

The relationship between central serotonergic activity and insulin sensitivity in healthy volunteers

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Abstract

In order to determine whether central serotonin (5-HT) activity is related to sensitivity of insulin receptors, 19 healthy volunteers with normal basal glycemia and HbA1c were studied. The relationship between prolactin response to D-fenfluramine (Δ PRL) in a challenge test and metabolic clearance rates (MCR) of glucose during the hyperinsulinemic–euglycemic clamp technique was evaluated. Δ PRL had been chosen as a correlate of central 5-HT activity. Two levels of insulin concentration of approximately 70 mU/l (MCR_{submax}) and 2000 mU/l (MCR_{max}) were used in a clamp, each for a duration of 120 min. A negative correlation was found between Δ PRL and MCR_{submax} ($r = -0.55$, $P < 0.02$) and between Δ PRL and MCR_{max} ($r = -0.51$, $P < 0.03$). We did not find any correlation between the prolactin response to D-fenfluramine and body weight, body mass index (BMI) or waist and hip circumference (WHR). The data support the hypothesis of a close connection between 5-HT activity in the brain and peripheral sensitivity to insulin. The possible physiological mechanisms of this connection are discussed. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Serotonin activity in the brain is often studied in relation to the regulation of affectivity and mood disorders. The study of sensitivity to insulin, on the other hand, focuses on the mechanism of insulin resistance, hyperinsulinemia, diabetes mellitus type 2 and atherogenesis. There are data suggesting a close correlation between the central serotonin activity and peripheral insulin sensitivity. Depressed patients, for example, show poorer utilization of glucose in the glucose tolerance test (GTT) (Van Praag and Leijnt, 1965; Mueller et al. 1968; Wright et al. 1976). Both the glucose and insulin levels in the GTT are elevated in depressed patients (Winokur et al., 1988). This could mean that a lower tolerance to glucose in these patients results from a decreased peripheral sensitivity to insulin rather than from a lower production of insulin in beta cells of the pancreas. The hypothesis of a lower sensitivity to insulin in depressed patients is also supported by data showing a decreased hypoglycemic response to insulin in these patients (Freeman, 1946; Van Praag and Leijnt, 1965; Wright et al. 1976; Casper et al. 1977). We hypothesize that there is a relationship between glucose metabolism and monoaminergic regulation in depression.

The idea of a reciprocal relation between sensitivity to insulin and central serotonin activity has also been supported by the influence of certain drugs on glucose metabolism. Substances which increase central serotonin activity, such as the 5-HT agonist D-fenfluramine or the selective serotonin re-uptake inhibitor fluoxetine, increase peripheral sensitivity to insulin and clinically improve the condition of insulin-non-dependent diabetes mellitus (Pestell et al., 1989; Potter van Loon et al., 1991; Scheen et al., 1991).

In our study, we tested the relation between central serotonergic activity and peripheral sensitivity to insulin in healthy volunteers with normal glycemia and a normal level of glyco-hemoglobin (HbA1). Only men were included in the study because varying estrogen levels in women influence the basal level of prolactin and, apparently, the serotonin activity as well (McBride et al., 1990; O'Keane et al., 1991a).

We used the D-fenfluramine challenge test (FF-test) to estimate central serotonergic activity. The FF-test measures changes in the prolactin plasma levels (Δ PRL) after the administration of the serotonin agonist D-fenfluramine. The stimulated level of prolactin thus reflects the central serotonergic activity. Previously in psychiatric research, either 60 or 30 mg of D-fenfluramine have been used (Coccaro and Kavoussi, 1994; Goodwin et al., 1994). Some authors (Goodwin et al., 1994) consider a dose of 30 mg insufficient. The dose of 60 mg is generally reported to be high enough to increase prolactin levels significantly (McBride et al., 1990; Muldon et al., 1996).

Peripheral sensitivity to insulin was determined using the hyperinsulin euglycemic clamp. This test assesses the glucose supply, which is necessary for maintaining normal glycemia after a standardized administration of insulin. Using these methods, the following hypotheses were tested:

1. There is a direct correlation between central serotonin activity and a peripheral sensitivity to insulin.
2. Body Mass Index and abdominal obesity are related to central serotonin activity and a sensitivity to insulin.

