ROLE OF DIFFERENT PRECURSORS
IN THE SYNTHESIS OF LIVER GLYCOGEN DURING CORTISOL ADMINISTRATION

By
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ABSTRACT

The rate of label incorporation of various precursors into liver glycogen and exhaled carbon dioxide was investigated in rats 3, 6, 24 and 48 hours after cortisol injection. The glycogen concentration in the liver attained its maximum 24 hours after the hormone injection, whereas the total radioactivity of glycogen from [1-14C] glucose, [1-3-14C] lactate and [1,2-14C] glycerol reached the highest level during 3 to 6 hours and then remained within the subnormal limits 24 hours after the injection. The label incorporation from [1-14C] glycine into liver glycogen reached a maximum 6 to 24 hours after the hormone injection, coinciding in time with the maximum increase of the glycogen concentration in the liver. Hence, amino acids play a more important role in glycogen formation as compared with other precursors; thus they seem to be the main substrates in cortisol-induced gluconeogenesis. Cortisol produced no effect on [1,2-14C] glycerol oxidation, but the hormone increases oxidation of other labelled substrates involved in exhaled carbon dioxide.

Numerous investigations with labelled substrates show that under the influence of glucocorticoids, glycogen may be formed in the liver from various sources, viz.: glucose (Ashmore et al. 1961; Glenn et al. 1961; Segal & Lopez 1963; Moriwaki & Landau 1963; Kendysh 1969; Haynes & Lu Yang Sieng 1969), pyruvate (Renold et al. 1956; Ashmore 1960; Glenn et al. 1961; Segal & Lopez 1963; Kendysh 1970a), lactate (Oyi & Shreeve 1966), glycerol (Moriwaki & Landau 1963; Oyi & Shreeve 1966), amino acids (Segal & Lopez 1963; Mori-
waki & Landau 1963; Otto 1965; Oyi & Shreve 1966; Nadkarni & Deshpande 1968), Krebs cycle and other metabolites (Ashmore 1960; Glenn et al. 1961). This diversity of precursors yet similar increase in their incorporation into liver glycogen after glucocorticoid injection allowed of the conclusion that glucocorticoids stimulate all the pathways and stages of glycogen synthesis, without showing any specific selectivity with regard to precursors (Segal & Lopez 1963; Moriwaki & Landau 1963). However, the results suggesting this conclusion were obtained during several hours after glucocorticoid injection and cannot, therefore, reflect the kinetic picture of the hormonal effect at later periods of time. The present investigation demonstrates that under the influence of cortisol, amino acids are more important precursors of glycogen than the metabolites of carbohydrate or lipid origin.

MATERIALS AND METHODS

The experiments were carried out on non-inbred male rats weighing on the average 190 g. Before decapitation the animals were fasted for 24 hours, water being supplied ad libitum. Cortisol (Gedeon - Richter) in the form of fine crystalline suspension was injected intraperitoneally at a dose of 10 mg per rat. The animals were decapitated 3, 6, 24 and 48 hours after the hormone injection. [1-14C] glucose and [1,2-14C] glycerol were injected intraperitoneally at doses of 26 and 30 μCi respectively, per rat 90 min before sacrifice and [1-3-14C] lactate and [1-14C] glycine were injected intraperitoneally in doses of 10 and 28 μCi per rat 60 min before sacrifice. More prolonged exposure if possible (90 min) for [1-14C] glucose and [1,2-14C] glycerol was used because of their slower oxidation to carbon dioxide. The specific activity of the labelled substrates was in the region of 10 to 20 mCi/g. The labelled substrates were received from firma Isotope, USSR. Immediately after the injection of the labelled substrates the rats were placed into a chamber with a closed ventilation system in which the radioactivity of exhaled carbon dioxide was measured directly. Measurements of carbon dioxide radioactivity were performed continuously at intervals of 5 min. The installation used was a modification of the method of Wang & Willis (1965). In order to improve the shape of the differential curve, the ionization chamber was replaced by a flow cell equipped with two end-window Geiger-Müller counters and ancillary electronic devices. The liver glycogen was isolated according to Good et al. (1933) and measured by the method of Nelson (1944). Glycogen radioactivity was determined by the Geiger-Müller end-window counter with a mica window of 1.2 mg/cm², self-absorption being taken into account. The results obtained were treated statistically, by means of Student’s t-test. The difference was taken as significant at P < 0.05. Vertical lines in the Figures below indicate the standard error.

RESULTS

As seen in Fig. 1, glycogen production in the liver under the influence of cortisol varies in a peculiar manner. A statistically significant increase in the
Fig. 1.
Glycogen concentration in the liver after cortisol injection. Each point represents the average of 45 rats. ○ - control; ● - cortisol.

Fig. 2.
Effect of cortisol on [1-14C] glucose incorporation into liver glycogen. Ordinate: total radioactivity of glycogen, per cent of injected dose. Abscissa: interval after cortisol injection, hours. Each point represents the average of 10 rats. ○ - control; ● - cortisol.
Fig. 3.
Effect of cortisol on [1-3-14C] lactate incorporation into liver glycogen. See Fig. 2.
Each point represents the average of 7 rats.

