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Chapter 1

STREPTOZOTOCIN AS A TOOL TO INDUCE CENTRAL PATHOLOGY AND COGNITIVE IMPAIRMENT IN RODENTS

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia and leads to irreversible cognitive and memory impairment. By 2050 more than a 100 million people are expected to be affected by AD. Furthermore, AD cannot be prevented, it has no successful treatment and the ultimate neurotoxic mechanisms have not been completely elucidated. Pathological hallmarks of AD include: neurofibrillary tangles, which are intraneuronal deposits composed primarily of abnormally-phosphorylated tau protein; extracellular senile plaques, mainly composed of beta-amyloid peptide (Aβ), and neuronal and synaptic loss. On the other hand vascular dementia (VaD) is the second cause of dementia, and comprises a great variety of pathological features, including small vessel disease or white matter alterations, among others. Moreover the borderlines between AD and VaD are blurred and in many patients coexist simultaneously. In order to study the onset, evolution and therapeutic alternatives to treat AD, many transgenic mice engineered to overproduce Aβ or increase tau phosphorylation have been developed although only about 5-10% of the cases are familiar AD patients. On the other hand whereas age remains the main risk factor to suffer AD and VaD, multiple epidemiological studies reveal that diabetes mellitus is also a risk factor to suffer these diseases. In this sense streptozotocin (STZ) has been largely used for the last four decades as a pharmacological approach to induce type 1 diabetes (T1D) in rodents, and to study related metabolic alterations as well as the effect of different therapeutic agents. However in recent years central complications associated with diabetes are getting a great deal of attention, and central neuronal abnormalities, learning and memory impairment, central vascular dysfunction or blood brain barrier alterations have been observed after

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systemic administration of STZ. Moreover central administration of STZ, by icv injection, has been used as a model of sporadic AD to induce central alterations, such as tau phosphorylation, neurodegeneration, blood brain barrier dysfunction or cognitive impairment, as observed in AD and VaD patients. Taking into account these considerations in this study we will review recent literature where central alterations, resembling AD and VaD, have been described, both after systemic and central administration of STZ, and we will analyze the utility of STZ as a model of sporadic AD.

1. INTRODUCTION

1.1. Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of dementia, accounting for ~50-70% of all dementia cases, and leads to irreversible cognitive and memory impairment. In 2006 about 26.6 million people suffered from AD all over the world, with an associated cost of ~220 million euros (Wimo et al., 2006), and in 2050 more than a 100 million people are expected to be affected by AD (Brookmeyer et al., 2007). Neuropathological features include: 1) neurofibrillary tangles, 2) senile plaques and 3) synaptic loss (Hyman & Trojanowski 1997). Neurofibrilary tangles are intraneuronal deposits composed primarily by abnormally phosphorylated tau protein. Tau is phosphorylated at different sites as disease progresses and phosphorylation state of tau results from a coordinated balance between kinase-mediated phosphorylations of tau and dephosphorylation by protein phosphatases (Clodfelder-Miller et al., 2006), that leads to neurofibrillary tangle formation and deposition. Tau deposition increases in parallel with the duration and severity of the illness (Hyman & Gomez-Isla 1996), however previous studies also indicate that neurofibrillary tangles are the latest pathological feature in the development of the illness (Woodhouse et al., 2005, Gong et al., 2010). Senile plaques, mainly composed by peptide beta-amyloid (Aβ), are a pathological feature extremely frequent in AD brains although Aβ can also deposit as cerebral amyloid angiopathy (CAA) surrounding brain vessels. Aβ is a 39-43 aminoacid peptide derived of the progressive cleavage of the Aβ precursor protein (APP) by β and γ secretases. Pathology and, dynamic formation and remodelation of senile plaques, as well as the specific implication of Aβ are not fully understood, although Aβ in different aggregation states, as well as compact senile plaques, are neurotoxic both in AD and experimental animal models (Urbanc et al., 2002), and they have been suggested to induce synaptic loss and dystrophic neurites (Brendza et al., 2005, Larson et al., 1999, Lombardo et al., 2003). Senile plaques have also been associated with abnormal neuritic curvature (Craft 2009, Knowles et al., 1999, Meyer-Luehmann et al., 2008, Spires et al., 2005) and may alter cortical synaptic integration (Stern et al., 2004). Neuronal loss is the pathological feature that best correlates with duration and severity of the illness. In this sense neuronal loss of >50% is observed in the associative cortex in AD patients (for review see (Serrano-Pozo et al., 2011, Gomez-Isla et al., 1997).

Although the ultimate neurotoxic mechanisms have not been completely elucidated recent evidence supports a biphasic evolution of AD, including an amyloid dependent and amyloid-independent stages of AD. Following this idea in an initial phase disruption of the neuropil, loss of dendritic spines, remodelling of neurites, and inflammatory responses would derive from soluble oligomeric and fibrilar Aβ accumulation, the second phase would consist

of the further development of tangles, synaptic and neuronal loss (Hyman 2011). At present AD has no successful treatment and current pharmacological approaches are limited to anticholinesterase drugs, such as donezepil, and glutamatergic antagonists like memantin. On the other hand active Aβ imunization has been widely explored, and senile plaques and Aβ remain a major therapeutic target, however the incidence of meningoencephalitis in AD immunized patients (6%) has significantly limited this approach (Orgogozo et al., 2003). At this point we cannot obviate that senile plaques and Aβ levels only characterize parts of a complex combination of pathological features, and the presence of soluble microscopic oligomeric forms of Aβ, could underlined cognitive alterations (He et al., 2013), since they likely contribute to the progressive neural system failure that occurs over decades (Serrano-Pozo et al., 2011).

