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Research Article

Cimetidine Elimination from the Cerebrospinal Fluid of the Rat

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The major goal of this study was to develop a small animal model that could be used to assess quantitatively the clearance of cimetidine from the cerebrospinal fluid (CSF) under relatively physiologic conditions. In addition, we addressed questions related to the pathways involved in the elimination of cimetidine from the CSF. We administered high and low bolus doses of cimetidine together with inulin, as a marker of bulk flow, into the lateral ventricle of anesthetized rats and sampled CSF from the cisterna magna. Principles of linear pharmacokinetic systems were applied to the data to obtain clearances from the CSF. The clearance of inulin was 2.02 ± 0.22 μl/min, which is in excellent agreement with the CSF production rate of 2.2 μl/min in anesthetized rats. The clearance of cimetidine from the CSF following the administration of a low dose was 11.8 ± 3.1 μl/min, which is in good agreement with the cimetidine CSF clearance in the rat obtained previously in studies using the technique of ventriculocisternal perfusion. A 32% decrease in the CSF clearance of cimetidine ($P < 0.05$) was observed when the high dose was administered, suggesting that CSF elimination is saturable. The clearance of inulin was unaffected by the high dose of cimetidine. This study demonstrates that the technique of lateral ventricle injection and sampling from the cisterna magna is useful in quantitatively assessing the elimination of compounds from the CSF in the rat under relatively physiologic conditions.

KEY WORDS: cimetidine; cerebrospinal fluid; clearance; choroid plexus; pharmacokinetics; transport.

INTRODUCTION

Cimetidine, a histamine H_2-receptor antagonist, is used clinically in the treatment of hypergastric acid secretory disorders (1). Although the drug elicits few serious side effects in normal patients, in selected patient populations the use of cimetidine is associated with an increased incidence of central nervous system (CNS) toxicity (1,2). Patients with hepatic impairment often exhibit signs of CNS toxicity ranging from lethargy to coma which are reversible upon discontinuation of the drug (2). These toxicities have been associated with an elevated concentration of cimetidine in the cerebrospinal fluid (CSF) relative to its concentration in plasma (CSF:P ratio) (3). In normal patients, the CSF:P ratio of cimetidine at steady state is 0.18 and in patients with cirrhosis the ratio is 0.5 (4). Two hypotheses may explain the elevated CSF:P ratio: an enhanced penetration of the drug into the CSF or an impaired elimination from the CSF. Compounds may be eliminated from the CSF by bulk flow of CSF, by simple diffusion, or by transport systems located in the choroid plexus of the brain (Fig. 1) (5). Inhibition of any of these pathways may cause an accumulation of substances in the CSF.

Jonsson *et al.* (6,7) and Ziemniak *et al.* (8) have studied the penetration of cimetidine into the CSF in humans and dogs, respectively. Suzuki *et al.* (9), using a ventriculocisternal perfusion technique, were able to demonstrate that cimetidine may be transported from the CSF to the blood by a saturable transport system. Further studies from this last group have identified the choroid plexus as a site of saturable transport (10).

We wished to test the hypothesis that the elimination of cimetidine from the CSF is impaired in patients with liver disease. To test this hypothesis, it was first necessary to develop an animal model in which the clearance of cimetidine from the CSF could be quantitated under relatively unperturbed conditions. Previously, ventriculocisternal perfusion has been employed to assess clearances of compounds from the CSF (11,12). Although the technique is particularly advantageous in quantitating clearances from the CSF under steady-state conditions, it involves the use of artificial per-

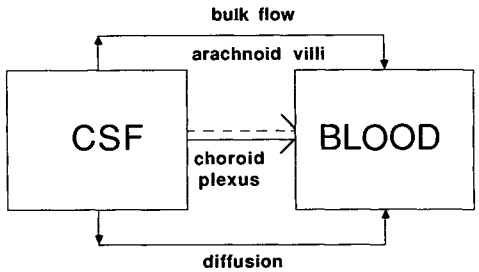

Fig. 1. A schematic drawing of the routes by which a compound may be eliminated from the cerebrospinal fluid (CSF).

