Daily Rhythms of Liver-Function Indicators in Rabbits

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Abstract: Serum concentrations of urea and cholesterol were used as indicators of liver function in rabbits in an investigation of the responsiveness of the daily rhythm of liver function to phase shifts in the environmental light-dark cycle. Locomotor activity was simultaneously monitored by actigraphy. Serum urea and cholesterol concentrations exhibited robust daily rhythmicity with opposite phases. Both indicators of liver function phase shifted in response to phase shifts of the light-dark cycle. The phase shifts in liver function appeared to be parallel to phase shifts in locomotor activity, but the data were not sufficiently strong to confirm that the liver relies on the secondary effect of photic phase shift on ingestive behavior in order to be phase-shifted by light as previously indicated by gene expression studies in laboratory rodents.

Key words: circadian rhythm, liver function, entrainment, Oryctolagus cuniculus.

Liver function, like the functions of practically all organs of the body, exhibits daily rhythmicity [1, 2]. Because urea and cholesterol are major products of liver function [3], blood concentrations of these substances can be used as indicators of liver function. Studies specifically examining enzymes involved in cholesterol synthesis and in the conversion of cholesterol to bile salts have confirmed the presence of daily rhythmicity [4].

Daily rhythmicity could be a mere result of rhythmicity in feeding. In a previous study on goats, however, we showed that the daily oscillation of serum cholesterol concentration persists in animals kept without food for several days [5], which indicates that hepatic rhythmicity must be endogenously generated. Indeed, gene-expression studies have shown that hepatic clock genes are expressed rhythmically in liver explants [6–8].

Although hepatic rhythmicity is present in the absence of feeding schedules, the time of feeding can synchronize the hepatic rhythms. Studies of clock gene expression have indicated that the rhythm of hepatic function can be synchronized by the time of feeding, but not by the environmental light-dark cycle [7–10]. This is surprising because most functions previously studied were found to respond more strongly to shifts of the light-dark cycle than to shifts in the time of feeding [11].

In the present study, we investigated the rhythms of serum urea and cholesterol concentrations (as indicators of liver function) in rabbits submitted to phase shifts of the environmental light-dark cycle. Our two goals were to characterize the rhythmicity of serum urea and cholesterol concentrations in rabbits and to evaluate the ability of the environmental light-dark cycle to synchronize these rhythms.

METHODS

Five female rabbits (Oryctolagus cuniculus, Blue Vienna breed) were used as experimental subjects. They were 12 weeks old, each animal weighing 2.5 kg at the beginning of the study. They were individually housed in metallic cages (90 cm × 50 cm × 35 cm) in a room thermostatically maintained at 21 ± 1°C and were fed rabbit pellets and water ad libitum. The experiment was conducted in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences.

The lights in the experimental room (400 lux) were on for 12 h each day. For the first 21 days, they were turned on at 0800 and turned off at 2000. On day 46, the light-dark cycle was returned to the original schedule (lights on at 0800 and off at 0800). On day 46, the light-dark cycle was returned to the original schedule (lights on at 0800 and off at 0800). The experiment was terminated on day 68.

General locomotor activity of individual animals was monitored continuously at 5 min intervals by an activity datalogger (Actiwatch, Mini Mitter Co., Bend, OR) strapped to the animal’s neck. Blood samples were collected by venipuncture of the marginal vein of the auricular pavilion for 24 h in 4-h intervals on days 21, 23, 45, and 66. The samples were centrifuged at 1,500 × g for 30 min and frozen at −20°C until being analyzed for urea and cholesterol concentrations.