Mutations of APP, or the proteolytic enzymes that generate Aβ, presenilins 1 and 2, are responsible for most of the familiar AD cases. However the vast majority of AD cases (~95%) are sporadic (Harman 2006) and the ultimate causes have not been elucidated. Following this idea heterogeneous associated aspects and pathological features have been observed among patients (Bhat 2010) and, although age remains the main risk factor to suffer dementia, recent studies have focused on metabolic alterations such as hypertension (de Leeuw et al., 2002, Reitz et al., 2007), hypercholesterolemia (Pappolla 2008, Pappolla et al., 2003), hyperhomocysteinemia (Seshadri et al., 2002) or arteriosclerosis-related factors (Honig et al., 2005). Interestingly, hyperinsulinemia and diabetes display a strong association with dementia (Luchsinger et al., 2004, Schrijvers et al., 2010) and the risk factor to suffer AD seem to largely increase in diabetic patients (Craft 2009).

1.2. Vascular Dementia

Vascular dementia (VaD) has been traditionally considered the second most common cause of progressive and irreversible dementia; although Lewy body dementia is also considered the second leading cause of dementia by some experts (Gorelick & Nyenhuis 2013). VaD is an heterogeneous pathology that can range from multiple microinfacts to small vessel ischemic disease or to microvascular injury (Craft 2009, O'Brien 2006), all of which might be triggered and have been associated with Aβ deposition as CAA in cerebral blood vessels (Greenberg et al., 2008). Classification of VaD is based on clinical differences and pathological changes, however in practice it is difficult to classify a patient into a specific subtype of VaD, since they usually present mixtures or pathology that contribute to cognitive impairment (Chen et al., 2013a, Moorhouse & Rockwood 2008). The borderlines between AD and VaD are thus blurred and in many patients markers of vascular injury coexist with traditional AD hallmarks. Furthermore strokes and AD often occur concomitantly and pose risks for one another (Gorelick & Nyenhuis 2013). Folowing this idea, considerable evidence indicates that stroke increases the risk of dementia (Ivan et al., 2004). In some cases AD features might be promoted by a specific form of vascular injury; for example blood brain barrier dysfunction may affect Aβ transport between brain and periphery, and thereby contribute to parenchymal and neurovascular Aβ deposition (Craft 2009). On the other hand AD pathology may cause vascular injury, as when Aβ-induced inflammation damages the endothelium. The pathological consequences of vascular damage include alteration of functional markers, such as increased reactive oxygen species (ROS) or increased matrix-

metalloproteinases (MMPs) activity (Garcia-Alloza et al., 2009). All these processes have been related in a different extent to neuronal death (Brown et al., 2009, Zhang & Murphy 2007). Although the ultimate cause of VaD is not clear, many studies have pointed out the significant effect of metabolic disorders on VaD, and type 2 diabetes (T2D) might play a significant role at this level (for review see (Gorelick 2004, Roman 2005, Craft 2009)).

1.3. Diabetes Mellitus

Diabetes mellitus is one of the most prevalent diseases in western countries. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). Type 1 diabetes (T1D) is a autoimmune disease characterized by pancreatic β-cells apoptosis, lack of insulin and hyperglicemia (Lee 2013) however about 90% of all the cases are type 2 diabetes (T2D) (Gotz et al., 2009). Since T2D prevalence increases with age, the progressive aging of population seems to indicate that the "diabetes epidemic" will continue (Wild et al., 2004). T2D is characterized by an initial stage of insulin resistance and hyperglycaemia. In order to compensate this deficit, β-pancreatic cells respond by increasing insulin production. Insulin secretion occurs in tandem with amylin, also implicated in the control of glucose levels. When β-pancreatic cells are exhausted and can no longer overproduce insulin, T2D evolves. T2D is associated with other peripheral alterations such as hypertension, cardiovascular disease, dyslipidemia or hypercholesterolemia, that have been addressed in detail both in basic science studies (Jelinek et al., 2011, Russell & Proctor 2006) and in epidemiological and clinical studies (35; 37). In recent years more and more studies have focused on the relationship between diabetes and central nervous systems alterations, with special interest in neurodegeneration and dementia, although the mechanisms implicated have not been elucidated.

2. Link Between Diabetes and Alzheimer´s Disease and Vascular Dementia

Association between T2D and dementia has been studied in the last decades and in the Rotterdam study Ott et al., (Ott et al., 1996) observed that T2D patients had a relative risks of 1.4 to suffer AD, duplicating this value in case of VaD. Other epidemiological studies have supported these data (Luchsinger et al., 2004, Plastino et al., 2010). It also seems that a combination of vascular risk factors, such as heart disease, hyperlipidemia, hypercholesterolemia, smoking, or more relevantly T2D, increase the risk to suffer AD (Luchsinger et al., 2005). Moreover, insulin levels and insulin resistance seem to be the parameters that best correlate with a higher risk to develop AD (Schrijvers et al., 2010). On the other hand mouse models of T1D also display similar patterns of peripheral neuropathy, with decreased motor nerve conduction velocity, to those observed in AD models (Jolivalt et al., 2012). Some relevant links between diabetes and AD have contributed to the association of both pathologies and the description of a complex syndrome defined as type 3 diabetes (T3D) (de la Monte & Wands 2008, Steen et al., 2005). Among others: 1) Early studies by Frolich et al., (Frolich et al., 1998) showed that brain insulin receptor densities were altered in

