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Report

Effects of Hypothermia on Drug Absorption

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The *in situ* rat gut technique was used to study the effects of hypothermia on the intestinal absorption of a 1 mg/ml solution of sodium pentobarbital in 0.01 *M* phosphate buffer (pH 6.0). Male Sprague-Dawley rats weighing between 300 and 370 g were exposed to an atmosphere of helox (helium:oxygen, 80:20) at 0–4°C for 5 hr. This procedure lowers the rectal temperature of the rats from 38 to 20°C. The animals were prepared for surgery using ether as anesthetic after their rectal temperature reached 20°C. Water flux in and out of the intestinal lumen was estimated from tritiated polyethylene glycol 4000 concentrations in the perfusate. The disappearance rate constant of pentobarbital from the intestinal lumen was 0.0638 ± 0.007 min^{-1} for hypothermic rats, in comparison to 0.114 ± 0.0123 min^{-1} for normothermic rats.

KEY WORDS: hypothermia; drug absorption; pentobarbital.

INTRODUCTION

Absorption of drugs from the intestinal tract has been studied by the *in situ* rat gut technique (1). The absorption rate is known to be influenced by the composition of the gastrointestinal contents (2), the pH of the drug solution (3), the solvent composition (4), and the fasting state (5,6). A number of *in vivo* alterations which may affect the pharmacological response to drugs have been noted when hypothermia is induced in an animal. Prominent among these are a depressed metabolic rate (7,8) and altered hepatobiliary function (9,10). A temperature-dependent metabolism of sulfanilamide (11), as well as reduced uptake, metabolism, and excretion of procaine (12), has been observed in the isolated perfused, hypothermic rat liver. Altered pharmacokinetics and pharmacodynamics of drugs in the hypothermic cat (13) have been reported, along with changes in the pharmacokinetics of propranolol (14) and injected potassium (15). Finally, reduced metabolism of pentobarbital in an isolated hypothermic rat liver has been observed (16).

These observations have led us to suspect that physiological alterations produced by induced hypothermia in animals may lead to an altered pharmacokinetic and pharmacological response to pentobarbital and other drugs. Data are presented suggesting that the first-order disappearance rate constant from the gut of hypothermic rats is significantly lower than that of normothermic rats.

MATERIALS AND METHODS

Twelve male Sprague–Dawley albino rats, one rat per cage, weighing 300 to 370 g were fasted overnight prior to surgery. Drinking water was allowed *ad libitum*. Hypothermia was induced in six of the rats 5 hr prior to surgery using the method of Fisher and Musacchia (17). Briefly, the method consists of placing the animal in a plastic chamber which has an inlet for gas entry and an outlet for gas exit. The gas mixture, helox (helium:oxygen, 80:20), was delivered after mixing at appropriate ratios with a gas mixer. The helox flow was adjusted to 300 ml/min and the plastic chamber was placed in a 4°C walk-in cold room. The six animals kept in a helox atmosphere showed higher respiration rates and lower body temperatures than the six control rats maintained at room temperature and without helox. Each rat's rectal temperature (±0.1°C) was monitored with rectal temperature probes (Yellow Springs Instrument Co., Yellow Springs, Ohio; YSI Series 400). It has been suggested (17) that in the cold the greater thermal conductivity of helium brings about a heat loss greater than heat production; this lowers the body temperature of the animal from 38 to 20°C in 4 to 6 hr. Maintenance of hypothermia merely requires low ambient temperatures.

The drug solution containing 1 mg/ml sodium pentobarbital in 0.01 *M* phosphate buffer (pH 6.0) was prepared. This solution also contained carbon-14-labeled sodium pentobarbital (1000 dpm/100 μl ring 2-^{14}C) (New England Nuclear). Tritiated polyethylene glycol 4000 (500 dpm/100 μg) (New England Nuclear) was added to the solution as a nonabsorbable marker to detect volume changes within the intestinal lumen. The solution was stored in the refrigerator. Before the *in situ* rat gut surgery, the drug solution was warmed to the temperature of the rat's rectal temperature.

Absorption of pentobarbital from the intestinal tract was studied in both hypothermic and normothermic rats at room temperature using 10 ml of solution (1).

