

Effect of Serum Protein Binding on the Entry of Lidocaine into Brain and Cerebrospinal Fluid in Dogs

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To test the hypothesis that lidocaine passage into the central nervous system is a function of free rather than total lidocaine concentration, we examined the effect of serum protein binding on the distribution of lidocaine into brain and cerebrospinal fluid (CSF) in dogs. Six of the 13 dogs studied were pretreated with rifampin to induce a 4-fold increase ($P = 0.019$) in serum concentration of α_1 -acid glycoprotein, which is a major binding protein for lidocaine. The dogs were anesthetized and prepared surgically to obtain samples of cortical brain tissue or CSF from the cisterna magna. Lidocaine at a dose of 3 mg/kg was infused intravenously over 15 s. Arterial blood and brain cortex or CSF were sampled serially during a 60-min interval and analyzed for lidocaine content. Unbound or free fraction of lidocaine in serum was measured by equilibrium dialysis. Rifampin pretreatment led to a significant decrease in average serum free fraction of lidocaine, from 0.24 ± 0.08 to 0.080 ± 0.030 ($P < 0.001$). Total lidocaine concentration in serum was higher, but free lidocaine concentration was lower in the rifampin group. Equilibration of lidocaine between serum and brain or CSF was reached by 10 min after lidocaine administration. Rifampin-pretreated dogs had consistently lower partition ratios of lidocaine between brain and serum or between CSF and serum. A strong and positive correlation between time-averaged brain to serum or CSF to serum ratios and serum free fractions were observed ($r = 0.92$, $P < 0.001$ for brain; $r = 0.90$, $P < 0.01$ for CSF). Moreover, partition ratios expressed on the basis of serum free drug concentrations did not differ between control and rifampin-pretreated groups. The results of this study indicate that the distribution of lidocaine between serum and the central nervous system after intravenous adminis-

tration is governed by free drug concentration in the circulation. Therefore, the relationship of local anesthetic toxicity to drug concentration in blood should be considered in terms of free rather than total drug-concentration measurements. (Key words: Anesthetics, local: lidocaine. Brain: CSF. Pharmacokinetics: α_1 -acid glycoprotein; protein binding.)

SEVERAL CLINICAL REPORTS have suggested that central nervous system (CNS) toxicity from lidocaine or bupivacaine during continuous regional anesthesia or intravenous antiarrhythmic therapy correlates better with the concentration of free or unbound drug in serum than with total drug in serum.^{¶,**,††} This observation is consistent with the so-called "free drug hypothesis," which asserts that plasma protein binding of drugs restricts their movement across membrane barriers.

The validity of the free drug hypothesis has been challenged by recent experimental studies that showed that brain uptake of drugs and hormones may not always be limited to their equilibrium free fraction in the cerebral capillary blood.¹ In particular, Pardridge *et al.*² showed by a single-pass intracarotid injection technique in rats that while the albumin-bound lidocaine is restricted from transport through the blood-brain barrier, a portion of the α_1 -acid glycoprotein (AAG)-bound pool of lidocaine is apparently available for uptake into the brain. Similar findings have been reported for bupivacaine.³ AAG is a major carrier protein for the local anesthetics. Because AAG is an "acute phase reactant," its circulating level is increased to a variable extent during trauma, surgery, cancer, inflammation, and many other disease states.⁴ The involvement of protein-bound drug in brain capillary endothelial transport would mean that in addition to free drug, AAG-bound drug would contribute to the effects of local anesthetics in the CNS.

The experimental studies to date have examined the

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uptake of lidocaine or bupivacaine during a single passage of intracarotid injectate through the cerebral vasculature. It can be questioned whether the conclusions based on intracarotid injection studies can be extrapolated to clinical situations. The results of transient-uptake studies may not be applicable to a situation where distribution pseudoequilibrium between CNS and blood has been reached, such as after peripheral venous administration of lidocaine. Moreover, the function of capillary endothelium could be altered during experimental manipulation of the cerebral circulation. The purpose of the present study was to clarify the role of serum AAG in the CNS distribution of lidocaine following intravenous bolus administration. The distribution kinetics of lidocaine in brain parenchyma, cerebrospinal fluid (CSF), and blood were studied in dogs with either normal serum AAG or dogs pretreated with rifampin to cause elevation of serum AAG concentration.⁵

Materials and Methods

ANIMAL PREPARATION

The following protocol was approved by the Animal Care Committee of the University of Washington. Thirteen unmedicated mongrel dogs (weight 12–21 kg) were studied. Six dogs were given a 300-mg rifampin capsule orally twice per day for 14 days prior to the study day. The other 7 dogs were assigned to the control group without rifampin pretreatment.

On the study day, anesthesia was induced with halothane (~ 2.5%, inspired) and nitrous oxide (66%, inspired) in oxygen. The trachea was intubated; expired carbon dioxide was continuously monitored (Beckman Medical Gas Analyzer, model LB2, Beckman Instruments, Inc., Fullerton, CA); and ventilation was regulated by a servomechanism to maintain expired carbon dioxide at normocapnia. The right femoral artery was cannulated for arterial blood-gas analysis and continuous monitoring of systemic arterial pressure and heart rate. A urinary catheter was inserted; the right femoral vein was cannulated for saline and drug administration; and body temperature was monitored by a nasopharyngeal thermistor probe. Maintenance fluids were given by continuous infusion of saline at a rate of $4\text{--}6\text{ ml} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$.

