

Halothane Anesthetic Requirements Are Not Affected by Aminophylline Treatment in Rats and Dogs

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The authors determined the effects of aminophylline on the anesthetic requirements for halothane in rats and dogs. MAC for halothane was determined in rats ($n = 24$) before and after aminophylline, $100 \text{ mg} \cdot \text{kg}^{-1} \text{ ip}$, or an equal volume of saline. Because changes in central noradrenergic neurotransmission have been linked to drug-induced changes in the depth of the anesthetic state, we investigated the effect of aminophylline on the turnover of norepinephrine in discrete brain regions of halothane-anesthetized rats. To facilitate testing at steady-state aminophylline conditions and to permit frequent blood sampling, halothane MAC was determined in dogs ($n = 7$) before and after a therapeutic level of aminophylline ($15 \pm 2 \mu\text{g} \cdot \text{ml}^{-1}$) was obtained. Neither in the rats ($1.0 \text{ vs. } 1.0\%$) nor in the dogs ($1.04 \pm 0.14 \text{ vs. } 1.01 \pm 0.14\%$) was halothane MAC affected by aminophylline treatment. Commensurate with the lack of change of anesthetic depth, aminophylline treatment did not affect noradrenergic neurotransmission in the brain of halothane-anesthetized rats. Furthermore, the anticipated increase in circulating catecholamines following aminophylline treatment in dogs did not materialize. The authors conclude that halothane anesthetic requirements are not altered by aminophylline treatment, possibly because of the attenuation of the putative sympathomimetic effects of aminophylline by halothane. (Key words: Anesthetics, volatile: halothane. Pharmacology: aminophylline. Potency, anesthetic: MAC. Sympathetic nervous system: central noradrenergic transmission.)

ASTHMATIC PATIENTS have an increased risk of perioperative morbidity and mortality.¹ One reason for the adverse outcome in these patients is increased airway responsiveness to chemical, pharmacologic, and physical stimuli, including airway instrumentation. It is important, therefore, that these patients be adequately anesthetized, especially during the period of tracheal intubation, to prevent bronchospasm.²

Aminophylline, a methylxanthine derivative, is frequently employed in the perioperative management of asthmatic patients to promote bronchodilation.³ While

the precise pharmacologic mechanism for its bronchodilatory properties remains uncertain, it appears that inhibition of phosphodiesterase activity is not significant at clinically relevant concentrations.⁴ The adenosine receptor antagonist action of theophylline-containing compounds has been advanced as the likely mechanism for many of this drug's pharmacologic properties.⁵ The neurophysiologic actions of adenosine are largely inhibitory and primarily involve inhibition of excitatory transmitter release.⁶ Conversely, aminophylline's antiadenosine effect facilitates endogenous catecholamine release and increases biogenic amine turnover in the brain.^{7,8} A clinical manifestation of this central excitatory property is the appearance of seizures when toxic theophylline levels are achieved.⁹ Furthermore, aminophylline has been used both therapeutically¹⁰⁻¹² and experimentally¹³ for reversal of anesthetic-induced hypnotic states.

Because of the well-defined relationship between enhanced central noradrenergic activity and increased MAC,¹⁴ we have examined aminophylline's effect on the MAC for halothane in rats and dogs. Halothane was selected as the test agent because it is frequently used in the anesthetic management of asthmatic patients for its bronchodilatory action.¹⁵

Methods

This study was approved by the institution's Animal Use Subcommittee.

