

LIVER ARGINASE ASSESSED BY MEANS OF WESTERN BLOT TECHNIQUE: 24 HOURS COURSE IN RAT (*RATTUS NORVEGICUS*)

A. Costa, A. Assenza, S. Di Mauro, G. Piccione, G. Caola

Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali – Sezione di Fisiologia, Veterinaria; Università degli Studi di Messina

Arginase has been studied in an enormous variety of organisms and tissues, but few studies were carried out on the circadian rhythmicity of this enzyme. In one study arginase showed a rhythmicity with an increase in activity prior to a scheduled daily meal (Fuentes et al., 1990) and this increase seemed to persist during a fast suggesting an endogenous food entrainable rhythm while in another study (Kato et al., 1978) did not showed any rhythmic pattern. The aim of our study is to assess the circadian pattern of arginase, as liver-function indicator, in liver tissue of rats kept under artificial photoperiod. For our investigation 6 homozygous Wistar rats (*Rattus norvegicus*) carrying a Per1-luciferase transgene 70 days old per time-point were used. The subjects, exposed to a 12 hours photoperiod (light on at 06.00, light off at 18.00), were anesthetized with alotane and then killed every 3 hours for 24 hours and liver samples were collected and kept at -80°C until the analysis that was performed after homogenization, centrifugation and heat treatment of the obtained surmatant at 95 °C for 5 min. On individual samples Western Blot technique (based on electrophoretic precipitation of protein in 10 % SDS gel) was performed using anti-arginase and anti- β

-actin antibodies. β -actin was used as control because no rhythmicity was present at hepatic level. The experimental part was performed at Department of Biology - University of Virginia as described by the current laws on laboratory animal care issued by United States Government and ACUC (Animal Care and Use Committee). Data statistic elaboration for tissue samples was carried out on mean values because intragroup variance was not significant. On these values, one-way ANOVA and single cosinor method as described by Nelson (1979) were applied. Figure 1 shows arginase/actin ratio in liver. ANOVA analysis did not allowed us to underline a statistical significant difference of arginase in the different time-points ($F_{(7,40)}=0.7405$; $P>0.05$) and comparison between different time-points with Bonferroni's test did not showed a significant statistical difference for any of the different time-points. By applying the single cosinor method no rhythmic activity was present for arginase. In our study, arginase did not show any circadian pattern. Our results are in line with the data from Kato et al. (1978) which observed that, among the five urea cycle enzymes in the liver, only argininosuccinate syntetase showed circadian fluctuation in its activity.

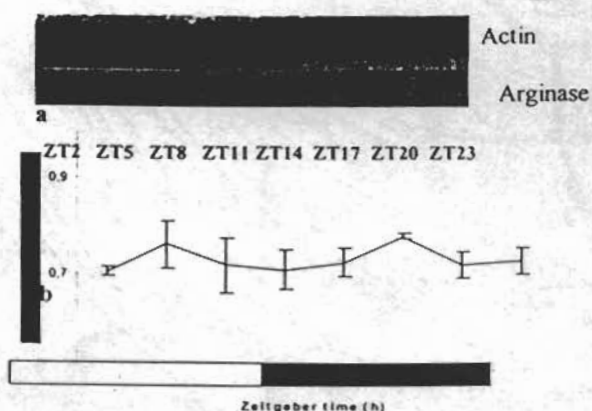


Figure 1 – Actin and Arginase electrophoretic migration by means of Western Blot in rat liver (a) and Arginase/Actin ratio (b) for the different time-points 3

References: Fuentes JM, Pascual MR, Salido G, Soler G, Madrid JA (1990) – Oscillation in rat liver cytosolic enzyme activities of the urea cycle. *Arch. Int. Physiol. Biochim. Biophys.*, 102; 237-241. Kato H, Mizutani-Funahashi M, Shiosaka S, Nakagawa H. (1978) – *J. Nutr.*, 7; 1071-7. Nelson W, Tong YL, Lee JK, Halberg F. (1979) – Methods for Cosinor-rhythmometry. *Chronobiol.*, 6; 305-323.