$P<0.05$	—
	20	$P<0.01$	$P<0.05$	—
	10	$P<0.01$	N.S.	—
	6	$P<0.01$	N.S.	—
	1	$P<0.10$	N.S.	—

Each mean heart rate response at the specified frequency is compared with all other means at that frequency, and probabilities of differences are noted.
* N.S. = Not significant.

Discussion

Effects of anesthetic agents on normal ganglionic transmission, reported here, are similar to those found by Larrabee and Holaday[12] in the cat. However, these authors used electrophysiologic methods, and, as a result, might not have been able to observe muscarinic transmission had it occurred. In contrast to the well-synchronized postganglionic response to preganglionic stimulation in a normal ganglion, the postganglionic activity following nicotinic block has been described as "asynchronous."[13] Such asynchronous activity, of course, cannot be evaluated quantitatively when mass recording electrodes are used.

Depression of impulse transmission in the system used in these studies may have been

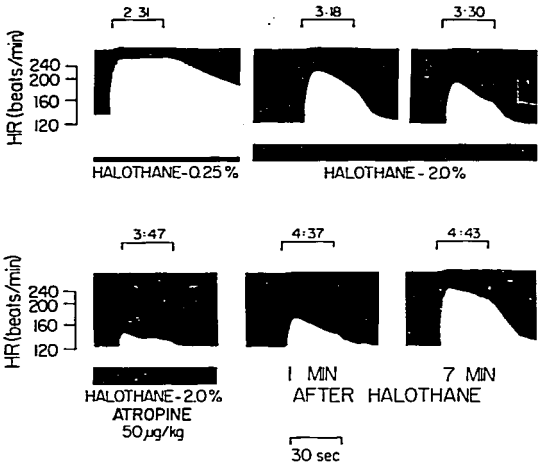

FIG. 7. Effects of halothane and atropine on response to high-frequency preganglionic stimulation. Cardiotachograph tracings with heart rate scale to the left. The nerve was stimulated for 30 seconds at a frequency of 40 per second at the times indicated over the horizontal bars. The inspired concentration of halothane is indicated beneath each panel. Panel four was obtained 6 minutes after the administration of atropine. Panels 5 and 6 show the results 1 minute and 7 minutes after discontinuation of 2 per cent halothane.

due to an action on any one or several of the components of the system. The main parameters that may be sensitive to drug action are: axonal conduction in pre- or postsynaptic nerves, release of transmitter from pre- or postsynaptic nerve endings, and excitatory transmitter action at the post-junctional membrane. Results of the stimulation of postganglionic nerves excluded the postganglionic system as a possible site of action, and, in fact, halothane and cyclopropane caused a degree of potentiation of the heart rate response to postganglionic stimulation. This potentiation of a sympathetic response was very similar to that observed by Flacke and Alper[9] with halothane during infusion of norepinephrine. It is possible, therefore, that potentiation of the response to nerve stimulation represents a sensitization to the postganglionic sympathetic transmitter.

The response to preganglionic stimulation was moderately depressed by the anesthetics in untreated preparations as well as in preparations pretreated with a nicotinic blocking agent, *i.e.*, with only the muscarinic system of transmission intact. However, administration of atropine, leaving only the nicotinic pathway intact, greatly increased the sensitivity to all anesthetic agents tested except nitrous oxide.

It appears unlikely that axonal conduction in the preganglionic fibers was more sensitive to depression than that in the postganglionic nerve where no evidence of depressant effect was observed.

This leaves the synaptic processes for consideration: transmitter release and postjunctional excitation. A difference in the activity of pharmacologically specific systems, such as observed here between the nicotinic and the muscarinic systems, is usually interpreted as evidence for a specific interaction between drug and tissue receptor. A specific effect of general anesthetics is difficult to conceive. However, it is possible that a nonspecific alteration, for example, a change in membrane permeabilities,[14] may have quite different consequences for different specific postsynaptic receptors. Our knowledge of the synaptic processes in the two pathways is insufficient to permit further elaboration. One possibility, however, comes to mind. The muscarinic system is activated at higher stimulation frequencies and may be more capable of sustained activity at such frequencies. Although the nicotinic system is normally capable of carry-

ing impulse traffic at high frequencies, it may be more susceptible to fatigue in the presence of anesthetics. This would provide an explanation for the effect of atropine which through elimination of the high-frequency muscarinic pathway would leave a nicotinic system more sensitive to fatigue at high frequencies.

This concept does not answer the question of whether the processes involved are pre- or postsynaptic. Recently, Gissen and his coworkers [15] have provided evidence that halothane has an effect upon transmitter release as well as upon postsynaptic sensitivity at the neuromuscular junction.

