Aus der Klinik und Poliklinik für Psychiatrie, Psychosomatik und Psychotherapie
der Universität Würzburg

Vorstand: Prof. Dr. J Deckert

Changes in gene expression of brain insulin system in STZ icv - damaged rats - relevance to Alzheimer disease

Inaugural-Dissertation
zur Erlangung der Doktorwürde der
Medizinischen Fakultät
der
Julius-Maximilians-Universität zu Würzburg

vorgelegt von
Jelena Osmanovic
aus
Zagreb, Kroatien

Würzburg, Februar 2008

Referent: Professor Dr. P. Riederer, Klinische Neurochemie
Koreferent. Professor Dr. med. A Fallgatter, Psychiatrie und Psychotherapie
Dekan: Professor Dr. med. M. Frosch

Tag der mündlichen Prüfung: 15.10.2008.

Die Promovendin ist Ärztin

Table of content

1 INTRODUCTION

1.1 Alzheimer's disease

Alzheimer's disease (AD) is the most common dementia in the elderly population (>65 years), associated with progressive neurodegeneration of the central nervous system (CNS) (Blennow et al., 2006). Clinically, AD typically begins with a subtle decline in memory and progresses to global deterioration in cognitive and adaptive functioning (Watson and Craft, 2004). On the pathophysiological level there are two types of AD; very rare autosomal dominant early-onset familiar type and very common late-onset sporadic type with still unknown etiology (Blennow et al., 2006). Early-onset familiar AD is caused by missense mutations in the amyloid precursor protein (APP) gene on chromosome 21, in the presenilin (PS) 1 gene on chromosome 14 and in the PS 2 gene on the chromosome 1 (Rocchi et al., 2003). Two main neuropathological hallmarks are found in the brain of patients with sporadic Alzheimer disease (sAD) and familiar AD: neurofibrillary tangles (NFT) and amyloid plaques. Neurofibrillary tangles consist of intracellular protein deposits made of hyperphosphorylated tau protein (Blennow et al., 2006). Tau protein is a microtubule-associated protein which is involved in stabilization and promotion of microtubules but when hyperphosphorylated it gains a toxic function which is lethal for the neurons (Iqbal et al., 2005). Extracellular amyloid plaques predominantly consist of aggregates of neurotoxic amyloid beta 1-42 (Aβ 1-42) generated in vivo by specific, proteolytic cleavage of APP (Blennow et al., 2006; Rocchi et al., 2003)

Classical and also leading amyloid cascade hypothesis assumes that pathological assemblies of amyloid beta (Aβ) are the primary cause of both AD forms and all other neuropathological changes (cell loss, inflammatory response, oxidative stress, neurotransmitter deficits and at the end loss of cognitive function) are downstream consequences of pathological Aβ accumulation (Rocchi et al., 2003). This hypothesis could explain the etiology of familiar AD, but the etiology of sAD still remains a mystery. Sporadic Alzheimer's disease has age (increased prevalence of sAD from below 1% at the age of 60 to 24-33% at the age of 85 and more), diabetes mellitus type 2 and apolipoprotein E4 (ApoE4) as risk factors, which may suggest that neurodegenerative lesions are compensatory phenomena, and thus manifestations of cellular adaptation to some chronic pathological changes in the brain (Ferri et al., 2005; Blennow et al., 2006). There is a growing body of evidence that changes in insulin and insulin receptor (IR) signaling cascade in the brain of people with sAD have an influence on the metabolism of APP and Aβ accumulation and in maintaining of balance between phosphorylated and nonphosphorylated tau protein (Gasparini et al., 2002; Hoyer, 2004; Frölich et al., 1998).

1.2 Insulin system in the brain

1.2.1 Insulin

Although insulin and IR are found throughout the CNS their physiological function is still generally unclear. The great majority of insulin in the brain is produced at the periphery in β pancreatic cells and is transported in to the

brain across the blood brain barrier by region specific saturable transport mechanism (Banks et al., 2004). Except at the periphery, a small proportion of insulin is synthesized in the brain. Insulin mRNA (one gene in humans and two genes in rats) is distributed in a highly specific pattern with the highest concentration in the pyramidal cell of the hippocampus and high concentration in the medial prefrontal cortex, entorhinal cortex and perirhinal cortex, the thalamus, the granular layer of the olfactory bulb and also in the hypothalamus (Devaskar et al., 1994).

1.2.2 Insulin receptor

To induce biological effects in the brain insulin binds to the specific brain insulin receptors (IR). In general the IR belongs to the receptor tyrosine kinase superfamily and it's made of two α-subunits and two β-subunits (Johanstone et al., 2003). There are two slightly different types of IR: a peripheral type on glial cells which is down regulated with insulin and a neuron-specific brain type with high concentration on neurons which is not down regulated with insulin. Also, the molecular mass of the α and β-subunits of the brain IR are a bit lower then that in the periphery. Besides these small differences no other changes were found *(*Adamo et al., 1989*).* Distribution of neuronal IR mRNA, throughout the brain shows a highly specific pattern, with the highest density found in the olfactory bulb, hypothalamus, cerebral cortex and hippocampus functionally involved in cognitive function, learning and memory (Marks et al., 1991).

1.2.3 Insulin receptor signaling pathway

IR signaling cascade in the brain is similar to the one at the periphery. There are two main parallel IR intracellular pathways, the phosphatidylinositol-3

kinase (IP-3K) pathway and the mitogen activated protein kinase (MAPK) pathway **(**Johanstone et al., 2003). When insulin binds to the extracellular α-subunit of IR it induces autophosphorylation of the intracellular β-subunit resulting in increased catalytic activity of the tyrosine kinase (Johanstone et al., 2003). Now activated IR becomes a docking site for the insulin receptor substrate (IRS), which then becomes phosphorylated on tyrosine residues. Insulin receptor substrate is now ready to bind various signaling molecules with SH2 domains; one of these molecules is phosphatidylinositol-3 kinase (PI-3K). After being activated, PI-3K induces phosphorylation and subsequent activation of protein kinase B (PKB/Akt), consequently activated PKB/Akt triggers glucose transporter 4 (GLUT4) and also phosphorylates the next downstream enzyme glycogen synthase kinase (GSK-3) which then becomes inactive (Johanstone et al., 2003). When activated GSK-3 becomes a very important enzyme which, within brain, is involved in whole range of processes (Kaytor and Orr, 2002). There are two closely related isoforms of GSK-3, GSK-3α involved in the metabolism of amyloid- peptides (Aβs), and the GSK-3β isoform involved in phosphorylation of tau protein (Kaytor and Orr, 2002.; Pei et al., 2003; Phiel et al., 2003). Therefore, metabolism of amyloid precursor protein (APP) and the formation of Aβ, as well the maintenance of the balance between phosphorylated/dephosphorilated tau protein, are under control of the insulin/IR signaling cascade (Fig. 1).

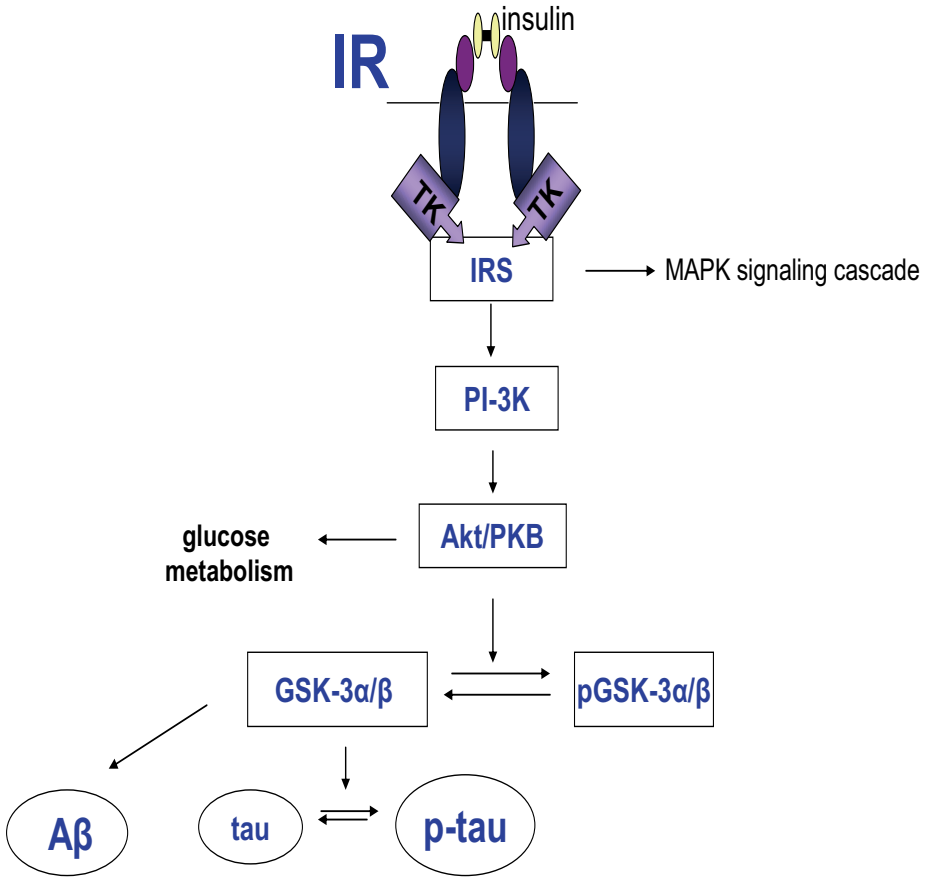

Fig. 1 Brain Insulin/Insulin receptor signaling cascade. **IR**- insulin receptor, **IRS**- insulin receptor substrate, **MAPK**- mitogen activated protein kinase, **IP-3K**- phosphatidylinositol-3 kinase, **Akt/PKB**- protein kinase B, **GSK-3**- glycogen synthase kinase, **Aβ**- amyloid beta peptid

1.2.4 Function of insulin in the brain

Regional specific distribution of insulin and IR in the brain gives insulin the opportunity to influence selective brain functions. Insulin/IR found within hypothalamus is involved in the regulation of the homeostasis of food intake

and reproduction (Park, 2001). In addition, insulin/IR localized in the hippocampus and cerebral cortex affects cognitive functions of learning and memory (Park, 2001; Zhao et al., 2004). There are several proposed mechanisms by which insulin can influence cognitive function and one of those is related to the regulation of brain glucose metabolism (Hoyer, 2004). Like in the periphery, in the CNS insulin is involved in the regulation of glucose/energy metabolism probably through the insulin-sensitive glucose transporter GLUT4, which is found in the hypothalamus, cerebral cortex and hippocampus, and shows overlapping with distribution of insulin/IR in brain structures that are involved in memory processes (Apelt et al., 1999). Glucose is the main source of the energy-rich compound acetyl-CoA and ATP (Garland and Randle, 1964.), but when influx of glucose in the brain is low, fatty acid or ketone bodies are used leading to reduction of production of acetyl-CoA and ATP (Garland and Randle 1964.). Acetyl-CoA is among others things used for the synthesis of the neurotransmitter acetylcholine which is found to be decreased in brains of people with sAD (Gibson et al., 1975). It appears that insulin/IR, glucose and acetylcholine have a relative direct association, but insulin/IR effect on memory may also interact with other neurotransmitters, for example via potentiation of NMDA receptor channels leading to increased in Ca^{2+} influx and long term potentiation (Skeberdies et al., 2001). Through the PI-3K pathway, it may be involved in long-term depression by internalization of glutamatergic AMPA receptors, or via recruiting of functional GABA receptors to the postsynaptic membrane (Man et al., 2000). Also, activation of the MAPK pathway during the process of learning may lead to the regulation of gene

expression involved in long-term memory storage. On the other hand short-term memory may be regulated by IR through its interaction with G-protein-coupled receptors (Zhao et al., 2004).

