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CENTRAL INSULIN RESISTANCE AS A TRIGGER FOR SPORADIC ALZHEIMER-LIKE PATHOLOGY: AN EXPERIMENTAL APPROACH

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Running title: Brain insulin system and experimental Alzheimer disease

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SUMMARY

A growing body of evidence implicates impairments in brain insulin signaling in early sporadic Alzheimer disease (sAD) pathology. However, the most widely accepted hypothesis for AD aetiology stipulates that pathological aggregations of the amyloid β (Aβ) peptide are the cause of all forms of Alzheimer's disease. Streptozotocin-intracerebroventricularly (STZ-icv) treated rats are proposed as a probable experimental model of sAD. The current work reviews evidence obtained from this model indicating that central STZ administration induces brain pathology and behavioural alterations resembling those in sAD patients. Recently, alterations of the brain insulin system resembling those in sAD have been found in the STZ-icv rat model and are associated with tau protein hyperphosphorylation and Aβ-like aggregations in meningeal vessels. In line with these findings the hypothesis has been proposed that insulin resistance in the brain might be the primary event which precedes the Aβ pathology in sAD.

INTRODUCTION

Although neuropathologically Alzheimer's disease (AD) is characterized by the accumulation of extracellular plaques, consisting primarily of a low molecular weight amyloid-β (Aβ) peptide, and intracellular neurofibrillary tangles of aggregated hyperphosphorylated tau protein, it is well documented that AD is not a single entity. Currently, the leading hypothesis assumes that pathological assemblies of Aβ are the cause of all forms of AD, whereas other neuropathological changes, including tau hyperphosphorylation, are downstream consequences of pathological Aβ accumulation (Hardy and Selkoe, 2002). However, the amyloid cascade hypothesis is consistent with only a very small proportion of all AD cases, that is those caused by missense mutations in three chromosomes (http://www.molgen.ua.ac.be/ADMutations/) leading to autosomal dominant familial AD with an early onset. In the great majority of AD patients disease is sporadic in origin (millions world-wide) with age and several susceptibility genes as risk factors, and is of late onset (Hoyer and Frölich, 2006). A growing body of evidence implicates impairments in the brain insulin signaling pathway in sAD pathology (Frölich et al, 1998; Hoyer, 1998; Hoyer and Frölich, 2006, de la Monte and Wands, 2005). Since insulin has been shown to affect both Aβ levels and tau hyperphosphorylation in the brain, this issue has recently emerged as a novel field of sAD ethiopathogenesis and therapy research (de la Monte et al., 2006; Hoyer, 2004). Due to the developmentally specific nature of sAD, its early stages being clinically unrecognisable, and brain analysis being possible only post mortem (frequently in only the severe late stage cases), brain neurochemistry that characterizes the initiation of this disease in humans is mostly unknown. Experimental models of sAD may provide clues to early brain changes in this disorder. This review presents the information gained to date in the experimental rat model of sAD which has paved the way to the new hypothesis, the implications of which may provide novel ethiopathogenic and therapeutic approaches in sAD research.

INSULIN IN THE BRAIN

Until the last three decades, the brain has not been thought of as an insulin-sensitive organ. The first evidence arguing against this hypothesis was the detection of immunoreactive insulin in dog cerebrospinal fluid (Margolis and Altszuler, 1967), suggesting that circulating insulin could cross the blood-brain barrier. The discoveries of insulin and insulin receptors (IR) in the brain that followed (Havrankova et al, 1978a,b) raised further questions about the origin of insulin in the brain, as well as physiological and pathophysiological role(s) of insulin and IR in this organ. An extensive review of the current knowledge of insulin and IR and their roles in the brain has been published previously (Hoyer and Frölich, 2006), and will be presented briefly here for the purpose of comparison with the data from human and experimental models of sAD.

It is a common belief that in the mature adult brain the majority of insulin originates from the periphery; that is it is transported from the circulation after secretion from the pancreatic β-cells. The transport of insulin across the blood brain barrier (BBB) is mediated via a saturable transport mechanism for which regional specificity in transporter distribution and kinetics has been reported (Banks, 2004). However, evidence has emerged that a smaller proportion of insulin is produced within the brain itself (Wozniak et al., 1993). Insulin gene expression and insulin synthesis have been demonstrated in both immature and mature mammalian neuronal cells (Schechter et al. 1992; Schechter et al.

1996; Schechter and Abboud 2001). In humans and in the chicken only one insulin gene is present, whereas in mice and rats insulin is produced by two independent genes that code for proinsulin I and II, both of which are localized to chromosome 1 (Todd et al. 1985). Insulin-1 and -2 mRNA were found to be distributed in a highly specific pattern with the highest density in the pyramidal cells of the hippocampus and high densities in medial prefrontal cortex, the entorhinal and perirhinal cortices, the thalamus and the granule layer of the olfactory bulb, as well as the hypothalamus (Devaskar et al., 1994; Grünblatt et al., 2006). Neither insulin mRNA nor synthesis of the hormone were observed in glial cells (Devaskar et al. 1994). The release of insulin from brain synaptosomes is stimulated by glucose (Santos et al. 1999).

Insulin signaling in the brain

Insulin in the brain binds to IRs which are abundantly but selectively distributed. Rodent studies have shown that the highest concentration of IRs is found in the nerve terminals of key brain regions, such as the olfactory bulb, hypothalamus, cerebral cortex, cerebellum and hippocampus (van Houten et al, 1979; van Houten et al, 1980; Unger et al, 1989; Abbott et al, 1999). IR mRNA is abundantly present in neuronal somata (Schwartz et al, 1992). The neuronal IR binds insulin in a highly specific and rapid manner (Raizada et al, 1988). It has been hypothesized that the differing distribution patterns of insulin-1 and IRs in the brain may suggest that IRs in different brain regions may use insulin from different sources, either peripherally or locally synthesized, for cell-to-cell communication and neuronal signal transduction (Zhao et al. 2004). The IR is a tetramer composed of two extracellular α-subunits and two intracellular β-subunits. The neuronal (brain) IR differs from the peripheral IR in that both the α and β subunits have a slightly lower molecular weight, and the neuronal IR is not down-regulated by insulin, which otherwise activates a similar signalling cascade (Adamo et al, 1989; Heidenreich et al, 1983). Binding of insulin to the IR α-subunit induces autophosphorylation of the β-subunit by phosphorylation of its intrinsic tyrosine residues 1158, 1162 and 1163, thus triggering tyrosine kinase activity (Fig. 1A) (Combettes-Souverain and Issad, 1998). The location of phosphotyrosine-containing proteins corresponds to IR distribution (Moss et al, 1990). The receptor's activation state is regulated by its phosphorylation state. Deactivation may be induced by the action of both phosphotyrosine phosphatase causing dephosphorylation of the β subunit (Goldstein, 1993) and by serine or threonine kinases causing phosphorylation at serine residues 1305 and 1306, and threonine residue 1348 respectively (Häring, 1991; Avruch, 1998). Insulin binding to the IR activates two parallel functional signal transduction cascades; one acting through the phosphatidylinositol-3 kinase (PI3K) pathway, and the other acting through the mitogen activated protein kinase (MAPK) pathway (Johnston et al., 2003). The former will be discussed later in the text. Briefly, tyrosine phosphorylation of IR β-subunits induces specific recruitment of proteins containing particular domains (SH2, PTB, etc.), amongst the most prominent of which are the proteins from the insulin receptor substrate family (IRS). It has been shown that IRS1 and the IR are co-expressed in particular brain regions, including the hippocampus (Baskin et al, 1994). Upon IR activation, the IRS becomes phosphorylated on tyrosine residues and capable of recruiting various specific (e.g. SH2) domain-containing signalling molecules; among them PI3K which becomes phosphorylated and consequently activated (Johnston et al., 2003). The activation of the PI3K pathway, in turn activates protein kinase B (Akt/PKB) (Fig. 1). The activated Akt/PKB triggers glucose transporter (such as GLUT4) translocation and consequently increases cellular glucose uptake (Johnston et al., 2003; Vannucci et al., 1998). Akt/PKB also phosphorylates (at the serine 9 residue) and consequently inactivates

both α and β cytosolic forms of glycogen synthase kinase-3 (GSK-3) (Cross et al., 1995). GSK-3 plays a key role in numerous cell functions, but only those that may be involved in sAD pathology will be briefly mentioned here. GSK-3α regulates the production of Aß peptides, the amyloid precursor protein (APP) derivatives (Phiel et al, 2003). The promotion of APP secretion from the intracellular to the extracellular space and the inhibition of its degradation by insulin-degrading enzyme is mediated by insulin and the tyrosine kinase activity of the IR (Gasparini et al, 2001). Furthermore, insulin signaling via activation of PI3K regulates APP release into the extracellular space (Solano et al, 2000). GSK-3β isoform is involved in tau-protein phosphorylation (Ishiguro et al., 1993). Tau-proteins belong to a family of microtubule-associated proteins that stimulate the generation and stabilization of microtubules within cells, and control axonal transport of vesicles (Stamer et al., 2002). Accumulation of hyperphosphorylated tau protein leads to the formation of neurofibrillary tangles. The phosphorylation and dephosphorylation of the tau protein is regulated by several protein kinases, including GSK-3β, and by several protein phosphatases, including PTP-1, -2A, -2B (Ishiguro et al, 1992; 1993). Prolonged exposure to insulin has been shown to induce down-regulation of glycogen synthase kinase-3ß activity and, thus, decreased phosphorylation of tau-protein (Cross et al, 1997; Hong and Lee, 1997).

Insulin's role in learning and memory

Evidence has been provided that brain insulin and the IR are functionally linked to improved cognition, in particular general and spatial memory, by up-regulation of insulin mRNA in the hippocampus and increased accumulation of the IR in hippocampal synaptic membranes (Zhao et al, 1999; for review Park, 2001; Zhao et al, 2004). Recent i*n vivo* evidence has demonstrated that the effect of intrahippocampal microinjection of insulin on spatial learning and memory in rats is dose-dependent, that is cognitive function is impaired with low insulin doses, unchanged with intermediate doses, and improved with high insulin doses (Moosavi et al, 2006). Although the exact mechanism(s) by which insulin could affect learning and memory is unclear, several pathways have been suggested, for example those related to glucose metabolism and the modulation of neurotransmission by different neurotransmitters. The overlapping distributions of insulin, the IR and the insulin-sensitive glucose transporter (GLUT) isoforms support the hypothesis of insulin-stimulated glucose uptake in selective brain regions, the hippocampus in particular (Apelt et al, 1999; McEwen and Reagan, 2004). Since hippocampal glucoregulatory activities contribute to cognitive function (Reagan, 2002), insulin modulation of glucose metabolism in this structure appears to be one of the key components of hippocampal vulnerability. Additionally, insulin is likely to modulate memory via other molecular events, such as increasing the probability of inducing long-term amplification, a molecular model of learning, by promoting N-methyl-D-aspartate receptor conductance (Wang and Salter, 1994), as reviewed elsewhere (van der Heide et al, 2006). Insulin may also modulate cognitive functions via its effects on neurotransmission, e.g. low doses of insulin can reverse the amnestic effects of cholinergic blockade (Blanchard and Duncan, 1997), and high levels of insulin reduce neuronal norepinephrine reuptake (Figlewicz et al, 1993). Thus the data suggests that normal insulin and IR signaling is a prerequisite for normal learning and memory function.