sporadic AD patients. Further studies have confirmed these findings, and extensive abnormalities in insulin and insulin-like growth factor (IGF) type 1 (IGF-1) and 2 (IGF-2) signaling mechanisms have been documented (Steen et al., 2005). 2) Central nervous system insulin receptors, located in astrocytes and neuronal synapses, are highly expressed in the basal forebrain, origin of the cortical and hippocampal cholinergic innervation. Cholinergic system seems to be of special vulnerability in AD, and cholinergic loss of the basal forebrain seems to be a good predictor of the clinical dementia in AD (Roberson & Harrell 1997, Schliebs & Arendt 2006, Walsh & Selkoe 2004). On the other hand insulin receptors are also expressed in relevant regions for learning and memory, such as cortex and hippocampus. This is consistent with evidence showing that insulin influences memory (Craft 2009), likely due to modulation of synaptic structure and function, long-term potentiation and central nervous system levels of neurotransmitters such as acetylcholine, of special relevance in AD (Roberson & Harrell 1997, Schliebs & Arendt 2006). 3) T2D progression correlates with pancreatic amylin deposition, in a similar way to Aβ deposition in AD brains. Moreover insulin, amylin and Aβ are degraded by neprelysin (NEP) and insulin degrading enzyme (IDE), and both NEP and IDE substrates can compete with each other. It has been postulated that an imbalance of substrates can affect the degradation rate of other substrates and possibly influence the pathogenesis of AD and T2D (Gotz et al., 2009). Following this idea it has been shown that insulin increases extracellular Aβ, both by increasing its secretion and by inhibiting its degradation by IDE (Qiu et al., 1998, Vekrellis et al., 2000). 4) Postmortem studies in human AD brains have shown reduced expression of the insulin protein and its mRNA levels, accompanied by reductions of IGF genes as well as their corresponding receptors (Lester-Coll et al., 2006, Steen et al., 2005). 5) Aβ oligomers may interfere insulin signaling in hippocampal neurons (Zhao et al., 2008). On the other hand insulin may regulate Aβ levels by modulation of β and γ secretases (Eckman & Eckman 2005, Farris et al., 2003). Accordingly, reduced brain insulin signaling increases Aβ levels in a mouse model of T1D (Jolivalt et al., 2008). 6) Also insulin seems to participate in cerebrovascular regulation by multiple mechanisms, including endothelium-dependent mechanisms, nitric oxide or cyclooxygenase activity, among others, linking metabolic disorders with VaD associated pathology (Correia et al., 2011). 7) Extensive evidence supports Aβ toxicity in different states of aggregation (Frydman-Marom et al., 2011, Meyer-Luehmann et al., 2008, Spires et al., 2005, Urbanc et al., 2002, Yao et al., 2011) and it seems that amylin, similarly to Aβ, can induce apoptotic cell death (Konarkowska et al., 2006, Matveyenko & Butler 2006). It is likely that amylin and Aβ aggregates alter cellular function by similar mechanisms, such as inducing ROS (Craft 2009). Following this idea hyperglycemia enhances the formation of advanced glycation end products, which by interacting with their receptor elicits the formation of ROS, that are also believed to be an early event in AD pathology (Guglielmotto et al., 2012). 8) Insulin has also been linked to tau phosphorylation, as a major pathological feature observed in AD, since insulin has been shown to activate kinases involved in tau phosphorylation such as GSK-3 (Yang et al., 2013b). Also, in a T1D animal model reduced brain insulin signaling results in increased tau hyperphosphorilation (Jolivalt et al., 2008). Altogether it seems clear that insulin plays a crucial role in maintaining brain normal activity, and alterations of insulin dependent functions could be associated with central pathological conditions, observed in sporadic AD and VaD (Correia et al., 2011, Craft 2009).

Overally, these data have lead to the description of a complex syndrome: type 3 diabetes mellitus (T3D) as a consequence of the relationship between T2D-AD-VaD (figure 1). Albeit

all the circumstantial links mentioned above, experimental data supporting a direct relationship between T2D, EA and VaD are limited, mostly because 1) studying the mechanistic relationship of insulin resistance to AD and VaD is hampered by the complexity of its measurement, 2) animal models are limited, and 3) diabetes is a complex disorder and so it is likely that multiple different, synergistic processes may interact to promote cognitive impairment (Craft, 2009; Strachan et al., 2008). Therefore the vast majority of studies are epidemiological, in which T2D is identified as a risk factor to suffer AD or VaD (Luchsinger et al., 2005, Luchsinger et al., 2004, Matsuzaki et al., 2010, Schrijvers et al., 2010). It should also be pointed out that studies with T2D patients and clinical diagnosis of AD or VaD have faced some challenges (for review see Craft, 2009): patients with diabetes are often presumed to have dementia of vascular origin and the effects of treatments for T2D may affect AD markers and vascular integrity (Craft 2009). Therefore in order to accurately delineate the pathogenesis of cognitive impairment in people with T2D, large-scale, prospective epidemiological studies are still required (Strachan et al., 2008).

