Prolactin has been claimed to be diabetogenic, because hyperprolactinemia is associated with decreased insulin binding in vitro and insulin resistance in vivo (4). The above mentioned statement is based on the findings obtained in human patients suffering from severe hyperprolactinemia. Cincotta and Meier (1) suggested that prolactin has a permissive role in supporting the hepatic lipogenic activities of insulin and that bromocriptine, adopaminergic agonist which inhibits prolactin secretion, can be used to reduce lipogenesis. When the prolactin is applied in the bromocriptine treated animals, hypolipidemic effect of bromocriptine is missing. Thus the above mentioned authors claimed the permissive role of prolactin in the lipid metabolism.

In our previous paper (2) we documented that terguride, i.e., dopaminergic agonist is potent to alleviate hyperlipidemia in obese and lean genetically hypertensive Koletsky rats. There remained to be solved the possible role of prolactin in the mentioned alleviation. It is a subject of the recent paper.

**Material and methods**

Experiments were performed in obese and lean genetically hypertensive rats of Koletsky type (3) of both sexes and in males and females rats of Wistar strain. Lean Koletsky SHR rats represent dominant non-obese homozygotes and heterozygotes whereas their obese siblings are recessive homozygotes. The abnormal animals were obtained by Koletsky (3) when mating spontaneously hypertensive rat (Okamoto -Aoki strain) with a normotensive Sprague-Dawley male rat. The genetically obese animals appeared after several generations of selective inbreeding of hypertensive off-springs of the original cross.

After weaning at the age of 30 days the animals were kept in groups of four and supplied with water and ST-lpelleted diet ad libitum.

**Plasma insulin**

Plasma insulin was determined by radioimmunoassay.

**Plasma prolactin**

Plasma prolactin was determined by radioimmunoassay using rat prolactin for standard curve and aspecific antibody to rat prolactin.

**Plasma lipids**

Blood sampled by cardiac puncture (in light ether anaesthesia at 7.00 after 14 h starvation) was centrifugated and the serum was stored in plastic tubes at -20 °C. Total plasma cholesterol and plasma triglycerides were determined enzymatically by Hitachi analyzer.

**Summary**

Plasma prolactin was measured in genetically hypertensive obese Koletsky rats, in their lean siblings and in normotensive rats of Wistar strain. Lean as well as obese females show hyperprolactinemia. The males of Wistar strain as well as obese rats and their siblings show comparable prolactinemia except lean males which show higher level than Wistar males. Sex dependence of prolactinemia is missing in the rats of Wistar strain. Long lasting terguride treatment decreases prolactinemia in obese as well as lean rats of both sexes. The drug showed decreased prolactinemia in the males of Wistar strain. When the group of rats are considered in correlation computation positive correlation can be documented between total plasma cholesterol and plasma prolactin. In obese females positive correlation was found between plasma insulin and plasma prolactin.

**Key words:** Koletsky SHR obese and lean rats; Prolactinemia; Terguride; Cholesterol; Triglycerides
Glucose tolerance
Blood was sampled to heparinized capillaries (from retrotubular plexus under light ether anaesthesia) before glucose loading (basal glycemia) as well as at 30, 60, 120 and 180 min after glucose loading. Glucose (3g/kg b.w. 30 min) was injected intragastrically after 14h starvation. Glycemia was analysed enzymatically (Oxochrom glucose, Lachema). Glycose tolerance was expressed as a sum of glycemia obtained 30,60,120 and 180 min after glucose loading (area under the glucose tolerance curve”).

Terguride treatment
The drug was applied i.p. in two daily doses (7.00 and 14.00) for 21 days (when laminea was investigated) or for 11 days only (when glucose tolerance was monitored). Terguride maleate was administered at a dose of 0.1mg/kg.

