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Effects of ZD7114, a selective β_3-adrenoceptor agonist, on neuroendocrine mechanisms controlling energy balance

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Abstract

Selective β_3-adrenoceptor agonists increase energy expenditure by increasing non-shivering thermogenesis in brown adipose tissue. The aim of this study was to investigate how changes in energy balance affect energy intake and interaction of peripheral metabolic feedback signals with central neuroendocrine mechanisms participating in the control of body energy balance. Expression of preproneuropeptide Y (preproNPY) mRNA in the arcuate nucleus and preprocorticotropin-releasing factor (CRF) mRNA in the paraventricular nucleus were measured by in situ hybridisation technique after 1 day, 1 and 5 weeks of treatment with ZD7114 ((*S*)-4-[2-[(2-hydroxy-3-phenoxypropyl)amino]ethoxy]-*N*-(2-methoxyethyl)phenoxyacetamide, 3 mg kg^{-1} day^{-1} in drinking water) in obese *fa/fa* Zucker rats. In addition, expression of leptin mRNA in epididymal fat and serum levels of leptin were analysed. Food intake, body weights, binding of GDP to brown adipose tissue mitochondria, plasma insulin and glucose were also measured. Treatment with ZD7114 significantly reduced weight gain and activated brown adipose tissue thermogenesis, but had no effect on food intake. Expressions of preproNPY or preproCRF mRNAs were similarly not changed by treatment with ZD7114. Furthermore, ZD7114 had no effect on plasma insulin or leptin and the expression of leptin mRNA in epididymal fat. However, statistically significant correlations were found between preproNPY and preproCRF mRNA expressions and brown fat thermogenic activity and plasma insulin levels in the ZD7114 treated rats, but not in the control rats. It is concluded that treatment with ZD7114 markedly activated brown fat thermogenesis, but did not affect neuropeptide Y (NPY) and CRF gene expression per se. However, the correlation analyses suggest that ZD7114 may modulate feedback connections of brown adipose tissue thermogenesis and plasma insulin with the hypothalamic neuroendocrine mechanisms integrating body energy balance.

Keywords: Brown fat; Zucker rat; β_3-adrenoceptor agonist; Neuropeptide Y; CRF (corticotropin-releasing factor); Leptin

1. Introduction

Selective β_3-adrenoceptor agonists are potential new drugs for treatment of obesity. They activate non-shivering thermogenesis in brown adipose tissue (Arch et al., 1984), which is the major site for cold- and diet-induced thermogenesis in rodents (Ricquier and Mory, 1984) and contributes to energy balance by dissipating excessive dietary caloric intake into heat (Rothwell et al., 1982). In several experimental rodent models of obesity, thermogenic activity of brown adipose tissue is lowered (Bray et al., 1989). Activation of brown adipose tissue thermogenesis by β_3-adrenoceptor agonists leads to increased energy expenditure and decreased weight gain in obese rodents (Arch et al., 1984; Holloway et al., 1992; Santti et al., 1994). Furthermore, chronic treatment improves insulin sensitivity (Holloway et al., 1992), but exact biochemical mechanisms contributing to this phenomenon are unknown.

Body energy balance is regulated by complex central and peripheral mechanisms. The major site for central regulation is the hypothalamus, where afferent signals from periphery and upper brain areas are integrated (Morley, 1987; Kaiyala et al., 1995). Several neurotransmitters and neuropeptides, including neuropeptide Y (NPY) and corticotropin-releasing factor (CRF), are involved in the hypothalamic regulation of body energy balance (Morley, 1987). NPY is the most potent naturally occurring orexi-

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genic agent (Morley, 1987). Its synthesis and release in the arcuate nucleus are increased in situations where food intake is restricted (Beck et al., 1990b; Brady et al., 1990; Pesonen et al., 1992a; Lewis et al., 1993). In addition, centrally administered NPY decreases energy expenditure by reducing brown fat thermogenesis and facilitating fat storage into the white adipose tissue (Billington et al., 1991). CRF has an opposite effect on energy balance: it suppresses food intake and increases energy expenditure (Rothwell, 1990).

Insulin and leptin play important roles in the peripheral feedback signalling. Leptin is a product of adipose tissue (Zhang et al., 1994) and acts through hypothalamic leptin receptors (Lee et al., 1996). It is thought to act as a feedback signal of the size of the body adipose mass to the energy balance controlling areas of the hypothalamus (Matson et al., 1996). NPY mediates, at least partly, the effects of leptin, since the administration of recombinant leptin reduces NPY and its mRNA levels in the hypothalamus of obese (*ob/ob*) mice which lack endogenous leptin (Stephens et al., 1995; Schwartz et al., 1996a) and in normal rats (Schwartz et al., 1996b; Wang et al., 1997). On the other hand, intracerebroventricular (i.c.v.) infusion of NPY increases adipose tissue leptin mRNA expression in normal rats suggesting a reciprocal regulation of leptin and NPY (Sainsbury et al., 1996). CRF seems also to be involved in mediating leptin effect as recombinant leptin elicits the release of CRF from the hypothalamus and adrenocorticotropin from the pituitary in superfused brain slice preparations (Raber et al., 1997) and i.c.v. administered leptin seems to increase CRF mRNA expression in the paraventricular nucleus in rats (Schwartz et al., 1996b). Insulin serves as another feedback signal from the periphery to the hypothalamus. When levels of insulin in the circulation are low, NPY activity is high in the hypothalamus, and increase in plasma insulin levels leads to decrease in NPY level (Schwartz et al., 1992). Central NPY infusion increases plasma insulin (Sainsbury et al., 1997). Insulin is also involved in the control of CRF and hypothalamo–pituitary–adrenal axis (Dallman et al., 1993). Thus, the hypothalamic regulatory mechanisms including NPY and CRF neurons, are affected by peripheral metabolic feedback signals, which, in turn, can be regulated by the same central mechanisms.

