SHORT COMMUNICATION

POST-HEPARIN LIPOLYTIC ACTIVITY AND TISSUE LIPOPROTEIN LIPASE ACTIVITY IN THE ALLOXAN-DIABETIC RAT

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SUMMARY

1. Alloxan-diabetic rats showed raised plasma triglyceride levels and low adipose tissue lipoprotein lipase activity compared with controls. Heart lipoprotein lipase activity appeared unaltered by the diabetic state.

2. Plasma post-heparin lipolytic activity was slightly but not significantly increased in the diabetic group. The significance of these findings is discussed.

Key words: alloxan diabetes, lipoprotein lipase, post-heparin lipolytic activity.

It is widely assumed that measurement of lipolytic activity in post-heparin plasma (PHLA) reflects the activity of the enzyme lipoprotein lipase in extrahepatic tissues, i.e. adipose tissue, muscle and the heart. Apart from the findings of low PHLA in patients with Fredrickson's type I (fat-induced) hyperlipoproteinaemia (Fredrickson, Ono & Davis, 1963), in which a defect in tissue lipoprotein lipase activity (LLA) is thought to occur, there is little evidence that PHLA reflects tissue LLA. This study was made to find out whether, in the rat, adaptive changes in PHLA and tissue LLA occur together. As there is evidence that adipose tissue is under hormonal control by insulin (Salaman & Robinson, 1966), we used the alloxan-diabetic rat as a model in which insulin deficiency occurs.

METHODS

Male Wistar rats weighing 200–250 g were injected intraperitoneally with alloxan (200 mg/kg). For each rat so treated, a control rat of similar weight was used. The rats, fed ad libitum, were killed 2 days later, at which time there was no significant difference in weight between the control and alloxan-injected animals. Fifteen minutes before the rats were killed, heparin (2000 units/kg) was injected intraperitoneally. This amount of heparin had been found in preliminary experiments to give maximal plasma PHLA. The animals were killed by cervical

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fracture and blood was taken, for estimation of glucose, triglyceride and PHLA, into tubes containing 76 mg of trisodium citrate as anticoagulant. The tubes were then inverted several times and kept in ice before centrifugation. Epididymal fat-pads and heart were dissected free and extracted with acetone and ether according to the method of Borensztajn, Otway & Robinson (1970). LLA was measured in these extracts by the method of Borensztajn et al. (1970), Intralipid being used as substrate. The reaction was monitored by the measurement of free fatty acid (Dole & Meinertz, 1960) at 0 and 60 min, over which time it was shown to be linear. Deoxyribonucleic acid (DNA) was measured in the homogenates of the acetone–ether extracts according to the method of Abraham, Scaletta & Vaughan (1972). Results were expressed as μmol of free fatty acid (FFA) h⁻¹ μg⁻¹ of DNA. PHLA was measured with a similar assay but with 1 ml of post-heparin plasma instead of tissue homogenate and omission of additional serum to activate the substrate, as post-heparin plasma already contains the necessary activating factors. Results were expressed as μmol of FFA h⁻¹ ml⁻¹.

Plasma triglyceride was measured by the method of Van Handel & Zilverschmidt (1957) and blood glucose by the method of Trinder (1969).

To avoid the possibility that the administration of heparin could have altered the relative activity of heart and adipose tissue LLA, similar experiments were carried out on a separate group of alloxan-diabetic and control rats without injection of heparin and measurement of PHLA.

**RESULTS AND DISCUSSION**

The diabetic rats (Table 1a) had raised plasma triglyceride levels compared with controls (P<0.02). Adipose tissue LLA was reduced in the diabetic group (P<0.0005), though heart LLA was not significantly different (P<0.1) compared with controls. Plasma PHLA was slightly, but not significantly, increased (P<0.1) in the diabetic group, compared with controls. The results for the rats not injected with heparin (Table 1b) were similar and indicate that the administration of heparin did not alter the relative activity of heart and adipose tissue LLA in the diabetic state.

These results indicate that, in alloxan-diabetic rats, raised plasma triglyceride levels occur in the presence of low adipose tissue LLA. Heart LLA seemed unaltered by the diabetic state. These results are in agreement with those of Schnatz & Williams (1963), though Kessler (1963) found reduced adipose tissue LLA and increased heart LLA in alloxan-diabetic rats. Reaven & Reaven (1973) have reported that, in the alloxan-diabetic rat, both hepatic triglyceride synthesis and release were reduced. This together with the above findings would suggest that decreased clearance of plasma triglyceride due to the low LLA of adipose tissue might be important in the aetiology of the raised plasma triglyceride levels seen in alloxan diabetes.

These results also show that in rats, in a situation where adipose tissue LLA is reduced, PHLA is not similarly reduced, being not significantly different from that in control animals. The measurement of PHLA is widely used as an index of tissue LLA. Conflicting results have been obtained from the measurement of PHLA in diabetes in man. Bagdade, Porte & Bierman (1968) reported a fall in PHLA associated with high plasma triglyceride levels after withdrawal of insulin in a group of insulin-requiring diabetics. Jones, Plotkin & Arky (1966), on the other hand, found no difference between PHLA in control subjects and insulin-requiring diabetics with and without hypertriglyceridaemia. They also found normal levels of PHLA in diabetics presenting with diabetic ketosis, which did not rise when this state had been corrected.
**Table 1. Blood glucose, plasma triglyceride, adipose tissue and heart lipoprotein lipase activity and plasma post-heparin lipolytic activity in paired control and alloxan-diabetic rats**

Mean values ± SEM are shown. LLA, Lipoprotein lipase activity; PHLA, post-heparin plasma; FFA, free fatty acid.

<table>
<thead>
<tr>
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<th>Blood glucose (μmol/l)</th>
<th>Plasma triglyceride (μmol/l)</th>
<th>Adipose tissue LLA (μmol of FFA h⁻¹ μg⁻¹ of DNA)</th>
<th>Heart LLA (μmol of FFA h⁻¹ μg⁻¹ of DNA)</th>
<th>PHLA (μmol h⁻¹ ml⁻¹)</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<td>Alloxan-diabetic</td>
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<td>(a) Rats injected with heparin (n = 11)</td>
<td>5·6</td>
<td>26·4</td>
<td>0·71 2·8</td>
<td>0·17 0·02</td>
<td>0·05 0·04</td>
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<td></td>
<td>±0·23 2·9</td>
<td>±0·14 0·01</td>
<td>±0·01 0·003</td>
<td>±0·006 0·01</td>
<td>±2·1 1·67</td>
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<tr>
<td>(b) Rats not injected with heparin (n = 5)</td>
<td>5·4</td>
<td>17·3</td>
<td>0·85 1·94</td>
<td>0·33 0·02</td>
<td>0·06 0·04</td>
</tr>
<tr>
<td></td>
<td>±0·5 5·0</td>
<td>±0·07 0·07</td>
<td>±0·07 0·004</td>
<td>±0·005 0·009</td>
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Rössner, Larsson-Cohn, Carlson & Boberg (1971) found that in women on oral contraceptive therapy there was an increase in plasma triglyceride levels accompanied by decreased PHLA, though the clearance of an intravenous fat-load was unaffected. La Rosa, Levy, Windmueller & Fredrickson (1972) have described a heparin-releasable triglyceride lipase in the liver and have suggested that plasma PHLA may emanate at least in part from this source. Our results indicate that, at least in the rat, PHLA does not seem to reflect adipose tissue LLA, which raises doubts as to its origin. It would seem that the assumption that plasma PHLA reflects tissue LLA in man requires validation.

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REFERENCES