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fusates and flow rates which might obscure real changes in the elimination of compounds from the CSF. Such changes may result from the accumulation of endogenous inhibitors in the CSF or alterations in bulk flow of CSF. Accordingly, the major goal of this study was to establish an animal model that could be used to assess quantitatively the clearance of cimetidine from the CSF under relatively physiologic conditions. In addition, we addressed the following questions related to cimetidine CSF elimination: (1) Is cimetidine eliminated from the CSF by pathways in addition to bulk flow? (2) Is cimetidine biotransformed in the CSF? and (3) Does the elimination of cimetidine from the CSF involve a saturable mechanism?

Our study demonstrated that the clearance of cimetidine from the CSF can be accurately quantitated following a single bolus injection of the drug into the lateral ventricle of the brain of a rat and sampling of CSF from the cisterna magna. Using this model, the pathways involved in the elimination of cimetidine from the CSF were characterized.

MATERIALS AND METHODS

Cannula Implantation

Male Sprague–Dawley rats (250–325 g) were anesthetized with an intramuscular dose of a mixture of ketamine (130 mg/kg) and acepromazine (1.6 mg/kg), and their heads were fixed in a stereotaxic device. Methods described by Myers (13) and Bouman and Van Wimersma Greidanus (14) for ventriculocisterna studies were used. Briefly, a midline scalp incision was made from the neck to a point between the eyes and the skull was exposed by removal of the periosteum. Using a Dremble Tool variable-speed drill with a No. 56-size drill bit, a hole was drilled into the frontal bone of the skull for placement of a cannula guide for injection into the lateral ventricle. Coordinates obtained from a stereotaxic atlas of the rat brain (15) were used to locate the lateral ventricle. A second hole was drilled just anterior to the interparietal–occipital bone suture at an angle, so that the occipital bone was used as a guide while lowering a cannula guide into the cisterna magna. Three additional holes were drilled for placement of 0-80 stainless-steel screws (0.031-in. length) to anchor the cannula guides to the skull. The three screws were mounted first and then a stainless-steel cannula guide (6.5-mm length) with a threaded Teflon pedestal was placed into the cisterna magna by hand and the space between cannula and skull was sealed with super glue. A cannula guide was then lowered into the brain to a point just above the right lateral ventricle using the electrode manipulator of the stereotaxic device and super glue was applied as a seal. Dental cement was applied to anchor the cannula guides to the skull and the anchor screws. Screw-capped cannula-dummy wires were screwed into the cannula guides to maintain a closed system, as well as to keep the cannula guides patent. The preparation was equilibrated for 45 min to allow CSF pressure to normalize.

Ventriculocisternal Procedure

Twelve animals were studied. Six animals received a high dose and six animals received a low dose of cimetidine. Five microliters of a solution containing ^{3}H-cimetidine (4–8 ng), ^{14}C-inulin (53–78 μg), a marker of bulk CSF flow, and unlabeled cimetidine (22.7 μg) in mock CSF (127.6 m*M* Na^+, 2.5 m*M* K^+, 1.3 m*M* Ca^{2+}, 1 m*M* Mg^{2+}, and 134.7 m*M* Cl^-, pH 7.33) (5,13) was administered to the six animals in the high-dose group. The six animals in the low-dose group received 5 μl of a solution containing ^{3}H-cimetidine (4–8 ng), ^{14}C-inulin (53–78 μg), and mock CSF without unlabeled cimetidine. Solutions were injected through the cannula guide into the lateral ventricle using a Hamilton microsyringe modified with a piece of stainless-steel tubing which allowed the bevel of the needle to extend only 1.5 mm beyond the length of the cannula guide. Samples of 5 μl were drawn from the cisterna magna, at 0, 2, 5, 10, 20, 40, 60, 90, 120, 150, and 180 min after injection, using another Hamilton microsyringe modified so that the needle tip reached only to the end of the cannula guide. CSF samples were placed into scintillation vials containing 10 ml of aqueous-based scintillant. ^{3}H and ^{14}C were assayed by liquid scintillation counting using dual-isotope monitoring on a Beckman LS7800 scintillation counter. Counting efficiencies for ^{3}H and ^{14}C were approximately 33 and 50%, respectively.