Table 1: Plasma prolactin effect of long lasting terguride treatment
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Terguride</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRM</td>
<td>3.41±(8)</td>
<td>2.00±(8)</td>
<td>0.00</td>
</tr>
<tr>
<td>NRF</td>
<td>2.79±(7E)</td>
<td>1.91±(8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHR</td>
<td>3.72±(6E)</td>
<td>1.44±(8)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHR-F</td>
<td>39.4±(24±)E</td>
<td>36.1±(24±)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHR-M</td>
<td>37.6±(35)</td>
<td>2.72±(0)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHRF</td>
<td>43.1±(01)E</td>
<td>5.2±(38)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 1: Mean±SD. Effect of long lasting terguride treatment on plasma prolactin in the rats of Wistar strain, in the genetically hypertensive obese rats of Koletsky rats and in their lean siblings. Abbreviations: NR - rats of Wistar strain, SHRF - genetically hypertensive obese rats of Koletsky type, SHRM - genetically hypertensive lean rats of Koletsky type. In brackets - number of rats in the group. Interstrain differences: c - P<0.02, d - P<0.01. Interstrain difference: D - P<0.00.

Table 2: Total plasma cholesterol: effect of long lasting terguride treatment
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Terguride</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRM</td>
<td>1.66±(24)</td>
<td>1.66±(24)</td>
<td>0.00</td>
</tr>
<tr>
<td>NRF</td>
<td>1.79±(46)</td>
<td>1.54±(16)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHR</td>
<td>1.80±(26)</td>
<td>1.84±(18)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHR-F</td>
<td>2.4±(24)E</td>
<td>1.99±(28)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHR-M</td>
<td>2.18±(37)E</td>
<td>2.39±(55)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHRF</td>
<td>2.5±(26)E</td>
<td>2.24±(30)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 2: Mean±SD. Total plasma cholesterol. Effect of long lasting terguride treatment. The abbreviations are the same as in Table 1.

Table 3: Plasma triglycerides: effect of long lasting terguride treatment
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Terguride</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRM</td>
<td>0.59±(17)</td>
<td>0.70±(21)</td>
<td>n.s.</td>
</tr>
<tr>
<td>NRF</td>
<td>0.62±(19)</td>
<td>0.60±(15)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHR</td>
<td>0.89±(15)</td>
<td>0.94±(19)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHR-F</td>
<td>0.86±(71)E</td>
<td>0.79±(15)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHR-M</td>
<td>3.5±(20)E</td>
<td>4.0±(7)</td>
<td>0.00</td>
</tr>
<tr>
<td>SHRF</td>
<td>3.72±(09)E</td>
<td>2.8±(40)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3: Mean±SD. Plasma triglycerides. Effect of long lasting terguride treatment. Abbreviations are the same as in Table 1.

Discussion
Cincotta and Meyer (1) were the first who directed the attention to the relationship between prolactin and lipid metabolism. They noted the fat reducing effect of bromocriptine in several strains of animals. Meier et al. (4) described that bromocriptine administration reduces body fat stores in obese postmenopausal females and alleviates hyperglycemias in type II diabetes in human patients. Scherthaner et al. (5) documented in human patients that severe hyperprolactinemia is associated with decreased insulin binding in vitro and insulin resistance in vivo. The above mentioned data suggest relationship between the prolactin on one side and the lipid and glucose metabolism on the other side. In our series of experiments there can be demonstrated possible correlation between total plasma cholesterol and prolac tinemia. When we consider the mean of individual groups of rats then we found r = -0.9049, P<0.02, n = 6 when parametric Pearson correlation coefficient is judged, and r = -0.8236, P<0.05, n = 6 when non-parametric Spearman correlation was calculated. We obtained similar correlation between prolac tin/prolactinemia and plasma triglycerides, but the correlation did not attain statistical significance (r = 0.1714, n = 6 when Spearman correlation was used, r = -0.5467, n = 6 when Pearson correlation was used.

