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Research Article

Pharmacodynamics of Zoxazolamine and Chlorzoxazone in Rats

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Zoxazolamine is used for the pharmacologic assessment of possible changes in oxidative enzyme activity (paralysis time test) in rodents, whereas one of its metabolites, chlorzoxazone, is used clinically as a skeletal muscle relaxant. In this investigation, the pharmacodynamics of the two compounds were characterized in normal adult rats to determine their suitability for studies of the kinetics of drug action in disease states. Upon i.v. infusion 5 min beyond the onset of loss of the righting reflex (LRR) and concomitant blood sampling, serum concentrations of either drug were higher at the onset than at the offset of LRR, suggestive of a distribution disequilibrium. When zoxazolamine was infused at three different rates to onset of LRR, the pharmacologic end point was reached in 10 to 53 min. Drug concentrations in serum and brain at onset of LRR increased with increasing infusion rate, whereas drug concentrations in cerebrospinal fluid (CSF) were infusion rate independent and essentially identical to CSF concentrations at offset of LRR. Similar experiments (five infusion rates) with chlorzoxazone revealed drug infusion rate dependence even of CSF concentrations at the onset of LRR; only at very slow infusion rates (onset of effect in ≥50 min) were onset concentrations in CSF essentially equal to offset concentrations. Neither drug produced measurable metabolite concentrations in the CSF. It is concluded that zoxazolamine but not chlorzoxazone distributes rapidly between CSF and the biophase, metabolites of either drug do not contribute measurably to the pharmacologic effect, and neither drug is subject to development of functional tolerance under the experimental conditions. Zoxazolamine is the more suitable agent for subsequent pharmacodynamic studies of disease effects in rats.

KEY WORDS: zoxazolamine; chlorzoxazone; paralysis time; loss of righting reflex; cerebrospinal fluid; pharmacodynamics.

INTRODUCTION

Zoxazolamine is widely used for a pharmacologic test (paralysis time) that serves as a convenient indicator of changes in *in vivo* cytochrome P-450 activity in rodents (1–3). It is partly metabolized to chlorzoxazone, which is used clinically to relieve localized muscle spasm (4). Both compounds are centrally acting skeletal muscle relaxants but zoxazolamine is no longer used clinically due to hepatotoxicity (5,6).

It is now evident from studies on patients and animal models that certain disease states can alter the pharmacodynamics (concentration–pharmacologic effect relationship) of various drugs that act on the central nervous system (7–13). Zoxazolamine and chlorzoxazone appeared to be promising candidates for the extension of these studies. To assess their usefulness for this purpose, it is necessary to determine if the concentration of these drugs in their biophase is in relatively rapid equilibrium with their concentration in one or more fluids or tissues that can be conveniently sampled (14). It must also be established if these drugs have active metabolites and if their pharmacologic effect is subject to time dependence due to rapid development of functional tolerance or for other reasons.

Drug concentrations in the biophase, i.e., at the site of drug action, can lag behind drug concentrations in plasma and certain other fluids and tissues, thereby preventing a direct correlation between drug concentration at these sampling sites and intensity of drug action (14–16). Certain disease states could, presumably, alter the distribution kinetics of a drug between its biophase and other body fluids and tissues. It is advantageous, therefore, to identify a sampling site for drug concentration determinations where the drug is in rapid equilibrium with the biophase. For this purpose, we infuse a drug intravenously at several different rates until the onset of a defined pharmacologic effect and look for a fluid or tissue where the drug concentration at the onset of a predefined pharmacologic effect is essentially independent of the infusion rate (14). If such infusion rate independence of the "effective" drug concentration can be demonstrated, it indicates also that active metabolites either do not exist or are present at subeffective concentrations and that development of functional tolerance does not occur in the time frame and under the conditions of the investigation or that it

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proceeds so rapidly (within minutes) as not to be observable under the experimental conditions.

