Pharmacology Biochemistry & Behavior, Vol. 14, pp. 283–286. Printed in the U.S.A.

Unimpaired Quinine Metabolism in Rats with Ventromedial Hypothalamic Lesions[1]

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Received 28 August 1980

GUNION, M. W., D. F. BROWN, R. H. PETERS AND D. NOVIN. *Unimpaired quinine metabolism in rats with ventromedial hypothalamic lesions.* PHARMAC. BIOCHEM. BEHV. **14**(3) 283–286, 1981.—Rats with lesions of the ventromedial hypothalamus (VMH) show a greater decrease in food intake than normal rats if fed diets adulterated with quinine. To determine if this hyperresponsivity is due to impaired quinine metabolism, *in vitro* rates of drug metabolism of female hooded rats with VMH lesions were measured. Determinations were made both under basal conditions and under conditions known to cause induction of drug metabolizing enzymes. VMH rats showed rates of drug metabolism virtually identical to controls under all conditions tested. These data suggest that VMH lesioned rats are not more sensitive to quinine dietary adulteration because of impaired quinine metabolism.

Ventromedial hypothalamus Lesions Drug metabolism Dietary quinine

LESIONS of the ventromedial hypothalamus (VMH) result in a syndrome of food intake and body weight changes. One of these changes is an enhanced responsivity to a commonly used dietary adulterant, quinine. It has long been known that rats with VMH lesions eat less of diets adulterated with this bitter-tasting alkaloid than do normal animals (e.g. [6,14]). This enhanced responsivity is not due to obesity alone [3,13], since VMH rats may simultaneously both eat and weigh less than normal animals fed the same quinine adulterated diet ([16,17]; see also [1, 5, 24]). The physiological basis of this enhanced responsivity to dietary quinine is not known.

Rats with VMH lesions are known to have profound metabolic alterations favoring anabolism [4,19]. Part of this shift toward energy storage includes the catabolism of proteins [9], making the constituent amino acids available for gluconeogenesis [25]. Some of the proteins so sacrificed are liver enzymes of the hepatic mixed function microsomal oxidation system [2]. This enzyme system has major responsibility for the metabolism of steroids and many drugs [2], and is known to be responsible for quinine degradation [21]. Knowledge of the functioning of this system in VMH rats may be important in understanding the effects of dietary quinine on vmh food intake, since dietary quinine causes decreased food consumption in normal rats at least in part by a nongustatory mechanism(s) ([7]; see also [12, 18, 23]). If VMH rats do have impaired drug metabolism, the postingestive effects of dietary quinine would be exacerbated. VMH rats might be required to restrict their quinine intake to match their decreased quinine excretion, ultimately reducing their consumption of quinine adulterated food.

In order to examine the possibility that VMH rats might eat less quinine adulterated food because of impaired quinine metabolism, we examined the drug metabolizing abilities of VMH and control rats using an *in vitro* test.

EXPERIMENT 1

A factor which must be considered here is that the hepatic mixed function microsomal oxidation system is an inducible system. That is, its activity increases (more of the constituent enzymes are synthesized) in the face of a sustained load [2]. It is possible that VMH rats may not be deficient in basal activity of this system, but may lack the ability to properly induce it. This inability might come about because amino acid sacrifice for gluconeogenesis would deplete the free amino acid pool from which the enzymes are synthesized [25]. Accordingly, relative rates of drug metabolism were measured in this experiment both under basal condi-

[1]Send reprint requests to R. H. P. Experiment 1 was performed at I. S. U. and Experiment 2 at U.C.L.A. Experiment 2 was supported by NIH Grant NS-07687 to D. N. During Experiment 2 M. W. G. was supported by a postdoctoral fellowship from the Mental Health Training Program of the Brain Research Institute. A preliminary report of this work was presented at the annual meeting of the Western Psychological Association, Honolulu, 1980.

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tions, and after a pharmacological challenge known to increase the activity of this enzyme system.

Method

Subjects. Subjects were adult female Long-Evans hooded rats (Blue Spruce Farms, Altamont, NY) weighing 256 to 295 g at the beginning of the experiment. They were housed in individual cages in a temperature controlled (23°C) room with a 12/12 hr light/dark cycle. They were given food and water as described below.

Surgery. Rats received bilateral VMH lesions under sodium secobarbital anesthesia (30 mg/kg IP; Myothesia®, Beecham, Bristol, TN), with methyl atropine (10 mg/kg IP) given to decrease respiratory complications. Lesions were made using a 30 gauge nichrome electrode insulated except for 0.5 mm at the conical tip. With the incisor bar set 5.0 mm above the interaural line, the tip of the electrode was positioned 0.5 mm above the floor of the calvarium, 5.8 mm anterior to the interaural line, and 0.7 mm lateral to the midline. Lesions were made by passing 2.0 mA anodal current for 20 sec between the electrode and a rectal cathode. Control surgery was performed by anesthetizing the rat, incising the scalp, and closing the wound.

