SHORT THESIS FOR THE DEGREE OF DOCTOR OF
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**Investigation of chronic atypical antipsychotic treatment on
metabolic control**

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Investigation of chronic atypical antipsychotic treatment on metabolic control

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The Examination takes place at the Library Room of Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, on the 28th of April, 2016, at 11 AM

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The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, on the 28th of April, 2016, at 1 PM

1. INTRODUCTION

Overweight and obesity shows prominent increase in the developed countries, and is associated with increased risk of various diseases such as type 2 diabetes, cardiovascular diseases and certain cancers. Above that they contribute to the increased mortality in obese patients, treating these illnesses is a huge burden on health care system and economy.

When energy intake and storage exceeds energy expenditure, the balance between the two tips towards intake which manifests in net weight gain. Energy balance is regulated by a complex system which consists of afferent pathways in the periphery that signal the central nervous system about the state of energy stores and which, in turn, initiates hunger or satiety to keep the body weight stable. In the developed societies, however, due to the sedentary lifestyle and the abundance of food, this balance is tipped, resulting in hyperphagia, overweight and insulin resistance, and eventually leading to various other diseases.

Patients with severe mental disorders, i. e. schizophrenia the prevalence of obesity is even higher compared to the general population, therefore they are at increased risk of diabetes and cardiovascular diseases. Furthermore, the therapeutically most efficient antipsychotics these patients are chronically treated with have severe metabolic side effects, including weight gain, insulin sensitivity impairment and dyslipidaemia. The mechanism with which these side effects develop is still unclear, therefore an efficient prevention or treatment is still difficult to achieve.

2. AIMS

The current experimental approach to explore the patomechanism of the atypical antipsychotics-related metabolic side effects primarily aim at the central targets of those drugs while how they might act on the periphery is mostly uncovered. Clozapine and olanzapine, atypical antipsychotics with the highest risk of weight gain cause meal pattern differences resembling those observed in CCK-1 receptor deficient rats.

Our first aim was to investigate whether the CCK-1 receptor mediated pathways play a role in the chronic clozapine treatment induced body weight gain and insulin resistance. The experiments were carried out on genetically CCK-1 receptor deficient rats. To the best of our knowledge, our study is the first that investigated the chronic effect of an atypical antipsychotic on this strain.

Our previous results demonstrated the role of CCK in the meal induced insulin sensitizing mechanism (MIS). We also provided evidence that olanzapine acutely doesn't impair MIS and that the levels of gastrointestinal peptides secreted during meal don't change in response to acute treatment. Our second objective therefore was to investigate whether MIS is kept after chronic olanzapine treatment, and whether chronic olanzapine would change the basal or postprandial levels of gastrointestinal peptides. We also aimed to investigate hepatic insulin resistance with 3-[3H]-glucose supplemented hyperinsulinaemic euglycaemic glucose clamp.

3. MATERIALS AND METHODS

Ethics

Study protocol was formally approved by the Animal Ethics Committee of the University of Debrecen. The experiments presented conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocols applied had been approved by the local ethical boards of the University of Debrecen, Hungary (08/2007 DE MÁB & 16/2007 DEMÁB).

Investigation of the metabolic effects of chronic clozapine treatment on CCK-1 receptor deficient Otsuka Long Evans Tokushima Fatty (OLETF) rats

Animals

The experiments were carried out on male Otsuka Long Evans Tokushima Fatty (OLETF) and Long Evans Tokushima Otsuka (LETO) rats, both of which were gorgeous gifts of the Tokushima Institute, Japan. The LETO rats served as a non-diabetic, non-obese control. Rats were housed in an animal room with 22 – 24 °C and

50 – 70 % relative humidity. The lighting was set to 12 h light and 12 h dark period. The animals were individually placed into a metabolic cage (3701M081, Tecniplast, Italy) and they were allowed to eat standard laboratory chow (S8106-S011 SM R/M-Z+H; ssniff Spezialdiäten GmbH, Germany) and drink tap water ad libitum throughout the 25 days experimental period.

Drug treatment protocol

After one week acclimatization period, both the LETO and OLETF rats were randomized into 2 groups with 8 animals per group (altogether 32 rats were used). One group of both LETO and OLETF rats were treated with once a day oral dose of 10 mg/kg clozapine (Sigma-Aldrich Magyarország Ltd, Budapest, Hungary) over 25 days (CLZ group). The other 2 groups were treated with vehicle and served as vehicle-treated control (CTRL group). The drug/vehicle administration was performed an hour before the dark phase of the daily cycle had stared. For oral drug treatment, the clozapine was dissolved in 25 μl of 1.2 N HCl, then it was further diluted with 0.9% NaCl solution and this solution was administered in a volume of 1 ml/kg body weight by oral gavage. If it was required, 1M NaOH was used to adjust the pH of the solution to approx. 5.5.

Metabolic measurements

Metabolic parameters were monitored daily by means of metabolic cage. In details, daily (24 h) body weight, food intake and water consumption as well as stool and urine production was determined every morning except at weekends when a 3-days average was calculated on the next Monday morning.

Beside the daily food consumption, the size and duration of the first meal after drug/vehicle administration was also determined. This part of the study was carried out on the 21st day of the treatment. In this case, the food was removed from rats and 6 hours later the animals were orally treated with the daily dose of the clozapine/vehicle. Two hours after the drug/vehicle administration the food hopper was replaced and the animals were left to eat ad libitum. A video surveillance system was used to record the eating behavior and the weight of the food hopper was recorded continuously. A meal was determined as the duration of food consumption when animals had eaten at least 1

kcal of chow and the intermeal interval was at least 5 minutes. After measuring the first meal size, the treatment schedule continued until the end of the experimental period according to as describe above, except that the daily metabolic parameters measured on the next day was neglected during the data analysis due to the 8 hours fasting.

The last determination of the metabolic parameters was done one day earlier than the insulin sensitivity determination was carried out (i.e. on the 24th day of the treatment schedule), since animals lost weight (approx. 20 g) due to 16 hours food restriction. On the other hand, the animals were let to access tap water ad libitum.

Feeding efficiency was calculated by means of dividing the changes in body weight by the amount of food consumed during the last 24 hours.

At the end of the experiments, animals were killed by exsanguination via the carotid artery. The arterial blood samples were collected, centrifuged (10.000 g for 2 minutes at 4 °C) and plasma samples were stored at -70 °C until subsequent analysis. In order to determine the adiposity, the perirenal, intraabdominal and epididymal white adipose tissue fat pads were cut out, weighted and expressed as the percentage of the total body weight.

