

University of Groningen

Pharmacological treatment of hyperinsulinaemia in rats depends on coping style

Boersma, Gretha; Benthem, Lambertus; van Dijk, Gertjan; Steimer, Thierry J.; Scheurink, Antonius

Published in:
European Journal of Pharmacology

DOI:
[10.1016/j.ejphar.2010.12.017](https://doi.org/10.1016/j.ejphar.2010.12.017)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Boersma, G., Benthem, L., van Dijk, G., Steimer, T. J., & Scheurink, A. J. W. (2011). Pharmacological treatment of hyperinsulinaemia in rats depends on coping style. *European Journal of Pharmacology*, 654(1), 122-127. DOI: 10.1016/j.ejphar.2010.12.017

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Endocrine Pharmacology

Pharmacological treatment of hyperinsulineamia in rats depends on coping style

Gretha Boersma^{a,*}, Lambertus Benthem^b, Gertjan van Dijk^a, Thierry J. Steimer^c, Anton J.W. Scheurink^a^a Department of Neuroendocrinology, University of Groningen, The Netherlands^b Department of Research and Development, AstraZeneca, Sweden^c Department of Psychopharmacology, University of Genève, Switzerland

ARTICLE INFO

Article history:

Received 25 August 2010

Received in revised form 4 December 2010

Accepted 11 December 2010

Available online 24 December 2010

Keywords:

Rosiglitazone

RU486

Glucocorticoid

PPAR γ agonist

Intravenous glucose tolerance test

ABSTRACT

Passive and proactive coping styles are associated with marked differences in behavioral and neuroendocrine responses. Previous studies revealed that the passive individuals are more prone to hyperinsulineamia. Likewise, we hypothesize that different coping styles may require different drugs to treat this. We tested this by treating passive and proactive rats (Roman Low Avoidance and Roman High Avoidance rats respectively) with either Rosiglitazone or with RU486. After eight days of treatment we performed and intravenous glucose tolerance test (IVGTT) and we compared the insulin and glucose levels with those measured during the IVGTT at baseline. Rosiglitazone improved insulin levels during an IVGTT in both passive and proactive coping styles. RU486, however, lowered insulin levels only in rats with a passive coping style. This study suggests that insight in the neuroendocrine differences between passive and proactive coping styles may provide an extra impulse to improve treatment of insulin resistance, since it allows the application of drugs targeted at the individual.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

The significance of personality, stress coping and other psychosocial factors for the development of insulin resistance and type 2 diabetes has become more evident in recent years (Feldman and Steptoe, 2003; Sovio et al., 2007; Yancura et al., 2006). The mechanisms underlying the interaction between psychosocial factors and metabolic pathologies, however, remain to be elucidated. One approach is to study these mechanisms in rodent lines with divergent stress coping and personality profiles. In our previous studies we have shown that rats selected for a passive strategy to cope with stress, the so-called Roman Low avoidance rats (RLA), have a higher sensitivity to develop signs indicative of the metabolic syndrome than proactively coping animals, the Roman High avoidance rats (Boersma et al., 2009). We confirmed these findings in passive and proactive littermates from an out bred wild-type Groningen (WTG) rat population. These WTG rats display a more moderate dispersion of coping styles and in these rats we again showed that more passive individuals had consistently higher proneness to develop insulin resistance than proactive individuals (Boersma et al., 2010).

Taken together, these studies indicate that the coping style of an individual plays an important role in the development of metabolic derangements. Likewise one may argue that different coping styles

may also respond differently to different treatments for metabolic disorders such as type 2 diabetes and the metabolic syndrome. We should therefore focus on custom made treatments for passive and proactive coping styles for treatment of insulin resistance. To this end, we decided to test the potential beneficial effects of two different drug treatments for hyperinsulineamia, Rosiglitazone and RU486, in both passively and proactively coping rats of the Roman selection lines.

In our first set of experiments focused on the effects of Rosiglitazone, a peroxisome proliferator-activated receptor gamma agonist, known to directly induce translocation of the glucose transporter type 4 (GLUT4) to the membrane (Saltiel and Olefsky, 1996; Spiegelman, 1998), and thereby increasing insulin sensitivity of the insulin receptor. This oral anti-diabetic agent is a commonly used treatment strategy for the metabolic syndrome and it has a good success rate in patients with type 2 diabetes (reviewed in Krentz and Bailey, 2005). Since Rosiglitazone directly improves the insulin signaling cascade circumventing possible differences in insulin receptor sensitivity, we assume that treatment with this drug will be equally effective in passive and proactive individuals.

The second drug, RU486, is specifically targeted at treating the hyperinsulineamia observed in passive coping style (Boersma et al., 2009). RU486 is a glucocorticoid receptor antagonist predominantly used in the treatment of diabetes associated with Cushing syndrome and glucocorticoid secreting tumors (Johanssen and Allolio, 2007). This therapeutic agent may be interesting since passively coping rats are characterized by moderate elevated glucocorticoid levels (Aubry et al., 1995; Boersma et al., 2009; Fernandez-Teruel et al., 2002; Gentsch et al., 1982). Elevated glucocorticoid levels, in turn, are associated with an increase susceptibility for insulin resistance. If elevated glucocorticoid

* Corresponding author. Department of Neuroendocrinology, University of Groningen, PO box 14, 9750 AA Haren, The Netherlands. Tel.: +31 50 363 2345; fax: +31 50 363 2331.