The above mentioned correlations do not exclude the possibility that prolactin takes a part in the regulative mechanism of lipid metabolism. The mentioned assumption is not in contradiction with sex dependent elevation of total plasma cholesterol in obese males as well as in lean Koletsky rats (see Table 2), where at the same time hyperprolactinemia can be demonstrated (see Table 1). In both cases cholesterol as well as prolactin is elevated in females. In the mentioned females terguride alleviates not only the hyperprolactinemia but decreases total plasma cholesterol as well.

Different picture can be found when we analyze plasma triglycerides. The sex dependence in the controls is not presented, but strain and substrain dependence is profoundly expressed (see Table 3). Triglycerides in obese rats are three or four times higher than in lean Koletsky rats. Strain dependence is expressed also in the terguride effect. Mentioned drug alleviates hyperprolactinemia only in obese females. As mentioned above hyperprolactinemia in lean as well as in obese Koletsky rats, hyperprolactinemia being elevated in females. Statistically significant hyperprolactinemia is observed only in males of all groups of rats except normotensive females.

Considering the total plasma cholesterol in controls (Table 2) sex dependence is obvious in lean as well as in obese Koletsky rats, elevation in is females. Sex dependence is apparent between normotensive and lean females in the last mentioned rats there is increase.

When we consider plasma triglycerides, strain dependence is obvious (Table 3). Thus lean Koletsky rats of both sexes show higher triglycerides than the rats of Wistar strain and at the same time they show lower triglycerides than obese Koletsky rats of both sexes. Sex dependence in triglycerides is not expressed.

Terguride alleviates triglycerides only in the obese male females of Koletsky rats.

Considering control animals in the results of glucose tolerance test, substrain dependence is apparent, obese of both sexes show elevated glucose intolerance. Terguride alleviates glucose intolerance in both substrains. But there is a strain dependence. While in obese rats decrease of the area under the curve represents 44% in both sexes, then in lean Koletsky rats this decrease is represented by 10% (males) or 11% (females).

We noted no relationship between prolactinemia and on glucose tolerance. Considering control animals in the results of glucose tolerance curve”. Glucose tolerance was not monitored in the rats of Wistar strain presented in this paper (the results of glucose tolerance test which were obtained in the other group of rats of Wistar strain can be found in paper No 2 - see references). Interstrain differences: B - P<0.05, D - P<0.01. The other abbreviations are the same as in Table 1.

Table 4: Glucose tolerance test. Effect of long lasting terguride treatment
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Terguride</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRM</td>
<td>334±39</td>
<td>484±32</td>
<td>0.05</td>
</tr>
<tr>
<td>SHR</td>
<td>542±33</td>
<td>492±31</td>
<td>0.05</td>
</tr>
<tr>
<td>SHR-M</td>
<td>744±206</td>
<td>531±46</td>
<td>0.01</td>
</tr>
<tr>
<td>SHR-F</td>
<td>523±20</td>
<td>523±20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4: Mean±SD. Plasma insulin. Long lasting terguride treatment. The abbreviations are the same as in Table 1.

Concluding remarks
We are grateful to Carl T. Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA, for providing us with terguride.

Acknowledgement
This paper was supported by Internal Grant Agency of Ministry of Health of the Czech Republic No 3684-3. The authors wish to thank Carl T. Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA, for providing the genetically hypertensive rats of Koletsky type.
Glucose tolerance
Blood was sampled to heparinized capillaries (from retrobulbar plexus under light ether anaesthesia) before glucose loading (basal glycaemia) as well as 30, 60, 120 and 180 min after glucose loading. Glucose (3g/kg b.w. 30 min) was applied intragastrically after 14th starving. Glycaemia was analysed enzymatically (Oxochrom glucose, Lachema). Glycose tolerance was expressed as a sum of glycaemia obtained 30, 60, 120 and 180 min after glucose loading ("area under the glucose tolerance curve").

Teruguride treatment
The drug was applied i.p. in two daily doses (7:00 and 14:00) for 21 days (when lipide was investigated) or for 11 days only (when glucose tolerance was monitored).

