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Pharmacology of Laudanosine in Dogs

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The authors determined the pharmacokinetics (including transfer into cerebrospinal fluid [CSF]) and the cardiovascular and central nervous system (CNS) effects of laudanosine, a metabolite of atracurium. Eight dogs were anesthetized with halothane; blood pressure and a fronto–occipital electroencephalographic lead were monitored. Laudanosine (1 mg·kg^{-1} iv) was administered as a bolus, and its concentrations in plasma, CSF, urine, and bile were determined by liquid chromatography. Three-compartment modeling of plasma laudanosine concentrations yielded an elimination half-life for laudanosine of 113 ± 24 min (mean ± SD) and a clearance of 25 ± 8 ml·kg^{-1}·min^{-1}. CSF concentrations of laudanosine were highest 5–10 min after iv injection of laudanosine and ranged in concentration from 208 to 572 ng·ml^{-1} (*i.e.*, 36–87% of the corresponding plasma concentrations). Unchanged laudanosine was found in urine (0.5–12% of injected dose) and bile (<0.1%); metabolites of laudanosine were found in both fluids. After a 6-h sampling period, dogs were hyperventilated with halothane (FI_{O_2} = 0.2) to a Pa_{CO_2} of 26–28 mmHg. Laudanosine was then administered 2 mg·kg^{-1} iv every 5 min. With cumulative doses of 2–8 mg·kg^{-1}, all dogs showed signs of "awakening" from anesthesia. Cumulative doses of 14–22 mg·kg^{-1} produced seizure activity in all animals. Mean arterial blood pressure decreased significantly to 86% of control levels at 1 min following administration of laudanosine (1 mg·kg^{-1} iv) and returned to control levels 4 min later. The authors conclude that laudanosine in dogs readily crosses the blood–brain barrier and can produce hypotension, signs of "awakening" from halothane anesthesia, and seizures. In addition, laudanosine is excreted unchanged by the kidney, and its metabolites are excreted by both the kidney and liver. (Key words: Anesthetics, volatile: halothane. Central nervous system: laudanosine. Cerebrospinal fluid: laudanosine. Metabolism: atracurium, laudanosine. Neuromuscular relaxants: atracurium. Pharmacokinetics: laudanosine.)

ATRACURIUM, A NONDEPOLARIZING neuromuscular blocking drug, has as a major metabolite, laudanosine, which is a known central nervous system (CNS) stimulant in animal models (fig. 1).[1] Recently, Fahey *et al.* found that following an iv dose of 0.5 mg·kg^{-1} of atracurium, up to 758 ng·ml^{-1} of laudanosine could be detected in the blood of patients with renal failure who were undergoing cadaver renal transplantation.[2] Elimination of laudanosine appeared to be delayed by abnormal renal function in these patients. The present study is designed to examine the pharmacokinetics of laudanosine (including transfer into the cerebrospinal fluid [CSF]) and to describe the cardiovascular and CNS effects of laudanosine in anesthetized dogs.

Materials and Methods

Eight mongrel dogs, weighing 20–35 kg, were studied with the approval of the University of California Committee on Animal Research. Anesthesia was induced with iv thiopental (10–15 mg·kg^{-1}), and the tracheae were intubated. Under halothane anesthesia, femoral artery catheterization was performed to measure arterial blood pressure and to provide access for blood sampling. Venous access was secured in a forelimb for administration of laudanosine and maintenance fluids (0.9% saline at 5–15 ml·kg^{-1}·h^{-1}). The head was shaved and metal screws were secured over the dura at three sites on one side of the head: the frontal, occipital, and temporal bones, the latter site to be used as a ground. The surrounding soft tissue was removed, and the screws were isolated from the remaining tissue. These three leads were then connected to a Grass® Model 7P1-A electroencephalographic (EEG) preamplifier (sensitivity, 150 μV·cm^{-1}), and the unilateral fronto–occipital EEG tracing was recorded on a polygraph. The electrocardiogram was also monitored. To sample CSF, an 18-gauge polyethylene catheter was placed percutaneously with an 18-gauge Touhy needle into the cisterna magna. A laparotomy was performed to insert cannulae into the common bile duct and the bladder for collection of bile and urine, and the laparotomy incision was closed.