E-mail addresses: g.j.boersma@rug.nl (G. Boersma), Bert.Benthem@astrazeneca.com (L. Benthem), gertjan.van.dijk@rug.nl (G. van Dijk), Thierry.Steimer@hcuge.ch (T.J. Steimer), A.J.W.Scheurink@rug.nl (A.J.W. Scheurink).

receptor stimulation indeed play a role in the presumed insulin resistance in RLA rats, we expect that blocking the glucocorticoid action with a glucocorticoid receptor antagonist, RU486, would obliterate differences in glucose homeostasis among RHA and RLA rats. Treatment with RU486 would therefore specifically improve insulin signaling in the RLA rats.

In summary, in the present study we hypothesize that different personalities may require different drugs for treatment of hyperinsulinemia. To this end, we treated proactive and passive rats with two different drugs and measured glucose and insulin responses to an intravenous glucose tolerance test before and after treatment. We hypothesize that Rosiglitazone will increase insulin sensitivity in both personality types and that RU486 will only be effective in the passive coping style.

2. Materials and methods

2.1. Animals

Adult male Roman High ($n = 16$) and Roman Low Avoidance rats ($n = 16$) with body weights between 300 and 400 g were used. The rats were obtained from a breeding colony at the Clinical Psychopharmacology Unit (APSI), University of Geneva, Switzerland. The Roman High and Low Avoidance rats (RHA and RLA, respectively) were originally selected by Bignami (Bignami, 1965) on the basis of their performance in a two-way active avoidance test. Rats with the most extreme coping styles were identified and selectively bred for many generations. This resulted in two sub-strains: Roman Low Avoidance rats with an extremely passive coping style and Roman High Avoidance rats with a proactive coping strategy (Driscoll et al., 1983). The passive coping RLA is characterized by low aggression levels, flexible behavioral patterns and a passive stress response, whereas the proactive RHA is characterized by high levels of aggression, rigid behavioral patterns and a proactive strategy towards stressors (Steimer et al., 1997).

All rats were housed individually in standard cages ($24 \times 24 \times 36$ cm), lab chow (Hope Farms, RMH-B knaagdier korrel, Arie Blok Diervoeding, Woerden, NL) and water were available ad lib. The room was controlled for temperature and humidity ($T = 20 \pm 2$ °C, humidity 60%) and was kept at a 12–12 h light–dark cycle (lights on = CT0). All animal experiments were approved by the local animal care committee.

2.2. Surgery

The rats underwent surgery to place two indwelling jugular vein catheters allowing continuous blood sampling in freely moving animals. Rats were sedated using an isoflurane- O_2/N_2O gas anesthesia. A silicon heart catheter (0.95 mm OD, 0.50 mm ID and 0.64 mm OD, 0.28 ID) was inserted into the right jugular vein and kept in place with a ligature. The catheter was pulled under the skin towards the skull where it was connected to a metal bow. This metal bow was fixed to the skull with dental cement and 4 small screws. The same procedure was repeated on the left side. During blood sampling or infusions a piece of tubing could be attached to the metal bow, hereby samples could be taken from conscious rats. In between experiments, the catheter was filled with a PVP/heparin solution preventing blood clot formation in the catheter (Steffens, 1969a). The animals were given 0.1 ml Finadine s.c. for analgesia and 0.25 ml penicillin s.c. to prevent infection. After surgery the rats were allowed to recover for at least 7 days.

2.3. Intravenous glucose tolerance test

After recovery from surgery, the rats were accustomed to the infusion and blood sampling procedure before the actual onset of the experiments (Steffens, 1969b). Then, an intravenous glucose toler-

ance test (IVGTT) was performed to measure the baseline responses in each individual animal. After the baseline IVGTT, the animals were treated with either Rosiglitazone or RU486 for eight days. A second IVGTT was performed at day 8, the last day of treatment. This within-subject experimental set-up allowed us to use each individual rat as its own control. During the intravenous glucose tolerance test (IVGTT) an infusion of 15 mg/min glucose was given in 3 ml saline solution over a 30 min period. This is a physiological dose that mimics the glucose response after a large meal (Strubbe and Bouman, 1978).

The experiments were performed in the middle of the light phase, between CT4 and CT6. Rats were denied access to their food from the beginning of the light phase until the end of the IVGTT; food was removed at CT0. Two baseline blood samples were taken before the start of the infusion ($t = -15$ and $t = -5$ min). The glucose infusion was given between $t = 0$ and 30 min, during and after infusion blood samples were taken at time points 5, 10, 15, 20, 25, 30, 35, 40, and 50 min. A total volume of 2.8 ml blood was taken and the loss of volume was substituted by saline infusion. Blood samples were kept on ice and stored in files with 10 μ l EDTA (0.09 g/ml). For glucose determination 50 μ l of full blood with 450 μ l heparin solution (2%) was stored at -20 °C. The remaining blood was centrifuged for 15 min and plasma was stored for insulin determination.