2. Methods

2.1. Study subjects

Nineteen healthy males (mean age 32.33 ± 11.11 years, mean weight 98.0 ± 17.96 kg) were included in the study. The Institute's ethical committee approved the study protocol, and informed consent was obtained from all subjects. One week before the FF-test, each subject underwent an examination including measures of weight, height and a basic biochemical screening. Normal glucose tolerance was confirmed by determining glyco-hemoglobin (HbA_{1c}). HbA_{1c} was measured using methods described by Fluckinger and Winterhalter (1976). Only men with HbA_{1c} in a normal range (3–6%) were included in the study. The body mass index (BMI) was calculated as weight/height² (kg/m²). Waist and hip circumference (WHR) was measured with patients in a supine position. Waist circumference was measured halfway between *crista* and *costa*. The hip circumference was measured over the widest part of the abdominal fat distribution and added together with the waist circumference, as suggested by Despres et al. (1991).

All subjects had normal basal levels of glucose (4.28 ± 0.58 mmol/l), and normal glyco-hemoglobin HbA_{1c} ($5.31 \pm 0.49\%$). The mean BMI was 28.53 ± 4.39 , and the range was 19.8–35.2. Six subjects (33%) were over the normal threshold of 30 for significant obesity. The mean WHR was 0.95 ± 0.06 .

2.2. The euglycemic glucose clamp

The euglycemic glucose clamp was performed on the first day of the study. We used a modification of the method (DeFronzo et al., 1979) for two levels of insulin (70 and 2000 mU/l), and each was performed over a period of 120 min. Euglycemia was maintained by an infusion of 20% dextrose dosed in response to plasmatic glycemia. The euglycemic clamp test started at 07:30 h, after an overnight fast. Before the test, subjects were placed in a supine position for 30 min. Two intracatheters were applied. One was placed in the antecubital vein. The second cannulated a dorsal hand vein in the opposite direction. The first was used for insulin and glucose infusions via infusion pumps (Infusomat-Secura, B. Braun Melsunger AG, Melsunger, FRO). The second was used for blood sampling. The subject's hand was kept in a heating box at 55°C to obtain arterialized venous blood. The examination lasted 4 h. Stepwise prime-continuous insulin infusions of 1 and 10 mU/kg/min of Humulin R recombination DNA origin, 40 IU/ml (Lilly France S.A., Fegersheim, France) were administered to rise acutely and to maintain the plasma concentrations of insulin at 70 and 1500 mU/ml in all examined groups.

The two insulin infusion steps lasted 120 min each. The plasma glucose concentration was maintained at 5.0 mmol/l by a variable infusion of 20% dextrose throughout the entire test period. The amount of infused solution was adjusted by negative feedback from glucose determinations, which were carried out every 5 min using a glucose analyzer (DeFronzo et al., 1979). For insulin sensitivity estimations, the glucose infusion rate was expressed as a steady state over the last 30 min of insulin infusion. Plasma insulin concentrations were measured both before the clamp and also every 10 min during the 90–120 min and 210–240 min periods. After the test procedure, subjects remained resting under medical supervision for at least 2 h.

2.3. Laboratory methods

Blood glucose was measured using the glucose oxidase method on a One-touch II system glucose analyzer (Lifescan Johnson & Johnson company, Milpitas, CA, USA) after proper calibration. Plasma immuno-reactive insulin (IRI) was determined using the AIA-600 Immunoassay Analyzer (Tosoh-Eurogenetics, Japan). The sensitivity of the assay was 0.1 ng/ml. The interassay coefficient of variation (c.v.) was 6.5–9.2%, and the intra-assay c.v. ranged between 4.5 and 9.1%. These laboratory measurements were performed at the Department of Biochemistry and Nuclear Medicine of the 3rd Faculty of Medicine, Charles University, Prague.

