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COMPARISON OF RAT AND MOUSE CIRCADIAN RHYTHM OF CHOLESTEROL-7α-HYDROXYLASE ACTIVITY

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SUMMARY

The circadian rhythm of the cholesterol-7α-hydroxylase activity previously described in the rat, is also detectable in various strains of mice. Nevertheless, its amplitude is much less pronounced. In both species, the phenomenon seems to be controlled by the glucocorticoids. The limited circadian variation in the mouse is more likely linked to (a) the reduced sensitivity of the mouse enzyme to the glucocorticoids and (b) the slightly longer biological half-life of mouse cholesterol-7α-hydroxylase.

INTRODUCTION

In 1969, we described the existence of an important circadian rhythm in the cholesterol-7α-hydroxylase activity of the adult rat liver [1]. This rhythm precipitated an extensive study which led us to discover the importance of adrenal hormones in the control of this phenomenon. This is supported by the following facts: (a) the daily fluctuations of the enzymatic activity are absent in the young rat and abruptly appear on the twentieth postnatal day, i.e. when the hypothalamo-adrenal axis reaches full maturity [2, 3]; (b) adrenalectomy and hypophysectomy completely abolish the circadian variation of the enzymatic activity [2]; (c) glucocorticoid injection [4, 5] and stress [5, 6] significantly induce the cholesterol-7α-hydroxylase activity in less than 2 h.

Exogenous factors, such as the lighting period and the food intake time should be considered as synchronizing agents of the rhythm [2, 5, 7]. By dissociating the lighting schedule and the feeding time, we have been able to conclude that this latter parameter, normally influenced by the lighting, is the main exogenous synchronizer of the cholesterol-7α-hydroxylase circadian rhythm [2]. The cholesterol-7α-hydroxylase activity half-life calculated from the spontaneous decay at the end of the night, is approximately three and one half hours, probably this explains why such rapid changes in the enzyme activity can be observed during a 24 h day. Finally, it is noteworthy to mention that the increase of enzyme activity (physiologically observed in the evening or obtained by glucocorticoid injection) is completely prevented by the administration of RNA or protein synthesis inhibitors [2].

The diurnal variation of cholesterol-7α-hydroxylase activity has been largely documented in the rat (for a review see reference 8). Except from a recent report on the African Green Monkey [9], no information is presently available on the existence of a similar phenomenon in other animal species. In this paper, we describe the presence of a limited cholesterol-7α-hydroxylase circadian rhythm in four different strains of mice as well as the relatively poor response of the mouse enzyme to glucocorticoid administration when compared to the rat hydroxylase.

MATERIALS AND METHODS

The experiments were performed on male Sprague–Dawley rats (200 g) and on adult C57Bl/6J, SWR/J, C_3HeB/FeJ and Balb/C mice. The animals were housed in an appropriate room where lighting was exclusively artificial, automatically turned on at 6 a.m. and off at 6 p.m. They received water and food (UAR A04, Villemoisson, France) *ad libitum*. Corticosterone, hydrocortisone and cycloheximide were obtained from Sigma (St. Louis, MO. U.S.A.) and administered intraperitoneally.

The cholesterol-7α-hydroxylase activity was measured on a 9000 *g* supernatant of a $\frac{1}{5}$ liver homogenate using a methodology described in detail elsewhere [10].

Inhibition of protein synthesis by cycloheximide was monitored 60 min after the injection of [^{3}H]-leucine by counting the radioactivity of the proteins precipitated by trichloracetic acid (TCA, 20%), extensively washed with TCA (10%) and redissolved in NaOH (0.5 N).

The protein concentrations were determined according to the method of Lowry *et al.*[11] with bovine serum albumin being used as the standard.

RESULTS

In a preliminary study, we have verified that the cholesterol-7α-hydroxylase assay was applicable to the same extent both to the rat and mouse enzymatic assay. The basic parameters previously described for the rat enzyme [2, 12] were also verified with the mouse enzyme. In particular, 0.3 mM was the optimal substrate concentration in the different strains of mice regardless of the time of sacrifice.