With the dog in the prone position, the head was fixed in a slightly elevated position using a stereotaxis frame. A midline scalp incision was made and both frontalis muscles reflected laterally. Over both cerebral hemispheres, the skull was thinned around the perimeter of a $4 \times 5\text{-cm}$ area to expose diploic veins, which were then occluded with bone wax. A midline incision was made over the occipital bone and cervical spines, and the posterior neck muscles were surgically separated to expose the atlantooccipital membrane. The inspired concentration of halothane was then decreased to 1.0%.

The dogs in each treatment group were divided into two subgroups for the sampling of either arterial blood and CSF (three rifampin-pretreated and three control) or arterial blood and cerebral tissue (three rifampin-pretreated and four control). Pilot studies indicated that CSF and brain tissue samples could not be obtained in every dog because the procedure for sampling of brain tissue inevitably released blood into the CSF. For the animals undergoing CSF sampling, a catheter was inserted through the atlantooccipital membrane, and its tip was placed in the cisterna magna to permit withdrawal of CSF. For the animals undergoing brain tissue sampling, the exposed $4 \times 5\text{-cm}$ area of cranium over each cerebral hemisphere was removed, and the dura was incised and reflected laterally to permit access to cortical tissue. Bleeding from cortical vessels was controlled by electrocautery. Drying of the cerebral cortex was minimized by application of saline and by covering the area with plastic film.

EXPERIMENTAL PROTOCOL

The experimental period began after blood chemistry and systemic hemodynamic variables had stabilized. Lidocaine 3 mg/kg was infused into the right femoral vein over 15 s. In both groups, arterial blood samples were obtained from the femoral artery cannula prior to administration of lidocaine and afterward at 1, 2, 3.5, 5, 7.5, 10, 15, 22.5, 35, 45, and 60 min. Serum was analyzed for total lidocaine concentration, free fraction of lidocaine, and concentrations of AAG and albumin. Simultaneous samples of either CSF or brain tissue were obtained at 2, 5, 10, 22.5, and 60 min after administration of lidocaine and analyzed for total lidocaine concentration. The volume of each cisternal CSF sample was 0.4 ml. For each brain tissue sample, the perimeter of a $2.5 \times 1.25\text{-cm}$ area was cauterized and then incised to a depth of 0.25 cm. The base of this block of tissue was separated from the underlying brain tissue using electrocautery, and the tissue sample was removed.

LIDOCAINE ANALYSIS

Concentration of lidocaine in serum (total including bound and unbound drug), CSF, or brain tissue extract was assayed according to a slightly modified gas chromatographic method of Mather and Tucker.⁶ Carbon disulfide was substituted for methylene chloride in the final solvent extraction step. Improved sensitivity to a level of $0.05\text{ }\mu\text{g/ml}$ was achieved using a nitrogen-phosphorous-sensitive flame ionization detector. The interassay coefficients of variation of the assay were less than 10% for lidocaine concentrations of 5 and $1\text{ }\mu\text{g/ml}$.

Brain tissue extracts were obtained by homogenizing 1 g tissue with 3 ml of 0.1 M phosphate buffer containing 1 μg internal standard (W12174, Astra Pharmaceutical

Products, Inc., Worcester, MA). The homogenate was centrifuged, and the supernatant was prepared for chromatographic analysis in the same manner as for serum and CSF. A 100- μ l aliquot was set aside for determination of hemoglobin content by spectrophotometry using the Hycel[®] cyanmethemoglobin reagent (Hycel, Inc., Houston, TX). The volume of blood adherent to the sampled tissue was estimated from the hemoglobin concentration in the extract. The lidocaine in contaminant blood was subtracted from brain tissue concentration measurements using the concentration of lidocaine in corresponding serum samples. Although the lidocaine concentration in blood is slightly higher than that in serum because of unequal partitioning into red cells, the effect of this error on the final brain lidocaine concentration data was minimal since the correction for residual blood content was less than 5%.

LIDOCAINE SERUM PROTEIN BINDING

A preliminary experiment revealed that binding of lidocaine to dog serum protein is saturable over the drug concentration range observed *in vivo*; *i.e.*, lidocaine free fraction is dependent on total drug concentration in serum. An *in vitro* protein binding isotherm (bound concentration *vs.* free concentration relationship) was determined for each dog by adding varying amounts of lidocaine to blank serum collected from the animal prior to study. Free concentration was measured by an equilibrium dialysis technique. Binding parameters determined from the *in vitro* isotherm was used to estimate the free lidocaine concentration corresponding to the total lidocaine concentration measured in each serum sample from the study. Details of the dialysis technique and numerical analysis of binding isotherm data are presented in the Appendix.

SERUM PROTEIN ASSAYS

AAG was measured by the colorimetric procedure of Abramson and Lutz,⁵ which quantifies the N-acetylnauraminic acid contained in the perchloric acid-nonprecipitable protein in serum. An average serum concentration of AAG for each dog was determined by assaying four serum samples collected at different times after drug administration. There was no significant variation in serum AAG during the blood-sampling period in both control and rifampin-treated dogs. Standard curves were constructed by dissolving known amounts of dog AAG (Sigma Chemical Co., St. Louis, MO) in saline over a concentration range of 0.2–2.0 mg/ml. The standard curves were linear over this range. The interassay coefficient of variation was 0.7% at 0.2 mg/ml and 1.5% at 1.0 mg/ml ($n = 4$).