The 0.1-ml perfusate samples withdrawn from the lumen were dispersed in 15 ml scintillator solution (Eastman Ready-To-Use II). Carbon-14 and tritium were quantitated in a liquid scintillation spectrometer (Beckman Model LS 7500) equipped with automatic quench compensation and

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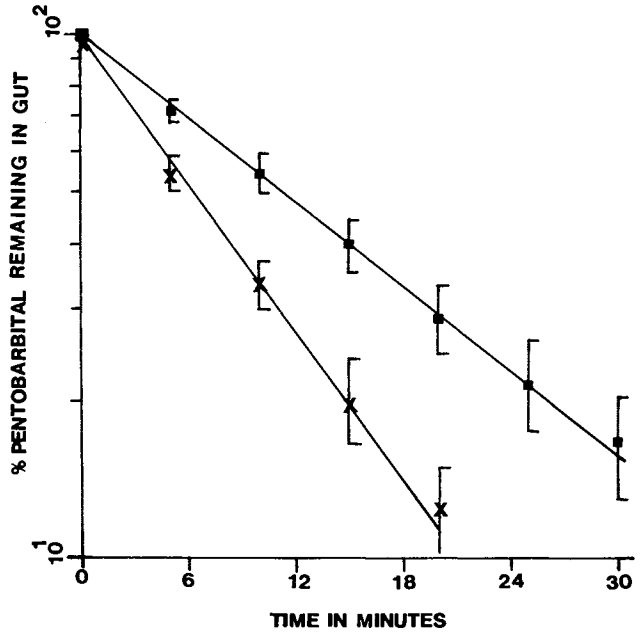

Fig. 1. Disappearance of ^{14}C-pentobarbital from the gut lumen for hypothermic (■) and normothermic (×) rats. The solid lines represent linear least-squares regression. Vertical bars represent one standard error of the mean value of six rat samples.

background correction. The samples were counted twice for 24 hr. In all cases the counting error was less than 1.5%.

RESULTS AND DISCUSSION

Figure 1 shows the percentage of pentobarbital remaining in the gut for hypothermic and normothermic rats over a 30-min time period. Concentrations of pentobarbital were corrected for fluid volume changes. For the first 30 min, all data could be described by first-order kinetics. The apparent rates of pentobarbital disappearance from the intestinal lumen of hypothermic rats were significantly decreased from normothermic rats (t test, $P < 0.01$). The rates of pentobarbital disappearance from intestinal lumen for individual hypothermic and normothermic (control) rats are shown in Tables I and II, respectively. The average rate of disappearance for hypothermic rats is 0.0638 ± 0.007 min^{-1}, which was significantly decreased in comparison to 0.114 ± 0.021 min^{-1} for normothermic rats.

Table I. Observed Pentobarbital Disappearance Rate Constants (Normothermia)

Rat label	Weight (g)	Fasting time (hr)	Rectal temp. (°C)	Disappearance constant (min^{-1})
M	352	15	34.5	0.077
O	345	15	36.5	0.093
Q	317	17	37.8	0.139
T	348	15	37.5	0.134
V	360	14	38.0	0.107
W	315	16	38.3	0.136
Average	339.5	15.3	37.1	0.114
SE	8.45	0.462	0.627	0.0116

Table II. Observed Pentobarbital Disappearance Rate Constants (Hypothermia)

Rat label	Weight (g)	Fasting time (hr)	Rectal temp. (°C)	Disappearance constant (min^{-1})
N	348	17	27.0	0.071
P	337	18	25.0	0.082
R	303	16	23.0	0.042
S	366	16	24.0	0.070
U	331	17	20.8	0.048
X	329	16	21.0	0.070
Average	335.7	16.7	23.5	0.0638
SE	9.39	0.365	1.07	0.0069

Figure 2 shows the relationship of the rate constant for the disappearance of pentobarbital from the intestinal lumen as a function of rectal temperature. A visible trend for a decreased pentobarbital disappearance due to hypothermia is apparent. The disappearance rate of pentobarbital decreases below 25°C and increases above 35°C; in the region between 25 and 30°C no apparent change in the disappearance rate constant is observed.

In an effort to explain the temperature effects on the disappearance rate constants an estimate of the temperature coefficient, Q_{10}, was obtained. The Q_{10} was estimated using Eq. (1):

$$Q_{10} = \frac{K_{(t+10)}}{K_t} \tag{1}$$

where K_t and K_{t+10} are the rate constants at two temperatures 10°C apart. In this experiment, the average temperature difference between hypothermic and normothermic rats