In order to appreciate the circadian rhythm variation of cholesterol-7α-hydroxylase in the mouse, the

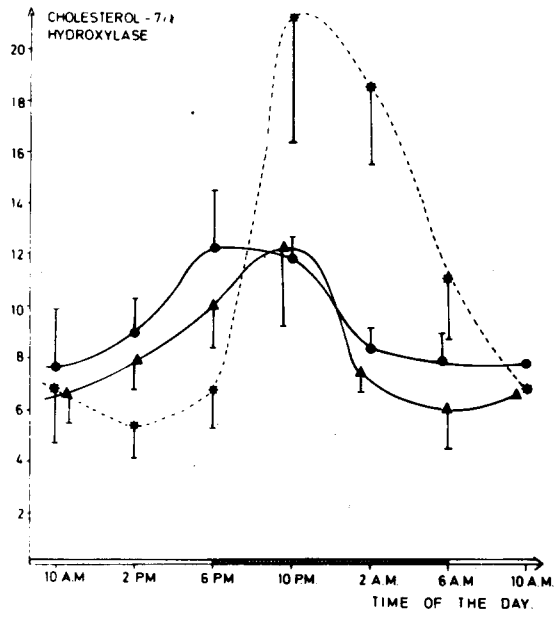

Fig. 1. Circadian evolution of cholesterol-7α-hydroxylase activity (pmol × min^{-1} × mg $protein^{-1}$) in the male Sprague–Dawley rats (*--*) and in the male (●——●) and female (▲——▲) C_3HeB/FeJ mice. The vertical bars indicate one standard deviation of the measurements made on six animals.

enzymatic assay was measured on both male and female C_3HeB/FeJ animals sacrificed every 4 h for a complete 24 h day.

Figure 1 shows that the limited circadian rhythm present in the C_3HeB/FeJ mice parallels the daily fluctuations observed in the rats. Similar results are obtained in the other strains of mice available in our laboratory (Table 1). The cholesterol-7α-hydroxylase activity is always higher at 10 p.m. than at 10 a.m., but the magnitude of the rhythm is much weaker in the mice compared to the rats. In effect, the amplitude of the rhythm never exceeded a factor of 2 between the maximum and minimum activity in the different strains of mice while it reaches a factor of 5 or 6 in the rats.

In order to further investigate the reasons for this important difference between two closely related rodent species, we injected mice with either glucocorticoids or cycloheximide. Our previous study in rats had in fact demonstrated that the enzyme was very rapidly induced by glucocorticoids and that its half-life was very short.

In a preliminary study, we have shown that the toxicity and the protein synthesis inhibitor effect of cycloheximide was very different in mice and rats. In particular, the lethal dose was about 25 mg/kg for the rats and about 500 mg/kg for the different strains of mice. That is why a 10 mg/kg dose giving an 85% protein synthesis inhibition and a 200 mg/kg dose giving a 92% inhibition were respectively chosen for rats and mice. Under these conditions, the mice appeared to be in better health than the rats.

The effect of this drug on cholesterol-7α-hydroxylase was a little more pronounced in the rats than in the mice (Fig. 2). From the decay of enzymatic activity during the first 3 h following the administration of cycloheximide, one can calculate the half-life of this enzyme. It is about 220 min in the male rats while about two times longer in the three strains of male mice. This half-life appears to be shorter in the female mice.

On the other hand, glucocorticoids, like hydrocortisone or corticosterone, have less effect on the cholesterol-7α-hydroxylase activity in the mice than in the rats (Table 2). A 2 mg/kg dose of these glucocorticoids significantly induces the rat enzyme without affecting that of the mouse. A 10 mg/kg dose affects both animal enzymes and this induction is very rapid as it is observed only 3 h after the injection.

It must be noted that the tested doses of the corticoids correspond several days physiological production. In fact, the purpose of this study is not to mimic a physiological state, but to study a pharmacological

Table 1. Cholesterol-7α-hydroxylase activity in different strains of mice and in Sprague–Dawley rats measured at 10 a.m. and 10 p.m.

Species	Strains	Sex	Cholesterol-7α-hydroxylase 10 a.m.	10 p.m.	P
Rats	Sprague–Dawley	M	6.78 ± 2.17	21.33 ± 4.94	<0.001
Mice	C57B1/6J	M	4.47 ± 0.85	8.66 ± 2.48	<0.01
	C57B1/6J	F	5.26 ± 1.39	12.32 ± 2.24	<0.01
	C_3HeB/FeJ	M	7.66 ± 2.26	11.95 ± 0.63	<0.01
	C_3HeB/FeJ	F	6.70 ± 1.28	12.29 ± 2.76	<0.01
	Balb/C	M	4.32 ± 0.83	8.41 ± 1.52	<0.001
	Balb/C	F	4.48 ± 0.99	8.75 ± 1.63	<0.01
	SWR/J	M	4.17 ± 0.72	7.87 ± 1.58	<0.01
	SWR/J	F	3.53 ± 0.91	7.67 ± 1.88	<0.01

Each series of results (pmol × min^{-1} × mg $protein^{-1}$) corresponds to the mean ± the standard deviation of determinations obtained from 6 animals. Statistical difference between values obtained at 10 a.m. and 10 p.m. (P) has been determined by Student's *t*-test.

