Hepatic lipase activity after liver denervation in hypothyroid rats

Anna-Lena Berg¹, Lars-Erik Hammarström², Per Hansson³, Torsten Holmin² and Peter Nilsson-Ehle³

Department of Medicine¹, Malmö General Hospital, University of Lund, Malmö, and Departments of Surgery²
and Clinical Chemistry³, University of Lund, Lund, Sweden

Abstract. Hepatic hilar denervation, hepatic vagotomy or sham operation were performed in hypothyroid rats. Activities of hepatic lipase were measured nine days after surgery. Sham operation in itself was associated with a decrease of hepatic lipase activity by about 40% compared with non-operated animals. Both hilar denervation and hepatic vagotomy were associated with increased hepatic lipase activity (40% and 35%, compared with sham-operated animals). Liver contents of norepinephrine were reduced by about 90% after hilar denervation, whereas hepatic vagotomy did not affect norepinephrine levels. No major changes in lipids and lipoproteins were noted.

Hepatic lipase (also designated salt resistant lipase) is an enzyme synthesized in hepatocytes and active at the capillary endothelium of the liver (1). It catalyses the interconversion of high density lipoprotein (HDL) subclasses and is also involved in the degradation of intermediate density lipoproteins (1). Metabolic control of hepatic lipase is mediated by hormones, such as insulin, gestagens (2-5), and thyroid hormones (6). Nervous regulation of hepatic lipase activity has also been demonstrated; rats denervated at the liver hilus had a 20% higher enzyme activity than sham-operated controls (7).

The interdependence between the thyroid gland and the autonomic nervous system is well established (8). The purpose of the present study was to investigate the effects of two denervation procedures, hepatic hilar denervation and hepatic vagotomy, on the regulation of hepatic lipase activity. To amplify the possible stimulatory effect of denervation (7) we conducted the experiment in hypothyroid rats, which have a decreased hepatic lipase activity (9).

Material and Methods

Animals and surgical procedures

Male Sprague-Dawley rats, weighing about 240 g, were used. They were housed in single cages on a 06.00-18.00 h light cycle at 23°C. The rats received chow ad libitum; no effect by hepatic hilar denervation on feeding was recorded in an earlier study (7). Prior to operation, all animals were provided with 6-n-propyl-2-thiouracil (PTU) in the drinking water (0.1 g/l) during 8 days. This regimen has been demonstrated to induce a hypothyroid state (10).

Animals were operated on under ether anesthesia. Hepatic hilar denervation was performed as previously described (11). Briefly, the hilar area was exposed, nerves were visualized by application of toluidine blue solution, and each discernible trunk was divided under the microscope.

The hepatic vagus branch (originating from the anterior trunk) was divided in the lesser omentum; the concomitant artery was spared (12). When present, the small hepatic branch, connected to the pyloric branch of the anterior vagus, was also divided. Care was taken to perform sham operations in an identical manner without actually dividing the nerves.

After the surgical procedure, the animals were kept for eight days with free access to chow and water containing PTU. Food intakes and body weights of all groups were recorded daily, and did not differ significantly. The rats were then fasted overnight, still having free access to water containing PTU. The animals were sacrificed by
CO₂ between 09.00 and 12.00 h on the ninth postoperative day. The thoracic cavity was immediately opened, and 7-9 ml of blood was drawn by cardiac puncture. The blood was allowed to clot, and serum was separated by centrifugation. A piece of the left liver lobe (155-200 mg wet weight) was removed and frozen at −70°C for later assay of hepatic lipase activity. Adjacent pieces of tissue were removed and frozen for determination of norepinephrine as a marker for sympathetic denervation (13), and neuropeptide Y and vasoactive intestinal peptide (VIP), which have been suggested as markers for parasympathetic denervation (14,15).

**Analytical methods**

Serum concentrations of free T₃ were determined by a radioimmunoassay kit (Amerlex-M) from Amersham International, UK.