This study was aimed to investigate how activation of brown adipose tissue thermogenesis with the β_3-adrenoceptor agonist (*S*)-4-[2-[(2-hydroxy-3-phenoxypropyl) amino]ethoxy]-*N*-(2-methoxyethyl)phenoxyacetamide (ZD7114) is reflected in changes in peripheral metabolic signals insulin and leptin and activity of hypothalamic neuropeptides NPY and CRF in genetically obese Zucker rat by measuring the expression of their mRNAs in the arcuate and the paraventricular nucleus, respectively. Brown fat mitochondrial GDP binding was measured as it is an established method to study the activity of brown fat thermogenesis (Trayhurn and Milner, 1989).

2. Materials and methods

2.1. Animals

A total of 48 obese *fa/fa* male Zucker rats were purchased from IFFA CREDO (L'Arbresle, France). The rats were approximately 10 weeks old in the beginning of the experiment. They were individually housed and maintained under a constant light–dark cycle (lights on from 0600 to 2000 h). The rats were provided with normal laboratory rat chow (SDS rat and mouse no. 1 maintenance diet, Whitham, UK) and had free access to drinking water.

2.2. Experimental design

The animals ($n = 48$) were divided into two groups matched with body weight and 24-h food intake. The treatment group received ZD7114 3 mg kg^{-1} day^{-1} dissolved in drinking water and the control rats received drinking water without ZD7114. Both the groups were further divided into three groups with different treatment durations. The first group was treated for 1 day, the second for 1 week and the third for 5 weeks either with ZD7114 or water only. The dose of ZD7114 was based on previous experiments where it was found to increase GDP-binding in chronically treated obese Zucker rats (Holloway et al., 1992). The bottles for drinking water were covered with aluminium film to protect the drug from light. The 24-h fluid intake was monitored and the concentration of ZD7114 in the drinking water was adjusted every second day to maintain its correct daily dose. Food intake was measured every second day by placing a weighted amount of pelleted food in the cage and calculating the amount consumed during 48 h. Body weights were also measured every second day. In the last day of the treatment, food was withdrawn from all animals at 0600 h. They were decapitated between 0900 and 1200 h. The brains were quickly removed and frozen in isopentane on solid carbon dioxide (CO_2). Blood was collected into prechilled EDTA tubes, whereafter plasma was separated. The epididymal fat was removed, weighted and frozen on solid CO_2. Intrascapular brown adipose tissue was dissected free from surrounding tissues and used for the preparation of mitochondrial fraction in order to analyse the binding of GDP. All the samples were stored at -70°C until analysed.

2.3. Analytical procedures

2.3.1. Hypothalamic preproneuropeptide Y and prepro-CRF mRNA expression

The brain was cut in a cryostat (at -18°C) into coronal 14-μm sections at the level of the arcuate nucleus (A4890) and the paraventricular nucleus (A5660) according to the co-ordinates in the stereotaxic atlas of König and Klippel

(1974). The in situ hybridisation procedure was performed as described earlier (Pesonen et al., 1992a). Brain sections were placed against Kodak X-OMAT AR film and exposed for 7–10 days at −70°C with intensifying screens and ^{14}C standards (American Radiochemicals, St. Louis, MO, USA). The autoradiography films were analysed with a OS/2 based image analysis system (MCID, Imaging Research, Ontario, Canada). For linear quantification ln(grey value of standard) was plotted against ln(radioactivity of ^{14}C standard). The grey values of each animal were converted to radioactivity per tissue equivalent, using the standard curve, and expressed as relative radioactivity units.