2.4. Data Analysis

The total amount of infused glucose was a measure of the glucose metabolized by cells during the clamp study. The effect of insulin was estimated as a metabolic clearance rate of glucose, calculated from the last 30 min of both insulin infusion steps, for example between 90 and 120 min (at a plasma insulin concentration of 70 mU/l- MCR_{submax}), and between 210 and 240 min (at a plasma insulin concentration of 2000 mU/l- MCR_{max}) (Gottesman et al., 1984). Sensitivity to insulin (SI) is expressed as the ratio $MCR_{submax}/MCR_{max} \times 100$ (%) (Pelikanova et al., 1994). Data were processed using a computerized statistical and graphical system developed for this purpose in BASIC language (Pelikanova et al., 1994).

2.5. Definition of variables

M_{submax} : glucose metabolized between 90 and 120 minutes (mg/kg/min). MCR_{submax} : metabolic clearance rate of glucose 90–120 min (ml/kg/min). M_{max} : glucose metabolized between 210 and 240 minutes (mg/kg/min). MCR_{max} : metabolic clearance rate of glucose 210–240 min (ml/kg/min).

M_{submax} and MCR_{submax} measure the insulin receptor response, the latter being the more sensitive parameter. M_{max} and MCR_{max} measure the postreceptor response of glucose. MCR_{max} is the more sensitive parameter (Rizza, 1981; DeFronzo, 1992). $SI = MCR_{submax}/MCR_{max} \times 100$ (%). SI shows the shift of the insulin dose–response curve.

2.6. The D-fenfluramine challenge test

The D-fenfluramine challenge test was performed 2 days after the euglycemic glucose clamp. The volunteers were requested to fast and refrain from smoking on the night before the test. Starting in the hospital at 07:00 h on the morning of the test, they remained at rest lying supine in the bed throughout the test period. At 07:45 h an IV catheter was inserted into a cubital vein and 500 ml of 5% glucose drip and 500 ml of saline drip were commenced to avoid the effects of a fast-induced hypoglycemia. To assay basal prolactin levels, 5 ml blood samples were drawn at 07:45 h. and at 08:00 h. At 08:00 h, 60 mg of D-fenfluramine was administered PO. Blood samples were drawn thereafter every hour for 4 h. The blood samples were centrifuged and stored in a freezer at 40°C. They were later analyzed for prolactin serum concentrations using RIA kits (Immunotech, France). The sensitivity of the assay was 0.5 ng/ml. The inter-assay coefficient of variation (c.v.) was 6.2–8% and the intra-assay c.v. ranged between 1.6 and 2.8%.

2.7. Definition of variables

Prolactin levels obtained at 08:00 h before the D-fenfluramine administration defined the prolactin baseline level (ng/ml). The peak value was the highest prolactin level following the D-fenfluramine challenge. *The prolactin response* (Δ PRL, ng/ml) was defined as the difference between the prolactin baseline level and the prolactin peak value.

2.8. Statistical analysis

The change in prolactin level after the administration of 60 mg of D-fenfluramine was appraised using the sign test. The Shapiro Wilk's test was used to assess the normality of values of prolactin increase. Correlations between Δ PRL and parameters of insulin sensitivity (glycemia, HbA_{1c}, M_{submax} , M_{max} , $\text{MCR}_{\text{submax}}$ and MCR_{max}) were assessed using the Spearman correlation coefficient. This test was also used for the relation between BMI (WHR) and Δ PRL and BMI (WHR), and the parameters of insulin sensitivity. A relationship between insulin sensitivity and BMI, and the prolactin response was estimated using multiple regression analysis. The parameters of insulin sensitivity served as a dependent variable, the prolactin response as an independent variable, and the BMI was used as a covariant in the analysis. A forward step-wise regression was used to get the best fitting model of prolactin response from the given set of predictors.

3. Results

Change in prolactin level after the administration of 60 mg of D-fenfluramine. The administration of 60 mg of D-fenfluramine led to a rise in the prolactin levels (sign test, $P \leq 0.01$); in two persons the PRL decreased. The average Δ PRL

following the administration of D-fenfluramine was 3.42 ng/ml. (± 4.00). Δ PRL values are not normally distributed (Shapiro Wilkov test, $P < 0.001$).