1.2.5 Insulin/IR regulation in the brain

The most important thing in the living organism is maintaining of homeostasis. Insulin/IR system homeostasis is maintained at the level of insulin production, storage, secretion, internalization and degradation as well as at the level of IR production and activation (Hoyer et al., 1993; Hoyer, 2004). The majority of experiments regarding insulin regulation are connected to its release, particularly in the hypothalamus (Gerozzissis et al., 2001; Orosco et al., 2001). Findings that insulin secretion in rat brain during depolarization condition depends on the calcium influx indicate that insulin in the brain is stored in the vesicles and its secretion is connected with neural activity (Wei et al., 1990). Also leptin and glucocorticoides were found to have influence on the insulin secretion directly or via other molecules (glucose, serotonin) (Niswender et al., 2004). Regarding insulin degradation, insulin degrading enzyme (IDE), a metalloendoprotease found with in cytosol, peroxisome, endosomes and on the cell surface was found to be the main enzyme for insulin degradation (Duckworth et al., 1988; Qiu et al., 2006). The gene of which is localized on chromosome 10q and its expression is tissue dependent with high concentration noted in brain (Farris et al., 2003; Cook et al., 2003). IDE degrades different substrates which can be divided in two bigger groups. The first group consists of substrates with high affinity for IDE (Km ≈ 0.01 μM) insulin, transforming growth factor α, atrial natriuretic peptide and insulin-

growth factor II. In contrast, the second group of substrates shows a lower affinity at Km >2 μM, and contains Aβ, APP, glucagons, insulin growth factor I, epidermal growth factor, β-endorphin (Farris et al., 2003). In normal conditions, brain IDE is the main degrading enzyme of the Aβ soluble fraction (Perez et al., 2000). In vitro inhibition of IP-3K abolished IDE upregulation by insulin causing Aβ assembly, indicating that excess of insulin can upregulate IDE to prevent Aβ assembly into the plaques (Zhao et al., 2004; Qiu et al., 2006). This indicates that low insulin level found in post mortem brain of patients with sAD can contribute to the Aβ pathology (Steen et al., 2005). Another level at which the insulin/IR balance is maintained is at the level of IR. Activation state of this tyrosine kinase receptor is regulated by the action of phosphotyrosine phosphatase and serin-threonine kinase (Goldstein, 1993). Glucocorticoids can desensitize IR by inhibition of phosphorylation of its tyrosine residues (Giorgino et al., 1993). Catecholamines were shown to decrease the activity of the receptor's tyrosine kinase by phosphorylation of the IR serine/threonine residues and can also decrease the insulin-induced tyrosine phosphorylation of the IRS1 and IRS2 (Häring et al., 1986). Tumor necrosis factor is another molecule involved in IR regulation by decreasing autophosphorylation of IR and tyrosine phosphorylation of IRS1 and IRS2 (Hoyer and Frölich, 2006) This all shows the complexity of insulin/IR regulation and points to the necessity for preserving its homeostasis which is important for memory.

1.3 Glucocorticoids and their interaction with the insulin system

One of the main endocrine systems involved in stress reaction is the hypothalamic-pituitary-adrenal axis (HPA-axis) (Lupein SJ. et al. 2005). When the organism is under psychological and physical treat the HPA-axis is activated. Corticotropin releasing factor (CRF) of the hypothalamus is the first to respond to stress, which triggers the release of the adrenocorticotropin hormone (ACTH) from the pituitary gland to the bloodstream. The last target is the adrenal gland in which ACTH stimulates the secretion of glucocorticoides (GCs; cortisol in humans, corticosterone in rats) (Lupein et al., 2005). Actions of GCs are mediated by two types of receptors; the mineralocorticoid (MR or type I) and glucocorticoids (GR type II) (de Kloet et al., 1998.). The MR receptors are found in the hippocampus, parahippocampal gyrus, entorhinal and insular cortices while GR receptors are distributed in the hypothalamus, hippocampus, parahippocampal gyrus and cortex (McEwen et al., 1999). Glucocorticoids with different effect are aiming to increase the energy substrates to the target organism giving the organism ability to cope, adapt and recover from the stress (Lupein et al., 2005). The glucocorticoid hormone cortisol is involved in numerous brain functions, including process of learning and memory, attention and perception with still unknown mechanism. When humans or rats are subjected to a high chronic level of GCs cognitive functions of learning and memory are found to be impaired, as well as there is a decrease in hypothalamus volume as compare to control (Landfied et al., 1978; Starkman et al., 1992). On a genetic level polymorphism in the gene coding for 11β-hydroxysteroid dehydrogenase I (β-HSD I) shows a 6-fold

increased risk for sAD (de Quervain et al., 2004), demonstrating a connection with memory losse and corticosteron level, because the β-HSD I in the brain acts as a reductase rescuing the GCs from its inert 11-keto form, thus amplifying the GCs action (de Kloet et al., 1998). Growing evidence shows that the basal tonus of the HPA-axis increases with aging leading to hypercortisolemia, what can finally compromise the function of the insulin/IR system (Lupein et al., 2005; Landfield et al., 2007). It was mentioned earlier in the text that GCs influence the insulin cascade at the level of IR causing its resistance by disregulation of its phosphorilation of the tyrosine residue (Giorgino et al., 1993). Thus, higher levels of GCs and lower activity of the insulin/IR signaling cascade found in AD could be the main mechanism by which corticosterone impairs cognition.

1.4 Changes of the brain insulin signaling cascade in sAD

Research of the brain insulin system has been more pronounced in the last decade, particularly regarding its function in the brain. There is a growing interest in finding the role of neuronal insulin signalling cascade in the brain, and off course in the brain of sAD. Recent literature data indicate that brain insulin deficiency and insulin resistance brain state are related to the late-onset sAD (de la Monte and Wands, 2005; Hoyer, 2004; Hoyer and Frőlich, 2005). The late-onset type of Alzheimer's disease is associated with glucose utilization abnormalities distributed all over the cerebral cortex, and particularly in structures with both high glucose demands and high insulin sensitivity (Henneberg and Hoyer, 1995). Neuronal glucose metabolism is under the

regulation of neuronal insulin, and abnormalities in the brain glucose metabolism found in Alzheimer's disease have been suggested to be induced at the level of insulin signal transduction (Hoyer, 2002; Hoyer and Frőlich, 2005). In line with this decreased brain insulin protein and its mRNA levels were found post mortem in the brain (frontal cortex, hippocampus and hypothalamus) of people with sporadic Alzheimer's disease (Craft et al., 1998; Steen et al., 2005), while IR density was found to be increased and tyrosine kinase activity decreased (Frolich et al., 1998; Steen et al., 2005). Interestingly, strikingly reduced expression of genes encoding insulin like growth factor-1 (IGF-1) and IGF-1 receptor has also been found in the frontal cortex, hippocampus and hypothalamus of patients with AD post mortem (Steen et al., 2005). Regarding the downstream IR signalling pathways, reduced levels of PI3-K have been found (Steen et al., 2005). Regional specificity of changes and difference in AD severity stage probably account for some inconsistency in results reported in relation to Act/PKB and GSK-3 alterations, whose phosphorylated form was mainly found to be decreased (Pei et al., 2003; Steen et al., 2005). Increased activity of GSK-3 found in hippocampus and hypothalamus could be related to decreased activity of Act/PKB found in the same regions (Steen et al., 2005). Regarding that the total amount of Akt/PKB and GSK-3 was found unchanged, alteration of the phosphorylation/dephosphorylation balance of these proteins seems to be involved (Steen et al., 2005). Hyperphosphorylation of tau in Alzheimer's disease has been found also to involve GSK-3 (Sengupta et al., 2006), and in line with increased activity of GSK-3 found in brains of AD patients post

mortem, increased level of phosphorylated tau protein was found in the frontal cortex of these patients (Steen et al., 2005). Recent data have pointed to another important enzyme, involved in tau dephosphorylation, the protein phosphatase 2A (PP2A), which can directly dephosphorylate tau (Planel et al., 2001). Immunoblotting analysis revealed a significant reduction in the total amount of PP2A in frontal and temporal cortices of sAD patients that matched the decrease in PP2A activity and lower PP2A expression in immunohistochemical studies (Sontag et al., 2004; Gong et al., 1995). Thus, it seems likely that hyperphosphorylated tau formation is the consequence of increased GSK-3β activity and/or decreased PP2A activity. As previously mentioned, GSK-3α regulates the production of Aβ (Phiel et al., 2003). APP derivatives Aβ peptides are generated intracellularly in the endoplasmic reticulum, and their accumulation is reduced by accelerating their transport to the plasma membrane. The promotion of APP secretion from the intra- to the extracellular space and the inhibition of its degradation by IDE is mediated by insulin and the tyrosine kinase activity (Gasparini et al., 2002; Zhao et al., 2004).

1.5 Streptozotocin intracerebroventricularly (STZ-icv) treated rats

Considering the fact that sAD has been recognised as an insulin resistant brain state, the STZ intracerebroventricularly (STZ-icv) treated rat has become the proposed experimental model of this disease (Hoyer, 2004; Lannert and Hoyer, 1998; Prickaerts et al., 1999). Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is a toxic substance which, for decades has

been used to induce experimental diabetes mellitus (DM). Streptozotocin enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA and generation of free radicals. DNA damage induces activation of poly ADP-ribosylation which leads to depletion of cellular NAD+ and ATP (Fig 2). When applied intraperitoneally in high doses (>65 mg/kg) STZ is toxic for insulin producing/secreting cells, which induces experimental diabetes mellitus type I. Low doses (40-60 mg/kg) of STZ given intraperitoneally damages IR and causes diabetes mellitus type II (Szkudelsky, 2001) (Fig. 3). STZ-icv administration of low doses (≤ 3 mg/kg) does not alter basal blood glucose or produce diabetes mellitus (Nitsch and Hoyer, 1991; Plaschke and Hoyer, 1993) but induces behavioural, neurochemical and structural changes that are similar to those found in sAD. STZ-icv treatment causes marked reduction in brain glucose/energy metabolism and shows a progressive trend towards oxidative stress (Duelli et al., 1994; Nitsch and Hoyer 1991; Lannert and Hoyer 1998). Regarding the structural changes, STZ-icv administration has been associated with astrogliosis and extensive cell loss. Also, deficit in the cholinergic transmission has been found in the brain of STZ-icv treated rats, but no morphological changes in cholinergic neurons could been found. As mentioned before in the text, sAD is now recognised as an insulin resistant brain state, therefore there is a growing interest in research in the insulin/IR system in the brain of STZ-icv treated rats. Regarding the enzymes downstream of the IR-PI3-K pathway, experiments have shown alterations of hippocampal GSK-3α/β in the experimental model of Alzheimer's disease, however, observed changes were of a greater extent in the phosphorylated

than in the non-phosphorylated form of GSK-3 (Salkovic-Petrisic et al., 2006) Also the changes were regionally specifically distributed; increased GSK-3 levels in the frontal cortex and unchanged GSK-3 levels in hippocampus, 1 and 3 months following the drug treatment (Salkovic-Petrisic et al., 2006). Regarding the phosphorylated GSK-3 (pGSK-3) form, levels in hippocampus were increased after 1 month, but decreased 3 months after the drug treatment, while in the frontal cortex, pGSK-3 was found to be decreased in both observational periods, 1 and 3 months following the STZ icv treatment (Salkovic-Petrisic et al., 2006). Akt/PKB levels were unchanged in hippocampus and decreased in frontal cortex 1 month following the STZ-icv treatment, while 3 months following the drug treatment Akt/PKB levels in hippocampus were decreased (Salkovic-Petrisic et al., 2006). All those neurochemical findings plus impairments of passive avoidance behaviour, and both, working and reference memory in STZ-icv rat model, demonstrate the resemblance of the STZ-icv rat model to the changes found in human sAD (Blokland and Jolles, 1993; Lannert and Hoyer 1998; Mayer et al., 1990; Prickaerts et al., 1999). Mechanism of this central action of STZ is still unknown but there is some evidence supporting the similarity to its peripheral action; GLUT2 and IR have been found regionally specifically distributed in the brain, also insulin is synthesised in the particular regions of the brain and depletion in ATP and increase in oxidative species has shown also regionally specific distribution. Due to the slow progression of sAD and postmortal diagnosis, initial pathophysiological changes and their further course in the human brain are unknown, making a search for the representative

experimental model additionally difficult. The STZ-icv rat model has a potential of being the good experimental model that gives us the possibility to follow up brain changes at different time points after the STZ-icv treatment.