Hepatic lipase activity in the liver tissue was determined as described by Hansson et al. (7). Briefly, the tissue was homogenized, and floating fat removed by centrifugation. The pellet, containing the membrane fraction, was suspended in buffer containing heparin (15 kU/l) in order to release the enzyme into the medium. The medium, which contains >80% of the hepatic lipase activity (7,16) was assayed for hepatic lipase activity by a specific method using a radiolabelled triglyceride emulsion as substrate (17), and protein was measured after precipitation by trichloroacetic acid (18). The protein content of the extract differed by less than 12%. Lipase activity was expressed as mU/mg protein.

Serum concentrations of cholesterol, triglycerides and HDL cholesterol were determined by enzymatic methods (Boehringer Mannheim GmbH, FRG); HDL cholesterol was measured after precipitation of VLDL and LDL by dextran sulphate and MgCl₂ (19). Serum lipoproteins were also studied by zonal ultracentrifugation of a pooled serum sample from each experimental group (20).

Statistical differences between the groups were evaluated by the Mann-Whitney U-test.

**Results**

All animals were hypothyroid (serum concentration of free T₃ <0.9 pmol/l). Sham operation decreased hepatic lipase activity by about 40% and reduced neuropeptide Y by about 20% (Table 1), but norepinephrine and VIP concentrations were not affected by sham operation (Table 1).

Hepatic hilar denervation increased hepatic lipase activity by about 40%, and hepatic vagotomy increased hepatic lipase activity by almost 35% (Table 1), as compared with sham operation. Hepatic lipase activities of these groups did not differ significantly from those recorded in the non-operated rats.

Liver concentrations of norepinephrine were reduced by about 90% after hilar denervation. Hepatic vagotomy did not affect norepinephrine levels (Table 1). The liver tissue of hilar denervated animals contained more VIP and less neuropeptide Y than sham-operated controls. On the other hand, neither VIP nor neuropeptide Y levels in vagotomized animals differed from those of sham-operated controls (Table 1). The increase in hepatic lipase activity in the two denervated groups was not accompanied by changes in cholesterol or

**Table 1.**

Hepatic lipase activities and neural transmitter marker concentrations in non- and sham-operated hypothyroid rats, and in hypothyroid rats subjected to hilar denervation or hepatic vagotomy (mean ± s.d., N=9).

<table>
<thead>
<tr>
<th></th>
<th>Non-operated</th>
<th>Sham-operated</th>
<th>Hilar denervated</th>
<th>Vagotomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic lipase activity (mU/mg protein)</td>
<td>13.6±1.6</td>
<td>8.2±2.7</td>
<td>11.6±1.2</td>
<td>11.0±2.0</td>
</tr>
<tr>
<td>Norepinephrine (pmol/g tissue)</td>
<td>262±100</td>
<td>186±75</td>
<td>19±14</td>
<td>180±82</td>
</tr>
<tr>
<td>VIP (pmol/g tissue)</td>
<td>0.20±0.052</td>
<td>0.21±0.062</td>
<td>0.28±0.050</td>
<td>0.22±0.049</td>
</tr>
<tr>
<td>Neuropeptide Y (pmol/g tissue)</td>
<td>4.4±1.0</td>
<td>3.4±0.7</td>
<td>2.2±0.3</td>
<td>3.3±1.0</td>
</tr>
</tbody>
</table>

a: p = 0.0023; b: p = 0.0339 compared with non-operated rats; c: p = 0.0046; d: p = 0.0305; e: p = 0.0002; f: p = 0.0132; g: p = 0.0013 compared with sham-operated rats.
non-HDL cholesterol concentrations (Table 2). Serum HDL cholesterol levels were slightly decreased in both the denervated groups. However, these changes did not reach statistical significance. Serum triglyceride levels were lower in the hilar denervated group (p=0.05; Table 2).

Zonal ultracentrifugations revealed no changes in concentration and composition of the major lipoprotein classes VLDL and HDL₂ in the two denervated groups compared with sham-operated controls (data not shown).

## Discussion

Neural regulation of liver metabolism is not fully understood. In this study we extend our previous investigations which demonstrated an increase in hepatic lipase activity in hilar denervated liver (7).

