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Effect of Terguride on Insulin Binding, Insulinaemia, Glucose Tolerance and Hyperlipaemia in Lean SHR Koletsky Rats

V. GOLDA, J. HILGERTOVÁ[1]

Institute of Experimental Neurosurgery, Hradec Králové and [1]Laboratory for Endocrinology and Metabolism, Charles University, Faculty of Medicine, Prague, Czech Republic

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Summary

Glucose tolerance, insulin binding to erythrocytes, insulinaemia, plasma total cholesterol, plasma triglycerides, weight of fat pads, food consumption and body weight changes were studied in genetically hypertensive lean Koletsky rats. Long-term treatment with dopaminergic agonist terguride (0.2 mg/kg/day) normalized glucose tolerance and increased the percentage of bound insulin to erythrocytes in both sexes. Terguride decreased insulinaemia, cholesterolaemia, fat pads and body weight only in female rats. Food consumption was not influenced by terguride over the injection period.

Key words

Glucose tolerance – Insulin binding – Insulinaemia – Fat pads – Plasma cholesterol – Plasma triglycerides – Terguride

Introduction

Obese, genetically hypertensive Koletsky (SHR/N-cp) rats as well as their lean siblings exhibit abnormalities in glucose tolerance (Golda and Cvak 1994). These abnormalities are accompanied by alteration in insulin binding to erythrocytes (Hilgertová *et al.* 1990). Insulin binding to erythrocytes was decreased in both obese and lean SHR/N-cp rats when compared to normotensive Wistar rats. On the other hand, basal plasma insulin was elevated only in the obese animals.

Glucose tolerance abnormalities are accompanied by hyperlipidaemia not only in the SHR/N-cp but also in their lean siblings (Golda and Cvak 1994). Moreover, glucose intolerance is a common trait of both subtypes and both sexes of our SHR rats. On the other hand, hyperlipidaemia is present especially in the lean SHR rats with a marked sex dependence in females.

Glucose tolerance abnormalities are alleviated by dopaminergic agonist terguride (Golda and Cvak 1994). It thus seemed to be well founded to look for possible drug-induced changes in insulin binding and plasma insulin under terguride treatment. In a series of experiments, our attention was directed towards the above mentioned parameters in lean SHR rats where the hyperlipaemia is present without obesity but only in females (Golda and Cvak 1994). We thus have a possibility to study the interrelationship between glucose intolerance in the presence (females) and absence (males) of concomitant hyperlipaemia.

Material and Methods

Animals

Experiments were carried out on lean genetically hypertensive SHR/N-cp rats (Koletsky 1975) of both sexes. Lean SHR/N-cp rats are dominant non-obese homozygotes and heterozygotes, whereas their obese siblings are recessive homozygotes (cp-cp). The abnormal animals were obtained by Koletsky (1975) when mating spontaneously hypertensive rat (Okamoto-Aoki strain) with normotensive Sprague-Dawley male rats. The genetically obese animals appeared after several generations of selective

inbreeding of hypertensive offsprings of the original crossed strain.

After weaning at the age of 30 days, the animals were kept in groups of four and supplied with water and DOS-2b (optimally balanced mixture of essential nutrients, vitamins and minerals) pelleted diet *ad libitum*. During the experiment, two animals were kept in a cage. Body weight, water and pellet intake was controlled daily (except weekends).

Terguride treatment

The drug was administered i. p. in two daily doses at 07.00 and 14.00 h for 21 days (when lipaemia, insulinaemia, insulin binding and body fat were assessed) or for 11 days (when basal glycaemia and glucose tolerance were monitored). Terguride maleate was administered in a dose 0.1 mg/kg i. p.

Insulin binding to rat erythrocytes

Plasma was separated from approximately 3 ml of heparinized blood drawn by cardiac puncture under the light ether anaesthesia. Erythrocytes were obtained by centrifugation in the Ficoll gradient, and incubated in the presence of constant amount of ^{125}I-insulin (33 pM) at 15 °C for 3 hours. The results were corrected for nonspecific binding. The details of the method were published previously (Hilgertová *et al.* 1990).