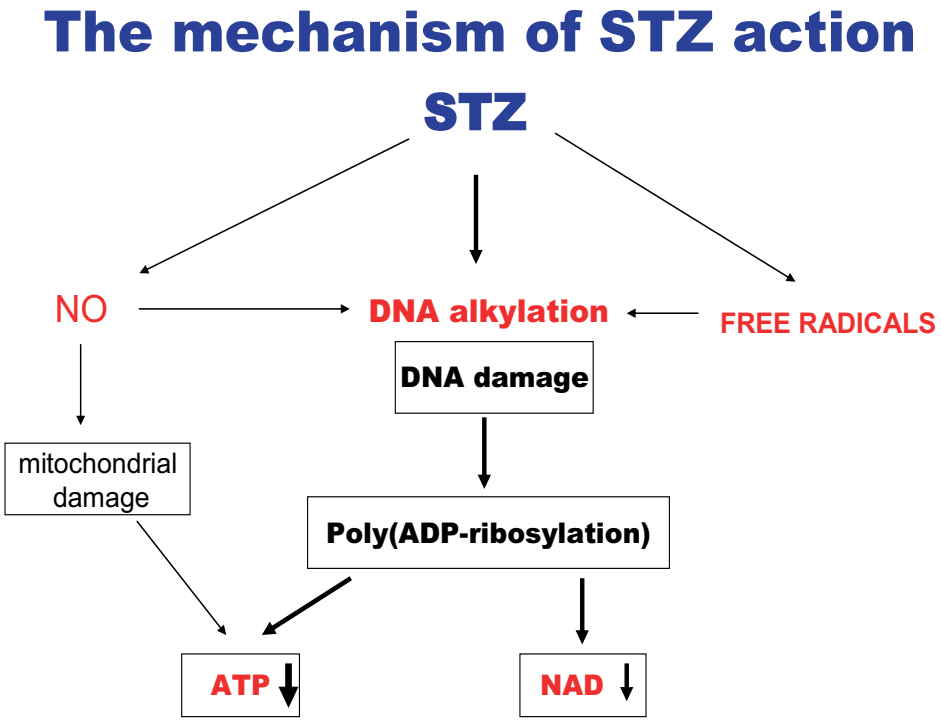

Fig. 2 Mechanism of streptozotocin (STZ) action**.** After entering the B cell via a glucose transporter (GLUT2) STZ causes alkylation of DNA and generation of free radicals and NO. Activation of poly ADP-ribosylation caused by DNA damage induces depletion of cellular NAD+ and ATP. Mitochondrial damage caused by NO leads to ATP-depletion as well.

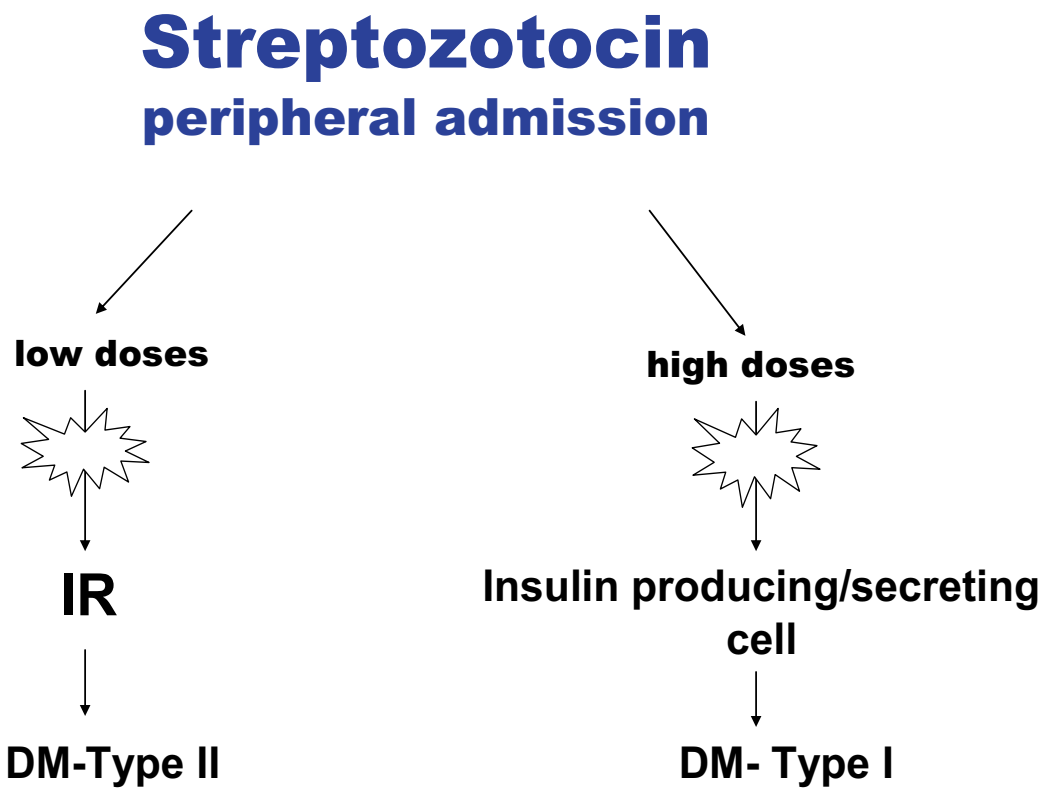

Fig. 3 Peripheral effect of streptozotocin (STZ). When applied intraperitoneally in low doses STZ damages IR and causes diabetes mellitus (DM) type II. High doses of STZ are toxic for insulin producing/secreting cells, which induces experimental DM type I.

2 HYPOTHESIS

Recent literature data suggests that sAD is associated with changes of brain insulin/IR signalling pathway which may lead to the hyperphosphorylation of tau protein and changes in Aβ metabolism (Hoyer, 2004; Lupein et al., 2005). Namely, activation of the brain IR triggers the intracellular signalling pathways which regulate phosphorylated state of tau protein and metabolism of Aβ. Thus, it could be hypothesized that alteration of the brain insulin system precedes Aβ pathology (Kaytor and Orr, 2002.; Pei et al., 2003; Phiel et al., 2003). However, the time course of changes in the brain insulin system cannot be investigated in humans. Additionally, if one assumes that altered brain insulin system is preceding the Aβ pathology, the question still remains what could cause brain insulin changes. One of the missing links could be plasma corticosterone levels. Basal activity of HPA-axis in sAD is increased leading to increased concentration of cortisol. Cortisol is one of the inhibitors of the IR tyrosine kinase activity and could possibly induce IR signaling dysfunction (Giorgino et al., 1993). Some literature data reports that stress-level of corticosteron administration increases Aβ formation and promotes tau accumulation in the animal model of sAD but the mechanisms underlying these effects are still unknown (Green et al., 2006). Under normal condition cortisol and insulin could act as antagonists; cortisol has catabolic effects and insulin has anabolic effects, so balance between insulin and cortisol might be important for the sAD pathogenesis (Landfield et al., 2007). In line with this finding, and assuming that peripheral action of STZ affecting IR is similar to that in the brain, STZ-icv rats are a representative experimental model of sAD.

As the model is not related to gene manipulation it could reliably demonstrate time development of changes in expression of genes of the brain insulin/IR system (3 months and 6 months after the STZ-icv treatment) and their relevance in sAD pathogenesis. Furthermore, we hypothesized that chronic treatment of rats with corticosterone as experimental model of chronic stress can decrease the activity of the brain insulin/IR system which, in line with physiological role of insulin in learning and memory, will lead to impairment of cognitive functions.

3 MATERIAL AND METHODS

3.1 STZ-icv treatment

3.1.1 Animals

Three-month-old male Wistar rats weighing 280-330 g (Department of Pharmacology, School of Medicine, University of Zagreb) were used throughout the study. . Animals were housed in the temperature controlled animal room (22.0±0.5ºC) and were kept on standardized food pellets and water *ad libitum*.

3.1.2 Drug treatments

For STZ-icv experiment, rats were randomly divided in 2 groups (5-6 per group) and given general anaesthesia (chloralhydrate 300 mg/kg, ip), followed by injection of STZ bilaterally into the lateral ventricle (2 μL/ventricle), according to the procedure described by Noble et al. (Noble et al., 1967). The following treatments were applied in a single dose: STZ (1 mg/kg, dissolved in 0.05M citrate buffer pH 4.5) in group I, and an equal volume of vehicle icv in group II. Animals were sacrified three and six months after the drug treatment. Brains were quickly removed, hippocampus cut out, immediately frozen and stored at -80 ^{O}C. STZ-icv-treated animals had no symptoms of diabetes and steady-state blood glucose level did not differ in comparison with control animals.

3.1.3 Morris Water Maze Swimming Test

Cognitive functions were tested in Morris Water Maze Swimming Test (Anger, 1991). Adaptation of rats to the experimental environment and behavioural activity was done during two days before the experimental trials. On the first of

these two days animals were subjected to 1 min of freely swimming in a pool (150x60 cm, 50 cm deep), with water temperature set at 25±1 ^{o}C, and on the second day rats were allowed to freely swim in the pool divided in four quadrants (I-IV). In the experimental trials, performed from day 1 to day 4, rats were thought to escape from water by finding an unseen rigid platform submerged about 2 cm below the water surface in quadrant IV. Stay on the platform was allowed for 15 s. One trial consisted of three starts, each from a different quadrant (I – III), separated by a 1-min rest period. Three consecutive trials were performed *per* day, separated by a 30-min rest period. After the third trial on day 4, the fourth trial was performed (starts from quadrants I-III) with a platform being removed from the pool, and the time spent in searching for the platform after entering quadrant IV was recorded. The cut off time was 1 min. Those rats who had no alterations in memory functions (control) were supposed to remember that the platform had previously been there, and, in line with that, to spend a long time swimming within quadrant IV, looking for the platform. In case of drug-induced deterioration of memory functions, rats were supposed to remember less intensively that the platform had been in quadrant IV, thus to spend less time in searching for the platform within this quadrant, in comparison with control rats.

3.2 Corticosterone subcutaneous (CTS-s.c.) treatment

3.2.1 Animals

Twelve-month-old male Wistar rats weighing 576±64 g (breeder: Thomae, Biberach, Germany) were used throughout the study. Animals were housed in

the temperature controlled animal room (22.0±0.5ºC) with a reversed light/dark cycle and were kept on standardized food pellets and water *ad libitum*.

3.2.2 Drug treatments

For the corticosterone treatment rats were divided in the 3 groups; group-I controls without the treatment (n= 8), group-II placebo rats treated with vehicle only (n=9), and group-III corticosterone treated group (n=9). In the group III rats received subcutaneous (s.c.) injections of corticosterone (Sigma, Germany) alternately on the right or left side of the neck at 26.8 mg/kg body weight (equivalent to 10 mg/day, in 1 ml of sesame oil). Literature data shows that this dose of corticosterone results in peak plasma non-stress levels within 24 h (Coburn-Litvak et al., 2003; Haugher et al., 1987). Before the corticosteron or vehicle treatment the animals were anaesthetized with 1.5 vol% halothane (O_2: N_2O = 30:70). Significant effect of daily short halothane anesthesia on corticosterone and on the HPA axis can be excluded (de Haan et al., 2002; Karuri et al., 1998). For placebo rats, 1 ml sesame oil alone as vehicle was also administered daily at 8--9 a.m. for 60 days (Stein-Behrens et al., 1994). The control group did not receive daily injections of corticosterone or vehicle and they were not anaesthetised. Rats were scarified after 60 days of corticosterone/vehicle treatment, brains were quickly removed, and cortex cut out, immediately frozen and stored at -80 ^{o}C.