Drug. Phenobarbital was used to induce the hepatic mixed function microsomal oxidation system. Phenobarbital readily induces this system [2], and has been shown to increase rates of quinine metabolism in the rat both *in vivo* and *in vitro* [21]. Phenobarbituric acid (Gaines Chemical Company, Pennsville, NJ) was added to distilled water (30 mg/ml) and brought into solution by the addition of a just sufficient amount of solid sodium hydroxide. Drug prepared in this manner caused no obvious discomfort upon injection. Control injections were of 0.9% saline.

Procedure. Prior to surgery all rats had ad lib access to both food (Teklad Mouse and Rat [4% fat], Winfield, IA) and tap water. Rats were deprived of food and water for the first 24 hr after surgery; at this time they again began ad lib access to both. Ad lib access to food was allowed in order to identify hyperphagic lesioned rats. After 7 days of ad lib feeding all rats were put on restricted food intake. Food was delivered onto the cage floor once daily, 8 hr into the light period. Control rats were given enough food to maintain them at their normal body weights. VMH rats were reduced back to normal body weights over a period of 7 to 10 days by food restriction. All rats were then maintained at normal body weight for the duration of the experiment. Daily food allowances were adjusted to allow 1% increases in body weight every two days for all rats, approximating the weight gains shown by control rats during the 7-day ad lib postsurgical period.

Drug injections began after 7 days maintenance at normal weights. Rats were injected twice daily, just after light onset and just before light offset. Half the VMH rats and half the control rats were given phenobarbital (30 mg/kg, IP); the other half of each surgical treatment received saline. These injections were given for 7 days. This drug dose and injection regimen are based on the procedure of Saggers *et al.* [21], who obtained a 50% decrease in plasma quinine half-life in normal male rats.

Enzyme assay. Rats were sacrificed by decapitation between 13 and 17 hr after the last injection. Lesioned brains were removed and saved in 10% Formalin for histology. Portions of liver were taken and immediately assayed for activity of drug metabolizing enzymes. The assay was based on the enzymatic dealkylation of p-nitroanisole to p-nitrophenol [15]. A 25% homogenate of liver (approximately 1.25 g tissue) was made using a cold buffer of 100 mM phosphate (pH 7.4), 20 mM nicotinamide, 4 mM glucose-6-phosphate, 0.4 mM nicotinamide adenine dinucleotide phosphate (sodium salt), and 10 mM magnesium chloride. The homogenate was centrifuged at 9000×g for 20 min at 4°C. Duplicate 1.0 ml aliquotes of the enzyme-containing supernatant were taken and added to prepared tubes containing the p-nitroanisole substrate (0.1 ml of 100 mM solution, 95% ethanol) and 2.0 ml of the buffer. Tubes were incubated for 60 min at 37°C, with vortexing every 15 min. After incubation the reaction mixture was deproteinated with 0.5 ml of 20% trichloroacetic acid, and centrifuged for 5 min in a clinical centrifuge. The supernatant was saved, and 1.0 ml of 1.0 N sodium hydroxide was added to it. This mixture was allowed to stand for 10 min. The soft white precipitate which formed was removed by centrifuging again for 5 min in the clinical centrifuge. Immediately after this centrifugation the absorbance of the yellowish supernatant (containing p-nitrophenol degraded from p-nitroanisole) was read at 410 nm. Tissue blanks were prepared by incubation without the p-nitroanisole substrate. Micromoles of p-nitrophenol produced were calculated from a regression line fit to the absorbance of p-nitrophenol standards (buffer as the solvent).

Histology. Following Formalin fixation brains were frozen and sectioned at 100 microns in the coronal plane [11]. Photographic enlargements of these unstained sections were used to assess the location and extent of tissue damage.

Results and Discussion

Histological. Correctly placed VMH lesions generally destroyed most or all of the area bounded rostrally by the anterior hypothalamus, caudally by the mammillary nuclei, laterally by the fornix, and dorsally by the dorsomedial nucleus. In several animals lesions spread laterally just past the plane of the fornix, and in one animal the lesions included much of the dorsomedial nuclei as well. Six animals had lesions which did not match this description, and data from these animals were excluded from the analysis. All VMH animals from which data were used gained more weight during the 7 day postoperative ad lib feeding period than the largest weight gain shown by any control animal (average gain of 6.4 g/day for VMH rats and 1.1 g/day for controls). Five control rats and 4 lesioned rats per drug condition were used in the analysis.

