meter of the cell in stained sections. The location of the enzyme in vivo suggested by this result is consistent with the association properties of the enzyme with the membrane in vitro.


The Effect of Hypophysectomy on the Glutathione—Insulin Transhydrogenase Activity of Rat Liver

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The liver has been shown to be one of the major organs involved in the degradation of insulin in the rat (Mirkvy, 1957). It has been shown that the enzymic degradation of insulin by rat liver takes place in a stepwise manner: first, a splitting of insulin at the disulphide bonds by glutathione—insulin transhydrogenase (GSH*-protein disulphide oxidoreductase, EC 1.8.4.2), and secondly, hydrolysis of the resulting A and B polypeptide chains to low-molecular-weight compounds (Varandani et al., 1972). This would suggest that GSH—insulin transhydrogenase is the rate-controlling enzyme in the degradation of insulin by the liver.

Elgee & Williams (1955) found that the degradation of 131I-labelled insulin in hypophysectomized rats was markedly decreased when compared with sham-operated animals. It is not possible, however, to ascertain from their published data whether these results can be explained in terms of an alteration in the activity of insulin-degrading enzymes. In the present study, we have investigated the effect of hypophysectomy on the activity of GSH—insulin transhydrogenase in rat liver.

GSH—insulin transhydrogenase activity was measured by a spectrophotometric method whereby transhydrogenase activity is coupled to that of glutathione reductase, the reaction rate being measured by following the oxidation of NADPH (Katzen & Stetten, 1962). Enzyme activity is expressed both in terms of activity per mg of protein and activity per mg of DNA.

Female albino Wistar rats weighing 250–300g were used in these experiments. Four rats were hypophysectomized by the para-pharyngeal route, four rats underwent a sham-hypophysectomy operation, and a third group of eight rats were untreated. The sham-hypophysectomy operation was carried out to create similar tissue disruption without removing any pituitary tissue. After death, the skulls of the hypophysectomized animals were inspected for the complete removal of the pituitary tissue. All three groups were maintained for 21 days under identical conditions with free access to food and water. The group of hypophysectomized animals lost 0.98±0.50g/day during the period after

* Abbreviation: GSH, reduced glutathione.
Table 1. *Effect of hypophysectomy on the liver GSH–insulin transhydrogenase activity, blood glucose concentration and serum insulin concentration of rats*

Each result is given as a mean ± s.d. for each group of animals. The untreated group consisted of eight animals, the other groups were of four animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body wt. (g)</th>
<th>Liver wt. (g)</th>
<th>GSH–insulin transhydrogenase activity</th>
<th>Blood glucose (mmol/l)</th>
<th>Serum insulin (m-i.u./l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>299 ± 18</td>
<td>8.88 ± 0.89</td>
<td>1.92 ± 0.21</td>
<td>210 ± 14</td>
<td>7.11 ± 0.35</td>
</tr>
<tr>
<td>Sham-hypophysectomized</td>
<td>285 ± 13</td>
<td>8.05 ± 0.35</td>
<td>1.93 ± 0.28</td>
<td>207 ± 19</td>
<td>6.94 ± 0.46</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>266 ± 26</td>
<td>6.90 ± 0.55</td>
<td>1.21 ± 0.19</td>
<td>140 ± 21</td>
<td>5.80 ± 0.84</td>
</tr>
</tbody>
</table>
surgery; the sham-operated rats gained 1.02±0.17 g/day during the same period. The animals were killed by decapitation, blood was collected and the serum and plasma were separated for insulin and glucose measurements respectively. Insulin concentrations were determined by a double-antibody procedure (Sønksen et al., 1972) with rat insulin (Novo Company, Copenhagen, Denmark) as a standard. The livers were quickly removed, weighed and homogenized in 0.25M-sucrose, containing 1 mM-EDTA. The 10% (w/v) homogenates were centrifuged at 12000 g (rav, 7.1 cm) for 10 min at 4°C and the supernatant was assayed for GSH–insulin transhydrogenase activity. Enzyme activity, plasma glucose and serum insulin concentrations of the treated and untreated animals are shown in Table 1.

Hypophysectomy resulted in a 47% decrease in enzyme activity, as compared with the untreated animals, when the results are expressed as activity per mg of protein, and a 33% decrease when expressed in terms of activity per unit of DNA. There was no significant difference in the activity of hepatic GSH–insulin transhydrogenase between the untreated and sham-operated rats. The plasma-glucose and serum-insulin concentrations were slightly lowered on hypophysectomy.

The experiment was repeated with male rats, each weighing approx. 300 g at the start of the experiment, when essentially the same pattern of results was obtained. It was noted, however, that the activity of GSH–insulin transhydrogenase in the livers of male rats weighing 150–300 g (approx. 10–16 weeks old) was significantly higher than in comparable females. Rats of this age are in the most active reproductive stages of their lives so that maximal hormonal differences between the sexes are to be expected.

The removal of the pituitary results in the withdrawal of the trophic hormones from the circulation. Of special interest was the fact that one of these is prolactin, for the activity of GSH–insulin transhydrogenase in mouse mammary gland has been shown to increase as lactation is established (Ferrier et al., 1973). When prolactin (N.I.H. S8, 2.5 i.u.) was injected subcutaneously, twice daily for 6 days, into hypophysectomized female rats, then the activity of the transhydrogenase returned to normal values.

Another trophic hormone, thyroid-stimulating hormone, may be a factor in the regulation of GSH–insulin transhydrogenase, for thyroidectomy results in a significant decrease in enzyme activity and this is restored after the injection of thyroxine (J. H. Thomas, P. Davey, A. Jones & J. S. M. Hutchinson, unpublished work). The activity of GSH–insulin transhydrogenase in rat liver is decreased in diabetes and in starvation and this correlates with the low insulin concentrations in these conditions (Thomas et al., 1973). One interpretation of these results was that the hepatic transhydrogenase is under feedback control by blood insulin (Thomas et al., 1973; Varandani et al., 1974). Endogenous insulin, however, is present in significant quantities in the hypophysectomized animals and thus it would seem from the results reported in this paper that this interpretation may be oversimplified and that there are additional controls over the activity of hepatic GSH–insulin transhydrogenase.

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