Albumin was measured by the bromocresol-green dye binding procedure using a commercial kit assay (procedure no. 631, Sigma). The standard curve was linear over

the relevant concentration range, 0.5–2.5 g/dl. Albumin determination was also carried out in four different samples from each dog to obtain an average serum albumin concentration.

DATA ANALYSIS

Clearance and steady-state volume of distribution were computed from the serum concentration–time data using statistical moments.⁷ The area under the serum concentration–time curve (AUC_t) and the area under the moment–time curve were computed using the LAGRAN program.⁸ Extrapolation of AUC and the area under the moment–time curve beyond the final sampling time were based on terminal half-lives estimated from log-linear regression of the last three or four serum concentration time points. The half-life estimates were subject to some error because the terminal sampling period was not much longer than the estimated half-life. However, the overall error to the moment estimates was acceptably small considering that the extrapolation accounted for less than 30% of the total AUC or area under the moment–time curve.

The area under the serum unbound concentration–time curve (AUC_u) was likewise computed from the predicted values for unbound concentration, assuming the terminal slope for total concentration and unbound concentration to be equal. The assumption is justified by the observation that the estimated unbound concentration for the 60-min sample was consistently lower than the estimated dissociation constant (K_d), which assures linear protein binding at and below the level of the last measured total concentration. A time-averaged serum free fraction (\bar{f}_u) for each dog was calculated from the ratio of AUC_u and AUC_t .

Area under the curve was also calculated for the brain tissue (AUC_{br}) and CSF (AUC_{csf}) data over the 60-min sampling period. These AUC estimates were used to compute a time-averaged partitioning ratio between brain tissue and serum or between CSF and serum.

Student's unpaired *t* test was used to compare all parameters between rifampin-pretreated and untreated dogs. A *P* value < 0.05 was considered statistically significant.

Results

Rifampin pretreatment significantly increased the circulating concentration of AAG in the dog. Serum AAG concentration in the control group ranged from 0.494 to 0.990 mg/ml, with a mean of 0.682 ± 0.174 mg/ml (see table A1 in Appendix for details). With the exception of one dog, each of the animals in the rifampin group had a serum AAG concentration greater than that observed in the control group. The reason for the failure to elicit

an induction of AAG in this dog is not known. The mean serum AAG concentration in the rifampin group (excluding the dog that did not respond), 2.67 ± 1.16 mg/ml, was four times greater than that in the control group. Serum albumin concentration did not differ between control and rifampin-pretreatment groups ($P > 0.05$).

Consistent with the increase in AAG, serum protein binding of lidocaine was significantly greater in the rifampin-pretreated dogs compared with the control animals. Because of the marked concentration-dependent binding of lidocaine to dog serum proteins, the mean serum free fraction data for the two groups were compared in a plot of estimated free fractions *versus* serum drug concentration (fig. 1). Mean serum free fraction varied from 0.1 to 0.6 over a concentration range of 0.5–15 $\mu\text{g/ml}$ in control dogs and from 0.05 to 0.3 over a concentration range of 1.0–28 $\mu\text{g/ml}$ in rifampin-pretreated dogs.

The effects of rifampin pretreatment on the concentration-time course of total lidocaine in serum are presented in the top panel of figure 2. An increase in the total circulating concentration of lidocaine was observed in the rifampin-pretreated animals. The mean AUC of total lidocaine (the AUC_t) increased by about 50%, from 154 ± 46 $\mu\text{g} \cdot \text{min/ml}$ in the control group to 235 ± 87 $\mu\text{g} \cdot \text{min/ml}$ in the rifampin group. This corresponded to a decrease in apparent clearance of lidocaine, from 18.2 ± 5.1 ml/min/kg in control dogs to 11.2 ± 4.1 ml/min/kg in rifampin-pretreated dogs ($P < 0.05$). There was also a marked decrease in steady-state distribution volume, from 0.97 ± 0.25 to 0.44 ± 0.24 l/kg ($P < 0.01$), which is consistent with the increase in serum protein binding of lidocaine.

As can be seen in the bottom panel of figure 2, serum free concentrations of lidocaine were, in contrast, less in

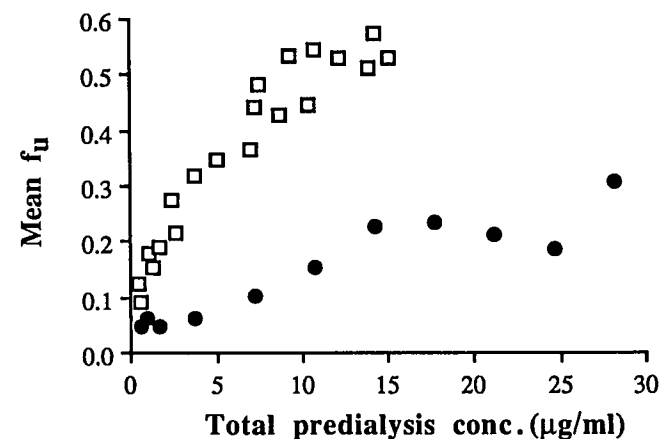


FIG. 1. Comparison of mean serum-free fraction (f_u) data over a range of total lidocaine predialysis concentration in control (□) and rifampin-pretreated (●) dogs. Each point represents the mean of seven dogs for the control group or six dogs for the rifampin-pretreated group.