Plasma lipids

Blood samples obtained by cardiac puncture (under light ether anaesthesia at 07.00 after 14 h fasting) were centrifuged and the serum stored at −20 °C. Enzymatic colorimetric method was used for determination of total plasma cholesterol (CHOD-PAP-Boehringer) and plasma triglycerides (GPO-PAP-TRIG-Boehringer). The estimations were made carried out on a Hitachi analyzer.

Basal glycaemia and glucose tolerance

Blood was sampled into heparinized capillaries (from retrobulbar plexus under light ether anaesthesia after 14 h fasting) before glucose loading (basal glycaemia) as well as 30, 60, 120 and 180 min after the glucose load. Glucose (3 g/kg b.w., 30 % solution) was applied intragastrically after the 14 h fast. Glycaemia was estimated enzymatically.

Statistics

The data were analyzed by Student's t-test. Statistical significance of value remoteness in the individual groups was controlled by the Dixon test (1971). The values reaching statistical significance of remoteness from the other values in a group we not considered in the t-test evaluation.

Table 1. Body weight, indices of body fat stores and food consumption

	Initial body weight (g)	% changes of b.w. during injection period	Epididymal and/or periuterine fat pad (g/100 g b.w.)	Food consumption (g/100 g b.w./day)
Control				
male (7)	280 ± 13	+4.76 ± 2.09	1.29 ± 0.30	7.77 ± 0.78
female (8)	193 ± 11	+2.82 ± 2.45	1.34 ± 0.22	8.47 ± 0.88
Terguride-treated				
male (8)	263 ± 24	+2.99 ± 3.09	1.14 ± 0.27	8.01 ± 0.95
female (8)	196 ± 80	+0.67 ± 2.46[b]	0.87 ± 0.16[d]	8.22 ± 1.36

Means ± S.E.M. are presented. The number of animals per group is in the brackets. The significance of values (by unpaired t-test) refers to the comparison between control and terguride-treated animals. [b]*P < 0.05,* [d]*P < 0.01.*

Results

Effect of terguride on body weight changes and fat pads (Table 1)

There is an apparent sex dependence of the terguride effect. Terguride reduced the periuterine fat pad and the body weight increase during the injection period only in females. This terguride effect was not accompanied by any changes in food consumption.

Effect of terguride on lipidaemia (Table 2)

A similar pattern of sex dependence was also found in the effect of terguride on total plasma cholesterol as in the changes of body weight and fat pads. The drug-induced decrease could only be found in females. Terguride had no effect on plasma triglycerides, while it decreased basal plasma glycaemia, but only in males (Table 2).

Table 2. Basal glycaemia and lipidaemia

	Basal glycaemia (mmol/l)	Triglycerides (mmol/l)	Total cholesterol (mmol/l)
Control			
male (7)	5.44±0.55	0.90±0.15	1.80±0.16
female (8)	4.67±0.60	0.96±0.37	2.68±0.22
Terguride-treated			
male (8)	4.89±0.49[b]	0.94±0.19	1.84±0.13
female (8)	5.06±0.49	0.79±0.15	1.99±0.28[d]

Means ± S.E.M. are presented. Abbreviations as in Table 1.

Table 3. Glucose tolerance

	30 min	60 min	120 min	180 min	"Area under curve"
Control					
male (7)	8.91±1.26	8.14±1.48	6.10±0.30	6.21±0.49	29.31±2.14
female (8)	10.61±1.87	8.03±1.43	6.38±1.26	5.22±0.77	30.52±3.13
Terguride-treated					
male (8)	6.87±0.88[d]	7.42±1.15	6.10±0.38	6.21±0.77	26.62±2.86[d]
female (8)	8.74±0.77[d]	6.27±1.15[d]	6.10±0.38	6.21±0.49	27.06±1.81[d]

Glucose concentration (mmol/l) is given as means±S.E.M. Abbreviations as in Table 1. "Area under curve" represents the sum of glycaemia monitored 30, 60, 120 and 180 min after the glucose loading.