Determination of the insulin sensitivity

The hyperinsulinaemic euglycaemic glucose clamp (HEGC) procedure was used to characterize the whole body insulin sensitivity. In brief, after an overnight fasting, rats were anesthetized with intraperitoneal injection of 50 mg/kg sodium thiopental (Thiopental Sandoz®, Sandoz Pharmaceutical PLC, Switzerland). After a middle sagittal surgical incision on the ventral surface of the neck, the trachea was exposed and a polyethylene tube was introduced into it to allow spontaneous breathing of the animals. Then the left jugular vein and the left carotid artery was exposed and cleaned from the adhering connective tissue. The insulin and glucose was infused as separate line of infusion through the two branches of the jugular vein, while the arterial cannula served for blood pressure monitoring as well as to obtain blood samples (0.1 ml) for subsequent blood glucose and plasma insulin determination. When the surgery was completed, a 30 min stabilization period was left, then continuous insulin infusion

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(Humulin R®, Eli Lilly, Indianapolis, IN, USA) at a rate of 6 mU/kg/min was commenced along with glucose infusion (20% w/v). The rate of glucose infusion was adjusted in such a way that euglycaemic (5.5 ± 0.5 mmol/l) blood glucose level could be maintained. Blood glucose concentration was determined by means of glucometer (Accu-Chek, Roche Diagnostics, Budaörs, Hungary) before and at 5 min intervals during the first 80 min and at 10 min intervals during the last 40 min of the HEGC experiment. In order to determine the fasting and steady state plasma level of insulin, additional blood samples were collected (0.5 ml, in 20 µl EDTA, 10 µl Trasylol; Bayer, Leverkusen, Germany) from the carotid artery immediately before the commencement of insulin infusion and during steady state of the HEGC, respectively. Blood samples were centrifuged (Centrifuge 5415R – Eppendorf GmbH, Germany) for 2 min at 4 °C and 10.000 g, then the plasma was aliquoted, frozen and stored at -70 °C for subsequent determinations.

Parameters used to characterize whole body insulin sensitivity

Glucose infusion rate

The average glucose infusion rate (mg/kg/min) – assuming that the exogenously administered insulin completely blocks the hepatic glucose production – equals with the glucose uptake of the peripheral insulin sensitive tissues (mainly the adipose tissue and striated muscle). In this cases the glucose infusion rate can be used to characterize the whole body insulin sensitivity.

Insulin sensitivity index

When insulin is unable to completely suppress hepatic glucose production (i.e. hepatic insulin resistance) the peripheral tissues take up not only the exogenously administered, but also the hepatic-derived glucose. In these cases the glucose infusion rate underestimates the whole body glucose uptake and the insulin sensitivity index - which is the quotient of the glucose infusion rate and the plasma insulin concentration during the steady state - is a more reasonable index of insulin sensitivity as it reveals the quantity of the glucose metabolized per unit of plasma insulin concentration.

Metabolic clearance rate of insulin

In insulin resistant state, the compensatory hyperinsulinaemia evolves by increased secretion and/or by decreased metabolic clearance rate of insulin. The relative contribution of metabolic clearance rate of insulin to hyperinsulinaemia can be established as the insulin infusion rate (mU/kg/min) divided by the difference between the steady state and fasting plasma insulin level and is expressed as ml/kg/min.

RNA extraction and reverse-transcription

Both LETO and OLETF rats were killed at the end of the HEGC, the skulls were exposed, the brains were removed and the hypothalamus was dissected. DNA-free total RNA was extracted by using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. The quality of the RNA samples was checked by electrophoresis through agarose gel stained with ethidium bromide and then the 18S and 28S rRNA bands were visualized under UV light. Concentration of total RNA was determined by SmartSpecTM Plus Spectrophotometer (Bio-Rad, Hercules, USA). Reverse transcription of DNase-treated total RNA (2 μg) using random primers (Invitrogen Life Technologies, Karlsruhe, Germany) and SuperscriptTM II RNase H-Reverse Transcriptase (Invitrogen) was carried out as described previously.

TaqMan® assay-based real-time PCR

The mRNA expression of target genes (CCK-1 and CCK-2 receptors) was measured by real-time PCR using TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, USA) and the ABI PRISM® 7900 HT Sequence Detection System (Applied Biosystems). The probes contain a 6-carboxy-fluorescein phosphoramidite (FAM dye) labeling at the 5' end of the probe and a minor groove binder plus a non-fluorescent quencher at the 3' end. TaqMan® Gene Expression Assay IDs were Rn00562164_m1 for CCK-1 receptor and Rn00565867_m1 for CCK-2 receptor, respectively. The expression of the housekeeping gene 18S rRNA served as the internal control. An 18S rRNA-specific primer and probe sequences were designed

using QPCR primer version 1.2. For 18S rRNA-specific primer and TaqMan probe sequences, see on Structural Biology and Bioinformatics Group homepage (http://fuel1.biochem.dote.hu/SBBG/). The oligonucleotide sequences for 18S rRNA were ordered from Bio-Science (Budapest, Hungary). The TaqMan PCR was performed on ABI 7900 HT Sequence Detector, using the standard cycling conditions recommended by the manufacturer (50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60°C for 1 min). Each well contained 10 µl PCR mixture, made from 2× TaqMan Gene Expression Master Mix, either 0.5 µl TaqMan inventoried assay (for CCK-1 or CCK-2 receptor) or oligos for 18S rRNA (500 nM forward and reverse primer each plus 100 nM probe) and 4.5 µl cDNA template. The TaqMan PCR for target (CCK-1R and CCK-2R) and reference (18S rRNA) genes was run from the same RT reaction. Three replicates were run for each gene for each sample in a 384-well format plate. No template controls were used to confirm that reagents were not contaminated. For mRNA expression measurements, the data were collected with ABI's Sequence Detector program. Quantification of relative expression levels with the ΔΔCT method was applied. Accordingly, data are presented as the fold change in gene expression normalized to an endogenous reference gene (18S rRNA) and relative to the untreated sample from LETO rat. Value of the relative fold change from vehicle treated control LETO rats was considered as 1 (100%).

Radioimmunoassay studies

Plasma insulin level was determined by means of radioimmunoassay (RIA) using commercially available insulin RIA kit (RK 400 M, Institute of Isotopes Budapest, Hungary). Both intra- and inter-assay variations were lower than 5%.

