SHORT COMMUNICATION

Lipolytic activities in post-heparin plasma in man measured with different substrate emulsions

B. VESSBY, J. BOBERG AND H. LITHELL
Department of Geriatrics, University of Uppsala, Uppsala, Sweden

(Received 24 June 1977; accepted 12 September 1977)

Summary

1. Post-heparin lipolytic activity in man has been studied by using a triglyceride substrate emulsion containing different emulsifiers.

2. The lipolytic activity measured was profoundly influenced by the type of emulsifier used in the substrate. Substrates stabilized by synthetic emulsifiers give higher lipolytic activity than Intraplipid, which contains egg phospholipids as emulsifiers. This difference was solely explained by higher salt-resistant lipase activities found with emulsions containing synthetic emulsifiers. The salt-inhibited lipase activity, which has properties as a lipoprotein lipase, was not influenced by the type of emulsifier.

3. When used under specified conditions Intraplipid seems to be virtually specific for extrahepatic post-heparin lipolytic activity.

Key words: emulsifier, lipoprotein lipase, post-heparin lipolytic activity, triglyceride substrate.

Introduction

Post-heparin plasma contains at least two different triglyceride lipase activities. By using affinity chromatography of post-heparin plasma on heparin-Sepharose eluted with a linear salt gradient, Ehnholm, Shaw, Greten, Langfelder & Brown (1974) were able to demonstrate one salt-resistant activity which did not need a serum cofactor for optimum activity and one serum-activated triglyceride lipase activity (EC 3.1.1.3, triacylglycerol lipase, glycerol ester hydrolase) (Ehnholm et al., 1974b). Although the latter enzyme activity showed inhibition characteristics similar to those of lipoprotein lipase (EC 3.1.1.34), e.g. from adipose or muscular tissue, the salt-resistant lipase activity has been shown to originate in the liver (La Rosa, Levy, Windmueller & Fredrickson, 1972; Assman, Krauss, Fredrickson & Levy, 1973; Ehnholm, Bensadoun & Brown, 1973).

The conflict between the results of earlier assays of post-heparin lipolytic activity may have been due to variation in amounts of more than one triglyceride lipase in plasma. Different assay systems may vary in specificity for the separate triglyceride lipase activities. The results are influenced by the composition and pH of the assay mixture (Ehnholm, Greten & Brown, 1974a), the dose of heparin injected (Boberg, 1972; Krauss, Levy & Fredrickson, 1974), the time between heparin injection and blood sampling (Boberg, 1972; Krauss et al., 1974) and the properties of the substrate (Datta & Wiggins, 1964; Boberg & Carlson, 1964; Krauss et al., 1974).

This study was undertaken to characterize how the type of emulsifier used in the substrate may influence the measurements of triglyceride lipase activities.

Materials and methods

Triglyceride substrates

Soya-bean oil emulsions containing 10% triglycerides and different emulsifiers were generously supplied by AB Vitrum, Stockholm, Sweden.


TABLE 1. Assay of post-heparin plasma lipolytic activity using triglyceride substrate emulsions containing 10% triglycerides and different emulsifiers

The concentration of each emulsifier is given in parentheses. Salt-resistant lipase activity is triglyceride lipase activity not inhibited by NaCl (1 mol/l) and salt-inhibited lipase activity is triglyceride lipase activity inhibited by NaCl (1 mol/l).

<table>
<thead>
<tr>
<th>Type of emulsifier</th>
<th>Triglyceride lipase activity (μmol/min)</th>
<th>Assay at 27°C</th>
<th>Assay at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total activity</td>
<td>Salt-resistant activity</td>
<td>Salt-inhibited activity</td>
</tr>
<tr>
<td>Phospholipids (1-2%) (Intralipid)</td>
<td>60</td>
<td>16</td>
<td>44</td>
</tr>
<tr>
<td>Myrj 52 (1%)</td>
<td>100</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>Tween 20 (0-195%)/Span 80 (0-455%)</td>
<td>92</td>
<td>60</td>
<td>32</td>
</tr>
<tr>
<td>Triton X-100 (1%)</td>
<td>112</td>
<td>64</td>
<td>48</td>
</tr>
<tr>
<td>Brij 35 (1%)</td>
<td>92</td>
<td>52</td>
<td>40</td>
</tr>
</tbody>
</table>

(Table 1). The detergents used were synthetic surfactants containing polyoxyethylene chains of diverging length in ether or ester bindings (Myrj 52, Tween 20/Span 80, Triton X-100, Brij 35). The emulsions were made in the same way as Intralipid (AB Vitrum) with the exception that no glycerol had been added. They were all made from the same batch of soya-bean oil and prepared at the same time during identical technical conditions.