3.2.3 Psychometric test parameters

To measure memory capacities and rat behavior, the psychometric holeboard memory test was performed according to Lannert & Hoyer (1998*)* and Plaschke et al. (1999). Habituation, training, and retesting of memory

measurements were performed in a defined holeboard box. This square closed-field area (70 x 70 x 40 cm) contained 16 holes on the flat in a 4 x 4 array. Each hole contained a metal cup (3.5 cm in diameter, 3 cm deep) which had a perforated bottom. The holes were of the same diameter as the outer diameter of the cups (4 cm), the inner diameter of the cups was 3.5 cm. On one side of the wall, a starting box was attached and separated from the testing area by means of a guillotine door which could be operated from a distance. For habituation, the rats were placed into the starting box and allowed to enter the testing area to explore the holeboard in which all 16 holes were baited with 50 mg of food pellet (Altromin, standard no. 1320, Lage, Germany). A trial started when the door was opened and ended when the rat had dipped into all 16 holes. Even if the rat did not find all food pellets, the trial ends after 10 minutes. After repeating the habituation process five times, the rats were trained to search in 4 out of 16 baited holes in a fixed order. The trial was terminated when the rat had found all food pellets or when 5 minutes had elapsed, whichever occurred first. Two training trials were performed each day. For (re)testing values, the rats were tested by the same procedure as during training, but different combinations of food holes were selected to avoid the possibility of habituation as experienced for the baited set of food holes during the training period. Working memory ratio (number of food rewarded visits / number of visits and revisits to the baited set of holes) and reference memory (number of visits and revisits to the baited set of holes / number of visits and revisits to all holes) were determined at the beginning and after the end of the corticosterone treatment and were calculated according to van der

Staay et al. (1990) and expressed as %. As example, the task for working memory was fulfilled by 100%, if all 4 out of 16 pellets were found without revisits of food- and non-food-baited holes. In addition, the latency time (time from the start of the experiment until the first food-rewarded hole was visited) and the whole run time (time to fulfill the task) were determined as behavioural determinants of locomotor activity. Each of the 16 holes supplied with food pellets was covered by a false bottom (a metal cup, 3 cm deep) to mask potential odour cues emanating from the “reward” in the baited holes. Thus, rats were unable to distinguish between baited and unbaited holes by olfactory stimuli.

3.2.4 Corticosteron determination

Plasma corticosterone concentrations were measured with a specific radioimmunoassay (RIA) after extraction according to a previously published method (Vecsei, 1979; Vollmayr et al., 2001). Briefly, 10 µl plasma was supplemented with 100 µl of 5% aqueous ethanol, tritium-labeled corticosterone (to determine individual loss), and the mixture was extracted with 1 ml of cyclohexane / dichloromethane (2:1, v/v). The organic extract was separated, evaporated to dryness, dissolved in 1 ml of 5% aqueous ethanol and quantified with a specific RIA. Intra-assay variation was 12.4%, inter-assay variation 14.3%. Each result was corrected for the individually determined procedural loss.

3.3 Quantitative real-time RT-PCR

Total-RNA extraction

Isolation of total RNA was done using RNeasy midi kit (Qiagen GmbH, Germany, Cat. No. 75144) for the hippocampal tissue and RNeasy maxi kit (Qiagen GmbH, Germany, Cat. No. 75162) for the cortical tissue. Isolation of total mRNA for each animal and brain region were separately. Original protocol was changed by adding an additional step in order to receive more pure DNA free total-RNA. The total RNA on the column was pretreated with DNase I (Qiagen GmbH, Germany, Cat. No. 79254) and the original protocol was continued in order to isolate total RNA from the tissue.

Q-PCR

Before performing the gene expression profile with real time RT-PCR, the total mRNA from each sample was reverse transcribed with random hexamer and oligo –dT primers using iScript ^{TM} cDNA Synthesis Kit (BioRad Labaratories; Cat. No. 170-8890). Transcribed cDNA was then used for the analysis of gene expression profile of IR, Insulin 1, Insulin 2, IDE and tau (Table 1.). The results for gene expression profile in the hippocampus of rats treated with STZ-icv were normalized to the house-keeping genes: beta actin (ACTB), ribosomal 18S (Rnr 1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 2). The house-keeping genes used for normalization of gene expression profile in the cortex of rats treated with CTS-s.c. were: actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-2-Mikroglobulin (B2m), ubiquitin (UBC) (Table 3.). All the house-keeping genes were tested for

the stability with the geNorm program (Vandesomple 2002), and according to the geNorm program a normalization factor was calculated.

For the RT-PCR iCycler iQ system (BioRad Co., Hercules, CA, USA) was used as described previously (Svaren et al., 2000). Transcribed cDNA from each sample was mixed with gene specific primer (200 nmol/L final concentration) and the QuantiTect SYBR Green PCR Kit (Qiagen GmbH, Germany, Cat. No. 204145). Real-time PCR was subjected to PCR amplification starting with one cycle at 95ºC for 15 minutes continuing with 34-45 cycles at 94ºC for 15 seconds. Annealing and detecting of specific fluorescent color at 55ºC for 30 seconds and extension at 76ºC for 30 seconds. The PCR reaction were run in the triplicates. The comparative threshold cycle (CT) method analyzed with BioRad iCycler iQ system program, was used to quantify the amplified transcripts. Standards cDNA were isolated from agarose gel using MiniEluet ^{TM} gel extraction kit (Qiagen Inc) or using Bio Rad Freeze N' Squeeze Kit. For each amplification product standard curves generated from 10-fold diluted pooled cDNA amplicons were used to determine efficiency and quantification.

Table 1 Gene of interest analyzed in both STZ-icv and CTS-s.c. treatment

Gene	***Gene symbol***	***Kit description***
Insulin receptor	*IR*	*Qiagen, Cat. No. QT 00198968*
Insulin 1	*Ins1*	*Qiagen, Cat. No. QT 00373303*
Insulin 2	*Ins2*	*Qiagen, Cat. No. QT 00177380*
IDE	*IDE*	*Qiagen, Cat. No. QT 00191464*
tau	*Mapt-tau*	*Qiagen, Cat. No. QT 00174797*

Table 2 House-keeping genes for STZ-icv treatment

Gene	***Gene symbol***	***Kit description***
Actin beta	*ACTB*	*Qiagen, Cat. No. QT 00193473*
Ribosomal 18S	*Rnr1*	*Qiagen, Cat. No. QT 00199374*
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	*Qiagen, Cat. No. QT 00199633*

Table 3 House-keeping genes for CTS-s.c. treatmant

Gene	***Gene symbol***	***Kit description***
Actin beta	*ACTB*	*Qiagen, Cat. No QT 00193473*
Beta-2-Mikroglobulin	*B2m*	*Qiagen, Cat. No QT00176295*
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	*Qiagen, Cat. No QT00199633*
Ubiquitin	UBC	*Qiagen, Cat. No QT 00372596*

4 RESULTS

4.1 STZ-icv treated rats

4.1.1 RT-PCR

STZ- icv 3 months after the treatment

The effect of STZ-icv treatment (3 mg/kg) on the gene expression profiles of IR, Insulin 1 (Ins1), Insulin 2 (Ins2), IDE and tau protein mRNA in the hippocampus 3 months after the treatment compared with control is shown in Fig. 4. In the hippocampus 3 months after the STZ-icv treatment a significant decrease in the expression of IR mRNA was found (-19%, $p< 0.05$). Expression of Ins1 was significantly down-regulated (-84.5%, $p<0.05$), mRNA expression of Ins2 and tau-protein was unchanged compared to control respectively while a significant down-regulation of IDE mRNA (-19.25%, $p<0.05$) was also found in hippocampus.

STZ-icv 6months after the treatment

The effect of STZ-icv treatment on the gene expression profiles of IR, Insulin 1, Insulin 2, IDE and tau protein mRNA in the hippocampus 6 months after the treatment compared with control is shown in Fig. 5. Expression of IR mRNA stayed significantly down-regulated in hippocampus of STZ-icv treated rats (-25.7%, $p<0.05$) 6 months after the STZ treatment. Again, the expression of tau protein and Ins2 mRNA was unchanged. STZ-icv treatment caused a significant decrease by 57% ($p<0.05$) in the expression of Ins1 mRNA and a significant decrease by 38% ($p<0.05$) in the expression of IDE mRNA in comparison with control.

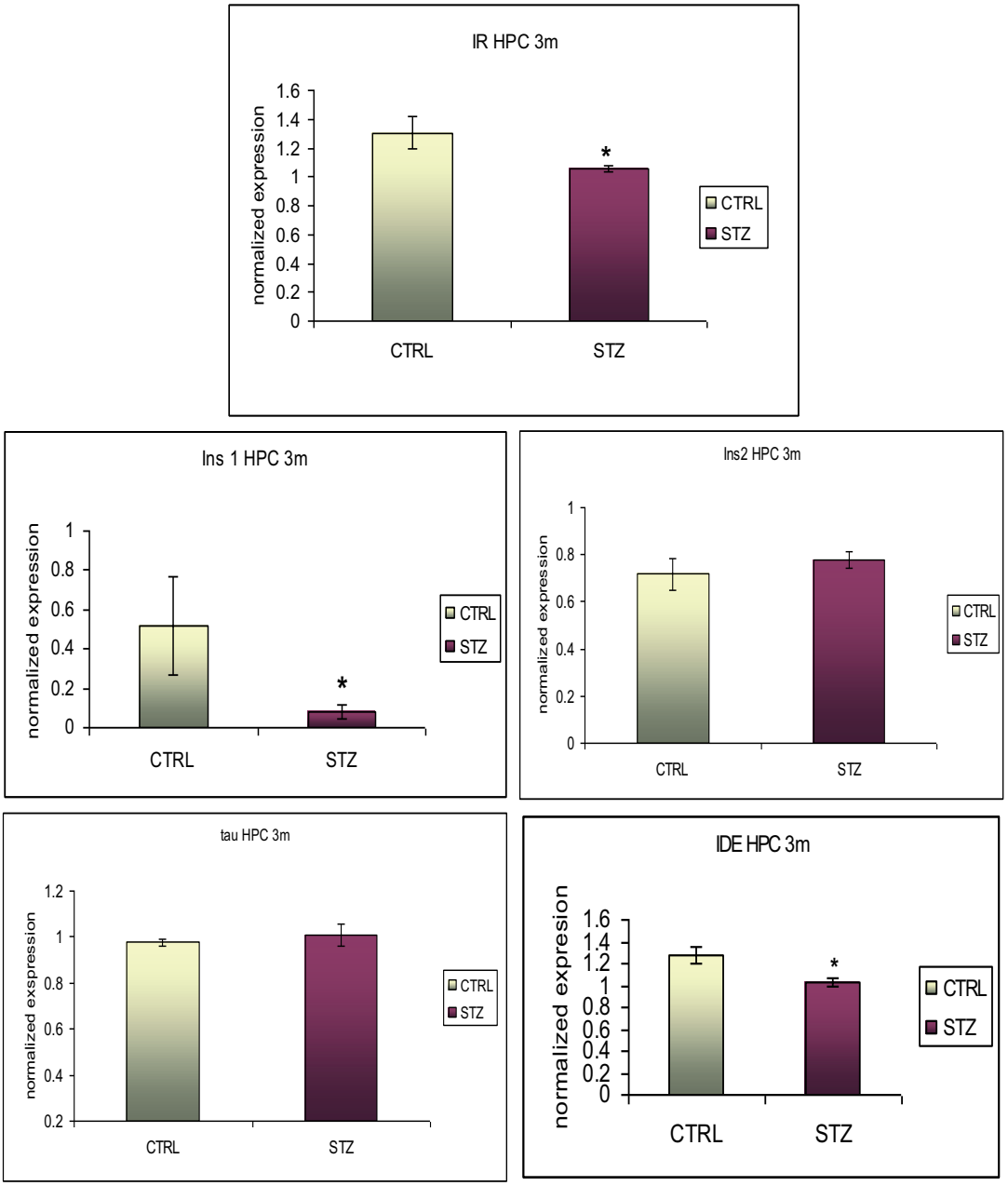

Fig. 4 Normalized gene expression of: **IR**-insulin receptor, **Ins1**-insulin-1, **Ins2**-insulin-2, tau protein and **IDE**-insulin degrading enzyme in hippocampus 3 months after the streptozotocin (**STZ**) treatment in comparison to control (**CTRL**). Mann-Whitney U-test; $p<0.05$

