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Central Monaminergic Neuronal Effects on Minimum Alveolar Concentrations (MAC) of Halothane and Cyclopropane in Rats

R. A. Mueller, M.D., Ph.D., R. D. Smith, Ph.D.,† W. A. Spruill, B. S.,‡ G. R. Breese, Ph.D.§*

The effect of interference with central catecholamine- or serotonin-containing neurons on the response of rats to inhalation anesthetics was explored. Interference with catecholaminergic function by inhibition of amine synthesis with α-methyltyrosine alone or combined with destruction of these neurons by 6-hydroxydopamine reduced brain concentrations of norepinephrine and dopamine without altering serotonin concentrations. These alterations decreased the minimum alveolar concentration (MAC) of halothane when tail-clamping was used as the test stimulus. Similar small reductions of halothane MAC were produced in rats given 5,6-dihydroxytryptamine and p-chlorophenylalanine, which decreased brain serotonin without altering norepinephrine or dopamine concentrations.

When catecholaminergic or serotoninergic neuronal function was similarly disrupted in rats later exposed to cyclopropane, no alteration in MAC was observed. It is suggested that disruption of central amine-containing neurons may lower the MAC's of depressant anesthetics only, and not excitatory anesthetics. (Key words: Anesthetics, volatile, halothane; Anesthetics, gases, cyclopropane; Serotonin, brain levels; Sympathetic nervous system, brain catecholamine levels; Brain, catecholamine levels; Potency, anesthetics, MAC.)

* Associate Professor, Anesthesiology; Assistant Professor, Pharmacology.

† Postdoctoral Fellow, Biological Sciences Research Center, Department of Psychiatry. Current address: Parke Davis and Company, Ann Arbor, Michigan.

‡ Predoctoral Student, Department of Pharmacology.

§ Associate Professor, Psychiatry and Pharmacology.

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SEVERAL YEARS AGO, Miller and co-workers[1] suggested that the MAC of a given anesthetic might vary with the activity of central noradrenergic nerves. This postulate was based on the observation that reserpine and alpha-methyldopa, both agents that interfere with the integrity of central amine-containing nerves, lowered the minimum alveolar concentration (MAC)[2] of halothane in the dog. In addition, the monoamine oxidase inhibitor, iproniazid, which interferes with amine inactivation and thus increases brain amine concentrations, produced a slight but significant increase in the MAC for cyclopropane in rats. More recently this proposal was strengthened by the work of Johnston and co-workers, which indicated that the MAC of halothane was higher in dogs given amphetamine, an agent known to increase the turnover of central norepinephrine.[3] Since none of these agents acts exclusively on catecholamine-containing neurons,[4-6] but all alter serotonin-containing neurons as well, it seemed that a more direct approach, such as selectively destroying those central neurons that contain the proposed neurotransmitters, might give a clearer indication of the importance of these nerves in determining the sensitivity of the animal to an anesthetic.

Two agents that destroy amine-containing nerves were employed. They were 6-hydroxydopamine, which produces acute degeneration of central noradrenergic nerve terminals,[7,8] and 5,6-dihydroxytryptamine, an agent producing selective degeneration of serotonin-containing nerve terminals.[9,10] Both these agents have chemical configurations similar enough to those of the naturally occurring amines to ensure that they are selectively accumulated in these neurons. Once taken up, these neurotoxic compounds produce chemical destruction of the nerve terminals.

Since inhalation anesthetics can be divided into two general groups, those producing an initial excitatory change in neuronal activity and those which progressively depress electrical phenomena,[11,12] it is possible that amine-containing neurons are important in altering the response to anesthesia with only one type of anesthetic. Therefore,a representative member of each class of central depressants should be explored. The purpose of the present study was thus to determine the relative importance of central serotonin- and catecholamine-containing neurons in determining the sensitivities to the anesthetic effects of both a progressively depressant (halothane)[13] and an initially excitant (cyclopropane)[14,15] inhalational anesthetic. The present studies demonstrate that only the depressant effect of halothane appears to be dependent upon central amine-containing neurons, and that the loss of either serotonin or norepinephrine and dopamine may contribute to the increased sensitivity of rats to this anesthetic.