2.4. Rosiglitazone treatment

Eight RHA and eight RLA rats were treated with a dose of 4 mg/kg/day (Kramer et al., 2001) Rosiglitazone (AstraZenica, Mölndal, Sweden) for 8 consecutive days. Rosiglitazone was administered in the drinking water. The water intake of the rats was monitored for a week before the start of the experiment, and the concentration of Rosiglitazone was adjusted accordingly. Since RLA rats drink generally more than the RHA rats (Boersma et al., 2009 and Table 1), the actual concentration of Rosiglitazone was calculated on the basis of baseline water intake of each individual rat. On average, the RLA rats received 50 ± 3 mg/L and RHA rats 57 ± 2 mg/L Rosiglitazone solution. During treatment water intake of the rats did not change, which means that that each individual rat received 4 mg/kg/day of Rosiglitazone daily.

2.5. RU486 treatment

Eight RHA and eight RLA rats were treated with 20 mg/kg/day (Diaz et al., 2001) RU486 (11 β -[p-(Dimethylamino)phenyl]-17 β -hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) (mifepristone, Sigma-Aldrich Chemie, Zwijndrecht) for 8 consecutive days. RU486 was given subcutaneously at CT2 and CT14, both injections contained 10 mg/kg RU486 in 0.5 ml saline. Before the start of the treatment the rats were accustomed to the subcutaneous injections procedure; they received a single saline injection (0.5 ml/kg) for 4 consecutive days. The efficiency of the RU486 treatment was assessed by measuring corticosteron levels in the baseline plasma samples prior to the IVGTT.

Table 1

Body weight (BW), food intake (FI) and water intake (WI) of RLA and RHA rats before treatment and after treatment with either Rosiglitazone or RU486. ^aIndicates a significant difference with RLA rats (within treatment) $P < 0.05$ ^bIndicates a significant difference with baseline condition (within a strain) $P < 0.05$.

	Rosiglitazone		RU486	
	RLA	RHA	RLA	RHA
Baseline BW (g)	435.3 \pm 6.7	401.7 \pm 9.9	433.3 \pm 7.3	399.8 \pm 10.0
Change in BW (g)	43.5 \pm 6.3 ^b	47.5 \pm 5.4 ^b	40.6 \pm 6.9 ^b	53.2 \pm 7.3 ^b
Baseline FI (kcal/day)	97.48 \pm 3.87	96.45 \pm 3.21	96.81 \pm 3.51	97.32 \pm 4.02
Treatment FI (kcal/day)	98.70 \pm 4.44	97.17 \pm 2.50	90.10 \pm 2.17 ^b	89.73 \pm 2.52 ^b
Baseline WI (ml/day)	41.78 \pm 2.11	34.80 \pm 1.11 ^a	40.62 \pm 2.05	34.32 \pm 1.86 ^a
Treatment WI (ml/day)	41.28 \pm 2.28	35.86 \pm 2.31 ^a	40.73 \pm 2.62	33.58 \pm 4.06 ^a

2.6. Post mortem analysis

The rats were sacrificed after 8 days of treatment, one day after the last IVGTT. Three hours before lights off, blood samples were taken directly from the heart under isoflurane-O₂/N₂O gas anesthesia for determination of blood glucose, plasma insulin and leptin levels. Animals were hereafter sacrificed using an overdose of pentobarbital. Epididymal and retroperitoneal fat pads and the liver were taken out and weighed. The skin with the subcutaneous fat was removed from the carcass. Liver, skin, and carcasses were dried at 80 °C for 5 days. Fat content was determined by extracting the fat from tissue using a petroleum based Soxhlet fat extractor. After fat extraction the tissue was dried for 5 days again. The relation between dry tissue weight before and after fat extraction provides information on the fat content of the tissue.

2.7. Chemical analyses

Plasma levels of insulin and leptin were measured using commercial radioimmunoassay (RIA) kits (Linco Research). Blood glucose levels were determined using the ferry-cyanide method in a Technicon auto analyzer. Plasma corticosteron levels were measured using a commercial radioimmunoassay (RIA) (Biomedics).

2.8. Statistical analysis

Data are expressed as averages with standard error of the mean. Differences in food and water intake, body weight and baseline plasma levels between strains were determined using one-way ANOVA using strain as the between subjects factor. Differences in insulin and glucose levels before, during and after the IVGTT were examined using repeated measures ANOVA, again using strain as the between subjects factor. The area under the curves of both glucose and insulin responses (t=0 till t=30 min) were calculated and reported as the average area under the curve (AUC) with the standard error of the mean. The differences between the strains were determined using one-way ANOVA. The statistical differences between the strains in corticosteron levels before and after the treatment were assessed using a repeated measures ANOVA, with the strain as the between subjects factor, and time as the within subjects factor. A confidence interval of 5% was used.