3.1. Correlation between the results of the D-fenfluramine challenge test and the parameters of insulin sensitivity (Table 1)

A significant correlation was found between Δ PRL and parameters M_{submax} ($r = -0.519$, $P = 0.027$), MCR_{submax} ($r = -0.550$, $P = 0.018$, Fig. 1) and MCR_{max} ($r = -0.513$, $P = 0.0295$, Fig. 2). No correlation was found between Δ PRL and the basal glucose level ($r = -0.395$, $P > 0.1$), nor between Δ PRL and HbA_{1c} ($r = 0.22$, $P > 0.1$).

3.2. Correlation between body weight, BMI, WHR, and Δ PRL following the D-fenfluramine administration

No significant correlation between body mass ($r = 0.395$; $P = 0.105$), BMI ($r = 0.33$; $P = 0.17$), abdominal obesity measured as WHR ($r = 0.20$, $P < 0.42$), and age ($r = 0.0093$, $P = 0.97$) on the prolactin response to Dfenfluramine was found (Table 1).

3.3. Correlation between BMI, WHR and the parameters of insulin sensitivity

Statistically significant correlations were found between BMI and all parameters of insulin sensitivity and between WHR and M_{submax} ($r = -0.5006$, $P = 0.0407$), M_{max} ($r = -0.6593$, $P = 0.0040$) and MCR_{max} ($r = -0.941$ a $P = 0.0119$) (Table 2).

3.4. Multiple regression analysis

Multiple regression analysis revealed a significant negative association between prolactin response and M_{submax} ($R^2 = 0.625$; $F(2,15) = 12.503$; $P = 0.00064$), MCR_{submax} ($R^2 = 0.5769$; $F(2,15) = 10.230$; $P = 0.00158$), MCR_{max} ($R^2 = 0.4662$;

Table 1
Correlation between prolactin response after D-FF stimulation (Δ PRL, ng/ml) and other variables

Variable	Spearman correlation $r =$	P -level
Age	0.0093	0.9707
Body weight	0.3948	0.1049
BMI	0.3374	0.1708
WHR	0.2078	0.4233
M_{submax}	-0.5190	0.0272*
MCR_{submax}	-0.5500	0.0180*
M_{max}	-0.3370	0.1708
MCR_{max}	-0.5129	0.0295*
SI	-0.3333	0.1708

* $P \leq 0.05$.