Assay. VMH and control livers did not differ in their abilities to degrade p-nitroanisole (Fig. 1). Both basal (saline) and challenged (phenobarbital) metabolism rates were nearly identical for VMH and control animals, $F(1,20)=1.33$, $p>0.26$ for the Surgery main effect, and $F(1,20)=1.21$, $p>0.28$ for the Surgery×Drug interaction. Phenobarbital treatment did result in a greater than four-fold increase in the rate of p-nitrophenol formation, $F(1,20)=200.29$, $p<0.0001$.

These results are consistent with previous data. Using a similar *in vitro* test, Saggers *et al.* [21] found that phenobarbital treatment of similar dose and duration caused a threefold increase in the rate of quinine metabolism in male rats. Also, in preliminary work it was found that VMH and control rats did not differ in basal rates of drug metabolism.

While these data suggest that VMH rats do not differ from normal rats in their ability to metabolize quinine, an alternative explanation arises. It is possible that the dose of

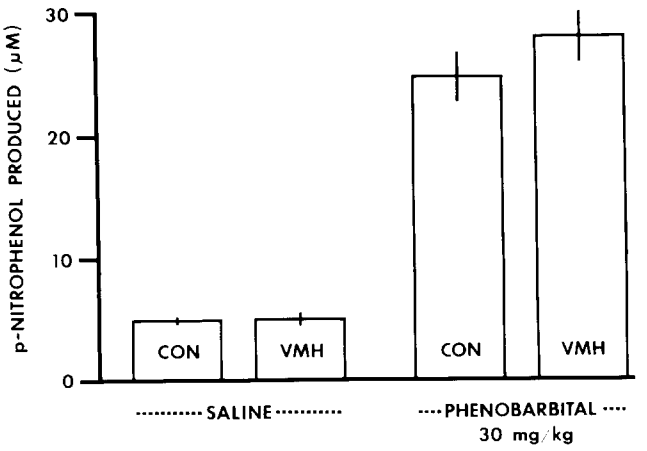

FIG. 1. Group mean rates of drug metabolism as indicated by production of p-nitrophenol by liver enzymes *in vitro*. Standard errors of the means are indicated by the vertical lines. Animals received either saline or phenobarbital for 7 days. Group abbreviations: CON—control, VMH—ventromedial hypothalamic lesions.

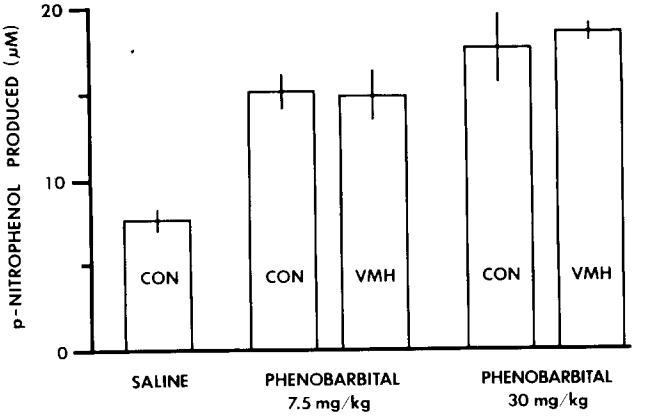

FIG. 2. Group mean rates of drug metabolism as indicated by production of p-nitrophenol by liver enzymes *in vitro*. Standard errors of the means are indicated by the vertical lines. Animals received either saline or phenobarbital for 2 days. Group abbreviations: CON—control, VMH—ventromedial hypothalamic lesions.

phenobarbital and/or the duration of treatment used here are not ones which might show such a difference even if it did indeed exist. The dose of phenobarbital used here caused a substantial increase in the rate of drug metabolism (fourfold), and may therefore be close to a ceiling effect [2]. It is also possible that while VMH and control rats might reach the same final levels of drug metabolism, they might do so at different rates. If VMH rats do not have as large a free amino acid pool to draw on as normal rats because of gluconeogenic demands, they might not be able to synthesize the required enzymes as quickly. In normal rats the induction of this enzyme system follows a negatively accelerating curve; for VMH rats this curve might be flattened.

EXPERIMENT 2

In order to examine these two possibilities the second experiment was performed. Experiment 2 was conducted identically to Experiment 1, except as noted below.

Method

Subjects were adult female hooded rats (Charles River, Boston, MA) weighing 252 to 293 g at the beginning of the experiment, and fed Purina rat chow (diet No. 5001, Ralston Purina, St. Louis, MO). Surgery was performed under sodium pentobarbital anesthesia (40 mg/kg, IP; Nembutal®, Abbott Laboratories, North Chicago, IL). With the skull flat between bregma and lambda, the electrode tip was placed 0.5 mm above the floor of the calvarium, 0.7 mm lateral to the sagittal suture, and 2.4 mm caudal to bregma. The electrode was a stainless steel insect pin (00) insulated with Epoxylite except for 0.5 mm at the conical tip. Lesions were produced by passing 1.0 mA anodal current for 15 sec between the electrode and a cathode attached to a saline-soaked gauze pad wrapped around the tail. Histological analysis was performed by microscopic examination of 60 μ thick sections stained with thionin.