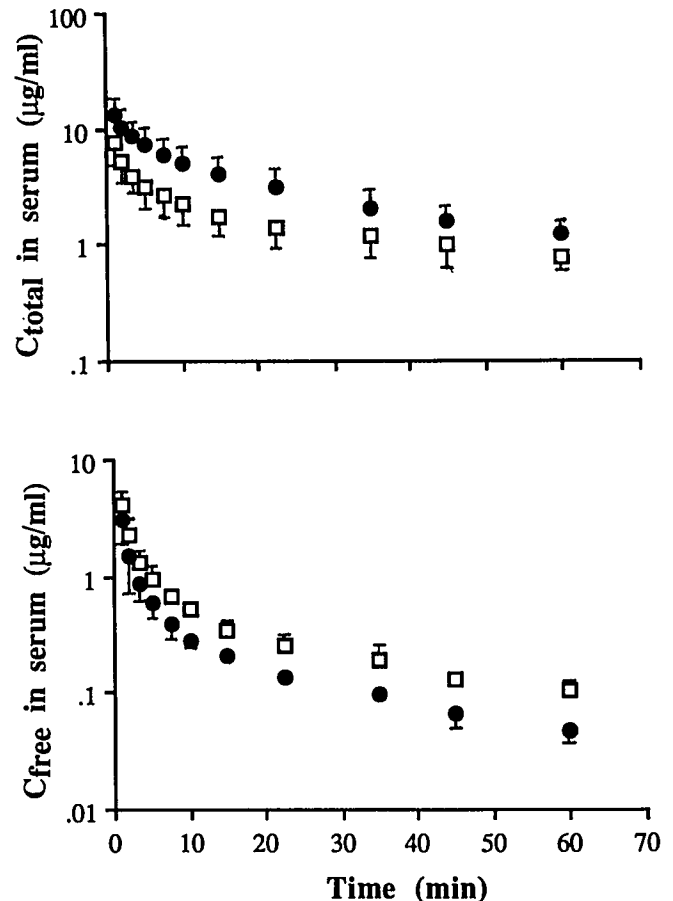


FIG. 2. Time course of total lidocaine concentration (C_{total}) (top) and free lidocaine concentration (C_{free}) (bottom) in serum of control (□) and rifampin-pretreated (●) dogs. Data are mean \pm SD.

the rifampin-pretreated animals compared to control animals. The mean AUC of free lidocaine (AUC_m) decreased by about 40%, from 33.6 ± 4.4 $\mu\text{g} \cdot \text{min} \cdot \text{ml}^{-1}$ in the control group to 20.9 ± 6.3 $\mu\text{g} \cdot \text{min} \cdot \text{ml}^{-1}$ in the rifampin group. The mean \bar{f}_u ($\bar{f}_u = \text{AUC}_m / \text{AUC}_t$) in the rifampin-pretreated dogs was only one third the value of that in the control dogs (0.24 ± 0.07 vs. 0.080 ± 0.030 ; $P < 0.001$). There was a statistically significant inverse correlation between AAG concentration and \bar{f}_u ($r = -0.835$, $P < 0.05$).

The time course of lidocaine distribution into the brain and CSF is shown in figures 3 and 4, respectively. Rifampin-pretreated dogs consistently had concentrations of lidocaine in brain that were less than these in the control dogs over the entire 60-min period (fig. 3, top). The lower brain concentrations in the rifampin group occurred in the presence of increased total concentrations of lidocaine in the circulation. This means that rifampin pretreatment resulted in much smaller brain-to-total serum concentration ratios (partition ratio) of lidocaine, illustrated in the center panel of figure 3. There was no overlap between