Materials and Methods

Male Sprague-Dawley rats, weights 250–500 g, were housed in a light–dark-cycled room (light 0700–1900) and were used for determination of anesthetic sensitivity at least four days after arrival from the supplier (Zivic-Miller, Alison, Pa.). It is generally agreed that one cannot measure alveolar gas tensions, and thus true MAC values may only be estimated.[16] Perhaps the recently developed techniques to estimate whole-brain tensions would provide the best index of depressant-agent activity,[17,18] especially if the drug treatments employed produced changes in ventilation or cardiac output. Thus, it is recognized that the technique of inflow gas sampling employed here is not ideal; however, some changes (age) are reflected by similar alterations in both inspired and end-expiratory concentrations.[16]

The method of Hoffman *et al.*[19] was employed to measure minimum anesthetic concentration (MAC), with slight modifications. MAC was determined for only one control and one experimental animal each day (randomizing the order of exposure), since determination of MAC with either halothane or cyclopropane required 3–4 hours for each rat. Halothane or cyclopropane (in air, 2 l/min) was passed through ½-inch-diameter conductive rubber tubing into a conductive plastic bag that enclosed the body of the animal. Each rat was initially placed in a small plastic restraining chamber, which was put into the bag. When the rat ceased struggling as anesthesia deepened, the chamber was removed to facilitate positioning the inflow over the animal's snout, with the rat's tail and hind quarters left protruding from the opposite end of the bag.

Animals were initially exposed to 15 per cent cyclopropane or 0.75 per cent halothane, and gas tensions were increased in steps of about 0.7 per cent (cyclopropane) or 0.06–0.09 per cent (halothane) using calibrated flowmeters or a Dräger vaporizer. Each increment was maintained for 30 minutes before the tail was clamped to determine the animal's response, such as movement of an extremity, tail movement, etc. Each increment in inspired anesthetic gas tension was checked with a flame-ionization gas chromatograph calibrated on the day of use. All values reported are percentages of dry gas at room temperature (22–25 C). Body temperature was monitored with a Yellow Springs thermometer and maintained at 35–36 C using a warming blanket to eliminate the effects of temperature on MAC.[20] The average (X) of the last concentration that still permitted movement and the concentration that first abolished movement in response to tail-clamping was calculated. Then anesthetic gas tensions were decreased and the mean (Y) between the first tension that permitted a response to reappear and that which had previously abolished the response recorded. MAC for the animal was recorded as the average of X and Y. It was assumed that the long time intervals permitted during each increment or decrement of anesthetic concentration and the above computation would minimize error due to slow equilibration of airway and brain anesthetic tensions.[18]

Animals were killed by cervical dislocation immediately after the first movement noticed with decreasing gas tensions, decapitated, and the whole brain removed. The brain was frozen on dry ice and stored at −80 C

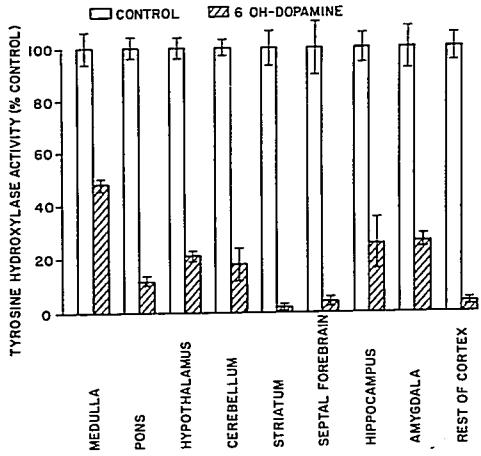

FIG. 1. Effects of 6-hydroxydopamine on tyrosine hydroxylase activity in various rat brain regions. Animals were pretreated with pargyline and 6-hydroxydopamine (6-hydroxydopamine) or pargyline alone (controls), as described in Methods. Vertical bars represent the means of 4–6 determinations ± SEM (brackets). All experimental values are significantly less than the corresponding control values ($P < .01$).

until an entire study (6–10 pairs of experimental and control rats) was completed (1–2 weeks). Brains were weighed frozen, and later homogenized in 10 ml ice-cold 0.1 N HCl containing 0.5 per cent ascorbic acid. Following removal of a 2-ml portion for determination of serotonin,[21] perchloric acid, 0.6 N, was added to the remainder of the homogenate, and the resulting supernatant was used for determination of norepinephrine[8,22] and dopamine.[23]

In order to measure noradrenergic and dopaminergic neuronal destruction by 6-hydroxydopamine, a group of rats not exposed to anesthesia was killed at a similar interval after 6-hydroxydopamine administration and dissected at 4 C into nine regions, as previously described.[24] Tyrosine hydroxylase was isolated by ammonium sulfate precipitation and enzymatic activity was measured by the method of Musacchio *et al.*[25] In those regions having little tyrosine hydroxylase per mg tissue, similar parts of three brains were combined to permit accurate determination of enzymatic content.