METHODS

Inbred male Lewis rats (LEW/CrlBR, Charles River Breeding Laboratories, Wilmington, Mass.), maintained on Charles River Rat-Mouse-Hamster Formula, were used in this investigation. The animals were allowed 7 to 12 days to adjust to the local animal facilities and to recover from possible stress incurred during transport. Before the investigation, the rats were housed individually in metal cages in an environment of controlled temperature (23–25°C) and alternating 12-h light (7 AM–7 PM) and dark cycles. Food and water were withdrawn in the morning on the day of the experiment. The animals used for the pharmacokinetic study had indwelling cannulas implanted in the right jugular vein and the left femoral vein under light ether anesthesia, 1 day before the study. The other animals had an indwelling cannula implanted in the right jugular vein, 3 or 4 days before the pharmacodynamic study.

To compare drug concentrations in serum at the onset and offset of loss of the righting reflex, either zoxazolamine or chlorzoxazone was infused into the femoral vein at a rate of 0.51 mg/min for 5 min beyond the onset of loss of the righting reflex. The infusion solution consisted of 10 mg zoxazolamine (Aldrich Chemical Co., Milwaukee, Wis.) in 1 ml normal saline (dissolved with the aid of 1 *N* HCl) or 10 mg chlorzoxazone (Sigma Chemical Co., St. Louis, Mo.) in 1 ml normal saline (dissolved with the aid of 1 *N* NaOH). The onset and offset of loss of the righting reflex were ascertained by placing the rats on their back and determining their ability to turn upright. They were not subjected to tail pinch or other nociceptive stimulus in this procedure. For each drug, blood samples (0.2 ml) from the jugular vein were obtained from four rats at the onset and offset of loss of the righting reflex, using a procedure that yields mainstream blood (14). The pharmacokinetics of the drugs were determined in six rats each by the serial collection of 12 blood samples per animal over 4.5 hr in addition to the samples obtained at the onset and offset of loss of righting reflex. The blood was replaced by the injection of an equal volume of citrated blood from a donor rat.

The effect of the infusion rate on the concentrations of zoxazolamine at the onset of loss of the righting reflex was determined in groups of 9 or 10 rats that received an i.v. infusion of zoxazolamine at one of three rates (0.206, 0.51, or 1.03 mg/min) by means of a Harvard infusion pump. The infusion was stopped immediately when the animals lost their righting reflex; the rats were then anesthetized with ether and samples of cerebrospinal fluid (CSF), blood, and brain were obtained, in that order.

Another 10 rats from the same shipment received an i.v. infusion of zoxazolamine at 0.51 mg/min for 5 min beyond the onset of loss of the righting reflex. Samples of CSF, blood, and brain were obtained immediately upon recovery of the righting reflex. CSF (100–200 μl within 1–2 min) was obtained by cisternal puncture (17), as much blood as possible was obtained from the abdominal aorta, and the animals were then decapitated by guillotine and the brain was removed within 2 to 3 min after the onset or offset of the pharmacologic effect.

The effect of the infusion rate on the concentrations of chlorzoxazone at the onset of loss of the righting reflex was determined in two separate experiments. In the first experiment, three groups of eight rats received an i.v. infusion of chlorzoxazone at one of three rates (0.227, 0.561, or 1.13 mg/min) until the onset of loss of the righting reflex. At that time, the infusion was stopped, the animals were anesthetized with ether and samples of CSF, blood, and brain were obtained, in that order. A fourth group of animals received an i.v. infusion of chlorzoxazone at 0.561 mg/min for 5 min beyond the onset of loss of the righting reflex, and samples of CSF, blood, and brain were removed immediately upon recovery of the righting reflex.

In the second chlorzoxazone experiment, two groups of 10 rats received an i.v. infusion of the drug at relatively slow rates (0.173 or 0.257 mg/min) and samples of CSF, blood, and brain were obtained at the onset of loss of the righting reflex.

