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Pharmacological treatment of hyperinsulineamia in rats depends on coping style

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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.

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

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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 hyperinsulineamia. 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 × 24 × 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 ± 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 cloth 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 tolerance 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 µl EDTA (0.09 g/ml). For glucose determination 50 µl of full blood with 450 µl 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 (11β-[p-(Dimethylamino)phenyl]-17β-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. [a]Indicates a significant difference with RLA rats (within treatment) $P<0.05$ [b]Indicates a significant difference with baseline condition (within a strain) $P<0.05$.

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