

Effects of Ablation of Serotonin or Norepinephrine Brain-stem Areas on Halothane and Cyclopropane MACs in Rats

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General anesthesia may result from interruption of neural transmission in discrete areas of brain rather than from a generalized depression of transmission. Preliminary experiments have demonstrated that anesthetics selectively alter neurotransmitter concentrations, or glucose metabolism, in specific regions in the brain. The authors questioned whether anesthetic requirement would be altered by destruction of selected nuclei. They found that bilateral destruction of a large norepinephrine cell-body area, the locus coeruleus in the rat, decreased halothane MAC from 1.13 to 0.78 per cent ($P \leq 0.01$) and cyclopropane MAC from 20.5 to 16.1 per cent ($P \leq 0.001$) compared with sham-operated littermates of equal weight. Ablation of the locus coeruleus did not change hypothalamic norepinephrine content, but decreased cortical norepinephrine levels by 80 per cent. Destruction of the ventral bundle, which supplies approximately 40 per cent of the norepinephrine in the central gray catecholamine area, decreased halothane MAC 35 per cent ($P \leq 0.001$) and cyclopropane MAC 16 per cent ($P \leq 0.01$). However, rats that had ventral-bundle lesions weighed 18 per cent less ($P \leq 0.001$) than controls. Ventral-bundle lesions decreased hypothalamic norepinephrine by 85 per cent without altering cortical norepinephrine. Lesions in the serotonin-rich nucleus raphe dorsalis decreased halothane MAC 25 per cent ($P \leq 0.02$) and cyclopropane MAC 16 per cent ($P \leq 0.01$). These lesions decreased hypothalamic serotonin content by 40 per cent, and cortical serotonin content by 80 per cent. Although destruction of individual nuclei significantly decreased anesthetic requirement, lesions in no one area altered MAC by more than 35 per cent. The hypothesis that general anesthesia results from discrete rather than generalized depression of transmission is thus supported, but not proven, by these experiments. (Key words: Anesthetics, gases: cyclopropane. Anesthetics, volatile: halothane. Brain: locus coeruleus; synapses; cortex, cerebral; hypothalamus. Potency, anesthetic: MAC. Sympathetic nervous system: catecholamines, norepinephrine. Serotonin.)

GENERAL ANESTHESIA may result from interruption of neural transmission in discrete areas of brain rather than from generalized depression of transmission. This hypothesis would be supported if a consistent

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neurotransmitter alteration (*e.g.*, release or altered content) in these areas were found with all anesthetics while other areas showed inconsistent changes. Such support was given by our demonstration that halothane and cyclopropane both consistently and selectively altered neurotransmitter content in a small fraction of brain nuclei.^{1,2} Cyclopropane or halothane anesthesia increases norepinephrine concentration in the locus coeruleus and central gray catecholamine area and increases serotonin levels in the nucleus raphe dorsalis, suggesting that anesthetic-induced depression of neurotransmitter release at specific sites in the brain may cause or modify anesthesia. Other evidence favoring this hypothesis is the very different metabolic changes seen in different regions of the brain during anesthesia.³

If anesthetic agents decrease the release of neurotransmitters in specific areas of brain and this decrease results in the state of anesthesia, then destruction of these specific regions should decrease anesthetic requirement. To test this hypothesis, we compared the anesthetic requirements of rats having destructive lesions in the locus coeruleus, ventral noradrenergic bundle, or nucleus raphe dorsalis with those of control animals.

Methods

All studies were performed on Sprague-Dawley rats weighing 150–200 g. Preliminary histologic studies were performed on 16 rats to determine standard stereotactic coordinates for placement of lesions. In 12 rats unilateral knife-cut or electrolytic lesions were made in the locus coeruleus, nucleus raphe dorsalis, or ventral noradrenergic bundle. Fourteen days later, using the fluorescence histochemical method of Falck and associates,⁴ localization of these brain areas was carried out in 12 rats with lesions plus an additional four rats without lesions.

All rats, both experimental and control, were anesthetized with ether and placed in a stereotactic apparatus.[¶] Using the determined stereotactic coordinates (outlined below), we made bilateral electrolytic lesions with a 26-gauge stainless steel electrode (coated with Teflon except for the exposed tip, which was 0.3 mm

¶ Kopf Instruments, Tujunga, California.

wide and 0.7 mm long). Electric current was applied with a Grass LM4 lesion-maker set at 3 mA for 25 sec.