3. Results

3.1. Body weight and food intake

Table 1 displays body weights and food intake of RLA and RHA rats during the baseline period and after treatment with either Rosiglitazone or RU486. At the start of the experiments, the RLA rats were somewhat but not significantly heavier than the RHA rats (P=0.072). Both Rosiglitazone and RU486 treatment reduced body weights in RLA and RHA rats (for Rosiglitazone RM-ANOVA $F_{3,21}=7,258$; RLA P=0.021; RHA p=0.007, and for RU486 RM-ANOVA $F_{3,21}=11,362$ RLA P=0.012; RHA P=0.001). There were no differences in food intake between RLA and RHA rats under baseline conditions. Treatment with RU486 significantly reduced food intake in both strains (RM-ANOVA $F_{1,21}=12,232$, P=0.016). Treatment with Rosiglitazone had no effect on the food intake. There were no differences between the strains in the effects of either Rosiglitazone or RU486 on both body weight and food intake. At baseline water intake was significantly higher in the RLA rats compared to RHA rats ($F_{3,21}=9.234$, P<0.01). Treatment with either RU486 or Rosiglitazone did not affect water intake.

3.2. Intravenous glucose tolerance test

Figs. 1 and 2 display the glucose and insulin responses to an IVGTT in chow fed RLA and RHA rats at baseline and after treatment with RU486 or Rosiglitazone. Fig. 3 provides the areas under the curve (AUC) of the insulin response.

There were no differences in glucose levels between RLA and RHA rats under any of the tested conditions, nor did either treatment affect glucose levels. Baseline plasma insulin levels were significantly higher in RLA rats in comparison to RHA rats ($F_{1,21}=11.095$, P=0.003). Treatment with Rosiglitazone significantly reduced the insulin response to an IVGTT in both RLA and RHA rats (at time points t=5, 10, 20, 25 and 30 min, P<0.05). Also the AUC for the insulin response was significantly reduced after Rosiglitazone treatment in both strains ($F_{1,21}=5,242$, P<0.05).

Treatment with RU486 significantly lowered the insulin response to an IVGTT in the RLA rats but not in the RHA rats. The reduction in the RLA rats was significant at time points t=5, 10, 20, 25 and 30 min (Fig. 2C) (ANOVA $F_{1,5}=8.210$, P<0.05). Also the area under the insulin curve was significantly lower in RLA rats after treatment with RU486 ($F_{1,21}=4,356$, P<0.05).

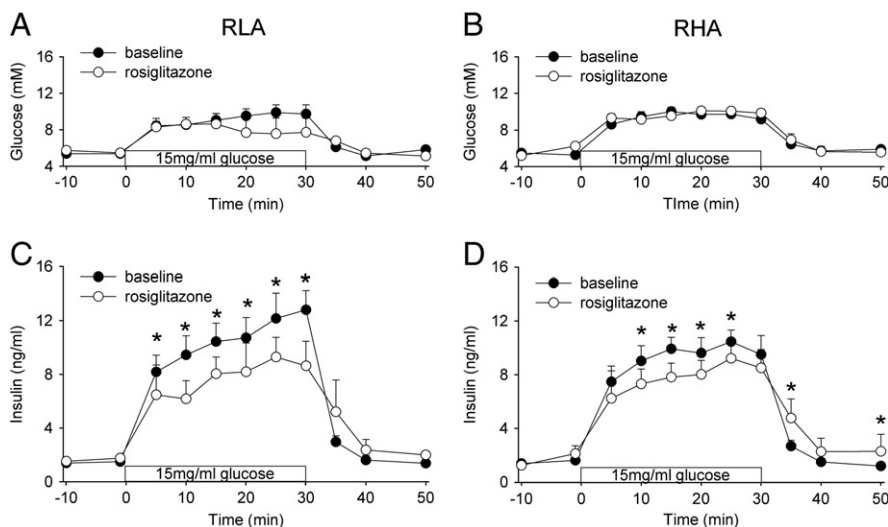


Fig. 1. Glucose and insulin levels before, during and after an intravenous glucose tolerance test in RLA and RHA rats under baseline conditions and after treatment with Rosiglitazone. A: Glucose response in RLA rats. B: Glucose response in RHA rats. C: Insulin response in RLA rats. D: Insulin response in RHA rats. Black symbols represent baseline samples, white symbols represent samples after Rosiglitazone treatment. * indicates a significant difference (P<0.05).