All the *in vivo* experiments were scheduled such that loss of the righting reflex occurred between 9 AM and 12 noon. Normal body temperature was maintained in all animals by placing the rats on isothermal pads (Deltaphase; Braintree Scientific Inc., Braintree, Mass.) because a preliminary study revealed that the infusion of zoxazolamine or chlorzoxazone can decrease the core body temperature by 1 to 3°C.

The protein binding of zoxazolamine and chlorzoxazone was determined by equilibrium dialysis of serum (0.3 ml) against an equal volume of 0.13 *M* sodium and potassium phosphate buffer, pH 7.4, containing zoxazolamine (5 mg/liter) or chlorzoxazone (8 mg/liter), respectively (to maintain the *in vivo* drug concentration in the serum phase). The dialysis was performed at 37°C for 4 hr in Plexiglas cells separated by a cellophane membrane with a molecular exclusion limit of 12,000 to 14,000 daltons (Visking dialysis tubing, Union Carbide, New York). Preliminary studies had shown that protein binding of the two drugs in rat serum is drug concentration independent over the range of 8 to 80 mg/liter for zoxazolamine and 9 to 100 mg/liter for chlorzoxazone.

Concentrations of zoxazolamine and chlorzoxazone in serum, dialysis buffer solution, CSF, and brain were determined by a modification of the high-performance liquid chromatographic assay of Griffeth *et al.* (1) with propranolol hydrochloride (Sigma) as the internal standard. A 10-μl solution of the internal standard in normal saline (200 mg/liter for zoxazolamine and 500 mg/liter for chlorzoxazone) was added to 50 μl of serum, followed by the addition of 100 μl of acetonitrile for precipitation of proteins. After centrifugation, 15 μl of the supernatant was injected onto a 30-cm μBondapak CN column, 10 μM (Waters Associates, Milford, Mass.), with a precolumn containing Bondapak Phenyl/Corasil packing, 37–50 μM. For the assay of 50 μl CSF or dialysis buffer solution, the concentration of the internal standard was reduced to 100 mg/liter for zoxazolamine and 200 mg/liter for chlorzoxazone, and the injection volume was 25 μl. The mobile phase consisted of 0.02 *M* sodium phosphate buffer/acetonitrile/triethylamine (79.5:20:0.5 by volume), final pH 5.8, and the flow rate was 1.2 ml/min. The effluent was monitored spectrophotometrically at 280 nm. Retention times were 4.8, 5.3, and 8.1 min for chlorzoxazone, zoxazolamine, and internal standard, respectively. Drug concentrations in brain were determined by

accurately weighing about 0.4 g of tissue from one hemisphere and homogenizing it with a motor-driven homogenizer and Teflon pestle for about 30 sec at 1000 rpm in 2 ml water and 100 μl internal standard solution (1 g/liter. To 250 μl homogenate, 500 μl acetonitrile was added and the mixture was vortexed and centrifuged. A 20-μl volume of the supernatant was injected onto the chromatographic column.

The calibration curves (peak area ratio versus concentration) for both zoxazolamine and chlorzoxazone were linear, with a negligible intercept in the concentration range of 10 to 100 mg/liter in serum, 2.5 to 20 mg/liter in dialysis buffer and CSF, and 25 to 150 mg/kg in brain. The detection limit was 0.5 to 5 mg/liters or mg/kg and the mean differences between duplicate assays of zoxazolamine and chlorzoxazone in serum, CSF, and brain were between 1.4 and 5%.

The presence of major metabolites in serum and CSF was also examined by high-performance liquid chromatography. Serum samples were mixed with an equal volume of acetonitrile, then vortexed, and 25 μl of the supernatant was injected onto a 15-cm μBondapak CN column. A 25-μl volume of CSF was directly injected onto the column. The mobile phase consisted of 0.02 *M* sodium phosphate buffer/acetonitrile/triethylamine (89.5:10:0.5 by volume), final apparent pH 6.5, and the flow rate was 1.5 ml/min. Retention times were 3.3, 3.7, 5.7, and 7.1 min for 6-hydroxychlorzoxazone (kindly supplied by McNeil Laboratories, Spring House, Pa.), 6-hydroxyzoxazolamine (McNeil), chlorzoxazone, and zoxazolamine, respectively, and their detection limit was 0.5 to 1 mg/liter.

