THE INDUCTION OF LIVER TYROSINE 2-OXOGLUTARATE TRANSAMINASE IN RATS BY IMMOBILIZATION

By

O. Hänninen and K. Hartiala

ABSTRACT

The immobilization of unfasted young female rats caused a linear increase in liver tyrosine 2-oxoglutarate transaminase activity reaching a four fold level within 12 hours. This was followed by a decrease. No gastric lesions were observed macroscopically in 26 hours, but in many cases these appeared after immobilization of at least 40 hours. The enzyme activity in the liver of rats with macroscopic gastric lesions was double or more than double the activity in immobilized rats with no lesions or in non-immobilized control animals. The increase was partially inhibited by pre-treating the animals 30 minutes before immobilization with a subcutaneous injection of actinomycin D. Adrenalectomized rats showed no increase in enzyme activity during immobilization.

The inducing effect of glucocorticoids on the liver tyrosine 2-oxoglutarate transaminase activity was first observed by Lin & Knox (1957). This enzyme reaches its maximal activity 4–5 hours after a single parenteral injection of cortisol, in contrast to alanine 2-oxoglutarate transaminase where maximum activity is observed after two days (Rosen et al. 1959, 1963). Tyrosine and some organic or even inorganic compounds have been found to induce liver tyrosine 2-oxoglutarate transaminase, but they are active only in animals with intact adrenals (Kenney & Flora 1961). Tryptophan and its analogues, especially serotonin and 5-hydroxy-tryptophan have, however, also been observed to act as inducers in adrenalectomized animals (Rosen & Milholland 1963). Sodium benzoate is also effective, and the increase in enzyme activity can be depressed by pre-treating the animals with puromycin or actinomycin D (Singer & Mason 1965).
The present report deals with the effect of an immobility stress on the liver tyrosine 2-oxoglutarate transaminase of young female rats investigated in order to determine whether the change in liver metabolism due to the activation of the adrenals coincided with the appearance of gastric lesions during immobilization (Bonfils 1964).

METHODS

Young female Wistar rats (90–100 g) fed ad libitum were used. Adrenalectomized rats were operated on seven days before use according to Grohman (1941) with the exception that one longitudinal midline incision was made. The animals were maintained by adding sodium chloride (1%o) to the drinking water. Rats were immobilized by femt fixation with plaster in a net cylinder for periods of varying duration in a quiet room (22° C). Actinomycin D (obtained from Merck, Sharp & Dohme Research Laboratories, West-Point, Pa.) was injected subcutaneously 30 minutes before immobilization. The glucocorticoid effect on the enzyme activity was controlled by injecting Cortone Acetate (Merck, Sharp & Dohme, Nederlander, N. V., Haarlem) intramuscularly three hours before the analysis.

The rats were stunned and bled immediately after liberation (at 9–12 a.m.), and the most ventral lobe of the liver was transferred to crushed ice. After cooling, the specimen was weighed on a torsion balance and homogenized with three times its weight of cold 0.15 M KCl solution for 15 seconds (24 000 rpm) in an Ultra-Turrax homogenizer. The tyrosine 2-oxoglutarate transaminase was determined in duplicate (cf. Rosen et al. 1963) from this total homogenate, about 20 minutes after the death of the animal, by adding 0.1 ml of the tissue homogenate to 0.75 ml of buffered substrate solution (prepared immediately before use from stock solutions as follows: 3 ml of 0.1 M 2-oxoglutarate solution, pH 7.4; 1 ml of 2.8 mM pyridoxal phosphate solution (Sigma Chemical Co., St. Louis) both kept at 4° C and 27 ml of a 50 mM sodium phosphate buffer, pH 7.4 kept at 20° C, and 25 µmoles of crystalline diethyldithiocarbamate (Merck AG, Darmstadt). The reaction was started by adding 0.1 ml of a 10 mM L-tyrosine suspension (Hoffman-La Roche, Basel) (20° C) and stopped after 15 minutes by adding 0.1 ml of 50%o (w/w) trichloracetic acid. The control mixtures were incubated without any tyrosine, which was only added after the trichloracetic acid. The protein precipitate was separated by centrifuging and the supernatant completely transferred to tubes together with 2 ml of a solution made up of one part of 3%o freshly made ammonium molybdate solution (5 N HCl) and two parts of a solution containing 0.5%o potassium dihydrogen phosphate in water. The measurement was carried out one hour later at 660 mµ in a Unicam spectrophotometer. The p-hydroxyphenylpyruvic acid reference was obtained from Hoffman-La Roche, Basel.

RESULTS

The plaster immobilization was followed by a rapid increase in the liver tyrosine 2-oxoglutarate transaminase activity. This linear increase reached its maximum after twelve hours of immobilization and was followed by a decrease of activity (Fig. 1). A new increase was observed, if the immobilization
The activity of tyrosine 2-oxoglutarate transaminase in the liver during the immobilization of rats; restraint in hours and 
$\text{REA} = \text{relative enzyme activity}$ when the mean activity in the controls (varying from 0.12 to 0.21 $\mu g$ of 
$p$-hydroxyphenylpyruvate liberated/min $\cdot$ mg of wet liver) was taken as 1. Each circle represents a group of five animals. The values of the standard error of the mean are shown.

was continued. In this case, however, some of the animals displayed only a control level of enzyme activity (Table 1).

