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Influence of Ketamine, Phenylcyclidine, and Phenobarbital on Cholesterol Metabolism in Rats[1] (39231)

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Phenobarbital treatment has been shown to increase the hydroxylation of a wide variety of steroids, among them testosterone, estrone, and various corticosteroids (1). Phenobarbital has also been found to increase 7α hydroxylation of cholesterol (2, 3) and 12α hydroxylation of 7α-hydroxy-4-cholesten-3-one (4). The use of phenobarbital in the sedation of primates has been essentially abandoned and replaced by either phenylcyclidine (Sernylan; [1-(1-phenylcyclohexyl)piperidine·HCl] or ketamine·HCl [Ketaset; [*dl*(2-*O*-chlorophenyl)-2-(methylamino) cyclohexanone·HCl]. Since all three compounds have been used for sedation, it was of interest to compare their effects on some phases of lipid metabolism in the rat. Our results are the basis of this communication.

Materials and methods. Male Wistar rats, allowed stock diet and water *ad libitum,* were given daily ip injections of either phenobarbital (100 mg/kg), phenylcyclidine (1 mg/kg), ketamine (1 mg/kg), or saline. The drugs were dissolved in 0.9% sodium chloride and given in dosages similar to those used for primate anesthesia. After 6 days the rats were killed by decapitation.

Serum cholesterol was determined by the method of Pearson *et al.* (5) and serum triglycerides by the method of Levy and Keyloun (6). One aliquot of liver was saponified in 15% alcoholic KOH, the lipids were extracted with petroleum ether, and total cholesterol (5) and triglycerides (6) were determined. For determination of cholesterol biosynthesis, liver slices (0.5 g) were incubated under oxygen for 3 hr in 5 ml of phosphate buffer (pH 7) containing 0.0006 *M* $MgCl_2$, 0.03 *M* nicotinamide, and either 0.5 μCi of sodium [1-^{14}C]acetate or 0.5 μCi of [2-^{14}C]mevalonic acid. The reaction was stopped by addition of hot 15% alcoholic KOH. The cholesterol was extracted into petroleum ether and isolated as the digitonide (7). The radioactivity of the digitonide was determined by liquid-scintillation spectrometry. The cholesterol content of each incubation mixture was determined. The saponification mixture remaining after extraction of the nonsaponifiable sterols was acidified to pH 1 with HCl, and the free fatty acids extracted with ether. Aliquots of the ether extracts were taken to dryness under N_2, and the radioactivity was determined directly.

One portion of liver was homogenized in 0.25 *M* sucrose containing 0.075 *M* nicotinamide and 2.5 m*M* EDTA. Washed microsomes were prepared according to the method of Shefer *et al.* (8). For assay of 7α hydroxylation, [4-^{14}C]cholesterol (0.15 μmole) solubilized in Tween 20 was incubated in potassium phosphate buffer pH 7.4, 0.167 mmole; $MgCl_2$, 11 μmole; $NADP^+$, 3 μmole; glucose-6-phosphate, 6 μmole; glucose-6-phosphate dehydrogenase, 51 U; and 0.2 ml of the microsomal preparation. The final volume was 0.5 ml. After incubation by shaking at 37° for 30 min, 15 vol of methylene dichloride–ethanol (5:1) were added, the steroids were extracted and separated by thin-layer chromatography on Silica Gel G (9, 10). The bands corresponding to 7α hydroxycholesterol and cholesterol were scraped from the plate and assayed for radioactivity by liquid-scintillation spectrometry.

The radioactive substrates were purchased from the New England Nuclear Corp., Boston, Massachusetts.

Results and discussion. The results of two experiments are presented in Tables I and II. The initial weight of the rats was 250–300 g. In both experiments phenobarbital

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TABLE I. EFFECT OF SEDATIVES ON LIPID METABOLISM IN RATS (EXPERIMENT 1).