AN EXPERIMENTAL RAT MODEL OF sAD: STREPTOZOTOCIN-INTRACEREBROVENTRICULARLY TREATED RATS

Given the complex nature of AD, it is difficult to establish an experimental animal model that would faithfully mimic the developmental pathology of this disease in humans. Frequently exploited are *transgenic Tg2576 mice* that over express the Swedish mutation of the human APP and demonstrate a progressive, age-related cortical and hippocampal deposition of Aβ plaques (Hsiao et al, 1996). Transgenic Tg2576 mice however, represent a model of AD induced by gene manipulation, and therefore, are unlikely to be representative of the sporadic type of this disease. Given the presence of the IR and insulin, as well as the possibility of its synthesis in the brain, and of disturbed insulin signal transduction in human sAD (Hoyer and Frölich, 2006), an experimental rat model was developed by using the drug streptozotocin (STZ).

STZ treatment

STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)) is a betacytotoxic drug which, following peripheral (parenteral) administration at high doses, selectively destroys insulin producing/secreting β cells in the pancreas, and causes type I diabetes mellitus in adult animals (Szkudelski, 2001). Type II diabetes can also be induced in rats by parenteral injections of STZ on the day of birth, resulting in a mild basal hyperglycemia, an impaired response to the glucose tolerance test, and a loss of β GSK-3 plays a key role in numerous cell functions, but only those that may be involved in cell sensitivity to glucose, 10 weeks post-injection (Szkudelski, 2001). Treatment with low to moderate doses of STZ in short-term experiments causes insulin resistance (Blondel and Portha 1989) via a decrease in autophosphorylation (Kadowaki et al. 1984) and an increase in total number of IRs, but with little change in phosphorylated IR-β subunit (Giorgino et al. 1992), and maintained insulin-immunoreactive cells in the pancreas generating a transient diabetes mellitus (Rajab et al. 1989; Ar'Rajab and Ahren 1993). Considering the presence of insulin (from both periphery and brain) and IRs in the brain, an experimental rat model was developed by using STZ applied intracerebroventricularly (icv) in doses of up to 100 times lower (per kg body weight /b.w./) than those used peripherally to induce an insulin resistant brain state (Nitsch and Hoyer, 1991; Duelli et al, 1994; Lannert and Hoyer, 1998). Central STZ administration caused neither systemic metabolic changes nor diabetes mellitus. In the past 17 years, since the first literature report of central STZ application, STZ has been administrated mostly in doses ranging from 1-3 mg/kg b.w., injected 1-3 times, either uni- or bi-laterally into the lateral cerebral ventricles (Table 1). Identical biochemical changes were found in the left and right striatum after administration of STZ to the right lateral cerebral ventricle only (Salkovic et al., 1995), suggesting that STZ-icv induced effects are not related to the direct non-specific toxic effect of STZ at the site of drug administration and that differing effects following uni- or bi-lateral application of STZ are not to be expected. In some of the experiments however, a wide variation in the susceptibility of individual animals has been demonstrated as a characteristic feature of STZ-icv treatment (Blokland and Joles, 1993; 1994; Prickaerts et al., 2000). Most experiments with STZ-icv applications used Wistar rats, with only a few utilising the Sprague-Dawley (Shoham et al., 2006) or Lewis strains (Prickaerts et al., 2000). Male animals were used in all experiments. Recently, bilateral intra-cortical administration of low STZ doses (40 μg/kg) to three-day-old rat pups has been reported (Lester-Coll et al., 2006; de la Monte et al., 2006).

STZ mechanism of action

The mechanism of central STZ action and its target cells/molecules have not yet been clarified but a similar mechanism of action to that in the periphery has been recently

suggested. In the periphery, STZ selective β cell toxicity results from the drug's chemical structure which allows it to enter the cell via the GLUT2 glucose transporter. The predominant site of GLUT2 localization is the pancreatic β cell membrane (Szkudelski, 2001). *In vitro* studies have also demonstrated that GLUT2 itself is a key target molecule for STZ as the drug reduces GLUT2 protein expression in a concentration-dependent manner (Gai et al, 2004). GLUT2 may also be responsible for the STZ induced effects in the brain as GLUT2 also is reported to have regional specific distribution in the mammalian brain (Brant et al, 1993; Leloup et al, 1994; Ngarmukos et al, 2001; Arluison et al, 2004a; Arluison et al, 2004b). The neuronal localization of GLUT2 is relatively similar to that of glucokinase (GLUT2 coupled with glucokinase participate in the glucose sensing mechanism of β-cells), supporting the hypothesis that GLUT2 is expressed by brain neurons involved in glucose sensing (Arluison et al, 2004a; Arluison et al, 2004b). However, since GLUT2 localization in the brain does not entirely parallel that of glucokinase at the quantitative level (Li et al. 2003), participation of GLUT2 in functions other than glucose sensing in the brain has been suggested (Arluison et al. 2004b; Arluison et al. 2004a). Following peripheral administration STZ causes alkylation of β-cell DNA which triggers activation of poly ADP-ribosylation, leading to depletion of cellular NAD+ and ATP (Szkudelski, 2001). Decreased levels of ATP have also been reported following STZ-icv treatment (Nitsch and Hoyer 1991; Lannert and Hoyer 1998). The chemical structure of STZ also suggests this compound may produce intracellular free radicals, nitric oxide (NO) and hydrogen peroxide (Szkudelski, 2001); indeed evidence of increased oxidative stress has been found in the brain of STZ-icv treated rats (Table 1). Possible STZ effects on insulin producing/secreting and insulin sensitive cells within the brain will be discussed later in the text. The exact intracellular effects stimulated by icv administration of STZ are likely to be elucidated only following identification of the brain cells targeted by this compound.

NEUROCHEMICAL, STRUCTURAL AND BEHAVIORAL CHANGES IN THE STZ-ICV RAT MODEL

Glucose/energy metabolism

Glucose is the principal source of energy production in the brain, and undisturbed glucose metabolism is critical for normal functioning of this organ. Brain glucose, and its metabolism, has been investigated from 3 weeks following STZ-icv administration (Table 1), where concentrations of glucose and ADP, as well as glycogen levels, were increased in the cerebral cortex (Nitsch and Hoyer 1991), and glucose utilization was significantly decreased (44%) (Pathan et al, 2006). Further, 6 weeks following STZ-icv treatment, reduced glucose utilization (up to 30%) was found in 17 of 35 brain areas, particularly the frontal, parietal, sensory motor, auditory and entorhinal cortex and in all hippocampal subfields (Duelli et al. 1994). In addition, significant decreases in activities of glycolytic key enzymes were found in the brain cortex and hippocampus 3 and 6 weeks post- STZ-icv administration (Plaschke and Hoyer 1993) resulting in diminished concentrations of the energy-rich compounds ATP and creatine phosphate (Nitsch and Hoyer 1991; Lannert and Hoyer 1998). This fall in cerebral ATP, GTP and creatine phosphate levels was significantly improved by 40-day subcutaneous treatment with estradiol or intraperitoneal injection with the antioxidant coenzyme Q10, in parallel with the initial administration of STZ (Lannert et al., 1998; Ishrat et la., 2006). Decreased glucose utilization in hippocampal and cortical tissue was significantly, and dose-dependently, increased by 2-week long oral treatment with the peroxisome proliferator activated γ receptor (PPARγ)

agonist, pioglitazone, applied from 5 days before to 9 days after the STZ-icv treatment (Pathan et al., 2006). PPARγ agonists are approved as oral hypoglycemic agents used in the treatment of insulin resistance in type 2 diabetes, but have also demonstrated some neuroprotective effects (Santos et al., 2005; Bordet et al., 2006).

Cholinergic transmission

Investigations of cholinergic transmission in STZ-icv treated rats are important as abnormalities in the central cholinergic system affect learning and memory (Spencer and Lal, 1983). A decrease in choline acetyltransferase (ChAT) activity has been consistently found in the hippocampus of STZ-icv treated rats as early as 1 week following drug treatment and is still present 3 weeks post-injection (Hellweg et al, 1992; Blokland and Jolles, 1993; Blokland and Jolles, 1994; Prickaerts et al, 1999; Terwel et al, 1995) (Table 1). This is followed by a significant increase in acetylcholinesterase (AChE) activity (Sokusare et al, 2005; Ishrat et al, 2006). A decrease in hippocampal ChAT activity was completely prevented by 2-weeks of orally administered acetyl-L-carnitine, which acts by enhancing the utilization of alternative energy sources (Prickaerts et al., 1995; Terwel et al., 1995). Chronic administration of cholinesterase inhibitor drugs reduced AChE activity in a dose dependent manner, in STZ-icv treated rats regardless of whether treatment began 1 week prior to, in parallel or 13 days after STZ-icv administration (Sonkusare et al., 2005; Shoham et al, 2006). AChE activity was inhibited in the cortex but not in the hippocampus (Shoham et al, 2006), and concomitant administration with the calcium channel blocker, lercanidipine, potientated a decrease in AChE activity (Sonkusare et al., 2005). Changes in both ChAT and ACheE in the hippocampus were prevented by chronic intraperitoneal treatment with the antioxidant coenzyme Q10 (Ishrat et al., 2006). Intra-cortical administration of STZ to rat pups was followed by reduced expression of ChAT and increased expression of the AChE gene, a response which could not be prevented by any of the three subtypes of PPAR agonists (Lester-Coll et al., 2006; de la Monte et al., 2006).

Oxidative stress

Evidence of increased oxidative stress has been found in whole brain homogenates in particular brain regions of STZ-icv treated rats (Table 1). Estimations of oxidative stress induced by STZ-icv treatment commonly ultilise the measurement of malonaldehyde levels (MDA), a product of lipid peroxidation used as an indicator of free radical generation, and glutathione levels, an endogenous antioxidant that scavenges free radicals and protects against oxidative stress, or immunohistochemically for nitrative stress. Significant elevations of MDA levels and decreased glutathione levels have been found in the brain of STZ-icv treated rats (Sharma and Gupta, 2001a; Sharma and Gupta, 2002; Ishrat et al, 2006; Pathan et al, 2006). A progressive trend towards oxidative stress has been found 1, 7 and 21 days following STZ-icv administration (twice 3mg/kg injections) to 4 month old rats (Sharma and Gupta, 2001a). Oxidative stress was found also in the brain of 1 year old rats, 3 weeks following a lower single STZ-icv dose of 1.5 mg/kg (Ishrat et al., 2006). STZ-icv generates NO (Szkudelski, 2001), and oxidative-nitrative stress was found 1 and 8 weeks following a single 3mg/kg STZ-icv dose (Shoham et al., 2006). Nevertheless it appears that generation of NO by NO synthase is not involved since inhibition of this enzyme has not prevented STZ-induced responses (Prickaerts et al., 2000). Chronic treatment with the antioxidant coenzyme Q10 starting from the day of STZ-icv treatment significantly reduced all parameters of oxidative stress (Ishrat et al., 2006). A similar effect has been reported for other antioxidants (Sharma and Gupta, 2001a; 2002).