The administered doses of the drugs were calculated as the product of the infusion time and the rate of infusion and were normalized for body weight. The results of the experiments were assessed statistically by one-way analysis of variance followed by the Newman–Keuls test where appropriate. Bartlett's test was used to assess homogeneity of variances. The Kruskal–Wallis test, followed by a Tukey-type multiple comparison analysis (18), was used in case of heteroscedasticity. The statistical significance of differences between two groups was determined by Student's *t* test or by the nonparametric Mann–Whitney *U* test when the variances were unequal. The total area under the serum concentration–time curve obtained in the pharmacokinetic experiment (average extrapolated area, 40% of the estimated total for zoxazolamine and 2% for chlorzoxazone) was used to calculate the time-averaged total clearance.

RESULTS

Serum concentrations of zoxazolamine and chlorzoxazone at the onset and offset of the defined pharmacologic effect, loss of the righting reflex (LRR), were determined by slowly infusing either drug until the loss of LRR and continuing the infusion for 5 min beyond that point. For each drug, the concentrations at the onset of the effect were significantly higher than the concentrations at the offset of LRR (Table I). The doses of the two drugs required to produce LRR were similar but the serum concentrations of chlorzoxazone associated with this effect were more than twice as high as the corresponding concentrations of zoxazolamine (Table I). The serum concentration profiles of the two drugs differed substantially (Fig. 1); the time-averaged serum clearance determined from these concentration data was 7.22 ± 1.01 ml min^{-1} kg^{-1} for zoxazolamine and 9.75 ± 0.35 ml min^{-1} kg^{-1} for chlorzoxazone (mean ± SD; $N = 6$ for each drug). The zoxazolamine clearance estimate is subject to considerable uncertainty because the extrapolated portion of the area under the concentration versus time curve is about 40% of the total estimated area and the pharmacokinetics of this compound in rats are known to be nonlinear (2). The results of this investigation show that the pharmacokinetics of chlorzoxazone in rats are also nonlinear.

Infusion of zoxazolamine to LRR at three different rates, with the onset of the effect at about 10, 20, and 50 min, showed increasing drug concentrations in serum and brain at the onset of the effect with increasing infusion rates (Table II). On the other hand, drug concentrations in the CSF at the pharmacologic end point were independent of the infusion rate. When the zoxazolamine infusion was continued for 5 min beyond the onset of LRR, the concentrations of drug in the CSF at the offset of LRR were essentially identical to the onset concentrations (Table III). This was also true for the serum concentrations but the reader is

Table I. Serum Concentrations of Zoxazolamine and Chlorzoxazone at Onset and Offset of Loss of the Righting Reflex (LRR) in Rats[a]

Variable	Zoxazolamine			Chlorzoxazone		
	Group I	Group II	Combined groups	Group I	Group II	Combined groups
No. of animals	6	4	10	6	4	10
Body weight (g)	197 ± 9	200 ± 7	198 ± 8	198 ± 3	204 ± 4	200 ± 4
Total dose (mg/kg)	67.2 ± 4.6	67.8 ± 2.4	67.5 ± 3.7	76.9 ± 3.8	73.7 ± 5.2	75.6 ± 4.5
Onset time of LRR (min)	21.0 ± 1.4	21.6 ± 0.8	21.2 ± 1.2	24.7 ± 1.9	24.4 ± 2.0	24.6 ± 1.8
Offset time of LRR (min)	114 ± 20	120 ± 28	117 ± 22	70.0 ± 11.4	57.5 ± 10.6	65.0 ± 12.3
Serum concentration (mg/liter)						
At onset	35.0 ± 7.1	38.7 ± 6.7	36.5 ± 6.8	90.6 ± 9.5	87.1 ± 5.6	89.2 ± 8.0
At offset	23.4 ± 2.8*	24.3 ± 1.0*	23.8 ± 2.2*	58.8 ± 8.6*	61.4 ± 7.9*	59.8 ± 8.0*
Time-averaged serum clearance (ml/min/kg)	7.2 ± 1.0	—	—	9.8 ± 0.4	—	—