Cimetidine Metabolism Studies

To study cimetidine metabolism in the CSF, 5 μl of a solution containing only ^{3}H-cimetidine (4–8 ng) in mock CSF was injected into the lateral ventricle of a single rat. Five-microliter samples were drawn from the cisterna magna at various times after administration of the drug. Two and one-half microliters of each sample was spotted onto a thin-layer chromatography (TLC) plate previously spotted with about 8 μg of unlabeled cimetidine. The remaining 2.5 μl of each sample was placed in 10 ml of scintillant. The TLC plates were developed in an ethylacetate:methanol:NH_4OH (5:1:1) solvent system, a modification of the assay method described by Bavin *et al.* (16), and the sections associated with cimetidine (R_f = 0.65) and with its sulfoxide metabolite (R_f = 0.40) were scraped. R_f values for cimetidine and cimetidine sulfoxide were empirically determined using pure compounds. The scraped sections were placed into vials containing 1 ml of water and 10 ml of scintillant and the radioactivity in the samples was monitored by single-label scintillation counting.

Data Analysis

To ascertain the clearances of cimetidine (CL_{CIM}) and inulin (CL_{IN}) from CSF we used a model-independent method where CL = *D*/AUC. *D* represents the dose of the compound and AUC represents the area under the CSF concentration versus time curve from time zero to infinity. AUC was calculated from the data by the trapezoidal rule (17) and extrapolated to time infinity by C_z/λ_z, where C_z is the computer-generated estimate of the concentration of the last sample and λ_z is the terminal elimination rate constant. To obtain λ_z the CSF concentration versus time data were fit to a mono- or biexponential equation using DRUGMODEL on the PROPHET computer system. In these studies the extrapolated AUC was less than 20% of the total AUC. Clearances, CL_{IN}, CL_{CIM}, and CL_{CIM^*}, the CSF clearance of cimetidine when the dose of unlabeled drug was administered, were compared statistically by groups. Statistical analysis

for differences between cimetidine and inulin clearances within each group was performed using Student's paired t test. Intergroup differences between CL_{CIM} and CL_{CIM^*} were ascertained using Student's unpaired t test. A P value of less than 0.05 was considered statistically significant.

Materials

Sprague–Dawley rats were obtained from Batin and Kingman Inc., Fremont, Calif. Ketaset and acepromazine were products of Bristol Laboratories, Syracuse, N.Y., and TechAmerica, Elwood, Kans., respectively. The stereotaxic device is a product of David Kopf Instruments, Tujunga, Calif. Stainless-steel screws, cannula guides, and cannula-guide dummy wires were purchased from Plastic Products Co., Inc., Roanoke, Va. QuickGel super glue is a product of Loctite Corporation, Cleveland, Ohio. Dental cement was purchased from Lang Dental Mfg. Co. Inc., Chicago, Ill. ^{3}H-Cimetidine (sp act, 10.6 Ci/mmol) and ^{14}C-inulin (sp act, 6.88 mCi/mmol) were purchased from Amersham Corp., Arlington Heights, Ill. Unlabeled cimetidine was purchased from the Sigma Chemical Company, St. Louis, Mo. Cimetidine sulfoxide was generously provided by Smith Kline & French (Philadelphia, Pa.). The scintillant, ScintiVerse II, and EM Reagents (silica gel 60 precoated, 0.2-mm layer thickness, aluminum backing) TLC support, and all solvents, which were of analytical grade, were purchased from Fisher Scientific Co., Fairlawn, N.J. The scintillation counter used in this study was a Model LS7800, a product of Beckman Instruments, Fullerton, Calif.

RESULTS

Semilogarithmic plots of the CSF concentration of inulin and cimetidine versus time from experiments in a representative rat that received the low dose (A) and in a representative rat that received the high dose (B) are shown in Figure 2. The CSF concentrations of inulin declined in an apparent log-linear fashion in these animals and in the other animals. Peak concentrations of inulin were achieved at about 10 min after injection. Cimetidine concentrations in the CSF declined biexponentially in these animals as well as in the other animals, with peak concentrations occurring at about 5 min.

Figure 3 depicts the AUC of cimetidine obtained in the animals that received a low dose of the drug plotted against dose. AUC increased linearly with dose (r = 0.98; $P <$ 0.001), suggesting that linear pharmacokinetic principles are applicable to this model. The clearance of cimetidine, calculated as the reciprocal of the slope of the regression line, was 10.7 μl/min.