Morphology

STZ-icv administration has been associated with certain brain morphological changes in the brain as early as 1 week following a single drug dose (Shoham et al., 2006), and in both ≥1 year and 4 month old rats (Terwel et al., 1995; Prickaerts et al., 2000; Shoham et al., 2003; 2006) (Table 1). Glial fibrillary acidic protein (GFAP), a marker of astrogliosis, a stereotypic reaction of astrocytes to neuronal damage (Prickaerts et al., 1999), has been found to be increased in both brain homogenates and tissue sections (Prickaerts et al., 1999; 2000). Increased GFAP immunocytochemical staining was mainly located in peri- and paraventricular regions including the septum, fornix and fimbria, striatum and hippocampus, suggesting that altered hippocampal function could result from an impaired innervation and direct damage to this region (Prickaerts et al., 2000; Shoham et al., 2003). Inflammatory processes and myelin and axonal neurotoxicity has been reported following STZ-icv treatment. Severely affected STZ-icv treated rats had not only astrogliosis, but also extensive cell loss, inferred from the increase in the volume of the ventricular system (Prickaerts et al., 2000; Shoham et al., 2003). Interestingly, one week after a single STZ-icv 3mg/kg dose, no change in the number or morphology of cholinergic neurons was detected in the basal forebrain nuclei, medial septum, diagonal band or the nucleus basalis magnocellularis and there was no change in the density of cholinergic terminals in the hippocampus (Shoham et al., 2006). Not withstanding, AChE activity was increased, a phenomenon the authors explained as a reduction in synaptic function associated with increased GFAP expression in activated astrocytes. Astrogliosis was prevented by a chronic treatment with ladostigil, a cholinesterase and monoamine oxidase-B inhibitor with neuroprotective effects (Shoham et al., 2006). At the ultrastructural level, 3 weeks following STZ-icv administration a significant enlargement of the trans-Golgi segment in the rat cerebral cortex was found, which did not resemble the Golgi atrophy found in the brain of sAD patients, but the authors suggested that considering the proamyloidogenic processing of beta-amyloid precursor protein may occur preferentially in the trans-Golgi segment, the observed early response of neuronal ultrastructure to desensitisation of the IR may predispose cells to form Aβ-amyloid deposits (Grieb et al., 2004). Differences in morphological changes in the brain in human sAD and the STZ-icv rat model could be related to direct STZ actions.

Reduced expression of neuronal- and oligodendroglia-specific genes and increased expression of genes encoding GFAP and microglia-specific proteins were also found in rat pups administered with STZ intra-cortically (STZ-ic) (Lester-Coll et al., 2006). Some authors have suggested the hypoplasia and degeneration of the cerebellum found in STZ-ic treated rat pups, unlike findings in AD, are related to the early postnatal development of the cerebellum in rodents. PPAR agonists produced receptor subtype- and region-dependent positive therapeutic effects in STZ-ic treated pups with PPAR-δ agonists being the most effective (PPAR δ>α>γ subtype effectiveness) in preservation of hippocampal and temporal lobes (de la Monte et al., 2006).

Learning and memory

STZ-icv treated rats consistently demonstrate deficits in learning, memory, and cognitive behaviour (Table 1). Cognitive deficits are long-term and progressive, observed as early as 2 weeks after STZ-icv administration and are maintained up to 12 weeks post treatment (Lannert and Hoyer, 1998; Salkovic et al., 2006, Shoham et al., 2006; Grünblatt et al,

2006). They are found regardless of age in both 1-2 year and 3-month old rats, and also after either a single 1 or 3mg/kg injection or multiple 1mg/kg STZ-icv injections (Mayer et al, 1990; Pathan et al, 2006; Lannert and Hoyer, 1998; Weinstock and Shoham, 2004; Salkovic-Petrisic et al., 2006, Shoham et al., 2006; Grünblatt et al, 2006), although some STZ-icv dose-dependency has been suggested with lower STZ doses inducing less severe cognitive deficits (Blokland and Joles, 1994; Prickaerts et al., 2000; Grünblatt et al, 2006). The correlation between spatial discrimination performance in the Morris task and the decrease in hippocampal ChAT activity which resembles the relationship between cognitive and biochemical cholinergic changes observed in AD has been found in STZ-icv treated rats (Blokland and Jolles, 1993; Blokland and Jolles, 1994). However, results of the effectiveness of chronic acetyl-L-carnitine treatment in the prevention of hippocampal ChAT activity and abolishing memory deficits in the Morris water maze swimming (MWM) test, are inconsistent (Terwel et al., 1995) (Prickaerts et al., 1995). Interestingly, it has also been demonstrated that STZ-icv induced development of reactive gliosis and oxidative stress 1 week post-treatment, preceded the induction of memory deficits at 3 weeks post-treatment (Shoham et al, 2006, Sharma and Gupta, 2001a), where no signs of neuronal damage or any reduction in specific cholinergic markers were detected in the cortex or hippocampus (Shoham et al., 2006).

Although the exact mechanism by which STZ-icv treatment damages cognitive function remains unknown, all changes discussed above; energy deficits, reduced activity of choline acetyltransferase (cholinergic deafferentiation), induction of oxidative stress and direct neurotoxic damage found in the fornix, anterior hippocampus and periventricular structures, may form the biological basis for the marked reduction in learning and memory capacities. Concordingly, memory deficits were reported to be prevented by chronic treatment with several types of drugs with differing mechanisms of action (as reviewed by Weinstock and Shoham, 2004); (I) drugs generating alternative energy sources such as acetyl-L-carnitine (Prickaerts et al., 1995); (II) cholinesterase inhibitors such as donepezil and ladostigil (possessing also monoamine oxidase B inhibition and neuroprotective activity which also prevent gliosis and oxidative stress (Sonkusare et al., 2005; Shoham et al., 2006); (III) estradiol which prevents reduction in cerebral ATP (Lannert et al., 1998); (IV) antioxidants such as melatonin, resveratrol and coenzyme Q10 which prevent an increase in free radical generation (Sharma and Gupta, 2001b; 2002; Ishrat et al., 2006). Treatment with the NO synthase inhibitor L-NAME had no protective effect on cognitive deficits in STZ-icv treated rats (Prickaerts et al., 2000).

BRAIN INSULIN AND THE IR SIGNALING CASCADE IN STZ-ICV RAT

Although alterations of the brain insulin system are the focus of human sAD research (Hoyer and Frölich, 2006), investigations in experimental models of this neurodegenerative disorder are rare, particularly those exploiting central STZ administration (Table 2 and 3). We have previously reported changes in the brain insulin and tau/Aβ system following the bilateral application of a single or multiple 1mg/kg STZ dose into the lateral cerebral ventricles of adult ≥3 month old rats (Salkovic-Petrisic et al., 2006; Grünblatt et al., 2004, Grünblatt et la., 2006) and the group of de la Monte et al. (Lester-Coll et al., 2006, de la Monte et al., 2006) has reported changes in the brain insulin system following bilateral intra-cerebral 40μg/kg STZ dose to three-day-old rat pups. Since only the abstract of a Chinese paper is available regarding brain immunohistochemical analysis of tau protein and Aβ expression, 3 weeks following STZ-icv treatment (Chu and Quian, 1995), there is little data for a reliable interpretation of their findings.

In STZ-icv treated adult rats region-specific alterations of the brain insulin system (including insulin, the IR and downstream IR signaling cascade) were identified, and these changes are progressive beyond the STZ-icv treatment period (Table 2) (Salkovic-Petrisic et al., 2006; Grünblatt et al., 2006). A decrease in the expression of the insulin gene 1 and 2, as well as the IR gene, was identified in the hippocampus and frontoparietal cortex 12 weeks following drug treatment. The IRβ protein was decreased in the frontoparietal cortex and hypothalamus, but the levels of phosphorylated IRβ (p-IRβ) were increased and tyrosine kinase activity was unchanged in these regions, whereas in the hippocampus IRβ protein levels were decreased, but p-IRβ levels, as well as tyrosine kinase activity, were increased. Downstream from the PI3-K signaling pathway, hippocampal Akt/PKB remained unchanged at 4 weeks and decreased by 12 weeks post-treatment, whereas in the frontoparietal cortex Akt/PKB expression was decreased 4 weeks and increased by 12 weeks post STZ-icv treatment. Total GSK-3 levels were unchanged whereas the p-GSK-3/GSK-3 ratio in the hippocampus was decreased 12 weeks following STZ-icv treatments, suggesting a change in GSK-3 activity. In line with this finding, as a downstream target of the IR signaling cascade, increase in the expression of tau protein and the p-tau/tau ratio were found in the hippocampus 4 and 12 weeks following STZ-icv treatment, respectively, and Aβ-like aggregates were absent 4 weeks following drug treatment, but were found in leptomeningeal capillaries 12 weeks post STZ-icv treatment (Table 3).

Although the investigated parameters in the brain and the direction of changes identified following the intra-cortical (ic) application of STZ (decreased mRNA expression of insulin, the IR and IGF-1R, decreased pGSK-3/GSK-3 ratio) are similar to those induced by STZ-icv administration (Table 2 and 3) (Lester-Coll et al., 2006; de la Monte et al., 2006), the results are not quite comparable for several reasons; (I) Intra-cortical administration of STZ in the rat pups could be expected to induce more non-specific localized tissue damage than administration of STZ into the cavity of the lateral cerebral ventricles of adult rats. (II) Sensitivity of brain neurons to STZ toxic effects could be expected to differ in the three-day-old pups and adult or old rats, at least due to differences in the DNA excision repair processes known to be important for STZ intracellular toxicity (Szkudelski, 2001). (III) Strain differences in the susceptibility to STZ-induced diabetes have been reported (Rodrigues et al., 1997), and two different rat strains were used in icv treatment of adult/old rats (Wistar strain, most frequently used in other STZ-icv treatment experiments) and ic treatment of rat pups (the Long Evans strain is generally used for the investigation of retinal complications of diabetes (Puro, 2002). (IV) Brain regions investigated were not completely comparable; frontoparietal cortex, hippocampus and hypothalamus in STZ-icv, and temporal lobe, cerebellum and hypothalamus in STZ-ic treatment. The region-specific STZ-induced changes in the brain, including the difference in changes observed in the hippocampus in comparison with the cerebral cortex (Salkovic-Petrisic et al., 2006), could be masked in STZ-ic treated pups where temporal lobe (including hippocampal formation) was biochemically analysed. (V) Time dependency of changes could not be concluded from experiments with STZ-ic treated rat pups as these were measured at only a single post-treatment period of 4 weeks (de la Monte et al., 2006) or three post-treatment periods were investigated (7, 14, and 21 days) but results were reported only for the 14-day period (Lester-Coll et al., 2006). Some changes were not observed at 4 weeks but appeared 12 weeks following STZ-icv treatment, like those of tau protein and Aβ (Salkovic-Petrisic et al., 2006), but were already present two or four weeks after STZ-ic treatment (Lester-Coll et al., 2006; de la Monte et al., 2006) (Table 3). Some additional parameters of tau protein and the Aβ system (tau and APP mRNA expression)

were investigated in STZ-ic treated pups with a discrepancy between significantly decreased tau mRNA and unchanged tau protein expression observed in the hypothalamus (de la Monte et al., 2006).