Following these procedures, anesthesia was maintained with halothane, 0.6–0.8% end-tidal (FI_{O_2} = 1.0), and ventilation was controlled to maintain Pa_{CO_2} between 35 and 40 mmHg. Esophageal temperature was maintained at 36.0–38.0° C by surface warming. After a period of at least 30 min, each dog was given laudanosine, 1 mg·kg^{-1}, as an iv bolus. Arterial blood samples were obtained prior to and at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, and 360 min following injection. Blood samples were centrifuged, and the plasma was stored. CSF samples (1 ml) were obtained prior to and at

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2, 5, 10, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min following injection. Bile and urine samples were collected over 30-min periods during the total blood sampling period. All body fluid samples were stored at −20° C until analyzed.

At the end of the 6-h blood sampling period, all animals were hyperventilated to a Pa_{CO_2} of 26–28 mmHg with halothane, 1% inspired (FI_{O_2} = 0.2), to decrease their seizure threshold. Laudanosine was then administered in 2 mg · kg^{-1} iv doses every 5 min until seizure activity was noted by abrupt appearance of a high-voltage, high-frequency EEG tracing and/or observation of purposeless, uncoordinated, spastic movements of all extremities. Animals were then paralyzed with vecuronium bromide, 0.4 mg · kg^{-1} iv, to rule out electromyographic interference of the EEG. Additional 2 mg · kg^{-1} iv doses of laudanosine were administered until EEG evidence of seizures was again observed. Thiopental was administered iv in incremental doses (12.5 mg each) to determine the dose necessary to abolish seizure activity.

Laudanosine was extracted from biologic samples using C_{18}-Sep-Paks^R (Waters Associates, Milford, MA); N-methyl-laudanosine was used as the internal standard. Concentrations of extracted laudanosine were determined using isocratic cation-exchange liquid chromatography (Partisil-10-SCX; Whatman, Inc., Clifton, NJ) with a mobile phase of 0.06 M sodium sulfate (*p*H 2.0):acetonitrile (65:35) at 2 ml · min^{-1}.[3] The assay was linear to 10 $\mu g \cdot ml^{-1}$ and sensitive to a laudanosine concentration of 10 ng · ml^{-1} with a coefficient of variation of 3% at 8 $\mu g \cdot ml^{-1}$ and 9% at 15 ng · ml^{-1}. Two- and three-compartment models were fit to plasma laudanosine concentrations *versus* time data. Best fit was determined statistically by the methods of Boxenbaum *et al.*[4] Using standard formulas, the following pharmacokinetic variables were estimated: rapid and slow distribution half-lives, elimination half-life, volume of the central compartment, volume of distribution at steady state, and total plasma clearance.[5] Transfer of laudanosine into CSF was expressed as the ratio of CSF and plasma concentrations of laudanosine. Urine and bile were assayed quantitatively for laudanosine and qualitatively for metabolites of laudanosine.

Values for mean arterial blood pressure and heart rate prior to the initial 1 mg · kg^{-1} dose of laudanosine were compared by repeated measures analysis of variance and Dunnett's test, with values obtained 1, 5, and 10 min after injection.[6] A value of $P < 0.05$ was considered statistically significant.