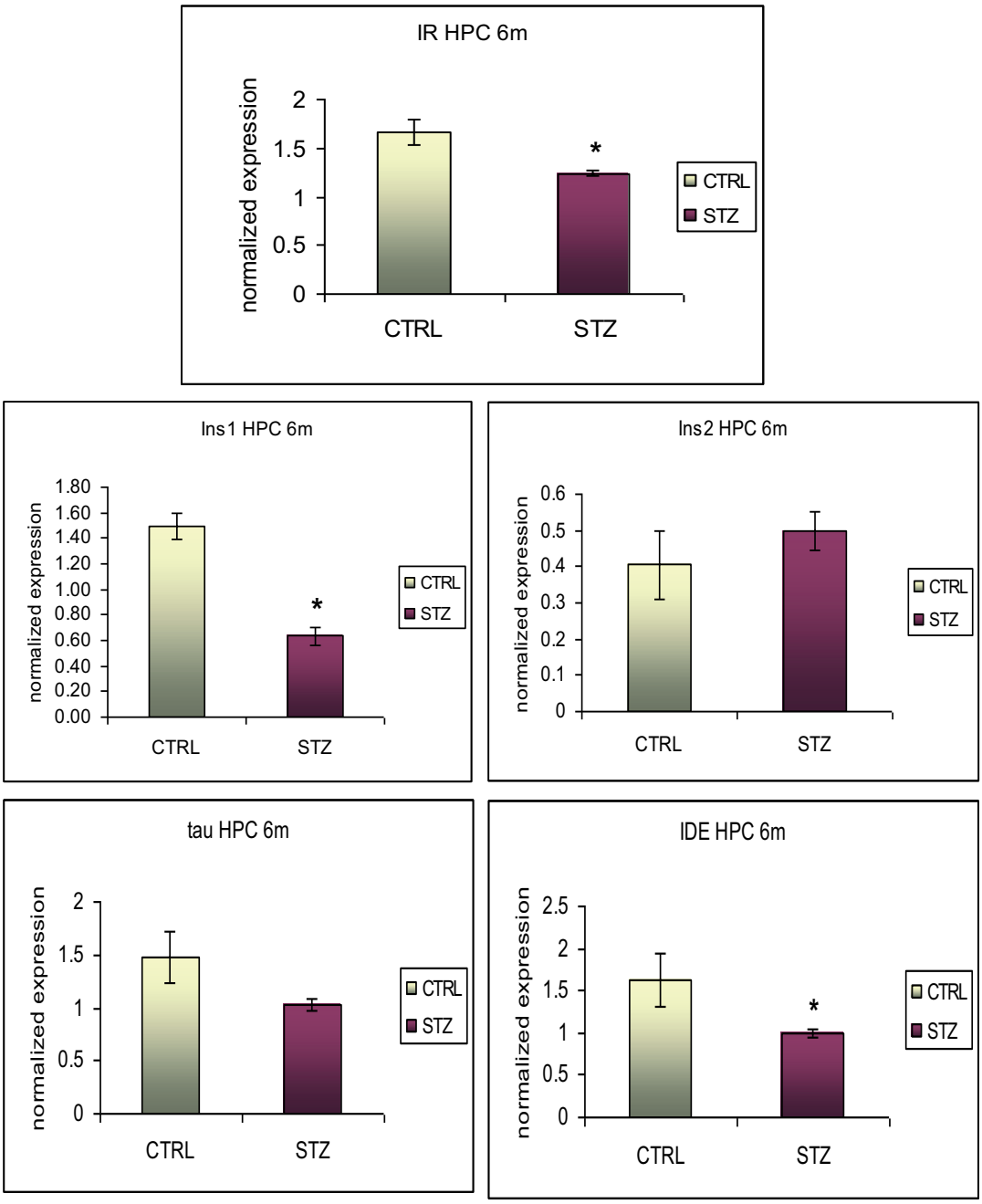

Fig. 5 Normalized gene expression of: **IR**-insulin receptor, **Ins1**-insulin-1, **Ins2**-insulin-2, tau protein and **IDE**-insulin degrading enzyme in hippocampus 6 months after the streptozotocin (**STZ**) treatment compared to control (**CTRL**). Mann-Whitney U-test; $p<0.05$

4.1.2 Learning and memory function in STZ-icv treated rats

Control rats have spent more time searching for the removed platform which shows that cognitive function of learning and memory in these rats is preserved. In contrast STZ-icv treated rats spent a less time swimming in the right quadrant looking for the platform indicating reduction in cognitive functions. STZ-icv treated rats did more mistakes in looking for the removed platform than control ones, thus they have a problem in remembering where the platform was. These STZ-icv induced cognitive deficits were found as early as 2 weeks after the treatment and were persisting 1,2,3,4 and 5 months following the STZ-icv treatment (Fig. 6).

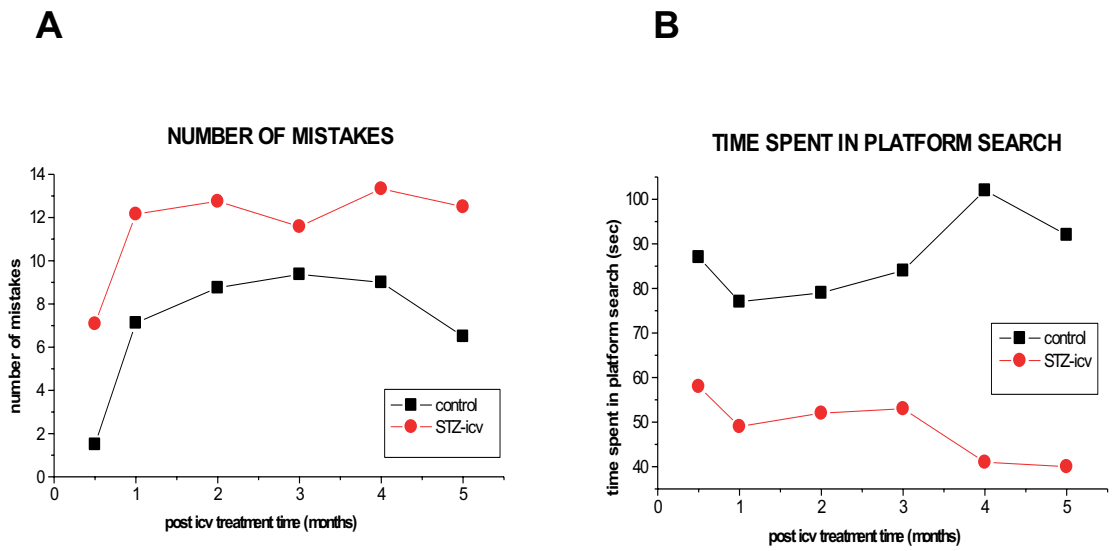

Fig. 6. Memory functions in the Morris water maze swimming test of rats treated with streptozotocin intracerebroventricularly (STZ-icv). **A** Mistakes were measured when the rat had entered the wrong quadrant i.e. quadrant without the platform. **B** The better the memory had been preserved, the longer the rats were searching for the platform and vice versa.

4.2 CTS- s.c. treated rats

4.2.1 RT-PCR

The effect of CTS-s.c. treatment on the gene expression profiles of IR, Insulin 1, Insulin 2, IDE and tau protein mRNA in the cortex after the corticosterone (CTS) treatment compared with control is shown in Fig. 7. Expression of IR mRNA was significantly down-regulated in CTS treated rats by 25.5% compared to untreated control i.e. control without stress, $p<0.05$. However, no significant difference was found between the vehicle (control with stress) and CTS-treated rats, while a significant difference was found between the untreated (stress free) and vehicle (with stress) control groups (25.5%). In all groups Ins2 mRNA expression stayed unchanged. CTS treatment caused a significant decrease by 57.5% ($p<0.05$) in the expression of Ins1 mRNA and a significant increase by 28% ($p<0.05$) in the expression of tau protein mRNA in comparison with untreated controls. Expression of IDE mRNA showed significant decrement in CTS treated rats compared to vehicle treated control (i.e. control exposed to acute stress of injection) as measured 60 days after the CTS treatment. When compared to untreated control (stress free) expression in CTS treated rats was decreased but because of the big intergroup variability this decrement was not significant. In both untreated (stress free) and vehicle control (with stress) no changes in the expression of IDE mRNA was observed.

4.2.2 Cognitive function in CTS treated rats

Daily corticosterone injection led to significant changes in rat behavior shown in Table 4a and 4b (Plaschke et al. 2006- with permission). After corticosterone treatment the total running time for the food search was increased from 116 ±

57 sec to 246 ± 88 sec ($p<0.05$) as shown in Table 4a. In addition, the latency time increased from 3.1± 4.9 sec to 12.4 ± 11.8 sec after two months of daily corticosterone administration. Both memory capacities, working and reference memory were markedly reduced after chronic corticosterone treatment in comparison to control rats (Table 4b). Working memory fell from 76 ± 24 % to 31 ± 20 % in corticosterone-treated rats ($p<0.05$), while reference memory was reduced from 57 ± 13 % to 38 ± 14 % ($p<0.05$).Placebo-treated rats demonstrated a significant increase in total run time from 116 ± 57 to 262 ± 86 sec ($p<0.05$), while the increase in latency time from 3.1 sec ± 4.9 to 6.5 ± 6.6 sec was not statistically significant. No significant changes in working (from 61± 22 % to 58 ± 45%) and reference memory (from 66 ± 14 % to 58 ± 23%) were observed in placebo-treated rats. In control animals without any treatment, the time to fulfil the holeboard task showed no significant changes (from 116 ± 57 sec to 97 ± 48 sec). Also, no changes in the working and reference memory capacity were found.

Table 4 Behavioural changes at the beginning and after the end of the corticosterone treatment. (Plaschke et al. 2006- with permission).

a)

	LOCOMOTOR ACTIVITY		
	TOTAL RUNNING TIME (SEC)		
	CTS (n=8)	PLACEBO (with stress, n=8)	CONTROL (n=12)
1st day	116±57	116±57	116±57
after 60thday	246±88*	262±86*	97±48

b)	MEMORY CAPACITIES			
	WORKING MEMORY (%)		REFERENCE MEMORY (%)	
	CTS (n=8)	PLACEBO (n=8)	CTS (n=8)	PLACEBO (n=8)
1st day	76±2	61±22	57±13	66±14
after 60th day	31±20*	58±45	38±14*	58±23

*p≤0.05 vs. 1st day

4.2.3 CTS concentrations in plasma

A significant increase in the plasma level of CTS treated rats after the 60 days of treatment confirms that treatment had induced chronic stress in treated animals. A tendency of increase of CTS in placebo control group was observed but due to high intragroup variability it was not statistically significant (Table 5).