Modest data has been reported regarding the therapeutic effects of drugs on brain insulin system alterations induced by central STZ application, i.e. only the effects of PPAR agonists in STZ-ic treated rat pups were investigated (de la Monte et al., 2006). These drugs demonstrated some therapeutic effects with respect to the insulin/IR signaling cascade dysfunction, but it is import to note that the effects were region- and PPAR subtype-specific. Not all PPAR-subtype agonists showed positive effects on STZ-induced damage and some of the alterations (insulin mRNA and p-GSK-3/GSK-3 ratio) did not respond to any of the PPAR-subtype agonists used (Tables 2 and 3). Although the PPAR-δ subtype agonists were the most effective (this subtype was found to be most abundant both in rat and human brain) (de la Monte et al., 2006), they were incapable of normalizing the changes to APP mRNA in the temporal lobe in contrast to the PPAR-α and –γ subtype agonists. Also, it has to be kept in mind that the positive therapeutic effects of PPAR agonists in STZ-ic treated pups on IR dysfunction (as well as on cholinergic transmission and neuronal damage), were obtained 4 weeks after a single intraperitoneal injection given on the same day as STZ. Evidence for the entry of these PPAR agonist substances through the blood brain barrier, which is of vital importance for their effects within the brain after only a single intraperitoneal injection, has not been provided by authors. Further experimentation is needed to clarify the meaning of these findings in respect to the mechanism of PPAR agonists since the positive therapeutic effects of treatment with the chronic PPAR-γ agonist rosiglitazone (which probably does not pass the blood brain barrier in a significant amount) (Pedersen and Flynn, 2004) on cognitive deficits in both humans with sAD and transgenic Tg2576 Alzheimer mice, are suggested to be related to an improvement of peripheral insulin resistance which positively affects cognition (Watson et al., 2005; Pedersen et al., 2006; Landreth, 2006). Such a mechanism does not seem likely in 3 day-old rat pups which do not develop peripheral insulin resistance since decreased blood glucose levels were found 14 days following the STZ-ic administration (Lester-Coll et al., 2006).

INSULIN RESISTANT BRAIN STATE AS A PROBABLE TRIGGER FOR sAD PATHOLOGY

Convincing evidence indicates that central STZ administration induces alterations resembling those found in sAD patients (Hoyer and Frölich, 2006; Cole and Frautschy, 2006; Qiu and Folstein, 2006; Watson and Craft, 2006; Wada et al., 2005). Similarities between human sAD and the STZ-icv model have been noted at three different levels; (I) biochemically, in the region-specific decrease in glucose utilization, reduction in cholinergic transmission and activation of the markers of oxidative stress damage, (II) morphologically, in neuronal damage and loss in hippocampal volume and associated structures accompanied by astroglyosis and neuronal inflammation, and finally, (III) behaviourally, manifested as progressive learning and memory deficits. However, regardless of these similarities, none of these biochemical alterations were able to provide a missing link that could connect all these changes to give a clue to the primary event which could initiate the pathological hallmarks of sAD, hyperphosphorylated tau protein in neurofibrillary tangles and aggregated Aβ peptide in amyloid plaques. Region-specific and, for some parameters, time-progressive, changes of the rat brain insulin system following STZ-icv treatment seem to be the missing link. Disturbances in insulin action, IR function

and downstream signaling pathways have been found post mortem in the brain of sAD patients (Hoyer and Frölich, 2006; Cole and Frautschy, 2006; Qiu and Folstein, 2006; Watson and Craft, 2006; Wada et al., 2005), suggesting a condition of brain insulin resistance, very similar to that found in STZ-icv and STZ-ic treated rats (Salkovic-Petrisic et al., 2006; Grünblatt et al., 2006, de la Monte et la., 2006).

The STZ-icv treated rat model has provided additional evidence that a progression of the brain insulin resistant state over time leads to Alzheimer-like tau protein and Aβ pathology. Three major questions arise from this hypothesis; (1) what could trigger the brain insulin resistant state in sAD and at which point in the insulin/IR/downstream signaling cascade; (2) how is the brain insulin resistant state connected to pathological changes in the brain; and (3) what are the implications of this hypothesis in relation to the drug treatment of sAD?

One of the main risks for sAD is aging, associated with increased cortisol action due to a shift in the hypothalamic pituitary-adrenal (HPA) axis to an increased basal tone (Cizza et al., 1994, for review Hoyer, 2004), frequently reported in AD patients (Raber, 1998). Cortisol may be a candidate for compromising the function of the neuronal IR via its dysregulation of the phosphorylation site of tyrosine residues in the receptor, and noradrenaline (found increased in cerebro-spinal liquid in sAD patients) may desensitise the neuronal IR by phosphorylation of serine/threonine residues (Fig. 1B) (Häring et al., 1986; Giorgino et al., 1993, reviewed by Hoyer, 2004). Desensitisation of the IR in sAD is suggested by findings of up-regulated IR density associated with reduced activity of IR tyrosine kinase (Frölich et al., 1998). This point seems to be the only major difference between sAD and the STZ-icv rat model regarding the brain insulin system. Human data contradicts the findings of decreased or unchanged expression of the IRβ subunit, and the increased or unchanged expression of the tyrosine-phosphorylated IRβ subunit and tyrosine kinase activity seen in the STZ-icv model (Grünblatt et al., 2006). However, this inconsistency could be related to the possible peripheral (e.g. glucocorticoid-induced) origin of the neuronal IR alterations in sAD and its lack in STZ-icv treated rats in which direct STZ-induced damage could be involved instead. On the other hand, data from STZ-icv model studies may point to an imbalance between the IR tyrosine phosphorylation and dephosphorylation under pathological conditions. This is in agreement with a generally known phenomenon of insulin receptor signaling dysfunction (i.e. an insulin resistant state) which may be caused when tyrosine phosphorylation, and/or when tyrosine dephosphorylation fails (Goldstein, 1993), and/or when serine/threonine phosphorylation is increased and maintained at a high level (Häring, 1991; Avruch, 1998) as induced by the cytokine, tumor necrosis factor-α (Hotamisligil et al., 1994). There is some evidence that failing receptor dephosphorylation may inhibit autophosphorylation activity (Lai et al., 1989) and that with time, tyrosine-phosphorylated IRs become inaccessible to phosphatases, therefore allowing persistence of tyrosine kinase activity (Paolini et al., 1996). The activity of the protein tyrosine phosphatase (PTP) subtype 1B known to negatively regulate the function of the IR has not been particularly investigated in AD, but the likelihood of a decrease in function cannot be ruled out. Namely, IR autophosphorylation/dephosphorylation has been reported to be mediated by reactive oxygen species; ie. exposure to hydrogen peroxide could lead to oxidative activation of IR tyrosine kinase activity and oxidative inactivation of PTP (Droge, 2005). STZ generates reactive oxygen species (Szkudelski, 2001), and similarly aging and AD are associated with oxidative stress in the brain (Barja, 2004). However, increased IR tyrosine activity does not necessarily lead to increased IR signal transduction throughout the cell, if

intracellular downstream pathway signaling elements are affected, which is the case both in the animal model (Fig. 1B) (Salkovic-Petrisic et al., 2006; Lester-Coll et al., 2006) and in humans (Hoyer and Frölich, 2006).

Interestingly, the activity of the PTP enzyme was found to be regulated by insulin (Kenner et al. 1993), and the activity of another protein phosphatase subtype, PP2A, has been found largely decreased in the mouse brain 3 days following peripheral STZ administration (Clodfelder-Miller et al., 2006), but also in the human AD brain (Gong et al., 1995). PP 2A and 2B are involved in phoshorylation/dephosphorylation regulation of tau protein (Gong et al., 1994a,b). Thus, insulin resistant brain state, via IR signaling pathway dysfunction, may with time lead to the tau hyperphosphorylation directly through the PI3-GSK-3β pathway, or through the PP 2A pathway. Importantly, the PI3-GSK-3α pathway may also lead to Aβ pathology, as demonstrated in STZ-icv adult rats (Salkovic-Petrisic et al., 2006) and STZ-ic rat pups (Lester-Coll et al., 2006) (Fig. 1B), due to insulin-dependent production of APP derivatives, β-amyloid peptides (Phiel et al., 2003). The time-dependent development of Aβ pathology has been clearly shown in the STZ-icv rat model with no pathological signs visible 4 weeks following STZ-icv treatment, and Aβ-like intracellular aggregates are visible 12 weeks afterwards (Salkovic-Petrisic et al., 2006). This is in agreement with what has been recently proposed, namely that intracellular, rather than extracellular accumulation of β-amyloid is an initiating factor in the pathogenesis of AD (Oddo et al., 2003). Low brain insulin levels reduce the release of Aβ from intracellular compartments into extracellular compartments where clearance is believed to occur. On the other hand, once generated, both derivatives of APP, ß-amlyoid (1-40) and ß-amlyoid (1-42) decrease the affinity of insulin for its receptor, resulting in reduced receptor autophosphorylation (Xie et al., 2002), closing the circle back to IR signaling dysfunction. Although insulin protein concentration in the brain has not been measured directly in STZ-icv (ic) rats, it could be assumed that brain-derived insulin formation is reduced according to the downregulation of insulin mRNA expression both in animals (Grünblatt et al., 2006; Lester-Coll et al., 2006) and humans (Steen et al., 2006). However, this may be compensated by peripherally formed insulin, due to undisturbed pancreas function after central STZ administration (Lester-Coll et al., 2006), as the main source of brain insulin is the pancreas, assuming that the pathobiochemistry induced after STZ-icv injection, is mainly due to dysfunction of the IR and downstream signalling. Data from sAD post mortem studies have demonstrated stronger insulin-immunoreactivity in neocortical pyramidal neurons but unchanged insulin and c-peptide biochemical levels compared to the age-matched controls (Frölich et al., 1998).

Convincing evidence indicates that central STZ administration induces brain pathology and behavioural alterations resembling those found in sAD patients. Additionally, alterations of the brain insulin system found in this experimental model support the hypothesis that central insulin resistance might be the primary event which precedes Aβ pathology in sAD. Further studies are necessary to clarify this issue and its implications in relation to the drug treatment of sAD.