Teruguride maleate was administered at a dose of 0.1mg/kg.

Results
Considering prolactin in the control animals strain dependence is apparent, i.e. SHR lean rats show higher plasma prolactin than rats of Wistar strain. When comparing the obese and lean Koletsky SHR rats no differences in prolactinemia were found.

Profound sex dependence in prolactinemia was found in females. Abbreviations: NR - rats of Wistar strain, SHR - genetically hypertensive obese rats of Koletsky type, SHR-O - genetically hypertensive obese rats of Koletsky type. Interstrain differences: c - P<0.05, d - P<0.01. Intersex differences: D - P<0.05.

Table 2: Total plasma cholesterol: effect of long lasting terguride treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Terguride</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR-M</td>
<td>1.46±0.2(8)</td>
<td>1.60±0.2(8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>NR-F</td>
<td>1.79±0.6(8)</td>
<td>1.54±0.6(8)</td>
<td>0.01</td>
</tr>
<tr>
<td>SHR-M</td>
<td>1.80±0.6(8)</td>
<td>1.84±0.6(8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHR-F</td>
<td>2.49±0.8(8)</td>
<td>1.99±0.8(8)</td>
<td>0.3</td>
</tr>
<tr>
<td>SHR-O-M</td>
<td>2.18±0.7(8)</td>
<td>2.39±0.5(8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHR-O-F</td>
<td>2.56±0.7(10)</td>
<td>2.24±0.3(10)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 3: Plasma triacylglycerides: effect of long lasting terguride treatment

Discussion
Cincotta and Meyer (1) were the first who directed the attention to the relationship between prolactin and lipide metabolism. They described the fat reducing effect of bromocriptine in several strains of animals. Meier et al. (4) described that bromocriptine administration reduces body fat stores in obese postmenopausal females and alleviates hyperlipemia in type II diabetes in human patients.

The above mentioned data suggest relationship between prolactin on one side and the lipide and glycide metabolism on the other side. In our series of experiments there can be demonstrated positive correlation between total plasma cholesterol and prolactinemia. When we consider the mean of individual groups of rats then we found r = 0.9049, P<0.02, n = 6 when parametric Pearson correlation coefficient is judged, and r = -0.8286, P=0.01, n = 6 when non-parametric Spearman correlation was calculated. We obtained similar correlation between plasma prolactin and plasma triglycerides, but the correlation did not attain statistical significance (r = 0.1885, P>0.05 when Spearman correlation was used. 

We found the above correlations do not exclude the possibility that prolactin takes part in the regulative mechanism of lipid metabolism. The mentioned assumption is not in contradiction with sex dependent elevation of total plasma cholesterol in obese as well as in lean Koletsky rats (see Table 2), where at the same time hyperprolactinemia can be demonstrated (see Table 1). In both cases cholesterol as well as prolactin is elevated in females. In the mentioned females terguride alleviates not only the hyperprolactinemia but decreases total plasma cholesterol as well. 

Table 4: Glucose tolerance test. Effect of long lasting terguride treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Terguride</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-M</td>
<td>333±39(8)</td>
<td>484±32(8)</td>
<td>0.05</td>
</tr>
<tr>
<td>SHR-F</td>
<td>542±36(8)</td>
<td>493±33(8)</td>
<td>0.05</td>
</tr>
<tr>
<td>SHR-O-M</td>
<td>764±206(10)</td>
<td>531±86(10)</td>
<td>0.01</td>
</tr>
<tr>
<td>SHR-O-F</td>
<td>653±202(10)</td>
<td>517±79(10)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Intersex differences: B - P<0.05, D - P<0.01.

Acknowledgement
This paper was supported by Internal Grant Agency of Ministry of Health of the Czech Republic No 368-4-1. The authors wish to thank Carl T. Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA, for providing the genetically hypertensive rats of Koletsky type.
References


Doc. MUDr. PhDr. Věroslav Golda, CSc., Institute of Experimental Neurosurgery, Charles University, Faculty of Medicine and Teaching Hospital, 500 05 Hradec Králové, Czech Republic.