Table 5 Corticosterone levels measured in plasma (Plaschke et al. 2006- with permission). with permission)

Treatment	**Plasma corticosterone** [µg/100ml]
Control *(n = 12)*	15.4 ± 5.4
Vehicle sesame oil *(n=8)*	27.7 ± 10.4
CTS *(n=8)*	32.4 ± 8.4*

*p≤0.05 vs control

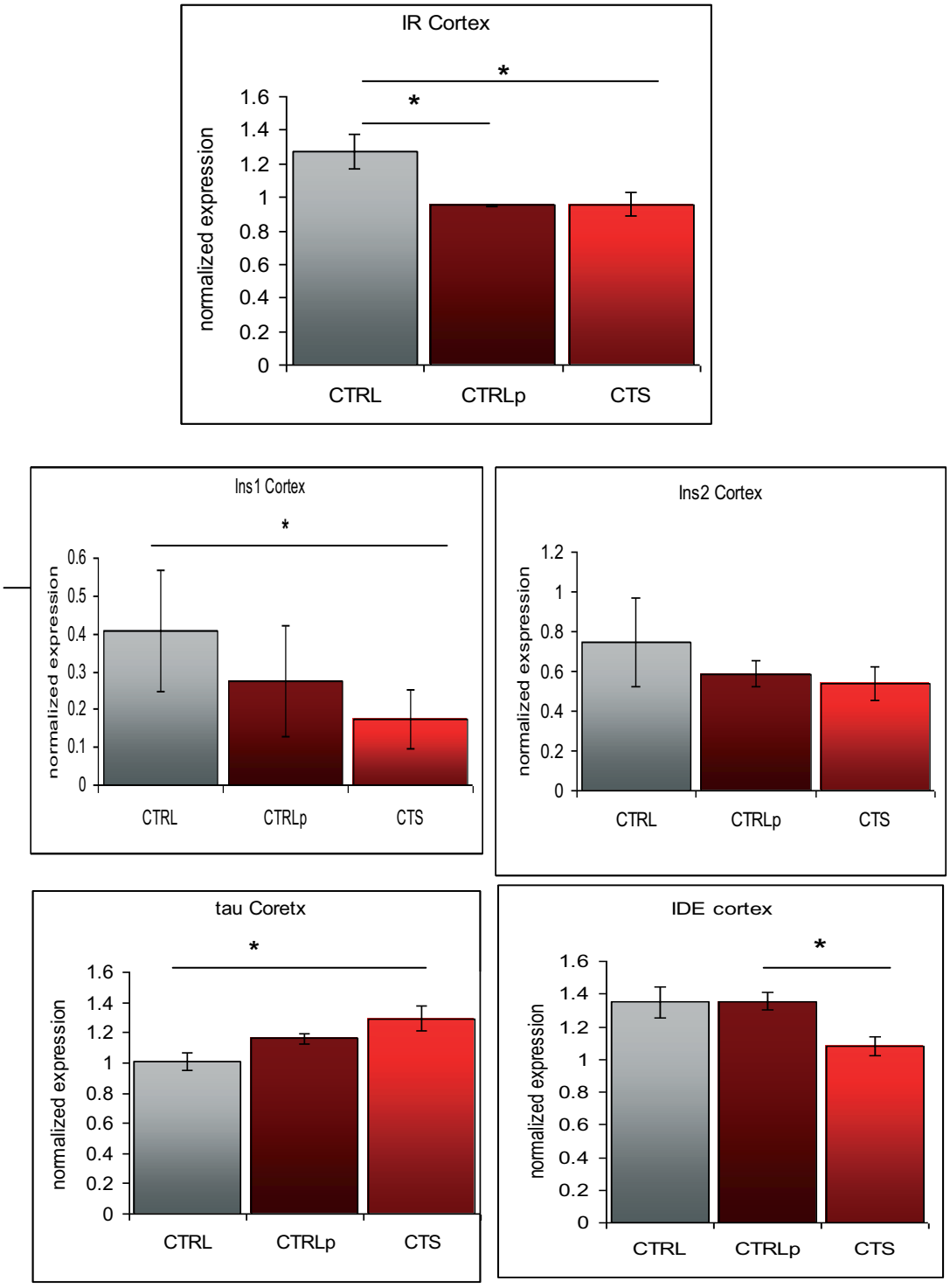

Fig. 7 Normalized gene expression of: **IR**-insulin receptor, **Ins1**-insulin-1, **Ins2**-insulin-2, tau protein and **IDE**-insulin degrading enzyme in cortex of corticosterone (**CTS**) treated rats, placebo control (**CTRLp**) and untreated control (**CTRL**). Mann-Whitney U-test; *p<0.05

Table 6 Summary of the brain insulin system gene expression data in STZ-icv rat model of sAD and the corticosterone chronic “stress” model

	STZ		**CTS**
	3 months	6 months	2 months
Ins1	↓	↓	↓
Ins2	≈	≈	≈
IR	↓	↓	↓
IDE	↓	↓	↓
tau	≈	≈	↑

↓ decreased
↑ increased
≈ unchanged

DISCUSSION

To explore the link between chronically elevated plasma levels of corticosterone and changes in the brain insulin/IR system, and to determine the effect of STZ-icv treatment on brain insulin/IR system, we investigated the expression of the insulin pathway genes in the hippocampus of STZ-icv treated rats and in the cortex of corticosterone s.c. treated rats.

When given peripherally STZ enters the cell trough GLUT-2 receptors and causes damage of DNA and generates free radicals (Szkudelsky, 2001). We can assume that the same mechanism of action takes place after a central (icv) application of STZ since the evidence of oxidative stress and reduced ATP levels in the STZ-icv rat model were reported (Sharma and Gupta, 2001). It is well established that when given intracerebroventriculary STZ induces cognitive deficits associated with changes in neuronal glucose metabolism and dysfunction of cholinergic neurotransmission (Lannet and Hoyer, 1998; Lester-Coll et al., 2006). All those mechanisms are a very important part of processes underlying learning and memory. These findings indicate that a changed brain insulin/IR signaling pathway plays a major role in learning and memory.

We have found a decrement of insulin gene expression in the STZ-icv rat model. In contrast to humans, rats have two genes for insulin Ins1 and Ins2, but our studies have shown that STZ-icv treatment has significantly decreased only expression of the Ins1gene. Considering the fact that the difference in function between Ins1 and Ins2 in rat brain is not yet clear, we can only speculate that the role of Ins1 is far more important for the physiological function of insulin in the rat brain. Our results of a decremeased synthesis of brain insulin are in the

line with findings of down-regulation of this gene in the brain of STZ intra cerebral treated young rats (Lester-Coll N. et al., 2006) and what is most important is the fact that the same results of down-regulation of the gene encoding insulin was found post-mortem in brains of people with sAD (Steen et al., 2005). Additionally, this finding is important because it shows that only small amounts of insulin are synthesized in the brain compared to the amount which is transported in the brain from periphery; thus insulin synthesized in the brain could have an important role in maintaining physiological functions of brain insulin. The present study shows down-regulation of IR mRNA expression in the STZ-icv rat model which could lead to the changes downstream of the brain insulin signaling cascade. In the STZ-icv rat model decrement of Akt/PKB and increase in activity of GSK-3α/β 3 months after the STZ-icv treatment was found regardless the STZ dose; 1mg/kg or 3mg/kg (Salkovic-Petrisic et al., 2006). Our findings goes in line with the results in humans showing decreased mRNA expression of IR, decreased level of PI-3K, activated Akt/PKB, increased GSK-3 activity and amyloid precursor protein mRNA expression in the brain of people with sAD (Steen et al., 2005). Consequently, we can assume that changes in brain IR synthesis and changes found in enzymes downstream the insulin signaling cascade can lead to hyperphosphorylation of tau protein and changes in Aβ metabolism.

Decreased IDE mRNA expression found in this study further supports involvement of the brain insulin system dysfunction in the development of Aβ pathology. Additionally, accumulation of Aβ found in the meningeal capillaries 3 months after the STZ-icv treatment goes in line with our findings of decreased

synthesis of IDE which is the main Aβ degradation enzyme (Salkovic-Petrisic et al., 2006; Zhao et al., 2004). Our results are in agreement with data of IDE deficiency reported in patients with sAD, caused by variations in the gene encoding IDE which are associated with the severity of clinical symptoms (Qiu et al., 2006).

However, these changes were not followed by alterations of gene expression of tau protein found in STZ-icv rat model. With respect to human sAD, literature data concerning the expression of tau protein show that splicing regulation of the tau gene and the relative expression of tau isoforms are not significantly changed in sporadic cases of the Alzheimer disease (Boutajangout et al., 2004). Both findings in humans and the STZ-icv rat model suggest that tau hyperphosphorylation and formation of neurofibrillary tangles is more likely to be caused by a misbalance between phosphorylation/dephosphorylation of tau protein i.e. a misbalance between the kinase/phosphatase homeostasis. STZ-icv treatment induces a changes similar to changes found in sAD, and therefore provides supportive evidence that sAD may be caused in part by neuronal insulin resistance.

One of the additionally findings in patients with AD is increased level of plasma corticosterone (de Leon et al., 1988). Normal secretion of corticosterone shows a daily rhythm with peak concentrations in the early morning and in the late afternoon. This rhythm is diminished in sAD because of the increased basal level of cortisol (de Leon et al., 1988). Such chronical elevation of cortisol may disturb the negative feedback regulation of the HPA-axis and may lead to the brain insulin resistance and its affecting the neuronal Ca^{2+} homeostasis (Hoyer

and Frölich, 2006). It is reported that high circulating levels of glucocorticoide (GS) can affect cognition and reduce memory (McEwen, 2005), however the question of whether a chronically increased corticosterone level affects the cortex IR/insulin system has not been answered yet. Research regarding high glucocorticoide level and its connection to impaired cognitive function were mostly done on rat hippocampal tissue, reporting dendritic remodeling/atrophy (McEwen, 1999), up-regulated expression of both NR2A and NR2B subunit mRNA of glutamate NMDA receptors (Weiland et al., 1997) and down-regulation of $5HT_{1A}$ mRNA in the dentate gyrus (Meijer and De Kloet, 1998). Another interesting data was found after a bolus injection of cortisol in addition to cognitive decline and that was suppressed glucose reuptake in the temporal lobe (De Kloet et al., 1998). Other data showed that GCs inhibited glucose transport approximately by 15-30% in both primary and secondary hippocampal astrocyte cultures (Virgin et al., 1991). Cognitive decline found in humans treated with GS and in individuals with Chushing syndrome suggests that stress levels of GCs indeed affect cognition (De Kloet et al., 2005). Bearing in mind that when the activated insulin/IR signaling pathway increases the glucose reuptake in the brain and has a beneficial effect on learning and memory functions, one can come to the logical conclusion that the missing link between high GCs levels found in sAD and the cognitive decline is caused by the change of the brain insulin/IR system. One group of authors has recently found a down-regulation of hippocampal insulin pathway genes in aged, cognitively impaired rats suggesting that insulin/IR may be decreased as a cause of increased GS action as found in aging (Landfield et al., 1978).

In our experiments we observed significant reduction in the rats working and reference memory after 60 days of daily corticosterone injections. Paralelly in the placebo group (sesame oil) changes in rat locomotor performance were detected associated with elevated plasma levels of corticosterone, demonstrating that 60 day lasting placebo daily injection may also have some stress effect on the handled animals. Decreased expression of IR mRNA in the corticosterone treated rats was observed in comparison to the untreated control but also a significant decrease was observed in the placebo treated rats compared to controls suggesting that beside the chronical stress induced by daily corticosterone injection, acute stress due to the injection procedure and handling of the animals may have the same effect (Horner et al., 1991). The influence of acute stress induced by the injection procedure (i.e. acute daily increase in cortisol plasma level) has been seen in some of the measured parameters. Namely, a significant decrease in IR and Ins1 mRNA in corticosterone treated animals compared to untreated controls was accompanied by, in general, a similar decrease in these genes expression in placebo treated animals compared to untreated ones, although the statistical significance could not been reached due to the a high intra group variability. In the corticosterone treated rats tau mRNA expression was found to be up-regulated compared to untreated controls. In the placebo group tau mRNA stayed the same as in untreated controls suggesting that acute stress induced by injection do not affect tau expression. Our results are in line with the literature as in sAD both a decrease in IDE levels and an increase in peripheral glucocorticoid levels have been documented (de Leon et al., 1988; Zhao et al.,

2004). In nonhuman primates (Macaca nemestrina) exposure to the high-dose of glucocorticoid cortisol (hydrocortisone acetate) reduced both IDE protein levels in the inferior frontal cortex and IDE mRNA levels in the dentate gyrus of the hippocampus (Kulstad et al., 2005). In this study IDE mRNA expression was significantly decreased after corticosterone treatment compared to placebo control, but the decrease compared to untreated controls was not statistically significant. Placebo control and the untreated control group showed no difference in IDE mRNA expression suggesting that the acute stress of injection has no influence on IDE expression.

The data presented here indicate that prolonged elevation of corticosterone significantly disrupts hippocampal-sensitive behavior (working and reference memory) suggesting that cerebral energy deficits and control of gene expression might play a role. Additionally decreased expression of Ins1, IR, IDE mRNA and unchanged expression of Ins2 mRNA, was found in both STZ-icv and CTS treated rats showing similarity in those two models (Table 6). This indicates that increased levels of plasma corticosterone, often found in sAD patients, with time leads to changes in the brain insulin system and IR signaling pathway. On the other hand possible differences at the level of tau mRNA expression may indicate that obviously insulin is not the only factor involved in the regulation of its homeostasis. Also, results presented here goes in line with our hypothesis that changes found in brains of people with sAD may not be and are not the consequence of changes in Aβ pathology.