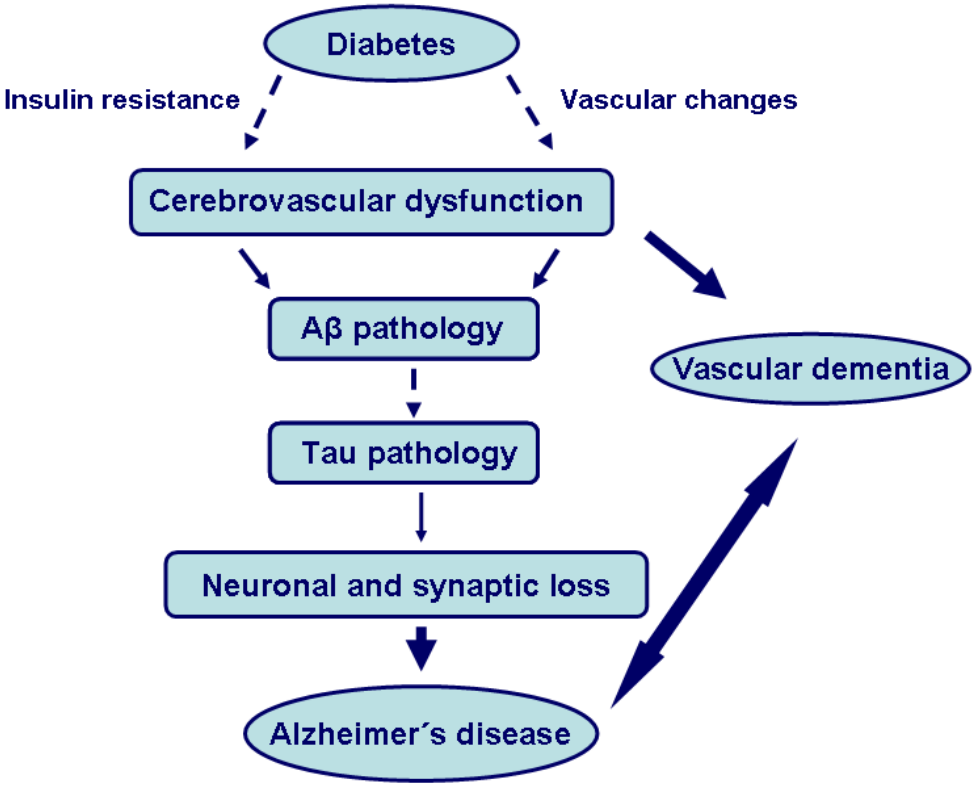

Figure 1. Schematic review or the relationship between diabetes, Alzheimer's disease and vascular dementia. Adapted from Bhat, 2010. © 2010 The Author. Journal of Neurochemistry © 2010 International Society for Neurochemistry.

3. TRANSGENIC AND KNOCK-OUT ANIMAL MODELS OF TYPE 2 DIABETES, ALZHEIMER´S DISEASE AND VASCULAR DEMENTIA

To our knowledge, there are only a handful of studies where an effort has been made to set T2D and AD together. Takeda et al., (Takeda et al., 2010) have developed a mixed T2D and AD model, crossing a model of T2D: ob/ob mice (leptin-defficient mice) or NSY mice, and a model of AD: APP23 mice, that presents Aβ deposition with aging. This study supports the cross-talk between T2D and AD. T2D worsened cognitive performance as well as cerebrovascular inflamation and CAA in the generated mice, whereas parenchymal Aβ burden was not altered. On the other hand Takeda et al., (2011) also showed that AD may interfere with natural evolution of T2D, since cross-bred mice showed an accelerated diabetic phenotype compared with ob/ob mice, suggesting that cerebrovascular changes and alteration in brain insulin signaling might play a pivotal role in the relationship between T2D and AD. Whereas an extremely novedous and promising animal model, mice were used at relatively young ages and little is stated about long-term effects, while both T2D and AD are chronic diseases closely influenced by age. In fact APP23 mice need long life span to develop the illness. A recent study (Hiltunen et al., 2012) has also observed learning impairment in pancreatic IGF-2 overexpressing mice crossed with APPswe/PS1dE9 mice. APP/PS1 mice (Jankowsky et al., 2004) are a relevant and widely used model of AD, that shows robust Aβ deposition by 6 months of age (Garcia-Alloza et al., 2006b). However IGF-2 overexpressing mice are a limited model of T2D, since they display mild hyperglycemia, and only about 30% of the mice develop overt diabetes when fed a high fat diet (Devedjian et al., 2000). These aspects may account for the fact that significant characteristics of AD, such as Aβ burden, are not affected in IGF-2XAPP/PS1 mice (Hiltunen et al., 2012). Also a recent approach includes crossing db/db mice (leptin receptor KO mice) as a model of T2D with APPswe/PS1dE9 mice (Jimenez-Palomares et al., 2012). Although to our knowledge these mice have not have been characterized at central level, it seems that the presence of the APP/PS1 transgenes is enough to worsen metabolic parameters in heterozygous db/db mice, that otherwise would not present metabolic dysfunction.

Whereas extremely useful all these models are based on transgenic and knock-out mice and only a small number of AD cases (Serrano-Pozo et al., 2011) and diabetes, as well as associated metabolic alterations (Saxena et al., 2007, Weedon & Frayling 2007), are of familiar origin. Therefore exploring other animal models s might be of great value in the study of the relationship between diabetes, AD and VaD.