Rats given 6-hydroxydopamine received 200 μg in 25 μl of a saline solution containing 0.5 per cent ascorbic acid via the cisterna magna,[26] 30 minutes after pargyline, 40 mg/kg ip; a second dose of 6-hydroxydopamine was given two weeks later without pargyline pretreatment. Control animals were given pargyline 30 minutes before intracisternal administration of 25 μl of the ascorbic acid vehicle. Animals that received 6-hydroxydopamine in this way manifested severe hypophagia and hypodipsia after the second injection,[27] necessitating manual nutritional supplementation for 7–10 days. The animals were used for anesthetic exposure after allowing at least three weeks after the second injection of 6-hydroxydopamine for complete recovery.

Animals given 5,6-dihydroxytryptamine received 75 μg in 25 μl of saline solution intracisternally 10–14 days before use.[12] The methyl ester hydrochloride of α-methyltyrosine (250 mg/kg, ip) was administered in 1 ml/kg saline solution 30 minutes prior to placing the rat in the anesthetic apparatus, and the time of sacrifice was thus 3–3½ hours after administration of this tyrosine hydroxylase inhibitor. The methyl ester hydrochloride of p-chlorophenylalanine (100 mg/kg was administered intraperitoneally 48 hours, 24

TABLE 1. Effects of Inhibitors of Central Amine-containing Neurons on Halothane MAC

Amine(s) Primarily Affected	Drug	Number of Rats	MAC Difference (Control–Experimental), Per Cent
Norepinephrine and dopamine	α-Methyltyrosine	6	0.13* ± 0.03
	6-Hydroxydopamine	4	0.06 ± 0.03
	α-Methyltyrosine + 6-hydroxydopamine	6	0.27† ± 0.04
Serotonin	p-Chlorophenylalanine	7	0.17* ± 0.04
	5,6-Dihydroxytryptamine	6	0.12* ± 0.03
	p-Chlorophenylalanine + 5,6-dihydroxytryptamine	5	0.32† ± 0.05

Indicated groups were treated with intracisteral injection of 6-hydroxydopamine or 5,6-dihydroxytryptamine and/or intraperitoneal injection of α-methyltyrosine or p-chlorophenylalanine, as described in Methods. Values represent the mean difference ± standard error of the difference. Control groups had minimum atmospheric concentrations (MAC) values ranging from 1.27 ± 0.04 to 1.23 ± 0.03 per cent.

* $P < 0.05$.

† $P < 0.01$.

hours, and 30 minutes before the start of anesthetic exposure, as above. The 6-hydroxydopamine, 5,6-dihydroxytryptamine, α-methyltyrosine methyl ester HCl, and p-chlorophenylalanine methyl ester HCl were purchased from Regis Chemical Company, Chicago, Illinois.

The MAC for each experimental rat was compared with that for the control animal run the same day by means of a t test for paired data.[28] Brain amine concentrations were compared with a t test for unpaired data.

Results

In confirmation of earlier findings,[8] intracisternal administration of 6-hydroxydopamine was found to decrease rat brain tyrosine hydroxylase, an enzyme located only within catecholamine-containing neurons (fig. 1). This decrease probably reflects the loss of a proportionate number of catecholamine-containing (norepinephrine and dopamine) neurons and is accompanied by a comparable decrease in brain catecholamines. The extent of neuronal destruction varies in different brain regions, probably as a result of variable penetration of the drug to the neurons from the cerebrospinal fluid. The protocol employed in the present study produced more marked decreases in enzymatic activity of the cortex and striatum and the least decrease in the medulla.

Dependence of Halothane MAC on Brain Amines

Since the initial investigation indicated a relationship between amine depletion and anesthetic MAC employed halothane as the anesthetic,[1] this correlation was re-explored in the rat, using 6-hydroxydopamine to lower amine concentrations. However, central 6-hydroxydopamine administration failed to alter the MAC of halothane significantly (table 1). Catecholamine content was not measured in this group of rats, but 6-hydroxydopamine consistently reduces whole-brain norepinephrine and dopamine (See table 4, also references 6, 8, 27, and 30). To determine the effect of inhibition of transmitter synthesis of catecholamine-containing neurons, rats were given α-methyltyrosine, an inhibitor of tyrosine hydroxylase.[29] In contrast to findings in 6-hydroxydopamine-treated rats, administration of α-methyltyrosine significantly lowered MAC (table 1) and decreased brain norepinephrine to 50 per cent of control and dopamine to 22 per cent by the time administration of the anesthetic was terminated (table 2).