Effect of long-term olanzapine treatment on meal-induced insulin sensitization and on gastrointestinal peptides in female Sprague-Dawley rats

Animals, study design & drug treatment

Thirty six female, Sprague-Dawley rats weighing 175-200 g (obtained from Innovo Ltd, the local distributor of Charles River Laboratories International, Inc.) were used throughout the study. Female rats were preferred as a model of antipsychotic-induced

metabolic dysregulation because these drugs exhibit more consistent metabolic abnormalities than males. After arrival, animals were habituated to the new environment for a week. Rats were housed in an animal room with 22 – 24 °C and 50 – 70 % relative humidity. The lighting was set to 12 h light and 12 h dark period (lights off at 7 am). The animals were individually placed into a metabolic cage (3701M081, Tecniplast Corp., Buguggiate, Italy) and they were allowed to eat standard laboratory chow (S8106- S011 SM R/M-Z+H; ssniff Spezialdiäten GmbH, Germany) and drink tap water ad libitum throughout the acclimatization period.

A week later, animals were divided into two main groups, the control as well as the olanzapine-treated group with 18 animals per group. At the end of the treatment period the control and olanzapine treated groups were randomly assigned into 2 subgroups. Animals in either subgroup were used in the hyperinsulinaemic euglycaemic glucose clamp (HEGC) measurement (n=6), while the other subgroup was used to determine postprandial insulin sensitizing mechanism by means of the rapid insulin sensitivity test (RIST; n=12). The RIST group was further divided in two according to the prandial state (i.e. fasted and re-fed; n=6-6). The group of re-fed rats were fasted overnight similarly to others, but 2 hours before the initiation of the RIST procedure they were left to eat ad libitum until anaesthesia was induced.

Due to the substantially shorter plasma half-life of olanzapine (obtained from Sigma-Aldrich Ltd, Budapest, Hungary) in rats than in humans we intended to achieve continuous, stable plasma concentration of olanzapine instead of some high peak concentrations after drug intake. Because of stability concerns, the osmotic minipump is not appropriate for long-term administration of olanzapine, so we administered olanzapine via the drinking water. For this purpose, olanzapine was dissolved in a minimum quantity of 1 M HCl and then diluted to the required concentration with tap water. If necessary, 1 M NaOH was used to adjust the pH of this solution to approx. 5.5. The amount of olanzapine in the drinking water was adjusted according to the daily water intake as well as body weight during the course of the study to maintain a daily olanzapine intake of approximately 2 mg/kg. This drug administration protocol lasted for 24 days. Previous studies had demonstrated that this relatively low dose of olanzapine was able to effectively induce weight gain in female Sprague-Dawley rats.

Furthermore, this olanzapine dose and route of administration have been demonstrated to maintain plasma olanzapine level in the human therapeutic range. Moreover in our experiment it seemed that the low olanzapine concentration did not cause bitter flavour of the drinking water, which was suggested by the equal amount of water consumed daily by the control and olanzapine treated animals. The control group received tap water.

Measurement of daily food and water consumption, stool and urine production

Daily food intake and water consumption as well as stool and urine production in rats having free access to standard laboratory chow and tap water were determined by means of metabolic cages (3701M081, Tecniplast, Italy) throughout the study.

Measurement of body weight and body composition

The body weight of animals was scaled every day in order to determine the amount of olanzapine which had to be dissolved in their drinking water. The first determination was used for assignment of rats into treatment groups and the last measurement was used for evaluation of the effect of treatment on body weight. At the end of the experiment, animals were killed by exsanguination via the carotid artery. Then intraabdominal (including perirenal) and inguinal white adipose tissue (WAT) fat pads were cut out and weighed. Total body adiposity was characterized either by the sum of the perirenal, intraabdominal and inguinal WAT fat pad's weight expressed in grams (g) or by the ratio of the total WAT to body weight expressed in percentage (%).

Insulin sensitivity determination

Whole body insulin sensitivity was determined by means of hyperinsulinaemic euglycaemic glucose clamp (HEGC) method. Animals were fasted for 16 hours before start of the experiment, but their access to water (including the olanzapine) was not restricted. Anesthesia was induced with intraperitoneal injection of 50 mg/kg thiopental (Thiopental® Sandoz, Sandoz GmbH, Kundl, Austria), then the animals were placed on a heatable operating table and their core temperature was maintained at 37.0 – 37.5°C during the experiment. The trachea was cannulated and rats were let to breathe freely. A

jugular vein and a carotid artery was cannulated for glucose (20% dextrose) and insulin (Humulin R®, Eli Lilly, Indianapolis, IN, USA) administration as well as for blood sampling, respectively. From t = -60. min a tracer amount of tritiated glucose (3-[3H]-glucose; Perkin-Elmer, Waltham, MA) was infused as a primed-continuous infusion (5 μCi bolus, followed by 0.075 μCi/min) through the jugular vein in order to determine the basal hepatic glucose output. At time t = 0. min, a continuous insulin infusion was commenced at a constant rate of 3 mU/kg/min over 120 minutes. Blood glucose level was determined every 10 minutes by means of glucometer (Accu-Chek, Roche Diagnostics, Budaörs, Hungary) and euglycaemia (5.5 ± 0.5 mmol/l) was maintained by adjusting the rate of exogenous glucose infusion. Blood samples for blood glucose and for plasma insulin determination were taken from the carotid artery. When blood glucose level had stabilized for at least 20 minutes, we defined this condition as steady state. This occurred within 90 minutes of starting the insulin infusion. In the steady state, 4 additional blood samples (0.5 ml in 20 μl EDTA, 10 μl Trasylol; Bayer, Leverkusen, Germany) were taken for plasma insulin and tritiated glucose determination. Blood samples were centrifuged (Centrifuge 5415R – Eppendorf GmbH, Germany) for 2 min at 4 °C and 10.000 g, then the plasma was aliquoted, frozen and stored at -70 °C for subsequent determinations.

Calculations

The calculation of GIR, ISI, MCRI and HOMA-IR is described above.

Hepatic glucose production

Basal hepatic glucose production (bHGP) was calculated by using the plasma samples obtained at t = 0. min. At that time, the rate of glucose appearance (Ra) equals the rate of glucose disappearance (Rd) and the hepatic glucose production (HGP) is calculated by dividing the [3-3H]-glucose infusion rate (dpm/min) by the plasma [3-3H]-glucose specific activity (dpm/mg). In the insulin-stimulated state the calculation is similar, however the effect of exogenously infused insulin must be taken into account. Plasma samples obtained at t = 120. min were used for this calculation. During the steady state of the HEGC the Rd equals the rate of glucose uptake. The Ra equals the rate of insulin-stimulated HGP (iHGP) plus the exogenous glucose infusion rate (GIR).

Calculation of Ra comes from the equation used above and the GIR is given by the rate of the infusion pump. Accordingly, the iHGP equals Ra minus exogenous GIR. Plasma [3-3H]-glucose activity was measured on the supernatants of Ba(OH)2 and ZnSO4 precipitates of plasma samples after evaporation to dryness in order to eliminate tritiated water.