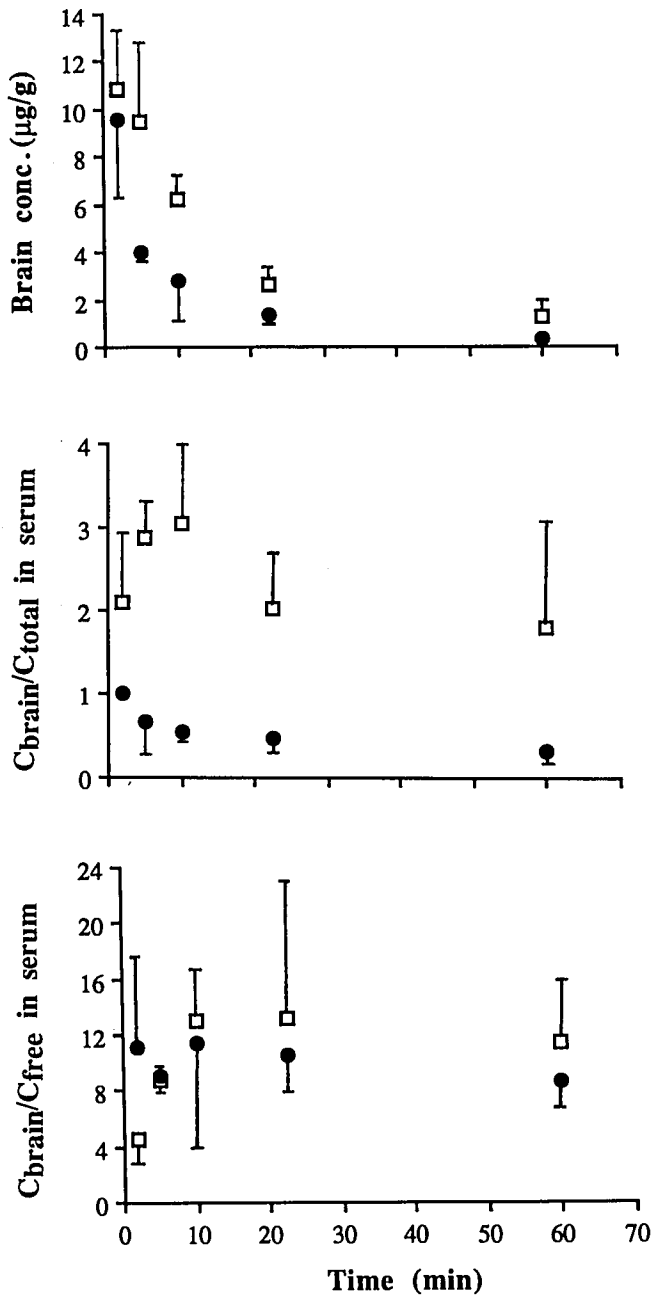


FIG. 3. Time course of lidocaine distribution into the brain of control (□) and rifampin-pretreated (●) dogs. Data are mean \pm SD. *Top*: Absolute brain concentration. *Center*: Partition ratios of brain concentration (C_{brain}) to total serum concentration (C_{total}). *Bottom*: Partition ratios of brain concentration to free serum (C_{free}) concentration.

the two groups in the partition ratios of individual animals at any of the time points. However, when the partitioning of lidocaine between brain tissue and serum is expressed on the basis of free concentrations in serum, there was no longer a significant difference between the two groups (fig. 3, bottom). The same pattern of results was observed in the CSF distribution of lidocaine (fig. 4). In both brain

and CSF, the partitioning ratios based on free serum drug concentration reached a steady value by 10 min after the intravenous injection of lidocaine, indicating a rapid attainment of a distribution equilibrium between drug in the CNS and in circulation.

Another approach to analyzing the influence of serum protein binding on the CNS distribution of lidocaine is

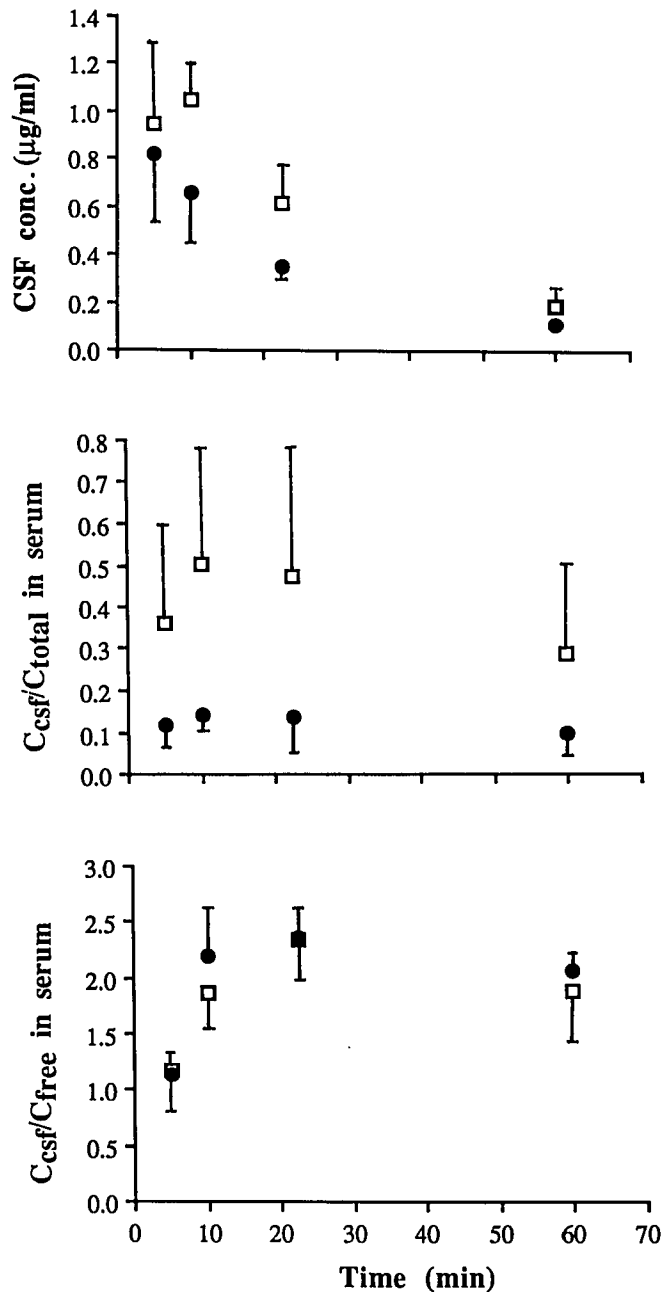


FIG. 4. Time course of lidocaine distribution into the cerebrospinal fluid (CSF) of control (□) and rifampin-pretreated (●) dogs. Data are mean \pm SD. *Top*: Absolute CSF concentration. *Center*: Partition ratios of CSF concentration (C_{csf}) to total serum concentration (C_{total}). *Bottom*: Partition ratios of CSF concentration to free serum concentration (C_{free}).

to correlate the time-averaged partition ratio for brain, represented by the quotient of AUC_{br} and AUC_t (AUC_{br}/AUC_t), with the \bar{f}_u in individual animals. An analogous correlation for the CSF data, *i.e.*, between AUC_{csf}/AUC_t and \bar{f}_u , was also examined. The time-averaged CNS partition ratios based on total drug in serum all were less in the rifampin-pretreated dogs. As shown in figure 5, there was an excellent correlation between AUC_{br}/AUC_t and \bar{f}_u ($r = 0.92, P < 0.005$) and likewise between AUC_{csf}/AUC_t and \bar{f}_u ($r = 0.90, P < 0.01$) when data from control and rifampin groups were pooled.

