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Mini review

Amyloid cascade hypothesis: is it true for sporadic Alzheimer's disease

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Abbreviations:

AD	– Alzheimer's disease;
sAD	– sporadic AD;
APP	– amyloid precursor protein;
Aβ	– amyloid-β peptide;
PS1/PS2	– presenilin 1 / 2;
IR	– insulin receptor;
TK	– tyrosine kinase;
IRS	– insulin receptor substrate;
PI3K	– phosphatydilinositol-3 kinase;
Akt/PKB	– Akt/protein kinase B;
GSK-3	– glycogen synthase kinase-3;
IDE	– insulin degrading enzyme;
STZ	– streptozotocin;
Icv	– intracererbroventricular

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder in which reliable early clinical diagnosis is impossible. Early-onset familial AD form, caused by mutations of genes involved in Aβ pathology, and prevailing late-onset sporadic AD (sAD) having age, diabetes type 2 and apolipoprotein E4 as risk factors, demonstrate convergent clinical (memory loss) and neuropathological (amyloid β /Aβ/ and tau protein) changes. Leading amyloid cascade hypothesis assumes that Aβ pathology is the primary cause of both AD forms, whereas other neuropathological changes are just downstream consequences. Transgenic mice AD models that are most widely used for AD pathophysiology research are designed to express human Aβ-production proteins containing different mutations from their birth. Because of that transgenic mice could represent familial AD forms only, while for the sAD, the streptozotocin-intracerebroventricularly (STZ-icv) treated rats were proposed. STZ is a substance selectively toxic for peripheral insulin producing/secreting cells and insulin receptor (IR). STZ-icv application induces AD-like changes; cognitive deficits, reduction in brain glucose/energy metabolism and cholinergic transmission, as well as gliosis and oxidative stress. Additionally, STZ-icv treatment induces time-dependent development of brain IR signaling cascade dysfunction leading to increased activity of glycogen synthase kinase-3 which results in Aβ (angiopathy) and tau (hyperphosphorylation) pathology. These findings suggest that development of insulin resistant brain state precedes and triggers Aβ pathology in sAD, challenging thus the amyloid cascade hypothesis when sAD is concerned. Further research is necessary to clarify this possibility of sAD ethiopathogenesis since it may reveal new AD therapeutic strategies towards to disease-modifying drugs.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease clinically characterized by progressive memory loss. Clinical diagnosis of AD, particularly in its early stage, is actually an exclusion one and there is no direct objective and reliable diagnostic test for this disease which is why currently approved therapy is focused to the management of already present symptoms of AD. Most crucial for developing disease-modifying therapeutic strategies (not to mention preventive ones) is to understand the precise mechanisms by which the different pathological lesions originate, keeping in mind the divergent causes of AD. Namely, early-onset familial AD is inherited in autosomal dominant manner, caused by missense mutations in three chromosomes (http://www.molgen.ua.ac.be/ADMutations/) and genes related

to amyloid pathology (amyloid precursor protein gene /APP/, presenilin 1 /PS1/ and presenilin 2 /PS2/ gene), while late-onset sporadic AD (sAD) has age, diabetes type 2 and several other susceptibility genes (e.g. Apolipoprotein E4 /ApoE4/) proposed as risk factors). In spite of that, these two causally different forms converge in their main clinical (dementia) and neuropathological features, extracellular senile plaques composed of insoluble amyloid beta (Aβ) fibrilles, and intraneuronal neurofibrillary tangles composed of hyperphosphorylated form of microtubule associated protein tau. Currently leading amyloid cascade hypothesis assumes that pathological assemblies of Aβ are the cause of both AD forms, whereas other neuropathological changes are downstream consequences of pathological Aβ accumulation *(2)*. Since the brain analysis, the only reliable method for proving this hypothesis is possible post mortem only, and frequently in the severe late stage of AD cases, brain neurochemistry that characterizes the initiation of this disease in humans is mostly unknown. To understand fundamentally the complexity of the brain from the biochemical and physiological aspects, one needs not only *in vitro* models but also animal AD models which should be validated for known and general accepted characteristics of AD, behavioural defects, primarily in cognitive processes and memory loss, and brain lesions, primarily Aβ aggregation and/or plaques formation. By comparing some of the pathological aspects of currently the most exploited animal AD model, transgenic mice, and the less exploited streptozotocin-intracerebrovetricularly treated (STZ-icv) rat model, this review is aimed to provide the clue why the amyloid cascade hypothesis should be taken with caution when speaking about the ethiopathogenesis of sporadic AD form.

