Anesthesiology
63:584–588, 1985

Laudanosine (A Metabolite of Atracurium) Increases the Minimum Alveolar Concentration of Halothane in Rabbits

Wei-zhong Shi, M.D.,* Mark R. Fahey, M.D.,† Dennis M. Fisher, M.D.,‡ Ronald D. Miller, M.D.,§
Claver Canfell, M.S.,¶ and Edmond I. Eger II, M.D.**

The authors hypothesized that laudanosine, a metabolite of atracurium and a central nervous system stimulant, might increase the minimum alveolar concentration (MAC) of halothane. An initial study in five rabbits anesthetized with halothane used a two-compartment model to produce estimates of pharmacokinetic variables for laudanosine. These estimates were used to determine the rates of infusion that would produce steady state plasma concentrations of laudanosine of approximately 200, 400, and 800 ng·ml^{-1}. Subsequent infusion of laudanosine in eight rabbits produced mean (±SD) steady state plasma concentrations of laudanosine of 234 ± 56, 457 ± 66, and 873 ± 105 ng·ml^{-1}. The control value for MAC of halothane was 1.08 ± 0.28%. At the lowest steady state plasma laudanosine concentration, MAC did not significantly differ from control (MAC = 1.15 ± 0.23%, $P < 0.1$). However, at 457 and 873 ng·ml^{-1}, laudanosine significantly increased the MAC of halothane by 23% and 30%, respectively. Infusion with saline in two additional rabbits did not affect MAC. Therefore, at the plasma concentrations of laudanosine found in humans after administration of atracurium, laudanosine increased the MAC of halothane in rabbits. (Key words: Anesthetics, volatile: halothane. Brain: laudanosine. Neuromuscular relaxants: atracurium. Pharmacokinetics: laudanosine.

LAUDANOSINE, a major metabolite of the nondepolarizing neuromuscular blocking agent atracurium, causes central nervous system (CNS) excitation in rabbits.[1] At a plasma concentration below that causing convulsions, laudanosine appears to "awaken" dogs lightly anesthetized with halothane.[2] These characteristics suggest that laudanosine might increase anesthetic requirement. This study determined the effect of laudanosine on the minimum alveolar concentration (MAC) of halothane in rabbits.

This article is accompanied by an Editorial. Please see: Standaert FG: Magic bullets, science, and medicine. ANESTHESIOLOGY 63:577–578, 1985.

Methods

Approval was obtained from the University of California Committee on Animal Research to study 15 nonfasting, unpremedicated, New Zealand white male rabbits (2.5–3.0 kg). Anesthesia was induced with halothane in oxygen. Animals breathed spontaneously through an endotracheal tube inserted via tracheostomy; Pa_{CO_2} ranged from 35 to 40 mmHg. Anesthesia was continued with halothane (at an inspired concentration of 1%) in oxygen. Cannulae were inserted into a carotid artery for blood sampling and an ear vein for administration of laudanosine. A unilateral fronto-occipital electroencephalogram (EEG) was obtained through subcutaneous needle electrodes and was recorded on a polygraph. Rectal temperature was kept at 39 to 40° C by using surface warming.

A solution of laudanosine (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin) was prepared by dissolving it in water and adjusting the *p*H to 6.5 with HCl. The solution was filtered and stored at 4° C.

To obtain pharmacokinetic data for laudanosine, five rabbits were given an iv bolus (3.0 mg·kg^{-1}) of laudanosine. Blood samples were obtained in heparinized syringes 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after administration of laudanosine. After centrifugation, the plasma was stored at −20° C. Laudanosine levels were determined using C_{18}-Sep-Pak® extraction (Waters Associates, Milford, Massachusetts) of plasma samples and isocratic (35% acetonitrile) ion-exchange liquid chromatography with fluorescent detection.[3] This assay is sensitive to 10 ng of laudanosine per milliliter of plasma and has a coefficient of variation of 9% at a laudanosine concentration of 15 ng/ml plasma. The data for plasma laudanosine values *versus* time were fit to a two-compartment pharmacokinetic model. Standard formulas were used to calculate values for distribution half-life, elimination half-life, clearance, volume of the central compartment, and volume of distribution at steady state.[4]

* Visiting Research Fellow in Anesthesia; Lecturer in Anesthesia, Peking Medical College, Beijing, Peoples Republic of China.

