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Report

Kinetics of Drug Action in Disease States. XXXIX. Effect of Orally Administered Activated Charcoal on the Hypnotic Activity of Phenobarbital and the Neurotoxicity of Theophylline Administered Intravenously to Rats with Renal Failure

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The central nervous system (CNS) sensitivity to the hypnotic (general anesthetic) action of phenobarbital and to the neurotoxic (convulsive) action of theophylline is greater in rats with acute renal failure than in normal animals, consistent with clinical observations. In the case of phenobarbital, this increased sensitivity can be produced in normal rats by infusion of a solution of the lyophilized dialysate of serum from rats with renal failure. It was hypothesized that the relevant constituent(s) of this dialysate may circulate between the blood and the intestinal lumen and that it (they) can be adsorbed by orally administered activated charcoal and thereby removed from the body. If so, treatment of renal failure rats with activated charcoal should partly reverse the increased CNS sensitivity to phenobarbital and to other drugs similarly affected. Accordingly, rats with renal failure produced by bilateral ligation of ureters were given an aqueous suspension of activated charcoal, about 1 g per kg body weight, orally every 8 hr for six doses. Uremic controls received equal volumes of water. About 2 hr after the last dose, the animals were infused i.v. with phenobarbital to onset of loss of righting reflex or with theophylline to onset of maximal seizures. In the phenobarbital study, charcoal treatment partly reversed the hypothermia associated with renal failure and caused a reduction of creatinine and total bilirubin concentrations in serum. The cerebrospinal fluid (CSF) concentration of phenobarbital at onset of loss of the righting reflex was significantly higher in charcoal treated rats than in their controls. In the theophylline experiment, charcoal treatment had no significant effect on the measured biochemical variables but caused a large increase in the dose and concentrations of theophylline required to produce maximal seizures. In both experiments, administration of activated charcoal caused a reversal of the hyperalgesia associated with renal failure, as determined before drug administration by tail flick latency. These results are consistent with the hypothesis that oral administration of activated charcoal can cause a reduction in the concentration of the circulating endogenous substance(s) that alters the pharmacodynamics of certain drugs in renal failure.

KEY WORDS: phenobarbital; theophylline; renal failure; activated charcoal; pain sensitivity; convulsions; loss of righting reflex.

INTRODUCTION

The hypnotic or general anesthetic action of phenobarbital and the neurotoxicity of theophylline are more pronounced in renal failure. This has been demonstrated rigorously in rats on the basis of lower than normal drug concentrations in the cerebrospinal fluid (CSF) at onset of loss of righting reflex produced by an i.v. infusion of the barbiturate (1) and at onset of maximal seizures upon infusion of theophylline (2). These results are consistent with clinical observations, namely a reduced induction dose requirement for thiopental anesthesia in uremic patients (3) and a higher incidence of seizures in theophylline-overdosed patients with impaired renal function (4).

The blood of rats with renal failure contains apparently elevated concentrations of one or more dialyzable substances that, when administered to normal rats, increase their sensitivity to the central nervous system (CNS) depressant action of phenobarbital (5). If this material can diffuse, like many drugs, from the blood into the lumen of the gastrointestinal tract and from there back into the circulation, then an interruption of this cycle should enhance the elimination of this material from the body and thereby reverse, at least in part, the increased CNS sensitivity to the barbiturate in renal failure. It has been found that repeated oral administration of activated charcoal, an effective adsorbent, can

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increase substantially the systemic clearance of certain intravenously administered drugs, including phenobarbital (6) and theophylline (7) in man and in rats (8). Consequently, a similar maneuver appeared promising as a means for reducing the concentration of the endogenous, dialyzable, barbiturate-potentiating material in the blood and tissues of rats with renal failure.

This investigation was designed to determine the effect of repeated oral administration of activated charcoal on the concentrations of phenobarbital and theophylline in the serum, brain, and CSF of rats with experimental renal failure at onset of loss of righting reflex and of maximal convulsions, respectively. Inasmuch as renal failure is often associated with hypothermia which is reversible upon hemodialysis (9), indicating a causative role of the dialyzable substance(s), the effect of treatment with activated charcoal on body temperature was also assessed. Finally, the effect of charcoal treatment on the experimental pain threshold was determined because the pain sensitivity of rats is increased during severe renal dysfunction or renal failure (10).

METHODS

Male Lewis rats (LEW/CrlBR, Charles River Breeding Laboratories, Wilmington, MA), weighing approximately 190 g when received and maintained on Charles River Rat-Mouse-Hamster Formula, were used in this investigation. The animals underwent surgery for bilateral ligation of ureters (11) and implantation of an indwelling cannula in the right jugular vein (12) under ether anesthesia. The animals were then housed individually in wire-mesh cages, with food and water freely available, in a temperature-, light-, and humidity-controlled room. During the 48 hr after surgery, the rats received 200 mg activated charcoal (SuperChar, Gulf Bio-Systems Inc., Dallas, TX) in water (total volume 1 ml) every 8 hr for six doses by stomach tube under very light ether anesthesia. Control rats received only water according to the same schedule.