Rapid Insulin Sensitivity Test (RIST)

The RIST was performed in order to investigate changes in postprandial whole body insulin sensitivity after long-term olanzapine administration. In brief, after induction of anaesthesia (50 mg/kg thiopental ip.) animals were placed on a heatable operating table. From midline incision, the trachea was exposed and cannulated then the left carotid artery and left jugular vein were also cannulated for blood sampling and insulin/glucose administration, respectively. Immediately after the insertion of the cannula in the carotid artery a blood sample (0.5 ml in 20 μl EDTA, 10 μl Trasylol; Bayer, Leverkusen, Germany, 5 μl DPP4 inhibitor, Merck KGaA, Darmstadt, Germany) was drawn for metabolic hormone determination. After a 30 min post-surgery stabilization period, arterial blood samples were taken from the carotid artery every 5 minutes for blood glucose determination. When the blood glucose value of three consecutive determinations was stable it was referred to as the control. Then 50 mU/kg bolus insulin (Humulin R®, obtained from Eli Lilly, USA) was infused over 5 min. At the same time, a continuous glucose infusion was also started. The blood glucose level was determined every 2 minutes and the rate of glucose infusion was adjusted accordingly to maintain the previously determined control value. The total amount of infused glucose (expressed as mg/kg body weight) required to counteract the hypoglycaemic effect of 50 mU/kg insulin produced the RIST index as an indicator of whole body insulin sensitivity.

Determination of blood hormone levels

From the frozen plasma samples the plasma level of insulin, GLP-1 (active), GIP (total), pancreatic polypeptide, peptide YY (total), ghrelin (active), leptin, amylin (active) was determined by means of MILLIPLEX MAP Rat Metabolism Panel (RGT-88K-08, EMD Millipore Corp., Billerica, MA, USA) according to the manufacturer's

instructions. The intra- and inter-assay variability coefficient for these hormones were 4-13% and 5-17%, respectively.

Data handling and statistics

All data were analyzed with paired t-test when it was possible, otherwise one-way analysis of variance (ANOVA) followed by a modified t-test for repeated measures according to Bonferroni's method was used.

4. RESULTS

Investigation of the metabolic effects of chronic clozapine treatment on CCK-1 receptor deficient Otsuka Long Evans Tokushima Fatty (OLETF) rats

Effect of chronic clozapine treatment on body weight and body composition

At the commencement of the study, there were no statistically significant differences in the body weights between the vehicle and clozapine treated groups of either LETO or OLETF rats. The initial weight of LETO rats were 367±23 g and 367±15 g in the vehicle and clozapine group, respectively. According to our expectation, the initial body weight of OLETF rats lacking functional CCK-1 receptor was slightly higher than that of LETO, but no significant differences were observed between the OLETF groups and their body weights were 388±7 g and 402±9 g in the vehicle and clozapine treated group, respectively. Moreover, the OLETF rats gained significantly more weight than the LETO during the treatment period. On the other hand, the clozapine treatment had no effect on the body weight gain of either LETO or OLETF rats.

By comparing the amount of white adipose tissue fat pads of the LETO and OLETF rats, we found that the OLETF rats had significantly more white adipose tissue mass than that of the LETO. Moreover, this was also true when the total amount of white adipose tissue mass was correlated to the total body weight. In this comparison the percentage amount of white adipose tissue mass (both total and correlated) was also

significantly more in the clozapine treated LETO and OLETF animals than that of their vehicle treated counterparts.

Effect of chronic clozapine treatment on feeding

There were no significant differences between the daily food consumption of LETO and OLETF rats and clozapine administration resulted in only a slight, but non-significant tendency of increased daily food consumption. Consequently, there were no significant changes in the cumulative food intake during the study period between the vehicle and clozapine treated groups of LETO or OLETF rats. Contrarily, when the size and duration of the 1st meal was determined, the stimulatory effect of clozapine treatment on food consumption was revealed. Both in LETO and OLETF rats, the clozapine administration increased the duration as well as the size of the first meal. When the feeding efficiency of the vehicle and clozapine treated rats was compared we did not find significant alteration between these two groups.

Effect of chronic clozapine treatment on glucose metabolism and insulin sensitivity

Fasting blood samples were obtained immediately before the insulin infusion of the HEGC was commenced. These blood samples were used to determine the fasting blood glucose and plasma insulin level as well as to calculate the HOMA-IR index. We found that both the fasting blood glucose and plasma insulin level was significantly higher in OLETF than in LETO rats. On the other hand, the clozapine treatment did not modify either the fasting blood glucose or plasma insulin level. Accordingly, the HOMA-IR index was elevated in the OLETF rats compared to LETO, but clozapine treatment failed to modify it.

During the steady state of the HEGC, the average glucose infusion rate in the vehicle treated LETO group was significantly higher than that of the vehicle treated OLETF group. However, clozapine treatment did not decrease the average glucose infusion rate either in the clozapine treated LETO or OLETF rats. Similarly to glucose infusion rate, the insulin sensitivity index of vehicle treated OLETF rats was significantly reduced compared to vehicle treated LETO rats. Moreover, the insulin

sensitivity index indicated that clozapine treatment resulted in insulin resistance both in LETO and OLETF rats. The metabolic clearance rate of insulin was similar in both vehicle treated LETO and OLETF groups. On the other hand, the clozapine administration decreased the metabolic clearance rate of insulin in both LETO and OLETF rats.

Changes in hypothalamic mRNA expression of CCK receptors

The relative fold change of mRNA of CCK-1 receptor was significantly higher in clozapine treated than in vehicle treated LETO rats. On the other hand, the expression of CCK-1 receptor level was undetectable in OLETF rats. In vehicle treated OLETF rats the mRNA of hypothalamic CCK-2 receptor was significantly higher than that of vehicle treated LETO. On the other hand clozapine treatment induced an almost 10 times increment in mRNA expression of CCK-2R in LETO rats, but in OLETF rats the clozapine treatment reduced the relative fold change of mRNA of CCK-2R.

Effect of long-term olanzapine treatment on meal-induced insulin sensitization and on gastrointestinal peptides in female Sprague-Dawley rats

Effect of olanzapine treatment on daily food and water consumption, stool and urine production

There was no significant difference between the control and olanzapine-treated groups regarding either the daily food intake or the daily water consumption. There was no significant difference between the control and olanzapine-treated rats regarding their daily stool and urine production.