Table 4. Insulin binding to erythrocytes and insulinaemia

	% of bound insulin ($7x10^9$ cells)	IRI pmol/l
Control		
male	2.05±0.92 (6)	243±77 (7)
female	1.89±0.53 (7)	178±14 (7)
Terguride-treated		
male	3.19±0.70 (8)[d]	179±49 (8)[c]
female	2.66±0.52 (7)[d]	176±80 (7)

Data are means±S.E.M., abbreviations as in Table 1.

Effect of terguride on glucose tolerance (Table 3)

Terguride reduces hyperglycaemia after glucose loading in males as well as in females (see "area under the glucose tolerance curve"). The effect of terguride became evident 30 and 60 min after glucose loading in the females. In the males, a statistically significant effect of terguride was attained only 30 min after glucose loading. The effect of terguride on basal plasma glycaemia has already been mentioned above.

Effect of terguride on insulin binding and on insulinaemia (Table 4)

Terguride increased insulin binding to erythrocytes in males and females. On the other hand, terguride decreased insulinaemia only in males.

Discussion

The basic findings of the present experiments have shown that the mechanism of improved glucose tolerance can be sought at the insulin receptor side. We found (Table 4) that terguride potently increased insulin binding to erythrocytes and also influenced the glucose tolerance (Table 3). In the same animals which were studied in the present paper (lean Koletsky SHR males), Zorad found a higher percentage of bound insulin in the fat cells of epididymal pads after terguride treatment. Thus, the same effect of terguride was found in insulin-sensitive (fat cells) and insulin-insensitive (erythrocytes) tissues. The obtained data suggest the possibility of a causal relationship between the alleviation of genetically based glucose intolerance and the increase of insulin binding to tissues under the terguride treatment. The proper mechanism of terguride effect on insulin binding on erythrocytes and adipocytes still remains to be solved. Meier-Ruge *et al.* (1980) mentioned a possible effect of ergot alkaloids on the cell membrane. According to these authors, ergot alkaloids are potent agents for inducing membrane stabilization. These data suggest that ergot alkaloids potently influence some basic cell membrane mechanisms and that they could be useful for controlling the influence of terguride on some erythrocyte membrane properties. The usefulness of a study of the terguride effect on biomembranes in SHR rats is emphasized by the fact that these rats exhibit biomembrane abnormalities (Yamori *et al.* 1984).

Nevertheless, the results of our recent experiments offer insight in the problem of mutual relationship between glycide and lipid disturbances. When sex dependence of terguride effect is taken into account then the relative independence of effect of this drug on insulin binding and glucose tolerance on one side (sex dependence is missing) and on total plasma cholesterol and fat pads on the other side (sex dependence is profoundly expressed) is apparent. These findings show that the mechanisms regulating glucose tolerance and those governing total plasma cholesterol and body fat are relatively independent.

Our data are to some extent comparable with the effects of hypolipidaemic drugs when triglycerides and cholesterol on one side and insulin sensitivity on the other side are taken into consideration (for a review see Škrha 1995). It was found that the hypolipidaemic drug simvastatin alleviates hypercholesterolaemia but insulin sensitivity is unchanged. Similar data were obtained when triglycerides and insulin sensitivity are considered. Bezafibrate, etofylin-clofibrate or fenofibrate alleviate hypertriglyceridemia but insulin sensitivity remains without changes. The last mentioned data (Škrha 1995) support the assumption arising from our recent findings, i.e. relative independence of regulative mechanism of lipid and glycide metabolism.

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Reprint requests
Dr. V. Golda, Institute of Experimental Neurosurgery, Faculty Hospital, 500 36 Hradec Králové, Czech Republic.