Discussion

The experimental technique to increase selectively the AAG fraction of serum proteins in the dog was a major consideration in the design of this study. Pretreatment with rifampin offered a reliable means of inducing the hepatic production of AAG without inflicting pain, illness,

or injury upon the animal. The 4-fold increase in serum AAG concentration obtained with rifampin pretreatment was comparable to that observed in many pathophysiologic states such as myocardial infarction, various types of malignancies, and inflammatory diseases.⁹ Also, the 2-week course of treatment with rifampin had no effect on serum albumin.

Another major aspect of the study design was the choice of bolus intravenous administration of lidocaine. One reason for selecting intravenous bolus injection of lidocaine was to investigate the effect of an increase in AAG binding on the initial uptake rate and on the eventual equilibration of lidocaine in the CNS. Because the serum binding of lidocaine was highly concentration-dependent, bolus dosing offered the opportunity to examine the partitioning behavior of lidocaine over a wide range of serum free fractions. Bolus intravenous injection also most closely resembles the effects of accidental vascular administration during attempted regional block, the mode of local anesthetic toxicity of most concern to anesthesiologists.

The results of our study led to the following conclusions: 1) binding of lidocaine to serum AAG restricted the entry of lidocaine into brain tissue and CSF; and 2) the extent of distribution of lidocaine was entirely governed by the equilibrium free fraction of drug in blood or serum. This is mainly supported by the observation that brain and CSF partition ratios, based on total drug in serum, were lower in the rifampin-pretreated dogs than in control dogs, whereas the corresponding ratios based on free drug in serum did not differ between the two groups. In addition, there was an excellent linear correlation between time-averaged partition ratios (based on AUC) and \bar{f}_u .

The earlier study, by Pardridge *et al.*,² showed that the AAG-bound fraction of lidocaine was available for uptake into brain tissue during the first passage of an intracarotid injectate through the cerebral capillaries in the rat. If this had held true in the present study, the partition ratios, based on free-drug concentration in serum, should have been greater in the rifampin-pretreated dogs than in control dogs, because a greater amount of drug was bound to serum AAG in the former group. Also, a curvilinear relationship between AUC_{br}/AUC_t and \bar{f}_u would have been predicted. Such a plot would have a discernible y-intercept (*i.e.*, distribution into the CNS persists even as serum free fraction approaches zero). None of these exceptional features was noted in our data (fig. 5). Therefore, contrary to the findings of Pardridge *et al.*,² the free drug hypothesis appears to be fully operative for the CNS distribution of lidocaine after intravenous bolus administration.

The apparent discrepancy in the conclusions of our study and that of Pardridge *et al.*² is probably related to differences in the experimental conditions. An analogous

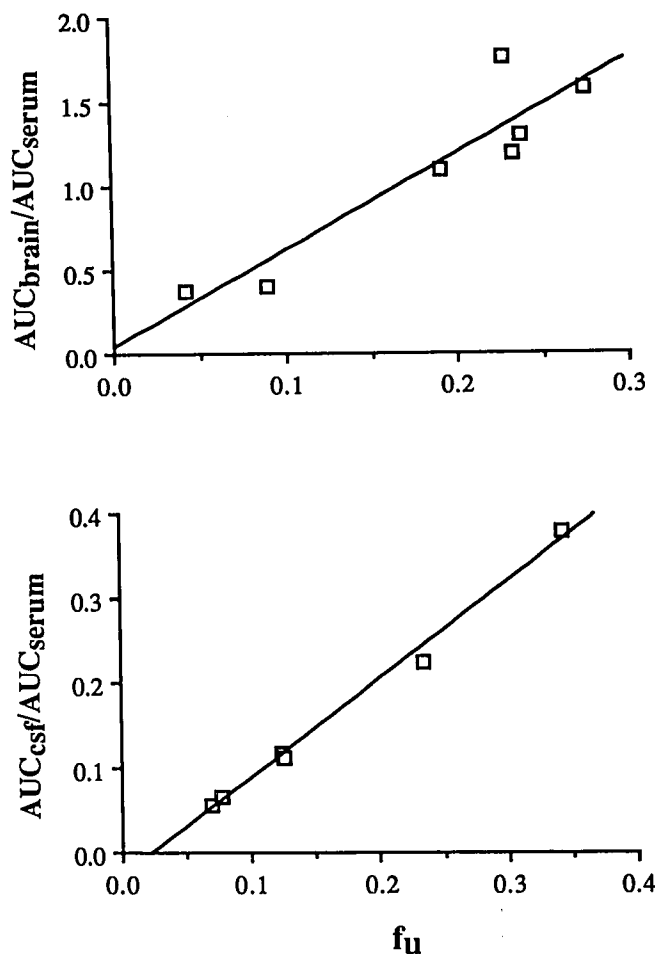


FIG. 5. Correlation of time-averaged partition ratio for brain (*top*) and cerebrospinal fluid (*bottom*) based on area under concentration time (AUC) data with the time-averaged serum free fraction (\bar{f}_u).