Table 2. Cholesterol-7α-hydroxylase activity in different strains of mice and in Sprague–Dawley rats 0, 3 and 6 h after administration of hydrocortisone in water (2 or 10 mg/kg) and corticosterone in medicinal oil (2 or 10 mg/kg)

Species	Strain	Sex	Treatment	Cholesterol-7α-hydroxylase Delay = 0 h	Delay = 3 h	Delay = 6 h
Rats	Sprague Dawley	M	Hydrocortisone (2 mg/kg)	6.64 ± 1.84	10.92 ± 2.35 (*)	10.86 ± 1.54 (*)
			Hydrocortisone (10 mg/kg)	6.64 ± 1.84	12.87 ± 3.42 (*)	14.36 ± 2.51 (*)
			Corticosterone (2 mg/kg)	5.75 ± 0.99	11.07 ± 1.86 (*)	13.97 ± 2.02 (*)
			Corticosterone (10 mg/kg)	5.75 ± 0.99	13.15 ± 2.09 (*)	15.38 ± 1.73 (*)
Mice	C57B1/6J	M	Hydrocortisone (10 mg/kg)	4.22 ± 0.45	5.60 ± 1.48 (NS)	5.47 ± 0.98 (*)
	C57B1/6J	F	Hydrocortisone (10 mg/kg)	5.79 ± 1.36	8.01 ± 1.60 (*)	7.76 ± 1.12 (*)
	SWR/J	M	Hydrocortisone (10 mg/kg)	5.39 ± 1.72	7.95 ± 2.06 (*)	7.69 ± 2.63 (NS)
	SWR/J	F	Hydrocortisone (10 mg/kg)	4.70 ± 1.58	9.46 ± 1.85 (*)	8.37 ± 0.86 (*)
	C_3HeB/FeJ	M	Hydrocortisone (2 mg/kg)	8.27 ± 1.28	9.11 ± 0.99 (NS)	8.85 ± 1.37 (NS)
	C_3HeB/FeJ	M	Hydrocortisone (10 mg/kg)	8.27 ± 1.28	11.20 ± 1.20 (*)	10.49 ± 2.68 (*)
	C_3HeB/FeJ	F	Hydrocortisone (2 mg/kg)	8.75 ± 1.91	8.69 ± 1.75 (NS)	7.97 ± 2.54 (NS)
	C_3HeB/FeJ	F	Hydrocortisone (10 mg/kg)	8.75 ± 1.91	12.07 ± 2.18 (*)	11.27 ± 2.33 (*)
	C_3HeB/FeJ	M	Corticosterone (10 mg/kg)	7.86 ± 1.53	12.06 ± 1.75 (*)	12.59 ± 2.13 (*)
	C_3HeB/FeJ	F	Corticosterone (10 mg/kg)	8.17 ± 1.82	11.38 ± 1.91 (*)	13.07 ± 3.16 (*)

Values (pmol × min^{-1} × mg $protein^{-1}$) are given as means ± standard deviation of the results from six animals. An asterisk indicates $P < 0.05$ when compared to the controls.

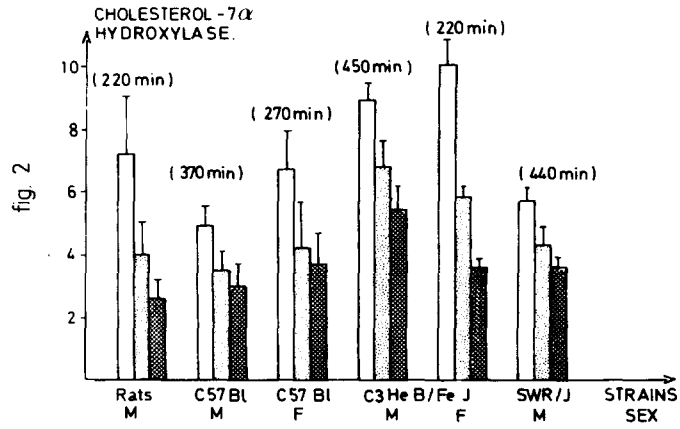

Fig. 2. Decay of cholesterol-7α-hydroxylase activity (pmol × min^{-1} × mg $protein^{-1}$) after one injection of cycloheximide in water (10 mg/kg to rats and 200 mg/kg to mice). Areas correspond respectively to the enzymatic activities of control (□), animals treated for 3 h (▨), and animals treated for 6 h (■). The vertical bars indicate one standard deviation of the measurements made on six animals. Values in brackets correspond to the half-life of the enzyme calculated from the decay of activity between 0–3 h following cycloheximide administration.

response to potential inducers. The absence of response in the mice thus strengthens our conclusion that the physiological circadian variations of glucocorticoid concentration or production does not influence the cholesterol-7α-hydroxylase activity in this animal.

