The Effects of Cyclopropane, Halothane and Diethyl Ether on the Cerebral Metabolism of Serotonin in the Rat

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The effects of cyclopropane, halothane and diethyl ether on cerebral levels of serotonin (5-HT) and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were studied in rats. The turnover rate of cerebral 5-HT was measured, using steady-state kinetics after inhibition of monoamine oxidase or blockade of acid transport, during exposure to 15 per cent cyclopropane, 0.75 per cent halothane, or 3 per cent diethyl ether. Cerebral content of 5-HT increased slightly (14 per cent) during cyclopropane anesthesia; decreased (17 per cent) with halothane; and did not change with diethyl ether. 5-HIAA levels increased with all three anesthetics, especially with diethyl ether (100 per cent), probably due to a decrease in its rate of removal from the brain. The turnover rate of brain 5-HT remained unchanged with cyclopropane and halothane, but increased considerably with diethyl ether.

SEROTONIN (5-hydroxytryptamine, 5-HT), a biogenic amine in plant and animal tissues, has been postulated to act as a neurotransmitter in the mammalian central nervous system.[1] Feldberg and his co-workers [2, 3] showed that in cats and dogs, serotonin, norepinephrine and epinephrine, by their actions in the anterior hypothalamus, are involved in thermoregulation. Serotonin injected into the anterior hypothalamus or into the cerebral ventricles produced shivering, constriction of cutaneous blood vessels and a rise in body temperature. Norepinephrine or epinephrine, similarly injected, reduced body temperature and stopped shivering.

Anderson and Bonnycastle [4] and Bonnycastle *et al.*[5] observed approximately twofold increases in serotonin levels in rat brain during anesthesia with a number of barbiturates, diethyl ether, chloralose, and other central nervous system depressants such as chloral hydrate, meperidine and alcohol. These authors posulated that the increase in cerebral serotonin levels was an effect of the state of central nervous system depression. On the other hand, Paasonen and Giarman [6] found no changes in cerebral serotonin levels in rats anesthetized with hexobarbital and diethyl ether.

The present study was undertaken to evaluate the effects of three commonly used inhalation anesthetics, cyclopropane, halothane and diethyl ether, on the metabolism of serotonin in the brain. The levels of serotonin and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were determined fluorometrically. The turnover rate, or rate of synthesis of serotonin, was also measured using steady-state kinetics during anesthesia with these agents.

Methods

Male Sprague-Dawley rat weighing approximately 200 Gm. each were used in all experiments. Groups of animals were placed in transparent plastic boxes (capacity 14 l., with inlet and exhaust ports at opposite ends). Compressed air or anesthetic mixture was de-

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livered at a rate of 3 l./min. from an Ohio series 3000 kinetometer with calibrated flowmeters and a Vernitrol vaporizer. Twenty-five per cent oxygen, with the balance nitrogen, was used as the diluent gas to facilitate distinction of the effect of the anesthetic on cerebral serotonin metabolism from that of oxygen.[7] Concentrations of anesthetics that abolished the righting reflex were chosen: cyclopropane, 15 per cent; halothane, 0.75 per cent; diethyl ether, 3 per cent. Rectal temperatures, measured just before sacrificing the animals were found to vary within 2° C. of control values (37–38° C.).

During inhalation of different anesthetic mixtures, groups of at least five rats each were sacrificed at intervals by decapitation. Brains were immediately removed and frozen until assayed. The whole brain content of serotonin and 5-HIAA was determined according to the methods of Bogdanski *et al.*[8] and Udenfriend *et al.*[9] respectively, using an Aminco-Bowman spectrophotofluorometer.

Student's *t* test was applied to assess the significance of the differences between the means of control and experimental groups.

The rate of turnover of cerebral serotonin was studied, using steady-state kinetics according to the method of Neff *et al.*[10] After one to four hours of anesthesia, an acid transport-blocking agent, probenecid (Benemid, 200 mg./kg.), was injected intraperitoneally. Exposure to the anesthetic agent was continued. Groups of animals were sacrificed 0, 15, 30 and 60 minutes after the injection of probenecid and cerebral 5-HIAA levels measured. These values were used to calculate the rate of accumulation of 5-HIAA, reflecting the rate at which serotonin was being oxidized to 5-HIAA at the time of probenecid administration. Alternatively, a monoamine oxidase inhibitor, pargyline (Eutonyl, 75 mg./kg.) was injected intraperitoneally and the initial rate of accumulation of serotonin was determined. For control experiments, rats were exposed to air in the plastic boxes before and after injection of probenecid or pargyline. The fractional rate constant of accumulation of 5-HIAA (after probenecid) or that of accumulation of serotonin (after pargyline) was derived from

TABLE 1. Effects of Anesthetic Agents on Cerebral Serotonin and 5-Hydroxyindoleacetic Acid in the Rat

