The diurnal rhythm of liver glycogen phosphorylase: correlating changes in enzyme activity and enzymic protein

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The diurnal rhythm of phosphorylase activity in mouse liver extracts was correlated with the 24 h fluctuations in phosphorylase protein. This last measurement was made using rocket immunoelectrophoresis. The peak activity of phosphorylase appeared coincident (at 18:00 h) with the greatest amount of phosphorylase protein detected. Conversely, the lowest activity measured and lowest enzymic protein content both occurred at 02:00 h. Regression analysis revealed a significant positive correlation between enzyme activity and protein. Thus changes in the cellular concentration of this enzyme are implicated in the diurnal rhythm of liver glycogen.

Diurnal rhythm *(Mouse liver)* *Glycogen phosphorylase* *Glycogen* *Rocket immunoelectrophoresis*

1. INTRODUCTION

Liver glycogen levels were first shown to undergo a diurnal rhythm by Forsgren [1] and were subsequently demonstrated to be under the control of food intake and the light-dark cycle [2–5]. Glycogen synthase activity, the rate-limiting enzyme of glycogenesis, also undergoes a 24 h rhythm [4,6,7]. In [6], glycogen synthase activity peaked around 12:00 h and was lowest during the night. Furthermore, they showed that if they treated the animals with cycloheximide, the steady rise in glycogen synthase activity that normally took place throughout the morning did not occur. From these results, the authors suggested that this rhythm was due to changes in the amount of enzyme present.

Phosphorylase, the rate-limiting enzyme of glycogenolysis, also undergoes a significant daily rhythm. In rodents fed either ad libitum or during the dark cycle only, peak activities occur during the last half of the light period [4,7,8]. Whether this enzyme activity flux is due to changes in the actual amount of enzymic protein is not known. Here, we have examined the changes in phosphorylase protein that occur over a 24 h period and have correlated this with the changes in activity. Phosphorylase protein was measured in liver extracts by rocket immunoelectrophoresis utilizing specific antibodies against this enzyme.

2. MATERIALS AND METHODS

Male C57BL/KsJ mice were allowed free access to food and water and kept under a constant 12 h light : 12 h dark cycle. The mice were killed at 4-h intervals over a 24 h period by decapitation and their livers were rapidly excised and frozen in liquid nitrogen.

Methods for the preparation of liver homogenates, assaying of total phosphorylase activity, purification of mouse liver phosphorylase, production and characterization of the antiserum, and description of the rocket immunoelec-

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trophoresis have been described [9]. The protein content was determined by the method of Lowry et al. [10].

[^{14}C]Glucose 1-phosphate was obtained from New England Nuclear. Glucose 1-phosphate, type III rabbit liver glycogen, AMP, type V agarose and electrophoretic film (Gelbond) were obtained from Sigma. All other chemicals were of reagent grade.

3. RESULTS

The antiserum used in this study has been previously shown to be monospecific for phosphorylase and to recognize both (i.e. active and inactive) forms of the enzyme [9]. Fig.1 illustrates a typical rocket immunoelectrophoresis gel used for the determination of phosphorylase protein. It should be noted that the rockets showed only one precipitin line using either purified phosphorylase or a liver extract. Furthermore, the areas underneath the rockets of the standards were linear with the quantity (ng) of phosphorylase protein applied to the wells.

The results of the phosphorylase protein determinations are shown in fig.2A. The amount of phosphorylase protein rose from 7.09 ± 0.23 μg/mg protein at 06:00 h to 8.30 ± 0.06 μg/mg protein at 18:00 h. During the same period, total phosphorylase activity (measured by the high substrate assay [11]), increased from 305 ± 6 to 366 ± 17 units/mg protein (fig.2B). The lowest phosphorylase protein content (5.86 ± 0.22 μg/mg protein) and lowest phosphorylase activity (217 ± 14 units/mg protein) occurred at

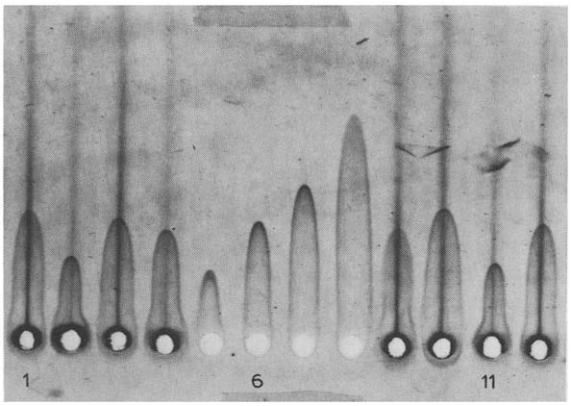

Fig.1. Rocket immunoelectrophoresis using phosphorylase antiserum. 4-μl samples were electrophoresed through a 1% agarose gel containing 0.9% (v/v) antiserum. All samples applied contained 1% (v/v) Triton X-100. Wells 5–8: 150, 300, 450 and 600 ng of purified phosphorylase, respectively. Wells 1–4 and 9–12; 3000 × g (10 min) supernatants of liver homogenates. Wells 1 and 12, 2 and 11, 3 and 10, and 4 and 9 are duplicates. Details for the electrophoresis and handling of the gel are described in [9].

