Increased triglyceride secretion rate and hyperinsulinaemia in ventromedial hypothalamic lesioned rats in vivo

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Abstract. The present study aimed to measure triglyceride secretion rate (TGSR) into the circulation in ventromedial hypothalamic (VMH) lesioned rats. Average gain of body weight in VMH lesioned rats was 72 ± 6 g (mean ± s.e., n = 9) in a week; significantly greater than that in controls (6 ± 2, n = 8, P < 0.001). TGSR was determined under hexobarbital anaesthesia in fasted rats by measuring the increase in plasma concentration after the triglyceride removal mechanism was blocked by injecting Triton WR-1339. TGSR in VMH lesioned rats was 500 ± 37 mg/dl of plasma/h; markedly higher than that in controls (239 ± 12, P < 0.001). Serum insulin concentration in VMH lesioned rats was 2.26 ± 0.32 ng/ml; significantly higher than that in controls (0.85 ± 0.08, P < 0.001). There was a positive correlation between serum insulin concentration and TGSR in VMH lesioned rats (r = 0.709, P < 0.05). The increased secretion rate of triglyceride in VMH lesioned rats is discussed in connection with the development of obesity in these rats.

Ventromedial hypothalamic (VMH) obesity produced by destruction of the ventromedial region of the hypothalamus is a consequence of excessive fat deposition in various organs. For the development of VMH obesity, several mechanisms have been suggested (Bray & York 1979).

We have indicated that neurally mediated hyperinsulinaemia is the primary factor in development of this obesity by showing that VMH lesioned rats that received pancreatic transplants developed neither hyperinsulinaemia nor obesity (Inoue et al. 1977a, 1978). Recently we also showed that lipoprotein lipase (LPL) in postheparin plasma increased in VMH lesioned rats. We suggested that the increase reflected increased adipose tissue LPL activity through hyperinsulinaemia, and this accelerated the uptake of plasma triglyceride into adipose stores, to contribute one factor to the cause of hypothalamic obesity (Inoue & Murase 1982). The process probably proceeds along with the increase in triglyceride secretion by the liver. Such a possibility has been suggested in VMH lesioned rats in vitro (Karakash et al. 1977), however, this has not yet been examined in vivo.

In the present study, we measured triglyceride secretion rate (TGSR) indirectly with the use of Triton in VMH lesioned rats (Otway & Robinson 1967). Because food intake affects TGSR (Otway & Robinson 1967), we examined TGSR in rats deprived of food for 14 h. Under such conditions exogenous triglyceride is absent in the plasma and endogenous triglyceride secretion can be selectively measured.

Materials and Methods

Female Sprague-Dawley rats weighing approximately 220 g were used. The animals were divided into two groups; a VMH lesioned group (n = 9) and a control

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group \((n = 8)\). Electrolytic lesions of bilateral VMH were produced by a method previously described \(\text{[Inoue et al. 1977b]}\). Sham-operated rats were used as controls. One week after VMH lesions, the rats, which were fasted overnight, were subjected to the following experiments: under ip hexobarbital anaesthesia \((50 \text{ mg/kg})\), 0.7 ml of blood samples were taken from the subclavian venous plexus for measurement of plasma triglyceride and serum insulin. Then, 120 mg of Triton WR-1339 \(\text{[Nakarai Chemical Co. Tokyo, Japan]}\), a non-ionic detergent, was injected iv. Post-Triton samples were collected 45 and 90 min after the Triton injection.

The samples and their timings were used to determine TGRS by the following equation:

\[
\text{TGRS} = \frac{\text{TG}_{45} - \text{TG}_0}{45} + \frac{\text{TG}_{90} - \text{TG}_0}{90} \times 60
\]

\(\text{TG}_0\), \(\text{TG}_{45}\) and \(\text{TG}_{90}\) indicate triglyceride concentrations in plasma collected 0, 45 and 90 min after Triton injection. TGRS is usually calculated by multiplying this formula by the plasma volume, but we omitted this step in the present study since we found that VMH lesioned rats did not have increased plasma volume after increase in body weight \(\text{[Yamakado et al. 1981]}\).

Immunoreactive insulin was determined by the method of Hales & Randle \(\text{[1963]}\) using rat crystal insulin as a standard. Plasma triglyceride was determined enzymatically \(\text{[Bucolo & David 1973]}\). Comparisons were evaluated by Student’s \(t\)-test with the minimum level of significance at \(P < 0.05\).

### Results

**Measurement of TGRS**

Triton inhibits the removal of triglyceride from the circulation by interrupting the contact of lipoprotein triglyceride with LPL. In preliminary experiments, Triton at a concentration of \(2 \text{ mg/dl}\) suppressed postheparin lipolytic activity in vitro by 84%. When LPL activity is blocked by Triton in vivo, plasma triglyceride concentration increase because the liver continues to secrete triglyceride into the circulation.