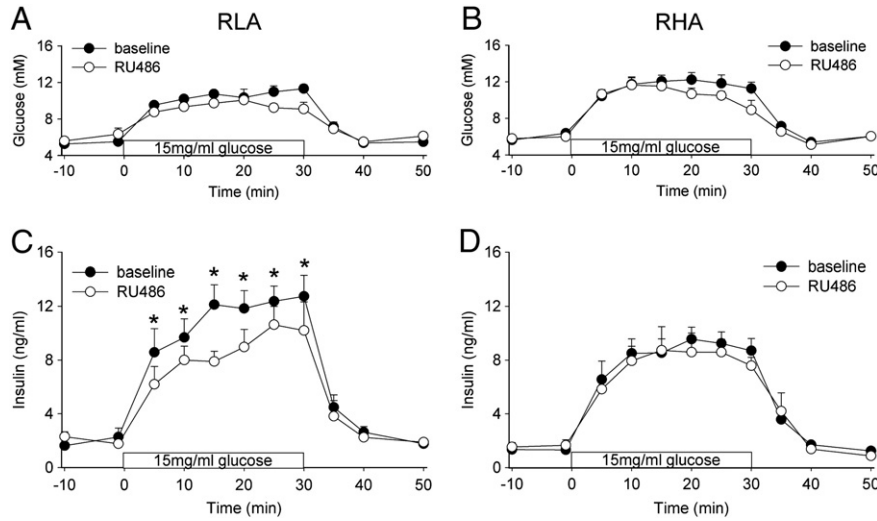


Fig. 2. Glucose and insulin levels before, during and after an intravenous glucose tolerance test in RLA and RHA rats under baseline conditions and after treatment with RU486. A: Glucose response in RLA rats. B: Glucose response in RHA rats. C: Insulin response in RLA rats. D: Insulin response in RHA rats. Black symbols represent baseline samples. White symbols represent samples after RU486 treatment. * indicates a significant difference ($P < 0.05$).

To compare the effect of the treatments between the strains we calculated the differences in the area under the curve before and after treatment (day 0 versus day 8) for each individual rat. The differences

were significant (reduction in the area under the insulin curve in RLA rats: -41.6 ± 16.6 , in RHA rats: -3.9 ± 8.3 ng/ml insulin* 30 min, ANOVA $F_{1,11} = 5.654$, $P < 0.05$). There were no significant differences between the strains with respect to the effect of Rosiglitazone (reduction in the area under the insulin curve for RHA rats: -33.6 ± 15.6 and for RLA rats: -51.4 ± 21.3 ng/ml insulin* 30 min, ANOVA $F_{1,11} = 2.238$, $P = 0.089$).

3.3. Corticosterone levels

Fig. 4 displays the plasma corticosterone levels at baseline and after treatment with RU486 or Rosiglitazone. Baseline corticosterone was significantly elevated in the RLA rats when compared to the RHA rats ($F_{3,21} = 5,242$, $P < 0.01$). Treatment with RU486 significantly lowered plasma corticosterone levels in both RLA and RHA rats ($F_{3,21} = 3,842$, $P < 0.01$). The reduction in corticosterone levels after treatment was significantly larger in the RLA rats compared to the RHA rats (ANOVA $F_{1,11} = 6.342$, $P < 0.05$). After treatment with RU486 there were no differences between the strains in corticosterone levels. Rosiglitazone treatment did not change corticosterone levels in either strain.

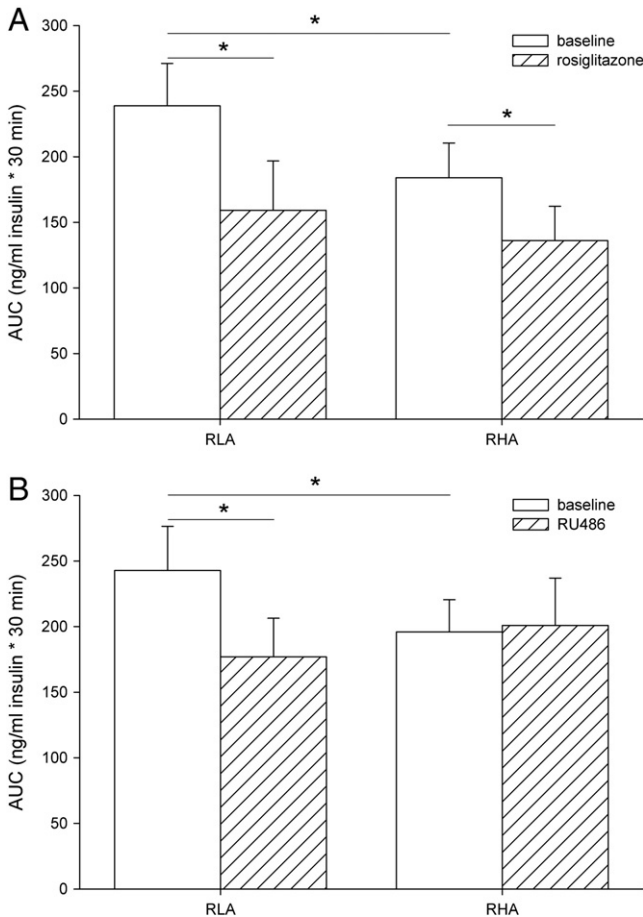


Fig. 3. The area under the curve of the insulin responses ($t = 0$ until $t = 30$) during a 30 min infusion of glucose (15 mg/min) under baseline and treated conditions. RLA and RHA rats A: The response in rats under baseline and Rosiglitazone treated conditions. B: The response in rats under baseline and RU486 treated conditions. White bars represent baseline conditions, hatched bars represent treated conditions. * indicates a significant difference ($P < 0.05$).