Effect of olanzapine treatment on body weight and body composition

At the beginning of the study, the 2 main groups, i.e. the control and the olanzapine treated groups were created by randomization of the rats which yielded non-significant difference between the groups regarding their initial body weight. The body weight of the control and olanzapine treated group was 213 ± 6.6 g and 214 ± 12 g, respectively. However, at the end of the study, olanzapine treatment resulted in significant body

weight gain (p<0.05) compared to control groups and the end-study body weight was 272 ± 13 g and 263 ± 12 g in the control and olanzapine treated groups, respectively. By measuring the intraabdominal (including perirenal) and inguinal WAT fat pads, we found significant difference (p<0.05) between the control and olanzapine-treated groups, and the weight of the total WAT in the control and olanzapine-treated groups was 5.0 ± 0.8 g and 9.0 ± 0.9 g, respectively. Similarly, the ration of WAT to body weight showed significant difference (p<0.05) between the control and olanzapine-treated rats and the respective values were 1.45 ± 0.13 % and 2.32 ± 0.2 % in the control and olanzapine treated groups.

Effect of olanzapine treatment on insulin sensitivity

Long-term olanzapine administration yielded insulin resistance in healthy female rats. This insulin resistance is characterized by elevated HOMA-IR value (p< 0.05) and reduced GIR (p< 0.05) needed to maintain euglycaemia during hyperinsulinaemic clamp procedure as well as by the reduced ISI (p< 0.05) as a measure of whole body insulin sensitivity. The MCRI (p< 0.05) was also decreased in response to chronic olanzapine treatment. The bHGP was significantly (p< 0.05) elevated in the olanzapine treated rats indicating hepatic insulin resistance in the fasted state. In the insulin-stimulated state, the iHGP was significantly (p< 0.05) reduced in both control and olanzapine-treated animals compared to the corresponding basal values. Moreover, the hepatic glucose production was significantly higher in the olanzapine-treated animals than in control rats during the insulin stimulated state.

Effect of olanzapine treatment on postprandial insulin sensitivity

In fasting state control rats showed low RIST index indicating preprandial insulin resistance which improved significantly (p< 0.05) after meal. In olanzapine-treated animals similar results were obtained, a relatively low insulin sensitivity before and significantly (p< 0.05) enhanced insulin sensitivity after meal. Comparing the control and olanzapine treated animals we found no significant differences either in the fasting or in the postprandial state, suggesting that the olanzapine treatment did not modify the postprandially activated endogenous insulin sensitizing mechanism.

Effect of olanzapine treatment on blood glucose and on plasma level of metabolic hormones

The fasting blood glucose level was significantly ($p < 0.05$) elevated in the olanzapine-treated group compared with the controls. The fasting plasma insulin level was also significantly ($p < 0.05$) higher in rats treated with olanzapine. Accordingly, the HOMA-IR indicated fasting insulin resistance. Fasting plasma leptin level was elevated and the fasting plasma ghrelin was reduced significantly ($p < 0.05$) in the olanzapine-treated rats. On the other hand, there was no significant difference in the fasting plasma level of the other hormones (GIP, amylin, GLP-1, PYY, PP) measured.

In the re-fed groups of control and olanzapine-treated animals the plasma insulin, leptin and GIP levels showed significant elevation ($p < 0.05$) compared to the corresponding fasting groups. Surprisingly, the postprandial plasma ghrelin level decreased only in the control, but not in olanzapine-treated animals. The plasma level of amylin, GLP-1, PYY and PP did not change in response to meal.

5. DISCUSSION

Investigation of the metabolic effects of chronic clozapine treatment on CCK-1 receptor deficient Otsuka Long Evans Tokushima Fatty (OLETF) rats

In the present study we demonstrated that despite of the failure of clozapine administration to induce obesity in either LETO or OLETF rats, the clozapine induced significant elevation in the body's white adipose tissue mass (the sum of perirenal, intraabdominal and epididymal white adipose tissue fat pads) both in LETO and OLETF rats. The lack of effect of clozapine treatment on daily food consumption and feeding efficiency can explain that there were no significant differences between the vehicle and clozapine treated groups in respect of their body weight gain. On the other hand, clozapine administration did influence the feeding pattern of both LETO and OLETF

rats, since it increased the size and duration of the 1st meal after drug administration. Furthermore, the chronic clozapine treatment reduced the insulin sensitivity index of both LETO and OLETF rats, characterized by elevated plasma insulin level due to the reduced metabolic clearance rate of insulin. Finally, we demonstrated that clozapine treatment increased the hypothalamic CCK-1 and CCK-2 receptor mRNA expression in LETO rats, while a significant decrement in CCK-2 receptor mRNA was observed in OLETF rats.

It is proposed that atypical antipsychotics can induce weight gain via their stimulatory action on feeding which is characterized by hyperphagia due to increased meal size and meal duration. Beside the large number of neurotransmitters and their receptors which is supposed to participate in the hyperphagic effect of atypical antipsychotics, our attention turned to the CCK. The CCK was the first gastrointestinal peptide that role in the regulation of satiety was discovered. Both the exogenously administered and endogenously released - in response to meal - CCK can reduce food intake by decreasing the meal size and meal duration. These effects of CCK on feeding pattern are very similar to that of observed with atypical antipsychotics administration. On the other hand, a study investigating the effect of olanzapine – another atypical antipsychotics with significant effect on weight gain – on the CCK-mediated satiety revealed that acute olanzapin treatment increased the meal size, but this effect was independent of CCK.

Our present study demonstrated that chronic clozapine administration induced substantial changes in the adiposity, irrespective of the presence of the functionally intact CCK-1 receptor. Although the body's fat content elevated significantly in response to the clozapine treatment, there were no differences in the body weight gain of either vehicle or clozapine treated LETO or OLETF rats. This is in conflict with the clinical observations, since clozapine is known as one of the atypical antipsychotics which can induce the utmost weight gain in schizophrenic patient. On the other hand, by reviewing the literature pertaining to the atypical antipsychotics-induced obesity in rodents, a much conflicting data can be found. Early reports demonstrated that chronic administration of atypical antipsychotics failed to induce obesity in rodents irrespective of their gender, while others found that the effect is gender specific and is confined to

only females. A very recent report, using subcutaneous electrical microinfusion pump in male Sprague – Dawley rats were able to reveal that olanzapine can induce adiposity in spite of the lack of increased food intake or body weight gain. Besides our present findings support this latter observation, it adds further insight into the effect of clozapine on the feeding pattern. We demonstrated that although clozapine treatment failed to increase the daily total food intake as well as the body weight or cumulative body weight gain, it did increase the size and duration of the 1st meal after the drug administration both in LETO and OLETF rats indicating that there is no direct interaction between the clozapine and CCK-1 receptor mediated pathways. This finding supports the previous observation of van der Zwaal who revealed that acute administration of olanzapin increases meal size independent of CCK.

