Kinetics of Drug Action in Disease States. XL. Effect of the Dialyzable Component(s) of Uremic Blood on Theophylline Neurotoxicity in Rats

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INTRODUCTION

Diseases can alter the relationship between the concentration and the intensity of action of a drug, i.e., its pharmacodynamics. Early clinical reports suggested that uremic patients are more sensitive to barbiturates than are patients with normal renal function (1). Further, animal studies have shown that the cerebrospinal fluid (CSF) concentration of phenobarbital required to produce loss of righting reflex is lower in rats with renal failure than in rats with normal renal function (2). Contrary to earlier indications (1), the altered pharmacodynamics were not due to accumulation of urea (3) but could be reproduced in normal rats by administration of a serum dialysate of rats with renal failure (4). Because of the role of one or more low molecular weight components in the increased barbiturate susceptibility, the ability of orally administered activated charcoal (5) to reverse the increased sensitivity of renal failure rats to the central nervous system (CNS) depressant action of phenobarbital was tested (6). Theophylline was also studied because its neurotoxicity (convulsant action) was substantially increased in renal failure (7). Indeed, treatment of renal failure rats with activated charcoal produced a significant reduction in the neurotoxicity of theophylline as reflected by a substantial increase in the cerebrospinal fluid concentration of this drug required to produce seizures (6). The modifying effect of activated charcoal administration is thought to be caused by adsorption of low molecular weight blood components that diffuse to the intestinal lumen and that ordinarily would be reabsorbed. Consequently, this investigation was designed to determine the effect of dialyzable components of blood from rats with renal failure, when administered to rats with normal renal function, on the susceptibility of the latter animals to the convulsant action of theophylline.

METHODS

Male, inbred Lewis rats (Charles River Breeding Laboratories, Wilmington, MA), weighing approximately 200 g and maintained on Charles River Rat-Mouse-Hamster Formula, were used in this investigation. They had indwelling cannulas implanted in the right jugular vein and left femoral vein under light ether anesthesia, 1 day before the pharmacodynamic study.

Male Wistar rats, weighing about 400 g, were used as donors of uremic blood. Acute renal failure was produced by bilateral ligation of ureters (two tight ligatures around each ureter and the ureters cut between the ligatures) under light ether anesthesia. Forty-eight hours later, the blood was withdrawn from the aorta of these animals. Serum was separated and the urea nitrogen concentration in serum of each rat was measured to confirm uremia. Similarly, blood was obtained from untreated rats that served as donors for one of the control groups. The individual sera from the uremic and normal donor animals were then combined into a uremic and a normal serum pool, respectively. Portions of these combined sera were placed in dialysis bags made from cellulose tubing of 3.3-cm flat width (Visking; Union Carbide Corp., Chicago, IL), with a molecular exclusion limit of 12,000 to 14,000 daltons. Each bag was immersed in 3.3 vol of water at 4°C (contained in a glass cylinder and stirred with a magnetic stirrer) for 24 hr.

The aqueous phase (dialysate) was then removed and replaced by the same volume of water for another 24 hr. The dialysates were then combined into uremic and normal dialysate pools, respectively, and they were lyophilized (Unitrap II; Virtis Co., Gardiner, NY). The residues were weighed and dissolved in a small volume of distilled water (7.75% of the respective pooled serum volume), the solution was centrifuged to remove undissolved material, and the supernatant was used as the reconstituted dialysate infusion solution.

To determine the effect of the dialyzable components of uremic blood on the concentration of theophylline at onset of maximal seizures, normal rats were placed in individual cages (after having been deprived of food, but not water, overnight) and infused through the femoral vein with either saline solution, reconstituted serum dialysate from normal rats, or reconstituted serum dialysate from rats with renal failure at a rate of 0.0206 ml/min. Fifteen minutes later, an infusion of theophylline (as aminophylline; Sigma Chemical Co., St. Louis, MO, in solution equivalent to 100 mg theophylline/ml) through the jugular vein was started at a rate of 1.03 mg/min and continued until the onset of maximal seizures (8). At that time, samples of CSF, blood (for serum), and brain were obtained, in that order. The concentrations of theophylline were determined by high-performance liquid chromatography after selective extraction (8).

The rectal temperatures were measured before and 15 min after the start of the dialysate infusion. The rats were then placed on isothermal pads to maintain their body temperature during the theophylline infusion and the continuing serum dialysate infusion.

Serum concentrations of creatinine were determined with the Sigma Test Kit 555-A (Sigma Chemical Co., St. Louis, MO) and urea nitrogen concentrations were determined with commercially available reagent solutions (No. 47952 and No. 4754; Pierce Chemical Co., Rockland, IL). The experimental results were analyzed by one-way analysis of variance followed by the Duncan test when differences were noted.

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RESULTS

The compositions of the serum and serum dialysate solutions are shown in Table I. Compared to normal serum, the uremic serum had much higher creatinine and urea nitrogen concentrations. These differences were reflected by the composition of the reconstituted dialysate solutions. The uremic dialysate had a much higher content of solids than the dialysate of serum from normal rats (Table I).

Based on assays of serum from blood obtained at the pharmacologic end point (i.e., after infusion of both serum dialysate and theophylline solution), creatinine and urea nitrogen concentrations were appreciably higher in the group of rats infused with uremic serum dialysate than in the two control groups (Table II). There was no significant difference in the rectal temperature of the three groups, either before or during infusion of normal saline or serum dialysate solutions (Table II).