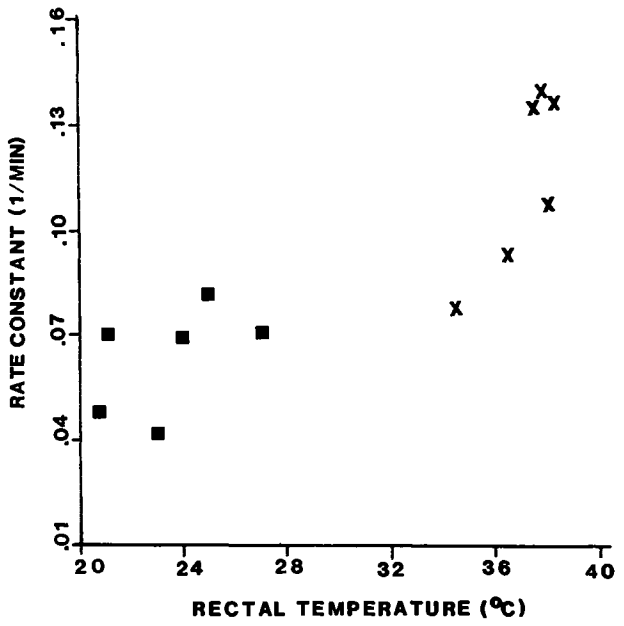

Fig. 2. Effect of rectal temperature on the disappearance rate constant of ^{14}C-pentobarbital from the gut lumen. No apparent change in the value of the disappearance rate constant is observed between 25 and 35°C.

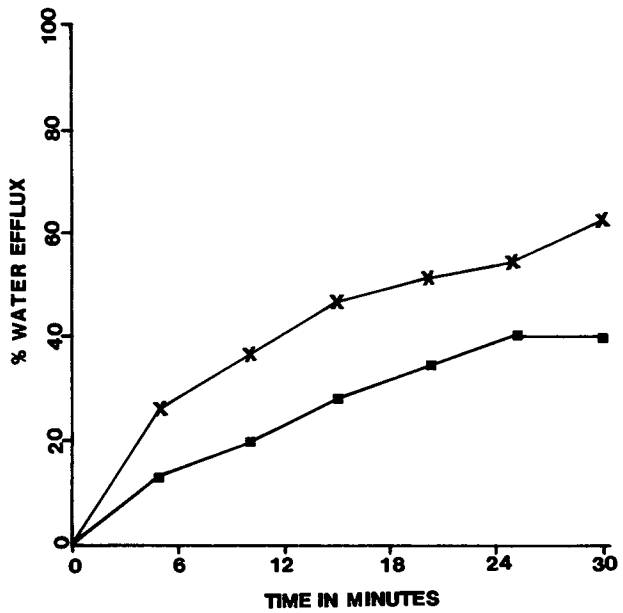

Fig. 3. Water efflux over 30 min for hypothermic (■) and normothermic (×) rats.

is 13.6°C. Thus, the Q_{10} can be estimated by dividing the rate constant for normothermic rats by the rate constant of hypothermic rats and then calibrating the temperature differences. The estimated value for Q_{10} is 1.32. This indicates that the thermosensitivity of the physiologic absorption process is smaller than that of the metabolic and rhythmical processes, which usually exhibit a Q_{10} of 3, but greater than that of simple physical processes such as diffusion, which has a Q_{10} of 1. The rates of disappearance of hypothermic animals were decreased to about 60% of the rates of disappearance of normothermic animals. The results suggest that in disease states where temperature is a factor, drug absorption may be affected if the patient is in a hypothermic state. This may possibly be explained in terms of changes in membrane permeability, enzyme activity, and blood perfusion to the intestinal membrane.

Water flux into and out of the intestinal lumen was monitored for the purpose of correcting drug concentration. However, the water flux estimates can also serve as an indicator of the influence of hypothermia on membrane permeability. Water flux was estimated as the percentage change over 5-min intervals by detecting the concentration change of the nonabsorbable ^{3}H-PEG 4000 marker. Water efflux was observed for all the studies. Figure 3 shows that water efflux was significantly greater for normothermic rats than for hypothermic rats. The cumulative water efflux was reduced by 64% under hypothermic conditions. It has been observed in our laboratories that reducing the rectal temperature by 1°C will decrease the water efflux by 5 to 7%. These changes in water flux may be due to changes in membrane permeability and/or changes in blood perfusion to the intestine during hypothermia. The results of this study suggest that the transfer of drugs from the blood to other tissues may be significantly altered during hypothermia. Elderly people have lower warmth and cold thresholds. Rectal temperatures of elderly people can vary between 21 and 32°C (17). Thus, the absorption process may be significantly influenced. Further studies are necessary to understand better the effect of hypothermia and its possible relationship to the altered pharmacokinetics of drugs in the elderly.

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