Results

For seven of the eight dogs, a three-compartment model best fit the data for plasma concentration of laudanosine (fig. 2). The values obtained were (mean ± SD)

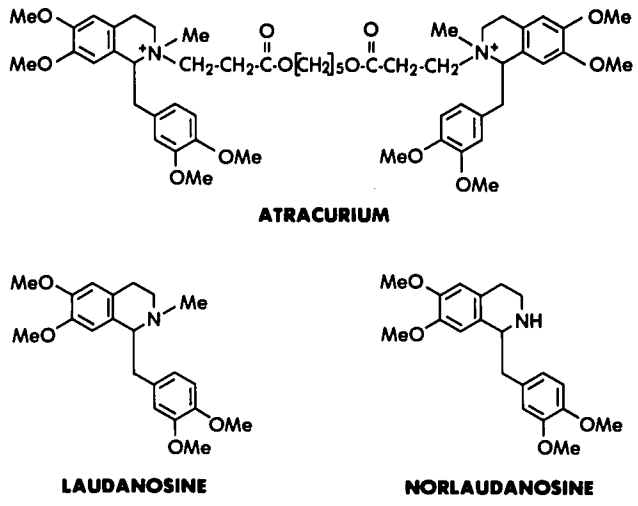

FIG. 1. Structures of atracurium, laudanosine, and norlaudanosine.

0.8 ± 0.2 min for rapid distribution half-life, 14.1 ± 1.6 min for slow distribution half-life, 113.3 ± 24.3 min for elimination half-life, 0.3 ± 0.2 l · kg^{-1} for volume of the central compartment, 2.8 ± 0.9 l · kg^{-1} for volume of distribution at steady state, and 25 ± 8 ml · kg^{-1} · min^{-1} for total plasma clearance. Plasma laudanosine concentrations could not be measured in the eighth dog because of contamination of the blood samples. Valid CSF samples were obtained in only five of eight dogs. In the three remaining dogs, placement of the catheter in the cisterna magna of two resulted in blood contamination of collected CSF samples, thereby invalidating CSF:plasma laudanosine comparisons, and technical problems prevented CSF sampling in the third. Peak values for CSF laudanosine concentrations were obtained 5–10 min after iv injection of laudanosine, and ranged from 208–572 ng · ml^{-1} (table 1). Comparison with the corresponding plasma concentrations of laudanosine yielded CSF:plasma laudanosine ratios of 0.37–0.87.

Laudanosine, 1 mg · kg^{-1}, produced signs of "awakening" from halothane anesthesia in two animals (dogs 3 and 4). These signs included tremors of the limbs, positive eyelid reflex, and swallowing and appeared intermittently 2–10 min after administration of laudanosine. Under conditions of hypocarbia, repetitive administration of laudanosine (2 mg · kg^{-1}), resulted in signs of "awakening" in all animals at cumulative laudanosine doses of 2–8 mg · kg^{-1}. Seizure activity characterized by tonic/clonic movements of the entire body and high-frequency, high-voltage EEG recordings occurred at cumulative doses of 14–22 mg · kg^{-1} (fig. 3). Seizures were of short duration and were followed by a period of minimal EEG activity. Following vecuronium-induced neuromuscular blockade, laudanosine was again repetitively administered, and again induced repeated episodes of seizure activity according