† Assistant Professor of Anesthesia.

‡ Assistant Professor of Anesthesia and Pediatrics.

§ Professor and Chairman of Anesthesia and Professor of Pharmacology.

¶ Staff Research Associate in Anesthesia.

** Professor of Anesthesia and Vice Chairman for Research.

Received from the Departments of Anesthesia and Pharmacology, University of California, San Francisco, California 94143. Accepted for publication May 1, 1985. Supported in part by the National Institutes of Health (NIH GMR01-26403-05), the Institute on Aging (Program Project 1P01 AG03104-01), and the Anesthesia-Pharmacology Research Foundation.

Address reprint requests to Dr. Fahey.

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/63/6/584/311138/0000542-198512000-00006.pdf by guest on 26 May 2022

The effect of laudanosine on the MAC of halothane was determined by studying eight rabbits.[5] Each MAC determination was done in duplicate with clamping of the tail as the stimulus.[6] End-tidal concentrations of halothane were measured with a Beckman LB-2® infrared analyzer. In eight rabbits, a control value for MAC was determined and an infusion of laudanosine begun at rates calculated to produce steady state plasma concentrations of approximately 200, 400, and 800 $ng \cdot ml^{-1}$. These three concentrations of laudanosine span the range of laudanosine concentrations (93–758 $ng \cdot ml^{-1}$) found in renal failure patients who had received a single, 0.5 $mg \cdot kg^{-1}$ iv dose of atracurium.[7] Minimum alveolar concentration for halothane was determined at each of these concentrations. Two control rabbits were studied in a similar fashion, except that saline was infused rather than laudanosine, to confirm the stability of MAC in the animal preparation.

Laudanosine was administered with the use of the two-infusion technique of Wagner, based upon the mean values for pharmacokinetic variables obtained from the first five rabbits.[8] The two-infusion technique consists of a rapid infusion followed by a slower infusion. Because three steady state plasma levels of laudanosine were sought, the total number of infusions per rabbit was six. The first rapid infusion was administered at a rate (R) equal to the product of the desired steady state plasma concentration (200 $ng \cdot ml^{-1}$), clearance (in $ml \cdot kg^{-1} \cdot min^{-1}$), and a factor determined from the elimination half-life and the desired duration for the rapid infusion. This infusion was followed immediately by the first slow infusion, administered at a rate (S) equal to the product of the desired steady state plasma concentration (200 $ng \cdot ml^{-1}$), and the clearance. Using the principle of superposition, the rate of the second rapid infusion was equal to the sum of R and S.[4] The second slow infusion was equal to twice S, so that a steady state plasma concentration of approximately 400 $ng \cdot ml^{-1}$ could be reached. Similarly, the third rapid infusion, designed to reach steady state plasma concentrations of laudanosine of approximately 800 $ng \cdot ml^{-1}$, was administered at a rate equal to twice the sum of R and S, and the third slow infusion at a rate of four times S. Thirty minutes after the start of each slow infusion, blood samples were obtained for determination of laudanosine levels, and halothane MAC was determined. Additional blood samples obtained after completion of determination of halothane MAC were also analyzed for laudanosine.

After the determination of halothane MAC at the highest concentration of laudanosine (~800 $ng \cdot ml^{-1}$), the infusion of laudanosine was terminated. Two hours later, a blood sample was obtained to determine the plasma concentration of laudanosine, and MAC was determined again.

The control value for MAC of halothane, the value for MAC of halothane during the three steady state infusions of laudanosine, and the value for MAC of halothane 2 h after stopping the infusions were compared using repeated-measures analysis of variance.[9] Intragroup comparisons were performed using the Student-Newman-Keuls test.[10] In addition, the control value for MAC was compared by unpaired t test with previously published values for the MAC of halothane in rabbits.[6,11] Statistical significance was said to exist if $P < 0.05$.

The remaining two rabbits were studied in a similar fashion except that saline rather than laudanosine was infused to confirm that the MAC of halothane was stable under the experimental conditions.