Shortly after administration of the last dose of charcoal suspension or water in the morning of the second day after ureter ligation, the rats were tested for pain sensitivity by the tail flick procedure (13). Briefly, the animals were placed in Plexiglas holders and 1 hr later the distal third of the tail was immersed in water at 49 ± 0.05°C. The time of occurrence of a characteristic contraction of the tail into a circle was determined and this determination was repeated 30 and 60 min later. The three results were averaged to determine tail flick latency.

After the last tail flick test, the rats were removed from the Plexiglas holder and their rectal temperature was determined. Sodium phenobarbital (40 mg/ml in distilled water) was infused into the jugular vein at a rate of 0.0206 ml/min until the onset of loss of righting reflex, which was determined without the application of a nociceptive stimulus. CSF, blood (for serum), and brain were obtained at that time under ether anesthesia (14) and were assayed later for phenobarbital by HPLC (15). Toward the end of the infusion, when continued monitoring indicated a decrease of body temperature, the rats were placed on isothermal pads to maintain normal body temperature. After the experiment, the rats were dissected and inspected for gross abnormalities (which were not found).

Another group of rats (obtained in a separate shipment about 2 weeks after the first group) received an intravenous infusion of theophylline (as aminophylline, 100 mg/ml distilled water) at a rate of 1.03 mg theophylline/min until the onset of maximal seizures (16). Unless they had already expired, the rats were immediately anesthetized with ether at that time and CSF, blood, and brain were obtained for subsequent determination of theophylline concentrations by HPLC after selective extraction (16) and serum protein binding measurements.

Serum protein binding of the drugs was determined by equilibrium dialysis at 37°C against an equal volume of 0.13 *M* sodium and potassium phosphate buffer solution, pH 7.4, containing phenobarbital, 200 mg/L, or theophylline, 270 mg/ml. The dialysis was performed for 6 hr (phenobarbital) or 3 hr (theophylline) in Plexiglas cells separated by a cellulose membrane with a molecular exclusion limit of 12,000 to 14,000 daltons (Visking, Union Carbide, New York).

Biochemical measurements of serum constituents were performed by standard procedures as previously described (1). Cholesterol concentration in serum was measured with a commercial kit (No. 352-20, Sigma Chemical Co., St. Louis, MO).

The results of the investigation were analyzed statistically by the unpaired Student's *t* test. The Mann–Whitney test was used in case of heteroscedasticity.

RESULTS

The rats used in the phenobarbital experiment are described in Table I. They were hypothermic before being placed on heating pads, and their serum creatinine and urea nitrogen concentrations were elevated above the usual corresponding concentrations in animals with normal renal function (1,2). Treatment with activated charcoal was associated with a small but statistically significant increase in body temperature and decrease in serum creatinine concentration and a significant decrease in serum total bilirubin concentration.

The serum, brain, and CSF concentrations of phenobarbital at the onset of loss of righting reflex are summarized in Table II. The CSF concentrations were significantly higher in charcoal-treated than in untreated rats with renal failure. There was a statistically significant correlation between the CSF concentration of phenobarbital and the rectal temperature of charcoal-treated and control rats taken together ($r = 0.43$, $n = 21$, $P < 0.001$). Mean concentrations of total (free and bound) phenobarbital in serum and brain were also higher in charcoal-treated than in untreated animals but these differences were not statistically significant. Serum protein binding of phenobarbital was not significantly altered by charcoal administration (Table II). The CSF concentration of phenobarbital in untreated normal rats under similar experimental conditions is about 80 mg/L (17), i.e. almost twice that of untreated rats with renal failure.

The rats used in the theophylline experiment were somewhat larger than those used in the phenobarbital study but otherwise exhibited similar biochemical characteristics

Table 1. Description of Male Lewis Rats Used to Study the Effect of Activated Charcoal on the Pharmacodynamics of Phenobarbital in Renal Failure[a]