In our experiments, the fact that anesthetics depressed transmission through both nicotinic and muscarinic pathways (figs. 2, 3, 5, 6) suggests a common mode of action, perhaps a decreased output of transmitter from the presynaptic terminals. Also, the observation that depression of transmission was greater the higher the frequency of nerve stimulation implies a presynaptic rather than postsynaptic site of action.

The experiments with hemicholinium were designed to test one possible presynaptic mechanism, since hemicholinium is known [10] to block the synthesis of acetylcholine. Our results demonstrate no obvious synergism between hemicholinium and the anesthetics. However, this does not rule out the possibility that anesthetics may affect presynaptic mechanisms other than acetylcholine synthesis, for example, impulse conduction in the fine preganglionic nerve twigs or release of acetylcholine from the nerve terminals.

In experiments reported elsewhere,[11] we observed that physostigmine potentiated muscarinic but not nicotinic transmission when transmitter release was decreased by pretreatment with hemicholinium. Physostigmine exerted the same type of action in the present experiments, thus supporting the concept of a presynaptic site of action of anesthetics. However, we must conclude that at present the available evidence is not sufficient to settle the question of the site of action of the anesthetics on ganglionic transmission.

The relative ganglionic-depressant potencies of the agents studied did not parallel their anesthetic potencies. While the range of concentration of halothane and diethyl ether was of the right order of magnitude, the potency of cyclopropane was less than the anesthetic potency of this agent, and no effect was observed with intravenous barbiturates or nitrous oxide.

It is possible that the effects of ether and halothane on sympathetic ganglia contribute to the overall effect of these agents on the magnitude of autonomic efferent tone, but clinical evidence regarding this possibility does not exist. The doses of atropine which were found to potentiate so markedly the blocking effect of the anesthetics are close to those used clinically. However, there appears to be some variation in the relative importance of the muscarinic ganglionic pathway in different species and in different ganglia. For example, Trendelenburg [16] found only a small muscarinic component in the cat's superior cervical ganglion, and the place of muscarinic ganglionic transmission in man is not known.

There must be considerable caution in interpreting the possible significance of these experiments for the neurophysiologic mechanisms of general anesthesia, although some of the basic requirements are fulfilled. The concentrations employed were in the same range as those producing clinical anesthesia, and the effects were easily reversible. The magnitude of the observed effects was far greater than required to postulate a role in central mechanisms, because even a small effect on a single synapse may be important when multiplied by a large number of synaptic steps. Also, features such as the influence of frequency of activation and the relative specificity of the drug action may relate to the differential sensitivity to anesthetics of some central nervous system functions in comparison to others. Nevertheless, this type of evidence remains inconclusive and its significance for the mechanism of general anesthesia must await a far better understanding of central nervous system functions.

Summary

The effects of several anesthetics, diethyl ether, halothane, cyclopropane and nitrous oxide, on impulse transmission in the stellate ganglion were investigated in spinal dogs,

Their actions on the ganglion were studied by comparing the effects of electrical stimulation of pre- and postganglionic nerves upon increase in heart rate. Complete curves relating frequency of stimulation to heart rate response were constructed. The effects of the anesthetics were studied in untreated preparations, after administration of atropine, and after administration of maximal doses of a nicotinic blocking agent, hexamethonium or mecamylamine.

Diethyl ether in inspired concentrations of 10 or 15 volumes per cent (blood concentration between 85 and 150 mg./100 ml.) did not affect the response to postganglionic stimulation. The response to preganglionic stimulation was depressed by the same concentrations. There was a moderate shift to the right in the frequency-response curves in untreated preparations and after complete block of nicotinic ganglionic transmission. Administration of atropine (0.05 mg./Kg.) caused a marked additional shift of the frequency-response curves and a depression of the maximal response.

The results with halothane in inspired concentrations between 0.25 and 2.0 volumes per cent (concentrations in blood between 4 and 35 mg./100 ml.) were similar with regard to stimulation of preganglionic nerves. In addition, halothane caused a potentiation of the response to postganglionic stimulation at higher frequencies of stimulation (6 to 40 stimuli/sec.).

No effects were observed with nitrous oxide in concentrations up to 80 volumes per cent. Cyclopropane had only a slight effect at a concentration of 20 volumes per cent, but produced the same changes as described for halothane when a concentration of 40 per cent was given.

The results can be interpreted as indicating an action of the effective agents in either or both of two possible areas: a) on the release of transmitter from preganglionic nerve terminals and b) on the sensitivity of the postganglionic sites to the transmitter agent.

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