2.3.2. Leptin mRNA expression

The RNA for the determination of leptin mRNA content was extracted from epididymal fat by acid guanidinium–phenol–chloroform method described earlier (Chomczynski and Sacci, 1987). Levels of leptin mRNA were measured using slot-blot hybridisation manifold (Schleicher & Schuell, Dassel, Germany). Oligoprobe of 33 nucleotides (5′-GGTCTGAGGCAGGGAGCAGCTCTTG-GAGAAGGC-3′) was used (KeboLab, Stockholm, Sweden). The leptin probe was labelled with [α-^{32}P]dCTP using the DNA 3′-end labelling kit (Boehringer Mannheim, Mannheim, Germany). To determine the amount of RNA loaded into each well, the membrane was also control probed hybridising it with a 28S ribosomal cDNA fragment. The hybridisation membrane was Gene Screen Plus (Du Pont NEN, Boston, MA, USA). The prehybridisation solution contained 50% (v/v) formamide, 0.5% (w/v) sodium dodecyl sulphate (SDS), 6 × SSPE (0.06 M sodium phosphate, 0.006 M EDTA), 5 × Denhardt's and 0.2 mg ml^{-1} denatured salmon sperm DNA. For the hybridisation the labelled probe was added. Prehybridisation (1.5 h) and hybridisation (23 h) were performed at 37°C. Washes were performed twice in 0.03 M sodium citrate (2 × SSC) plus 0.1% SDS at room temperature for 5 min and twice in 0.1 × SSC plus 0.1% SDS at 48°C for 15 min. For rehybridisation of the membrane, the probe was removed by boiling in 10 mmol l^{-1} Tris–HCl, 1 mmol l^{-1} EDTA and 1% SDS. For autoradiography, the membrane was placed against Kodak XAR-5 film and exposed at −70°C. Slot-blots were quantitated with the MCID image analysis system. The amount of leptin mRNA was expressed in relation to the amount of 28S mRNA. The specificity of hybridisation with the leptin oligo probe was determined by Northern blot analysis of brown fat total RNA after formaldehyde gel electrophoresis (data not shown). Hybridisation conditions were identical in the slot-blot and Northern hybridisations.

2.3.3. Plasma leptin

Plasma leptin was analysed with rat leptin RIA kit (Linco Research, St. Charles, MO, USA).

2.3.4. [^{3}H]GDP binding

Binding of [^{3}H]GDP to brown adipocyte mitochondria was measured as described earlier (Santti et al., 1994). In brief, the fresh brown fat pads were minced, diluted in 250 mM ice cold sucrose buffer and homogenised. The homogenate was used for immediate preparation of mitochondria with differential centrifugation. The binding of [^{3}H]GDP was determined by incubating mitochondria in a basic medium containing 100 mM sucrose, 20 mM TES (*N*-tris-[hydroxymethyl]-methyl-2-aminoethane-sulphonic

Table 1
Final weight, weight gain, weight of epididymal white fat, cumulative food intake, plasma insulin and glucose, binding of GDP to brown fat mitochondria, mitochondrial protein and mRNA expressions (relative density units) of preproNPY in the arcuate nucleus and preproCRF the paraventricular nucleus in obese male Zucker rats treated with control or ZD7114 (3 mg kg^{-1} day^{-1} p.o.) for 1 day, and 1 or 5 weeks

	1 day		1 week		5 weeks	
	Control	ZD7114	Control	ZD7114	Control	ZD7114
Final weight (g)	313 ± 4	325 ± 5	361 ± 9	358 ± 7	467 ± 7	445 ± 7[a]
Weight gain (g)	1.6 ± 0.4	1.4 ± 0.5	30 ± 1.5	26 ± 1.3	150 ± 7	129 ± 6[d]
Epididymal fat (g)	5.7 ± 0.2	5.8 ± 0.5	6.7 ± 0.3	6.8 ± 0.5	9.9 ± 0.6	9.2 ± 0.3
Cumulative food intake (g)	n.d.	n.d.	188 ± 5	188 ± 3	1019 ± 27	1000 ± 25
Plasma insulin (ng ml^{-1})	12.5 ± 2.0	14.7 ± 2.6	13.0 ± 1.8	10.1 ± 1.8	16.4 ± 2.3	11.2 ± 0.7
Plasma glucose (mmol l^{-1})	7.8 ± 0.2	7.7 ± 0.1	7.4 ± 0.2	7.4 ± 0.2	7.9 ± 0.2	7.8 ± 0.2
GDP-binding (pmol g^{-1} tissue)	192 ± 36	554 ± 62[c]	254 ± 45	1149 ± 68[c]	61 ± 18	536 ± 63[c]
GDP-binding (pmol mg^{-1} protein)	197 ± 26	278 ± 21[a]	211 ± 35	281 ± 16[a]	214 ± 23	200 ± 13
Brown fat protein (mg lobe^{-1})	0.5 ± 0.1	1.0 ± 0.1[b]	0.6 ± 0.1	2.3 ± 0.2[c]	0.1 ± 0.03	1.8 ± 0.2[c]
NPY mRNA	0.18 ± 0.01	0.19 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
CRF mRNA	0.16 ± 0.01	0.15 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01

$n = 8$ in each group.
Values are mean ± S.E.M.
n.d. = not detected.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ compared to control in the same timepoint, two-way ANOVA followed by contrasts.
[d] $P = 0.015$, ZD7114 and control treatment differ significantly, two-way ANOVA.