[a] The rats were infused i.v. with zoxazolamine or chlorzoxazone at 0.51 mg/min for 5 min beyond the onset of LRR. The pharmacokinetics of the drugs were determined in six animals (group I) by serial blood collection over 4.5 hr. Blood samples were obtained from other rats (group II) only at the onset and offset of loss of righting reflex. Results are reported as mean ± SD.

* Significantly different from drug concentrations at the onset of LRR ($P < 0.01$).

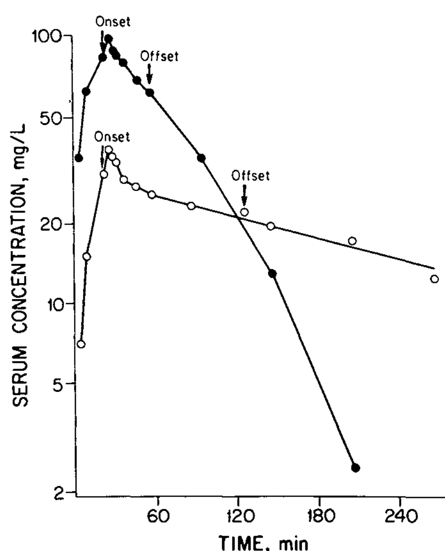

Fig. 1. Serum concentrations of zoxazolamine (○) and chlorzoxazone (●) in individual rats after i.v. infusion of the drug at a rate of about 2.5 mg min^{-1} kg^{-1} until 5 min after loss of the righting reflex. The arrows show the times of onset and offset of the pharmacologic effect.

Table III. Serum, Brain, and CSF Concentrations of Zoxazolamine at Recovery of the Righting Reflex in Rats[a]

Body weight (g)	208 ± 9
Total dose (mg/kg)	68.3 ± 18.0
Serum concentration (mg/liter)	
Total drug	21.3 ± 2.1
Free drug	5.9 ± 0.9
Brain concentration (mg/kg)	101 ± 13
CSF concentration (mg/liter)	5.2 ± 0.7
Free fraction in serum × 100	27.4 ± 2.5

[a] Rats were infused i.v. with zoxazolamine, 0.51 mg/min, for 5 min beyond the onset of loss of the righting reflex (total infusion time, 28.0 ± 8.1 min). Recovery occurred at 61.9 ± 31.5 min after the infusion was stopped. Results are reported as mean ± SD (N = 10).

referred to the Discussion for special considerations concerning the interpretation of the serum concentration data.

Infusion of chlorzoxazone at three different rates, producing LRR in about 10, 20, and 54 min, showed that onset time concentrations of the drug not only in serum and brain, but also in CSF, increased with increasing infusion rates (Table IV). The serum-free fraction of chlorzoxazone was about 0.11, considerably smaller than that of zoxazolamine (about 0.27). When the infusion of chlorzoxazone was continued until 5 min after the onset of LRR and drug concentrations at the return of the righting reflex were determined (Table V), it was found that the drug concentration in the CSF was essentially identical to that at the onset of LRR in the rats that received the slowest infusion of chlorzoxazone (Table IV). For this reason, the infusion rate experiment was extended to an even slower infusion rate, with onset of the effect in 86 min on the average (Table VI). Since this required the use of a new shipment of rats, the lowest infusion rate used in the first experiment was repeated. For this purpose, the nominal infusion rate was adjusted slightly to account for the small difference in body weight of the two groups of animals. The CSF concentrations in the two experiments were almost identical. Considering the result of the second experiment only, serum and brain chlorzoxazone concentrations at onset of LRR were significantly higher at the higher infusion rate, whereas CSF concentrations did not differ.

