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Terguride Attenuates Prolactin Levels and Ameliorates Insulin Sensitivity and Insulin Binding in Obese Spontaneously Hypertensive Rats

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Summary

Glucose tolerance, serum insulin, insulin receptors in epididymal fat tissue, circulating total cholesterol and triglyceride concentrations as well as serum prolactin were studied in obese and lean spontaneously hypertensive rats (SHR) of both sexes. Obese animals displayed insulin resistance and elevated insulin and triglyceride concentrations. Moreover, in obese rats the increased mass of epididymal fat tissue was accompanied with decreased capacity of high affinity binding sites of insulin receptors in the tissue plasma membranes. Terguride treatment lowered prolactin serum levels which was accompanied by ameliorated insulin sensitivity in obese animals of both sexes. In addition, terguride treatment decreased serum insulin and triglyceride concentrations in obese females and at the same time enhanced the affinity of high affinity insulin binding sites. Our results show that obesity in SHR is associated with a decreased capacity of insulin receptors and that prolactin may play a role in obesity-induced insulin resistance, particularly in female rats.

Key words

Prolactin • Obese SHR • Insulin binding • Fat tissue • Insulin sensitivity.

Introduction

The obese SHR, a rat strain originally developed by Koletsky (1975), represents an animal model of obesity and hyperinsulinemia with genetically hypertensive background. As we reported previously (Golda and Petr 1988, Golda and Cvak 1994), both obese and lean Koletsky SHR display impaired glucose tolerance. This glucose intolerance is accompanied by decreased insulin binding and internalization in hepatocytes and erythrocytes when compared to normotensive Wistar rats (Hilgertová *et al.* 1990). On the other hand, hyperinsulinemia is present only in obese SHR. In addition, the obese rats show markedly elevated levels of plasma triglycerides. Since adipose tissue is the main source of plasma free fatty acids for synthesis of

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triglycerides in the liver, we were interested in studying the state of insulin receptors in fat tissue. Insulin receptors could reflect the sensitivity of the adipose tissue to insulin which is an important negative regulator of free fatty acid production. In spite of recently reported data on reduced insulin receptor signaling in muscles and the liver of obese SHR (Friedman *et al.* 1997), the data describing insulin receptors in the adipose tissue of these rats are still lacking.

A further aim of our study was to examine the effect of terguride, a partial dopaminergic agonist, on serum prolactin levels, fat tissue mass, plasma insulin, glucose tolerance and adipose tissue insulin receptors. We have already reported that terguride reduced hyperlipidemia and normalized glucose tolerance in contrast to bromocriptine which only had a hyperlipidemia-reducing effect (Golda and Cvak 1994). Both drugs are dopamine receptor ligands although with different agonist/antagonist activity depending on their exogenous concentration (Golda and Cvak 1994). Nevertheless, it has repeatedly been shown that different types of dopaminergic agonists ameliorated obesity and associated metabolic dysfunction (Cincotta and Meier 1989, Golda and Cvak 1994, Cincotta *et al.* 1997). The mechanism of this effect of dopaminergic receptor stimulation is not yet fully understood.

Material and Methods

Animals

Obese and lean SHR of the NIH-derived substrain of Koletsky rats (Wexler *et al.* 1980) were bred in the animal facility at the Institute of Experimental Neurosurgery, Hradec Králové (Golda and Petr 1988). After weaning at the age of 30 days, the animal were kept in groups of four with free access to standard pelleted laboratory diet ST-1 (VELAZ, Prague, Czech Republic). During the experiments two animals were kept in each cage.

Terguride treatment

Terguride (transdihydrolisuride) maleate (Galena, Opava, Czech Republic) was administered i.p. in two daily doses (each 0.1 mg/kg) at 7:00 h and 14:00 h for 21 days. Glucose tolerance was determined after 11 days of terguride treatment (Golda and Cvak 1994). All other determinations were performed after the termination of experiment i.e. 21 days.