A total of five groups was used in this experiment. VMH and control rats received 7.5 or 30 mg/kg IP sodium phenobarbital (total of 4 groups; Luminal®, Eli Lilly, Indianapolis, IN; these doses correspond to doses of 6.8 and 27.2 mg/kg of phenobarbital, respectively, as prepared in Experiment 1). An additional group of control animals was given 0.9% saline. Rats received these injections for 2 days. The twice-daily injections were given 3 hr into the light and dark phases of the diurnal cycle.

Results and Discussion

Histological. Lesions were confined to the area of the ventromedial and arcuate nuclei. Lesions rarely extended laterally past the fornix or dorsally into the dorsomedial nuclei. Such intrusions were always unilateral. The lesions generally spared the anterior pole of the ventromedial nucleus, and often entered the premammillary area. There was a marked tendency for one lesion to be smaller than its contralateral counterpart. The total area of bilateral destruction was much less than in the lesions of Experiment 1. All lesioned rats used in this experiment gained more weight during the postsurgical ad lib food period than the largest gain of any control rat during the same period (average gain of 5.2 g/day for VMH rats and 0.8 g/day for controls). There were 6 rats in each group receiving saline or 7.5 mg/kg phenobarbital; 5 control rats and 4 VMH rats received 30 mg/kg phenobarbital.

Assay. Again, VMH and control livers did not differ in their rates of p-nitroanisole degradation (Fig. 2). Analysis of variance on data from phenobarbital treated rats showed no signficiant effect of surgical treatment, $F(1,17)=0.00$, $p>0.9$ for the Surgery main effect, and $F(1,17)=0.26$, $p>0.6$ for the Surgery×Dose interaction. Both doses of phenobarbital caused significant increases in the rate of drug metabolism. Seven and five tenths mg/kg resulted in a 90% increase over saline control, $t(16)=5.73$, $p<0.01$, and 30 mg/kg gave an increase of 134% over saline control, $t(13)=6.71$, $p<0.001$. The difference between the two doses was also significant, $F(1,17)=4.87$, $p<0.05$. Thus a significant effect of dose was obtained.

Comparison of these data with those of Experiment 1 also indicates a clear effect of duration of treatment. Two days of 30 mg/kg produced a 134% increase over control (Experi-

ment 2), while 7 days of this treatment produced a 422% increase over control (Experiment 1).

GENERAL DISCUSSION

VMH lesioned rats are well known to be more responsive than normal animals to the food intake-suppressing effect of dietary quinine. The data presented here suggest that this effect is not due to an impaired ability to metabolize this drug, since there is no indication of impaired VMH quinine metabolizing ability in any of the four combinations of dose and treatment duration employed. This is despite the fact that the experimental procedure slightly favored such an outcome. Under once-daily feeding schedules, like the one employed in these experiments, VMH rats eat their allotted food much more quickly than controls (e.g. [10,22]). In the experiments reported here rats were sacrificed 18 to 22 hr after the last delivery of food. At sacrifice VMH rats would be expected to be actively degrading proteins for their constituent amino acids, having long ago eaten all their food. Control animals would not be degrading proteins to the same extent as VMH rats, having finished their food more recently (indeed, some control rats had a small amount of food remaining at sacrifice). Coupled with the increased gluconeogenic demand VMH rats placed on their free amino acid pools, it could have been expected that control rats would have had a larger proportion of previously synthesized drug metabolizing enzymes still intact at sacrifice than VMH rats.

While other data ([7]; see also [12, 18, 23]) indicate that quinine has postingestive effects which alter food intake, it is not clear what these effects are. Quinine can produce toxic effects both by peripheral action (altered gastric secretion, gastrointestinal irritation, abdominal pain) and by direct central action (nausea, tinnitus) [20]. Any of these toxic effects, or others, could interact with the altered physiology [4,19] of VMH rats to produce an enhanced suppression of food intake. It is also possible that nontoxic effects of quinine may alter food intake. For example, it has been shown *in vitro* that quinine can alter both insulin release from isolated pancreatic islets and islet glucose oxidation [8]. Such effects could interact with lesion induced metabolic alterations [4,19] to produce changes that would ultimately suppress VMH food intake. Such changes need not be "toxic" in nature. Finally, since quinine can produce central effects directly, reason indicates quinine might also have direct effects on feeding-related systems.

ACKNOWLEDGEMENTS

We wish to thank Donald Dyer for his advice, Arthur Campfield and Frederick Fox for the use of their equipment, and Michael Tordoff for his comments on the manuscript. We also wish to thank Percy Pyne of Gaines Chemical Company for the gift of phenobarbituric acid.

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