No measurable concentrations of chlorzoxazone and 6-hydroxyzoxazolamine (after zoxazolamine administration) or 6-hydroxychlorzoxazone were found in the serum, brain, and CSF samples obtained in this investigation, nor was there any chromatographic evidence of any other drug metabolites in these samples.

DISCUSSION

Zoxazolamine and chlorzoxazone are centrally acting skeletal muscle relaxants of the benzazole type that inhibit reflex pathways within the spinal cord (19). Although the specific receptor(s) mediating the effects of these com-

Table II. Effect of Infusion Rate on Serum, Brain, and CSF Concentrations of Zoxazolamine in Rats at Onset of Loss of the Righting Reflex[a]

Variable	Infusion rate: 0.206 mg/min	0.51 mg/min	1.03 mg/min
No. of animals	10	9	10
Body weight (g)	203 ± 18	197 ± 11	202 ± 9
Infusion time (min)[b]	52.8 ± 7.6*	19.9 ± 1.4*	10.2 ± 0.7*
Total dose (mg/kg)	53.5 ± 4.9	51.5 ± 2.2	51.9 ± 3.5
Serum conc. (mg/liter)			
Total drug[b]	19.4 ± 2.1*	21.5 ± 1.4*	23.8 ± 2.4*
Free drug[b]	4.9 ± 0.6*	6.0 ± 1.2*	6.9 ± 0.9*
Brain conc. (mg/kg)[b]	97.1 ± 10.8*	110 ± 7	116 ± 10
CSF conc. (mg/liter)	5.3 ± 0.4[c]	5.2 ± 0.8	5.6 ± 0.5
Free fraction in serum × 100	25.3 ± 2.0	28.3 ± 6.5	28.9 ± 4.5

[a] Results are reported as mean ± SD.
[b] Infusion rate had a significant effect on the results ($P < 0.001$ by one-way analysis of variance).
[c] $N = 8$ due to failure to obtain CSF from two rats.
* Significantly different from the results of the other two groups ($P < 0.05$).

Table IV. Effect of Infusion Rate on Serum, Brain, and CSF Concentrations of Chlorzoxazone in Rats at Onset of Loss of the Righting Reflex[a]

Variable	Infusion rate		
	0.227 mg/min	0.561 mg/min	1.13 mg/min
Body weight (g)	209 ± 8	214 ± 11	215 ± 9
Infusion time (min)[b]	54.2 ± 3.5*	19.7 ± 1.8*	9.9 ± 1.3*
Total dose (mg/kg)[b]	59.0 ± 3.0*	51.6 ± 3.7	52.0 ± 5.1
Serum conc. (mg/liter)			
Total drug[b]	59.1 ± 4.5*	68.6 ± 9.0	75.9 ± 7.6
Free drug[b]	6.8 ± 0.9*	7.8 ± 1.1	8.3 ± 0.8
Brain conc. (mg/kg)[b]	58.3 ± 3.5*	65.2 ± 4.0*	73.0 ± 6.6*
CSF conc. (mg/liter)[b]	6.4 ± 0.4*	7.5 ± 0.4*	8.2 ± 0.6*
Free fraction in serum × 100	11.5 ± 1.6	11.5 ± 1.7	11.0 ± 1.1

[a] Results are reported as mean ± SD ($N = 8$).
[b] Infusion rate had a significant effect on the results ($P < 0.025$ by one-way analysis of variance).
* Significantly different from the results of the other two groups ($P < 0.05$).

pounds has not been isolated or identified, it is known that the benzazole effects are qualitatively different from those of diazepam, barbiturates, and ethanol (20) even though the former, like all centrally acting muscle relaxants, have sedative activity (6). Zoxazolamine and chlorzoxazone have been found to decrease striatal dopamine turnover in rats, whereas diazepam, pentobarbital, and ethanol had no such effect (20). Both benzazoles are eliminated almost entirely by biotransformation, mainly to 6-hydroxy compounds that are rapidly conjugated with glucuronic acid (21–23). The hydroxylated metabolites are stated to have little or no muscle relaxant activity in mice and rats (21,22) but no experimental details in support of that contention have been reported. Zoxazolamine is also metabolized to chlorzoxazone, but only to a very minor degree (21).