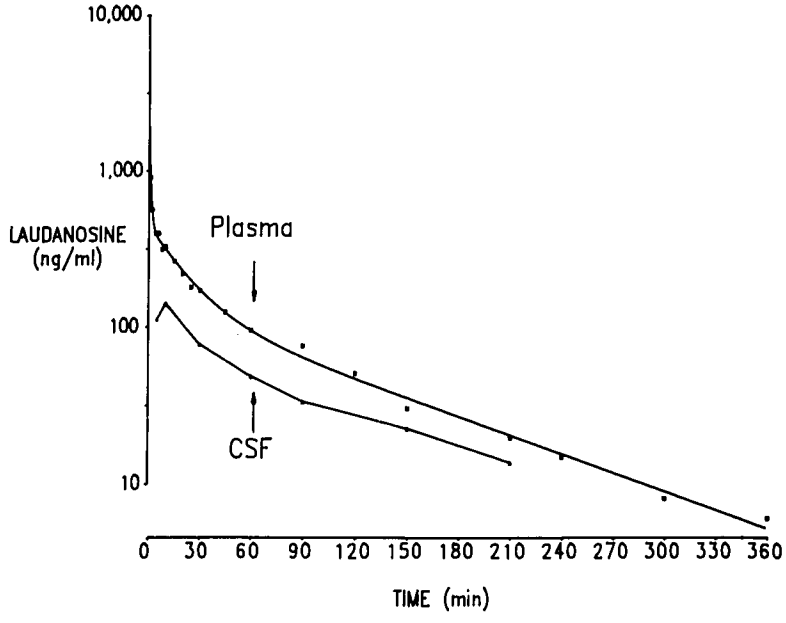

FIG. 2. A plot of time and concentrations of laudanosine (ng/ml) in plasma and CSF in one study animal. The plasma curve represents the fitted three-compartment model.

to EEG criteria. Thiopental, in total iv doses of 12.5–25.0 mg, easily terminated seizure activity. Plasma and CSF samples obtained in one animal at the time of seizure revealed laudanosine concentrations of 10.99 $\mu g \cdot ml^{-1}$ and 3.42 $\mu g \cdot ml^{-1}$, respectively.

Mean arterial blood pressure decreased significantly from a control pressure of 101 ± 17 mmHg (SD) to 86 ± 24 mmHg during the first minute following laudanosine administration ($0.025 < P < 0.05$). By 5 min, the mean arterial blood pressure had returned to control levels. Heart rate did not change significantly.

Analysis of urine and bile samples collected during the first 4 h of the 6-h blood sampling period indicated the presence of unchanged laudanosine in urine (0.5–12% of the injected dose) and in bile (trace amounts, <0.1% of the injected dose). Urine and bile also contained several polar glucuronidated metabolites of laudanosine. Although we were unable to quantify these metabolites, we detected them in the plasma of all seven dogs and in the CSF of four of the five dogs from whom CSF samples were obtained. Metabolites appeared in plasma 2–4 min after iv administration of laudanosine, but not in CSF until 60 min after laudanosine administration.

Discussion

The pharmacokinetic analysis of laudanosine excretion in the dog revealed certain properties of this compound. The large volume of distribution at steady state of 2.8 $l \cdot kg^{-1}$ demonstrates that laudanosine is distributed widely throughout the body of the dog, a finding consistent with the high solubility of laudanosine in ethanol and ether.[7] The elimination half-life of laudanosine of 113 min is five times greater than that of atracurium in humans[3] and six

TABLE 1. Plasma and Cerebrospinal Fluid (CSF) Concentrations of Laudanosine ($ng \cdot ml^{-1}$)

Dog		Min after Laudanosine Administration										
		2	5	10	30	60	90	120	150	180	210	240
1	Plasma	836	590	584	254	141	112	75	45	73	29	22
	CSF	—	166	208	114	71	49	—	33	—	20	—
3	Plasma	1944	1122	871	306	147	217	214	177	205	171	315
	CSF	364	406	302	160	113	96	—	—	—	—	—
4	Plasma	1239	788	454	274	164	120	100	75	65	55	40
	CSF	137	233	258	176	133	96	70	—	—	—	37
5	Plasma	777	498	346	154	92	61	54	40	35	28	25
	CSF	324	329	290	138	135	69	—	49	—	—	26
8	Plasma	1094	661	405	210	106	68	50	42	34	28	24
	CSF	—	572	333	174	113	57	45	37	35	—	—

times greater than that of atracurium in the cat.[8] This suggests that laudanosine may be excreted more slowly in humans, and presumably in dogs, than the parent drug, atracurium.