Original Article

DEVELOPMENT OF LIPID AND GLYCOSE ABNORMALITIES IN GENETICALLY HYPERTENSIVE OBESE KOLETSKY RATS AND IN THEIR LEAN SIBLINGS

Věroslav Golda, Jiřina Hilgertová

Institute of Experimental Neurosurgery, Charles University, Faculty of Medicine and Teaching Hospital, Hradec Králové; (Head: doc. MUDr. J. Náhlovský, CSc.) Laboratory for Endocrinology and Metabolism, Charles University, Faculty of Medicine, Prague; (Head: prof. MUDr. V. Schreiber, DrSc.).

Summary: Experiments were performed in the genetically hypertensive Koletsky rats and in their lean siblings at the age of two and three months. In the study of development of glycide and lipid abnormalities animal represents control for itself. At the age of two months Koletsky obese rats show relative to their lean controls a decrease in plasma triglycerides (males +84%, females +52%) and insulin (males +89%, females +20%). During one month plasma triglycerides increased in lean males +9%, in lean females 0%, but in obese males +21%, in obese females +139%. Considering insulinemia similar results were obtained. Thus during one month insulin elevates in lean males +10%, in lean females +23%, but in obese males +80%, in obese females +144%. During one month glucose intolerance is elevated as well only in obese rats. Total plasma cholesterol during period of one month shows no changes in both substrains of rats. Similar picture can be found in basal glycemia. In all groups of rats no changes were registered except one, i.e., obese females show decrease. Considering the strain differences in basal glycemia then at age of one as well as two months obese of both sexes show elevation. As to the body weight at the age of two as well as three months there is increase in obese rats. The changes of body weight during one month are expressively higher in obese rats.

Key words: Development of glycide and lipid abnormalities; Koletsky obese and lean SHR rats; Insulinemia; Glucose tolerance; Triglycerides; Cholesterol; Basal glycemia

Introduction

Developmental changes of abnormalities in genetically hypertensive obese Koletsky rats were originally described by Koletsky (8). He analyzed the body weight from two to ten months of age in the obese rats and in their lean siblings. The author monitored development of plasma triglycerides from two to twelve months. In the period from two months to four months triglycerides elevate from 1.98 mmol/l to 4.67 mmol/l. Total plasma cholesterol in this same period elevated from 2.49 mmol/l to 3.74 mmol/l. The author did analyzed sexual differences. At recent time one group of authors turn their attention to developmental aspect of metabolic abnormalities in the rats which can be viewed as a potential model of type II diabetes. Ionescu et al. (7) studied blood glucose tolerance in genetically obese fa/fa rats at 6- to 7-wk and 13- to 14-wk-old lean and obese (fa/fa) rats. They found that glucose intolerance became more pronounced with the duration of the syndrome. Moreover, the 6- to 7-wk obese rats showed normal and even higher beta-cell responsiveness to intravenous or oral glucose. In contrast, the 13- to 14-wk obese rats presented a decreased beta-cell responsiveness to such stimuli. Thus the beta-cell function of obese rats worsens with time. The mentioned authors came to the conclusion that insulin resistance, elevated basal glycemia, and abnormal glucose tolerance can be viewed as a potential model of type II diabetes.

Data accumulated in our paper (3) when we monitored plasma triglycerides, basal glycose and glucose tolerance in genetically hypertensive obese Koletsky rats and data presented in 1989 (6) when we demonstrated hyperinsulinemia in the mentioned Koletsky obese rats suggest that this type of rats can be judged as a potential genetically based animal model of diabetes II.

DeFronzo et al. (1) when summarizing our recent knowledge in the ethiology of non-insulin dependent diabetes mellitus (NIDDM) came to the conclusion that at the earliest stages of development of NIDDM there is elevated secretion of insulin.