The immobilization was without effect in adrenalectomized rats (Fig. 2). Adrenalectomy lowered the resistance of the animals against immobilization to a considerable degree and three out of ten rats died during an overnight experiment. Cortisone acetate effectively increased the tyrosine 2-oxoglutarate transaminase activity when given three hours before the analysis (Fig. 2).

Actinomycin D pre-treatment inhibited the increase of enzyme activity during immobilization, but did not abolish it completely (Fig. 2).

During the present experiments no macroscopic gastric lesions were found when the immobilization time was less than 26 hours. Rats immobilized for 40 hours or more showed gastric bleedings and ulcerations in many cases (Table 1). The animals were fed until the start of the experiments, which explains why the stomach was not empty. The increase in liver tyrosine 2-oxoglutarate transaminase seems to precede the appearance of gastric lesions.
Table 1.
The liver tyrosine 2-oxoglutarate transaminase activity and the macroscopic gastric lesions during prolonged immobilization of rats. Enzyme activity expressed as liberated p-hydroxyphenylpyruvate (± the values of the standard error of the mean).

<table>
<thead>
<tr>
<th>Groups (number of rats in each group)</th>
<th>p-hydroxyphenylpyruvate μg/min · mg of wet liver</th>
<th>Immobilization time in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Controls (5, 5, -)</td>
<td>0.120 ± 0.006</td>
<td>0.112 ± 0.008</td>
</tr>
<tr>
<td>Immobilized: no macroscopic lesions,</td>
<td></td>
<td></td>
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<tr>
<td>( -, 3, 5)</td>
<td></td>
<td></td>
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<tr>
<td>one or more ulcerations, gastric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bleeding (5, 3, 6)</td>
<td>0.294 ± 0.019</td>
<td>0.387 ± 0.041</td>
</tr>
</tbody>
</table>

DISCUSSION

The adrenals seem to play an essential role in the increase in the activity of liver tyrosine 2-oxoglutarate transaminase during immobilization, since this is abolished by adrenalectomy. The decrease in enzyme activity might indicate the beginning of an exhaustion in the mechanism causing increased enzyme activity. The high enzyme activity in rats developing macroscopic gastric lesions seems, however, to exclude a deficiency at the hepatic cell level.

Actinomycin D failed to block completely the increase of enzyme activity during an overnight immobilization. The injection as such acts as a stressor due to pain and fear; the control animals were similarly treated, but not immobilized. It is possible that the dose was somewhat too low, or that the release of the drug from the injection area was too slow. Probably the increase in the enzyme activity was, at least in part, due to an increase in the amount of the enzyme, since actinomycin D is known to block protein synthesis mainly at the messenger ribonucleic acid level (Kirk 1960, see also Laszlo et al. 1966; Mialhe-Voloss et al. 1966).

If a comparison is made within the groups described in Table 1, the liver enzyme activity was clearly higher in those showing than in those without macroscopic gastric lesions. Presumably the rats developing gastric ulcerations start a new high excretion of glucocorticoids whereas the others accept the immobilization. All the tissue analyses were carried out during daytime, but
The effect of actinomycin D and adrenalectomy on the induction of liver tyrosine 2-oxyglutarate transaminase by immobilization and the effect of cortisone acetate on the enzyme activity without immobilization in rats. Shaded columns represent the controls, A = 8 h immobilization, B = 90 μg of actinomycin D subcutaneously 30 min before the 8 h immobilization, C = adrenalectomy 7 days before the 8 h immobilization and D = 10 mg of cortisone acetate intramuscularly 3 hours before the analysis of enzyme activity. Each group consisted of five pairs of animals except in C, which consisted of 10 controls and 7 immobilized animals. The values of the standard error of the mean are shown. The enzyme activity is given as μg of liberated p-hydroxyphenylpyruvate/min · mg of wet liver.

Rats are most active at night (e.g. Hänninen 1966). This together with the dietary arrangements, which delayed the formation of gastric lesions, probably had an effect on the observed time distribution in the phases of the liver tyrosine 2-oxoglutarate transaminase activity. Up to 90 per cent of fasted rats showed gastric lesions after 24 hours of immobilization (Bonjils 1964; Häkkinen et al. 1966).

The tyrosine 2-oxoglutarate transaminase probably has some function in the metabolism of emergency, because its activity fluctuates very rapidly (Rosen et al. 1963; Singer & Mason 1965). These changes can also be induced with ease by changing the endogenous production of corticoids, as exemplified by the increase in enzyme activity during immobilization of the animal.
ACKNOWLEDGEMENT

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