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CIRCADIAN OSCILLATION IN RAT LIVER TYROSINE AMINOTRANSFERASE ACTIVITY AFTER CHLOROFORM INHALATION

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Abstract—Determination of tyrosine aminotransferase activity in the liver of rats exposed for 2 min to a chloroform atmosphere showed an adaptive increase in the activity of the enzyme, with a maximum plateau between 4–6 hr after initiating the stress. The amplitude of the adaptive response differed in terms of the circadian rhythm of the enzyme, the periods of intense basal activity being characterized by the lowest reactivity.

The existence of circadian fluctuation in the activity of some enzymes linked to the metabolism of amino acids has been reported as a demonstrated fact in the literature and, hence, implies strict control of the factor time in studies on the mechanisms of enzymatic regulation [1–6]. Recent investigations in our laboratory [7] have shown the adaptive increase of hepatic tyrosine aminotransferase (1-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) (TAT) activity in all cases in which the rats were exposed to the action of stress factors (irradiation, hypoxia, burns, immobilization on the back, phenobarbital, ether, etc.), which suggested that the induction of TAT activity may be considered, at least for the rat, as one of the components of the alarm reaction of the organism.

As the activity of hepatic TAT presents a very clearly outlined circadian rhythm [1–4], we considered it of interest to study the relationship between the induction capacity of TAT and its basal activity levels at different moments in the day.

Male Wistar rats weighing 100 ± 10 g were used in the experiment. The experiments were carried out in November, the animals being kept in natural light–darkness conditions (light from 6 to 18 hr). The animals were stressed by exposure to a chloroform atmosphere achieved in glass jars of 15 l., covered with metal grating on which cotton wool imbibed with 4 ml chloroform was laid. After exactly 2 min the animals were taken out of the chloroform atmosphere in a preanaesthetic state and sacrificed 4 hr after starting the stress. The control groups were handled in exactly the same way, except for the presence of chloroform.

The liver was collected immediately, weighed and cold homogenized in 0·14 M KCl. TAT activity was evaluated directly in the homogenate after removal of the material sedimenting for 15 min at 3000 *g*, by determining *p*-OH-phenylpyruvate in alcaline medium after incubation of the samples for 30 min at 37° [8]. The results were expressed in μmoles of *p*-OH-phenylpyruvate/g moist tissue per hr.

The animals' exposure to the chloroform atmosphere was followed by a progressive increase of TAT activity during 4–5 hr when it reached a peak plateau, the value of which was 3-fold higher than the initial one; later the enzyme activity decreased slowly gaining after some hours the level of the control group (Fig. 1). The evolution in time of the TAT induction caused by chloroform was very similar with that initiated by other inductors [9].

The repeated stressing of the animals 2 hr after starting the first stress was followed by a supplementary increase of the enzyme activity. In this case the response was not an arithmetical summing up of the effects of the two separate stresses but the result of a non-linear law of the saturation curve (Fig. 2).

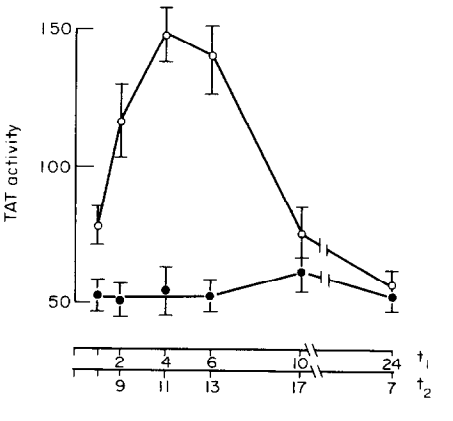

Fig. 1. Increase of TAT activity after chloroform stress t_1 = hours after starting the stress; t_2 = time of the day (●) control; (○) chloroform stress. There were eight rats per group and means and S.E.M. are indicated.

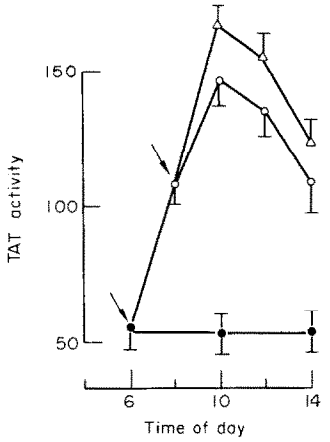

Fig. 2. TAT induction after repeated chloroform stress (●) control; (○) animals stressed once; (△) animals stressed twice. Arrows indicate the moment when the stress is started. There were six animals per group and means and S.E.M. are indicated.

The amplitude of the TAT adaptive response to chloroform stress is highly dependent upon the circadian rhythm of the enzyme. As shown in Fig. 3a, the basal level of TAT in rat varies within broad limits in the course of 24 hr.

The light period was characterized by a minimal basal activity which increased progressively at the same time as darkness began to set in, reaching maximum values between 22–24 hr. This peak meant a 4-fold increase of the minimal basal level of the enzyme. The second half of the darkness period was characterized by the slow decrease of enzyme activity to its minimal level. Our results concerning the extent of the night peak of the TAT activity and the period when it took place were similar to those obtained by other authors [1–3].

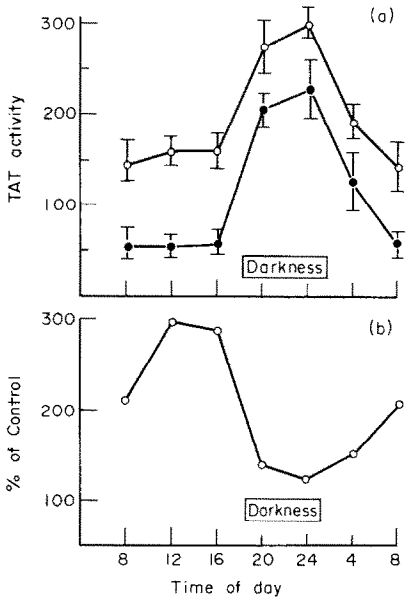

Fig. 3. Circadian rhythm of TAT activity in control (●) and chloroform treated (○) rats (a). The induction of enzymatic activity in the stressed rats is also expressed for each time-point as a percentage of the corresponding control values (b). Chloroform stress was performed 4 hr before the enzymatic activities were determined. Eight animals per group were used and means and S.E.M. are indicated.

The TAT response to the chloroform stress had different values depending on the circadian oscillation of its basal activity. The most obvious effect was observed during the day, when the amplitude of TAT response revealed a 3-fold increase of the minimal level of its activity specific to this period; this value is lower than the maximal night level of TAT basal activity. Throughout the darkness period the amplitude of TAT response to the chloroform stress was far lower and it seemed to follow the same non-linear law which could be observed in the case of two repeated stresses treated in Fig. 2. This is why, if the TAT induction capacity was related to the level of the controls' basal activity in the same hour, one found the existence of a circadian rhythm of the enzyme reactivity which was inversely proportional to its circadian oscillations (Fig. 3b).

The possible existence of certain close relationships between the circadian rhythm of certain enzymes and their induction capacity has been suggested, without being sustained by concrete facts [2]. If the adaptive response of TAT is favourable to the organism, the effect of a stressor appears to have a lesser influence when it acts during the period of intense reactivity, which corresponds, according to our opinion, with the period of minimal basal activity. This is supported, at least in part, by the data indicating that the circadian cycle does influence the effectiveness and lethality of some drugs, the most resistant period being the beginning of the light period, when the rat is least sensitive to toxins [10].

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