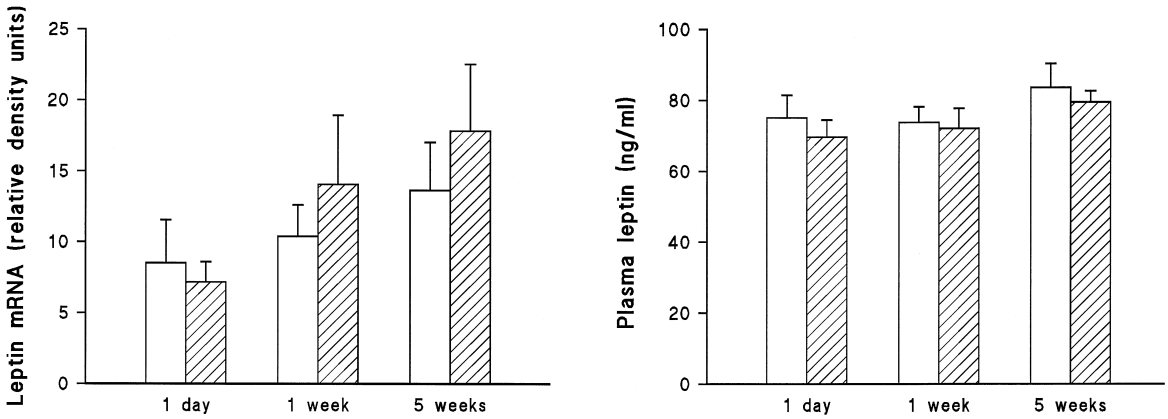

Fig. 1. Expression of leptin mRNA in epididymal fat (left panel) and concentration of plasma leptin (right panel) after 1 day, 1 or 5 weeks of treatment with ZD7114 (3 mg kg^{-1} day^{-1}, hatched bars) or control (open bars) in obese Zucker rats ($n = 8$ in each group). Values are the mean ± S.E.M. No statistically significant difference emerged between the ZD7114 treated and control rats.

acid), 1 mM EDTA, 10 mM choline chloride, 2 μM rotenone, [^{14}C]sucrose and 10 μM [^{3}H]GDP. Protein content of mitochondrial suspensions were assayed according to the method of Peterson (1977).

2.3.5. Plasma insulin and glucose

Plasma insulin was measured with rat insulin RIA kit supplied by Novo BioLabs, Bagsvaerd, Denmark. Plasma glucose was analysed with glucose oxidase method with an Analox GM 7 measuring device (Analox, London, UK).

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) revealing the effect of time and treatment was used. If a significant difference in the interaction term was noted, ZD7114 and control groups were compared with contrast analysis at

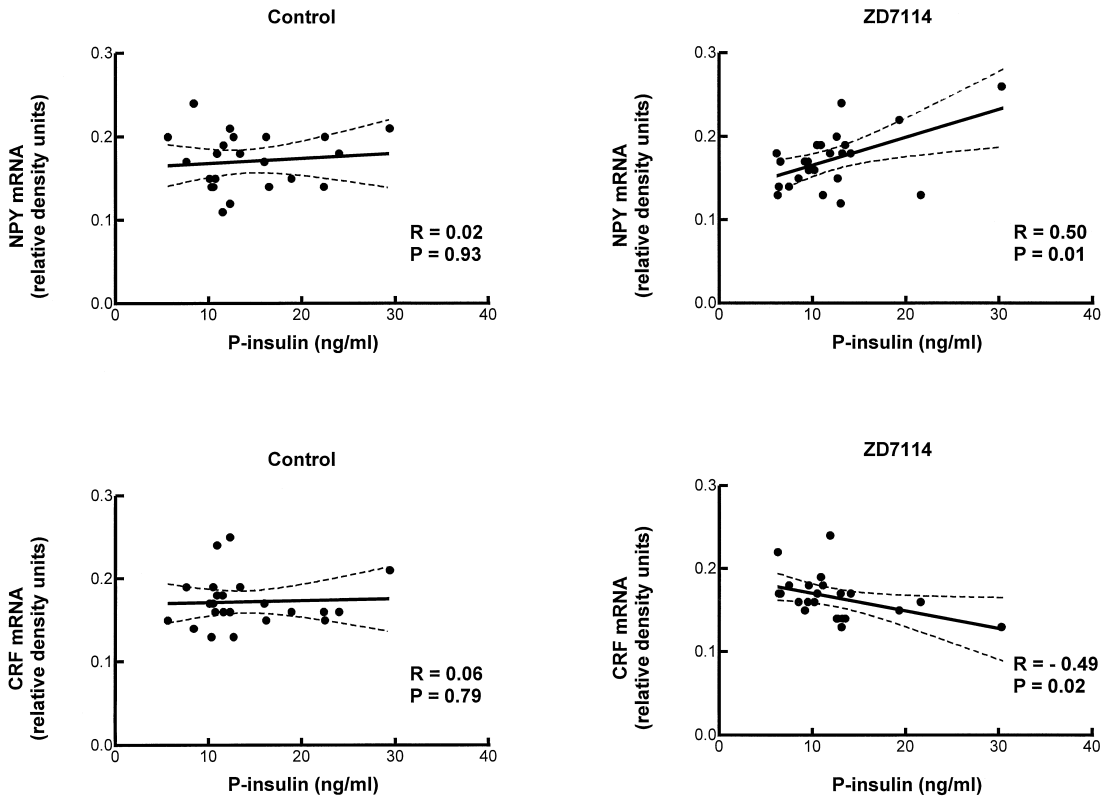

Fig. 2. Correlation between plasma insulin and mRNA expressions of preproNPY in the arcuate nucleus or preproCRF in the paraventricular nucleus after control (left panels) and ZD7114 (3 mg kg^{-1} day^{-1}, right panels) treatments for 1 day, 1 or 5 weeks in obese Zucker rats. R and P values from Pearson's correlation test, plasma insulin and preproCRF mRNA expression values used in statistical analysis after logarithmic transformation.

each time point. The Pearson's correlation coefficients were computed to reveal correlations between two variables. Logarithmic or square root transformations of the data were used when necessary to normalise the data. The calculations were performed using the BMDP software (BMDP Statistical Software, programs 7D, 4V and 6D, Los Angeles, CA, USA). A *P*-value less than 0.05 was considered statistically significant.