Figure 4A depicts the paired clearances of cimetidine and inulin obtained in the six rats that received a low dose of cimetidine. Cimetidine was cleared significantly faster than inulin (mean ± SD; 11.77 ± 3.13 vs 2.04 ± 0.22 μl/min; P = 0.001). These results suggest that cimetidine may be eliminated from CSF by a route that is supplementary to bulk flow. Figure 4B shows the paired clearances of cimetidine and inulin in the six animals that received a high dose of cimetidine. Again, the clearance of cimetidine was significantly greater than that of inulin (8.04 ± 2.07 vs 2.01 ± 0.23 μl/min; P = 0.001).

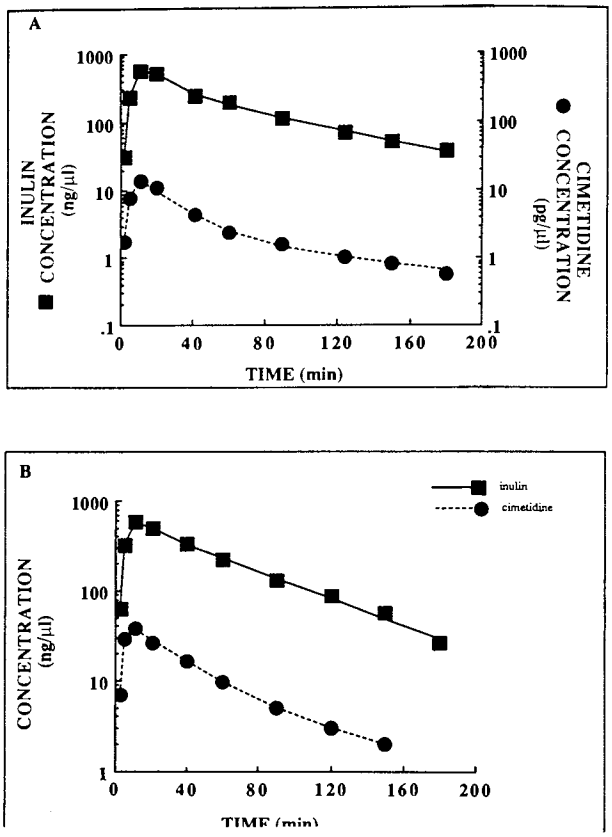

Fig. 2. (A) A plot of inulin (■) and cimetidine (●) concentrations in CSF in a single animal that received inulin and a low dose of cimetidine. Cimetidine and inulin concentrations differ by an order of magnitude as indicated beside figure symbols. (B) A plot of inulin (■) and cimetidine (●) concentrations in CSF in a single animal that received inulin and a high dose of cimetidine. Cimetidine and inulin concentrations are the same order of magnitude.

The clearances of cimetidine in the animals that received high or low doses of cimetidine are shown in Table I. Cimetidine was cleared significantly slower in the animals that received the high dose of cimetidine (8.04 ± 2.07 vs 11.8 ± 3.1 μl/min; $P < 0.05$), whereas inulin clearances were not affected by the high dose of cimetidine (2.04 ±

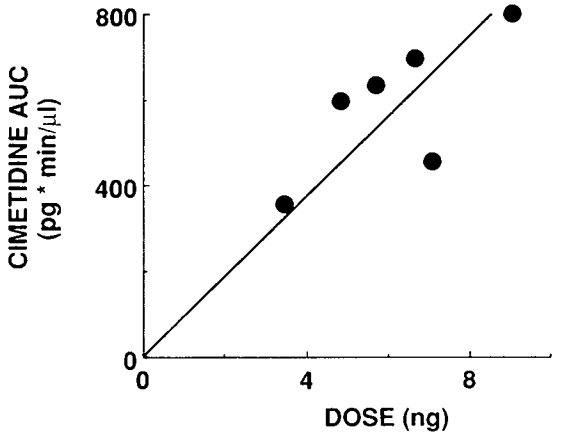

Fig. 3. Plot of area under the curve (AUC) vs dose in the six animals that received inulin and a low dose of cimetidine. The slope of the regression line is 0.0935 min/μl (r=0.98; $P < 0.001$).