Analysis of urine and bile for laudanosine has shown that the kidney has a greater role than the liver in the excretion of unchanged laudanosine. This is in contrast with the results of Neill and Chapple who demonstrated in two cats given atracurium that only 3.2% of the dose was excreted in the urine as laudanosine, while 7.5% was excreted in bile.[8] The difference in animal species studied may be one reason for this discrepancy. A more important reason may be that our study used a specific and sensitive assay for determining laudanosine concentrations, while the Neill and Chapple study used a less specific and more qualitative liquid chromatographic comparison of metabolite concentrations. Our results are consistent with evidence obtained in humans indicating that patients with renal failure have higher laudanosine levels following an iv bolus of atracurium than do patients with normal renal function.[2,9]

The liquid chromatographic assay used in our study enabled detection of metabolites of laudanosine in plasma, urine, bile, and CSF. Identification of these metabolites will require further study, but preliminary investigations by our group suggest that they are demethylated derivatives of laudanosine.[10] *In vitro* studies of laudanosine metabolism in rabbit liver microsomal preparations conducted in our laboratory have shown that laudanosine undergoes enzymic N-demethylation to norlaudanosine (fig. 1); other mono-demethylated and di-demethylated metabolites have been found but not characterized.[11] Study is underway to characterize these metabolites to identify each of them.

The concentrations of laudanosine we found in CSF paralleled those in plasma, suggesting that laudanosine readily crosses the blood–brain barrier to exert CNS effects. Metabolites of laudanosine were also found in CSF. Further work is needed to identify the laudanosine metabolites present in CSF and their possible CNS activity.

The appearance of signs of "awakening" soon after the initial dose of laudanosine (1 $mg \cdot kg^{-1}$) was an unexpected finding. Prior to administration of laudanosine, the dogs had been anesthetized with halothane (1% inspired in oxygen; similar to that used during the administration of laudanosine) for several hours for a laparotomy without evidence of "awakening." However, signs of "awakening" following laudanosine administration have appeared in rabbits, in whom plasma concentrations of laudanosine of 457 and 873 $ng \cdot ml^{-1}$ increased the minimum alveolar concentration (MAC) for halothane by 23% and 30%, respectively.[12] In addition, Lanier *et al.* have demonstrated in the dog that atracurium, 1 $mg \cdot kg^{-1}$, produced "cerebral stimulation" that was defined as a change in the EEG pattern from "anesthetized" to "awake," presumably through the CNS effects of laudanosine.[13] The clinical significance of the CNS effect of laudanosine must await studies of anesthetic requirement in surgical patients receiving atracurium. Until then, clinicians should remain alert to the possible CNS stimulating effects of atracurium.[14]