Serum insulin, prolactin and lipids

Three-month-old animals were sacrificed by decapitation without anesthesia at 7:00 h after a 14-hour fast. Serum was collected for analysis of insulin, prolactin, cholesterol and triglyceride concentrations. Insulin was determined by a human radioimmunoassay kit (Cis bio international, Solupharm) using rat insulin (Novo Nordisk) as standard. Prolactin was measured by radioimmunoassay using double antibody technique. ^{125}I-rPRL of specific activity 1280 kBq/μg was purchased from NEN^{TM} Life Science Product (CA, USA). Reference preparation and specific antibodies were kindly provided by NHPP Ogden BioService (MD, USA). The incubation was performed at room temperature for 24 hr followed by an overnight precipitation phase at 4 °C. Results are expressed in term of rPRL-RP-3 standard. The mean error of the assay was 8.6 %.

Cholesterol and triglyceride concentrations were estimated enzymatically by a Hitachi automatic analyzer using the Boehringer Mannheim (Germany) kits for triglycerides GPO-PAP and cholesterol CHOD-PAP.

Glucose tolerance

Blood was rapidly sampled (within 30 s) into heparinized capillaries from the retrobulbar plexus under light ether anesthesia before glucose loading (basal glycemia) as well as 30, 60, 120 and 180 min after glucose loading. Glucose (3 g/kg b.w., 30 % solution) was applied intragastrically after 14 h fasting. Our pilot experiments showed the same blood glucose levels during glucose tolerance estimation when comparing blood samples from the retrobulbar plexus under short-lasting anesthesia with sampling from the tail or decapitation without anesthesia. Glycemia was estimated by a kit based on the oxochrome method (BIO-LA-TEST, Oxochrom, Lachema, Brno, Czech Republic). Glucose tolerance was expressed as the area under the glycemic curve (AUC).

Fat tissue plasma membrane preparation

Epididymal or perigonadal fat tissue was dissected immediately after decapitation, weighed and stored in liquid nitrogen until use. The frozen tissue was homogenized in 10 volumes of 10 mM Tris-HCl, 250 mM sucrose, 1 mM phenylmetylsulfonyl fluoride (Sigma Chemicals), 1 mM benzamidine (Sigma Chemicals) buffer, pH 7.4 using Polytron homogenizer. All steps following homogenization were carried out at

4 °C. The homogenate was centrifuged at 2000 x *g* for 15 min to sediment nuclei and cellular debris. The fat cake was removed from the top of the supernatant and the supernatant was further centrifuged at 16 000 x *g* for 15 min. The resulted pellet (designated as the crude plasma membrane preparation) was resuspended in 50 mM Tris-HCl, pH 7.6 and used for insulin binding immediately after protein determination.

Insulin binding

The binding experiments were performed in Eppendorf tubes containing 50 µg of plasma membrane proteins, 100 µl of mono-^{125}I-(Tyr A14)-insulin (0.2 nM final concentration), 50 µl of native monocomponent porcine insulin (Novo) in increasing concentrations 1 pM to 1 µM. This mixture was completed with 300 µl of 0.1 mM Tris-HCl, pH 7.6 binding buffer with 2 mM N-ethylmaleimide (Sigma), 1 mM $CaCl_2$ and 0.1 % bovine serum albumin, proteinase free (Sigma Chemicals). Total binding was determined in the absence of nonlabeled insulin. Incubation was carried out at 4 °C for 21 h (Macho *et al.* 1999). The binding was stopped by centrifugation at 4 °C (16 000 x *g*, 10 min) and immediate aspiration of the supernatant. The bottom part of the Eppendorf tube containing the membrane pellet was cut off and counted in a gamma-counter. The obtained competition data were analyzed with the LIGAND program (Munson and Rodbard 1984).

Mono-^{125}I-(Tyr A14)-insulin was prepared using the lactoperoxidase method of iodination (Jorgensen and Larsen 1980, Zórad *et al.* 1985).

Statistical analysis

Results are expressed as means ± S.E.M. One-way ANOVA and the Bonferroni post-test were used for statistical analysis.

Table 1. Body weight, fat tissue mass and lipemia of experimental animals.