Results

Two-compartment modeling of plasma laudanosine data yielded mean values (±SD) of 5.2 ± 2.3 min for distribution half-life, 47.3 ± 16.9 min for elimination half-life, 0.8 ± 0.4 $l \cdot kg^{-1}$ for volume of the central compartment, 2.1 ± 1.0 $l \cdot kg^{-1}$ for volume of distribution at steady state, and 41.9 ± 6.7 $ml \cdot kg^{-1} \cdot min^{-1}$ for clearance. All five rabbits, who were given laudanosine 3 $mg \cdot kg^{-1}$, had uncoordinated motor activity accompanied by high amplitude and high-frequency EEG activity that began 30 s after administration of laudanosine, lasted 30 to 60 s, and ended spontaneously.

Rates for the three rapid infusions were calculated as being 42, 48, and 96 $\mu g \cdot kg^{-1} \cdot min^{-1}$; each infusion lasted 10 min. Rates for the three slow infusions were calculated as being 6, 12, and 24 $\mu g \cdot kg^{-1} \cdot min^{-1}$; the length of these infusions depended on the time needed to perform duplicate determinations of MAC. For most animals, plasma concentrations of laudanosine during steady state infusions changed less than 15% during determinations of MAC (table 1). In rabbit 3, the concentration of laudanosine decreased 38% during the third slow infusion; in rabbit 8, the concentration of laudanosine increased 25% during the first slow infusion. Mean (±SD) concentrations of laudanosine during the three slow infusions and before MAC determinations were 234 ± 56, 457 ± 66, and 873 ± 105 $ng \cdot ml^{-1}$.

The mean (±SD) control value for MAC of halothane in eight rabbits was 1.08 ± 0.28%, which was significantly different from the value of 0.82 ± 0.25% ($P < 0.02$) published by Davis *et al.*[6] and the value of 1.39 ± 0.23% ($P < 0.02$) published by Drummond.[11] The initial steady state concentration of laudanosine tended to increase MAC (9% above control), but this was not statistically significant ($0.05 < P < 0.10$). However, at steady state concentrations of 457 and 873 $ng \cdot ml^{-1}$, laudanosine significantly increased the mean (±SD) value for MAC of

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/63/6/584/311138/0000542-198512000-00006.pdf by guest on 26 May 2022

TABLE 1. Plasma Concentrations of Laudanosine ($ng \cdot ml^{-1}$) during Three Infusions

Relation to MAC Determinations	First Slow Infusion		Second Slow Infusion		Third Slow Infusion		Two Hours after End of Third Slow Infusion
	Before	After	Before	After	Before	After	Before
Rabbits							
1	235	243	596	536	994	948	47
2	347	344	468	501	998	947	32
3	286	279	452	483	803	496	<10
4	218	225	446	489	896	877	54
5	216	235	365	414	765	702	31
6	204	192	414	445	770	676	50
7	173	184	446	457	782	787	<10
8	194	243	471	446	975	847	46
Mean ± SD	234	243	457	471	873	785	33
	56	51	66	38	105	155	22

halothane by 23 ± 13% ($P < 0.001$) and 30 ± 19% ($P < 0.001$), respectively (fig. 1). These increases differed significantly from control but not from each other (table 2). MAC values were not significantly different from control values ($P > 0.50$) 2 h after the end of the third slow infusion of laudanosine, when the mean (±SD) concentration of laudanosine was 33 ± 22 $ng \cdot ml^{-1}$. Minimum alveolar concentration changed less than 3% in the two rabbits given saline infusions over 4 h. During determinations of MAC, no animals had EEG or clinical evidence of seizure activity.