Furthermore, clozapine-induced significant elevation in the CCK-1 and CCK-2 receptor mRNA in the LETO rats suggests that the clozapine could have an effect on the hypothalamic level of the food intake regulation in healthy species. On the other hand, in our experiment the clozapine administration reduced the hypothalamic CCK-2 receptor mRNA expression in OLETF rats, an opposite effect that was observed in LETO rats. One possible explanation for this confusing result could be that a functional compensation between the CCK-1 and CCK-2 receptors exists and the absence of CCK-1 receptor may facilitate the CCK-2 receptor signaling in OLETF rats. However, satiety and hunger are subtle regulated processes in which several other peptides are involved and the elimination of one component could result in unforeseen consequences such as the reverse effect of clozapine on the mRNA expression of the CCK-2R in OLETF rats.

The lack of effect of the clozapine on body weight could be explained by the dose used. One can speculate that the 10 mg/kg daily dose induced sedation and consequent decrease in the overall activity of the animal including the feeding. Moreover, the lower physical activity can accompanied with decreased muscle mass which could explain the lack of body weight gain in spite of the enhanced adiposity. On the other hand, the clozapine-induced weight gain was usually studied in rats in a dose range of 7.5-12 mg. Another explanation could be that during the rest of the day animals were able to compensate by shorten the intermeal interval or increasing subsequent meal sizes. However, to clarify the precise effect of clozapine on feeding pattern requires additional

experiments, but now this was out of the primary scope of the present investigation. Finally, the gender specific effect of clozapine treatment cannot be ruled out, since several lines of evidences suggest, that female rats have greater liability to weight gain in response to therapy with atypical antipsychotics than male rats and most rodent models used to study the detrimental effect of atypical antipsychotics on weight gain had been carried out in female.

In our study the fasting blood glucose and plasma insulin level did not differ significantly between the vehicle and clozapine treated animals indicating that there was no significant difference between the baseline (i.e. fasting) insulin sensitivity of the treatment groups. On the other hand, our study clearly demonstrated that insulin sensitivity index reduced significantly in clozapine-treated rats during clamped hyperinsulinaemia produced by hyperinsulinaemic euglycaemic glucose clamping, the golden standard method for determination of insulin sensitivity. Using HEGC for characterization of the whole body insulin sensitivity, the M value is the commonly used marker. On the other hand if hepatic insulin resistance occurs, the glucose infusion rate of its own underestimates the entire peripheral glucose uptake, since additional glucose originated from the liver is taken up by the insulin sensitive tissues. In these cases, the insulin sensitivity can be compared more precisely if we determine the amount of glucose taken up by the insulin sensitive tissues per unit of plasma insulin concentration. However, it must be noted that there is distinct conclusion regarding the insulin sensitivity depending on the level of plasma insulin. On the other hand this is in accordance with the clinical observation that the first sign of the development of type 2 diabetes is the impaired glucose tolerance without changes in the fasting blood glucose or plasma insulin level. Here we demonstrated that although the fasting blood glucose and plasma insulin levels were unchanged after 25 day clozapine treatment, but during hyperinsulinaemia (at a level that usually occurs after meal) the insulin sensitive tissues of the clozapine treated rats took up less glucose per unit of plasma insulin concentration than that of the vehicle treated animals, indicating a decreased insulin sensitivity index. We suggest that decreased metabolic clearance rate of insulin contributes significantly to the reduced insulin sensitivity index.

These results also support those preclinical and clinical observations that atypical antipsychotics-induced insulin resistance can develop independent of obesity. On the other hand, it is worth mentioning, that the white adipose tissue mass of the rats treated with atypical antipsychotics elevated significantly and the role of the adipose tissue-derived hormones (e.g. resistin, leptin, TNFα, IL-6, adiponectin) in the development of insulin resistance is not questionable. There could be at least two possible explanations that unchanged glucose infusion rate was combined with reduced insulin sensitivity index and metabolic clearance rate of insulin in response to clozapine treatment. In the first case, it is assumed that clozapine induce peripheral insulin resistance without affecting hepatic insulin sensitivity. In this case the hepatic glucose output would be entirely suppressed by the hyperinsulinaemic euglycaemic clamp and the peripheral tissues (e.g. skeletal muscle, white adipose tissue) of vehicle and clozapine treated LETO or OLETF rats would take up similar amount of glucose. So, the reduced metabolic clearance rate of insulin can be viewed as a compensatory mechanism that enables the sufficiently high plasma insulin level to offset the peripheral insulin resistance. The other putative mechanism that clozapine treatment resulted in hepatic insulin resistance as proposed by others. In this case the peripheral tissues of clozapine treated animals take up more glucose than they do in vehicle treated rats, since the amount of exogenously administered glucose (i.e. the glucose infusion rate) was similar and this was supplemented by additional glucose which entered the circulation from the liver. That is the higher plasma insulin due to the reduced metabolic clearance rate of insulin ensures that peripheral tissues will be able to take up the extra glucose originating from the liver. Additional experiments that include determination of hepatic glucose production and peripheral glucose uptake by means of radiotracer administration should answer these questions.

Finally, it should be noted, that several other hormones and transmitters are involved in the regulation of food intake, body weight and insulin sensitivity as well as atypical antipsychotics modulate multiple transmitter systems. Our ongoing study using female Sprague-Dawley rats supports the previous findings that clozapine elevates preprandial level of ghrelin, a hunger-stimulating hormone. However, in order to find the primary signaling pathway which is responsible for the clozapine-induced metabolic derangement requires additional experiments.

Taken together, we can draw a conclusion that although there is significant parallelism in the changes of feeding pattern as well as insulin sensitivity caused by either CCK-1 receptor blockade or treatment with atypical antipsychotics and there are evidences that the atypical antipsychotics and CCK-mediated pathways share common routes in the control of satiety, here we failed to demonstrate the role of CCK-1 receptor in the clozapine-induced weight gain and insulin resistance. Furthermore, we demonstrated that the impairment of the metabolic clearance rate of insulin and concomitant elevation in plasma insulin level can explain the observed insulin resistance.