situation has been reported recently for diazepam.¹⁰ Using the intracarotid injection technique, Jones *et al.*¹⁰ found that in the presence of serum albumin, the effective exchangeable fraction of diazepam in the cerebral capillaries during single-pass uptake by rat brain was greater than the *in vitro* equilibrium free fraction. However, the involvement of the albumin-bound drug pool was not confirmed in a subsequent steady-state brain distribution study by Dubey *et al.*¹¹ Specifically, they found an excellent agreement between steady-state diazepam concentration in the brain interstitial fluid as measured by a microdialysis probe and free concentration determined either *in vivo* by placement of the probe in the vena cava or *in vitro* by equilibrium dialysis. This was true in both normal and genetically analbuminemic rats. Dubey *et al.* concluded that the earlier findings by Jones *et al.*¹⁰ of an apparent uptake of the albumin-bound fraction of diazepam reflected a nonequilibrium phenomenon.¹¹ Although our study was not carried out under steady-state conditions, pseudoequilibrium in distribution was achieved between the CNS and serum within 10 min after intravenous bolus administration; partition ratios between serum and brain and between serum and CSF reached a steady value by 10 min after lidocaine administration.

The assumption of the free drug hypothesis implies that the clinical effects of lidocaine and other local anesthetic agents should be correlated with free rather than total concentration in serum. This prediction is certainly supported by the observations of Denson *et al.*¹² of bupivacaine CNS toxicity in patients with terminal cancer pain. Despite the very high total serum bupivacaine concentration observed in this group of patients ($> 3 \mu\text{g/ml}$), CNS toxicity was not uniformly evident. When free concentration of serum bupivacaine was measured, the distinction between symptomatic and asymptomatic patients became clear in that the former group had free concentrations exceeding $0.25 \mu\text{g/ml}$, whereas all patients in the latter group had free concentrations well below $0.25 \mu\text{g/ml}$. De Rick *et al.*¹² also showed, in an epinephrine-induced ventricular ectopy model in the dog, that the antiarrhythmic effect of lidocaine correlates significantly with the steady-state free concentrations of lidocaine in plasma and not with the total plasma drug concentrations. It appears that the restrictive effect of serum protein binding also extends to the myocardial distribution of xylydide anesthetics.

In summary, serum protein binding is the principal determinant of CNS distribution of lidocaine. The existing clinical literature concerning local anesthetic toxicity is based upon the measurement of total, rather than free, local anesthetic concentration, with few exceptions. Because AAG concentration is highly variable, total local anesthetic concentration cannot be expected to predict free concentration accurately. Therefore, any observed

relationships between total concentration and pharmacologic effects or toxicity may be misleading. Free rather than total local anesthetic concentration should be considered whenever circulating local anesthetic concentrations are measured.

Appendix

SERUM PROTEIN BINDING MODEL

Binding of lidocaine to dog serum protein was markedly concentration-dependent (fig. 1). Serum free fraction of lidocaine varied from 0.2 to 0.6 over the expected drug concentration range of 0.1–10 $\mu\text{g/ml}$ after intravenous administration of a 3-mg/kg dose of the drug. In this instance, direct *ex vivo* determination of free fraction in serum samples containing low concentrations of lidocaine ($< 1.0 \mu\text{g/ml}$) posed a technical problem. Even the addition of the minimum amount of radiolabeled lidocaine needed for radioactivity assay ($\sim 0.28 \mu\text{g/ml}$) would increase the total lidocaine concentration in serum sufficiently to change the equilibrium free fraction. Moreover, a dilution effect is inevitably introduced by dialysis in that the total drug concentration in the serum compartment after dialysis is less than that in the original sample. Therefore, the measured post-dialysis free fraction would always be lower than actual (predialysis) free fraction. For this reason, *in vitro* binding measurements were performed in blank serum collected from each dog at lidocaine concentrations covering the range of total serum concentrations observed in each dog after the 3 mg/kg intravenous bolus of lidocaine. The *in vivo* equilibrium free fraction for each serum sample was then predicted from the total drug concentration measurement obtained from the gas-chromatographic assay, using a set of binding parameters generated from the *in vitro* binding data.

Equilibrium dialysis was performed in 0.5-ml Plexiglas cells. Spectrapore 2 cellophane membrane (Spectrum Medical Industries, Los Angeles, CA) with a molecular-weight cutoff between 12,000 and 14,000 d was placed between the two halves of each cell. The membrane was prepared by soaking in distilled water for several hours with frequent rinsing, followed by soaking overnight in a phosphate buffer (0.134 M, pH 7.4) at 4° C prior to use. One half of the dialysis cell was filled with 0.25 ml of the phosphate buffer containing a known concentration of lidocaine, and the other half was filled with 0.25 ml blank dog serum preadjusted to pH 7.4. ¹⁴C-lidocaine (NEN, Du Pont Co.—Biotechnology System, Wilmington, DE; specific activity 55.0 mCi/mmol, purity 96%), 3.6×10^4 dpm or 70 ng, was added to the buffer side. The cells were rotated at 15 rpm in a 37° C water bath. Upon achieving equilibrium (3 h), 0.1-ml aliquots of the serum and buffer dialysate were mixed with 5 ml scintillation fluid (Ready Safe, Beckman Instruments, Fullerton, CA), and radioactivity was counted in a Packard 2000 CA Tri-Carb liquid scintillation counter.