4. PHARMACOLOGICAL MODEL: STREPTOZOTOCIN

Taking into account previous considerations, including the necessity to have animal models that reproduce sporadic AD, as well as the close relationship between T2D and AD-VaD, the use streptozotozin (STZ) (2-deoxy-2-(3-(methyl-3-nitrosoureido))-D-glucopyranose) might provide a different approach to address these aspects. STZ is a β-pancreatic cells toxin isolated from the bacterium Streptomycetes achromogens (Nugent et al., 2008). STZ has been classic pharmacological tool to induce in rodents both T1D and T2D, depending on the administration protocols used (Patel & Bhadada 2013, Ding et al., 2013, Clodfelder-Miller et

al., 2006). In this sense peripheral administration at high doses completely destroys β-pancreatic cells, causing T1D, lower doses of STZ cause insulin resistance by damaging insulin receptors, resembling T2D (Salkovic-Petrisic et al., 2013a). It also seems that as a T2D models, a more complete version of the disease is observed when STZ is administered in combination with long-term high fat diet (Srinivasan et al., 2005, Zhang et al., 2008). It has been suggested that this might be more representative of westernized diet-associated diabetes, while an alternative to genetic models (Nugent et al., 2008). STZ has been widely used as a tool to induce diabetes, and central alterations associated to the model have also been observed, both after peripheral and central administration of the drug, resembling alterations observed in dementia processes, including AD and VaD. However it has been in the last two decades that central STZ has been considered as a suitable model for T3D, after icv injection (de la Monte & Wands 2008, Salkovic-Petrisic et al., 2013a).

4.1. Alzheimer´S Disease and Vascular Dementia-Like Complications Associated to Peripheral Administration of STZ

STZ has been a classical model to induce diabetes, therefore initial observations regarding central pathology respond to the peripheral administration of the drug used to induce T1 or T2D. STZ itself cannot pass through the blood-brain barrier due to the absence of the STZ transporter GLUT2 at this level. Therefore, the systemic-injection consequences relay on the effect of systemic hypoinsulinemia on the brain (Park 2011). Whereas outcomes at central level have been quite reproducible, many different administration protocols have been used in previous studies, ranging from various medium doses (50 mg/Kg) (Ramos-Rodriguez et al., 2013) to a single very high dose of STZ (70 mg/kg) (Revsin et al., 2009). As previously stated insulin resistant conditions do not only affect peripheral tissues, but also brain function can be significantly affected. In this sense original studies on brains from peripherally STZ injected mice already showed some pathological features, including focal accumulation of collagen fibrils in the basement membranes of arteriole and capillary walls (Mukai et al., 1980), similar to those observed in some variants of VaD in humans (Burke et al., 2013, Craggs et al., 2013). Following this idea, peripherally administered STZ also impairs endothelium function, and it has also been considered a model of VaD (Sharma & Singh 2011, Sharma & Singh 2010). More recently it has been observed that vascular alterations in C57Bl6 mice treated with STZ lead to spontaneous small vessel hemorrhages (Ramos-Rodriguez et al., 2013) as observed in figure 2, showing a severe case of VaD-like vascular dysfunction. On the other hand oxidative stress related macro and microgangiopathy may induce neuronal damage caused by chronic intracellular hyperglycaemia, leading to increased production of ROS in the brain, and reducing antioxidant capacity in STZ treated rats (Tahirovic et al., 2007) as observed in AD animal models and Alzheimer's patients (Dumont & Beal 2011, Garcia-Alloza et al., 2010, Garcia-Alloza et al., 2006a). Also, random degeneration of neuronic cells has been observed in STZ treated rats (Mukai et al., 1980) and a significant cortical atrophy has been observed in STZ treated mice (Ramos-Rodriguez et al., 2013), supporting similar findings to those detected in AD patients. In humans estimates of tissue damage, or loss in characteristically vulnerable brain regions, such as the hippocampus and entorhinal cortex are predictive of progression of middle cognitive impairment to AD (Frisoni et al., 2010).

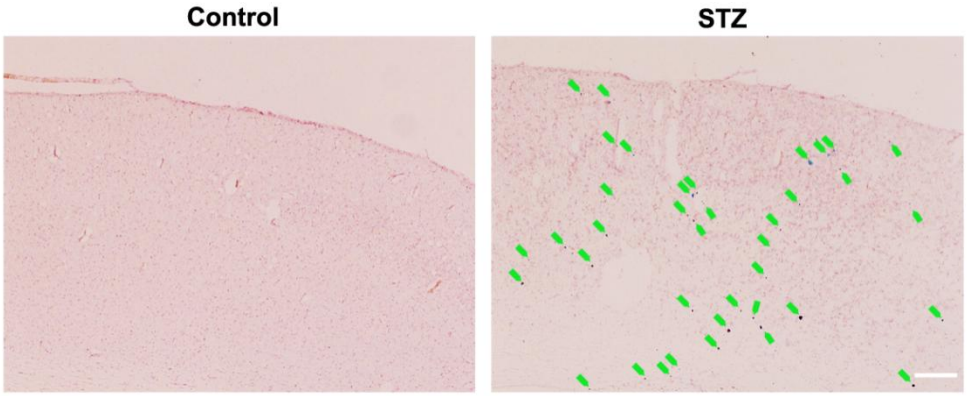

Figure 2. Illustrative example of the presence of hemorrhages in the cortex of C57Bl6 mice 8 weeks after ip STZ injection (50 mg/Kg, 5 consecutive days) stained with Prussian blue, and counterstained with neutral red. Green arrows point at hemorrhages and a great amount of spontaneous bleeding can be observed in STZ treated mice. Scale bar=250 μm.

Other studies have also reported that insulin deficiency, after STZ administration to amyloid models of AD may worsen AD-like pathological features. In this sense, STZ may accelerate amyloid pathology in APP mice, by increasing total Aβ levels and senile plaques deposition (Jolivalt et al., 2010). Further assessment in other AD mice have shown similar results, and increased senile plaques deposition has been observed in APP/PS1 mice as well as enhanced APP processing, including increased levels of APP695 protein, BACE1 and APP cleavage fragments (sAPPα, sAPPβ and CTFs) (Wang et al., 2010). A similar profile has been observed in 5xFAD mice, in which increased APP processing, due to higher BACE1 levels, has been observed accompanied by increased Aβ40 and Aβ42 levels (Devi et al., 2012). Also, in a mouse model presenting both Aβ and tau pathology (3xTg-AD mice) an increase in brain APP and Aβ levels was observed after peripheral STZ challenge (Li et al., 2010), supporting the capacity of STZ to interfere with APP processing.