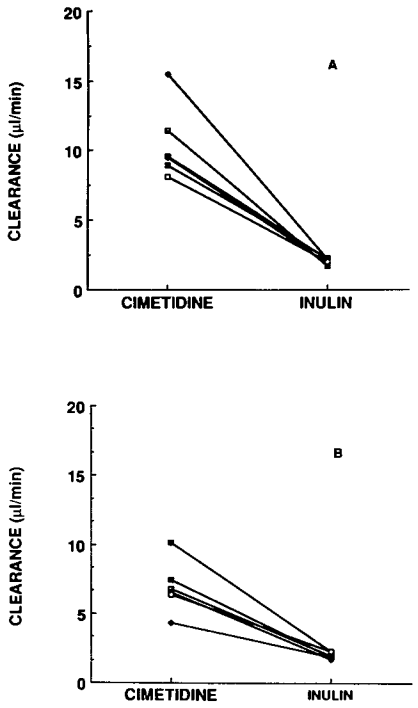

Fig. 4. Plots showing paired relationships between cimetidine and inulin clearances in the six animals that received a low dose of cimetidine (A) and in the six animals that received a high dose of cimetidine (B).

0.22 vs 2.01 ± 0.23 µl/min; $P > 0.05$). These results suggest that at least one route by which cimetidine is eliminated from the CSF is saturable.

The extent of biotransformation of cimetidine to its sulfoxide metabolite in the CSF was studied using thin-layer chromatography. The sulfoxide-to-cimetidine ratio in all animals was very low and was felt to be mostly an artifact of the assay rather than metabolism occurring in the CSF. The AUC of the sulfoxide contributed less than 10% to the AUC calculated from the total radioactivity.

DISCUSSION

An alteration in the elimination of cimetidine from the CSF could be responsible for the increased CSF:P ratios observed in patients with liver disease. Figure 1 illustrates the pathways available for the elimination of compounds from CSF. Any substance that is soluble in CSF will be eliminated with the normal turnover of CSF via bulk flow passage through the arachnoid villi. Additionally, given the correct concentration differential, substances possessing the necessary permeability properties will leave the CSF by simple diffusion. Facilitated or active transport systems located in the choroid plexus epithelium of the ventricles will contribute to the elimination of substances with specific physical and chemical properties (5,18–20).

The major goal of this study was to establish an animal model that could be used to assess quantitatively clearances of compounds from the CSF under relatively unperturbed physiologic conditions. Although the technique of ventriculocisternal perfusion has been widely used to quantitate clearances of compounds from the CSF (5,11), we felt it had

Table I. Cimetidine CSF Clearances in the Rat

Animal number	Dose (ng)	Clearance (µl/min)	Animal number	Dose (µg)	Clearance (µl/min)
1	7.59	10.34	7	17.06	7.90
2	8.19	18.03	8	16.67	7.55
3	7.67	11.15	9	16.62	8.64
4	3.90	11.17	10	4.58	5.22
5	6.54	10.46	11	13.63	11.55
6	5.75	9.46	12	14.48	7.36
Mean[a]		11.77			8.04
SD		3.13			2.07

[a] $P < 0.05$.

particular limitations related to the use of artificial perfusates and flow rates. Accordingly, we used an animal model that involved administering the compound via a bolus injection into the lateral ventricle of an anesthetized rat followed by sampling of CSF from the cisterna magna. Clearance of the compound from the CSF was then determined by applying pharmacokinetic principles of linear systems. This method for ventriculocisternal study of the distribution and elimination of compounds from the CSF has been described previously (21–28). Clearance concepts have also been applied to this method by Levin *et al.* (23,24,26–28), who performed studies of drug distribution and elimination from the CSF in beagle dogs. To date studies in smaller animals have relied solely on half-life determination to assess CSF elimination (21). Clearances have not been determined.

In this study we used three criteria to determine whether clearances could be accurately quantitated in the rat model. First, a basic assumption in using the equation CL = D/AUC is that AUC increases linearly with dose, i.e., the system is linear. In six animals, a range of low doses of cimetidine was used. Accordingly, we plotted AUC versus dose and obtained a good linear relationship (Fig. 4), suggesting that the principles of linearity were valid for this model.

Second, we compared the inulin clearances obtained in this study to the rate of CSF production in the rat. Inulin is a large (MW 5000) polar compound. Elimination of this compound from the CSF is therefore presumed to occur almost exclusively by the bulk flow pathway (21). Presumably, under normal physiologic conditions the rate of CSF production should approximate the rate of bulk flow of CSF through the ventricular system. The mean inulin clearance obtained in this study was 2.04 µl/min, which is in excellent agreement with the value of CSF production in the anesthetized rat of 2.2 µl/min obtained from ventriculocisternal perfusion studies (5,9). A similar relationship between inulin clearance and CSF production rate was observed in the dog (23,24,26–28).