RATS

Twenty-four male Sprague-Dawley rats, weighing 250–300 g, were placed in a 1,000 l plexiglass chamber for determination of MAC. All animals had access to food and water up to the time of study. Halothane was vaporized with compressed air as the carrier gas and oxygen were added to maintain the chamber oxygen content between 21–25%. For approximately 5 min the exposure chamber was "charged" with a high halothane concentration by introducing 3–5 ml boluses of liquid halothane into the chamber *via* a sealed injection port.¹⁶ The gas flow through the vaporizer was then adjusted and the anesthetic concentration maintained at a level estimated to be 70–80% of MAC. Halothane concentrations in the chamber were determined at 15-min intervals by gas

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TABLE 1. Effects of Aminophylline on Halothane MAC and Plasma Catecholamines (mean \pm SD)

	Control	Aminophylline
Rat MAC (%)	1.0	1.0
Dog MAC (%)	1.04 \pm 0.14	1.01 \pm 0.14
Epinephrine (pg \cdot ml ⁻¹)	331 \pm 104	300 \pm 103
Norepinephrine (pg \cdot ml ⁻¹)	218 \pm 42	182 \pm 34

chromatography while trends were followed with an infrared analyzer. Body temperature in selected animals was monitored with a Yellow Springs Tele-Thermometer® and maintained at 38° C with a warming mattress. After a 2-h equilibration period, MAC was determined by applying a clamp to the rat's tail, which had been positioned outside of the exposure chamber. Because the first tail clamping was performed at a chamber concentration of halothane that was below the presumed MAC value for this strain and age of rat,¹⁶ most of the animals moved and the chamber halothane concentration was adjusted upward by \pm 5% in the following manner. The inflow halothane concentration was increased by approximately 40% and after 10 to 15 min, when the chamber halothane concentration had approached the desired level, the inflow concentration was decreased to approximate the new chamber concentration. The concentration in the chamber was now held constant by fine adjustments of the inflow halothane concentration for at least 20 min. This

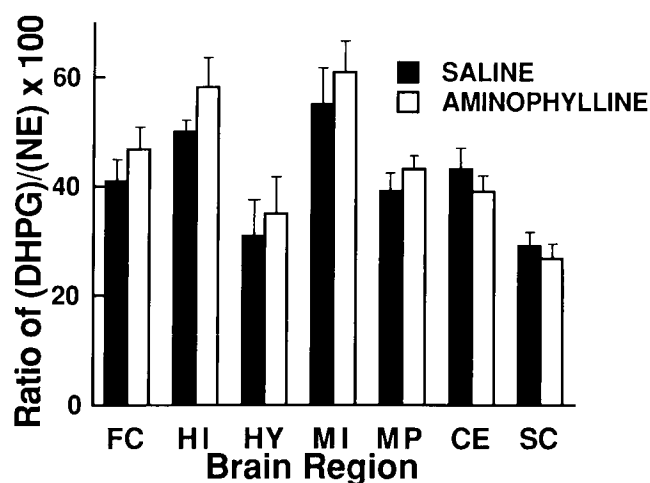


FIG. 1. Comparative effect of aminophylline, 100 mg \cdot kg⁻¹, (n = 9) or saline (n = 9) ip on steady-state turnover of norepinephrine in discrete regions of rat brain in halothane-anesthetized rats. The ratios of the concentration of dihydroxyphenylethyleneglycol (DHPG) to the concentration of norepinephrine (NE) for each brain region are represented as means \pm SEM. No significant differences were noted by unpaired *t* test.

“plateau” in halothane concentration, as reflected by continuous infrared analysis, was confirmed by gas chromatography on two samples taken 15 min apart. A period of not less than 35 min elapsed between successive tail clampings. MAC represented the inspired anesthetic concentration at which 50% of the animals failed to move in response to tail clamping.¹⁶ One-half of each of the responding and nonresponding cohorts received aminophylline 100 mg \cdot kg⁻¹ ip while the other half received an equal volume of saline (ip). The MAC determination was repeated at 30-min intervals for two further determinations, and blood was sampled for theophylline by the fluorescence polarization immunoassay technique (Abbott Laboratories Diagnostic Division, North Chicago, IL) 60 min after administration.

