Pharmacology Biochemistry & Behavior, Vol. 11, pp. 359–361. Printed in the U.S.A.

Serum Phenobarbital and Barbital Concentrations in Rats on a Limited Food Regimen[1]

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Received 11 June 1979

TANG, M., C. E. LAU AND J. L. FALK. *Serum phenobarbital and barbital concentrations in rats on a limited food regimen.* PHARMAC. BIOCHEM. BEHAV. 11(3) 359–361, 1979.—Serum barbiturate levels were tracked for several hours in food deprived rats given 40, 60 or 80 mg/kg (SC) of either phenobarbital or barbital. All 3 phenobarbital doses produced peak serum drug levels at 1.5 hr postinjection. Serum barbital concentrations peaked 3 hr after both 60 and 80 mg/kg barbital doses while the 40 mg/kg dose produced a stable low drug level throughout the 4 postinjection hours. For both barbiturates administered, 40 mg/kg doses produced lower serum drug levels than either 60 or 80 mg/kg. The differences in serum drug concentrations between the latter two dose levels were inconsistent over time.

Barbiturate serum level Barbital Phenobarbital Effect of food deprivation

Chronic studies in behavioral pharmacology often require that repeated drug doses be given to animals maintained on limited food rations. With the advent of microanalytic technology, it is now possible to track serum drug concentrations over time in particular animals. Furthermore, repeated sampling is desirable when animals are under a chronic dosing schedule, such as in drug tolerance studies, to determine if a slowly eliminated drug is accumulating in the blood.

In the present study, rats were reduced to 80% of their free-feeding body weights by limiting daily food rations and their serum barbiturate levels were tracked for a few hours. These data were gathered in order to determine, in weight-reduced animals, the best postdrug injection times at which to start behavior sampling in terms of peak serum barbiturate levels.

METHOD

Animals

Eight, male, albino rats of the Holtzman strain were used. Mean body weight was 372 g at the start of the experiment. They were housed in individual Acme stainless steel cages in a temperature-controlled room with a 12-on-12-off lighting cycle (7 a.m.–7 p.m. on). The food used was Purina Laboratory Chow, pelleted, and water was available continuously.

Drugs

Injection solutions for both sodium phenobarbital (J. T. Baker Chemical Co.) and sodium barbital (Sigma Chemical Co.) were prepared by dissolving 60 mg of the sodium salt in 1 ml of isotonic saline. All injections were administered subcutaneously (SC) into the loose skin at the back of the neck and were never greater than 1 ml in volume. Solutions were prepared immediately before each injection.

Procedure

The 8 animals, randomly selected from the rat colony, were assigned to two barbiturate groups: phenobarbital and barbital (N=4 each). Animals in both groups were reduced slowly to 80% of their free-feeding body weights by limiting food rations (2–3 weeks) and maintained at that weight level for the duration of the experiment.

At 9:00 a.m. each day, animals were weighed and food rations necessary to maintain each at 80% body weight were given. After the animals had remained in this food deprivation condition for at least 3 months, a series of barbiturate injections was administered. Doses of 40, 60 and 80 mg/kg of sodium phenobarbital were given to animals in the phenobarbital group while the same doses of barbital were administered to animals in the barbital group. On injection days, animals were injected immediately following body weight determinations. Tail blood samples were obtained at 1, 1.5, 2, 3, or 4 hr postinjection, and food rations were given immediately following the last blood sampling. The dose level for a particular injection was selected randomly and in cases where more than one injection at the same dose level was given to the same animal the mean was used as the value for that animal. Injections were separated by at least 10 days.