Effect of long-term olanzapine treatment on meal-induced insulin sensitization and on gastrointestinal peptides in female Sprague-Dawley rats

The present study is the extension of our previous one in which we demonstrated that single oral dose of olanzapine did not modify the post-prandially activated insulin sensitizing mechanism termed meal-induced insulin sensitization in healthy female rats, however it was able to reduce fasted-state insulin sensitivity. In addition to investigating the effect of chronic olanzapine treatment on fasted-state insulin sensitivity, to our knowledge this is the first report of long-term olanzapine treatment on postprandial insulin sensitivity. Here we demonstrated that the meal-induced insulin sensitization remained unaffected in spite of the 24 days olanzapine administration, but it caused fasted-state insulin resistance. The latter is induced by hepatic insulin resistance and decrease in the metabolic clearance rate of the insulin. Furthermore, olanzapine resulted in significant weight gain and enhanced white fat pad mass without obvious increment in daily food-intake. Olanzapine treatment increased fasting plasma insulin and leptin levels according to the developing fasted-state insulin resistance and increased fat mass, respectively. Fasting, pre-prandial plasma active ghrelin level was lower in the olanzapine treated group and meal failed to reduce plasma ghrelin level.

The meal-induced insulin sensitization is an endogenous adaptive mechanism which helps to adapt to postprandial glucose challenge. Meal-induced activation of this mechanism results in facilitated glucose uptake mainly in the skeletal muscle by enhancing insulin sensitivity. However, if dysfunction of this mechanism persists for

long-term, type 2 diabetes-like state can develop. Data obtained in the present study allow to hypothesize that pharmacological activation of the meal-induced insulin sensitization could be therapeutically exploited in patients treated with olanzapine. At the same time, the data presented here do not give us sufficient explanation for lack of effect of olanzapine on meal-induced insulin sensitization. The post-prandially activated insulin sensitizing mechanism is atropine-sensitive and we expected that olanzapine due to its high muscarinic receptors affinity could inhibit this mechanism acutely. Surprisingly, olanzapine failed to modulate meal-induced enhancement in insulin sensitivity neither after single oral dose nor after long-term administration. In order to solve this conflicting observation, i.e. the lack of effect of olanzapine on the atropine-sensitive postprandial insulin sensitization additional experiments are required. The assumption that subtype selectivity of the muscarinic receptors is responsible for this observation is only speculative.

Investigating the fasted-state insulin sensitivity our results add further insight into the putative mechanism of the olanzapine-induced insulin resistance. In fasted animals we found significantly elevated plasma insulin and glucose levels, which could be the consequence of the increased hepatic glucose output. The presence of insulin resistance is further supported by the reduced GIR and ISI obtained during the clamped hyperinsulinaemia. Using [3-3H]-glucose for hepatic glucose production determination we demonstrated both basal and insulin-stimulated hepatic insulin resistance which could contribute to the reduced GIR and ISI. On the other hand, and this is another original observation of this study, we suggest that reduced metabolic clearance of insulin from the plasma could also play a role in the developing insulin resistance. These data are in accordance with a previous study that described the effect of long-term olanzapine administration on fasted-state insulin sensitivity. On the other hand, most studies investigated the effect of single dose of olanzapine on the whole body insulin sensitivity because it is suggested that acute treatment with AAP is able to model the effect of long-term drug treatment. However, both acute and chronic studies using either olanzapine, clozapine or other AAP suggest that hepatic insulin resistance is the key element in the olanzapine-induced whole body insulin resistance.

Our data regarding the effect of olanzapine on body weight gain are in accordance with the results of recent studies that have demonstrated the olanzapine-induced body weight gain in female rats. Due to the significantly shorter plasma half-life of olanzapine in rats than in humans we have administered olanzapine via drinking water instead of using osmotic minipumps, daily sc. or ip. injections or training the animals for self-administration via cookie-dough. Other research groups using drinking water as a vehicle also reported positive results regarding the metabolic action of olanzapine both in male and female rats. In addition to body weight gain olanzapine treatment elicited increase in the adiposity as determined by means of the measuring the weight of perirenal, intraabdominal and inguinal WAT. Our observations support previous findings that olanzapine increases intra-abdominal WAT in rats. A surprising result of our study was that gain in body weight and adiposity were not accompanied with increased food consumption. This finding is in contrast to the majority of previous animal studies on the olanzapine-induced hyperphagia, on the other hand, reports of olanzapine-induced weight gain and increased adiposity without hyperphagia are also available. One putative explanation for the olanzapine-induced obesity and adiposity in the absence of hyperphagia could be the reduced energy expenditure due to reduced skeletal muscle work. Indeed, olanzapine induces sedation and reduces locomotor activity in the dose we used.

In order to get detailed insight into the putative hormonal background of the olanzapine-induced metabolic alterations we determined the fasting plasma insulin, leptin, ghrelin, GIP, GLP-1, amylin, PYY and PP levels. Although the plasma levels of GLP-1, amylin, PYY and PP did not show significant alteration between the control and olanzapine treated groups, significant elevation of plasma insulin and leptin level and significant reduction of plasma ghrelin level was detected in the olanzapine treated animals. In the postprandial state, the plasma ghrelin level reduced significantly in vehicle-treated rats, but no change was observed in olanzapine-treated animals. These hormonal changes could be the indicators of the olanzapine induced metabolic derangements. The elevated fasting plasma insulin could refer to the increased hepatic glucose output and hepatic insulin resistance. During the steady state of the clamp procedure (although the same insulin infusion rate was applied in both treatment groups) plasma insulin level was higher in the olanzapine treated animals which is

responsible for the reduced ISI. The MCRI's data suggest that the elevated plasma insulin level -at least in part- occurred due to the reduced insulin removal from the plasma. Whatever the precise mechanism of the elevated plasma insulin is, our results are in accordance with animal and human studies which reported hyperinsulinaemia in response to olanzapine administration.

The fasting plasma leptin level was significantly elevated in olanzapine treated rats, but there was no significant difference in the postprandial leptin value between the control and olanzapine treated groups. The elevated fasted plasma leptin is the sign of increased adiposity, since correlation between the white adipose tissue mass and plasma leptin level is strongly established both in rodents and humans. Moreover, human data suggest that olanzapine-induced obesity is accompanied with elevated plasma leptin level. Impairment in leptin regulation early in the treatment with second generation antipsychotics should have impact on the co-medication of psychotic patient with insulin resistance and type 2 diabetes and there is a high risk of metabolic dysfunction in these patients irrespective of the specific psychotropic drug treatment administered.