	Initial body weight (g)	**Final body weight (g)**	**Fat tissue mass (g/100 g b.w.)**	**Triglycerides (mmol/l)**	**Cholesterol (mmol/l)**
Males					
Control (7)	270±13	292±11	1.29±0.12	0.90±0.04	1.80±0.11
Control + Terguride (7)	266±22	275±18	1.17±0.09	0.93±0.08	1.82±0.22
Obese (8)	347±23*	409±17	3.00±0.11***	3.10±0.40*	2.22±0.14
Obese + Terguride (8)	353±30*	406±16***	3.00±0.13***	3.90±0.71**	2.28±0.22
Females					
Control (6)	190±10	207±14	1.24±0.19	0.79±0.08	2.72±0.04
Control + Terguride (6)	190±8	192±12	0.88±0.10	0.76±0.09	2.01±0.11*$^{++}$
Obese (6)	379±9***	489±22***	2.70±0.21**	3.40±0.12***	2.37±0.14
Obese + Terguride (6)	386±29***	440±19***	3.00±0.24***	2.80±0.13***$^{+}$	2.33±0.13

*Data are means ± S.E.M. Control rats are lean SHR of Koletsky type, obese animals are obese SHR. The number of animals per group is indicated in brackets. Initial body weight is the body weight of rats before terguride treatment. Final body weight is the body weight of rats at the end of the experiment, i.e. after 21 days of terguride treatment. Significant differences against the corresponding controls: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, significant effects of terguride: $^{+}$ $p<0.05$, $^{++}$ $p<0.01$.*

Results

Obese animals of both sexes had significantly higher body weight in comparison to their controls and terguride treatment did not change the body weight in any group of animals (Table 1). Relative epididymal fat tissue mass was almost three times higher in obese SHR in comparison with lean animals and was not affected by terguride treatment (Table 1.).

Similarly, serum triglyceride concentrations were elevated by more than three times in obese rats of both sexes (Table 1). On the other hand, cholesterol levels were not significantly different between obese and lean animals. In lean SHR, terguride was without any

effect on fat tissue mass and lipemia, except for the cholesterol lowering effect in females. In obese animals, dopamine receptor stimulation by terguride significantly lowered serum triglyceride concentrations in females (Table 1).

All studied groups of animals exhibited comparable glycemia (Table 2). Despite that, the obese SHR had markedly elevated serum insulin levels (Table 2). Terguride treatment significantly diminished hyperinsulinemia in obese females only, although the same but insignificant tendency was also seen in males. The glucose tolerance expressed as the AUC parameter was significantly impaired in obese males in comparison to their lean controls. A tendency to a slight increase was also observed in obese females (Table 2). Terguride treatment normalized the AUC in obese animals of both sexes (Table 2), while it was without any effect on glycemia, insulinemia and AUC in lean animals.

Table 2. Serum glucose, insulin, prolactin and glucose tolerance of experimental animals.