The doses of zoxazolamine (Z) and chlorzoxazone (C) required to produce LRR in rats are approximately equal; the Z/C dose ratio in the experiment described in Table I is 0.89. This is in very good agreement with the reported dose ratio of 0.93 for the ED_{50} of these drugs when administered i.p. to Wistar rats (24). The substantial difference between the onset and the offset serum concentrations of the two drugs (Table I) suggests either a distribution disequilibrium between the serum and the biophase (i.e., biophasic drug concentrations lag behind the serum concentrations) or a gradual accumulation of the active metabolite(s).

Table V. Serum, Brain, and CSF Concentrations of Chlorzoxazone at Recovery of the Righting Reflex[a]

Variable	Value
Body weight (g)	215 ± 9
Total dose (mg/kg)	67.9 ± 7.5
Serum concentration (mg/liter)	
Total drug	60.9 ± 9.1
Free drug	6.4 ± 0.8
Brain concentration (mg/kg)	60.8 ± 3.6
CSF concentration (mg/liter)	6.5 ± 0.6
Free fraction in serum × 100	10.6 ± 1.1

[a] Rats were infused i.v. with chlorzoxazone, 0.561 mg/min, for 5 min beyond the onset of loss of the righting reflex (total infusion time, 26.0 ± 3.2 min). Recovery occurred at 29.5 ± 6.9 min after the infusion was stopped. Results are reported as mean ± SD ($N = 8$).

Determination of the infusion-rate dependence of zoxazolamine concentrations at the onset of LRR (Table II) revealed that there is indeed an initial disequilibrium of the drug between the serum and the biophase as well as between the brain (as a whole) and the biophase. Since the brain is a heterogeneous tissue, there may be regions (possibly subcellular) where there is no such disequilibrium. The zoxazolamine concentration in the CSF at the onset of LRR was independent of the infusion rate and essentially equal to the offset concentration (Tables II and III). This indicates that zoxazolamine in the CSF equilibrates rapidly with zoxazolamine in the biophase and therefore reflects concentrations of the drug at the site of action. It indicates also that metabolites of the drug do not contribute significantly to its pharmacologic activity, either by reason of intrinsic lack of activity or due to subeffective concentrations. The results also indicate that zoxazolamine was not subject to development of acute tolerance in the time frame of the study.

There is an apparent inconsistency in the serum concentration data of zoxazolamine in Tables I to III. The onset serum concentrations in Table II are lower than the onset serum concentrations in Table I and very similar to the offset serum concentrations in Table III. The reason is technical.

Table VI. Effect of Relatively Slow Infusion Rates on Serum, Brain, and CSF Concentrations of Chlorzoxazone in Rats at Onset of Loss of the Righting Reflex[a]

Variable	Infusion rate	
	0.173 mg/min	0.257 mg/min
Body weight (g)	233 ± 11	229 ± 7
Infusion time (min)	85.9 ± 7.7	50.1 ± 5.3*
Total dose (mg/kg)	64.0 ± 4.5	55.9 ± 4.8*
Serum concentration (mg/liter)		
Total drug	59.2 ± 5.5	64.1 ± 3.4*
Free drug	5.7 ± 0.8	6.5 ± 0.6*
Brain concentration (mg/kg)	46.8 ± 4.2	53.0 ± 5.5*
CSF concentration (mg/liter)	6.2 ± 0.6	6.6 ± 0.4
Free fraction in serum × 100	9.6 ± 0.8	10.1 ± 0.7

[a] Results are reported as mean ± SD ($N = 10$).
* Significantly different from the results at the lower infusion rate ($P < 0.05$).