Hours of Exposure	Cyclopropane			Halothane			Diethyl Ether		
	5-HTP (μg./Gm. ± S.E.)	*p*	5-HIAA (μg./Gm. ± S.E.)	5-HTP (μg./Gm. ± S.E.)	*p*	5-HIAA (μg./Gm. ± S.E.)	5-HTP (μg./Gm. ± S.E.)	*p*	5-HIAA (μg./Gm. ± S.E.)	*p*
0	0.50 ± 0.01 (6)*	<0.02	0.40 ± 0.01 (6)	0.51 ± 0.02 (5)	<0.05	0.40 ± 0.01 (6)	0.47 ± 0.01 (6)	N.S.	0.40 ± 0.01 (6)	<0.001
1	0.47 ± 0.02 (6)	N.S.	0.42 ± 0.01 (6)	0.50 ± 0.01 (5)	<0.05	0.53 ± 0.01 (5)	0.48 ± 0.02 (6)	N.S.	0.45 ± 0.02 (6)	<0.001
2	0.51 ± 0.02 (6)	<0.02	0.48 ± 0.003 (6)	0.45 ± 0.02 (5)	<0.05	0.46 ± 0.01 (5)	0.49 ± 0.02 (6)	N.S.	0.51 ± 0.01 (6)	<0.001
3	0.50 ± 0.02 (6)	<0.02	0.44 ± 0.01 (6)	0.43 ± 0.01 (5)	<0.05	0.48 ± 0.00 (3)	0.47 ± 0.02 (6)	N.S.	0.51 ± 0.01 (6)	<0.001
4	0.57 ± 0.03 (6)	<0.05	0.45 ± 0.01 (6)	0.43 ± 0.02 (5)	<0.05		0.43 ± 0.01 (6)	<0.001	0.69 ± 0.02 (6)	<0.001

* Numbers of animals shown in parentheses.

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TABLE 2. Effects of Cyclopropane, Halothane and Diethyl Ether on the Rate of Turnover of Serotonin in Rat Brain

Anesthetic	Probenecid Method				Monoamine Oxidase Inhibitor Method			
	Hours of Exposure	Turnover Rate (μg./Gm./hour ± S.E.)	k (hr^{-1})	P	Hours of Exposure	Turnover Rate (μg./Gm./hour ± S.E.)	k (hr^{-1})	P
Control	0	0.28 ± 0.01	0.70		0	0.39 ± 0.03	0.88	
Cyclopropane (15 per cent)	1	0.32 ± 0.02	0.60	N.S.	2	0.42 ± 0.03	0.73	N.S.
Halothane (0.75 per cent)	4	0.26 ± 0.02	0.65	N.S.	3.5	0.36 ± 0.03	0.70	N.S.
Diethyl Ether (3 per cent)	4	0.56 ± 0.03	0.84	<0.001	3	0.59 ± 0.02	1.19	<0.001

the relationship

$$k = \frac{\text{rate of accumulation} \pm \text{S.E.}}{[\text{concentration}_0] \pm \text{S.E.}},$$

[concentration$_0$] being the steady-state level of 5-HIAA or serotonin at the time of injection.

In order to exclude the possible effect of hypercarbia, which might be present during anesthesia, cerebral levels of serotonin, 5-HIAA, and turnover rate of serotonin were measured after exposure to a mixture of 5 per cent carbon dioxide, 25 per cent oxygen and 70 per cent nitrogen for one to four hours.

Results

The effects of cyclopropane, halothane and diethyl ether on cerebral levels of serotonin and 5-HIAA after two to five hours of anesthesia are summarized in table 1. In various groups of control animals kept in plastic boxes but breathing air, the levels of serotonin ranged from 0.47 ± 0.01 and 0.51 ± 0.02 μg./Gm. (mean ± S.E.). Cyclopropane produced a slight but significant increase (14 per cent, $P < 0.02$) at two hours. Halothane, on the other hand, caused a progressive decrease in the serotonin levels; after four and five hours of anesthesia these were approximately 16–17 per cent less than that of control ($P < 0.01$ and < 0.05, respectively). Diethyl ether had no appreciable effect.

All three anesthetics increased the brain 5-HIAA levels significantly. Changes during cyclopropane and halothane anesthesia were moderate (20 per cent). The increase during diethyl ether anesthesia was pronounced, for 5-HIAA levels were doubled after four hours of exposure.

Results of studies of the turnover rate of cerebral serotonin are summarized in table 2. As measured by the rate of accumulation of 5-HIAA after probenecid injection, or that of serotonin after pargyline injection, the turnover rate of serotonin was not significantly affected by cyclopropane or halothane.

Diethyl ether significantly increased the turnover rate of cerebral serotonin. With the

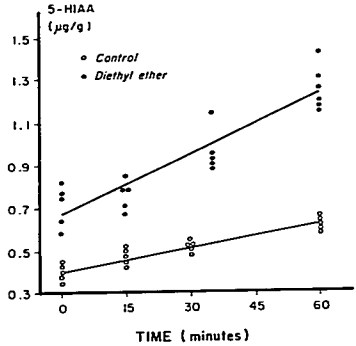

FIG. 1. Effect of four hours of exposure to 3 per cent diethyl ether on the turnover rate of rat cerebral serotonin, measured as the rate of accumulation of 5-hydroxyindoleacetic acid (5-HIAA). Probenecid (200 mg./kg.) was injected intraperitoneally at zero time. The turnover rate in the control group was 0.28 μg./Gm./hour; the turnover rate after diethyl ether was 0.56 μg./Gm./hour ($P < 0.001$).