TABLE 2. Effects of α-Methylthyrosine and 6-Hydroxydopamine on Brain Amines

Group	Number of Rats	Brain Amines		
		Norepinephrine (ng/g)	Dopamine (ng/g)	Serotonin (μg/g)
α-Methyltyrosine	6	79† ± 19	139* ± 18	0.32 ± 0.025
Control	6	157 ± 38	619 ± 39	0.36 ± 0.04
6-Hydroxydopamine + α-methyltyrosine	6	43† ± 8	96† ± 10	0.62 ± 0.03
Control	6	308 ± 43	897 ± 60	0.63 ± 0.03

Experimental animals were given only α-methyltyrosine methyl ester hydrochloride intraperitoneally or 6-hydroxydopamine intracisternally five weeks before the α-methyltyrosine. Control animals received equal amounts of vehicle at the same time and by the same route, as described in Methods. The difference between brain amine contents of the two control groups of animals reflects the older age of the rats that served as controls for the administration of both 6-hydroxydopamine and α-methyltyrosine, and the known progressive increase in brain monoamines with age. Values represent means ± SEM.

* $P < .05$.

† $P < .01$.

To test the possibility that residual neurons remaining after 6-hydroxydopamine treatment might compensate for the deficit in neural function that follows loss of some neuronal units,[30] rats treated with 6-hydroxydopamine were given the tyrosine hydroxylase inhibitor in addition. This combined treatment, which depleted norepinephrine and dopamine much more severely than either drug alone (to 14 and 11 per cent of control, respectively; table 2), further decreased the MAC of halothane (table 1).

The effect of inhibition of serotonin neuronal function on halothane MAC was examined after treatment with 5, 6-dihydroxytryptamine and p-chlorophenylalanine. Even though serotonin was reduced by only 38 per cent (table 3), 5,6-dihydroxytryptamine significantly decreased the MAC of halothane (table 1). Inhibition of serotonin synthesis by p-chlorophenylalanine sufficient to reduce brain serotonin to 28 per cent of control (table 3) also decreased the MAC of

TABLE 3. Effects of Serotonergic Neuron Inhibition by p-Chlorophenylalanine and 5,6-Dihydroxytryptamine on Brain Amines

Group	Number of Rats	Brain Amine Concentration		
		Norepinephrine (ng/g)	Dopamine (ng/g)	Serotonin (μg/g)
p-Chlorophenylalanine	8	192 ± 24	387 ± 50	0.17† ± 0.02
Control	8	249 ± 94	352 ± 45	0.60 ± 0.10
5,6-Dihydroxytryptamine	5	244 ± 15	397 ± 54	0.34† ± 0.06
Control	5	252 ± 15	469 ± 43	0.54 ± 0.02
p-Chlorophenylalanine + 5,6-dihydroxytryptamine	5	214 ± 9	357 ± 56	0.21† ± 0.04
Control	5	287 ± 39	494 ± 35	0.62 ± 0.10

Animals received p-chlorophenylalanine, 5,6-dihydroxytryptamine, or both, as described in Methods. Rats were killed immediately after the first response to tail compression was elicited as halothane inspired concentration was being decreased. Values represent means ± SEM.

† $P < .01$ relative to control group.

TABLE 4. Brain Norepinephrine and Cyclopropane MAC in Rats given 6-Hydroxydopamine

Group	Whole-brain Norepinephrine (mg/g)	Cyclopropane MAC (Per Cent)
Control	598 ± 20	19.0 ± 0.6
6-Hydroxydopamine	98† ± 12	18.7 ± 0.8

Rats were given 6-hydroxydopamine or saline solution (controls) intracisternally, as described in Methods. All animals were killed immediately after the first response to tail compression was elicited as cyclopropane inspired concentration was being decreased. All values represent means ± SEM. The mean difference in MAC (control–experimental) and standard error of the difference were 0.3 ± 0.6 per cent, for eight pairs of determinations.

† $P < .001$.

halothane significantly (table 1). When p-chlorophenylalanine was given in rats previously treated with 5,6-dihydroxytryptamine a more dramatic decrease in MAC was observed (table 1). This combination of neural destruction and synthesis inhibition had decreased brain serotonin to 34 per cent by the time the MAC of halothane was determined (table 3). None of the drug treatments that interfered with serotonin neuronal function altered the content of whole-brain norepinephrine or dopamine.