We chose to perform the experiments in hypothyroid animals for two reasons. Firstly, hypothyroid rats with low hepatic lipase activities (9) might be a better model for studying increases in hepatic lipase activities caused by denervation. Secondly, the experimental model made it possible to investigate the relations between thyroid and neural regulation of hepatic lipase activity. It is well established that thyroid hormones have a stimulatory effect on hepatic lipase activity in vivo (6), and that they interact especially with catecholamines (8).

Owing to the risk of re-innervation (sprouting), it is conceivable that metabolic experiments on denervated tissue should be performed within 10 days after surgery. At this time point, metabolic effects of the surgical trauma might still be present. By including both non-operated and sham-operated controls it was possible to observe the effects of surgery as such. The decrease in hepatic lipase activity after sham operation may be an effect of traumatic stress or of the acute phase reaction developing after surgery (21). These data emphasize the importance of selecting proper control groups.

As previously shown (7), hilar denervation is associated with a marked decrease in hepatic noradrenaline content. Neuropeptide Y and VIP have been proposed as markers for sympathetic and parasympathetic innervation, respectively (22,15). There was a decrease in neuropeptide Y contents after hilar denervation, although less marked than the decrease in catecholamine content. By contrast, it seems that VIP (under these conditions) is not a good marker of parasympathetic denervation, since the hepatic content of the peptide was unrelated to the actual surgical procedures done.

Both denervated groups demonstrated significantly higher hepatic lipase activities than the sham-operated controls. The increase was more marked than that seen in euthyroid hilar denervated animals (7). There are two possible interpretations of these results: It is possible that our data reflect a general regulatory function of neural impulses on HL activity; however, it cannot be excluded that denervation influences hepatic lipase activity.

### Table 2.
Serum lipid and lipoprotein concentrations in non-operated and sham-operated hypothyroid rats, and in hypothyroid rats subjected to hilar denervation or hepatic vagotomy (mean ± so; N = 9).

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>S-cholesterol (mmol/l)</td>
<td>3.06±0.32</td>
<td>3.16±0.49</td>
<td>3.13±0.25</td>
<td>2.98±0.31</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>2.10±0.10</td>
<td>2.06±0.30</td>
<td>1.96±0.17</td>
<td>1.88±0.29</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/l)</td>
<td>1.08±0.33</td>
<td>1.18±0.25</td>
<td>1.18±0.19</td>
<td>1.10±0.13</td>
</tr>
<tr>
<td>S-triglyceride (mmol/l)</td>
<td>0.59±0.19</td>
<td>0.71±0.13</td>
<td>0.56±0.12a</td>
<td>0.67±0.16</td>
</tr>
</tbody>
</table>

a: p = 0.05 compared with sham-operated rats.
activity mainly by preventing the decrease of hepatic lipase activity occurring after sham operation. In both cases, alterations in blood flow to the liver may be involved. Although there is no experimental evidence of changes in blood flow after liver denervation, it has been demonstrated that there is a 3- to 4-fold increase in blood loss after standardized liver resection in the rat if the resection is preceded by denervation at the liver hilus (23).

It should be noted that both hilar denervation and vagotomy affect hepatic lipase activity similarly, despite widely different effects on hepatic norepinephrine content. Therefore, hepatic noradrenergic innervation does not seem to be involved in the regulation of hepatic lipase activity in this experimental model. However, our data do not exclude an involvement of circulating catecholamines (24), since plasma concentrations of norepinephrine and epinephrine were not measured. Our findings could be related to the lack of distinctly separated autonomous innervation of the liver (25), i.e. that parasympathetic and sympathetic innervation are not supplied in anatomically distinct structures.

We did not register any significant differences in plasma lipoprotein concentrations between the experimental groups. Probably, the differences in hepatic lipase activity are too discrete to affect the lipoprotein pattern. The hepatic lipase system, like the lipoprotein lipase system, normally operates well below substrate saturation (26), and therefore more marked reductions in enzyme activity probably are necessary to affect lipoprotein metabolism.

The present study further emphasizes the complexity of neural regulation of hepatic function. In particular, hepatic vagotomy needs to be studied further with regard to chemical markers and metabolic effects.

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Dr Peter Nilsson-Ehle,
Department of Clinical Chemistry,
University Hospital,
S-221 85 Lund,
Sweden.