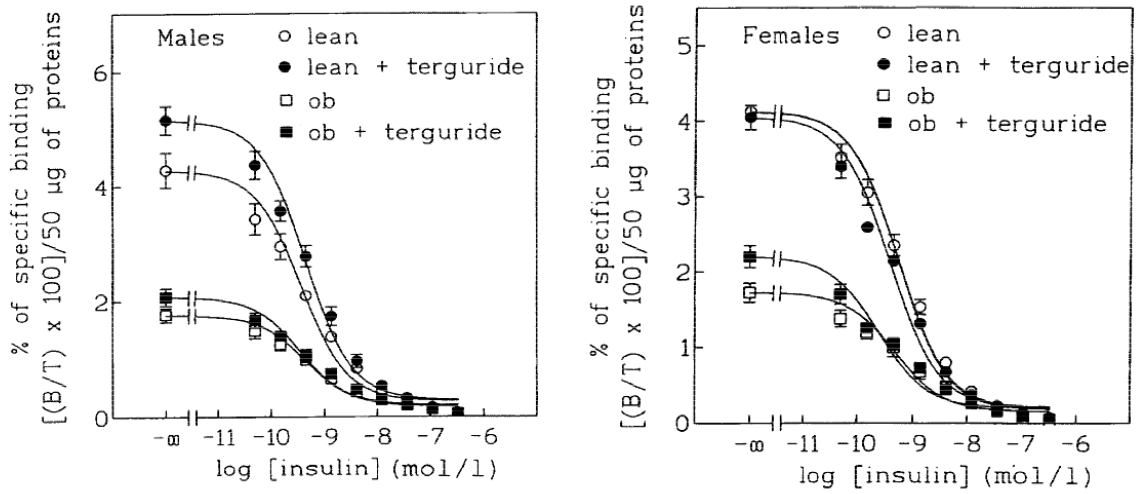
	Glucose (mmol/l)	**IRI (pmol/l)**	**AUC (mmol/l) x h**	**PRL (ng/ml)**
Males				
Control (7)	5.45±0.22	246±26	29.3±0.8	18.3±2.8
Control + Terguride (7)	4.90±0.22	174±19	26.5±1.1	$1.6\pm0.2^{+++}$
Obese (8)	4.62±0.28	953±181**	44.3±3.9**	13.3±1.4
Obese + Terguride (8)	4.07±0.21	641±87	$29.0\pm1.0^{++}$	$2.9\pm0.4^{++}$
Females				
Control (6)	4.84±0.19	177±6	29.2±1.2	41.1±12.4
Control + Terguride (6)	5.28±0.04	165±9	26.7±0.8	21.2±5.3
Obese (6)	4.62±0.42	575±72***	34.8±2.7	50.7±16.4
Obese + Terguride (6)	4.35±0.44	$258\pm23^{++}$	$25.8\pm0.8^{+}$	$4.8\pm1.0^{+}$

*IRI – serum immunoreactive insulin, AUC – glucose tolerance expressed as area under the glycemic curve, PRL – serum prolactin levels. Values are expressed as means ± S.E.M. Significant differences against the corresponding controls: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, significant effects of terguride: $^{+}$ $p<0.05$, $^{++}$ $p<0.01$, $^{+++}$ $p<0.001$. For other legend see Table 1.*

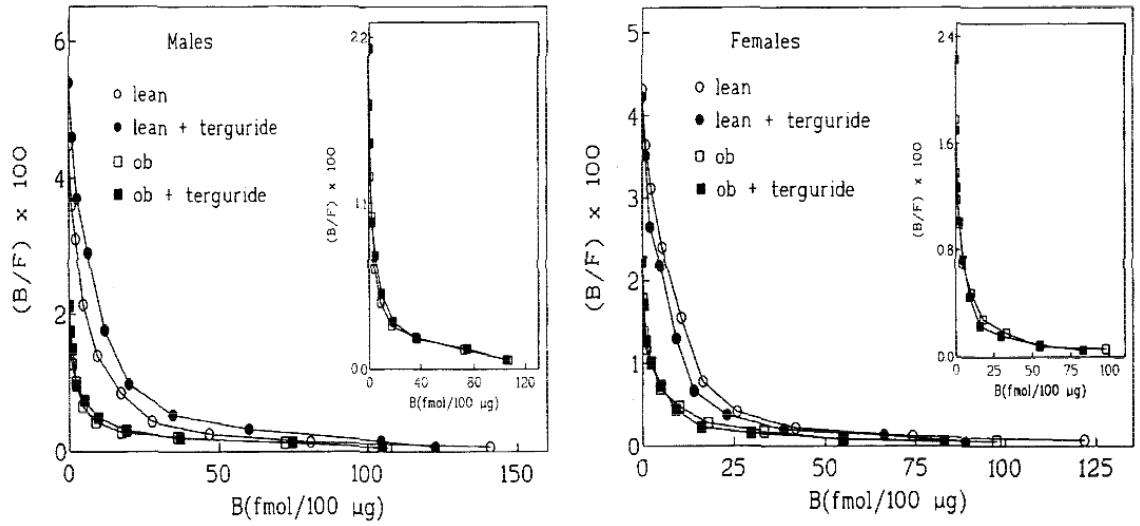
Prolactin levels did not differ significantly between obese and lean SHR of both sexes (Table 2). Considering the sex differences, the obese females had significantly higher prolactin levels than obese males ($p<0.05$). Terguride treatment significantly decreased serum prolactin in controls, obese males and obese females with the same tendency in lean females (Table 2).