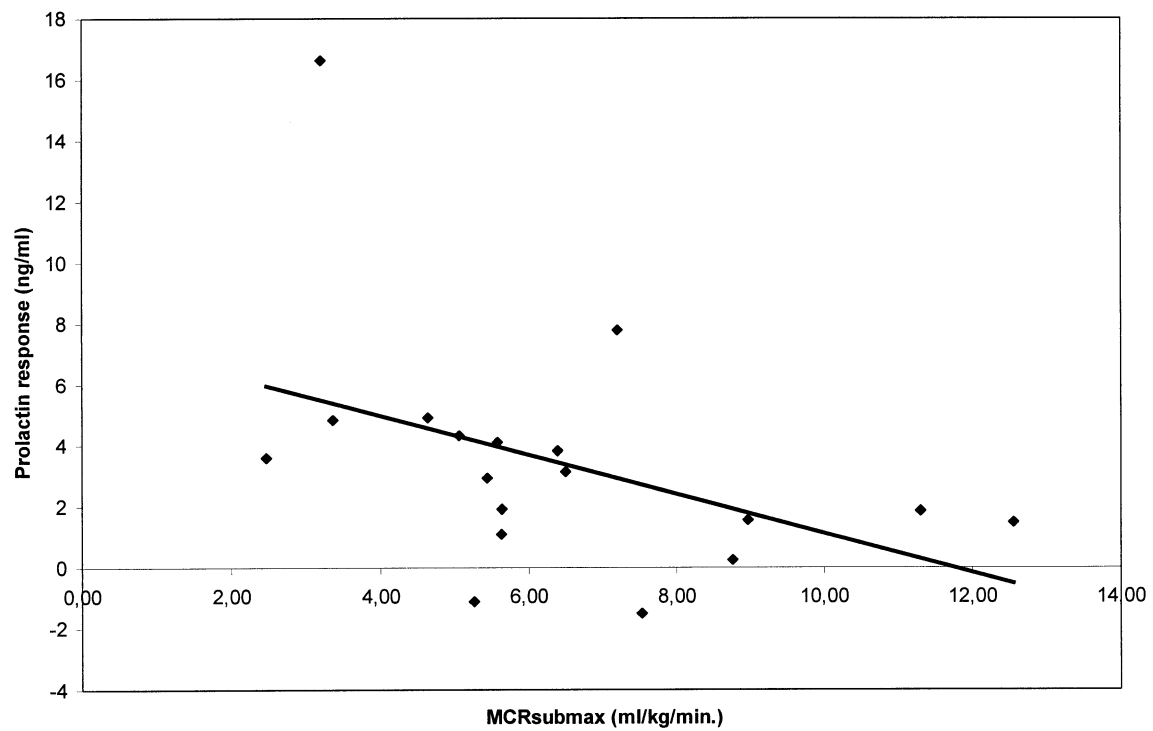


Fig. 1. The correlation between prolactin response in the D-fenfluramine test and the metabolic clearance rate of glucose at a plasma insulin concentration of 70 μ U/ml (MCR_{submax}). $R = -0.5500$, $P = 0.018$.

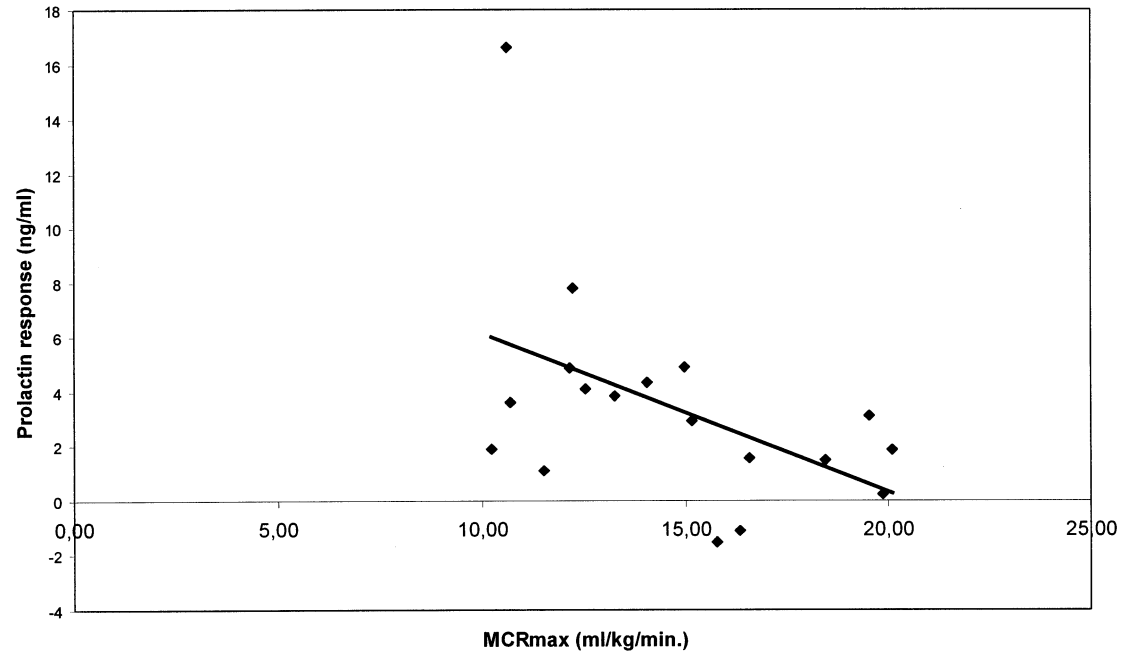


Fig. 2. The correlation between prolactin response in the D-fenfluramine test and the metabolic clearance rate of glucose at a plasma insulin concentration of 2000 $\mu\text{U}/\text{ml}$ (MCR_{max}). $R = -0.5129$,