In the experiment described in Table I, the blood sample at the onset of the effect was taken while infusion of the drug was ongoing. Conversely, in the experiment summarized in Table II, the drug infusion was stopped at the onset of the effect, the animals were anesthetized with ether, CSF was obtained, and then the blood sample was obtained. Zoxazolamine pharmacokinetics in rats exhibit a very prominent plasma distribution phase, with a distribution half-life of only about 4 min (1). Thus, in the approximately 2-min interval between the end of the infusion and blood sampling, drug concentrations in plasma can decrease appreciably. For example, in the experiment reported in Table I, serum zoxazolamine concentrations at the end of the infusion were 42.0 ± 7.4 μg/ml, whereas 2 minutes later they had decreased to 34.7 ± 4.6 μg/ml (mean ± SD; $N = 6$). The difference would have been even greater had the infusion been stopped 5 min earlier (i.e., at onset of LRR), because the relative disequilibrium of the drug in the body would have been more pronounced. Similar considerations apply to chlorzoxazone.

Another difference between the two experiments could also have contributed to differences in onset concentration. In the experiment represented by Table I, blood for serum was obtained from the jugular vein, whereas in the experiment represented by Table II, the blood was taken from the abdominal aorta (i.e., the arterial system). In both cases, the drug was infused into a vein. Thus, blood was sampled near the input site in the first experiment and more distally (i.e., after some distribution of the drug to tissues) in the second experiment.

Unlike zoxazolamine, chlorzoxazone exhibited infusion-rate dependence of onset concentrations not only in serum and brain but also in CSF (Table IV). However, the CSF concentrations at onset produced by the slowest drug infusion (0.227 mg/min) were almost identical to the offset concentrations (Table V), suggesting that drug equilibration between the biophase and the CSF requires between 20 and 54 min under the experimental conditions. This was confirmed when, upon even slower infusion of the drug, CSF concentrations at the onset of LRR did not decrease further (Table VI). These results indicate that metabolites do not contribute measurably to the pharmacologic effect of chlorzoxazone and that the drug is apparently not subject to functional tolerance development, at least under the experimental conditions. Clearly, chlorzoxazone in CSF equilibrates much more slowly with its biophase than does zoxazolamine. The reasons for this difference may include the more pronounced protein binding and lower lipid solubility (as judged by chromatographic retention time) of the former, assuming that equilibration of these drugs between blood, CSF, and the biophase is by passive diffusion. Zoxazolamine is a weak base with a pK_a of about 3.25 (25), whereas chlorzoxazone is a weak acid with a pK_a of 8.3 (26); both compounds are almost entirely nonionized at physiologic pH.

The offset concentrations of zoxazolamine in serum and brain determined in this investigation (21.3 μg/ml and 101 ± 12 μg/g; Table III) are similar to those reported by Kato *et al.* (27) for female Wistar rats (about 30 μg/ml and 80 μg/g) and the offset serum concentrations reported by Griffeth *et al.* (1) for male Fischer-344 rats (15 μg/ml). A comparison of the offset concentrations of zoxazolamine and chlorzoxazone in CSF (a fluid with a very low protein concentration and therefore negligible protein binding) obtained in this investigation, 5.2 ± 0.7 and 6.5 ± 0.6 μg/ml, respectively, shows that the two drugs are almost equipotent with respect to their ability to cause LRR.

The duration of LRR produced by zoxazolamine is referred to in the literature as the paralysis time, whereas the duration of LRR produced by barbiturates is designated the sleeping time. We have not been able to observe any difference in the behavior or appearance of rats treated with either zoxazolamine or chlorzoxazone or with phenobarbital or heptabarbital in doses sufficient to produce LRR. We believe that in all cases LRR is due to a hypnotic effect, albeit by different mechanisms (20), and not caused by muscular paralysis in the case of the benzazoles. These conclusions are consistent with those of Hoffmeister (28), who examined the effect of an immobilizing dose of zoxazolamine on the electroencephalogram of rabbits and determined that the inability of the animals to move was due to narcosis rather than paralysis.

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