Lesions of the locus coeruleus were made in ten rats. With the incisor 3.0 mm below the interauricular line, lesions were bilaterally placed 1.7 and 2.2 mm caudal to the interauricular line, 1.15 mm lateral to the midline, and 6.5 mm ventral to the dura. Lesions in the nucleus raphe dorsalis were similarly placed in ten rats. The animal's head was positioned in the stereotaxic apparatus with the nose angled 5 degrees below the horizontal. An electrode was then inserted in the midline at the level of the interauricular line. The tip of the electrode was lowered 6.5 mm down from the dural surface and the lesion made. Bilateral ventral-bundle lesions were made in 40 rats. With the nose angled 15 degrees below the horizontal, bilateral lesions were made using a knife with a 1.1-mm cutting edge. Coordinates used were 2.5 mm caudal to the interauricular line, 0.8 mm lateral to the midline, and 8.7 mm ventral to the dura. Thirty-one control animals were treated similarly except that bilateral electrolytic lesions of the same dimensions as the lesions in the locus coeruleus were placed randomly in the frontal cortex. In addition, 20 additional control animals that were simply anesthetized with ether were included in the study.

Eighteen to 22 days after the placement of the lesions, MAC for cyclopropane in oxygen was determined according to the method of Eger *et al.*,⁵ as modified for rats by White *et al.*⁶ The animals were anesthetized in a clear, cylindrical chamber. Because of the low solubility of cyclopropane in blood, end-tidal cyclopropane concentrations were assumed to equal inspired (*i.e.*, chamber) concentrations when the latter had not changed for 20 min. End-tidal halothane, cyclopropane and carbon dioxide samples were analyzed with a Beckman LB 2 infrared analyzer. Rectal temperature was monitored and maintained at 37.0 ± 1.0 C with a heating pad. MAC was the anesthetic concentration midway between that permitting and that preventing movement in response to an alligator clip applied to the distal half of the tail for one minute. MAC was the mean of three determinations made at least one hour and no more than four hours apart.

The MAC for halothane in the same animals was determined seven days later. For this determination, tracheostomies were performed on all animals and end-tidal samples obtained from a side-arm sampling line of the tracheostomy tube as previously described.⁶ Because of a high mortality rate in the animals with lesions in the ventral bundle during and shortly after cyclopropane anesthesia, different animals with that lesion were used to determine MACs for halothane and for cyclopropane. Immediately after the deter-

mination of MAC for halothane, the animals were sacrificed by decapitation and the brains quickly removed and frozen on dry ice. To verify placement of lesions, 60- μ m coronal sections through the areas of the lesions were examined under a microscope for completeness and accuracy in 50 per cent of all animals. Large areas in all regions of the brain were dissected using the method of Glowinski and Iverson.⁷ Norepinephrine and serotonin in these regions were assayed by the radioisotopic enzymatic method of Henry as described by Coyle *et al.*⁸ and Saavedra *et al.*⁹

Results

Norepinephrine concentration decreased 75 per cent in the cortices of animals with lesions of the locus coeruleus and 85 per cent in the hypothalami of animals with lesions of the ventral bundle (table 1). Serotonin concentration decreased 77 per cent in the cortices of animals having lesions of the nucleus raphe dorsalis. No other significant decrease was found for any area with any lesion.

Lesions of the locus coeruleus, nucleus raphe dorsalis, and ventral bundle all decreased MACs for cyclopropane and halothane (table 2). For each agent the effects of the three lesion were quantitatively similar (*i.e.*, 16–21 per cent decreases in MAC for cyclopropane and 25–35 per cent decreases in MAC for halothane. The decrease in MAC for halothane tended to be greater than that for cyclopropane. No alteration in MAC occurred in the sham-operated animals with cortical lesions.

Discussion

Qualitatively, these results support the hypothesis that an effect on discrete rather than general areas of the brain may cause anesthesia. When we destroyed areas where we had previously demonstrated consistent neurotransmitter content changes with halothane or cyclopropane, we found decreases in anesthetic requirements. In contrast, destruction of randomly selected areas of cerebral cortex had no effect on anesthetic requirement. Animals with each lesion had a greater percentage decrease in MAC for halothane than for cyclopropane. We have no explanation for this consistent difference.

In this study, we tried to destroy three areas. Two of those areas were readily destroyed: the serotonergic cell body area near the third ventricle, the nucleus raphe dorsalis, and the locus coeruleus, a brain-stem area rich in norepinephrine. The latter nucleus supplies a large proportion of the cerebral and cerebellar norepinephrine content.¹⁰ In the brain stem around the third ventricle we tried to destroy the central gray

TABLE 1. Effects of Destruction of Specific Brain-stem Nuclei on Halothane MAC and Norepinephrine and Serotonin Concentrations in Large Areas of Brain*

	MAC for Halothane (Per Cent)	Norepinephrine ($\mu\text{g/g}$)		Serotonin ($\mu\text{g/g}$)	
		Cortical Lesion	Hypothalamic Lesion	Cortical Lesion	Hypothalamic Lesion
Control	1.13 \pm 0.03 (20)	0.37 \pm 0.04 (10)	2.00 \pm 0.08 (10)	0.44 \pm 0.04 (10)	0.50 \pm 0.08 (10)
Percentage decrease from control					
Locus coeruleus	31 \pm 4† (9)	75 \pm 5* (5)	<5 (5)	<5 (5)	<5 (5)
Ventral bundle	35 \pm 4† (19)	<5 (10)	86 \pm 2† (10)	<5 (10)	<5 (10)
Nucleus raphe dorsalis	25 \pm 4† (9)	<5 (5)	<5 (5)	77 \pm 9† (5)	20 \pm 10 (5)
Small areas of cortex	-0.01 \pm 3 (22)	<5 (11)	<5 (11)	<5 (11)	<5 (11)

* Data are means \pm SEM. Number of animals in each group is indicated in parentheses.