When tau pathology has been assessed in AD mice after STZ treatment, slight differences have been observed among animal models. Whereas no effect was detected in 3xTg-AD mice (Li et al., 2010), a significant increase in tau phosphorylation at the threonine 231 site (part of the microtubule-binding domain), and at Ser199/202 (a marker for neurofibrilary tangles), has been observed, probably mediated by reduced GSK3β phosphorylation at the recognized inactivating site (ser 9) (Jolivalt et al., 2010). Tau hyperphosphorylation increases as disease progresses, in particular at pathologic sites, such as pS422, concomitant with neurofibrillary tangle formation. Increased tau phosphorylation at this residue has been observed in pR5 mice, that express the longest human tau isoform carrying the pathogenic P301L mutation (Ke et al., 2009). In this animal model a significant increase in pS422 phosphorylation was detected in the amygdala, indicating that more NFTs may have been induced by the STZ treatment than are normally found at this age. Further assessment of neurofibrillary tangles by Gallyas silver impregnation revealed advanced deposition of fibrillar tau in STZ-treated pR5 mice (Ke et al., 2009). Following this idea rats on high fat diet, high protein and high glucose diet, in combination with intraperitoneal injection of STZ presented an increase in tau hyperphosphorylation, decreased AKT activation, and GSK-3β over-activation (Yang et al., 2013a).

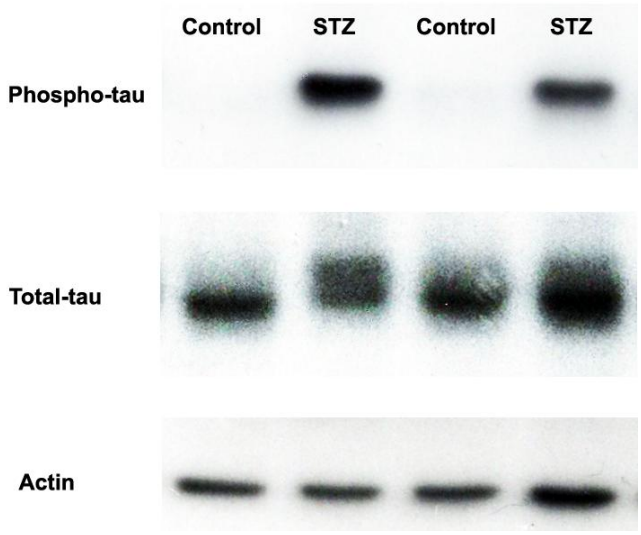

Figure 3. Example of cortical tau hyperphosphorylation by western blot in C57Bl6 mice, 8 weeks after ip STZ injection (50 mg/Kg, 5 consecutive days), using 4T8 antibody. Whereas total tau and actin levels remained unchanged a significant increase in phosphotau is observed in STZ treated mice.

Since phosphorylation state of tau results from a coordinated balance between kinase-mediated phosphorylations of tau and dephosphorylation by protein phosphatases Clodfelder-Miller et al., (Clodfelder-Miller et al., 2006) carried out an extensive assessment of different tau phosphorylation sites and observed accompanied changes in tau kinases, including inhibition of GSK3, by increased phosphorylation on serine-9, as well as increased phosphorylation of both p38 and JNK, indicating activation of these two kinases in mice (Clodfelder-Miller et al., 2006). Also phosphatase PP2A activity, that dephosphorylates multiple residues of tau, was significantly reduced. This study also showed that the whole process, leading to increased tau phosphorylation, is mediated by insulin, since insulin administration after short-term insulin deficiency, provoked by STZ treatment, reduced tau hyperphosphorylation at selective sites (Clodfelder-Miller et al., 2006). The observed changes in phosphatases and kinases, accompanied by the increased phosphorylation of tau on multiple sites, suggested that STZ may impair the natural history of tau, contributing to tau hyperphosphorylation (Clodfelder-Miller et al., 2006). When STZ has been administered to wildtype animals, (Ramos-Rodriguez et al., 2013) a significant increase in tau phosphorylation has also been observed, and although no tangles have been observed, these data support the inductive role of STZ in tau pathology as observed in figure 4.

Although Aβ and tau pathology, as well as neuronal and synaptic loss are the pathological features of AD, cognitive impairment remains the main manifestation of the disease, and following this idea previous studies have also focused on learning and memory alterations associated to peripheral administration of STZ. In this sense it has been well established that STZ-induced diabetes also impaired learning and memory abilities in rodents when tested in different paradigms, including active avoidance tests (Flood et al., 1990, Alvarez et al., 2009) or the Morris water maze test (Ramos-Rodriguez et al., 2013), where significant learning and memory dysfunction has been observed, with altered pathways along the acquisition phase of the test, as observed in figure 5. The close relationship between diabetes and Alzheimer has also been explored in AD mice after STZ treatment, and

significantly worse performances in learning and memory processes have been observed (Wang et al., 2010), supporting a synergistic effect between both pathologies. In combination with electrophysiological studies, learning and memory disabilities observed in STZ-treated mice seem to be mediated by alterations in long-term potentiation and depression (Biessels et al., 1996, Artola et al., 2005), whereas hippocampal neurogenesis and synaptic plasticity also seems to be affected (Stranahan et al., 2008). It has also been observed that detected deficits might be recovered after insulin administration, and many other pharmacological approaches have been also shown to have some beneficial effects, including antioxidants such as Ginkgo biloba extract, vitamin E or resveratrol (Hoyer et al., 1999, Tuzcu & Baydas 2006, Schmatz et al., 2009, Comin et al., 2010), non-steroidal anti-inflammatory drugs such as aspirin (Wang et al., 2011) or histone deacetylases ihnibitors such as sodium butyrate (Sharma & Singh 2011).