3. Results

There was a statistically significant reduction in weight gain after treatment with ZD7114 (treatment: $P = 0.015$, time: $P < 0.001$, interaction: $P = 0.25$, two-way ANOVA, Table 1). The final weights are shown in Table 1. Cumulative food intake was not changed after 1 and 5 weeks treatments (treatment: $P = 0.62$, time: $P < 0.001$, interaction: $P = 0.65$, Table 1). Food intake after 1 day treatment was not measured. Feed efficiency was higher in rats treated for 1 week than rats treated for 5 weeks, and tended to be lower in the ZD71114 treated groups than control (1 week, control: 0.16 ± 0.01, 1 week, ZD7114: 0.13 ± 0.01, 5 weeks, control: 0.12 ± 0.02, 5 weeks, ZD7114: 0.10 ± 0.02; treatment: $P = 0.07$, time: $P = 0.006$, interaction: $P = 0.76$). The weight of epididymal fat was similar in both groups at all timepoints (treatment: $P = 0.54$, time: $P < 0.001$, interaction: $P = 0.49$, Table 1). Plasma insulin levels were reduced by 22% in the rats treated with ZD7114 for 1 week and by 32% after 5 week treatment. However, the effect of ZD7114 on plasma insulin was not statistically significant (treatment: $P = 0.16$, time: $P = 0.31$, interaction: $P = 0.15$, Table 1). Plasma glucose was not changed by ZD7114 (treatment: $P = 0.97$, time: $P = 0.052$, interaction: $P = 0.87$, Table 1).

Binding of GDP to brown fat mitochondria expressed as pmol g^{-1} of brown adipose tissue was increased in ZD7114 treated rats at all timepoints. This effect occurred already after 1 day of treatment. The binding was higher after 1 week and declined after 5 weeks (treatment: $P < 0.001$, time: $P < 0.001$, interaction: $P < 0.001$, Table 1). Likewise, treatment effect was significant, when binding of GDP was expressed as pmol mg^{-1} of mitochondrial protein, but the level of binding did not change over time (treatment: $P = 0.007$, time: $P = 0.30$, interaction: $P = 0.08$, Table 1). The content of mitochondrial protein was increased after treatment with ZD7114 (treatment: $P < 0.001$, time: $P < 0.001$, interaction: $P < 0.001$, Table 1).

The expression of leptin mRNA in epididymal fat was not changed by treatment with ZD7114 (treatment: $P = 0.63$, time: $P = 0.16$, interaction: $P = 0.84$, Fig. 1). Simi-