Serum free fraction (f_u) was calculated from the ratio of radioactivity in buffer to serum. Total concentration (C_t) of lidocaine on the serum side at dialysis equilibrium was calculated by the specific activity method of Giacomini *et al.*¹³ Given the values of f_u and C_t , unbound (C_u) and bound (C_b) concentrations of lidocaine were calculated. The C_b and C_u data for each dog were

TABLE A1. Protein Content and *In Vitro* Binding Parameters for Lidocaine in Serum from Control and Rifampin-pretreated Dogs

| Dog | AAG (mg/ml) | Albumin (g/dl) | B _{max} (μg/ml) | K _d (μg/ml) | k |
|------------------------------------|-------------|----------------|--------------------------|------------------------|-------|
| Control | | | | | |
| 1 | 0.643 | 2.43 | 1.60 | 0.343 | 0.360 |
| 2 | 0.578 | 2.08 | 2.11 | 0.256 | 0.313 |
| 3 | 0.659 | 2.89 | 2.24 | 0.285 | 0.350 |
| 4 | 0.844 | 2.41 | 3.46 | 0.293 | 0.380 |
| 5 | 0.494 | 2.39 | 1.17 | 0.288 | 0.362 |
| 6 | 0.990 | 2.24 | 4.49 | 0.259 | 0.378 |
| 7 | 0.569 | 2.00 | 2.23 | 0.298 | 0.272 |
| Mean | 0.682 | 2.35 | 2.47 | 0.289 | 0.345 |
| SD | 0.174 | 0.29 | 1.14 | 0.029 | 0.039 |
| Rifampin-pretreated | | | | | |
| 8 | 3.68 | 2.27 | 13.13 | 0.324 | 0.610 |
| 9 | 1.10 | 2.26 | 5.52 | 0.411 | —* |
| 10 | 2.99 | 2.24 | 12.92 | 0.449 | —* |
| 11 | 3.73 | 2.08 | 16.26 | 0.358 | —* |
| 12† | 0.632 | 2.24 | 2.18 | 0.267 | 0.289 |
| 13 | 1.85 | 1.75 | 8.07 | 0.424 | 0.203 |
| Mean | 2.67 | 2.12 | 11.2 | 0.393 | 0.367 |
| SD | 1.16 | 0.22 | 4.31 | 0.051 | 0.215 |
| <i>P</i> value compared to control | 0.019 | >0.05 | 0.012 | 0.0091 | >0.05 |

B_{max} = maximum binding capacity; K_d = dissociation constants of the saturable site; k = linear binding constant for the nonsaturable site.

* Linear (low-affinity) binding site is absent.

† Dog 12 not included in the mean.

fitted to either a one-site or two-site binding model equation using the BMDP nonlinear regression program.

$$C_b = B_{max} \cdot C_u / (K_d + C_u) \quad (1)$$

$$C_b = B_{max} \cdot C_u / (K_d + C_u) + kC_u \quad (2)$$

where B_{max} = maximum binding capacity for the saturable site, expressed in milligrams of protein-bound lidocaine per milliliter of serum; K_d = equilibrium dissociation constant of the saturable site; and k = linear binding constant for the nonsaturable (*i.e.*, very low affinity) binding site.

Once the serum protein binding parameters were estimated for each dog, free lidocaine concentration corresponding to the measured total drug concentration in each serum sample was predicted by numerical solution of the above equation.¹⁴

In order to evaluate the accuracy of predicted C_u values, equilibrium dialysis was performed on two serum samples from each dog taken at an early and late time point, usually at 5 and 35 min after lidocaine administration. Based on the postdialysis C_b, C_u values predicted from the protein-binding isotherm for that dog were compared to the measured C_u. A plot of measured C_u values versus predicted C_u values was linear with a correlation coefficient of 0.981 (n = 26). There was a slight tendency toward underpredicting the C_u values. However, the slope of the line was 1.06 (95% confidence interval 0.967–1.14), indicating that the overall accuracy in the prediction of C_u values from the fitted binding isotherms was within the expected analytical error of 10%.

The results of the computerized model analyses are summarized in table A1. In all control dogs and in three of the six rifampin-pretreated dogs, a two-site model, comprised of one saturable and one nonsaturable site, was needed to characterize the binding isotherm. For the remaining three rifampin-pre-

treated dogs, only a single saturable binding site was evident. For the remaining three rifampin-pretreated dogs, only a single saturable binding site was evident. Rifampin pretreatment increased the maximum binding capacity (B_{max}) of the saturable site by an average of 4-fold in five of the six dogs. The dog that failed to exhibit an induction of AAG also did not show an increase in B_{max}. The values of B_{max} showed an excellent correlation with the serum concentration of AAG (r = 0.986, P < 0.001), combining data from the control and rifampin-pretreated groups. This suggests that the saturable binding site identified by the model analysis probably represented AAG. Rifampin pretreatment caused a small but statistically significant increase of the K_d from 0.289 ± 0.029 μg/ml to 0.393 ± 0.05 μg/ml (P < 0.01). The exact reason for an apparent change in binding affinity is not understood. However, it is known that serum AAG exhibits considerable microheterogeneity.⁴ Therefore, serum composition of the variant forms of AAG may be altered by rifampin induction. Also, a recent study by Krauss *et al.*¹⁵ suggested that lidocaine binding is dependent on an interaction between AAG and albumin in serum. The apparent change in dissociation constant may have resulted from the shift in the concentration ratio of AAG to albumin after rifampin pretreatment. The estimates of binding constants for the nonsaturable site, which are also listed in table A1, were not statistically different between the two groups.

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