AMYLOID CASCADE HYPOTHESIS

The amyloid cascade hypothesis proposes that gradual aberrant accumulation of Aβ initiates a complex, multistep cascade of neuropathological events that leads to development of both AD forms, familial and sporadic one *(2)*.

Aβ is a 4 kDa protein that exhibits microheterogeneiety in amino acid sequence and in a variety of biophysical states. In a physiological condition, most of Aβ peptide is in the form of Aβ1-40 residues while less than 5% of the newly generated Aβ ends at residue 42, forming long form of Aβ1-42 peptide which is more prone to aggregation than Aβ1-40 form and is initiating formation of pathological oligomers, fibrils and plaques *(3)*. Oligomers and fibrils appear to be the most potent neurotoxins while the end stage senile plaques are relatively inert. Although it has been traditionally thought that extracellular Aβ aggregates in the form of senile plaques are the main pathogenic species, recent literature data recognizes that intraneuronal accumulation of the oligomeric non-fibrillar Aβ form precedes and contributes to the extracellular pathology *(4)*. Aβ is generated from mature APP being metabolized by two competing pathways, α-secretase pathway resulting in non-toxic products, and β-secretase leading to products which may be substrate for γ-secretase generating Aβ1-40/42 *(3)*. In physiological condition, the production and clearance of Aβ are balanced but in pathological case of increased production of total Aβ or increased Aβ1-42/Aβ1-40 ratio or in case of decreased Aβ degradation/clearance, Aβ1-42 levels are elevated. The production of more aggregatable Aβ1-42 form can be elevated by mutations in three different genes, APP, PS1 and PS2 that cause familial AD while decreased Aβ clearance can appear due to decreased expression of e.g. enzyme responsible for its removal, the insulin degrading enzyme (IDE), as found in sporadic AD *(3)*. Regardless the primary cause and clinical form of AD, the amyloid cascade hypothesis proposes that both conditions lead to Aβ1-42 accumulation, oligomerization and plaque formation, which further initiates a whole range of pathological cascade effects; microgliosis and astrocytosis, inflammatory response, oxidative stress, neuronal/neuritic dysfunction, cell death, neurotransmitter deficits, and finally, memory loss. In parallel, oxidative stress and neurotransmitter deficits induce kinase/phosphatase activity imbalance which at the level of tau protein (microtubule-associated protein that stimulates the generation and stabilization of microtubules within cells, and control axonal transport of vesicles /5/) results in accumulation of hyperphosphorylated tau protein and formation of neurofibrillary tangles which contribute to memory loss.

TRANSGENIC MICE MODELS

Transgenic mice are produced by the introduction of a human gene sequence into the mouse genome, resulting in expression of a human protein and they have played the revolutionary role in AD research *(as reviewed elsewhere 4, 6–8)*. The first transgenic mice model that developed AD features was the one which reproduced amyloid deposition by expressing human APP containing mutations associated with the early-onset familial AD form *(9)*. Evidence for the central role of APP in AD pathogenesis comes from the findings that it is a direct precursor of amyloid peptides and that the mutations in APP cause overproduction of amyloid peptides and development of the early-onset familial AD form *(10)*. This first APP transgenic mice (named »PDAPP«) demonstrated development of plaques around 6 months of age, accompanied by findings of dystrophic neurites, synaptic loss, gliosis and cytoskeletal abnormalities like accumulation of phosphorylated neurofilaments and tau but not in a form of neurofibrillary tangles *(7)*. Additional lines of mice expressing different mutant human APP transgene have been reported afterwards demonstrating time-dependent development of similar neuropathological characteristics among which the most widely used one has become the Tg2576 mouse *(11)*. It has become clear that such gene manipulations could generate different amyloid pathology, like transgenic mice (APP23) with predominant severe Aβ accumulation within the capillary wall, i.e. cerebral congophillic amyloid angiopathy (CAA) *(12)*. Among factors influencing the particular