DISCUSSION

Cholesterol-7α-hydroxylase is the first and rate limiting step in the biliary acid biosynthetic pathway [13]. The biliary salts are in fact absolutely required for digestion and absorption of dietary lipids in the bowel. In the rat, the cholesterol-7α-hydroxylase activity is induced just before the animal begins eating; thus the biliary salts are released in the intestine at the exact time that they are needed. Compared to the rat, the mouse synthesizes biliary acids at a more constant rate, assuming that cholesterol-7α-hydroxylase is also the rate limiting enzyme. This phenomenon might be related to the fact that unlike the mouse, the rat does not possess a gallbladder. Thus, at the moment of food intake, the mouse would expulse its biliary content into the intestinal lumen whereas the rat requires prior preparation by increasing the rate of biliary salt biosynthesis.

Compared to the rat, the behavior of mouse cholesterol-7α-hydroxylase is not qualitatively different as both animal enzymes possess the same biochemical parameters, a circadian rhythm, a short half-life and are induced by glucocorticoids.

The differences are only quantitative: mouse cholesterol-7α-hydroxylase has a slightly longer half-life, responds less to low dose injections of hydrocortisone or corticosterone and presents a less important circadian rhythm.

Thus, the circadian rhythm of cholesterol-7α-hydroxylase activity observed in the rat does not appear to be a particular phenomenon associated with an animal which does not possess a gallbladder, but is on the contrary, a more general phenomenon.

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REFERENCES

1. Gielen J., Van Cantfort J., Robaye B. and Renson J.: Rythme circadien de la cholestérol-7α-hydroxylase chez le rat. *C.R. Acad. Sci. (Paris)* **269** (1969) 731–732.
2. Gielen J., Van Cantfort J., Robaye B. and Renson J.: Rat liver cholesterol-7α-hydroxylase. III. New results about its circadian rhythm. *Eur. J. Biochem.* **55** (1975) 41–48.
3. Van Cantfort J.: Evolution postnatale de l'activité de la cholestérol-7α-hydroxylase chez le rat. *C.R. Acad. Sci. (Paris)* **274** (1972) 2593–2595.
4. Van Cantfort J.: Contrôle par les glucocorticoïdes de l'activité circadienne de la cholestérol-7α-hydroxylase. *Biochimie* **55** (1973) 1171–1173.
5. Gielen J. E., Van Cantfort J. and Kremers P.: Genetic and hormonal regulation of steroid hydroxylases and drug metabolizing enzymes in rat liver. *Arch. Toxicol.* **36** (1976) 255–266.
6. Van Cantfort J.: Influence du stress sur l'activité de la cholestérol-7α-hydroxylase. *C.R. Acad. Sci. (Paris)* **277** (1973) 345–348.
7. Van Cantfort J.: Circadian rhythm of cholesterol-7α-hydroxylase activity in genetically blind rats. *J. Interdiscipl. Cycle Res.* **5** (1974) 89–94.
8. Myant N. B. and Mitropoulos K. A.: Cholesterol-7α-hydroxylase. *J. Lipid. Res.* **18** (1977) 135–153.
9. Hulcher F. H., Margolis R. D. and Bowman D. J.: Cholesterol-7α-hydroxylase of pigeon liver microsomes. *Biochim. biophys. Acta* **529** (1978) 409–418.
10. Van Cantfort J., Renson J. and Gielen J.: Rat liver cholesterol-7α-hydroxylase. I. Development of a new

assay based on the enzymic exchange of the tritium located on the 7α position of the substrate. *Eur. J. Biochem.* **55** (1975) 23–31.
11. Lowry O., Rosebrough N., Farr A. and Randall R.: Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
12. Van Cantfort J. and Gielen J.: Cholesterol-7α-hydroxylase. II. Biochemical properties and participation of endogenous cholesterol in the assay *in vitro*. *Eur. J. Biochem.* **55** (1975) 33–40.
13. Danielsson H., Einarsson K. and Johansson G.: Effect of biliary drainage on individual reactions in the conversion of cholesterol to taurocholic acid. *Eur. J. Biochem.* **2** (1967) 44–49.