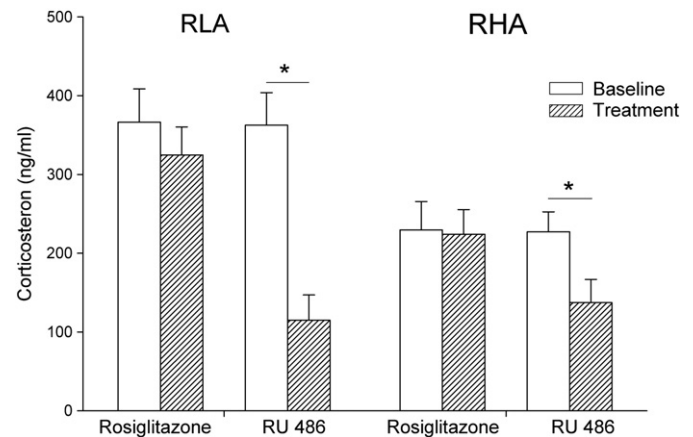


Fig. 4. Corticosterone levels in RLA and RHA before and after treatment with either Rosiglitazone or RU486. White bars represent untreated baseline conditions, hatched bars represent treatment conditions. * indicates a significant difference ($P < 0.05$).

3.4. Body composition

Body composition was determined at the last day of treatment; the results of the analysis are summarized in Table 2. The body weights of the RLA rats were significantly higher compared to the RHA rats in both treatment groups ($F_{3,25} = 2.925$, $P < 0.05$). Total fat mass of RLA rats was higher than the fat mass of RHA after both treatments ($F_{3,25} = 3.052$, $P = 0.032$). RU486-treated RLA rats had more adipose tissue distributed in their epididymal compartment when compared to RU486 treated RHA rats ($F_{3,25} = 4.851$, $P = 0.009$). There was no difference in the amount of retroperitoneal fat mass between RLA and RHA rats treated with RU486. After treatment with Rosiglitazone the RLA rats had more fat distributed in their epididymal compartment than RHA rats ($F_{3,25} = 3.899$, $P = 0.018$). The amount of retroperitoneal adipose tissue after Rosiglitazone treatment was not different between RLA and RHA rats. Within each strain, there were no differences in the body composition of rats treated with Rosiglitazone compared to those treated with RU486.

4. Discussion

The present study investigated the effectiveness of two different drugs for treating hyperinsulinaemia in rat strains that were selected for either a passive or a proactive coping style. It was found that RU486 was only effective in reducing plasma insulin levels in passively coping rats whereas the effect of Rosiglitazone on insulin was similar in both rat strains.

For the general anti-diabetic agent, Rosiglitazone, we expected a comparable effect in passive and proactive individuals. Our study confirmed this: treatment with Rosiglitazone reduced the insulin response to an IVGTT in both RLA and RHA rats. As mentioned before, passive individuals are characterized by a hyperinsulinaemic response to an IVGTT (Boersma et al., 2009). Therefore, the effect of Rosiglitazone on plasma insulin seemed somewhat larger in the passive RLA rats. Treatment with Rosiglitazone was very effective in reducing the insulin response in the RLA rats to a level that was similar to that of the RHA rats. It was not surprising that Rosiglitazone improved insulin responses in both the passive and proactive individuals in the present study. Thiazolidinediones, like Rosiglitazone, are thought to increase insulin sensitivity by activation of PPAR gamma, which in turn leads to an increased translocation of the glucose transporter 4 (GLUT4). This increased availability of GLUT 4 then facilitates glucose transport into the cell. Several studies have shown the beneficial effects of Rosiglitazone (Kramer et al., 2001) although the exact mechanism by which the drug may increase GLUT4 translocation in skeletal muscle cell remains to be elucidated. It seems that Rosiglitazone affects the insulin receptor cascade directly and may circumvent most of the differences in origin of hyperinsulinaemia in the RLA rat.

The second drug that was tested in the present study is RU486, commonly used for treating insulin resistance in patients in which type 2 diabetes is secondary to chronically increased glucocorticoid levels, such as Cushing syndrome (Johanssen and Allolio, 2007).

Table 2

Body fat distribution of RLA and RHA rats treated with either Rosiglitazone or RU486. ^aIndicates a significant difference with RLA rats (within treatment) $P < 0.05$.