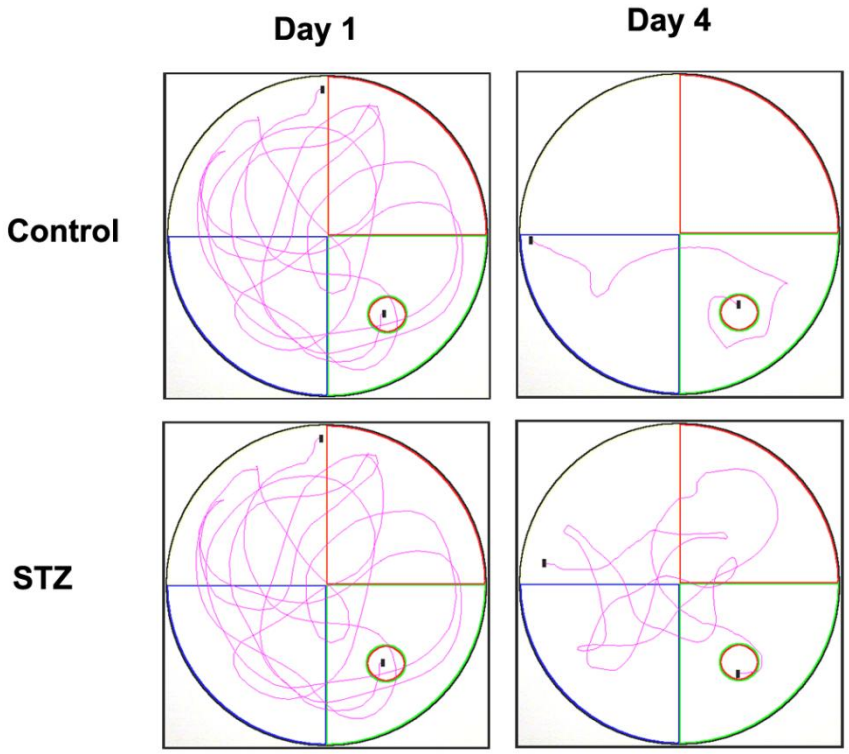

Figure 4. Representative Morris water maze pathways during the first day and the last day of the acquisition phase, both in C57Bl6 control and STZ treated mice (50 mg/Kg ip injection,5 consecutive days). Significantly longer pathways can be observed in STZ treated mice to reach the hidden platform, as an indication of the impairment of these animals to learn and remember the localization of the hidden platform.

4.2. Alzheimer´S Disease and Vascular Dementia-Like Complications Associated To Intracerebroventricular Administration of STZ

As shown above, peripheral administration of STZ reproduces or aggravates some of the pathological features observed in AD and VaD, however central delivery of STZ has also been explored, in order to provoke central diabetes, without affecting pancreatic cells.

Intracerebroventricularl (icv) STZ delivering has been used and considered as a model of sporadic AD or T3D, in which it is observed a brain type of non-insulin dependent diabetes, or cerebral diabetes (de la Monte & Wands 2008, Lester-Coll et al., 2006). As previously stated STZ cannot cross the BBB although since GLUT2 receptor are distributed heterogeneously in the mammalian brain, therefore when icv administered, STZ can selectively reduce insulin central levels without affecting insulin and glucose levels in the periphery (Park 2011). Therefore mechanisms, implications and consequences of peripheral or central administration of STZ might be significantly different (Salkovic-Petrisic et al., 2013a). After central STZ administration IGF-1 receptors are reduced, supporting that insulin signaling is impaired in a similar way to that observed in AD patients, where both insulin production and insulin resistance are altered in the brain (Steen et al., 2005, Park 2011). Central STZ administration has been widely used in the last few years, specially in rats, and a recent study systematically compares doses and post-administration end points showing that although at extremely low doses, when compared with peripheral administration, 1mg/kg of STZ icv injected might not be enough to induce cognitive deficits required in the model (Mehla et al., 2013).