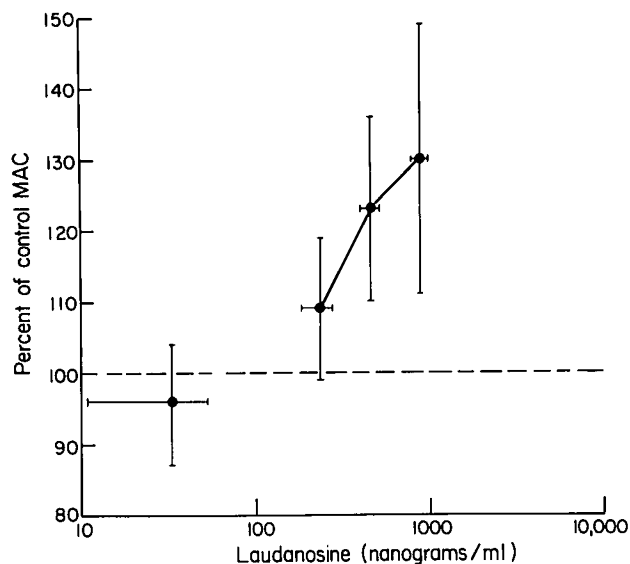

FIG. 1. In eight rabbits given infusions of laudanosine designed to produce steady state plasma concentrations of approximately 200, 400, and 800 $ng \cdot ml^{-1}$, the MAC of halothane increased progressively (three highest points). Two hours after discontinuing infusion, concentrations of laudanosine decreased to 4% of the highest value and MAC returned to control levels (lowest point). All values represent mean ± SD.

Discussion

The MAC of halothane determined in this study is larger than that published by Davis *et al.*[6] The only difference in methods between the two studies was that Davis *et al.* allowed only 5 min of equilibration time after changing the inspired halothane concentration before stimulating their rabbits, whereas an equilibration time of 15 min was used in this study. Since Davis *et al.* began their estimation of MAC at a concentration that suppressed response, they may have underestimated the concentration at which a response would first appear (*i.e.*, the brain would still be at a higher partial pressure of anesthetic than that indicated by the end-tidal sample). The time constant for rabbit cerebral cortex, assuming a blood flow of 61 $ml \cdot min^{-1}$ for each 100 g of tissue and a partition coefficient of 2.3, is 3.8 min.[12,13] The time needed to reach 95% equilibration between blood and cerebral cortex partial pressure of halothane is three time constants,

TABLE 2. Minimum Alveolar Concentration of Halothane for Rabbits Given Infusions of Laudanosine or Saline

Rabbit No.	Before Infusion (Control)	During Slow Infusion #1	During Slow Infusion #2	During Slow Infusion #3	Two Hours after End of Third Infusion
Laudanosine					
1	1.56	1.56	1.86	1.93	1.55
2	0.83	1.04	1.12	1.17	0.67
3	0.83	1.02	1.22	1.40	0.77
4	1.39	1.42	1.56	1.57	1.48
5	0.95	1.06	1.20	1.20	0.95
6	0.86	0.86	0.92	0.92	0.79
7	1.00	1.03	1.17	1.23	0.99
8	1.19	1.24	1.44	1.60	1.18
Mean ± SD	1.08	1.15	1.31	1.38	1.05
	0.28	0.23	0.30	0.32	0.33
Saline					
1	1.29	1.28	1.28	1.27	1.29
2	0.84	0.84	0.85	0.85	0.85

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/63/6/584/311138/0000542-198512000-00006.pdf by guest on 26 May 2022

or 11.4 mins.[13] Indeed, statistical comparison by unpaired *t* test of the end-tidal concentrations of halothane, at which responses to tail clamp first appeared, confirmed that the end-tidal concentrations in the Davis *et al.* study were significantly lower ($P < 0.01$) than those found in this study. There was no difference in the end-tidal concentrations that abolished response to stimulus ($P < 0.50$).

The value for the MAC of halothane determined in this study was significantly less than that of Drummond. A 20-min equilibration time was used in the Drummond study, as well as continuous sampling of halothane concentrations. Continuous sampling, as opposed to obtaining aliquots of end-tidal exhaled gas, is more likely to allow contamination of end-tidal samples by inspired gas. This is particularly true in the rabbit, where respiratory rate is rapid and tidal volumes are small. Perhaps contamination of end-tidal halothane determinations by inspired gas may explain the higher value for the MAC of halothane reported by Drummond.

In this study and in that of Davis *et al.* and Drummond, MAC was noted to vary widely among animals. However, in the individual animal, MAC appears to be stable (*e.g.*, in this study, animals given saline infusions had steady MAC values). Thus, the changes in MAC observed in this study can be explained by the central nervous system (CNS) effects of laudanosine; and other explanations related to experimental design are unlikely.