Dependence of Cyclopropane MAC on Brain Amines

In spite of the marked reduction of brain catecholamine content by 6-hydroxydopamine, no change in the MAC of cyclopropane was observed (table 4). Furthermore, preliminary experiments suggested that no decrease in cyclopropane MAC occurred in other rats after administration of both 6-hydroxydopamine and α-methyltyrosine. Animals that received 5,6-dihydroxytryptamine intracisternally to destroy central serotonin-containing neurons[31] did not manifest any change in cyclopropane MAC (table 5).

Since ablation of a portion of either catecholamine- or serotonin-containing neurons was not associated with a significant alteration in cyclopropane MAC, an additional group of rats was given reserpine to interfere simultaneously with the functions of both types of neurons.[32] Although reserpine produced a decrease of norepinephrine to 3 per cent of normal 24 hours later (table 6), cyclopropane MAC remained unaltered.

Discussion

The present evidence of a slight reduction in halothane MAC in rats whose catecholaminergic neuronal function is altered without alteration in brain serotonin concentration confirms the earlier results of Miller *et al.*[1] Their initial studies indicated an increase in anesthetic susceptibility in rats given drugs that should have altered several central amine-containing neurons, although they interpreted the changes in anesthetic susceptibility solely in terms of central nervous system norepinephrine-containing neurons. Although reserpine and α-methyldopa, the two agents found to decrease the MAC of halothane in dogs, do produce dramatic effects in noradrenergic

TABLE 5. Brain Amines and Cyclopropane MAC after 5,6-Dihydroxytryptamine

Group	Brain Amines			Cyclopropane MAC (Per Cent)
	Norepinephrine (mg/g)	Dopamine (mg/g)	Serotonin (μg/g)	
Control	442 ± 45	748 ± 60	0.65 ± 0.21	19.7 ± 0.2
5,6-Dihydroxytryptamine	424 ± 16	723 ± 52	0.32* ± 0.12	19.0 ± 0.4

Rats received 5,6-dihydroxytryptamine or saline solution (controls) intracisternally, as described in Methods. All animals were killed immediately after the first response to tail compression was elicited as cyclopropane inspired concentration was being decreased. All values represent means ± SEM. The mean difference in MAC (control–experimental) and standard error of the difference were 0.7 ± 0.9 per cent, for six pairs of determinations.

* $P < .05$.

nerve function, they also perturb dopamine- and serotonin-containing neurons.[4,5] In assessing the disruption of neuronal function, experiments involving the use of tissue concentrations of amine must be interpreted with caution, since actual utilization of the amine may be decreased, unaltered, or increased. Unfortunately, however, it is not possible to obtain an estimate of transmitter release or turnover of amine in a single animal *in vivo*. Thus, amine concentrations are used as an index of neuronal alteration only; these numbers may not reflect the true integrity of function in the specific neuronal type. However, it seems likely that a drug or procedure that disturbs only one amine concentration without altering others is somewhat selective in its actions.

As reflected by both whole-brain amines and regional decreases in tyrosine hydroxylase, the effect of 6-hydroxydopamine is confirmed in the present study. However, in spite of this destruction, the brain appears to be able to recover some function by resorting to alternative pathways or by the development of supersensitivity. The development of supersensitivity to dopamine after 6-hydroxydopamine has already been proposed.[33] Another mechanism that compensates for the loss of some neuronal units is increased utilization or hyperactivity of the remaining nerves of that fiber system.[34] Such an effect would produce an increased rate of synthesis and release of amines from the remaining nerves in an attempt to overcome the decrease in rate of transmitter release. This may account for the increased susceptibility of 6-hydroxydopamine-treated rats to α-methyltyrosine, found previously when a decrease in performance task execution was used as an indicator of catecholamine neuronal function.[30] In the present study, although only one high dose of α-methyltyrosine was used, the effects on MAC may have reflected potentiation of its effects on resistance to anesthesia.