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REFFERENCES

Abbott MA, Wells DG, Fallon JR (1999) The insulin receptor tyrosine kinase substrate p 58/53 and the insulin receptor are components of CNS synapses. J Neurosci 19:7300-7308

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

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

Arluison M, Quignon M, Thorens B, Leloup C, Penicaud L (2004a) Immunocytochemical localization of the glucose transporter 2 (GLUT2) in the adult rat brain. II. Electron microscopic study. J Chem Neuroanat 28:137-146

Arluison M, Quignon M, Nguyen P, Thorens B, Leloup C, Penicaud L (2004b) Distribution and anatomical localization of the glucose transporter 2 (GLUT2) in the adult rat brain--an immunohistochemical study. J Chem Neuroanat 28:117-136

Ar'Rajab A, Ahren B (1993) Long-term diabetogenic effect of streptozotocin in rats. Pancreas 8:50-57

Avruch J (1998) Insulin signal transduction through protein kinase cascades. Mol Cell Biochem 182:31-48

Banks WA (2004) The source of cerebral insulin. Eur J Pharmacol 490:5-12

Barja G (2004) Free radicals and aging. Trends Neurosci 27: 595-600

Baskin DG, Schwartz MW, Sipols AJ, D'Alessio DA, Goldstein BJ, White MF (1994) Insulin receptor substrate-1 (IRS-1) expression in rat brain. Endocrinology 134:1952-1955

Blanchard JG, Duncan PM (1997) Effect of combinations of insulin, glucose and scopolamine on radial arm maze performance. Pharmacol Biochem Behav 58:209-214

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

Blokland A, Jolles J (1994) Behavioral and biochemical effects of an ICV injection of streptozotocin in old Lewis rats. Pharmacol Biochem Behav 47:833-837

Blondel O, Portha B (1989) Early appearance of in vivo insulin resistance in adult streptozotocin-injected rats. Diabete Metab 15:382-387

Bordet R, Ouk T, Petrault O, Gele P, Gautier S, Laprais M, Deplanque D, Duriez P, Staels B, Fruchart JC, Bastide M (2006) PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases. Biochem Soc Trans 34:1341-1346

Brant AM, Jess TJ, Milligan G, Brown CM, Gould GW (1993) Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. Biochem Biophys Res Commun 192:1297-1302

Chu WZ, Qian CY (2005) Expressions of Abeta1-40, Abeta1-42, tau202, tau396 and tau404 after intracerebroventricular injection of streptozotocin in rats. Di Yi Jun Yi Da Xue Xue Bao 25:168-170

Cizza G, Calogero AE, Brady LS, Bagdy G, Bergamini E, Blackman MR, Chrousos GP, Gold PW (1994) Male Fischer 344/N rats show a progressive central impairment of the hypothalamic-pituitary-adrenal axis with advancing age. Endocrinology 134: 1611-1620

Clodfelder-Miller BJ, Zmijewska AA, Johnson GV, Jope RS (2006) Tau is hyperphosphorylated at multiple sites in mouse brain in vivo after streptozotocin-induced insulin deficiency. Diabetes 55:3320-3325

Cole GM, Frautschy SA (2007) The role of insulin and neurotrophic factor signaling in brain aging and Alzheimer's disease. Exp Gerontol 42:10-21

Combettes-Souverain M, Issad T (1998) Molecular basis of insulin action. Diabetes Metab 24:477-489

Cook DG, Leverenz JB, McWellan PJ, Kuslstad JJ, Ericksen S, Roth RA, SChellenberg GD, Jin LW, Kovacina KS, Craft S (2003) Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. Am J Pathol 162:313-319

Cross DAE, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated protein kinase. Nature 378:785-789

Cross DA, Watt PW, Shaw M, von der Kaay J, Downes CP, Holder JC, Cohen P (1997) Insulin activates protein kinase B, inhibits glycogen synthase kinase-3 and activaties glycogen synthase by rapamycin-sensitive pathways in skeletal muscle and adipose tissue. FEBS Lett 406:211-215

de la Monte SM, Tong M, Lester-Coll N, Plater M Jr, Wands JR (2006) Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: relevance to Alzheimer's disease. J Alzheimer Dis 10:89-109

de la Monte SM, Wands JR (2005) Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. J Alzheimers Dis 7:45-61

Devaskar SU, Giddings SJ, Rajakumar PA, Carnaghi LR, Menon RK, Zahn DS (1994) Insulin gene expression and insulin synthesis in mammalian neuronal cells. J Biol Chem 269:8445-8454

Ding A, Nitsch R, Hoyer S (1992) Changes in brain monoaminergic neurotransmitter concentrations in rat after intracerebroventricular injection of streptozotocin. J Cereb Blood Flow Metab 12:103-109

Droge W (2005) Oxidative aging and insulin receptor signaling. J Gerontol A Biol Sci Med Sci 60:1378-13785

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

Figlewicz DP, Bentson K, Ocrant I (1993) The effect of insulin on norepinephrine uptake by PC12 cells. Brain Res Bull 32:425-431

Frölich L, Blum-Degen D, Bernstein HG, Engelsberger S, Humrich J, Laufer S, Muschner D, Thalheimer A, Turk A, Hoyer S, Zochling R, Boissl KW, Jellinger K, Riederer P (1998) Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. J Neural Transm 105,**:**423-438

Gai W, Schott-Ohly P, Schulte im Walde S, Gleichmann H (2004) Differential target molecules for toxicity induced by streptozotocin and alloxan in pancreatic islets of mice in vitro. Exp Clin Endocrinol Diabetes 112: 29-37

Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, Xu H (2001) Stimulation of β-amyloid precursor protein trafficking by insulin reduces intraneural β-amyloid and requires mitogen-activated protein kinase signalling. J Neurosci 21:2561-2570

Giorgino F, Almahfouz A, Goodyear LJ, Smith RJ (1993) Glucocorticoide regulation of insulin receptor and substrate IRS-1 tyrosine phosphorylation in rat skeletal muscle in vivo. J Clin Invest 91:2020-2030

Giorgino F, Chen JH, Smith RJ (1992) Changes in tyrosine phosphorylation of insulin receptors and a 170,000 molecular weight nonreceptor protein in vivo in skeletal muscle of streptozotocin-induced diabetic rats: effects of insulin and glucose. Endocrinology 130:1433-1444

Goldstein BJ (1993) Regulation of insulin receptor signalling by protein-tyrosine dephosphorylation. Receptor 3:1-15

Gong CX, Grundke-Iqbal I, Iqbal K (1994a) Dephosphorylation of Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A. Neuroscience 61:765-772

Gong CX, Singh TJ, Grundke-Iqbal I, Iqbal K (1994b) Alzheimer's disease abnormally phosphorylated tau is dephosphorylated by phosphatase-2B (calcineurin). J Neurochem 62:803-806

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

Grieb P, Kryczka T, Fiedorowicz M, Frontczak-Baniewicz M, Walski M (2004) Expansion of the Golgi apparatus in rat cerebral cortex following intracerebroventricular injections of streptozotocin. Acta Neurobiol Exp (Wars) 64:481-489

Griffin RJ, Moloney A, Kelliher M, Johnston JA, Ravid R, Dockery P, O'Connor R, O'Neill C (2005) Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer's disease pathology. J Neurochem 93:105-117

Grünblatt E, Hoyer S, Riederer P (2004) Gene expression profile in streptozotocin rat model for sporadic Alzheimer's disease. J Neural Transm 111:367-386

Grünblatt E, Salkovic-Petrisic M, Osmanovic J, Riederer P, Hoyer S (2006) Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. J Neurochem doi:10.1111/j.1471-4159.2006.04368.x

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297:353-356

Häring HU, Kirsch D, Obermeier B, Ermel B, Machicao F (1986) Decreased tyrosine kinase activity of insulin receptor isolated from rat adipocytes rendered insulin-resistant by catecholamine treatment in vitro. Biochem J 234:59-66

Häring HU (1991) The insulin receptor: signalling mechanism and contribution to the pathogenesis of insulin resistance. Diabetologica 34:848-461

Havrankova J, Roth J, Brownstein M (1978a) Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272:827-829

Havrankova J, Schmechel D, Roth J, Brownstein M (1978b) Identification of insulin in rat brain. Proc Natl Acad Sci USA 75:5737-5741

Heidenreich KA, Zahniser NR, Berhanu P, Brandenburg D, Olefsky JM (1983) Structural differences between insulin receptors in the brain and peripheral target tissues. J Biol Chem 258:8527-8530

Hellweg R (1994) Trophic factors during normal brain aging and after functional damage. J Neural Transm Suppl 44:209-217

Hellweg R, Nitsch R, Hock C, Jaksch M, Hoyer S (1992) Nerve growth factor and choline acetyltransferase activity levels in the rat brain following experimental impairment of cerebral glucose and energy metabolism. J Neurosci Res 31:479-486

Hong MF, Lee VMY (1997) Insulin and insulin-like growth factor-1 regulate tau phosyphorylation in cultured human neurons. J Biol Chem 272:19547-19553

Hotamisligil GS, Murray DL, Choy LN, Spiegelmann BM (1994) Tumor necrosis factor α inhibits signalling from the insulin receptor. Proc Natl Acad Sci US 91:4854-4858

Hoyer S (1998) Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. J Neural Transm 105: 415-422

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 function and insulin signal transduction in sporadic Alzheimer's disease, in Research Progress in Alzheimer's Disease and Dementia, Vol. In Press (Sun M. K., ed.). Nova Science, New York

Hoyer S, Lannert H, Noldner M, Chatterjee SS (1999) Damaged neuronal energy metabolism and behavior are improved by Ginkgo biloba extract (EGb 761). J Neural Transm 106:1171-1188

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 274:99-102

Ishiguro K, Shiratsuchi A, Sato S, Omori A, Arioka M, Kobayashi S, Uchida T (1993) Glycogen synthase kinase 3-beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. FEBS Lett 325:167-172

Ishiguro K, Takamatsu M, Tomizawa K, Omori A, Takahashi M, Arioka M, Uchida T, Imahori K (1992) Tau protein kinase I converts normal tau protein into A68-like component of paired helical filaments. J Biol Chem 267:10897-10901

Ishrat T, Khan MB, Hoda MN, Yousuf S, Ahmad M, Ansari MA, Ahmad AS, Islam F (2006) Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats. Behav Brain Res 171:9-16

Johnston AM, Pirola L, Van Obberghen E (2003) Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. FEBS Lett 546:32-36

Kadowaki T, Kasuga M, Akanuma Y, Ezaki O, Takaku F (1984) Decreased autophosphorylation of the insulin receptor-kinase in streptozotocin-diabetic rats. J Biol Chem 259:14208-14216

Kenner KA, Hill DE, Olefsky JM, Kusari J (1993) Regulation of protein tyrosine phosphatases by insulin and insulin-like growth factor I. J Biol Chem 268:25455-25462

Lackovic Z, Salkovic M (1990) Streptozotocin and alloxan produce alterations in rat brain monoamines independently of pancreatic beta cells destruction. Life Sci 46:49-54

Lai WH, Cameron PH, Doherty JJ, 2nd, Posner BI, Bergeron JJ (1989) Ligand-mediated autophosphorylation activity of the epidermal growth factor receptor during internalization. J Cell Biol 109:2751-2760

Landreth G (2006) PPARγ agonists as new therapeutic agents for the treatment of Alzheimer's disease. Exp Neurol 199: 245-248

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

Lannert H, Wirtz P, Schuhmann V, Galmbacher R (1998) Effects of Estradiol (-17beta) on learning, memory and cerebral energy metabolism in male rats after intracerebroventricular administration of streptozotocin. J Neural Transm 105:1045-1063

Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing-Jallat P, Ferre P, Penicaud L (1994) Glucose transporter 2 (GLUT2): expression in specific brain nuclei. Brain Res 638:221-226

Lester-Coll N, Rivera EJ, Soscia SJ, Doiron K, Wands JR, de la Monte SM (2006) Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. J Alzheimers Dis 9:13-33

Li B, Xi X, Roane DS, Ryan DH, Martin RJ (2003) Distribution of glucokinase, glucose transporter GLUT2, sulfonylurea receptor-1, glucagon-like peptide-1 receptor and neuropeptide Y messenger RNAs in rat brain by quantitative real time RT-PCR. Brain Res Mol Brain Res 113:139-142

Margolis RU, Altszuler N (1967) Insulin in the cerebrospinal fluid. Nature 215:1375-1376

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 BS, Reagan LP (2004) Glucose transporter expression in the central nervous system: relationship to synaptic function. Eur J Pharmacol 490:13-24

Moosavi M, Naghdi N, Maghsoudi N, Asl SZ (2006) The effect of intrahippocampal insulin microinjection on spatial learning and memory. Horm Behav 50:748-752