Metabolic parameters	Saline[a]	Test compound		
		Phenobarbital[a]	Phenylcyclidine[a]	Ketamine[a]
Weight gain (g)	7 ± 5[b]	$-6 \pm 7_a$[c]	7 ± 5	$15 \pm 6_a$
Liver weight (g)	$9.8 \pm 0.8_b$	$11.9 \pm 0.3_{bcd}$	$9.8 \pm 0.6_c$	$10.4 \pm 0.5_d$
Liver (as % body weight)	$2.92 \pm 0.18_e$	$3.67 \pm 0.10_{efg}$	$2.92 \pm 0.10_f$	$3.02 \pm 0.11_g$
Cholesterol				
Serum (mg/dl)	136 ± 5	144 ± 9	120 ± 7	124 ± 6
Liver (mg/100 g)	567 ± 17	583 ± 6	552 ± 7	562 ± 10
S + L pool (mg)	68.7 ± 3.3	83.6 ± 1.7	66.0 ± 3.3	70.8 ± 3.2
Triglycerides				
Serum (mg/dl)	$47.9 \pm 4.9_h$	$36.5 \pm 1.6_{hi}$	$37.1 \pm 1.9_j$	$47.4 \pm 3.1_{ij}$
Liver (mg/100 g)	762 ± 62	813 ± 43	730 ± 52	823 ± 50
S + L pool (mg)	80.9 ± 10.5	101.0 ± 6.8	75.5 ± 7.4	90.1 ± 6.7
Cholesterol synthesis from Mevalonate (dpm $\times 10^3$)	$7.40 \pm 1.30_k$	$2.99 \pm 0.73_{klm}$	$8.29 \pm 1.01_l$	$9.45 \pm 1.94_m$
Cholesterol 7α hydroxylase				
pmole/mg protein	10.43 ± 1.44	$14.44 \pm 1.67_n$	11.15 ± 1.16	$8.85 \pm 1.88_n$
pmole/liver	$6627 \pm 1010_o$	$15087 \pm 1953_{opq}$	$7380 \pm 569_p$	$5450 \pm 933_q$

[a] Number of animals, six.
[b] Standard error.
[c] Values bearing same subscript are significantly different.

TABLE II. EFFECT OF SEDATIVES ON LIPID METABOLISM IN RATS (EXPERIMENT 2).

Metabolic parameters	Saline[a]	Test compound		
		Phenobarbital[b]	Phenylcyclidine[b]	Ketamine[a]
Weight gain (g)	9 ± 1[c]	8 ± 2	9 ± 1	10 ± 2
Liver weight (g)	12.4 ± 0.7	$14.8 \pm 1.5_a$[d]	$11.9 \pm 0.7_a$	12.6 ± 0.7
Liver weight (% body weight)	$4.19 \pm 0.17_b$	$510 \pm 0.35_{bcd}$	$3.99 \pm 0.09_c$	$4.07 \pm 0.12_d$
Liver protein (mg/g)	$240 \pm 20_e$	$231 \pm 32_f$	221 ± 35	$140 \pm 17_{ef}$
Cholesterol				
Serum (mg/dl)	135 ± 5	127 ± 5	132 ± 4	129 ± 6
Liver (mg/100 g)	530 ± 10	$520 \pm 3_g$	$500 \pm 20_h$	$552 \pm 8_{gh}$
S + L pool (mg)	77.7 ± 3.4	88.0 ± 8.1	71.0 ± 2.7	81.2 ± 5.3
Triglycerides				
Serum (mg/dl)	55.8 ± 5.1	46.6 ± 3.2	60.3 ± 5.5	65.0 ± 4.6
Liver (mg/100 g)	$750 \pm 41_k$	$668 \pm 66_i$	$620 \pm 27_{jk}$	$777 \pm 44_{ij}$
S + L pool (mg)	99.2 ± 10.0	104.5 ± 18.1	$78.9 \pm 4.0_l$	$103.6 \pm 9.1_l$
Cholesterol synthesis				
Acetate (dpm $\times 10^4$)	4.36 ± 0.22	4.24 ± 0.37	3.78 ± 0.24	2.93 ± 0.65
Mevalonate (dpm $\times 10^4$)	$1.95 \pm 0.06_{mn}$	$1.46 \pm 0.11_{mop}$	$1.96 \pm 0.10_o$	$2.25 \pm 0.10_{np}$
Fatty acid synthesis				
Acetate (dpm $\times 10^4$)	3.57 ± 0.48	2.91 ± 0.29	3.36 ± 0.44	2.98 ± 0.29
Cholesterol 7α hydroxylase				
pmole/mg protein	11.89 ± 2.90	$18.28 \pm 3.07_q$	$10.39 \pm 1.24_q$	13.15 ± 3.14
pmol/liver	4259 ± 931	$7854 \pm 1375_{rs}$	$3322 \pm 242_r$	$4030 \pm 973_s$