[1]Supported by PHS grants DA1110 and AA00253.
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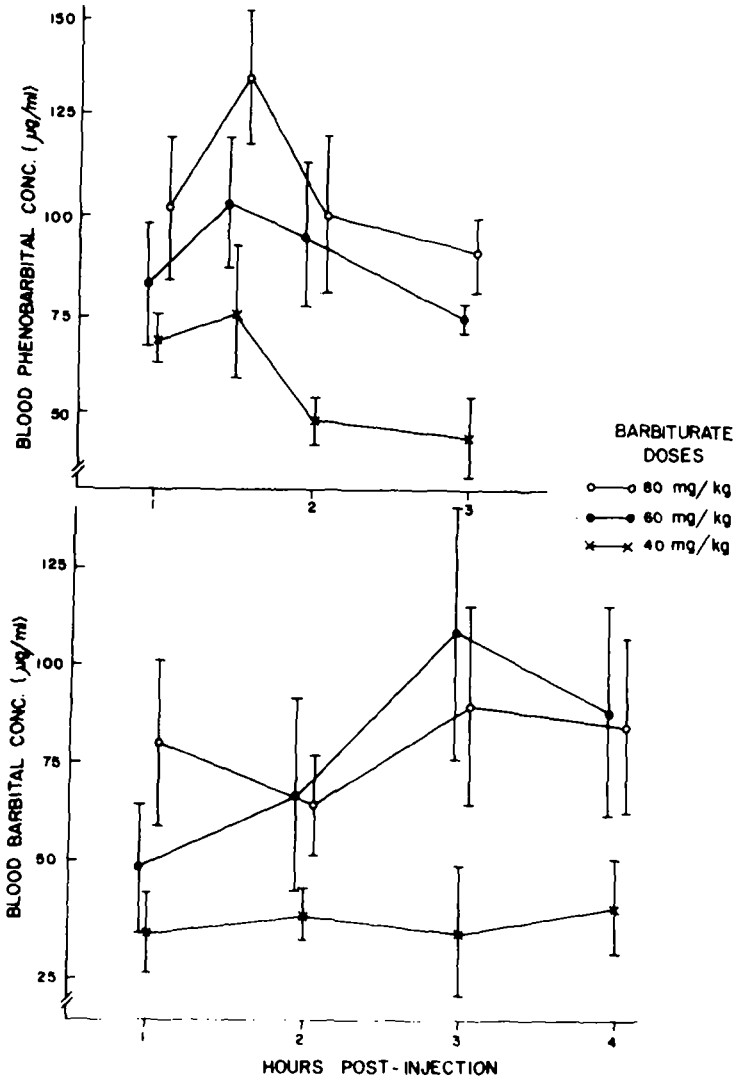

FIG. 1. Serum barbiturate concentrations of food deprived rats given 40, 60 or 80 mg/kg (SC) of either barbital or phenobarbital.

Blood Sampling

Each animal was removed from its home cage and brought into an adjacent room for blood sampling. For the first sample of the day, approximately 4 mm was removed from the tip of the tail with a guillotine; subsequently, samples were obtained by removing a suture that was tied around the tail. A 100 μl sample of blood was collected into a 100 μl disposable micro-pipette (Corning); the tip of the tail was tied with size 0 suture, and the animal was returned to its home cage. The length of these glass pipettes was precut to fit into a micro-centrifuge (Clay Adams micro-hematocrit centrifuge). The micro-pipettes were sealed at the cut end with plastic putty (Sherwood Critoseal) and put into the micro-centrifuge. Care was taken that the inside surface of the cut end was absolutely dry before putting on the putty to prevent sample loss during centrifuging. Samples were centrifuged for 5 min. Each sample tube was cut off at the junction where the clear serum separated from the red cell precipitate. The serum was transferred directly into a 50 μl glass pipette by tilting the cut end of the serum pipette towards the end of the empty pipette. Both ends of the 50 μl pipette were then sealed with putty and parafilm. The samples were frozen immediately for later analyses.

Sample Analysis

Serum barbiturate levels were determined by chromatographic analysis using a method described previously [3].

RESULTS AND DISCUSSION

Availability of a microanalytic technique enabled the tracking of barbiturate blood levels over time after a single injection in the same animal given various dosages. This is particularly important since we observed great variability in the time course of blood barbiturate concentration between animals, while relative consistency was found within each animal.

In animals given free access to both food and water, a single 60 mg/kg intraveinous dose of either phenobarbital or barbital produced initial high plasma drug concentrations that declined rapidly during the first 30 min after which a much slower rate of drug disappearance persisted for the next few hours [4]. A large intraperitoneal dose of barbital (200 mg/kg) resulted in a continuous decrease in plasma drug level from 1.5 to 10 hr [2], while a smaller dose (50 mg/kg) of phenobarbital produced a peak plasma drug level within 1 hr post-injection and remained relatively stable for the next 5 hours [1]. In the present study, a subcutaneous route of administration was chosen to avoid lesioning the peritoneal cavity with repeated exposure to the highly alkaline barbiturate solutions. Figure 1 shows the mean serum concentrations of phenobarbital (upper) and barbital (lower) in rats maintained on a limited food regimen. For all 3 doses of phenobarbital administered, serum drug concentrations appear to be highest at 1.5 hr postinjection. In the case of barbital, serum barbital levels after 60 or 80 mg/kg did not peak until 3 hr after the injection; the 40 mg/kg dose produced a stable low serum drug concentration throughout the 4 hr period. For both barbiturates used, a 40 mg/kg injection resulted in blood levels that were lower than when either 60 or 80 mg/kg was given. The differences between the 60 and 80 doses were not consistent over time.

The slow disappearance rates of both barbital and phenobarbital from the blood (see Fig. 1) have important implications in the design of behavioral experiments involving chronic administration of these drugs. The half life of phenobarbital in other animals maintained on a similar food-limiting regimen was found to be 9 hr, and a single injection of 80 mg/kg phenobarbital produced serum levels as high as 108 μg/ml (mean=92) when measured 24 hours postinjection.

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