Moss AM, Unger JW, Moxley RT, Livingston JN (1990) Location of phosphotyrosine-containing proteins by immunocytochemistry in the rat forebrain corresponds to the distribution of insulin receptor. Proc Natl Acad Sci USA 87:4453-4457

Muller D, Nitsch RM, Wurtman RJ, Hoyer S (1998) Streptozotocin increases free fatty acids and decreases phospholipids in rat brain. J Neural Transm 105:1271-1281

Ngarmukos C, Baur EL, Kumagai AK (2001) Co-localization of GLUT1 and GLUT4 in the blood-brain barrier of the rat ventromedial hypothalamus. Brain Res 900:1-8

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

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracelullar Aβ and synaptic dysfunction. Neuron 39: 409-421

Paolini R, Serra A, Kinet JP (1996) Persistence of tyrosine-phosphorylated FcepsilonRI in deactivated cells. J Biol Chem 271:15987-15992

Pathan AR, Viswanad B, Sonkusare SK, Ramarao P (2006) Chronic administration of pioglitazone attenuates intracerebroventricular streptozotocin induced-memory impairment in rats. Life Sci 79:2209-22016

Pedersen WA, Flynn ER (2004) Insulin resistance contributes to abberant stress responses in the Tg2576 mouse model of Alzheimer's disease. Neurobiol Disease 17: 500-5006

Pedersen WA, McMillan PJ, Kulstad JJ, Leverenz JB, Craft S, Haynatzki GR (2006) Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice. Exp Neurol 199: 245-248

Pei JJ, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, Winblad B, Cowburn RF (1999). Distribution of active glycogen synthase kinase 3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. J Neuropathol Exp Neurol 58:1010-1019

Pei JJ, Khatoon S, An WL, Nordlinder M, Tanaka T, Braak H, Tsujio I, Takeda M, Alafuzoff I, Winblad B, Cowburn RF, Grundke-Iqbal I, Iqbal K (2003) Role of protein kinase B in Alzheimer's neurofibrillary pathology. Acta Neuropathol (Berl) 105:381-392

Perez A, Morelli L, Cresto JC, Castano EM (2000) Degradation of soluble amyloid beta-peptides 1-40, 1-42, and the Dutch variant 1-40Q by insulin degrading enzymes from Alzheimer disease and control brains. Neurochem Res 25:247-255

Phiel CJ, Wilson CA, Lee VMY, Klein PS (2003) GSK-3α regulates production of Alzheimer's disease amyloid-β peptides. Nature 423:435-439

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 Dev Neurosci 11:477-483

Prickaerts J, Blokland A, Honig W, Meng F, Jolles J (1995) Spatial discrimination learning and choline acetyltransferase activity in streptozotocin-treated rats: effects of chronic treatment with acetyl-L-carnitine. Brain Res 674:142-146

Prickaerts J, De Vente J, Honig W, Steinbusch H, Ittersum MMV, Blokland A, Steinbusch HW (2000) Nitric oxide synthase does not mediate neurotoxicity after an i.c.v. injection of streptozotocin in the rat. J Neural Transm 107:745-766

Prickaerts J, Fahrig 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

Puro DG (2002) Diabetes-induced dysfunction of retinal Muller cells. Trans Am Ophthalmol Soc 100: 339-352

Qiu QW, Folstein MF (2006) Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer's disease: review and hypothesis. Neurobiol Aging 27: 190-198

Raber J (1998) Detrimental effects of chronic hypothalamic-pituitary-adrenal axis activation. Mol Neurobiol 18: 1-22

Raizada ML, Shemer J, Judkins JH, Clarke DW, Masters BA, Le Roith D (1988) Insulin receptors in the brain: structural and physiological characterization. Neurochem Res 13:297-303

Rajab AA, Ahren B, Bengmark S (1989) Islet transplantation to the renal subcapsular space in streptozotocin-diabetic rats: short-term effects on glucose-stimulated insulin secretion. Diabetes Res Clin Pract 7.197-204

Reagan LP (2002) Glucose, stress and hippocampal neuronal vulnerability. Int Rev Neurobiol 51:289-324

Rickle A, Bogdanovic N, Volkman I, Winbland B, Ravid R, Cowburn RF (2004) Akt activity in Alzheimer's disease and other neurodegenerative disorders. Neurochem 15:955-959

Rodrigues B, Cam MC, Kong J, Goyal RK, McNeill JH (1997) Strain differences in susceptibility to streptozotocin-induced diabetes: effects on hypertriglyceridemia and cardiomyopathy. Cardiovasc Res 34: 199-205

Salkovic M, Sabolic I, Lackovic Z (1995) Striatal dopaminergic D1 and D2 receptors after intracerebroventricular application of alloxan and streptozotocin in rat. J Neural Transm Gen Sect 100:137-145

Salkovic-Petrisic M, Lackovic Z (2003) Intracerebroventricular administration of betacytotoxics alters expression of brain monoamine transporter genes. J Neural Transm 110:15-29

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

Santos MS, Pereira EM, Carvaho AP (1999) Stimulation of immunoreactive insulin release by glucose in rat brain synaptosomes. NeuroChem Res 24:33-36

Santos MJ, Quintanilla RA, Toro A, Grandy R, Dinamarca MC, Godoy JA, Inestrosa NC (2005) Peroxisomal proliferation protects from beta-amyloid neurodegeneration. J Biol Chem 280: 41057-41068

Schechter R, Beju D, Gaffney T, Schaefer F, Whetsell L (1996) Preproinsulin I and II mRNAs and insulin electron microscopic immuno-reaction are present within the fetal nervous system. Brain Res 736:16-27

Schechter R, Whitmire J, Holtzelaw L, George M, Devaskar SU (1992) Developmental regulation of insulin in the mammalian central nervous system. Brain Res 582:27-37

Sharma M, Gupta YK (2001a) Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sci 68:1021-1029

Sharma M, Gupta YK (2001b) Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. Pharmacol Biochem Behav 70:325-331

Sharma M, Gupta YK (2002) Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. Life Sci 71:2489-2498

Shoham S, Bejar C, Kovalev E, Schorer-Apelbaum D, Weinstock M (2006) Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in rats. Neuropharmacology 52:836-843

Shoham S, Bejar C, Kovalev E, Weinstock M (2003) Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. Exp Neurol 184:1043-1052

Solano DC, Sironi M, Bonfini C, Solarte SB, Govoni S, Racchi M (2000) Insulin regulates soluble amyloid precursor protein release via phosphatidyl inositol 3 kinase-dependent pathway. FASEB J 14:1015-1022

Sonkusare S, Srinivasan K, Kaul C, Ramarao P (2005) Effect of donepezil and lercanidipine on memory impairment induced by intracerebroventricular streptozotocin in rats. Life Sci 77:1-14

Spencer DG, Lal H (1983) Effects of anticholinergic drugs on learning and memory. Drug Dev Res 3:489-502

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkov EM (2002) Tau blocks traffic organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol 156:1051-1063

Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease – is this type 3 diabetes? J Alzheimer's Dis 7:63-80

Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D, Jr (1992) Insulin in the brain: a hormonal regulator of energy balance. Endocr Rev 13:387-414

Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50:336-346

Terwel D, Prickaerts J, Meng F, Jolles J (1995) Brain enzyme activities after intracerebroventricular injection of streptozotocin in rats receiving acetyl-L-carnitine. Eur J Pharmacol 287:65-71

Todd S, Yoshida MC, Fang XE, McDonald L, Jacobs J, Heinrich G, Bell GI, Naylor SL, Sakaguchi AY (1985) Genes for insulin I and II, parathyroid hormone, and

calcitonin are on rat chromosome 1. Biochemical and Biophysical Research Communications 131:1175-1180

Unger J, McNeill TH, Moxley RT3rd, White M, Moss A, Livingston JN (1989) Distribution of insulin receptor-like immunoreactivity in the rat forebrain. Neuroscience 31:143-157

Van der Heide LP, Ramakers GMJ, Smidt MP (2006) Insulin signaling in the central nervous system: Learning to survive. Prog Neurobiol 79:205-221

van Houten M, Posner BI., Kopriwa BM, Brawer JR (1979) Insulin-binding sites in the rat brain: in vivo localization to the circumventricular organs by quantitative radioautography. Endocrinology 105:666-673

van Houten M, Posner BI, Kopriwa BM, Brawer JR (1980) Insulin binding sites localized to nerve terminals in rat median eminence and arcuate nucleus. Science 207: 1081-1083

Vannucci SJ, Koehler-Stec EM, Li K, Reynolds TH, Clark R, Simpson IA (1998) GLUT4 glucose transporter expression in rodent brain: effect of diabetes. Brain Res 797:1-11

Veerendra Kumar MH, Gupta YK (2003) Effect of Centella asiatica on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rats.Clin Exp Pharmacol Physiol 30:336-342

Wada A, Yokoo H, Yanagita T, Kobayashi H (2005) New twist on neuronal insulin receptor signaling in health, disease and therapeutics. J Pharmacol Sci 99: 128-143

Wang YT, Salter MW (1994) Regulation of NMDA receptors by tyrosine kinases and phosphatases. Nature 369:233-235

Watson G, Cholerton B, Reger M, Baker L, Plymate S, Asthana S, Fishel M, Kulstad J, Green P, Cook D, Kahn S, Keeling M, Craft S (2005) Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. Am J Ger Psychiat 13: 950-958

Watson GS, Craft S (2006) Insulin resistance, inflammation, and cognition in Alzheimer's disease: lessons for multiple sclerosis. J Neuro Sci 245: 21-33

Weinstock M, Kirschbaum-Slager N, Lazarovici P, Bejar C, Youdim MB, Shoham S (2001) Neuroprotective effects of novel cholinesterase inhibitors derived from rasagiline as potential anti-Alzheimer drugs. Ann N Y Acad Sci 939:148-161

Weinstock M, Shoham S (2004) Rat models of dementia based on reductions in regional glucose metabolism, cerebral blood flow and cytochrome oxidase activity. J Neural Transm 111:347-366

Wozniak M, Rydzewski B, Baker SP, Raizada MK (1993) The cellular and physiological actions of insulin in the central nervous system. Neurochem Int 22: 1-10

Xie L, Helmerhorst E, Taddel K, Plewright B, van Bronswijk W, Martins R (2002) Alzheimer's β-amyloid peptides compete for insulin binding to the insulin receptor. J Neurosci 22 (RC221):1-5

Yun SW, Gartner U, Arendt T, Hoyer S (2000) Increase in vulnerability of middle-aged rat brain to lead by cerebral energy depletion. Brain Res Bull 52:371-378

Zhao W, Chen H, Xu H, Moore E, Meiri N, Quon MJ, Alkon DL (1999) Brain insulin receptors and spatial memory. J Biol Chem 274:34893-34902

Zhao WQ, Chen H, Quon MH, Alkon DL (2004) Insulin and the insulin receptor in experimental models of learning and memory. Eur J Pharmacol 490:71-81

Table 1. Previous studies using central administration of streptozotocin in rats.