To determine the effect of aminophylline on central noradrenergic neurotransmission, a separate cohort of rats was anesthetized with 1.2% halothane. After 1 h, aminophylline (n = 9), 100 mg \cdot kg⁻¹, or an equal volume of saline (n = 9) was administered ip and the rats were killed by decapitation after a further 60 min. The brain was rapidly removed from the calvarium and dissected on ice into seven neuroanatomically and neurochemically specific brain regions, according to Holman *et al.*,¹⁷ and after weighing, the samples were stored at -70° C until analysis. The brain regions were frontal cortex (FC), hippocampus (HI), hypothalamus (HY), midbrain (MI), medulla-pons (MP), cerebellum (CE), and spinal cord (SC). The frozen brain tissue was homogenized in 0.2 M perchloric acid together with 2×10^{-9} M of 3,4-dihydroxybenzylamine, which served as an internal standard for the catecholamines.¹⁸ The extraction and subsequent analysis of the catecholamine neurotransmitter, norepinephrine (NE), and its principal deaminated metabolite, dihydroxyphenylethyleneglycol (DHPG), were performed by high-performance liquid chromatography with electrochemical (LC-EC) detection method¹⁸ modified to measure the hydrolyzed product of conjugated DHPG.¹⁹

DOGS

These experiments were extended to dogs to facilitate: 1) frequent blood sampling; 2) testing at steady-state theophylline levels; and 3) determination of end-tidal (alveolar) rather than inspired concentration. This last reason is particularly pertinent because aminophylline can alter ventilatory parameters, thereby changing the relationship between inspired and alveolar halothane concentrations. Anesthesia was induced with halothane in oxygen *via* mask in seven female dogs (14–30 kg). After induction, the trachea was intubated without the use of muscle relaxants. Nasal temperature, monitored continuously, was main-

tained at $37 \pm 1^\circ \text{C}$ with a heating pad. Ventilation was controlled to avoid respiratory acidosis and to achieve normocarbica. Catheters were placed percutaneously in the femoral artery and foreleg vein for continuous arterial blood pressure (BP) monitoring, blood sampling, and intravenous maintenance fluid and drug administration, respectively. Halothane concentration was determined by an infrared analyzer and continuously recorded along with arterial BP and end-tidal CO_2 . MAC for halothane was determined as described earlier.²⁰ After MAC was determined, aminophylline, $15 \text{ mg} \cdot \text{kg}^{-1}$, was administered intravenously over 20 min to achieve a plasma theophylline concentration of $15 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$, which was sustained by a continuous infusion at a rate of $25 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.²¹ These levels were confirmed by drug assay (see earlier discussion). After a 15-min equilibration phase, MAC was reassessed. Before and after aminophylline infusion, blood was sampled for catecholamine determination by LC-EC.¹⁸

For the rat experiment, MAC values were compared for statistical significance by analysis of variance (ANOVA) for repeated measures. The ratio of the concentration of the major metabolite to the parent monoamine concentration has been validated as a measure of steady-state neurotransmitter turnover and hence neurotransmission in monoaminergic pathways²² because:

$$(\text{DHPG})/(\text{NE}) = k_2/k_1$$

where k_1 is the rate constant for the elimination of DHPG, k_2 is the rate constant for the conversion of NE to DHPG, (NE) and (DHPG) are the concentrations of the parent neurotransmitter and its principal metabolite, respectively. Statistical differences in this ratio between the aminophylline-treated and saline-control animals was compared by the unpaired t test for each brain region. In the dog experiments, MAC and plasma catecholamine values before and after aminophylline treatment were analyzed by paired t test with Bonferroni correction for multiple comparisons. A P value of <0.05 was considered significant.

Results

In the rat experiment, MAC for halothane did not change after administration of aminophylline (table 1). The plasma theophylline concentration at the conclusion of testing was $112 \pm 7 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$. No change in the ratio of (DHPG)/(NE) was present in any brain region of the aminophylline-treated rats when compared with the saline controls (fig. 1). In the dog experiments, the MAC for halothane before and after a steady-state theophylline

TABLE 2. Effects of Aminophylline on MAP and Acid-Base Balance (mean \pm SD)

	Before	After
MAP	102.4 ± 11.6	100.6 ± 13.2
pH	$7.40 \pm .04$	$7.40 \pm .03^*$
PaO_2	558 ± 18.6	$551 \pm 14.9^*$
PaCO_2	35.4 ± 2.99	$36.9 \pm 2.41^*$

* Arterial blood gases at postaminophylline MAC.

level ($15 \pm 2 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$) was achieved, were similar (table 1). The plasma catecholamine values were not significantly different after theophylline administration (table 1). Similarly, cardiorespiratory parameters, including mean arterial pressure, pH, PaO_2 , and PaCO_2 , which may independently affect the MAC for halothane, were unchanged (table 2).