Fig. 4.
Effect of cortisol on [1,2-14C] glycerol incorporation into liver glycogen. See Fig. 2.
Each point represents the average of 7 rats.
glycogen concentration occurs after 3 hours, reaches a maximum at 24 hours and remains substantially high for 48 hours after hormonal injection. The total radioactivity of liver glycogen from [1-14C] glucose is elevated 3 and 6 hours after the injection and decreases significantly at later periods (Fig. 2). [1-3-14C] lactate incorporation into liver glycogen following cortisol injection develops in the same way (Fig. 3). Glycogen synthesis from [1,2-14] glycerol attains a maximum at 3 hours after the injection and then gradually decreases. However, 24 hours after the hormone injection the label incorporation into glycogen is still higher than the control value (Fig. 4). In contrast to other precursors, an increase of [1-14C] glycine incorporation into liver glycogen under the influence of cortisol takes a longer time to reach a maximum glycogen concentration (Fig. 5).

Fig. 6 and Table 1 give the data relevant with regard to the effect of cortisol on the oxidation of different labelled precursors to carbon dioxide. Cortisol exerts no effect on the rate of [1,2-14C] glycerol oxidation in either period tested. Three and 6 hours respectively after the hormone injection [1-14C] glucose and [1-3-14C] lactate oxidation show a temporary decrease followed by a significant increase. The rate of [1-14C] glycine oxidation to carbon dioxide increases significantly after 3 to 24 hours and decreases slightly 48 hours after the cortisol injection.
**DISCUSSION**

Our experimental finding demonstrate that different precursors are mobilized into liver glycogen in a peculiar way. When the highest increase in the label incorporation into glycogen from $[1^{-14}C]$ glucose, $[1-3^{-14}C]$ lactate and $[1,2^{-14}C]$ glycerol occurs before the maximum rise of the glycogen content in the liver, then $[1^{-14}C]$ glycine incorporation into glycogen after cortisol injections proceeds in parallel with the glycogen accumulation in the liver. It should be noted that during this period, i.e. 24 hours after the hormone injection, glycogen synthesis from other precursors is almost completely arrested. These data suggest that the mechanism of glycogen synthesis in the liver under the influence of glucocorticoids involves two consecutive stages. The first stage is the hormonal mobilization of accessible precursors into glycogen which are evidently derived from the blood; this stage reaches completion.
Table 1.
The total radioactivity of expired CO$_2$*.

<table>
<thead>
<tr>
<th>Time after cortisol administration, hours</th>
<th>[1,2-$^{14}$C] glycerol</th>
<th>[1-$^{14}$C] glucose</th>
<th>[1–3-$^{14}$C] lactate</th>
<th>[1-$^{14}$C] glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>cortisol</td>
<td>$P$</td>
<td>control</td>
</tr>
<tr>
<td>3</td>
<td>6.20</td>
<td>6.40</td>
<td>$&gt;0.05$</td>
<td>30.00</td>
</tr>
<tr>
<td></td>
<td>$\pm0.45$</td>
<td>$\pm0.50$</td>
<td></td>
<td>$\pm1.60$</td>
</tr>
<tr>
<td>6</td>
<td>5.85</td>
<td>5.80</td>
<td>$&gt;0.05$</td>
<td>31.60</td>
</tr>
<tr>
<td></td>
<td>$\pm0.45$</td>
<td>$\pm0.40$</td>
<td></td>
<td>$\pm1.65$</td>
</tr>
<tr>
<td>24</td>
<td>6.20</td>
<td>6.00</td>
<td>$&gt;0.05$</td>
<td>30.40</td>
</tr>
<tr>
<td></td>
<td>$\pm0.45$</td>
<td>$\pm0.42$</td>
<td></td>
<td>$\pm1.50$</td>
</tr>
<tr>
<td>48</td>
<td>8.60</td>
<td>8.45</td>
<td>$&gt;0.05$</td>
<td>30.80</td>
</tr>
<tr>
<td></td>
<td>$\pm0.65$</td>
<td>$\pm0.62$</td>
<td></td>
<td>$\pm1.60$</td>
</tr>
</tbody>
</table>

* Data expressed as per cent of injected dose.
between 6 to 24 hours after glucocorticoid injection. During this stage the role of various precursors in the glycogen synthesis follows a course indicating a simultaneous action of glycogenesis and gluconeogenesis processes. The next stage probably involves a preferential mobilization of amino acids from tissue sources. One of the main sources is the lymphoid tissue the metabolites of which resulting from its glucocorticoid-induced destruction, are actively involved in liver glycogen and blood glucose. These observations suggest therefore, that under the influence of glucocorticoids, gluconeogenesis from amino acids originating from the lymphoid tissue is responsible for the main bulk of glycogen (Kendysh 1970b; Kendysh & Moroz 1970).

It should be emphasized that there is no distinct correlation between substrate oxidation into carbon dioxide at the level of the organism. In fact, during the most intensive mobilization the oxidation of [1−3−14C] lactate decreases significantly, that of [1−14C] glucose and [1,2−14C] glycerol remains unchanged while oxidation of [1−14C] glycine increases substantially. In conclusion the following should be noted. In our preliminary experiments and other experiments (Nims & Geisselsoder 1960) it has been demonstrated that liver glycogen in rats fasted for 1–2 days is maintained at the minimum constant level but is greatly increased due to stress when fasting is continued for 3 or more days. Thus under the conditions of a continuous fasting the effect of injected hormones might be masked by secretion of endogenous glucocorticoids especially at later time periods when the injected hormones are no longer present in the body. Accordingly we used animals subjected to an interrupted fasting which allowed the estimation of the cortisol effect during a constant glycogen background. However, such an experimental pattern has its drawbacks. The glycogen production in this case under the influence of cortisol from endogenous sources occurs only at the period of 24 hours after the hormone injection, but 48 hours after cortisol administration glycogen is produced from both endogenous and alimentary sources. These peculiarities should be taken into account in the interpretation of our data.

REFERENCES

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