REFERENCE	STZ DOSE	AGE at STZ dose	TIME AFTER STZ TREATMENT		FINDINGS
			cognitive tests (learning & memory)	biochemical/ histological analyses (brain tissue)	
Mayer et al, 1990	1x1.5 mg/kg icv (unilat.)	1 year	11-13 days		↓ learning&memory
Lackovic and Salkovic, 1990	1x ≤20 mg/kg icv (unilat.)	2 months		1 week DA,NA,5-HT	↓ monoamine turnover rate ↑ monoamine content
Nitsch and Hoyer, 1991	1x1.25 mg/kg icv (unilat.)	1 year		3 weeks energy metabolism	↓ (CTX)
Ding et al, 1992	1x1.5 mg/kg icv (unilat.)	1 year		3 weeks monoamine content & turnover	↓ content (enthorhinal CTX-5HT,NA; frontal CTX, S- NA) ↑ 5HT turnover rate
Hellweg et al, 1992	1x1.5 mg/kg icv (unilat.)	1 year		1, 3 weeks NGF, ChAT	↓ NGF (1 w) – septum ↑ NGF (3 w) – HPC, CTX ↓ ChAT activity (HPC)
Blokland and Jolles, 1993	1x1.5 mg/kg icv (bilat.)	4 months	10-14 days	~20 days ChAT	↓ learning&memory in middle-aged ↓ ChAT activity (HPC)
Plaschke and Hoyer, 1993	2x1.25 mg/kg icv (bilat.) (3w) 3x1.25 mg/kg icv (bilat.) (6w)	1 year		3 and 6 weeks glycolitic enzymes	↓ glycolytic enzyme activity (CTX, HPC)
Blokland and Jolles, 1994	1x1.5 mg/kg icv (bilat.)	2 years	2 weeks	~20 days ChAT	no decline in learning & memory (active avoidance & open field test) ↓ ChAT activity (HPC)
Duelli et al, 1994	3x 1.5 mg/kg icv (bilat.)	1.5 year		6 weeks glucose utilization	↓ glucose utilization (CTX, HPC)
Terwel et al, 1995	1x1.5 mg/kg icv (unilat.)	18 months		3 weeks peptidases, dehydrogenases, ChAT	↓ ChAT activity (HPC) ↓ peptidase & dehydrogenase activity (septum)
Salkovic et al, 1995	1x0.5 mg/kg icv (unilat.)	2 months		1 week dopaminergic D1, D2 receptors, G-proteins	↓ D1 Bmax (striatum) ~ D2 Bmax, ~ Gs/Gi protein
Müller et al, 1998	1x 1.5 mg/kg icv (bilat.)	1 year		3 weeks free fatty acids phospholipids	↑ lipolysis (HPC, T-CTX)
Lannert and Hoyer, 1998	3x1 mg/kg icv (bilat.)	1 year	20, 40, 80 days	85 days energy metabolism	↓ learning&memory ↓ energy metabolism
Prickaerts et al, 1999	1x1.25 mg/kg icv (bilat.)	20 months	3 weeks	3 weeks astrogliosis marker ChAT	↓ learning&memory astrogliosis (S, HPC, septum) ↓ ChAT activity (HPC)
Hoyer et al, 1999	3x ~ 1 mg/kg icv (bilat.)	1 year	12 weeks	12 weeks energy metabolism	↓ learning&memory ↓ energy metabolism
Yun et al, 2000	1x1 mg/kg icv (bilat.)	15 months		1 month energy metabolism	↓ energy metabolism
Prickaerts et al, 2000	1x1.25 mg/kg icv (bilat.)	20 months	3 weeks	5 weeks astrogliosis NO/NOS (IHC)	partly ↓ learning&memory ↓ energy metabolism ↑ astrogliosis NOS is not involved in STZ induced toxicity
Sharma and Gupta, 2001	2x 3 mg/kg icv (bilat.)	~ 4 months (320-350 g)	17 days	1,7,21 days oxidative stress	↓ learning&memory ↑ oxidative stress (progressive)
Weinstock et al, 2001	1x1.5 mg/kg icv (unilat.) 3x0.5 mg/kg icv (bilat)	4 months	N.D.	N.D. morphology	↓ learning&memory ↑microgliosis, axonal degeneration, distorted morphology of axonal fibres
Sharma and Gupta, 2002	2x3 mg/kg icv (bilat.)	4 months	2.5 weeks	3 weeks oxidative stress	↓ learning&memory ↑ oxidative stress
Salkovic-	1x0.5 mg/kg	~ 2-3		1, 4 weeks	↓ DAT 4 w (VMB);

Petrisic and Lackovic, 2003	icv (bilat.)	months (150-200 g)		monoamine transporter mRNA	↓ NAT4 w (LC) ↑ NAT 1,4 w (A1)
Shoham et al, 2003	1x ~1.8 mg/kg icv (bilat.)	3.5-4 months (270-290 g)	40 days	40 days morphology	↓ learning&memory neurotoxicity to myelin and axons in fornix and HPC
Veerendra and Gupta, 2003	1x3 mg/kg icv (bilat.)	3 months	2, 3 weeks	3 weeks oxidative stress	↓ learning&memory ↑ oxidative stress
Grünblatt et al, 2004	3x 1mg/kg icv (bilat.)	1 year		6 weeks gene expression	altered gene expression (IGF-1R, GABA-R, glutamate transporter, potassium channels)
Grieb et al, 2004	2x ? (N.D.) icv (bilat.)	N.D.		3 weeks Golgi apparatus	↑ trans part of Golgi complex (F-CTX)
Sonkusare et al, 2005	2x1.5 mg/kg icv (bilat.)	4 months	2.5 weeks	3 weeks AChE	↓ learning&memory ↑ AChE activity (whole brain)
Chu and Qian, 1995	1x3 mg/kg icv (bilat.)	N.D.		3 weeks morphology, IHC - tau protein & amyloid beta (1-40; 1-42)	↑ expression of tau & amyloid beta (CTX, HPC)
Salkovic-Petrisic et al, 2006	1x1 mg/kg icv (bilat.)	3 months	1, 3 months	1, 3 months IR signaling cascade	↓ learning&memory at 1&3 m disturbed IR signaling pronounced at 3 m
Pathan et al, 2006	2x 3 mg/kg icv (bilat.)	~ 4 months (320-350 g)	14 days	21-25 days oxidative stress glucose utilization	↓ learning&memory ↑ oxidative stress ↓ glucose utilization
Shoham et al, 2006	1x 3 mg/kg icv (bilat.)	4 months (320-340 g)	2 , 4 weeks	1 , 8 weeks IHC – cholinergic markers, oxidative – nitrative stress	↓ learning&memory at 4 w gliosis at 1 w (CTX, HPC-CA1, septum, c. callosum); ↑ oxidative stress; unchanged ACh neurons (1, 8 w)
Ishrat et al, 2006	1x1.5 mg/kg icv (bilat.)	1 year	2 weeks	3 weeks oxidative stress, ATP, ChAT-AChe	↓ learning&memory ↑ oxidative stress (HPC,CTX) ↓ ATP (HPC, CTX) ↓ ChAT-↑AChE activity (HPC)
De la Monte et al, 2006	1x40 µg/kg (intracerebral –bilat.)	pups	1 month	1 month cholinergic markers NOS IR signaling	↓ learning&memory ↓ChATmRNA (TL,CB); AChEmRNA ↓TL; ↑HPT ↑NOS2,3mRNA (TL) altered IR signaling
Grünblatt et al, 2006	1x1 mg/kg 3x1 mg/kg icv (bilat.)	3-4 months	2 weeks, 2 months	3 months insulin and IR	↓ learning&memory at 2w,1 and 2 m altered insulin and IR

STZ - streptozotocin; icv – intracerebroventricular; unilat – unilateral; bilat – bilateral; w – week; m – month; DA – dopamine; NA – noradrenaline; 5-HT – serotonin; NGF – nerve growth factor; ChAT – choline acethyltransferase; AChE – acethyl cholinesterase; NO – nitric oxide; NOS – nitric oxide synthase; IR – insulin receptor; MAO – monoamine oxidase; IHC – immunohistochemistry; IGF-1R – insulin-like growth factor-1 receptor; GABA-R – gamma aminobutiric acid receptor; CTX – cerebral cortex; F-CTX – frontoparietal cerebral cortex; T-CTX – temporal cerebral cortex; S – striatum; HPC – hippocampus; VMB - ventral medial bundle; LC – locus coeruleous; TL – temporal lobe; CB – cerebellum; HPT – hypothalamus; ND – data not available; ↑ - increase; ↓ - decrease.

Table 2. Comparison of brain insulin system alterations in sporadic Alzheimer's disease and in an experimental model of this disease, streptozotocin-intracerebroventricularly treated rats.

BRAIN INSULIN SYSTEM	HUMAN	STZ-icv rat		
		time after treatment		therapy improvement
		≤1 month	3 months	
INSULIN				
mRNA	HPC&HPT&F-CTX&CB ↓ (1)		HPC↓ (Ins1) (4) F-CTX ↓ (Ins2) (4)	
		STZic pups: TL&HPT&CB ↓ (2,3)		STZic pups: PPAR agonists: NO (3)
protein	CTX↑/~ (5)			
IGF-1				
mRNA	F-CTX&HPT ↓ (1)			
		STZic pups: TL ↓;HPT&CB ~ (3)		STZic pups: PPAR agonists:YES (3)
IR				
mRNA	FHPC&HPT&F-CTX ↓ (1)		HPC↓ (4) F-CTX ↓(4)	
		STZic pups: TL&CB ↓ ; HPT~(2,3)		STZic pups: PPAR agonists:YES (partly) (3)
Protein (IRβ)	HPC ↓ (1)		F-CTX ↓ (4) HPC ↑ (4) HPT ↓ (4)	
p IRβ	HPC ↓ (1)		F-CTX ↑ (4) HPT ↑ (4)	
TK activity	↓ (5)		HPC ↑ (4)	
density (Bmax)	↑ (5)	STZic pups: TL&HPT&CB ↓ (2,3)		STZic pups: PPAR agonists: YES (partly) (3)
IGF-1R				
mRNA	HPC&HPT&F-CTX ↓ (1)	CTX, S ↓(6)		
		STZicv pups: HPT&CB ↓ ;TL~(2,3)		STZic pups: PPAR agonists: YES (partly) (3)
protein	HPC ↓ (1)			
density (Bmax)		STZic pups: TL ↑ (3)		STZic pups: PPAR agonists: NO (3)
IRS				
mRNA	F-CTX&HPC&HPT ↓ (IRS1) (1) HPC ↓ (IRS2) (1)	STZic pups: ↓ (2)		
p-IRS	HPC&HPT ↓ (IRS1) (1)			
Akt/PKB				
mRNA				
protein	HPC&HPT ~ (1) CTX ↓ (cytosol) (7)	F-CTX ↓ (8)	HPC ↓ (8) F-CTX ↑ (8)	
p-protein	HPC&HPT ↓ (1) F-CTX ↑ (9)			
pAkt/Akt ratio				
activity	T-CTX ↑ (soluble fraction) (10)			
GSK-3				
mRNA				
protein		HPC&CTX ~ (8)	HPC&F-CTX ~ (8)	

		STZic pups: TL&HPT ~ (3)		
p-protein		HPC ↑ (8)		
		STZic pups: TL&HPT ~ (3)		
pGSK-3/GSK-3 ratio		HPC ↑ (8) F-CTX ↓ (8)	HPC ↓ (8) F-CTX ↓ (8)	
		STZic pups: TL&HPT ↓ (3)		STZic pups: PPAR agonists: NO (3)
GSK-3α				
pGSK-3α	F-CTX ↑ (11)			
GSK-3β	HPC&HPT ~ (1)	STZic pups: ↑ (2)		
pGSK-3β	HPC&HPT ↓ (1) F-CTX ↑ (11)			
IDE				
mRNA	HPC&HPT~ (1)			
protein	HPC ↓ (12)			
activity (Aβ degrading)	↓ (13)			

STZ – streptozotocin; icv – intracerebroventricular; ic – intracerebral; IGF-1- insulin-like growth factor 1; IR – insulin receptor; IGF-1R- insulin-like growth factor 1 receptor; IRS - insulin receptor substrate; Akt/PKB- protein kinase B; GSK-3- glycogen synthase kinase 3; IDE- insulin degrading enzyme; p- phospho; Aβ – amyloid beta; HPC- hippocampus; HPT- hypothalamus; TL- temporal lobe; CB- cerebellum; F-CTX- frontoparietal cerebral cortex; PPAR – peroxisome-proliferator activated receptor; ↓ - decrease; ↑ - increase. Number of reference in brackets: 1) Steen et al., 2005; 2) Lester-Coll et al., 2006; 3) de la Monte et al., 2006; 4) Grünblatt et al., 2006; 5) Frölich et al., 1998; 6) Grünblatt et al., 2004; 7) Griffin et al., 2005; 8) Salkovic-Petrisic et al., 2006; 9) Pei et al., 2003; 10) Rickle er al., 2004; 11) Pei et al., 1999; 12) Cook et al., 2003; 13) Perez et al., 2000.