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probenecid method, the turnover rate in control animals breathing air was 0.28 ± 0.01 μg./Gm./hour. In animals exposed to 3 per cent diethyl ether for four hours the rate was 0.56 ± 0.03 μg./Gm./hour; the difference was highly significant. Figure 1 shows the rates of change in cerebral 5-HIAA levels after probenecid injection in controls and animals anesthetized with diethyl ether. Similar results were obtained using the pargyline method of measurement (*cf.* table 2).

Cerebral serotonin and 5-HIAA levels after one to four hours of exposure to 5 per cent carbon dioxide were essentially the same as the control values. Turnover rate of serotonin measured after one hour of exposure to carbon dioxide was not significantly different from that of control.

Discussion

The results of these experiments showed that cyclopropane, halothane and diethyl ether, administered in concentrations that abolished righting reflexes for periods as long as five hours, do not alter rat cerebral serotonin levels appreciably. Changes observed with cyclopropane and halothane, although significant, were less than 20 per cent of control values and in opposite directions. Cerebral serotonin levels remained unchanged during diethyl ether anesthesia. Studies with pentobaribtal (unpublished data) also showed no significant changes in brain serotonin contents, in contrast to the reports of Bonnycastle and his coworkers [4, 5] who found a twofold increase with most central nervous system depressants. Their suggestion that the cerebral serotonin level rises as a result of central depression is not supported by the results of the present study.

On the other hand, cerebral 5-HIAA levels increased with all three anesthetics, especially diethyl ether (100 per cent). Because the principal pathway for metabolism of cerebral serotonin is presumably through oxidative deamination by monoamine oxidase,[11] there is no significant precursor for 5-HIAA other than serotonin. An increase in 5-HIAA levels would mean either an increased rate of serotonin oxidation by monoamine oxidase or a decreased rate of 5-HIAA removal from the brain, or both. With cyclopropane and halothane the turnover rate (rate at which serotonin was synthesized or metabolized) was not significantly altered. Therefore, it would appear that these anesthetics partially blocked the efflux of 5-HIAA from the brain. It is not known whether 5-HIAA leaves the brain by simple diffusion or by active transport; indirect evidence favors the latter mechanism.[12]

Diethyl ether increased the turnover rate of cerebral serotonin. After four hours of anesthesia, when the brain 5-HIAA had reached a maximal level, complete blockade of 5-HIAA efflux from the brain by probenecid resulted in 5-HIAA accumulation at a rate double the control value (fig. 1). This indicates that oxidation of serotonin was accelerated. Because the serotonin level remained more or less unchanged, it can be assumed that the rate of synthesis was accelerated also. This was confirmed by the finding that the rate of serotonin accumulation after monoamine oxidase inhibition was faster than the control value (*cf.* table 2).

Diethyl ether also decreased the rate of 5-HIAA efflux from the brain. In another series of experiments,[7] 100 per cent oxygen increased the turnover rate of serotonin from a control value of 0.30 to 0.55–0.67 μg./Gm./hour. There was only a transient increase in 5-HIAA levels (20 per cent). In the present study the turnover rate of serotonin was increased about twofold but the 5-HIAA levels also doubled.

Hypercarbia may be excluded as a factor in changes in 5-HIAA levels and serotonin metabolism observed during anesthesia. Exposure to 5 per cent carbon dioxide had no apparent effect on levels of serotonin, 5-HIAA or serotonin turnover rate.

A number of mechanisms may be considered to explain the increase in the turnover rate of serotonin by diethyl ether: (1) an increase in the rate of tryptophan hydroxylation to 5-hydroxytryptophan (5-HTP), the rate-limiting step in the biosynthesis of serotonin [13]; (2) an increase in the rate of 5-HTP decarboxylation to serotonin; and (3) an increase in monoamine oxidase activity. Results of the present study provide no evidence to elucidate the possible site of action of diethyl ether. It may be speculated, however, that since hydroxylation of tryptophan is the rate-limiting

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step it is the mechanism most likely to be affected.

While it is not possible to decide from these results whether diethyl ether increases monoamine oxidase activity, anesthetics (including cyclopropane and halothane in the concentrations used) apparently do not inhibit this enzyme. This is of some significance, at least, in considerations of anesthetic action on the metabolism of biogenic amines, especially that of catecholamines.

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Kidney

RENAL FUNCTION Eighteen patients with normal renal function were investigated to determine the changes in urinary protein excretion associated with routine abdominal procedures. There was a real increase in urinary protein concentration and excretion at the time of and following surgery. This increase was transient, reaching a peak in the third or fourth postoperative 24-hour urine collection and returning to normal between the eighth and tenth postoperative days. Protein excretion was higher in patients who underwent major operations, which suggests that tubular handling of proteins was temporarily compromised. Whether there is actual cellular damage, or a metabolic change, is a matter of conjecture. (*Macbeth, W. A., and Pope, G. R.: Effect of Abdominal Operation upon Protein Excretion in Man, Lancet 1: 215 (Feb.) 1968.*)

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