Characteristics	No treatment (controls)	Charcoal treatment
No. of animals	10	13
Body weight (g)		
Before ureter ligation	191 ± 12	191 ± 10
After ureter ligation	201 ± 16	194 ± 12
Body temperature (°C)	32.8 ± 1.0	33.6 ± 0.9*
Hematocrit (%)	37.6 ± 3.5	35.2 ± 5.9
Serum creatinine (mg/dl)	5.91 ± 0.90	5.24 ± 0.41*
Serum urea nitrogen (mg/dl)	153 ± 16	149 ± 14
Serum alanine aminotransferase (IU/L)	21.0 ± 7.2	26.4 ± 13.1
Serum aspartate aminotransferase (IU/L)	64.3 ± 14.1	62.9 ± 23.8
Serum total bilirubin (mg/dl)	0.193 ± 0.063	0.138 ± 0.034*
Serum total protein (g/L)	66.5 ± 7.6	59.7 ± 8.8
Serum total cholesterol (mg/dl)	90.7 ± 10.1	88.3 ± 14.8

[a] The rats received 200 mg activated charcoal in water or only water by gavage every 8 hr for six doses. Results are reported as mean ± SD.
* Significantly different from controls by unpaired t test, $P < 0.025$.

(Table III). However, treatment with activated charcoal had no apparent effect on any of the measured characteristics, including body temperature and serum creatinine and serum bilirubin concentrations.

Renal failure rats treated with activated charcoal required a significantly larger dose and substantially higher concentrations of theophylline in serum, serum water (i.e., free drug), brain, and CSF to produce maximal seizures than did untreated rats with renal failure (Table IV). The serum protein binding of theophylline was significantly increased by charcoal treatment (Table IV). The CSF concentration of theophylline in untreated normal rats under similar experimental conditions is about 230 mg/L, i.e., about 70% higher than in the untreated rats with renal failure (18).

Rats used in the phenobarbital and theophylline studies had similar tail flick latency times when tested before administration of these drugs (Table V). Treatment with activated charcoal caused a small but statistically significant increase in latency, indicative of a decrease in their sensitivity to pain.

Table II. Effect of Orally Administered Activated Charcoal on Concentrations of Intravenously Infused Phenobarbital at Onset of Loss of Righting Reflex in Rats with Acute Renal Failure[a]

Variable	No treatment (controls)	Charcoal treatment
No. of animals[b]	10	12
Infusion time (min)	20.7 ± 2.1	22.0 ± 3.2
Dose (mg/kg)	85.9 ± 13.7	93.4 ± 13.1
Serum concentration (mg/L)		
Total drug	109 ± 20	115 ± 15
Free drug	79.8 ± 14.9	80.5 ± 12.4
Brain concentration (mg/kg)	50.2 ± 9.1	58.2 ± 12.7
CSF concentration (mg/L)	44.1 ± 6.1	49.8 ± 5.5 (11)*
Serum free fraction × 100	73.0 ± 2.4	69.8 ± 5.2

[a] Results are reported as mean ± SD.
[b] Unless stated otherwise in parentheses.
[c] Significantly different from controls, $P < 0.04$. CSF collection was unsuccessful in one rat.

DISCUSSION

Activated charcoal treatment was used in this investigation not as an antidote for the inhibition of drug absorption from the gastrointestinal tract or for enhancing the systemic elimination of drugs by gastrointestinal dialysis (19) but rather as an investigational tool to interrupt (if possible) the cycling of certain dialyzable endogenous substances between blood and gut lumen by adsorbing these substances and causing their elimination from the body by fecal excretion. In this context, the magnitude of the elicited effects is of less concern than the occurrence of effects and their direction. The usual dose of activated charcoal as an adsorbent in adult humans and in children is about 1 g/kg, and the same dose was used in this investigation.

Several of the physiologic perturbations associated with renal failure, including hypothermia (9), decreased drug-protein binding (20,21), impaired intestinal transport of butyric acid (22), decreased presystemic elimination of propranolol (23), and increased CNS sensitivity to barbiturates (1), appear to be due to the accumulation of dialyzable endogenous substances. Some pathophysiologic effects of uremia are thought to be caused by the accumulation of endogenous substances of 300 to 1500 daltons classified as middle molecules (24). It is likely that many of these materials can diffuse from the blood into the intestine and back. If this cycle can be interrupted by oral administration of activated charcoal and the systemic concentrations of some of these substances can thereby be decreased, then certain of the cited physiologic perturbations should be partly reversible by charcoal treatment. In fact, sorbents including activated charcoal have been used as adjunctive therapy in renal failure (25).