Laudanosine increases the MAC of halothane in rabbits by as much as 30% over control values. Intravenous administration of amphetamine,[14] cocaine,[15] or ephedrine[16] causes similar increases in the MAC of halothane in animals. However, there may be a ceiling to the effect of all these drugs. Concentrations of laudanosine above 400–800 $ng \cdot ml^{-1}$ may not increase MAC further. That a ceiling effect exists is suggested by the fact that in two rabbits, MAC did not increase as the concentration of laudanosine increased from 400 to 800 $ng \cdot ml^{-1}$ (table 2). The MAC for halothane returned to control levels 2 h after termination of the third slow infusion of laudanosine. This occurrence also supports the conclusion that laudanosine itself acts to increase MAC.

The elimination half-life of laudanosine in the rabbit reported in this study is similar to the rate of breakdown of atracurium determined *in vitro* in rabbit plasma.[17] This *in vitro* determination, using rabbit plasma at physiologic *p*H and temperature, presumably only measures the rate of Hofmann elimination of atracurium. The *in vivo* elimination half-life of atracurium in the rabbit, which has not been studied, may be shorter because of the contribution of ester hydrolysis and metabolic organ clearance of atracurium. In the dog, the value for the *in vivo* elimination half-life of laudanosine of 112 ± 25 min is four times longer than the *in vitro* value for atracurium.[2,17] It is also three times longer than the *in vivo* elimination half-life of laudanosine in the rabbit, which may be explained by *in vitro* evidence that laudanosine is rapidly demethylated by rabbit liver microsomes.[18] Although the elimination half-life for laudanosine has not been determined in humans, some evidence indicates that surgical patients, like dogs and rabbits, may excrete laudanosine more slowly than atracurium.[3,7] Further work is needed to determine the metabolism and route of elimination of laudanosine in humans.

The concentrations of laudanosine used in this study are similar to those measured in surgical patients with renal failure given a single dose (0.5 $mg \cdot kg^{-1}$) of atracurium.[7] This study has shown that such concentrations change halothane MAC in rabbits, which suggests that the same may happen in patients receiving atracurium. Clinically, it is possible that the incidence of awareness in patients who are lightly anesthetized and paralyzed (*e.g.*, a hypovolemic trauma patient) may be increased when atracurium is used for neuromuscular blockade.

Clinical studies suggest that other nondepolarizing relaxants possess an anesthetic-potentiating effect, and this may also be true for atracurium. Forbes *et al.*[19] found that pancuronium decreased the MAC of halothane in humans. Munson and Wagman[20] showed that iv administration of gallamine and *d*-tubocurarine in monkeys raised the seizure threshold to lidocaine. Neuromuscular blocking drugs also may decrease afferent input from muscle spindles to the reticular activating system.[21] This decrease in afferent input may make the brain more susceptible to depression from anesthetic agents. Thus, the effects of laudanosine on anesthetic requirement may be offset by the possible anesthetic-potentiating effects of atracurium-induced neuromuscular blockade or the administration of other CNS depressants. Further clinical studies are needed to explore these possibilities.

Laudanosine produced after atracurium administration probably would not produce motor evidence of seizure activity in surgical patients, since the neuromuscular paralysis from atracurium would preclude muscle movement. In addition, general anesthetics would tend to suppress CNS excitation. This study suggests that there is a spectrum of CNS effects from laudanosine, with increase in anesthetic requirement at low concentrations and seizure activity with higher plasma concentrations. The range of plasma concentration of laudanosine needed to produce seizures in animals has not been studied, but it might be possible with repeated administration of atracurium in patients that such concentrations of laudanosine could be reached. In addition, the absence of CNS depressants (*e.g.*, in postsurgical patients or in intensive care patients) or the presence of CNS pathology (*e.g.*, patients with seizure disorder or brain tumor) may lower the plasma concentration of laudanosine needed to produce seizure activity in humans. Studies using the EEG to mon-

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/63/6/584/311138/0000542-198512000-00006.pdf by guest on 26 May 2022

itor surgical and intensive care patients given atracurium have yet to be done.