[a] Number of animals, six.
[b] Number of animals, five.
[c] Standard error.
[d] Values bearing same subscripts are significantly different.

was hepatomegalic in comparison to the other treatments. It has a similar effect in the female mouse (11). Serum cholesterol levels were similar in all the experimental groups, but serum triglycerides were lower in the phenobarbital-treated rats and higher in the ketamine group than they were in the other two groups. Salvador *et al.* (11) found that phenobarbital depressed serum lipid levels in both male and female mice. Liver cholesterol levels were comparable in all groups in Experiment 1 and somewhat higher in the ketamine group in Experiment 2. Liver triglyceride levels were highest in the ketamine group, but the difference between the lowest levels (phenylcyclidine) and highest (ketamine) was 11% in the first experiment and 20% in the second. The data indicate that neither phenylcyclidine nor ketamine significantly affected serum or liver lipids.

In the first experiment, cholesterol bio-

synthesis from mevalonate was measured and found to be significantly depressed in liver slices from rats injected with phenobarbital. In the second experiment, both acetate and mevalonate were used as substrates. Cholesterogenesis from acetate was comparable for all groups but when mevalonate was the substrate in Experiment 2 biosynthesis was again significantly lower in the phenobarbital group. These results are at variance with those of Kato *et al.* (12) who found that phenobarbital treatment increased hepatic cholesterol synthesis from acetate by 42%. They treated the rats for 3 rather than 6 days, which might account for the discrepancy. Since only cholesterol synthesis from mevalonate was affected by phenobarbital treatment, the block is probably at the level of HMG-CoA reductase. Fatty acid synthesis from acetate was similar in all four groups. Again, only phenobarbital affected cholesterol synthesis in liver slices.

Cholesterol 7α hydroxylase activity was greater in the livers of the rats given phenobarbital. On the basis of specific activity (pmole/mg microsomal protein), the 7α hydroxylase activity in the phenobarbital-treated rats was 28, 23, and 39% greater than in the saline-, phenylcyclidine-, or ketamine-treated rats in Experiment 1, and 35, 43, and 28% greater in Experiment 2. On the basis of the whole liver, the phenobarbital treatment caused significantly greater 7α hydroxylation. These results are consistent with the findings of Shefer *et al.* (3) although the percentage increase of 7α hydroxylase activity in their hands was greater.

These data indicate that the use of phenylcyclidine or ketamine anesthesia does not fundamentally affect the parameters of the phases of lipid metabolism studied in these experiments.

Summary. The effects of ip injections of phenobarbital (100 mg/kg), phenylcyclidine (Sernylan; [1-(1-phenylcyclohexyl)-piperidine·HCl] (1 mg/kg), and ketamine (Ketaset; [dl(2-*O*-chlorophenyl)-2-(methylamino)cyclohexanone· HCl] (1 mg/kg) on lipid metabolism in rats were compared. This study was undertaken to determine whether the two sedatives currently used in primates share any of the undesirable effects of phenobarbital on lipid metabolism. All three compounds were administered to male Wistar rats for 6 days. Phenobarbital was hepatomegalic, stimulated 7α hydroxylation of cholesterol, and inhibited cholesterol synthesis by rat liver slices from mevalonate, but not acetate. The two other sedatives exhibited effects very similar to those observed in the controls. From our work in rats it is concluded that the use of Sernylan or Ketaset for sedation of nonhuman primates will not significantly affect these parameters of lipid metabolism.

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