Assay of triglyceride lipase activity in post-heparin plasma

For determination of post-heparin lipolytic activity in plasma heparin (5000 i.u./ml; AB Vitrum) was given in a dose of 100 i.u./kg body weight intravenously to a healthy man who had fasted overnight. A venous blood sample was taken 40 min after heparin injection. Post-heparin lipolytic activity was determined by the method of Boberg & Carlson (1964) and Boberg (1970). The assay was performed either at 27°C or 37°C in an ammonium buffer [31 vol. of aq. ammonia solution (0·1 mol/l), 30 vol. of ammonium chloride solution (0·1 mol/l)] containing 10% bovine albumin at pH 8·70. The concentration of the triglyceride substrate in the incubation mixture was 8 mmol/l.

Results

Table 1 shows the results from one experiment performed at 27°C and 37°C with different substrate emulsions. Similar results were achieved in several experiments. The assay was performed both without extra salt and in the presence of sodium chloride (1 mol/l) in the incubation mixture.

Post-heparin lipolytic activity measured with Intralipid was completely inhibited by sodium chloride (1 mol/l) when the assay was performed at 37°C. The total triglyceride lipase activity measured with Intralipid at 27°C was lower than at 37°C. However, a certain amount of the lipase activity was not inhibited by salt when measured at 27°C. The salt-inhibited lipase activity was approximately doubled when the incubation temperature was increased from 27°C to 37°C. When the plasma sample was preincubated with protamine sulphate (Krauss et al., 1974) for 10 min the lipase activity was inhibited to a similar extent as with sodium chloride (1 mol/l).

All emulsions stabilized by synthetic detergents showed similar properties both at 27°C and at 37°C, which were clearly different from those of the phospholipid-stabilized Intralipid. At 27°C Intralipid showed an activity of salt-resistant triglyceride lipase that was only 27% of the total triglyceride lipase activity, and this ranged from 57 to 68% with the other four emulsions. The total salt-resistant activity measured with these emulsions at 27°C was approximately four times that measured with Intralipid as a substrate. However, Intralipid and the four emulsions stabilized with synthetic detergents showed a similar salt-inhibited triglyceride lipase activity.

When the incubation temperature was 37°C the total triglyceride lipase activity was higher than at 27°C with all substrates. This was always due to an increase in salt-inhibited lipase activity, which approximately doubled when the incubation temperature was increased from 27°C to 37°C. With the emulsions stabilized by synthetic detergents the amount of salt-resistant lipase activity measured at 37°C was virtually unchanged compared with that at 27°C. The total lipolytic activity
was 60–80% higher with emulsions with synthetic detergents than with Intralipid. This difference was solely explained by the higher salt-resistant activity that was present with the emulsions stabilized by synthetic emulsifiers. With Intralipid as a substrate virtually all lipolytic activity was inhibited by sodium chloride (1 mol/l) when measured at 37°C. With the other four emulsions the salt-resistant lipase activity amounted to 32–44% of the total activity at the same temperature.

Discussion

In the past, different types of substrates have been used in assay systems for determination of lipoprotein lipase activity. The diverging results from studies where Triton X-100 (La Rosa et al., 1972; Krauss et al., 1974), Tween 60 (Datta & Wiggins, 1964), gum arabic (Ehnholm et al., 1974b) and phospholipids (Boberg & Carlson, 1964) have been used as emulsifiers, probably arise to some extent from the properties of the substrates. It is not clear why the type of emulsifier in the substrate so profoundly influences the determination of the enzyme activities. Electron-microscopy studies of the particle size of soya-bean-oil emulsions of Intralipid type prepared with various emulsifying agents did not indicate that particle size varies with the emulsifying system used (Jeppson & Schoefl, 1974). We have not yet defined the physico-chemical factors responsible for the differences in substrate properties between triglyceride emulsions containing different emulsifying agents.

Intralipid stabilized by egg phospholipids thus seems to have unique properties as triglyceride lipase substrate. In an assay performed at 37°C, with a plasma sample drawn 40 min after heparin injection as enzyme source, the lipase activity was virtually completely inhibited by sodium chloride (1 mol/l) or by preincubation with protamine sulphate. This is in accordance with earlier reports by Boberg & Carlson (1964) and Boberg (1970). This assay thus seems to measure extrahepatic triglyceride activity very specifically with inhibition properties which are characteristic for lipoprotein lipase. Our results are supported by Rogers, Barnett & Robinson (1976), who used Intralipid as a substrate, finding that at most 10% of the total lipolytic activity could be attributed to the salt-resistant lipase. Corey & Zilversmit (1977) have also reported on a glycerol-based trioleylglycerol and phosphatidylcholine emulsion similar to Intralipid, which seems to be specific for extrahepatic post-heparin lipolytic activity.

Acknowledgments

This work was supported by the Swedish Medical Research Council (grant no. B76-4679-02) and Vitrum AB, Sweden.

References