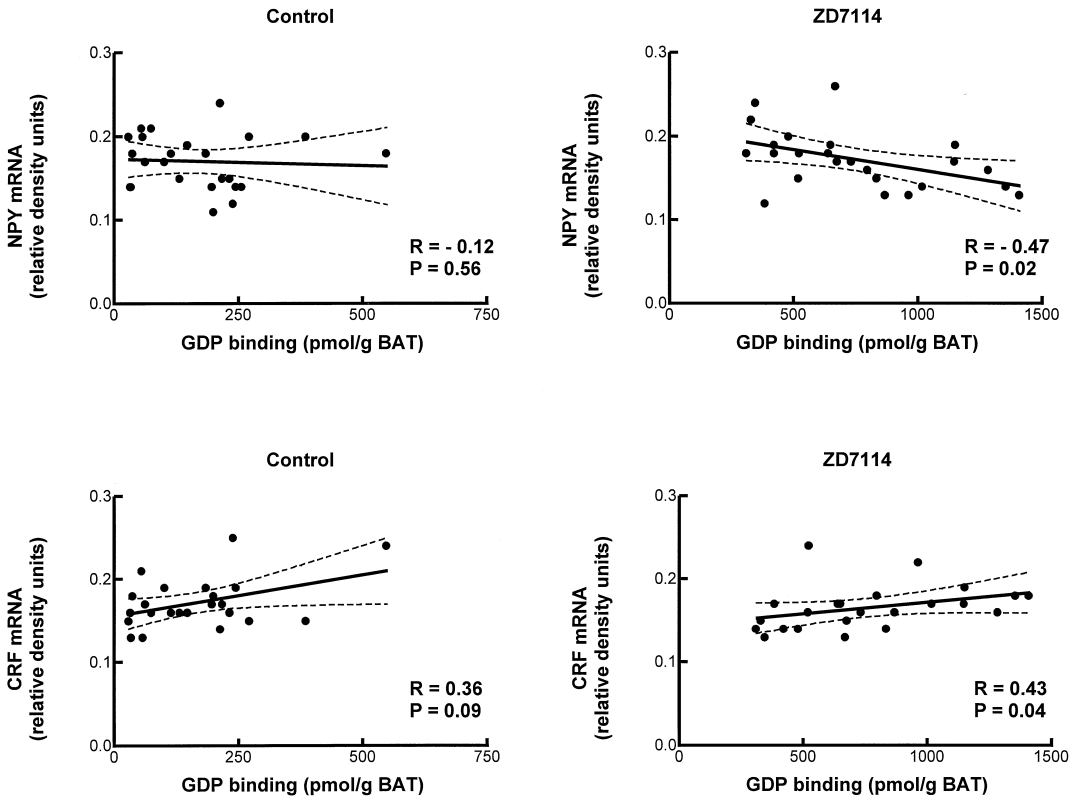

Fig. 3. Correlation between the GDP binding to brown fat mitochondria and mRNA expressions of preproNPY in the arcuate nucleus or preproCRF in the paraventricular nucleus after control (left panel) and ZD7114 (3 mg kg^{-1} day^{-1}, right panel) treatments for 1 day, 1 or 5 weeks. *R* and *P* values from Pearson's correlation test, GDP binding and preproCRF mRNA expression values used in statistical analysis after logarithmic transformation.

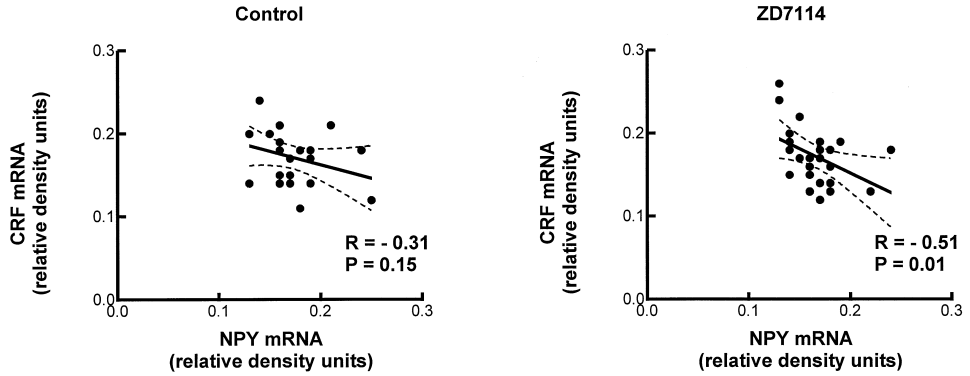

Fig. 4. Correlation between mRNA expressions of preproNPY in the arcuate nucleus or preproCRF in the paraventricular nucleus after control (left panel) and ZD7114 (3 mg kg^{-1} day^{-1}, right panel) treatments for 1 day, 1 or 5 weeks in obese Zucker rats. R and P values from Pearson's correlation test, preproCRF mRNA expression values used in statistical analysis after logarithmic transformation.

larly, ZD7114 had no effect on the level of leptin in plasma (treatment: $P = 0.17$, time: $P = 0.39$, interaction: $P = 0.94$, Fig. 1).

Treatment with ZD7114 did not change preproneuropeptide Y (preproNPY) mRNA expression in the arcuate nucleus (treatment: $P = 0.86$, time: $P = 0.03$, interaction: $P = 0.72$, Table 1). The expression was lower in the rats treated for 1 week with either ZD7114 or water only than in those treated for 1 day ($P = 0.03$) or 5 weeks ($P = 0.04$). Similarly, there was no difference in the expression of preproCRF mRNA in the paraventricular nucleus between ZD7114 treated and control animals (treatment: $P = 0.33$, time: $P = 0.02$, interaction: $P = 0.77$, Table 1). However, the expression was higher in rats treated for 1 week compared to those treated for 1 day ($P = 0.007$) or 5 weeks ($P = 0.04$).

There was no correlation between preproNPY and preproCRF mRNA and plasma insulin when all the rats were used in the analysis. However, when the control and ZD7114 treated rats were analysed separately a positive correlation between preproNPY mRNA and plasma insulin, and a negative correlation between preproCRF mRNA and plasma insulin were revealed in the ZD7114 treated rats, while no correlation was seen in the control rats (Fig. 2). Similarly, a negative correlation between preproNPY mRNA and GDP binding to brown fat mitochondria and a positive correlation between preproCRF mRNA and GDP binding were seen in the ZD7114 treated, but not in the control rats (Fig. 3). Similar correlation was seen between protein content of brown adipose tissue mitochondria and preproNPY mRNA ($R = -0.16$, $P = 0.46$ in control; $R = -0.42$, $P = 0.04$ in ZD7114 group) and preproCRF mRNA ($R = 0.27$, $P = 0.21$ in control; $R = 0.52$, $P = 0.01$ in ZD7114 group). Furthermore, preproNPY and preproCRF mRNA expressions correlated after treatment with ZD7114, but not after control treatment (Fig. 4).