The partial reversal of hypothermia and the slight lowering of serum creatinine and bilirubin concentrations by

Table III. Description of Male Lewis Rats Used to Study the Effect of Activated Charcoal on the Neurotoxicity of Theophylline in Acute Renal Failure[a]

Characteristics	No treatment (controls)	Charcoal treatment
No. of animals	10	15
Body weight (g)	222 ± 18	217 ± 20
Body temperature (°C)	34.1 ± 1.0	34.5 ± 0.8
Hematocrit (%)	40.1 ± 6.6	43.8 ± 4.5
Serum creatinine (mg/dl)	7.24 ± 1.16	7.51 ± 1.67
Serum urea nitrogen (mg/dl)	141 ± 13	132 ± 14
Serum total bilirubin (mg/dl)	0.166 ± 0.056	0.156 ± 0.025
Serum total protein (g/L)	54.3 ± 5.3	58.3 ± 6.6
Serum total cholesterol (mg/dl)	102 ± 18	113 ± 21

[a] The rats received 200 mg activated charcoal in water or only water by gavage every 8 hr for six doses. Results are reported as mean ± SD.

charcoal treatment in one of the two experiments suggest some enhanced elimination of endogenous substances via gastrointestinal dialysis in the uremic rats, albeit too little to be of physiologic importance. However, the direction of these changes is consistent. Moreover, there was also a significant reversal of the hyperalgesia caused by renal failure, an effect that suggests a causative role for the endogenous substance(s) that accumulates in renal failure and circulates between blood and gut. Cholesterol determinations were made as a supplemental part of this study to determine if charcoal has a hypocholesterolemic effect under the experimental conditions. It did not.

There is a disequilibrium of phenobarbital concentrations between serum or brain and its site of action in normal rats during drug infusion but not between CSF and the site of action (15). Assessments of the pharmacodynamics of phenobarbital in rats should therefore be focused on drug concentrations in the CSF (with the added advantage that these concentrations are those of unbound drug). The equilibrium of phenobarbital between CSF and the site of action is slower in renal failure but the possible bias introduced by this alteration is in a direction opposite to the phenobarbital concentration difference found in the present investigation (1). The partial reversal by charcoal treatment of the renal failure-associated increased sensitivity of rats to the hypnotic action of phenobarbital suggests that the endogenous dialyzable substance(s) responsible for this effect circulates between the blood and the lumen of the gastrointestinal tract.

Charcoal treatment had a striking effect on the neurotoxicity of theophylline. The CSF concentration of this methylxanthine causing convulsions is substantially lower in rats with renal failure than in normal rats (2). Activated charcoal reversed this alteration appreciably, consistent with the hypothesis that it may be caused or facilitated by an endogenous substance that circulates between the blood and the intestinal lumen. There is no evidence to suggest that this material is either identical to or different from that which causes increased sensitivity to the hypnotic action of phenobarbital in renal failure. Studies now in progress will address this issue.

Activated charcoal administration appears to be an effective treatment for theophylline intoxication in man (26–28). The most serious and often life-threatening clinical manifestation of theophylline intoxication is convulsions (16). Patients with impaired renal function appear to be particularly at risk (4). Considering the results of the present investigation, activated charcoal may not only inhibit the absorption and accelerate the elimination of theophylline from the body but also decrease the sensitivity of these patients to the neurotoxic effect of the drug.

The results of our pain threshold measurements suggest that the increased pain sensitivity of rats with renal failure is caused by the systemic accumulation of an endogenous sub-

Table IV. Effect of Orally Administered Activated Charcoal on Concentrations of Intravenously Infused Theophylline at Onset of Maximal Seizures in Rats with Acute Renal Failure[a]

Variable	No treatment (controls)	Charcoal treatment
Infusion time (min)	34.4 ± 12.7	47.8 ± 9.7***
Dose (mg/kg)	160 ± 58	227 ± 47***
Serum concentration (mg/L)		
Total drug	239 ± 68	316 ± 48***
Free drug	205 ± 61	249 ± 43*
Brain concentration (mg/kg)	163 ± 60	206 ± 37*
CSF concentration (mg/L)	137 ± 58	189 ± 38**
Serum free fraction × 100	82.6 ± 2.4	78.8 ± 3.8**

[a] Results are reported as mean ± SD; n as in Table III.
* Significantly different from controls, $P < 0.05$.
** Significantly different from controls, $P < 0.02$.
*** Significantly different from controls, $P < 0.005$.

Table V. Effect of Orally Administered Activated Charcoal on Tail Flick Latency of Rats with Acute Renal Failure[a]

Experiment	No treatment (controls)	Charcoal treatment
Phenobarbital	3.73 ± 0.36	4.53 ± 0.77*
Theophylline	3.39 ± 0.39	4.01 ± 0.48*

[a] The tail was immersed in water at 49°C shortly before administration of either phenobarbital or theophylline. Latency is expressed as seconds. Results are mean ± SD; n = 10 to 15.
* Significantly different from controls, $P \leq 0.006$.

stance(s) and that this hyperalgesia can be reversed, at least in part, by oral administration of activated charcoal, which presumably interrupts the cycling of the endogenous substance(s) between blood and the gastrointestinal tract and thereby lowers the systemic concentration(s).

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