Discussion

Acute aminophylline treatment did not change the MAC for halothane in rats or dogs (table 1). While cardiopulmonary performance parameters such as BP, PaO_2 , and PaCO_2 , which are known to affect MAC, were not assessed in the rat experiments, these were controlled for in the dog experiments (table 2). However, it is still possible in the rat experiment that any aminophylline-induced stimulatory effect could have been offset by concomitant hypotension, hypoxia, and hypercarbia.

Despite the fact that theophylline levels were high enough in the aminophylline-treated rats to expect an increase in noradrenergic neurotransmission,⁷ central norepinephrine turnover was unaltered by aminophylline during halothane anesthesia (fig. 1). We were also unable to corroborate any increase in circulating catecholamine concentration^{23,24} following aminophylline treatment in the halothane-anesthetized dogs (table 1). Because a strong correlation exists between norepinephrine levels in the blood and the cerebrospinal fluid (CSF),²⁵ we theorize that this lack of peripheral catecholamine release in the dog is, too, a reflection of an absence of change in central noradrenergic neurotransmission.

Miller *et al.*²⁶ have proposed that drug treatments or stimuli that increase norepinephrine at neuroeffector junctions in central noradrenergic pathways will decrease the sensitivity of the brain to anesthetic compounds reflected as increased MAC values. Thus, acute cocaine²⁷ or amphetamine¹⁴ administration increases MAC for halothane; conversely, reserpine,^{14,26} which decreases the neurotransmitter concentration, decreased the MAC.¹⁰⁻¹⁴ Activation of prejunctional adenosine receptors in central

noradrenergic pathways decreases norepinephrine release²⁸ and will enhance a barbiturate-induced hypnotic state.²⁹ Competitive antagonism of the adenosine receptors occurs at clinically relevant theophylline concentrations and will facilitate norepinephrine release and consequently will reverse barbiturate anesthesia.¹³ These changes, however, may not be reflected in all drug-induced anesthetic states, possibly because of underlying sympatholytic features of drugs such as halothane.³⁰ It is possible that the resistance of halothane anesthesia to reversal by the antiadenosine compound may be due to an adenosine-enhancing effect of halothane³¹ that overcomes the competitive antagonism. Alternatively, during halothane anesthesia all presynaptic mechanisms that facilitate norepinephrine release may be inhibited. Evidence in favor of this more global effect of halothane is the inability of a centrally active α_2 -adrenergic antagonist to affect MAC for halothane in dogs.³²

If we are to extrapolate these observations into the clinical setting, then reversal of the anesthetic state by aminophylline may not obtain to the same extent in the setting of anesthesia with the sympatholytic potent volatile agents. A change in the depth of the anesthetic state may be especially important in asthmatic patients because of the risk of provoking bronchospasm in a light anesthetic plane. Thus, the concurrent use of aminophylline with thiopental as an induction agent in an asthmatic patient may be problematic. However, the potential arrhythmogenic interaction of halothane and acutely administered aminophylline should also be considered when selecting the appropriate anesthetic agent for the asthmatic patient.³³

This study was designed to examine exclusively the effects of acutely administered aminophylline on the depth of the anesthetic state produced by halothane, while in the clinical setting aminophylline is more likely to be administered chronically. In a previously reported study that addressed the arrhythmogenic potential of acute *versus* chronic aminophylline administration in a canine halothane-epinephrine arrhythmia model, we observed a sensitization by acute but not by chronic treatment.³⁴ Whether a similar dependency on duration of aminophylline treatment exists for effects on anesthetic depth remains to be established.

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