Plasma leptin correlated with weight gain ($R = 0.30$, $P = 0.04$; control: $R = 0.29$, $P = 0.17$; ZD7114: $R = 0.31$, $P = 0.15$), weight of epididymal fat ($R = 0.43$, $P = 0.002$, control: $R = 0.32$, $P = 0.13$, ZD7114: $R = 0.59$, $P = 0.003$) and leptin mRNA expression in epididymal fat ($R = 0.50$, $P < 0.001$; control: $R = 0.53$, $P = 0.009$; ZD7114: $R = 0.51$, $P = 0.02$). There was no correlation between plasma leptin and plasma insulin ($R = 0.23$, $P = 0.11$; control: $R = 0.46$, $P = 0.02$; ZD7114: $R = -0.10$, $P = 0.65$), final body weight ($R = -0.09$, $P = 0.55$; control: $R = -0.13$, $P = 56$; ZD7114: $R = -0.04$, $P = 0.87$), GDP binding ($R = -0.26$, $P = 0.08$; control: $R = -0.34$, $P = 0.11$; ZD7114: $R = -0.10$, $P = 0.66$), preproNPY mRNA ($R = -0.04$, $P = 0.82$, control: $R = 0.19$, $P = 0.38$; ZD7114: $R = -0.29$, $P = 0.17$) or preproCRF mRNA ($R = -0.13$, $P = 0.38$; control: $R = -0.23$, $P = 0.29$, ZD7114: $R = -0.04$, $P = 0.86$).

4. Discussion

Selective β_3-adrenoceptor agonists decrease weight gain and increase non-shivering thermogenesis in brown fat. The aim of this study was to investigate if the change in energy expenditure changes energy intake and regulatory mechanisms of body energy balance. We found that treatment with ZD7114 had no effect on food intake or the hypothalamic preproNPY or preproCRF mRNA expression, expression of leptin mRNA or plasma leptin or insulin levels per se in obese Zucker rats. However, significant negative correlation between preproNPY mRNA and thermogenic activity in brown fat and a positive correlation between preproCRF mRNA and thermogenesis was found in the ZD7114 treated rats, but not in the control rats (Fig. 3). Similarly, ZD7114 induced a statistically significant correlation between plasma insulin and preproNPY and preproCRF mRNA expressions (Fig. 2). Furthermore, preproNPY and preproCRF expressions correlated in the ZD7114 treated, but not in the control rats (Fig. 4).

Despite of increased brown fat thermogenesis and reduced weight gain in the ZD7114 treated rats, food intake

was not compensatorily changed. In line with this, hypothalamic mRNA expressions of food intake regulating neuropeptides NPY and CRF were not changed. Food intake has been reported earlier to be unchanged in obese animals, but slight increase is seen in lean animals during chronic β_3-adrenoceptor agonist treatment (Arch and Wilson, 1996). In agreement with our NPY mRNA data, NPY protein content in the paraventricular nucleus was not changed by chronic BRL 35135 treatment in *fa/fa* Zucker rats (Santti et al., 1994) and no change was seen in the arcuate NPY mRNA after 24-h i.p. CL 316243 in lean mice (Mantzoros et al., 1996). ZD7114 increased GDP binding by 9-fold and decreased weight gain by 14%. However, the effect is modest compared to the effect seen after BRL 35135 treatment where GDP binding was increased by 45-fold and weight gain reduced by 19% and weight of epididymal fat significantly decreased (Santti et al., 1994). Although ZD7114 treatment tended to decrease plasma insulin levels the change was only modest and not statistically significant which contrasts data obtained by BRL 35135 (Santti et al., 1994).

ZD7114 is a potent and selective β_3-adrenoceptor agonist, which stimulates whole body oxygen consumption and brown adipose tissue thermogenesis at doses which have minimal effects on cardiovascular parameters in rat (Holloway et al., 1991). It has been shown to activate brown fat thermogenesis even in situations when it is lowered such as in obese Zucker rats and adult dogs (Champigny et al., 1991; Holloway et al., 1992). Furthermore, chronic treatment leads to decreased weight gain in these animals and improved glucose homeostasis, independent of weight loss, in animal models of non-insulin dependent diabetes (Holloway et al., 1992). In isolated brown adipocytes, ZD7114 stimulates oxygen consumption with efficacy comparable to isoprenaline, but in white adipocytes it is a partial agonist at β_3-adrenoceptors (Mayers et al., 1996). This may explain the rather weak metabolic effects of ZD7114 in obese animals.

Although preproNPY and preproCRF mRNA expressions were not changed, we found significant correlations between NPY, CRF, brown fat thermogenesis and plasma insulin in ZD7114 treated rats. First, brown fat thermogenic activity negatively correlated with preproNPY mRNA expression level and positively with preproCRF mRNA in ZD7114 treated rats, whereas no correlation was seen in the control rats (Fig. 3). The correlation is in accordance with the known effects of NPY and CRF on brown adipose tissue activity as i.c.v. injection of NPY decreases and CRF administration increases activity in brown fat independently of their effects on food intake (Rothwell, 1990; Billington et al., 1991). Therefore, the improvement in the defective brown fat thermogenesis in obese Zucker rats by ZD7114 may be reflected to central neuroendocrine control mechanisms.