In summary, laudanosine, at plasma concentrations present in surgical patients with renal failure given atracurium, increases the MAC of halothane in rabbits by as much as 30%. If these data apply to humans, the incidence of awareness in lightly anesthetized patients may increase during administration of atracurium.

Halothane (Fluothane®) was donated by Ayerst Laboratories.

References

1. Babel A: Etude comparative de la laudanosine et de la papaverine au point de vue pharmacodynamique. Rev Med Suisse Romande 19:657–688, 1899
2. Hennis PJ, Fahey MR, Miller RD, Canfell C, Shi W-Z: Pharmacology of laudanosine in dogs (abstract). ANESTHESIOLOGY 61: A305, 1984
3. Fahey MR, Rupp SM, Fisher DM, Miller RD, Sharma M, Canfell C, Castagnoli K, Hennis PJ: The pharmacokinetics and pharmacodynamics of atracurium in patients with and without renal failure. ANESTHESIOLOGY 61:699–702, 1984
4. Gibaldi M, Perrier D: Pharmacokinetics. New York, Marcel Dekker, 1975, pp 48–86, 297–300
5. Eger EI II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. ANESTHESIOLOGY 26:756–763, 1965
6. Davis NL, Nunnally RL, Malinin TI: Determination of the minimum alveolar concentration (MAC) of halothane in the white New Zealand rabbit. Br J Anaesth 47:341–345, 1975
7. Fahey MR, Rupp SM, Canfell C, Fisher DM, Miller RD, Sharma M, Castagnoli K, Hennis PJ: The effect of renal failure on laudanosine excretion in man. Br J Anaesth (In press)
8. Wagner JG: A safe method for rapidly achieving plasma concentration plateaus. Clin Pharmacol Ther 16:691–700, 1974
9. Glantz SA: Primer of Biostatistics. New York, McGraw-Hill, 1981, pp 250–261
10. Zar JH: Biostatistical Analysis. Englewood Cliffs, Prentice-Hall, 1974, pp 151–161
11. Drummond JC: MAC for halothane, enflurane, and isoflurane in the New Zealand white rabbit: And a test for the validity of MAC determinations. ANESTHESIOLOGY 62:336–338, 1985
12. Rosendorff C, Cranston WI: Application of the ^{133}xenon clearance method to the measurement of local blood flow in the conscious animal, Cerebral Blood Flow: Clinical and Experimental Results. Edited by Brock M, Fieschi C, Ingvar DH, Lassen NA, Schurmann K. New York, Springer-Verlag, 1969, pp 36–38
13. Eger EI II: Anesthetic uptake and distribution. Baltimore, Williams and Wilkins, 1974, pp 78–82
14. Johnston RR, Way WL, Miller RD: Alteration of anesthetic requirement by amphetamine. ANESTHESIOLOGY 36:357–363, 1972
15. Stoelting RK, Creasser CW, Martz RC: Effect of cocaine administration on halothane MAC in dogs. Anesth Analg 54:422–424, 1975
16. Steffey EP, Eger EI II: The effect of seven vasopressors on halothane MAC in dogs. Br J Anaesth 47:435–438, 1975
17. Neill EAM, Chapple DJ, Thompson CW: Metabolism and kinetics of atracurium: An overview. Br J Anaesth 55:23S–25S, 1983
18. Sharma M, Fahey MR, Castagnoli K, Shi W-Z, Miller RD: In vitro metabolic studies of atracurium with rabbit liver preparations (abstract). ANESTHESIOLOGY 61:A304, 1984
19. Forbes AR, Cohen NH, Eger EI II: Pancuronium reduces halothane requirement in man. Anesth Analg 58:497–499, 1979
20. Munson ES, Wagman IH: Elevation of lidocaine seizure threshold by gallamine. Arch Neurol 28:329–333, 1973
21. Hodes R: Electrocortical synchronization resulting from reduced proprioceptive drive caused by neuromuscular blocking agents. Electroencephalogr Clin Neurophysiol 14:220–232, 1962

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/63/6/584/311138/0000542-198512000-00006.pdf by guest on 26 May 2022