6-Hydroxydopamine renders the animal more irritable, and the rats require more morphine to achieve a given resistance to nociceptive stimuli.[35] The decrease in MAC found after 6-hydroxydopamine, therefore, is probably not related to any analgesic property, since morphine lowers MAC.[36] The catecholamine systems of the brain in general provoke excitation and locomotor activity, whereas interference with their function usually leads to depression and inactivity.[37] This general arousal function may be the mechanism which, when depressed by decreased catecholamine availability, renders the animal more susceptible to the depressant effects of halothane. Halothane itself may further depress the activity of this system, as reflected by decreased turnover or utilization of cerebral norepinephrine.[38] Such a proposal is of necessity very general, since MAC may reflect only the summation of changes in many systems.[39]

Administration of 5,6-dihydroxytryptamine produces decreased brain serotonin[9] and tryptophan hydroxylase.[31] However, the extent of destruction of serotoninergic neurons by 5,6-dihydroxytryptamine is less complete than that produced in catecholaminergic neurons by 6-hydroxydopamine. Nevertheless, a significant decrease in halothane MAC was observed. p-Chlorophenylalanine may reduce brain serotonin stores by at least three mechanisms,[40,41] 1) a decrease in uptake of

TABLE 6. Effects of Reserpine on Brain Norepinephrine and Cyclopropane MAC in Rats

Group	Number of Rats	Whole-brain Norepinephrine ng/g Brain	Cyclopropane MAC (Per Cent)
Control	19	1229 ± 90	18.1 ± 0.3
Reserpine, 1 day	6	401‡ ± 12	17.5 ± 0.4
Reserpine, 3 days	7	339† ± 60	19.1 ± 0.7
Reserpine, 6 days	6	610* ± 28	17.3 ± 0.2

Rats were given 10 mg/kg reserpine intraperitoneally at zero time, and MAC determined for cyclopropane at the indicated time intervals. Rats were killed after the first response to tail compression was elicited as cyclopropane inspired concentration was reduced. Values represent means ± SEM. Calculation of mean differences (control–experimental) and standard error of the difference revealed no significant effect on cyclopropane MAC.

* $P < .05$.
† $P < .01$.
‡ $P < .001$.

tryptophan, the serotonin precursor from the blood, 2) an inhibition of tryptophan hydroxylase by substrate competition, and 3) possibly the production of an abnormal, less active form of tryptophan hydroxylase. Thus, although the mechanism of inhibition of serotonin synthesis is much more complicated, the end result is an inhibition of serotonin synthesis that is similar to the effect of α-methyltyrosine on catecholamine synthesis. As with the catecholamine synthesis inhibition studies, the combined use of the selective neurotoxin 5,6-dihydroxytryptophan and serotonin synthesis inhibition produced a more dramatic decrease in halothane MAC than either treatment alone. Since p-chlorophenylalanine administration can antagonize morphine analgesia,[42] the decrease in halothane MAC would appear not to be the result of a primary effect on antinociceptive mechanics. Since p-chlorophenylalanine alone produces insomnia in rats,[43] it would appear not to possess meaningful sedative actions; indeed, in awake animals, an increase in aggressive behavior and an absence of normal EEG sleep patterns are observed.[44]

Other observations support an association between decreased amine availability and sensitivity to depressant anesthetics. Diazepam, which lowers the MAC of halothane in man,[45] also decreases turnover of central catecholamines[46] and serotonin in rats.[47] Eger found that hypoxia (to $Pa_{O_2} < 30$ mm Hg) decreased the MAC of halothane in dogs,[2] and Davis and Carlsson have recently demonstrated decreased synthesis of cerebral norepinephrine, dopamine, and serotonin as a result of exposure of rats to 5.6 per cent oxygen.[48]

Neither 6-hydroxydopamine nor 5,6-dihydroxytryptamine altered the cyclopropane MAC of rats, even though the extent of amine depletion appeared comparable to that produced in rats given halothane. Since reserpine produces drastic reductions in all three amines (norepinephrine, dopamine and serotonin) an attempt was made to determine whether this treatment would alter cyclopropane MAC. Since, again, no alteration in MAC was found after a dose of reserpine (10 mg/kg, ip) sufficient to deplete brain norepinephrine severely, it seems safe to conclude that these amine systems are not important in counteracting the neuronal changes produced by anesthetic concentrations of cyclopropane. The present findings with cyclopropane may be related to the lack of amphetamine effect on the MAC of fluroxene in rats, although the MAC of halothane was increased by amphetamine.[3] This may again reflect the unimportance of central amine-containing neurons in determining the resistance to a relatively excitatory anesthetic such as fluoroxene.[12]

Winters and Ferrar-Allado[11] and Clark and Rosner[12] have recently proposed that cyclopropane and halothane may represent agents at different points in the spectrum of anesthetic agents that produce, respectively, initial excitation as opposed to progressive depression of the CNS. Perhaps the integrity of central monoamine-containing neurons is important only in altering the response to depressant anesthetics and these neurons do not antagonize the initial promiment excitatory phenomena that characterize the effects of cyclopropane at light levels of anesthesia. This thesis can be accepted only when other anesthetic agents have been explored to test the importance of the effect of amine-containing neurons on the ability of the agents to produce anesthesia.