Second, NPY and CRF themselves correlated in the ZD7114 treated, but not in the control obese Zucker rats in this study (Fig. 4). NPY and CRF seem to interact in normal situations (Dallman et al., 1993; Sahu and Kalra, 1993), CRF administration i.c.v. was shown to decrease NPY mRNA levels in the hypothalamus of lean and obese Zucker rats (Bchini-Hooft van Huijsduijnen et al., 1993) and to antagonise the orexigenic effect of NPY (Morley et al., 1987). Inhibition of endogenous CRF activity by the CRF antagonist (Heinrichs et al., 1993) or by immunotargeted toxins (Menzaghi et al., 1993) enhanced feeding response to exogenous NPY. In obese Zucker rats these regulatory mechanisms and their connections are widely disturbed as they show increased NPY activity (Beck et al., 1990a; Sanacora et al., 1990; McCarthy et al., 1991; Pesonen et al., 1992a) and abnormalities in hypothalamo–pituitary–adrenal axis (Guillaume-Gentil et al., 1990; Pesonen et al., 1992b). After ZD7114 treatment these animals show a physiologically relevant negative correlation between the two neuropeptides with opposite effects on energy balance suggestive of a new homeostasis between NPY and CRF.

Third, ZD7114 treatment induced a positive correlation between plasma insulin and preproNPY mRNA and a negative correlation between plasma insulin and preproCRF mRNA whereas no correlation was seen in the control rats (Fig. 3). Plasma insulin is involved in the control of hypothalamic NPY (Schwartz et al., 1992) and CRF and hypothalamo–pituitary–adrenal axis (Dallman et al., 1993; Strack et al., 1995). Obese Zucker rats are insulin resistant and i.c.v. insulin fails to regulate NPY in these animals (Schwartz et al., 1991). The correlation could reflect restored link between periphery and the hypothalamus in the ZD7114 treated rats. Positive correlation is opposite to the inverse correlation between insulin and NPY reported earlier (Pickavance et al., 1996) and also in contrast to decreased NPY activity after i.c.v. insulin administration (Schwartz et al., 1991) in lean rats. However, in obese Zucker rats hyperinsulinemia, severe insulin resistance and the possible effect of the drug treatment on insulin sensitivity may complicate the interpretation of the data and thus no firm conclusions can be drawn without additional studies.

Correlation analysis should be interpreted with caution, since they do not prove a causal relationship between the parameters. Considering the complexity of the neuroendocrine mechanisms controlling food intake and energy expenditure, where most single factors affect each other and sometimes to opposite directions, correlation analysis may reveal important drug effects which would not necessarily be reflected in a single parameter per se. It is noteworthy that the observed statistically significant correlations between the measured parameters are in many respects physiologically relevant.

Activation of sympathetic nervous system and β_3-adrenoceptors has been demonstrated to inhibit leptin synthesis and release in adipose tissue in vitro and in vivo in lean animals (Moinat et al., 1995; Trayhurn et al., 1995,

1996; Giacobino, 1996; Mantzoros et al., 1996). The effect seems to be smaller in obese rodents (Trayhurn et al., 1996). In the present study, ZD7114 had no effect on leptin mRNA expression in epididymal fat or plasma leptin concentration in obese Zucker rats. It is possible that due to the defective leptin receptor in *fa/fa* rats (see below) the synthesis and release of leptin may be insensitive to changes in energy balance in obese Zucker rats. This is further supported by the lack of correlation between plasma leptin levels and hypothalamic preproNPY and preproCRF mRNA expressions. On the other hand, plasma leptin is known to correlate with size of white adipose tissue and plasma insulin level (Matson et al., 1996). In this experiment, the size of epididymal fat was not changed by ZD7114 treatment and similarly, there was no statistically significant change in plasma insulin, which could have contributed to the lack of effect on leptin. In fact, chronic BRL 35135 treatment, which significantly reduced both the weight of epididymal fat and plasma insulin level, decreased the plasma level of leptin in *fa/fa* rats (Savontaus et al., unpublished observation).

Obese *fa/fa* Zucker rats have a point mutation in the gene encoding the leptin receptor resulting in amino acid substitution in the extracellullar domain of the receptor (Iida et al., 1996; Takaya et al., 1996). The function of the mutated receptor is impaired, and obese Zucker *fa/fa* rats are therefore resistant to leptin (Matson et al., 1996). Use of the genetically obese *fa/fa* Zucker rat as a model to study interactions between peripheral metabolic feedback signals and central neuroendocrine mechanisms controlling energy balance should be considered in the right context. Although the obesity syndrome present in obese Zucker rats is rather complex and feedback connections from periphery to the hypothalamus are altered due to genetic abnormality in leptin receptor, this model is in many respects relevant to elucidate mechanisms of actions of anti-obesity drugs. First, these animals have a fully established obesity syndrome with many common characteristics of human obesity and non-insulin dependent diabetes. Second, leptin resistance is suggested to play a role in human obesity. Third, use of other genetically obese rodent models or, e.g., diet-induced obesity are also complicated with similar type of pitfalls. Finally, since regulation of energy balance is strongly state-dependent, anti-obesity drugs cannot be investigated in meaningful way using normal lean animals, if one would like to elucidate how such drugs affect mechanisms controlling altered metabolic balance of obesity.

It is concluded that although ZD7114 markedly activates brown adipose tissue thermogenesis, this does not result in altered NPY and CRF activities in obese Zucker rats. However, ZD7114 may modulate feedback connections between brown adipose tissue thermogenesis and plasma insulin with the hypothalamic neuroendocrine mechanisms integrating body energy balance.

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