Third, the clearance of cimetidine from the CSF obtained in this study was compared to that calculated from a ventriculocisternal perfusion study by Suzuki *et al.* (9). Using a perfusion flow rate of 17.4 µl/min, they obtained an extraction ratio for cimetidine of 0.354. Since clearance is equal to the product of flow rate and extraction ratio, a cimetidine clearance of 6.2 µl/min can be calculated from their data. This value is in reasonable agreement with the clear-

ance value of 11.8 μl/min obtained in our study, especially when one considers the lower flow rate in our study (2.04 μl/min), which may result in a higher clearance. Interestingly, in both studies there was an approximately 33% reduction in CSF clearance when high concentrations of cimetidine were present.

Thus it appears that application of pharmacokinetic principles of linear systems to data obtained following lateral ventricle injection and sampling from the cisterna magna is useful in quantitatively assessing clearances of cimetidine and inulin from the CSF of the rat. The model has the advantage of allowing CSF clearances to be ascertained under relatively physiologic conditions. Artificial perfusates and flow rates are eliminated.

In addition to establishing a working model to study cimetidine clearance from the CSF of the rat, we wished to address specific questions related to the pathways of elimination of cimetidine from the CSF. Four characteristics of the elimination of cimetidine from the CSF were examined in this study. First, we examined whether cimetidine is eliminated from the CSF by pathways in addition to bulk flow. Since cimetidine and inulin clearances were determined in each animal, it was possible to compare these clearances within animals. In each animal the clearance of cimetidine was significantly greater than that of inulin (Figs. 4A and B). Assuming that inulin is cleared almost exclusively by bulk flow, these data suggest that cimetidine is eliminated from the CSF via a pathway(s) in addition to the bulk flow pathway.

Second, we observed that the clearance of cimetidine, but not of inulin, was significantly decreased after administration of the high dose of cimetidine (Table I), suggesting that cimetidine is eliminated from the CSF by a saturable pathway or pathways. These results are consistent with results from previous studies demonstrating that cimetidine actively accumulates in choroid plexus tissue (10). In this study, we did not ascertain the nature of the transport process, nor did we determine whether one or more transport systems were involved in the elimination of cimetidine from the CSF of the rat. The notion of multiple transport pathways for cimetidine is consistent with the results of Gisclon *et al.* (29) in renal tissue and those of Suzuki *et al.* (10), which demonstrated inhibition of cimetidine uptake into choroid plexus tissue by organic anions, organic cations, and oligopeptides.

After a high dose of cimetidine, the clearance of cimetidine (8.04 ± 2.07 μl/min) was significantly greater than that of inulin (2.04 ± 0.22 μl/min), suggesting that there may be an unsaturable pathway for the elimination of cimetidine from the CSF. Cimetidine (pK_a 6.80) has an oil:water partition coefficient of 2.5 (14) and is about 77% nonionized at the pH of the CSF. The compound might therefore be expected to be eliminated from the CSF via lipophillic diffusion. This pathway could account for the difference between the decreased cimetidine clearance and the clearance of inulin. The disparity can also be explained by assuming that saturation of the transport system was incomplete. Incomplete saturation of cimetidine transport is arguable on the grounds that the concentrations of cimetidine in these experiments may not have been high enough to saturate the transport process completely.

An additional pathway for the elimination of a drug from the CSF is biotransformation (not shown in Fig. 1). We quantitated the major metabolite of cimetidine, cimetidine-sulfoxide, in the CSF and observed only small amounts. Thus, in agreement with Ziemniak *et al.* in dogs (8), our data suggest that there is no significant formation of this metabolite in the CSF of the rat.

In conclusion, the technique of bolus injection into the lateral ventricle and sampling from the cisterna magna used in these experiments provides a physiological method for studying elimination of substances from the CSF of the rat. Application of the pharmacokinetic principles of linear systems to quantitate clearances appears to be valid for cimetidine and inulin. The data suggest that cimetidine is eliminated from the CSF of the rat by pathways in addition to the bulk flow of CSF through the arachnoid villi. A saturable transport process appears to be involved and there is evidence that diffusion of the compound may occur. Further studies are ongoing in our laboratory to determine the nature of the saturable transport process and to address questions related to the elimination of cimetidine from the CSF in disease states.

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