The results of the theophylline infusion experiment are summarized in Table III. There were no significant differences between the three groups with respect to the total dose of infused theophylline required to produce maximal seizures or the theophylline concentrations in the serum, brain and CSF at the onset of maximal seizures.

DISCUSSION

Renal failure can lead to central nervous system dysfunction (9) and to increased sensitivity to the pharmacologic effects of drugs that act on the central nervous system (1,2,7). Uremic encephalopathy is generally ascribed to the retention and consequent accumulation of certain endogenous substances, the so-called uremic toxins (10) including "middle molecules" (11). Despite extensive and prolonged efforts by many investigators, these substances have not generally been identified and linked causatively to the physiologic perturbations of uremia. Using solvent-specific peritoneal dialysis and electroencephalographic monitoring of nephrectomized rats, Lipman *et al.* (12) recently concluded that uremic encephalopathy in this model is produced by an unknown, dialysable neurotoxin.

The effectiveness of orally administered activated charcoal in decreasing the sensitivity of rats with renal failure to the neurotoxic (convulsant) effect of theophylline suggests the presence of one or more proneurotoxic or theophylline-potentiating substances that diffuse from blood to the gastrointestinal lumen and ordinarily are reabsorbed into the general circulation. Charcoal adsorption appears to decrease the concentration of this material in blood and tissues.

The endogenous material is likely to be of low molecular weight and is probably eliminated in rats with normal renal function largely by renal excretion so that it accumulates in the blood of rats with renal failure. Hence, administration of a dialysate of blood from rats with renal failure to normal rats should increase their sensitivity to the convulsant action of theophylline. Our reasoning was guided by our previous studies on the effect of renal failure on the hypnotic action of phenobarbital (2–4): the sensitivity of normal rats to the hypnotic action of phenobarbital can be increased by an infusion of the concentrated serum dialysate from rats with renal failure (4).

Failure in the present study to alter the neurotoxicity of theophylline in normal rats by administration of a concentrated serum dialysate from rats with renal failure [prepared in a similar manner as in the phenobarbital study (4)] could be due to several reasons. The "dose" of the relevant endogenous material could have been insufficient. We administered, on average, the dialysate of serum from 4.45 renal failure rats to one normal rat, similar to the dose used in the phenobarbital study (4). As in the phenobarbital study, infu-

Table I. Description of Source and Composition of Serum Dialysate Solutions

Variable	Normal serum dialysate	Uremic serum dialysate
No. of donor rats	66	68
Total volume of serum (ml)	395	305
Serum creatinine (mg/dl)	0.56	8.95
Serum urea nitrogen (mg/dl)	16.0	215
Amount of solids recovered from dialysate (g)	3.70	6.12
Total volume of RDS[a] (ml)	30.6	23.6
Creatinine in RDS (mg/dl)	3.62	36.4
Urea nitrogen in RDS (mg/dl)	145	1939

[a] Reconstituted dialysate solution.

Table II. Description of Male Lewis Rats Used in this Investigation[a]

Characteristics	Infused with: Normal saline	Normal serum dialysate	Uremic serum dialysate
Body weight (g)	201 ± 17	203 ± 5	209 ± 15
Serum creatinine (mg/dl)**	1.07 ± 0.11	0.94 ± 0.14	1.84 ± 0.35*
Serum urea nitrogen (mg/dl)**	19.4 ± 4.2	20.6 ± 2.2	39.9 ± 6.6*
Rectal temperature (°C)			
Before infusion	38.2 ± 0.5	37.8 ± 0.6	38.2 ± 0.7
At 15 min after start of infusion	38.8 ± 0.4	38.4 ± 0.4	38.7 ± 0.7

[a] Results are reported as mean ± SD; $n = 10$ per group.
* Significantly different from the other two groups, $P < 0.05$.
** Significant difference between groups by analysis of variance, $P < 0.001$.

Table III. Effect of Dialyzable Component(s) of Uremic Serum on Theophylline Concentrations at Onset of Maximal Seizures[a]

Variable	Infused with[b]: Normal saline	Normal serum dialysate	Uremic serum dialysate
Infusion time (min)	57.5 ± 4.9	56.4 ± 6.3	59.3 ± 5.9
Total dose (mg/kg)	294 ± 16	276 ± 30	292 ± 14
Serum conc. (mg/L)	407 ± 44	392 ± 23	387 ± 24
Brain conc. (mg/kg)	294 ± 31	284 ± 26	279 ± 21
CSF conc. (mg/L)	242 ± 20	248 ± 16	245 ± 17

[a] Results are reported as mean ± SD; $n = 10$ per group. There were no statistically significant differences between groups.

sion of uremic serum dialysate approximately doubled serum creatinine and urea nitrogen concentrations. Another reason for the lack of effect could be that the relevant endogenous material is unstable and degraded rapidly during the serum dialysis, lyophilization, and/or reconstitution processes. Irrespective of these possibilities, the results of our investigation suggest that the endogenous material responsible for increasing the CNS stimulant action of theophylline and the endogenous material that increases the CNS depressant action of phenobarbital are not the same.

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