amyloid pathology manifestation were background strain, promoter, human APP mutation, expression levels and importantly, ratio of Aβ1-40:Aβ1-42 production *(7)*. The selective overproduction of a particular Aβ form has been further improved by introducing »multiple« transgenic lines, mouse strains that harbour different combinations of human genes. Double transgenic mice co-expressing human APP and Presenilin (PS1) demonstrate the increased production of Aβ1-42 accompanied by earlier development of amyloid plaques *(13),* while double transgenic mice co-expressing human APP and ApoE4 demonstrate higher Aβ1-40:Aβ1-42 ratio and substantial CAA development *(14)*. Therefore, transgenic mice models led to finding that changing of Aβ1-40:Aβ1-42 ratio in favour of Aβ1-42, shifts amyloid pathology from the vasculature to the parenchyma, i.e. from CAA to plaque formation. Furthermore, triple transgenic mice (3XTg-AD) co-expressing PS1, APP and tau mutations demonstrated plaque development from 6 months of age and tau pathology at the age of 12 months, indicating that APP and Aβ precede and directly influence neurofibrillary pathology development *(15)*. Furthermore, one of the main challenges in studies with transgenic mice AD models has been determining the onset of cognitive deficits and its molecular correlates by paying attention to small soluble Aβ and detergent-insoluble Aβ species, with aging found to be one of the important (negatively) interfering factors *(16)*. Experiments with triple 3XTgAD mice have shown that at age of 2 months no intra- and extra-cellular Aβ accumulation, as well as no cognitive deficits in Morris Water Maze Swimming test (MWM) could be found, while at age of 6 months, intraneuronal Aβ pathology, hippocampal synaptic deficits and cognitive deficits in MWM test were found, which progressed to development of plaques and tau pathology at age of 12 months *(4)*. Contrary to extensive research on behaviour, amyloid and tau pathology, gliosis and cell loss, biochemistry of various neurotransmitters and other signaling molecules have been quite neglected in transgenic mice AD models.

STZ-ICV RAT MODEL

STZ-icv model is produced by a single or multiple (up to 3 times within one month) injections of a cytotoxic drug streptozotocin, bilaterally into the lateral cerebral ventricle of an adult rat, first reported in 1990 *(17, 18)*. STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)) is a betacytotoxic substance which has been parenterally applied to cause experimental diabetes mellitus type I and II in rats and mice for many decades *(19)*. Contrary to that, central application of low STZ doses (up to 100 times lower than doses used parenterally) demonstrated development neither systemic metabolic changes nor diabetes mellitus but developed numerous behavioural, neurochemical and structural features that resembled those found in human AD *(20)*.

STZ-icv administration has been associated with certain brain morphological changes in the form of *astrogliosis* found as early as 1 week following the treatment regardless the age of animals *(21–24)*. At that time point, severely affected STZ-icv treated rats had also extensive *cell loss (22, 23)*. A progressive trend towards *oxidative stress* has also been found starting as early as 1 week following the STZ-icv administration *(25–27)*. Interestingly, one week after STZ-icv administration, no change in the number or morphology of cholinergic neurons could be detected in any of the brain regions including the hippocampus *(24),* although at that time point a *decreased cholinergic transmission* (decreased choline acetyltransferase and increased acetycholinesterase activity) has started to be persistently found later on in the hippocampus of STZ-icv treated rats *(21, 26, 28–31)*. *Decreased glucose/energy metabolism* particularly in cerebral cortical regions and hippocampus has been reported starting from 3 weeks following the STZ-icv administration *(27, 33–36)*. All these neurochemical and structural changes have been accompanied by long-term and progressive *deficits in learning and memory*, observed as early as 2 weeks after STZ-icv administration and reported to still persist 12 weeks post treatment *(24, 36–38)*.