6 SUMMARY

This research was aimed to evaluate the time-course of changes in the brain insulin and some elements of the insulin receptor (IR) signalling cascade in the streptozotocin-intracerebroventricullarly (STZ-icv) treated rats representing experimental model of sporadic Alzheimer's disease (sAD) and to compare them with effects of chronically increased corticosterone on the brain insulin system.

This study shows down-regulation in mRNA expression of insulin, insulin receptor (IR), and insulin degrading enzyme (IDE) but no changes were observed in the expression of tau mRNA in hippocampus of STZ-icv treated rats. Comparing these results to the ones found in corticosterone treated rats similarities at the level of insulin, IR and IDE mRNA expression can be assumed. In contrast tau mRNA expression in corticosterone treated rats were increased, data which are in line with sAD. Behavioural deficits were found in both STZ-icv and corticosterone treated rats.

In conclusion, these results demonstrate that many of the characteristic features of sporadic Alzheimer's disease (sAD) can be produced experimentally by impairing the insulin/IR signaling pathway combined with a chronic increase of corticosterone. This supports our hypothesis that sAD represents a neuro-endocrine disorder associated with brain-specific disregulation in insulin and IR signaling, caused in part by increased level of corticosterone. In line with that our study puts a question on the classical amyloid β (Aβ) hypothesis, supporting

the view of brain insulin system dysfunction as a trigger for the Aβ pathology in an experimental sAD model.

Zusammenfassung

Ratten, die intrazerebroventricular (icv) mit Streptozotocin (STZ) behandelt werden, eignen sich gut als Tiermodelle für die sporadische Alzheimererkrankung (sAD). In der hier vorgelegten Arbeit wurden Veränderungen bezüglich der Insulinkonzentration sowie einiger Bestandteile der Insulinrezeptor (IR) – Signalkaskade in Rattengehirnen, welche icv mit STZ behandelt wurden, zu verschiedenen Zeitpunkten untersucht. Die Auswirkungen von STZ auf die zerebrale IR-Signalkaskade wurden dann mit denen von chronisch erhöhten Corticosteronkonzentrationen verglichen.

In dieser Studie wurde im Hippocampus eine verminderte mRNA-Expression von Insulin, der IR sowie des insulinabbauenden Enzyms (IDE) nachgewiesen; bezüglich der tau-mRNA-Expression konnten jedoch in diesem Gehirnareal der mit STZ behandelten Ratten keine Veränderungen beobachtet werden. Die Resultate der Insulin-, IR- und IDE-mRNA-Expression fielen bei den mit Corticosteron behandelten Ratten ähnlich aus Im Gegensatz hierzu nahm die tau-mRNA-Expression bei Ratten, die mit Corticosterone behandelt wurden, zu, was auch für eine sAD kennzeichnend ist. Sowohl bei den mit STZ als auch bei den mit Corticosteronen behandelten Ratten konnten Verhaltensanomalien beobachtet werden.

Die in dieser Arbeit erzielten Resultate deuten darauf hin, dass viele Merkmale einer sAD experimentell durch eine Beeinträchtigung des Insulin/IR-Signalwegs sowie eine chronische Erhöhung der Corticosteronkonzentration hervorgerufen

werden können. Dies untermauert wiederum unsere Hypothese, dass es sich bei sAD um eine neuroendokrine Störung handelt, die mit gehirnspezifischen Fehlfunktionen in der Insulin/IR-Signalkaskade einhergeht, welche zum Teil durch erhöhte Corticosteronkonzentrationen ausgelöst werden können. Auf Grund der in dieser Arbeit erzielten Resultate stellt sich die Frage, ob β-Amyloid (Aβ) ein Auslöser oder eine Konsequenz einer sAD darstellt. Die hier vorgelegte Arbeit last den Schlus zu, dass bei sAD-Tiermodellen ein Zusammenhang zwischen primären Fehlfunktionen im zerebralen Insulinsystem und dadwol sekundär ausgeloster Aβ-Pathologie besteht. Weiterfübende Untersuchungen wird aber notwendig um diese Aussagen zu bestätigen.

7 LITERATURE

Adamo, M., Raizada, M.K., LeRoith, D., 1989. Insulin and insulin like growth factor in the nervous system. Mol. Neurobiol. 3, 71-100.

Anger, W.K., 1991. Animal test systems to study behavioral dysfunctions of neurodegenrative disorders. Neurotoxicology 12, 403-413.

Apelt, J., Mehlhorn, G., Schliebs, R., 1999. Insulin-sensitive GLUT4 glucose transporters are colocalized with GLUT3-expressing cells and demonstrate a chemically distinct neuron-specific localization in rat brain. J. Neurosci. Res. 57, 693-705.

Banks, W.A., 2004. The source of cerebral insulin. Eur. J. Pharmacol. 490, 5-12.

Blennow, K., de Leon, M.J., Zetterberg, H., 2006. Alzheimer's disease: seminar. Lancet 368, 387-403.

Blokland, A., Jolles, J., 1993. Spatial learning deficit and reduced hippocampal ChAT activity in rats after an icv injection of streptozotocin. Pharmacol. Biochem. Behav. 44, 491-494.

Boutajangout, A., Boom, A., Leroy, K., Brion, J.P., 2004. Expression of tau mRNA and soluble tau isoforms in affected and non-affected brain areas in Alzheimer's disease. FEBS Lett. 576, 183-189.

Craft, S., Peskind, E., Schwartz, M.W., Schellenberg, G.D., Raskind, M., Porte, Jr D., 1998. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. Neurology 50, 164-168.

Coburn-Litvak, P.S., Pothakos, K., Tata, D.A., McCloskey, D.P., Anderson, B.J., 2003. Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. Neurobiol. Learn.Mem. 80, 11-23.

Cook, D.G., Leverenz, J.B., McMillan, P.J., Kulstadt, J.J., Ericksen, S., Roth R.A et al. 2003. Reduced hippocampal insulin degrading enzyme in late onset Alzheimer's disease is associated with a lipoprotein E-ε 4 allele. Am. J. Pathol. 162, 313-319.

De Haan, M., van Herck, H., Tolboom, J.B., Beynen, A.C., Remie, R., 2002. Endocrine stress response in jugular-vein cannulated rats upon multiple exposure to either diethyl-ether, halothane/O2/N2O or sham anaesthesia. Lab. Animal 36, 105-114.

De la Monte, S.M., Wands, J.R., 2005. Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. J. Alzheimer Dis. 7, 45-61.

De Leon, M.J., McRae, T., Tsai, J.R., George A.E, Marcus, D.L., Freedman M et al. 1988. Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy. Lancet 2, 391-392.

De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joëls, M., 1998. Brain corticosteroid receptor balance in healt and disease. Endo. Rev. 19, 269-301.

De Kloet, E.R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nature 6, 463-475.

De Quervain, D.J.F., Poiriet, R., Wollmer, M.A., Grimaldi, L.M.E., Tsolaki, M., Streffer,J.R., 2004. Glucocorticoid-related genetic susceptibility for Alzheimer's disease. Human Mol. Gen. 13, 47-52.

Devaskar, S.U., Giddings, S.J., Rajakumar, P.A., Carnaghi, L.R., Menon, R.K., Zahn, D.S., 1994. Insulin gene expression and insulin synthesis in mammalian neuronal cells. J. Biol. Chem. 269, 8445-8454.

Duckworth, W.C., 1988. Insulin degradation: mechanism, products, significance. Endocrin. Rev. 9, 319-345.

Duelli, R., Schröck, H., Kuschinsky, W., Hoyer, S., 1994. Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. Int. J. Develop. Neurosci. 12, 737-743.

Farris,W., Mansourian, S., Chang, Y., Lindsay, L., Eckman, E.A., Frosch, M.P. et al. 2003. Insulin-degrading enzyme regulates the levels of insulin, amyloid-β protein and the β-amyloid precursor protein intracellular domain in vivo. Proc. Natl. Acad. Sci. USA 100, 4162-4167.

Ferri, C.P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. 2005. Prevalence of dementia: a Delphi consensus study. Lancet 366, 2112-2117.

Frolich, L., Blum-Degan, H-G., Engelsberger, S., Humrich, J., Laufer, S., Muschner, D., et al. 1998. Brain insulin and insulin receptors in aging and Alzheimer′s disease. J. Neural. Transm. 105, 423-438.

Gasparini, L., Netzer, W.J., Greengard, P., Xu, H., 2002. Does insulin dysfunction play a role in Alzheimer′s disease? Trends Pharmacol. Sci. 23, 288-293.

Garland, P.B., Randle, P.J., 1964. Regulation of glucose uptake by muscles. 10. Effects of alloxan-diabetes, starvation, hypophysectomy and adrenalectomy, and of fatty acids, ketone bodies and pyruvate, on the glycerol output and concentrations of free fatty acids, long-chain fatty acyl-coenzyme A, glycerol phosphate and citrate-cycle intermediates in rat heart and diaphragm muscles. Biochem. J. 93, 678-687.

Gerozzissis, K., Rouch, C., Lemierre, S., Nikolaidis, S., Orosco, M., 2001. A potential role of central insulin in learning and memory related to feeding. Cell Mol. Neurobiol. 21, 389-401.

Gibson, G.E., Jope, R., Blass, J.P., 1975. Decreased synthesis of acetylcholine accompanying impaired oxidation of pyruvic acid in rat brain minces. Biochem. J. 148, 17-23.

Giorgino, F., Almahfouz, A., Goodyear, L.J., Smith, R.J., 1993. Glucocorticoid regulation of insulin receptor and substrate IRS-1 tyrosine phosphorylation in rat skeletal muscule in vivo. J. Clin. Invest. 91, 2020-2030.

Goldstein, B.J., 1993. Regulation of insulin receptor signaling by protein-tyrosine dephosphorylation. Receptor 3: 1-5.

Gong, C.X., Shaikh, S., Wang, J.Z., Zaidi, T., Grundke-Iqbal, I., Iqbal, K., 1995. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. J. Neurochem. 65, 732-738.

Green, K.N., Billings, L.M., Roozendaal, B., McGaugh, J.L., LaFerla, F.M., 2006. Glucocorticoids increase amyloid –β and tau pathology in a mouse model of Alzheimer's disease. J. Neurosci. 26, 9047-9056.

Haugher, R.L., Millam, M.A., Catt, K.J., Aguilera, G., 1987. Differential regulation of brain and pituitary corticotropinreleasing factor receptors by corticosterone. Endocrinology 120, 1527-1533.

Häring, H.U., Kirsch, D., Obermaier, B., Ermel, B., Machicao, F., 1986. Decreaset tyrosine kynase activity of insulin receptor isolated from rat adippocytes rendered insulin-resistance by catecholamine treatment in vitro. Biochem. J. 234, 59-66.

Henneberg, N., Hoyer, S., 1995. Desensitization of the neuronal insulin receptor: a new approach in the etiopathogenesis of late-onset sporadic dementia of the Alzheimer type (SDAT)? Arch. Gerontol. Geriatr. 21, 63-74.

Horner, C.H., O'Regan, M., and Arbuthnott, E., 1991. Neural plasticity of the hippocampal (CA1) pyramidal cell-quantitative changes in spine density following handling and injection for drug testing. J. Anat. 174, 229-238.

Hoyer, S., Prem, L., Sorbi, S., Amaducci, L., 1993. Stimulation of glycolitic key enzymes in cerebral cortex by insulin. Neuroreport 4, 991-993

Hoyer, S., 2002. The brain insulin signal transduction system and sporadic (type II) Alzheimer's disease: an update. J. Neural. Transm. 109, 341-360.

Hoyer S. 2004 Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. Eur. J. Pharmacol. 490, 115-125.

Hoyer, S., Frölich, L., 2006. Brain insulin function and insulin signal transduction in sporadic Alzheimer disease. In Research Progress in Alzheimer's Disease and Dementia, (Sun M.K., ed), chapter 11, Nova Science, New York.

Iqbal, K., Alonso, A.C., Chen, S., Chohan, M.O., El-Akkad, E., Gong, C.X., et al. 2005. Tau pathology in Alzheimer disease and other tauopathies. Biochim. Biophys. Acta. 1739, 198-210.