Table 2

Correlation between BMI, WHR and the parameters of insulin sensitivity (M_{submax} , $\text{MCR}_{\text{submax}}$, M_{max} , MCR_{max} and SI)

Pair of variables	Spearman correlation $r =$	P -level
WHR vs M_{submax}	-0.5006	0.0407*
WHR vs $\text{MCR}_{\text{submax}}$	-0.4317	0.0835
WHR vs M_{max}	-0.6593	0.0040**
WHR vs MCR_{max}	-0.5941	0.0119*
WHR vs SI	-0.3124	0.2221
BMI vs M_{submax}	-0.6161	0.0065**
BMI vs $\text{MCR}_{\text{submax}}$	-0.5748	0.0126*
BMI vs M_{max}	-0.6698	0.0024**
BMI vs MCR_{max}	-0.6202	0.0060**
BMI vs SI	-0.4303	0.0746

* $P \leq 0.05$,

** $P \leq 0.01$.

$F(2,15) = 6.5497$; $P = 0.00903$) and for M_{max} ($R^2 = 0.4466$; $F(2,15) = 6.05121$; $P = 0.01183$).

3.5. The forward stepwise regression

The forward stepwise regression was used to get the best fitting model of prolactin response from the given set of predictors. In the first step the test included MCR_{max} as the best predictor ($R^2 = 0.230$; $F(1,16) = 4.777$; $P = 0.04405$); in the second step the age improved the predictive value of the model ($R^2 = 0.37810$; $F(1,15) = 4.56$; $P = 0.02837$). The P -level for age was under the statistical significance ($P = 0.078$) and the P -level for MCR_{max} decreased ($P < 0.0129$). In further steps, M_{max} (step 3) and body weight (step 4) were included in the regression, but the features of the model improved only slightly.

4. Discussion

Our finding of a correlation between insulin sensitivity parameters and WHR and BMI is in agreement with the well-known relationship between obesity, particularly the abdominal type, and a decreased sensitivity to insulin (Gerich, 1988; Young et al., 1989). In our study, we found a statistically significant negative correlation between glucose metabolized in the hyperinsulin euglycemic clamp (M_{submax} , $\text{MCR}_{\text{submax}}$ and MCR_{max}) and a prolactin response in the D-fenfluramine challenge test. This finding confirms a relationship between serotonergic activity and a sensitivity to insulin in healthy persons. As ΔPRL and the metabolic clearance rate of glucose correlated at both plasma insulin concentrations (70 mU/l for $\text{MCR}_{\text{submax}}$ and 2000 mU/l for MCR_{max}), we assume that both the receptor and post-receptor components of insulin sensitivity are somehow linked to 5-HT activity

(Gottesman et al., 1984). It is not easy to establish the way in which the insulin sensitivity is connected with 5-HT activity. In the FF-test, Δ PRL after D-fenfluramine administration is measured. D-fenfluramine is an indirect 5-HT agonist, which facilitates the pre-synaptic release of serotonin and blocks its reuptake. A lower Δ PRL after D-fenfluramine administration was found in depressed persons compared to healthy controls (Siever et al., 1984; Mitchell et al., 1990; O'Keane et al., 1991b; Malone et al., 1993; Coccaro and Kavoussi, 1994). However, in our previous study (Horáček et al., 1998), we found in healthy volunteers a positive correlation between Δ PRL after D-fenfluramine administration and parameters of depression and anxiety. So a 'physiological' decrease of mood in healthy controls seems to be connected with an increased prolactin response in the D-FF test. A possible explanation for this could be that the post-synaptic up-regulation of 5-HT receptors compensates for a mild decrease of pre-synaptic serotonin output in case of still 'physiological' sadness and anxiety. On the other hand, the blunted prolactin response in patients with major depression could be the consequence of a diminished presynaptic serotonin pool or of inactivated postsynaptic pathways, which cannot be compensated even with D-fenfluramine stimulation. Our findings of a negative correlation between insulin sensitivity and prolactin response in *healthy volunteers* suggest that a lower insulin sensitivity is connected with a decrease of central serotonergic activity.