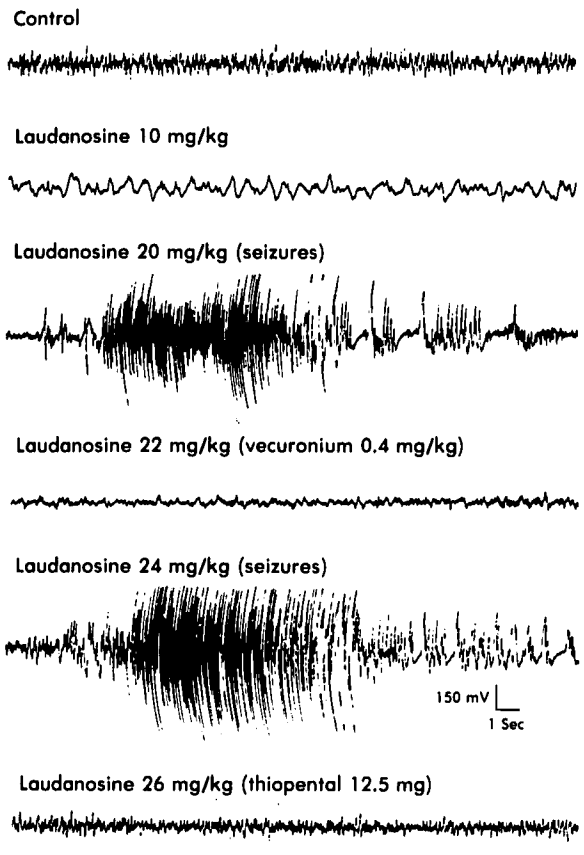

FIG. 3. EEG tracings from a unilateral fronto–occipital lead in a hyperventilated dog during halothane–air anesthesia after cumulative doses of laudanosine. Vecuronium was administered to eliminate EEG muscle artifacts; the absence of seizures at 22 mg/kg of laudanosine represents a postictal effect from the seizure produced at 20 mg/kg of laudanosine.

Seizure activity in the dog appeared after large cumulative doses of laudanosine. This finding is in agreement with that of Mercier and Mercier who demonstrated that consecutive iv doses of 9 and 10 $mg \cdot kg^{-1}$ of laudanosine were necessary to cause a change in the EEG to an "epileptic pattern" in one dog in their study.[15] The single-electrode EEG system used in our study cannot determine the precise origin of seizure activity, but CNS stimulation by laudanosine can be blocked with small doses of thiopental. This may explain why studies of laudanosine in animals anesthetized with large doses of long-acting barbiturates have not found any significant CNS effects.[16,17]

The CNS effects of laudanosine demonstrated in this study, although difficult to interpret, are of potential clinical importance. The conditions necessary to produce sei-

zures in study animals required large doses of laudanosine in the combined presence of hyperventilation, normoxia, and halothane anesthesia. Large concentrations of laudanosine, such as the plasma concentration of 11 $\mu g \cdot ml^{-1}$ that produced seizures in one study animal, are unlikely to result from atracurium administration in surgical patients. Studies of laudanosine production after a single 0.5 $mg \cdot kg^{-1}$ dose of atracurium have reported peak levels of 327 and 758 $ng \cdot ml^{-1}$ in normal and renal failure patients, respectively.[3] Prolonged infusions of atracurium in patients in intensive care have resulted in concentrations of laudanosine as high as 5.1 $\mu g \cdot ml^{-1}$.[18] However, it is important to realize that the sensitivity of surgical patients to laudanosine is not known. Animal studies have shown that some animal species, such as the rabbit, are highly sensitive to the CNS effects of laudanosine, while others, such as the dog used in this study, require large doses of laudanosine before major CNS effects can be elicited. Clinical studies are required to study the CNS effects of atracurium in humans and to explore how such variables as seizure disorders or type of anesthetic may alter the sensitivity of patients to these CNS effects.

At doses of 1 $mg \cdot kg^{-1}$, laudanosine decreased mean arterial pressure. Chapple and Clark studied the effects of this same dose in cats and found no significant changes in mean arterial pressure.[16] Differences in anesthetic technique (chloralose and pentobarbital *vs.* halothane) and species (cat *vs.* dog) may explain the difference in results. Ingram *et al.* demonstrated that laudanosine, 2 $mg \cdot kg^{-1}$, decreased mean arterial pressure and produced dysrhythmias in cats anesthetized with halothane and thiopental.[17] The decrease in mean arterial blood pressure produced by laudanosine may partially explain the hypotension induced by rapid administration of large doses of atracurium.[19] Laudanosine concentrations peak within 2 min after the administration of atracurium in surgical patients, and thus laudanosine would be at its highest concentrations at a time when atracurium-induced hypotension is most commonly observed.[2]

In conclusion, we found that laudanosine can easily cross the blood–brain barrier to produce CNS stimulation in the dog. Effects range from "awakening" from halothane anesthesia at doses of 1–8 $mg \cdot kg^{-1}$ to seizure at doses of 14–22 $mg \cdot kg^{-1}$. Laudanosine also decreases mean arterial blood pressure. The effects of laudanosine on CNS and cardiovascular physiology in dogs suggest further clinical study of atracurium to determine the effects of laudanosine in humans.

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