Johanstone, A.M., Pirola, L., Van Obberghen, E., 2003. Molecular mechanisms of insulin receptor substrate protein-mediate modulation of insulin signaling. FEBS Letters 546, 32-36.

Karuri, A.R., Engelking, L.R., and Kumar, M.S., 1998. Effects of halothane and methoxyflurane on the hypothalamic-pituitary-adrenal axis in rat. Brain Res. Bull. 47, 205-209.

Kaytor, M.D., Orr, H.T., 2002. The GSK3 beta signaling cascade and neurodegenerative disease. Curr. Opin. Neurobiol. 12, 275-278.

Kulstad, J.J., McMillan, P.J., Leverenz, J.B., Cook, D.G., Green, P.S., Peskind, E.R., et al. 2005. Effects of chronic glucocorticoid administration on insulin-degrading enzyme and amyloid-beta peptide in the aged macaque. J.Neuropathol. Exp. Neurol. 64,139-146.

Landfield, P.W., 1978. An endocrine hypothesis of brain aging and studies on brain-endocrine correlations and monosynaptic neurophysiology during aging. Adv. Exp. Med. Biol. 113, 179-199.

Landfield, P.W., Blalock, E.M., Chen, K.C., Porter, M.M., 2007. A new glucocorticoid hypothesis of brain aging: implications for Alzheimer's disease. Current Alzh. Res. 4, 205-212.

Lannert H., Hoyer, S., 1998. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behav. Neurosci. 112, 1199-1208.

Lester-Coll, N., Riviera, E.J., Soscia, S.J., Doiron, K., Wands. J.R., de la Monte, S.M., 2006. Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. J. Alzheimers Dis. 9, 13-33.

Lupein, S.J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T. et al. 2005. Stress hormones and human memory function across the lifespan. Psychneuroendocr. 30, 225-242.

.

Mann, H.J., Lin, J.W., Ju, W.H., Ahmadain, G., Liu, L., Becker, L., et al. 2000. Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependet receptor internalization. Neuron 25, 649-662.

Marks, J.L., Porte, D., Jr, Baskin, D.G., 1991. Localization of type I insulin-like growth factor receptor messenger RNA in the adult rat brain by in situ hybridization. Mol. Endocrinol. 5, 1158-1168.

Mayer, G., Nitsch, R., Hoyer, S., 1990. Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. Brain Res. 532, 95-100.

McEwen, B.S., 1999. Stress and hippocampal plasticity. Ann. Review. Neurosci. 22, 105-122.

McEwen, B.S., 2005. Glucocorticoids, depression and mood disorder: structural remodeling in the brain. Met. Clin. Exp. 54, 20-23.

Meijer, O.C., De Kloet, E.R., 1998. Corticosterone and serotonergic neurotransmission in the hippocampus: functional implications of central corticosteroid receptor diversity. Crit. Rev. Neurobiol. 12, 1-20.

Niswenderk, K.D., Baskin, D.G., Schwartz, M.W., 2004. Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. Trends. Endocrinol. Metab. 15, 362-369.

Nitsch, R., Hoyer, S., 1991. Local action of the diabetogenic drug streptozotocin on glucose and energy metabolism in rat brain cortex. Neurosci. Lett. 128, 199-202.

Noble, E.P., Wurtman, R.J., Axelrod, J., 1967. A simple and rapid method for injecting H3-norepinephrine into the lateral ventricle of the rat brain. Life Sci. 6, 281-291.

Orrosco, M., Gerozzissis, K., 2001. Macronutrient-inducet cascade of events leading to parallel changes in hypothalamic serotonin and insulin. Neurosci. Biobehav. Rev. 25, 167-174.

Park, C.R., 2001. Cognitive effects of insulin in the central nervous system. Neurosci. Biobehav. Rev. 25, 311-323.

Pei, J-J., Khatoon, S., An, W-L., Nordlinder, M., Tanaka, T., Braak, H., et al. 2003. Role of protein kinase B in Alzheimer ′s neurofibrillary pathology. Act. Neuropathol. 105, 381-392.

Perez, A., Morelli, L., Cresto, J.C., Castano, E.M., 2000. Degradation of soluble amyloid beta-peptides 1-40, 1-42, and a Dutch variant 1-40Q by insulin degrading enzyme from Alzheimer disease and control brains. Neurochem. Res. 25, 247-55.

Phiel, C.J., Wilson, C.A., Lee, V.M.-Y., Klein, P.S., 2003. GSK-3α regulates production of Alzheimer's disease amyloid-β peptides. Nature 423, 435-439.

Planel, E., Yasutake, K., Fujitas, S.C., Ishiguro, K., 2001. Inhibition of protein phosphatase 2A overrides tau protein kinase I/glycogen synthase kinase 3β and cyclin-dependent kinase 5 inhibition and results in tau hyperphosphorylation in the hippocampus of starved mouse. J. Biol. Chem. 276, 34298-34306.

Plaschke, K., Hoyer, S., 1993. Action of the diabetogenic drug streptozotocin on glycolytic and glycogenolytic metabolism in adult rat brain cortex and hippocampus. Int. J. Develop. Neurosci. 11, 477-483.

Plaschke, K., Yun, S.W., Martin, E., Hoyer, S., Bardenheuer, H.J., 1999. Interrelation between cerebral energy metabolism and behaviour in a rat model of permanent brain vessel occlusion. Brain Res. 830, 320-329.

Plaschke, K., Feindt, J., Djuric, Z., Heiland, S., Autschbach, F., Lewicka, S., et al. 2006. Chronic corticosterone-induced deterioration in rat behaviour is not paralleled by changes in hippocampal NF-kappaB-activation. Stress 9, 97-106.

Prickaerts, J., Fahring, T., Blokland, A., 1999. Cognitive performance and biochemical markers in septum, hippocampus and striatum of rats after an i.c.v. injection of streptozotocin: a correlation analysis. Behav. Brain Res. 102, 73-88.

Rocchi, A., Pellegrini, S., Siciliano, G., Murri, L., 2003. Causative and susceptibility genes for Alzheimer′s disease: a review. Brain Res. Bull. 61, 1-24.

Salkovic-Petrisic, M., Tribl, F., Schmidt, M., Hoyer ,S., Riederer, P., 2006. Alzheimer-like changes of protein kinase B and glycogen synthase kinase -3 in rat frontal cortex and hippocampus after the damage of insulin signaling pathway. J. Neurochem. 96, 1005-1015.

Sengupta, A., Grundke-Iqbal, I., Iqbal, K., 2006. Regulation of phosphorylation of tau by protein kinases in rat brain. Neurochem. Res. 31, 1473-1480.

Sharma, M., Gupta, Y.K., 2001. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sci. 68, 1021-1029.

Skeberdies, V.A., Lan, J., Zheng, X., Zukin, R.S., Bennett, M.V., 2001. Insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis. Proc. Natl. Acad. Sci. USA 98, 3561-3566.

Sontag, E., Luangpirom, A., Hladik, C., Mudrak, I., Ogris, E., Speciale, S., White C.L. 3rd., 2004. Altered expression levels of the protein phosphatase 2A ABalphaC enzyme are associated with Alzheimer disease pathology. J. Neuropathol. Exp. Neurol. 63, 287-301.

Starkman, M.N., Gebarski, S.S., Berent, S., Schteingart, D.E., 1992 Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol. Psychiatry 32, 756-765.

Steen, E., Terry, B.M., Rivera, E.J., Cannon, J.L., Neely, T.R., Tavares, R., Xu ,X., J., Wands, J.R., de la Monte, S.M., 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease – is this type 3 diabetes? J. Alzheimer Dis. 7, 63-80.

Stein-Behrens, B.A., Elliott, E.M., Miller, C.A., Schilling, J.W., Newcombe, R., and Sapolsky, R., M., 1992. Glucocorticoids exacerbate kainic acid-induced extracellular accumulation of excitatory amino acids in the rat hippocampus. J. Neurochem. 58, 1730-1735.

Svaren, J., Ehrig, T., Abdulkadir, S.A., Ehrengruber, M.U., Watson, M.A., Milbrandt, J., 2000. EGR 1 target genes in prostate carcinoma cell identified by microarray analysis. J. Biol. Chem. 275, 38524-38531.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. 50, 537-546.

Van der Staay, F.J., Van Nies, J., Raaijmakers, W., 1990. The effects of aging in rats on working and reference memory performance in a spatial holeboard discrimination task. Behav. Neural Biol. 53, 356-370.

Vecsei, P., 1979. Glucocorticoids: cortisol, cortisone, corticosterone, compound S, and their metabolites. In: Jaffe BM, Behrmann HR (Eds.) Methods of Hormone Radioimmunoassays. Academic Press: New York, 1979, pp 767-792.

Vollmayr, B., Faust, H., Lewicka, S., Henn, F.A., 2001. Brain-derived-neurotrophic-factor (BDNF) stress response in rats bred for learned helplessness. Mol. Psychiatry 6, 471-474.

Virgin, C.E.Jr., Ha, T.P., Packan, D.R., Tombaugh, G.C., Yang, S.H., Horner, H. C., Sapolsky RM., 1991.Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: implications for glucocorticoid neurotoxicity. J. Neurochem. 57, 1422-1428.

Watson, G.S., Craft, S., 2004. Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. Eur. J. Pharmacol. 490, 97-113.

Wei, L.T., Matsumoto, H., Rhoads, D.E., 1990. Release of immunoreactive insulin from rat brain aynaptosomes under depolarising conditions. J. Neurochem. 54, 1661-1665.

Wieland, N.G., Orchinik, M., Tanapat, K., 1997. Chronic corticosterone treatment induces parallel changes N-methyl-D- aspartate receptor subunit messenger RNA levels and antagonist binding sites in the hippocampus. Neuroscience 78, 653-662.

Qiu, W.Q., Folstein, M.F., 2006. Insulin, insulin degrading enzyme and amyloid-β peptide in Alzheimer's disease: review and hypothesis. Neurobiol. Aging 27, 190-198.

.

Zhao, W.-Q., Chen, H., Quon, M.J., Alkon, D.L., 2004. Insulin and the insulin receptor in experimental models of learning and memory. Eur. J. Pharmacol. 490, 71-81.

ACKNOWLEDGEMENT

I would like to thank Prof. Dr. Peter Riederer for giving me the opportunity to do my doctoral thesis in his laboratory. Also I thank Dr. Edna Grünblat for helping me to find my way in the laboratory.

To Prof. Dr. Melita Šalković-Petrišić I thank for giving me opportunity to come to Germany. She also gave me a big moral and professional help.

Prof. Dr. Zdravko Lacković I thank for promoting cooperation in research of joint PhD programs.

I thank Prof. Dr. J. Deckert and DAAD (Deutcher Akademischer Austausch Dienst) for financial help.

I am very grateful to Konstanza Plasche for giving me the tissue on which I have done the part of my doctoral thesis.

Also I am grateful to Gabi, Miryam and Božica for technical help, without them I would be lost.

Thanks to Ms. Gerlinde Kerscher for doing all those boring paper work for me.

At the end, thanks to my boyfriend who was very tolerant and full of understanding. Thank to my mother for being there for me when I needed her. Thanks to Carmen, Maria and Mario for making my stay in Germany easier and fun. Big Thanks to Lidija for moral support.

CURRICULUM VITAE

Name:		**JELENA OSMANOVIC**
Data and Place of Birth:		09. 09. 1979. Bjelovar, Croatia
Telephone:		*091/545-0098*
E-mail:		*josmanov@mef.hr*

Education:	1994-1998	Gymnasium
	1998-2004	School of Medicine, University of Zagreb, Croatia
	2005-	Postgraduate study "Biomedicine and health" School of Medicine, University of Zagreb
Internalship:	2004-2005	General Hospital „Sveti Duh“, Zagreb, Croatia
Employment:	2005	Department of Pharmacology, School of Medicine, University of Zagreb, Croatia
	2006-2007	I was working in the group of Prof. Riederer at the Poliklinik für Psychiatrie Psyhosomatik und Psychotherapie, Bayerische Julius-Maximilians Universität Würzburg from August 2006 until the October 2007.