	Rosiglitazone		RU486	
	RLA	RHA	RLA	RHA
Body weight (g)	391 ± 8.9	354 ± 9.6 ^a	392 ± 8.2	344 ± 9.7 ^a
Lean body mass (g)	186 ± 4.1	177 ± 6.3	187 ± 3.6	171 ± 4.0
Total body fat (%)	11.7 ± 1.77	9.5 ± 0.76 ^a	11.1 ± 0.77	9.5 ± 0.77 ^a
Epididymal fat (g)	5.1 ± 0.62	3.3 ± 0.16 ^a	4.5 ± 0.23	3.3 ± 0.24 ^a
Retroperitoneal fat (g)	6.7 ± 1.16	6.3 ± 0.67	6.6 ± 0.57	6.2 ± 0.57
Subcutaneous fat (g)	25.4 ± 4.9	18.3 ± 4.2	23.7 ± 2.3	19.6 ± 2.25
Liver weight (g)	17.6 ± 1.3	16.9 ± 1.5	18.4 ± 1.6	16.5 ± 1.8
Leptin (ng/ml)	4.0 ± 0.42	3.5 ± 0.58	3.9 ± 0.45	3.8 ± 0.66

Evidence in literature suggests that there are moderate differences between passive and proactive individuals in HPA-axis activity and baseline glucocorticoid levels (Gentsch et al., 1982). Therefore, we hypothesized that treatment with RU486 might be particularly effective in lowering plasma insulin levels in the passively coping RLA rats. The data obtained in the present study supported this hypothesis. Treatment with RU486 significantly lowered the insulin response to an IVGTT in the passively coping RLA rats and had no effect in proactive RHA rats.

Since RU486 was effective in attenuating hyperinsulinaemia in the passively coping rat one may assume that the effect was secondary to the effect of RU486 on circulating glucocorticoid levels. Indeed, the data of the present study revealed that: 1) corticosterone levels are increased in untreated RLA rats and 2) that treatment of RLA rats with RU486 normalized corticosterone (and insulin) to a level comparable to the proactive RHA rats. The data also suggest that moderately increased corticosterone levels may serve as a useful treatment strategy for hyperinsulinaemia in passive coping individuals. The underlying mechanisms are not well understood. Glucocorticoids are thought to induce insulin resistance through several pathways. First, glucocorticoids decrease the sensitivity of muscle glucose uptake to insulin by decreasing translocation of GLUT4 transporters to the membrane. Second, glucocorticoids can inhibit the rate of glucose phosphorylation (Dimitriadis et al., 1997). Finally, glucocorticoids affect insulin sensitivity indirectly by stimulation of distribution of adipose tissue in the visceral compartment (reviewed in (Peeke and Chrousos, 1995)).

Even though, the average body weight of the rats from the two strains did not differ in a statistically significant manner, RLA were heavier, and this is consistent with previous observations (Boersma et al., 2010; Rossi et al., 1997). Treatment with both RU486 and Rosiglitazone significantly lowered body weight in both the RHA and RLA rats but the difference between the strains remained unchanged throughout the experimental period. The decrease in body weight after RU486 can, in part, be explained by the concomitant decrease in food intake during treatment (Trocki et al., 1995). In contrast, Rosiglitazone treatment did not affect daily food intake suggesting that the reduction in body weight is secondary to increased energy expenditure rather than being caused by reduced energy intake. Interestingly, at baseline water intake was higher in the RLA, however, treatment with either RU486 or Rosiglitazone did not affect water intake in either strain. This suggests that the hyperinsulinaemic response in the RLA rats is not due to differences in water intake. Glucose levels either at baseline or during the IVGTT were not different for RLA and RHA rats. Treatment with either Rosiglitazone or RU486 did not affect glucose levels.

Total and epididymal fat mass were significantly different between RHA and RLA rats after both treatment with RU486 and Rosiglitazone. Due to the within-subject design of the present study, we have no data on the body composition in untreated animals. However, in a previous study with (untreated) RLA and RHA rats of similar age (Boersma et al., 2009, 2010), we already observed that total and epididymal fat mass are significantly higher in RLA in comparison with RHA rats. This means that the observed differences in body fat distribution in the present study are presumably not caused by direct effects of either RU486 or Rosiglitazone. Finally, there were no differences in liver weights or leptin levels between the RLA and RHA rats after either treatment with Rosiglitazone or RU486.

In conclusion, the data of the present study reveal that Rosiglitazone improves the insulin response to an IVGTT independent of the coping styles of the individual. In contrast, RU486, improves hyperinsulinaemia solely in the passive coping style, by targeting the specific hormonal characteristics of this coping style. We conclude that insight in the neuroendocrine differences between different coping styles may provide an extra and important impulse to improve treatment of hyperinsulinaemia.

Acknowledgements

We would like to thank Jan Bruggink for his excellent technical support. These studies were supported by an unrestricted research grant by AstraZeneca.