Table 3. Comparison of brain tau protein and amyloid beta alterations in sporadic Alzheimer's disease and in an experimental model of this disease, streptozotocin-intracerebroventricularly treated rats.

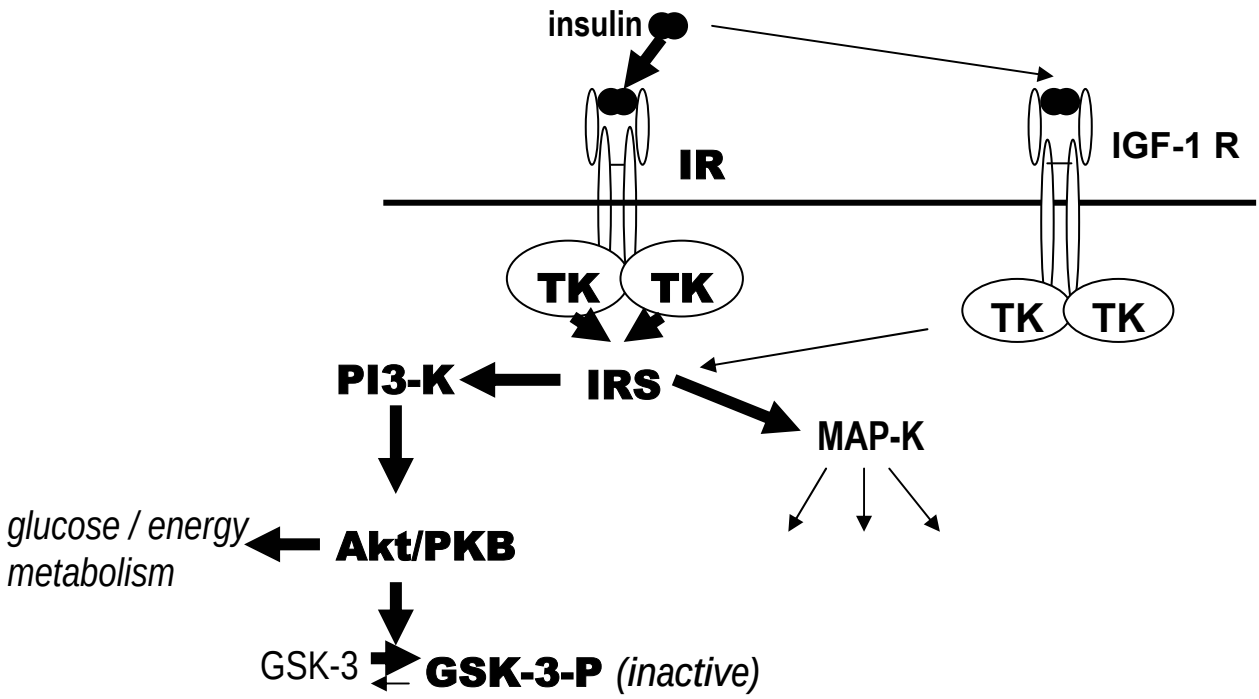
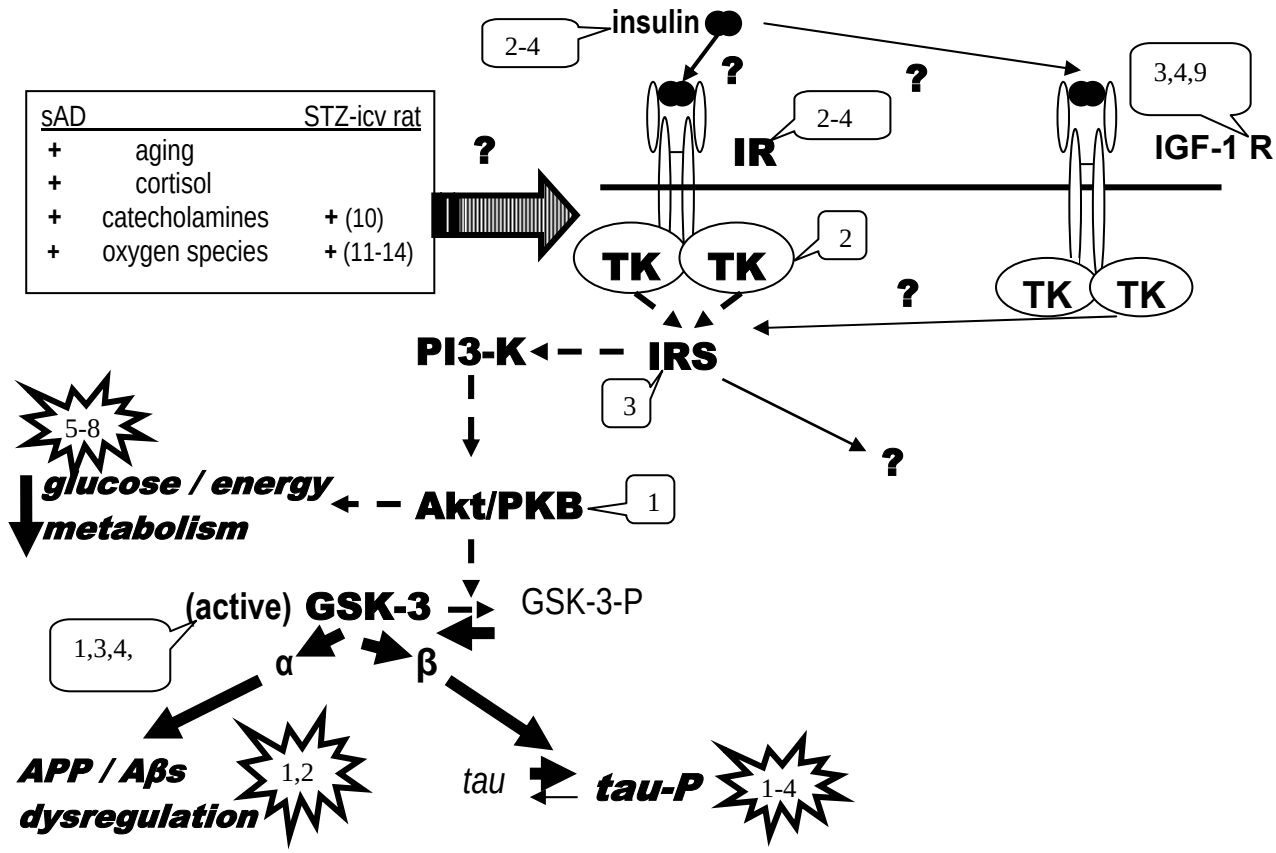
	HUMAN	STZ-icv rat		
		time after treatment		therapy improvement
		≤1 month	3 months	
Tau protein				
mRNA	HPC&HPT ↓ (1)	STZic pups: TL&HPT&CB ↓ (2,3)		STZic pups: PPAR agonists: YES (partly) (3)
protein	F-CTX ↑ (6)	HPC ↑ (4,5) ↑ (7))	HPC ↑ 4,5)	
		STZic pups: TL&HPT ~ (3)		
p-protein	F-CTX ↑ (6)	HPC~ (4)	HPC ↑ (4)	
		STZic pups: TL&HPT ↑ (2,3)		STZic pups: PPAR agonists: YES (3)
p-tau/tau ratio		STZic pups: TL&HPT ↑ (3)		STZic pups: PPAR agonists: YES (3)
APP				
mRNA	HPC&HPT ↑ (1)	STZic pups: TL&HPT ↑ (2,3)		STZic pups: PPAR agonists: YES (partly) (3)
protein				
Aβ				
Aβ 40		STZic pups: (2)		
Aβ 42		↑ (7)		
		STZic pups: (2)		
aggregates		absent (5)	in meningeal capillaries (5)	

STZ – streptozotocin; icv – intracerebroventricular; ic – intracerebral; p- phospho; APP- amyloid precursor protein; Aβ- amyloid beta; HPC- hippocampus; HPT- hypothalamus; TL- temporal lobe; CB- cerebellum; F - CTX- frontoperietal cerebral cortex; PPAR – peroxisome-proliferator activated receptor; ↑ - increase; ↓ - decrease. Number of reference in brackets: 1) Steen et al., 2005; 2) Lester-Coll et al., 2006; 3) de la Monte et al., 2006; 4) Grünblatt et al., 2006; 5) Salkovic-Petrisic et al., 2006; 6) Pei et al., 1999; 7) Chu and Quian, 2005.

Fig 1. Brain insulin receptor signaling cascade in physiological conditions (A) and in the insulin resistant brain state (B) induced by the streptozotocin-intracerebroventricular treatement. IR – insulin receptor; IGF-1R – insulin-like growth factor-1 receptor; TK – tyrosine kinase; IRS – insulin receptor substrate; MAP-K – mitogen activated protein kinase; PI3-K - phosphatidylinositol-3 kinase; Akt/PKB – protein kinase B; GSK-3 - glycogen synthase kinase-3; GSK-3-P – phosphorylated glycogen synthase kinase-3; APP – amyloid precursor protein; Aβ – amyloid beta; tau – tau protein; tau-P – phosphorylated tau protein; sAD – human sporadic Alzheimer's disease; STZ-icv – streptozotocin-intraverebroventricularly treated rats. Number of reference in brackets: (1) Salkovic-Petrisic et al., 2006; (2) Grünblatt et al., 2006; (3) Lester-Coll et al., 2006; (4) de la Monte et al., 2006; (5) Plaschke and Hoyer, 1993; (6) Duelli et al., 1994; (7) Lannert and Hoyer, 1998; (8) Pathan et al., 2006; (9) Grünblatt et al., 2004; (10) Lackovic and Salkovic, 1990; (11) Sharma and Gupta, 2001; (12) Pathan et al., 2006; (13) Shoham et al., 2006; (14) Ishrat et al., 2006.

BRAIN INSULIN RECEPTOR SIGNALING CASCADE

A) physiological condition



B) dysfunction – insulin resistant brain state