In our study the plasma ghrelin concentration was also modulated dual ways by olanzapine treatment. First, the fasting plasma ghrelin concentration was significantly lower in olanzapine treated rats. Second, the postprandial plasma level of ghrelin was significantly higher in olanzapine-treated rats, i.e. no reduction in postprandial plasma ghrelin level was observed in olanzapine-treated rats. According to the current leading hypothesis of the functional role of ghrelin, one would have expected an elevated plasma ghrelin concentration in the olanzapine treated animals. However, we found the opposite, the fasting ghrelin level was decreased in olanzapine-treated rats compared to controls. We speculate that the elevated fasted plasma insulin and leptin level would be responsible for the lower plasma ghrelin level as proposed by Tschop. This speculation is further supported by a literature review that suggested a tri-phasic effect of AAPs on plasma ghrelin level. This review report suggests that an initial upregulatory phase in plasma ghrelin level lasting for 1-2 weeks is followed by a downregulation lasting for 2-6 weeks in humans or 1 week in rodents before returning to the baseline level. We believe that the timing of our metabolic hormone determination coincided with the downregulatory phase that could explain the observed low plasma ghrelin level in the

olanzapine treated rats. Whatever the explanation on the effect of olanzapine on fasting plasma ghrelin level is, to the best of our knowledge our study is the first investigating the effect of meal on plasma ghrelin level in olanzapine treated rats. We found that 2 hours after the commencement of feeding the plasma level of ghrelin failed to show significant difference from the corresponding fasted value. This was an unexpected result since plasma ghrelin concentration should have decreased in response to meal. One putative explanation could be that in olanzapine treated rats the relative postprandial elevation in plasma insulin and leptin levels (49% and 88%, respectively) were lower than that of controls (166% and 464%, respectively). Another explanation could be that plasma ghrelin level returns earlier to its baseline level in olanzapine treated rats than in the drug-free controls. In addition to get detailed insight into the time course of changes in plasma ghrelin level after meal requires additional experiments.

Other metabolic hormones measured were not altered by feeding except GIP, however olanzapine treatment had no effect on this parameter suggesting that GIP does not participate in the metabolic effect of olanzapine. Regarding the other hormones we found that olanzapine failed to modify the plasma level of GLP-1, amylin, PYY or PP 2 hours after the commencement of feeding. On the other hand, it cannot be ruled out that olanzapine treatment could even alter these parameters, since blood samples for hormone determination were obtained approximately 2 hours after meal, so we have no information about the postprandial first 2 hours. Others revealed that in rats 2 hours after oral glucose challenge the plasma level of metabolically important hormones return to the baseline. Nevertheless, our results are in accordance with those clinical findings which also failed to reveal the effect of olanzapine on gut hormone secretion.

Taken together, our study is the first that investigated the chronic effect of olanzapine on meal-induced insulin sensitization. The fact that post-prandial insulin sensitivity remains functionally intact after long-term olanzapine treatment gives us hope for its pharmacological exploitation in order to treat or prevent the olanzapine-induced insulin resistance. Animal studies have demonstrated that activation of meal-induced insulin sensitization could be achieved either pharmacologically by e.g. acetylcoline, nitric oxide donors, or SAMEC (the combination of S-adenosylmethionine, vitamin E, and vitamin C) or nonpharmacologically by diet

management or promotion of exercise. Furthermore, our study emphasises the importance of the appropriate method selection to study the post-prandially activated insulin sensitizing mechanism. Hyperinsulinaemic euglycaemic glucose clamping is best suited for determination of steady-state insulin sensitivity, but the RIST method is preferred to investigate rapid, temporary change in the meal-related alteration in whole body insulin sensitivity. Using both HEGC and RIST method enabled us to reveal the distinct action of olanzapine on fasted-state and post-prandial insulin sensitivity, respectively.

6. SUMMARY

The present study was aimed to investigate the relationship between metabolic side effects associated with atypical antipsychotic treatment and peripherally released hormones that play a role in food intake and regulation of insulin sensitivity. Our further aim was to investigate whether chronic atypical antipsychotic treatment influences the meal induced insulin sensitizing mechanism.

Our first experiment was carried out on genetically cholecystokinin-1 (CCK-1) receptor deficient Otsuka Long Evans Tokushima Fatty (OLETF) rats and their genetically healthy counterparts. Chronic clozapine treatment increased short term food intake in both groups and though body weight was unchanged, adiposity increased which could contribute to the developed insulin resistance.

In our second experiment we investigated the effects of chronic olanzapine treatment on the meal induced insulin sensitization and the gastrointestinal peptide levels. Olanzapin increased body weight and adiposity and developed a significant insulin resistance. The reduction of ghrelin levels in parallel with hyperinsulnaemia and hyperleptinaemia suggests that leptin sensitivity isn't completely impaired, but it is possible that with longer treatment ghrelin levels would have increased significantly. The endogenous insulin sensitizing mechanism remained intact after olanzapine treatment.

New findings:

- Our study is the first to investigate the effects chronic atypical antipsychotic treatment on genetically CCK-1 receptor deficient animal model.
- Our results suggest that the metabolic derangements associated with chronic clozapine treatment develop independently from the CCK-1 pathway.
- Our results suggest that the meal induced insulin sensitization remains functionally intact after long-term olanzapine treatment, and its pharmacological exploitation gives us hope in order to treat or prevent the olanzapine-induced insulin resistance.

7. APPENDIX

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List of publications related to the dissertation

1. **Hegedűs, C.**, Kovács, D., Kiss, R., Sári, R., Németh, J., Szilvássy, Z., Peitl, B.: Effect of long-term olanzapine treatment on meal-induced insulin sensitization and on gastrointestinal peptides in female Sprague-Dawley rats.
   J. Psychopharmacol. 29 (12), 1271-1279, 2015.
   DOI: http://dx.doi.org/10.1177/0269881115602952
   IF:3.593 (2014)

2. **Hegedűs, C.**, Kovács, D., Drimba, L., Sári, R., Varga, A., Németh, J., Szilvássy, Z., Peitl, B.: Investigation of the metabolic effects of chronic clozapine treatment on CCK-1 receptor deficient Otsuka Long Evans Tokushima Fatty (OLETF) rats.
   Eur. J. Pharmacol. 718 (1-3), 188-196, 2013.
   DOI: http://dx.doi.org/10.1016/j.ejphar.2013.08.034
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List of other publications

3. Kovács, D., **Hegedűs, C.**, Kiss, R., Sári, R., Németh, J., Szilvássy, Z., Peitl, B.: Meal-induced insulin sensitization is preserved after acute olanzapine administration in female Sprague-Dawley rats.
   Naunyn-Schmiedebergs Arch. Pharmacol. 388 (5), 525-530, 2015.
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4. Lelesz, B., Tóth, G.K., Peitl, B., **Hegedűs, C.**, Drimba, L., Sári, R., Szilvássy, Z., Németh, J.:
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IF:2.36

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Effects of Cicletanine in Conscious Rabbits With Metabolic Syndrome.
J. Cardiovasc. Pharmacol. 60 (2), 208-218, 2012.
DOI: http://dx.doi.org/10.1097/FJC.0b013e31825c3c4c
IF:2.383

Total IF of journals (all publications): 17,932
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on
the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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