Different studies in rodents have shown that local administration of STZ results in central metabolic alterations (Hoyer et al., 1994, Lannert & Hoyer 1998, Labak et al., 2010), including a reduction of glucose utilization in different brain regions (Duelli et al., 1994) or alterations in enzymes implicated in glycolisis and glucogenolysis (Plaschke & Hoyer 1993). Altered glucose metabolism has also been observed, by high-resolution micro-PET 18F-deoxyglucose, in non-human primates after icv infusion of STZ and cortical distribution of glucose hypometabolism seems to resemble that observed at early stages of AD patients (Heo et al., 2011). These authors also detected an increase of sulcal markings by MRI at six weeks post-injection, suggesting brain atrophy, as observed in Alzheimer's patients. Previous studies have also shown neuronal damage and brain atrophy in rodents. In this sense shrinkage of the dorsal hippocampus and adjacent fornix has been observed after icv STZ injections, as well as ventricle enlargement and gliosis in myelinated periventricular brain structures (Weinstock et al., 2001, Shoham et al., 2003). Hippocampal neuronal density has also been observed to be reduced in STZ icv injected rats and it has been suggested that this effect could be mediated by cyclooxygenases 1 and 2 isozymes activity (Dhull et al., 2012). Other STZ studies have also reported septal and white matter atrophy. The basal forebrain, that includes the septum, seems to be a region deeply affected in AD (Teipel et al., 2005) and observed atrophy by MRI was accompanied by a significant reduction of neuronal density in this region, without affecting other brain areas as the striatum (Kraska et al., 2012). Functional manifestation of central alterations have also been observed in icv STZ injected models, and hippocampal synaptic transmission impairment and long-term potentiation alterations have been detected, associated to alterations in integrin-linked kinase-GSK-3-β signaling. This effect may decrease the trafficking and function of postsynaptic glutamate receptors; thereby, leading to synaptic deficits (Shonesy et al., 2010). Other indicative markers of central damage, similar to those also observed in AD patients, have also been detected after icv administration of STZ in rats, including increased oxidative stress and presence of ROS as well as brain reduced antioxidant capacity (Sharma & Gupta 2001, Lester-Coll et al., 2006, Tahirovic et al., 2007).

When tau pathology has been addressed, a significant increase of total tau and phospho tau levels have been observed in the hippocampus of STZ-treated rats (Salkovic-Petrisic et al., 2006, Grunblatt et al., 2007) and similar observations have been made in mice (Chen et

al., 2013b), without neurofibrillary tangles deposition. When icv STZ has been administered to a classical AD model as it is the tg2576 mouse, an increase of total tau protein, associated with decreased phosphorylated/total tau ratio has been observed (Plaschke et al., 2010) whereas in 3xTg-AD an increase in tau phosophorylation has been reported (Chen et al., 2013c), suggesting slight differences depending on the animal models and specific experimental approaches used. On the other hand Salkovic-Petrisic et al., have also shown the presence of Aβ aggregates stained with Congo Red both in blood vessels, resembling amyloid angiopathy, and cerebellar plaques in rats icv injected with STZ (Salkovic-Petrisic & Hoyer 2007). This group has recently shown that amyloid angiopathy deposition is observed in wildtype rats up to 9 months after STZ injections, and histological confirmation has included thioflavin S and Congo red staining, as well as in immunohistochemistry studies with anti-Aβ antibodies (Salkovic-Petrisic et al., 2011). Also other studies have shown increased Aβ immunoreactivity in the hippocampus from STZ treated rats (Shingo et al., 2012), as well as increased Aβ levels in different brain regions (Santos et al., 2012) supporting the capacity of the icv STZ model to provoke amyloid pathology, similar to that observed in AD patients. In a transgenic mouse model of AD (tg2576) increased cerebral aggregated Aβ fragments and congophilic amyloid deposits was observed supporting a synergistic effect between icv injected STZ and the overexpression of APP (Plaschke et al., 2010).

Apart from typical neuropathological features associated with AD and VaD, a great deal of attention has been paid to learning and memory alterations observed in icv STZ injected rodents in different paradigms, as well as to the role of therapeutic alternatives to revert observed deficits. In this sense icv injection of STZ in rats results in working and reference memory dysfunction in the holeboard task and the passive avoidance paradigm (Lannert & Hoyer 1998, Salkovic-Petrisic et al., 2013b). Similar alterations have been observed in the delayed non-matching to position task and in working and spatial memory assessed in the Morris water maze test (Prickaerts et al., 1999, Salkovic-Petrisic et al., 2013b), the Y-maze test and novel object recognition tests (Liu et al., 2013). Observed cognition impairment after icv administration of STZ to rats have also been reported in mice, and a worsening effect is observed when STZ is administered to transgenic AD models, such as 3xTg-AD mice (Chen et al., 2013c) or APP overexpressing mice (Plaschke et al., 2010). Since the lack of treatment for AD or VaD remains one of the most relevant issues in the study of the dementia processes, many therapeutic approaches have been used in the icv STZ injected model, and significant improvement of tau phosphorylation and cognitive impairment has been widely observed (for review see (Salkovic-Petrisic et al., 2013a). Following this idea, antioxidant treatments, including resveratrol (Sharma & Gupta 2002), curcumin (Ishrat et al., 2009) or catechin (Ejaz Ahmed et al., 2013) have been shown to improve cognition in STZ treated mice. Also, treatment with statins (Dalla et al., 2010, Tramontina et al., 2011) or ciclooxigenase inhibitors (Dhull et al., 2012) have been shown to recover cognitive impairment associated to icv STZ injection. Moreover presently approved drugs for AD may improve learning and memory dysfunction observed after STZ icv injection, including anticholinesterasic drugs (Sonkusare et al., 2005) and memantine (Ponce-Lopez et al. 2011). The fact that many different pharmacological approaches may help to recover cognitive impairment, and associated central dysfunction after icv STZ administration, supports the multiple and complex pathological pathways affected by STZ, in a similar way to those observed in AD patients.

Altogether, animal models for AD and VaD present evident limitations, and although transgenic mice have proved to be extremely useful to study associated pathology and cognitive impairment, icv injected STZ seems to provide a different approach that resembles biochemical, morphological and cognitive effects associated with an insulin-resistant brain state, similar to that observed in AD patients. On the other hand, since additional treatment options are urgently needed for AD population (for review see (Sabbagh & Cummings 2011) STZ might prove to be a rational animal model in the search of new therapeutic alternatives.

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