† Significantly different from control, $P < 0.001$.

catecholamine area, a norepinephrine terminal area bordering the nucleus raphe dorsalis.¹¹ We were unable to destroy this area without damaging other nuclei, so we settled for destroying the ventral noradrenergic bundle. This bundle supplies about 40 per cent of the noradrenergic terminals to the central gray catecholamine area.¹¹ MAC testing was performed at least 18 days after nuclear destruction, when neurotransmitter content in discrete areas of brain has stabilized.^{10,11} Previous studies have not disclosed any area of brain where norepinephrine or serotonin is increased after these destructive lesions.^{10,11} Since we were interested in controlling for previous surgical or anesthetic experience, the controls and the experimental animals experienced identical test conditions and anesthetic-surgical exposure times. A more sensitive control for each animal might have been that animal prior to nuclear destruction, but those controls would not have had the previous anesthetic-surgical experiences or the tracheostomy, in case of halothane MAC testing.

TABLE 2. Effects of Destruction of Specific Brain-stem Nuclei on MACs for Cyclopropane and Halothane*

Location of Lesion	Weight (g)	MAC for Cyclopropane (Per Cent)	MAC for Halothane (Per Cent)
Locus coeruleus	243 \pm 4	16.1 \pm 0.3‡ (10)	0.78 \pm 0.04‡ (9)
Nucleus raphe dorsalis	264 \pm 4	17.2 \pm 0.6† (10)	0.85 \pm 0.05‡ (9)
Ventral bundle	204 \pm 4‡	17.2 \pm 0.5‡ (20)	0.74 \pm 0.05‡ (19)
Sham cortical	255 \pm 4	20.5 \pm 0.5 (31)	1.13 \pm 0.03 (22)

* Data are means \pm SEM. Number of animals in each group is indicated in parentheses.

† Significantly different than control, $P \leq 0.01$, by group t test.

‡ Significantly different than control, $P \leq 0.001$, by group t test.

The MAC-lowering effects of these lesions may be interpreted as indicating an effect of diffuse alterations in neurotransmitter rather than blockades of release at crucial sites. Destruction of the three areas of brain had effects on norepinephrine or serotonin content in other parts of the brain, presumably at least in part as a result of axonal degeneration. Previous studies have associated noradrenergic neuronal depression with anesthesia. In dogs, depletion of central and peripheral catecholamines by administration of reserpine or alpha-methyl-dopa potentiates the action of anesthetics.¹² Conversely, pretreatment with a monoamine oxidase inhibitor, which interferes with the normal degradative metabolism of amines, increases anesthetic requirements for both halothane and cyclopropane.¹² Mueller *et al.*¹³ were able to confirm Miller's findings with halothane but not with cyclopropane in studies in rats using intraventricular injections of 6-hydroxydopamine to deplete brain amines. Increases in norepinephrine release after dextroamphetamine or cocaine increase halothane requirement.^{14,15} Although we were trying to look at only one part of the noradrenergic system by destroying the locus coeruleus, destruction of the locus coeruleus produces the same magnitude of change in anesthetic requirement as does pharmacologic destruction of the entire noradrenergic system.

Previous data regarding the effect of the serotonergic nervous system on anesthesia have been conflicting. In fact, pretreatment of dogs with a serotonin depletor did not alter halothane requirements.¹⁶ Miller *et al.*¹² found that anesthetic requirement increased when brain serotonin (and catecholamine) content was increased by administration of a monoamine oxidase inhibitor. The maximal change in anesthetic requirement produced by pharmacologic maneuvers altering total brain serotonin was duplicated in our study by destruction of the nucleus raphe dorsalis.

It should be noted that rats that had bilateral ventral-bundle lesions failed to gain weight and often died when given the same induction dose of anesthetic as controls. This situation was partially remedied by using a lower induction dose and longer induction time for all animals. The lesser weight gain of rats with ventral-bundle lesions does raise a question whether the decreased anesthetic requirement of these animals results from an inability to tolerate the lesion. Animals that had other lesions all gained weight comparable to controls (table 2).

Although consonant with a hypothesis that there are specific sites of anesthetic action, our results cannot be interpreted as unequivocal evidence for that hypothesis. Although destruction of the specific sites indicated above did decrease MAC, in no case did the average decrease exceed 35 per cent. Concomitant destruction of all three areas might have produced a greater change but was not attempted in this study. Perhaps lesions in areas rich in other putative transmitters such as acetylcholine, peptides, or amino acids will more dramatically influence anesthetic requirements without influencing animal "well-being."

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