The relationship between 5-HT activity and a sensitivity to insulin could be explained by several different mechanisms. Insulin in the periphery changes the spectrum of plasmatic amino acids: it increases tryptophan (Trp) and decreases other large neutral amino acids-LNAA (Val, Leu, Ile, Phe, Tyr). The LNAA compete with tryptophan for transport to the brain (Fenstrom and Wurtman, 1972; Curzon, 1985; Jamnicky et al., 1991). An insulin-related increase of plasmatic tryptophan is dependent on the sensitivity of insulin receptors. Fukagawa et al. (1987) found that euglycemicly administrated insulin leads, in older persons who usually have lower insulin receptor sensitivity, to a milder increase of tryptophan in comparison to younger controls. As tryptophan is the serotonin precursor, an increased availability of tryptophan may lead to the increase in the synthesis of serotonin. Moreover, insulin directly facilitates the transport of tryptophan through the blood-brain barrier (Crandall et al., 1981; Fukagawa et al., 1986; Malone et al., 1993). Insulin also exerts a direct effect on monoaminergic neurons in the brain. This is usually discussed in connection with the potentiation of noradrenergic neurotransmission (McCarthy, 1994).

In the opposite way, the central serotonin activity influences the peripheral sensitivity to insulin. Serotonin containing ventromedial and paraventricular hypothalamic nuclei regulate the appetite. The decrease of serotonin activity in this region can lead to a higher food intake with a consequent hypersecretion of insulin, which could finally lead even to insulin-resistance. In animal models, a 5-HT deficit led to the necessary addition of carbohydrates to food (Gerozis et al., 1993). Antidepressants and other serotonin increasing drugs decrease glycemia without any influence on insulin levels (Pestell et al., 1989; Scheen et al., 1991; Goodnick et al., 1995). This fact, supported also by the finding that the fenfluramine treatment

of patients with Type 2 diabetes mellitus normalizes glucose levels, could mean that stimulation of the 5-HT neurotransmission enhances the sensitivity to insulin (Goodnick et al., 1995).

In our sample, we did not find a significant correlation between body weight (BMI, WHR) and prolactin response after the D-fenfluramine challenge. It casts doubt upon the hypothesis of weight as a cause of the relationship between serotonin activity and insulin sensitivity, and supports the hypothesis of a direct interaction between both variables.

The connection between central 5-HT activity and glucose tolerance could be mediated by corticosteroids. Major depression is thought to be connected with a reduction of 5-HT tonus and also with an increase of HPA activity, expressed as non-suppression in the dexamethasone suppression test (Syvalahti, 1994). Corticoids are known to have anti-insulin and hyperglycemic effects and could participate in the development of glucose intolerance (Plat et al., 1996). Nevertheless, studies following cortisol levels and insulin sensitivity brought controversial results (Nathan et al., 1981; Winokur et al., 1988). A possible role of corticoids has been supported by the finding that higher cortisol levels were connected with blunted Δ PRL after the FF-challenges in depressed patients (Malone et al., 1993). In our study, however, we did not measure cortisol levels.

Our finding of a direct relationship between central 5-HT activity and sensitivity to insulin could have an important clinical impact. Our results are in accordance with the clinical trials using serotonergic agents in the treatment of insulin resistance (Pestell et al., 1989; Potter van Loon et al., 1991; Scheen et al., 1991). We also suggest that modalities which increase sensitivity to insulin can facilitate serotonin activity in the brain and thus exert some antidepressant effects.

5. Conclusions

Using the D-fenfluramine challenge test and the hyperinsulin euglycemic clamp, we have demonstrated the relationship between central serotonergic activity and peripheral sensitivity to insulin in healthy volunteers. We did not find a correlation between a prolactin response to the D-fenfluramine challenge and body weight. Consequently, we deduce that weight is not the factor mediating the relation between central 5-HT activity and insulin sensitivity.

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