References

- Aubry, J.M., Bartanusz, V., Driscoll, P., Schulz, P., Steimer, T., Kiss, J.Z., 1995. Corticotropin-releasing factor and vasopressin mRNA levels in roman high- and low-avoidance rats: response to open-field exposure. *Neuroendocrinology* 61, 89–97.
- Bignami, G., 1965. Selection for high rates and low rates of avoidance conditioning in the rat. *Anim. Behav.* 13, 221–227.
- Boersma, G.J., Scheurink, A.J., Wielinga, P.Y., Steimer, T.J., Benthem, L., 2009. The passive coping Roman Low Avoidance rat, a non-obese rat model for insulin resistance. *Physiol. Behav.* 97, 353–358.
- Boersma, G.J., Benthem, L., van, D.G., Steimer, T.J., Scheurink, A.J., 2010. Coping style predicts the (in)sensitivity for developing hyperinsulinemia on a high fat diet in rats. *Physiol. Behav.* 100, 401–407.
- Diaz, B., Barreto, E., Cordeiro, R., Perretti, M., Martins, M., Silva, P., 2001. Enhanced serum glucocorticoid levels mediate the reduction of serosal mast cell numbers in diabetic rats. *Life Sci.* 68, 2925–2932.
- Dimitriadis, G., Leighton, B., Parry-Billings, M., Sasson, S., Young, M., Krause, U., Bevan, S., Piva, T., Wegener, G., Newsholme, E.A., 1997. Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochem. J.* 321 (Pt 3), 707–712.
- Driscoll, P., Dedek, J., Martin, J.R., Zivkovic, B., 1983. Two-way avoidance and acute shock stress induced alterations of regional noradrenergic, dopaminergic and serotonergic activity in Roman high- and low-avoidance rats. *Life Sci.* 33, 1719–1725.
- Feldman, P.J., Steptoe, A., 2003. Psychosocial and socioeconomic factors associated with glycated hemoglobin in nondiabetic middle-aged men and women. *Health Psychol.* 22, 398–405.
- Fernandez-Teruel, A., Driscoll, P., Gil, L., Aguilar, R., Tobena, A., Escorihuela, R.M., 2002. Enduring effects of environmental enrichment on novelty seeking, saccharin and ethanol intake in two rat lines (RHA/Verh and RLA/Verh) differing in incentive-seeking behavior. *Pharmacol. Biochem. Behav.* 73, 225–231.
- Gentsch, C., Lichtsteiner, M., Driscoll, P., Feer, H., 1982. Differential hormonal and physiological responses to stress in Roman high- and low-avoidance rats. *Physiol. Behav.* 28, 259–263.
- Johanssen, S., Allolio, B., 2007. Mifepristone (RU 486) in Cushing's syndrome. *Eur. J. Endocrinol.* 157, 561–569.
- Kramer, D., Shapiro, R., Adler, A., Bush, E., Rondinone, C.M., 2001. Insulin-sensitizing effect of rosiglitazone (BRL-49653) by regulation of glucose transporters in muscle and fat of Zucker rats. *Metabolism* 50, 1294–1300.
- Krentz, A.J., Bailey, C.J., 2005. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs* 65, 385–411.
- Peeke, P.M., Chrousos, G.P., 1995. Hypercortisolism and obesity. *Ann. NY Acad. Sci.* 771, 665–676.
- Rossi, R., Driscoll, P., Langhans, W., 1997. Differential circadian eating patterns in two psychogenetically selected strains of rats fed low-, medium-, and high-fat diets. *Behav. Genet.* 27, 565–572.
- Saltiel, A.R., Olefsky, J.M., 1996. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 45, 1661–1669.
- Sovio, U., King, V., Miettunen, J., Ek, E., Laitinen, J., Joukamaa, M., Veijola, J., Jarvelin, M.R., 2007. Cloninger's Temperament dimensions, socio-economic and lifestyle factors and metabolic syndrome markers at age 31 years in the Northern Finland Birth Cohort 1966. *J. Health Psychol.* 12, 371–382.
- Spiegelman, B.M., 1998. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47, 507–514.
- Steffens, A.B., 1969a. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol. Behav.* 4, 833–836.
- Steffens, A.B., 1969b. Blood glucose and FFA levels in relation to the meal pattern in the normal rat and the ventromedial hypothalamic lesioned rat. *Physiol. Behav.* 4, 215–225.
- Steimer, T., la, F.S., Schulz, P.E., 1997. Neuroendocrine correlates of emotional reactivity and coping in male rats from the Roman high (RHA/Verh)- and low (RLA/Verh)-avoidance lines. *Behav. Genet.* 27, 503–512.
- Strubbe, J.H., Bouman, P.R., 1978. Plasma insulin patterns in the unanesthetized rat during intracardial infusion and spontaneous ingestion of graded loads of glucose. *Metabolism* 27, 341–351.
- Trocki, O., Baer, D.J., Castonguay, T.W., 1995. Comparison of effects of adrenalectomy and RU-486 in rats given a choice of maintenance diet and fat supplement. *Am. J. Physiol.* 269, R708–R719.
- Yancura, L.A., Aldwin, C.M., Levenson, M.R., Spiro III, A., 2006. Coping, affect, and the metabolic syndrome in older men: how does coping get under the skin? *J. Gerontol. B Psychol. Sci. Soc. Sci.* 61, 295–303.