A dose of 80 mg Triton increased plasma triglyceride secretion rate to its maximal level \(\text{(Fig. 1, left)}\). When 120 mg of Triton was injected, plasma triglyceride concentration rose linearly for at least 90 min \(\text{(Fig. 1, right)}\). Based on these data, we used 120 mg per rat of Triton and took blood samples at 45 and 90 min after Triton injection.

**Fig. 1.**

Effect of Triton WR-1339 on triglyceride secretion rate \(\text{(TGRS)}\). Left: TGRS dependence on Triton dose. Each point: mean \(\pm\) sd \((n = 3)\). Right: time dependence of serum triglyceride after 120 mg dose of Triton \(\text{(at arrow)}\). ● and ▲: two different rats; same conditions.
Body weight, serum insulin and plasma triglyceride concentrations, and triglyceride secretion rate (TGSR) in VMH lesioned and control rats.

<table>
<thead>
<tr>
<th>No.</th>
<th>Body weight</th>
<th>Serum insulin (ng/ml)</th>
<th>Plasma triglyceride (mg/dl of plasma/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td></td>
</tr>
<tr>
<td>VMH</td>
<td>9</td>
<td>225 ± 5*</td>
<td>297 ± 10**</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>222 ± 5</td>
<td>228 ± 4</td>
</tr>
</tbody>
</table>

VHM: ventromedial hypothalamic lesioned rats. * values are mean ± se. **P < 0.001.

Body weight, plasma triglyceride, serum insulin concentrations and TGSR

In 1 week after VMH operations, the average increase in body weight of VMH lesioned rats was 72 g, while in sham-VMH lesioned rats, it was 6 g (Table 1). No difference was found between the two groups in fasting plasma triglyceride concentration (Table 1). VMH lesioned rats had significantly higher serum insulin concentrations than controls (Table 1). TGSR of the VMH lesioned rats was 500 ± 37 mg/dl/h (mean ± se), which was significantly higher than that of the controls (239 ± 12) (P < 0.001) (Table 1).

There was a positive correlation between TGSR and serum insulin concentration in VMH lesioned rats (r = 0.709, P < 0.05), whereas no correlation was found in controls (r = −0.162) (Fig. 2).

Discussion

The present study demonstrates that VMH lesioned rats show 1) increase both in TGSR and serum insulin concentrations, and 2) a positive correlation between these two changes in VMH lesioned rats, but not in controls. The increased TGSR agrees with an in vitro study by Karakash et al. (1977) who showed that TGSR by the perfused liver of VMH lesioned rats increased.

In the present study VMH lesioned rats had a fasting plasma triglyceride concentration before Triton injection that was similar to that of control animals, although TGSR was increased. Our plasma triglyceride concentration data in VMH lesioned rats differ from those of Bernardis & Schnatz (1971) who showed elevated plasma triglyceride concentration in weanling VMH lesioned rats. The discrepancy between our results and theirs may arise from the difference in blood sampling methods; they took blood samples from rats fed ad libitum, while we sampled from rats after an overnight fast. We suggested previously that adipose tissue LPL activity is increased in VMH lesioned rats (Inoue & Murase 1982), and we presume now that the normal fasting plasma triglyceride concentration in VMH lesioned rats in the present study can be explained by assuming that the secreted triglyceride is taken up into adipose stores because of the high LPL and adequate capacity of the adipose tissue.
The increased TGSR should be endogenous in origin and not due to increased food intake, because our study was performed under fasted conditions.

There was a positive correlation between serum insulin concentrations and TGSR in VMH lesioned rats in the present study because hyperinsulinaemia enhances TGSR (Robertson et al. 1973). This triglyceride promoting effect of insulin has also been demonstrated directly by liver perfusion (Woodside & Heimberg 1976; Topping & Mayes 1972). The lack of correlation between serum insulin and TGSR in normal rats could be attributed to feedback through the VMH. Thus, increase of triglyceride could suppress insulin secretion through the VMH which would, in turn, limit triglyceride secretion to keep both factors at very low levels.

We have indicated that hyperinsulinaemia is of prime importance in the development of obesity in VMH lesioned rats (Inoue et al. 1978; Inoue & Bray 1979, 1980). The present study suggests that hyperinsulinaemia produced by VMH lesions increases TGSR. The increased TGSR coupled with accelerated triglyceride deposition into adipose stores (Inoue & Murase 1982) is probably an important factor in the development of obesity in VMH lesioned rats.

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References


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