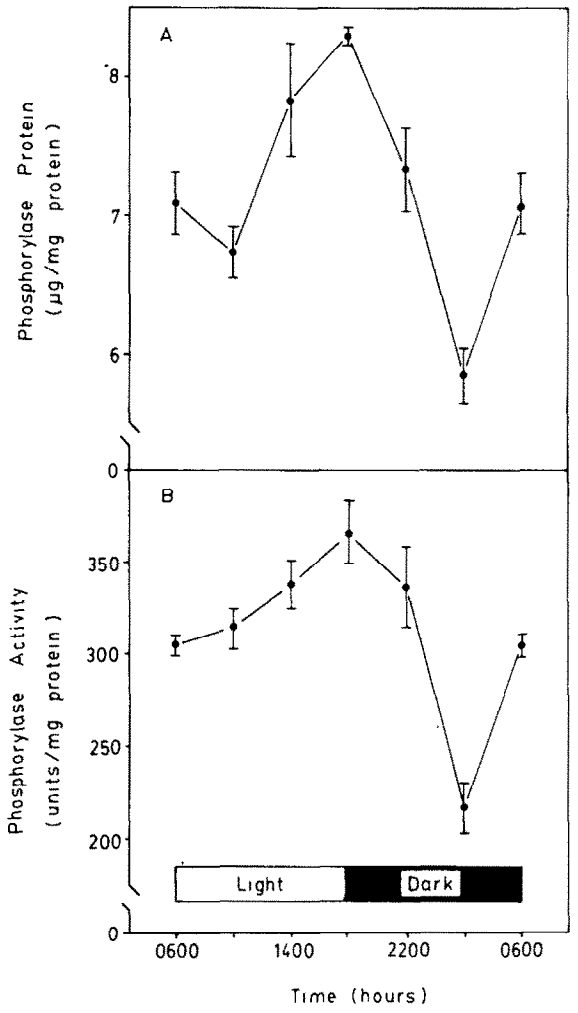

Fig.2. Diurnal rhythm of phosphorylase protein (A) and phosphorylase activity (B). Phosphorylase protein was measured by rocket immunoelectrophoresis. Total phosphorylase activity was measured by the high substrate assay [11]. Both measurements were made on 3000 × g (10 min) supernatants of 1:6 liver homogenates. Values shown are the mean ± SE of 6 mice.

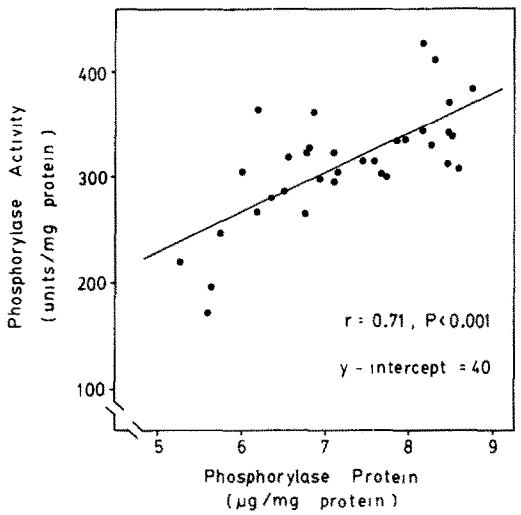

Fig.3. Correlation between total phosphorylase activity and phosphorylase protein. The points plotted represent values from individual mice and are the same values used to calculate the data in fig.2. Regression analysis was used to determine the correlation coefficient and regression line.

02:00 h. These 2 observations indicated that a correlation between phosphorylase activity and phosphorylase protein probably exists. When these values from individual samples were plotted, a significant positive correlation ($r = 0.71$, $p < 0.001$) was observed (fig.3).

4. DISCUSSION

The daily rhythm of liver glycogen in rodents is such that massive quantities of this polymer are stored during the dark hours as the animals actively feed, only to be depleted during the next light cycle [1–5,7]. This rhythm is accompanied by rhythms in glycogen synthase and phosphorylase activities [4,6,7].

Examination of the phosphorylase rhythm in particular reveals an explanation at the molecular level for the daily fluctuation of liver glycogen. During the light phase, when liver glycogen is being broken down, a rise in phosphorylase activity was observed to occur (fig.2B). This was shown in this study to be due to an increase in the cellular concentration of this enzyme (fig.2A). During the dark phase, when glycogenesis is in operation, phosphorylase activity decreased and was accompanied by a drop in enzymic protein (fig.2). The elevated plasma glucose concentrations that occur during this feeding period would not only promote glycogen synthesis but also produce an inactivation of any existing phosphorylase in the active *a* form [12]. It is obvious from the diurnal rhythm of phosphorylase protein that changes in the rates of synthesis and/or degradation of this enzyme are not constant through a light-dark cycle but instead are altered in such a manner as to change the cellular concentration of phosphorylase. The correlation coefficient of 0.71 (fig.3) indicates that the total phosphorylase activity expressed is heavily, but not entirely, dependent on the amount of enzyme present. In conclusion, this study provides direct evidence for the participation of changes in enzymic protein in the diurnal rhythm of liver phosphorylase, an enzyme important in the regulation of circulating glucose concentrations.

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