Insulin binding to epididymal fat tissue plasma membranes was very distinct in lean and obese SHR, as is documented by the displacement curves for both sexes (Figs 1 and 2). The total binding in obese animals decreased significantly by more than 50 % (Table 3). Transformation of the displacement data according to Scatchard (1949) and analysis of Scatchard data (Figs 3 and 4) using the model of two independent binding sites with high and low affinity (Munson and Rodbard 1984) revealed a profound decrease of the capacity of high affinity binding sites (R_1) for insulin without changes of their affinity in obese rats of both sexes (Table 3).

Terguride treatment shifted upright the averaged displacement curves in all experimental groups (Figs 1 and 2) suggesting an increase in receptor capacity and/or affinity. Scatchard analysis revealed that the effect of terguride was only significant in the group of obese females resulting in a significant elevation of the affinity of high affinity binding sites (K_1) (Fig. 4, Table 3). In agreement with the above findings, the Scatchard curve in obese SHR with terguride (Fig. 4, inset) was the only curve shifted up and left against its control (ob) curve. Terguride also tended to increase the affinity of insulin low affinity binding sites (K_2) in lean rats, but these changes were not significant due to the large scatter of K_2 data (Table 3).



Figs 1 (males) and 2 (females). *Averaged displacement curves for insulin binding to epididymal fat tissue plasma membranes. The points represent mean values ± S.E.M., n is the same as indicated in Tables 1, 2 and 3. ob – obese animals.*



Figs 3 (males) and 4 (females). *Averaged Scatchard curves of insulin binding to epididymal fat tissue plasma membranes. The points represent mean values, n is the same as indicated in Tables 1, 2 and 3. Inset shows the magnified Scatchard curves of obese (ob) groups.*

Discussion

Obese SHR used in our experiments originated from NIH-derived substrain of Koletsky rats (Wexler *et al.* 1980, Hansen 1983). However, taking into account the absence of significant differences in glycemia between obese and lean animals, our substrain resembles more the SHROB/Koletsky substrain (Friedman *et al.* 1997) than the NIH-derived Hansen's corpulent SHR/N-cp substrain (Marette *et al.* 1993). Furthermore, our rats show milder differences in insulinemia and lipemia between obese and lean animals than the SHROB and SHR/N-cp strains. Despite that, our data clearly indicate the presence of apparent signs of insulin resistance such as significant hyperinsulinemia, higher values of AUC and substantial downregulation of insulin receptors in the adipose tissue in our substrain of Koletsky rats. The inverse relationship between the insulin concentration and number of insulin receptors has been documented in several tissues in obesity, non-insulin-dependent diabetes and in other

Table 3. Parameters of insulin binding to adipose tissue plasma membranes.

	SB (%)	R_1 (fmol/100 μg)	R_2 (fmol/100 μg)	K_1 (l x mol^{-1} x 10^8)	K_2 (l x mol^{-1} x 10^8)
Males					
Control (7)	4.3±0.8	31.7±+5.8	470.7±111.8	14.8±4.3	0.053±0.013
Control + Terguride (7)	5.6±0.6	23.1±+3.2	274.7±37.3	21.3±4.6	0.33±0.16
Obese (8)	1.7±0.3*	9.7±+1.3**	301.8±64.2	14.7±2.8	0.10±0.02
Obese + Terguride (8)	2.1±0.4**	11.6±+2.9	250.4±33.8	16.6±2.4	0.12±0.03
Females					
Control (6)	4.1±0.1	30.9±+2.8	347.5±52.8	11.6±0.7	0.058±0.015
Control + Terguride (6)	4.1±0.3	22.4±+2.0	218.8±32.3	14.8±0.9	0.14±0.05
Obese (6)	1.8±0.3***	16.5±+3.9*	304.1±91.8	8.2±1.0	0.11±0.04
Obese + Terguride (6)	2.2±0.3**	11.9±+1.5	235.4±18.0	14.9±2.3$^+$	0.09±0.03