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References

1. Miller RD, Way WL, Eger EI II: The effects of alpha-methyldopa, reserpine, guanethidine and iproniazid on minimum alveolar anesthetic requirement (MAC). ANESTHESIOLOGY 29:1153–1158, 1968
2. Eger EI II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of potency. ANESTHESIOLOGY 26:756–763, 1965
3. Johnston RR, Way WL, Miller RD: Alteration of anesthetic requirement by amphetamine. ANESTHESIOLOGY 36:357–363, 1972
4. Gal EM, Millard SA: The relevancy of changes in cerebral 5-hydroxytryptamine to reserpine produced sedation. Biochem Pharmacol 17:2193–2203, 1968

5. Hess SM, Cornnamacher RH, Ozaki M, et al: The effects of α-methyl-meta-tyrosine on the metabolism of norepinephrine and serotonin *in vivo*. J Pharmacol Exp Ther 134:129–138, 1961
6. Breese GR, Cooper BR, Mueller RA: Evidence of serotonergic involvement in the actions of amphetamine. Br J Pharmacol (in press)
7. Ungerstedt U: 6-Hydroxydopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5:107–110, 1968
8. Breese GR, Traylor TD: Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence of selective degeneration of catecholamine neurons. J Pharmacol Exp Ther 174:413–420, 1970
9. Baumgarten HG, Bjorklund A, Lachenmayer L, et al: Long lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. Acta Physiol Scand suppl 373, 1971
10. Breese GR, Cooper BR, Grant LD, et al: Biochemical and behavioral alterations following 5,6-dihydroxytryptamine administration into brain. Neuropharmacology 13: 177–187, 1974
11. Winters WD, Ferrar-Allado T: The cataleptic state induced by ketamine: A review of the neuropharmacology of anesthesia. Neuropharmacology 11:303–315, 1972
12. Clark DL, Rosner BS: Neurophysiologic effects of general anesthetics. I. The electroencephalogram and sensory evoked potentials in man. ANESTHESIOLOGY 38:564–582, 1973
13. Mori K, Winters WD, Spooner CE: Comparison of reticular and cochlear multiple unit activity with auditory evoked responses during various stages induced by anesthetic agents. II. Electroenceph Clin Neurophysiol 24:242–248, 1968
14. Inamoto A, Fujita M, Oikawa T, et al: Relationship of arterial hypertension and EEG pattern of hippocampus during cyclopropane anesthesia. Jap J Anesthesiol 13:855–856, 1964
15. Mori K, Iwabuchi K, Kawamata M, et al: The neural mechanism of cyclopropane anesthesia in the rabbit. ANESTHESIOLOGY 36:228–237, 1972
16. White PF, Johnston RR, Eger EI II: Determination of anesthetic requirement in rats. ANESTHESIOLOGY 40:52–57, 1974
17. Wolfson B, Dorsch SE, Juo T, et al: Brain anesthetic concentration—a new concept. ANESTHESIOLOGY 36:176–180, 1972
18. Eger EI II, Bahlman SH: Is the end-tidal anesthetic partial pressure an accurate measure of the arterial anesthetic partial pressure? ANESTHESIOLOGY 35:301–303, 1971
19. Hoffman JC, Munson ES, DiFazio CA: Effects of acute hypo- and hyperthyroidism on cyclopropane requirement to (MAC) in the rat. ANESTHESIOLOGY 29:1094–1098, 1968
20. Eger EI II, Saidman LT, Brandstater B: Temperature dependence of halothane and cyclopropane anesthesia in dogs: Correlation with some theories of anesthetic action. ANESTHSIOLOGY 26:764–770, 1965
21. Bogdanski DF, Pletcher A, Brodie BB, et al: Identification and assay of serotonin in brain. J Pharmacol Exp Ther 117:82–88, 1956
22. Anton AH, Sayre DF: A study of the factors affecting the aluminum oxide–trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138: 360–375, 1962
23. Anton AH, Sayre DF: The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. J Pharmacol Exp Ther 145:326–336, 1964
24. Cooper BR, Howard JL, Grant LD, et al: Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. Pharmacol Biochem Behav 2:639–649, 1974
25. Musacchio JM, Julou L, Kety SS, et al: Increase in rat brain tyrosine hydroxylase activity produced by electroconvulsive shock. Proc Natl Acad Sci (USA) 63:1117–1119, 1969
26. Schanberg SM, Schildkraut JJ, Breese GR, et al: Metabolism of normetanephrine in rat brain: Identification of conjugated 3-methoxy-4-hydroxyphenylglycol as the major metabolite. Biochem Pharmacol 17:247–254, 1968
27. Breese GR, Smith RD, Cooper BR, et al: Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine. Pharmacol Biochem Behav 1:319–328, 1973
28. Steele RGD, Torrie JH: Principles and Procedures of Statistics. New York, McGraw-Hill, 1960
29. Spector S, Sjoerdsma A, Udenfriend S: Blockade of endogenous norepinephrine synthesis by α-methyltyrosine, an inhibition of tyrosine hydroxylase. J Pharmacol Exp Ther 147:86–95, 1965
30. Cooper BR, Breese GR, Howard JL, et al: Enhanced behavioral depressant effects of reserpine and α-methyltyrosine after 6-hydroxydopamine treatment. Psychopharmacologia 27:99–110, 1972
31. Baumgarten HG, Lachenmayer L, Scholossberger HG: Evidence for a degeneration of indolamine-containing nerve terminals in rat brain, induced by 5, 6-dihydroxytryptamine. Z Zellforsch 125:553–569, 1972
32. Carlsson A, Rosengren E, Bertler A, et al:

Effect of reserpine on the metabolism of catecholamines, Psychotropic Drugs. Edited by S Garattini, V Ghetti. Amsterdam, Elsevier, 1957

33. Ungerstedt U: Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol Scand 367:69–93, 1971
34. Agid A, Javoy J, Glowinski J: Dopaminergic neurone-hyperactivity of dopaminergic neurons after partial destruction of the nigro-striatal dopaminergic system. Nature [New Biol] 245:150–151, 1973
35. Elchisak MA, Rosecrans JA: Effect of central catecholamine depletion by 6-hydroxydopamine in morphine antinociception in rats. Res Comm Chem Pathol Pharmacol 6:349–353, 1973
36. Munson ES, Saidman LJ, Eger EI II: Effect of nitrous oxide and morphine on the minimum anesthetic concentration of fluroxene. ANESTHESIOLOGY 26:134–139, 1965
37. Weiss B, Laties VG: Behavioral pharmacology and toxicology. Annu Rev Pharmacol 9:297–326, 1969
38. Ngai SH, Neff NH, Costa E: The effects of cyclopropane and halothane on the biosynthesis of norepinephrine *in vivo*. ANESTHESIOLOGY 31:53–60, 1969
39. Shim CY, Andersen NB: Minimal alveolar concentration (MAC) and dose–response curves in anesthesia. ANESTHESIOLOGY 36:146–151, 1972
40. Koe BK, Weissman A: p-Chlorophenylalanine: A specific depletor of brain serotonin. J Pharmacol Exp Ther 154:499–516, 1966
41. Knapp SA, Mandell AJ: Parachlorophenylalanine—its three phase sequence of interactions with the two forms of brain tryptophan hydroxylase. Life Sci II, Part 1, 761–771, 1972
42. Tenen SS: Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. Psychopharmacologia 12:278–285, 1968
43. Mouret J, Bobillier P, Jouvet M: Insomnia following parachlorophenylalanine in the rat. Eur J Pharmacol 5:17–22, 1968
44. Sheard MH: The effect of p-chlorophenylalanine on behavior in rats: Relation to brain serotonin and 5-hydroxyindoleacetic acid. Brain Res 15:524–528, 1969
45. Perisho JA, Buechel DR, Miller RD: The effect of diazepam (Valium) on minimum alveolar anesthetic requirement (MAC) in man. Can Anaesth Soc J 18:536–540, 1971
46. Taylor KM, Laverty R: The effect of chlordiazepoxide, diazepam and nitrazepam on catecholamine metabolism in regions of the rat brain. Eur J Pharmacol 8:296–301, 1969
47. Chase TN, Katz RI, Kopin IJ: Effect of diazepam on fate of intracisternally injected serotinin-C^{14}. Neuropharmacology 9:103–108, 1970
48. Davis JN, Carlsson A: The effect of hypoxia on monoamine synthesis, levels and metabolism in rat brain. J Neurochem 21:783–790, 1973