*SB – insulin specific binding, R_1 and R_2 capacity of high and low affinity insulin receptors, respectively, K_1 and K_2 affinity of high and low affinity receptors, respectively. The results are expressed as means ± S.E.M. Significant differences against the corresponding controls: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, significant effects of terguride: $^+$ $p<0.05$. For other legend see Table 1.*

hyperinsulinemic states (Grunberg 1988). We found a downregulation of insulin receptors in adipose tissue in rats with hyperinsulinemic and normoglycemic obesity induced by neonatal monosodium glutamate treatment (Zorad *et al.* 1997, Macho *et al.* 2000). Similarly, the SHROB/Koletsky rats display about a 30 % reduction in insulin receptor levels in skeletal muscles and the liver presumably as a consequence of hyperinsulinemia (Friedman *et al.* 1997). Downregulated high affinity insulin receptors in adipose tissue of obese SHR may contribute to a lower antilipolytic action of insulin against stimulated lipolysis leading to a higher production of circulating free fatty acids by the fat tissue and consequently to increased triglyceride production in the liver. Interestingly, hypertriglyceridemia in our obese SHR was not accompanied with hypercholesterolemia, contrary to SHROB/Koletsky rats (Friedman *et al.* 1997) and Hansen's corpulent SHR (Wexler *et al.* 1980).

In comparison to the lean controls, the glucose intolerance expressed as AUC was higher in obese male SHR only. This seems to be in accordance with the tendency of higher serum insulin levels in this group in comparison to obese females.

The presence of hyperprolactinemia in our obese and lean SHR/Koletsky rats compared with normotensive Wistar rats was described in our previous paper (Golda and Jurčovičová 1998). This fact is in agreement with the data of Cincotta (Cincotta and Meier 1989, Meier *et al.* 1992, Cincotta and Meier 1995, Cincotta *et al.* 1997) and Schernhaner *et al.* (1985). It implicates the possible role of prolactin in mechanisms of insulin resistance. In addition, prolactin synergistically with glucose stimulates insulin gene expression (Petryk *et al.* 2000).

Dopaminergic inhibition of prolactin release and prolactin gene expression (Chuang *et al.* 1993) lead to a reduction of fat stores, decrease lipemia and ameliorates other obesity-associated metabolic dysfunction in several animal species (Cincotta and Meier 1989, Golda and Cvak 1994, Cincotta *et al.* 1997). In turn, severe hyperprolactinemia in human patients is associated with decreased insulin binding and insulin resistance (Schernhaner *et al.* 1985). In our studies, prolactin inhibition by terguride improved insulin resistance, hypertriglyceridemia, hyperinsulinemia and insulin binding mainly in obese females. Indeed, obese female SHR have the highest prolactin levels and a very high degree of prolactin inhibition after terguride treatment. In females, prolactin levels are under the regulatory influence of circulating estrogens which, besides the direct effect, also inhibit the tuberoinfundibular dopamine (Nedvídková *et al.* 2000). Terguride treatment apparently also eliminated the effects of estrogens. It thus seems that

prolactin may contribute to obesity-induced insulin resistance particularly in obese females. This statement is strengthened by the fact that in humans the most profound effect of dopaminergic inhibition of prolactin on the degree of body fat reduction was documented in postmenopausal women (Meier *et al.* 1992). Whether the mechanism of terguride action is based solely on prolactin inhibition or it also involves other consequences of dopamine receptor stimulation requires further investigation. Nevertheless, terguride, aside from its classical indications (e.g. hyperprolactinemia and acromegaly), can be considered as a potential drug for alleviation of obesity-induced metabolic abnormalities.

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