Presented data are quite convincing in demonstrating the resemblance of STZ-icv rat model to the human AD condition. However, contrary to the transgenic mice AD models in which particular pre-specified, known genes are targeted to design the specific gene-and feno-type, in STZ-icv rat the target is speculated to be similar to the peripheral one. In the periphery, in addition to generating free radicals, chemical structure of STZ allows it to be a substrate for the glucose transporter GLUT2, predominantly localized in the pancreatic β cell membrane, which leads to alkylation of β-cell DNA consequently activating poly ADP-ribosylation, resulting in depletion of cellular NAD+ and ATP and damaging the main β cell function – insulin production and secretion *(19)*. Several evidence supports possible similarity in the peripheral and central mechanism of action; (i) GLUT2 has been found regionally specifically distributed in the brain *(39–41),* (ii) insulin is synthesized in the particular brain regions, (iii) regionally specifically decreased levels of ATP have been reported following STZ-icv treatment *(33, 36)* as well as development of (iv) oxidative stress *(25)*. Furthermore, peripheral treatment with low to moderate doses of STZ can cause insulin resistance via damaging insulin receptor (IR) and its tyrosine kinase (TK) function and, as presented above, low STZ-icv doses induce alterations of brain IR and consequently insulin resistant brain state. Therefore, it could be assumed that, contrary to transgenic mice AD models, STZ-icv rat model is not related to manipulation of genes involved in APP/Aβ homeostasis, but is targeting the functioning of brain IR signaling cascade.

BRAIN INSULIN SYSTEM IN HUMAN AD, TRANSGENIC MICE AND STZ-ICV RAT MODELS

Brain insulin and the IR are functionally linked to improved cognition, particularly general and spatial memory, by up-regulation of insulin mRNA in the hippocampus

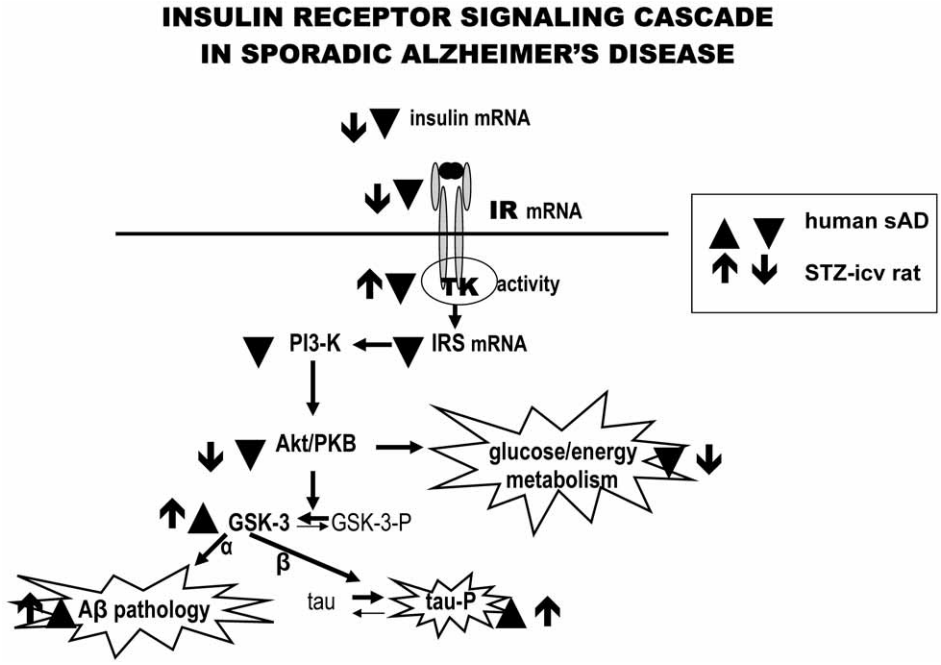

Figure 1. *Brain insulin receptor signaling cascade in sporadic Alzheimer's disease. General changes of brain insulin and IR signaling cascade reported in human sAD and its animal model, STZ-icv rats are presented as reviewed by Hoyer S, 2004 (1) and Salkovic-Petrisic & Hoyer, 2007 (20) in the reference list. sAD, sporadic Alzheimer's disease; Aβ, amyloid-β peptide; IR, insulin receptor; TK, tyrosine kinase; IRS, insulin receptor substrate; PI3K, phosphatydilinositol–3 kinase; Akt/PKB, Akt/protein kinase B; GSK-3, glycogen synthase kinase-3; STZ, streptozotocin; icv, intracererbroventricular.*

and increased IR accumulation in hippocampal synaptic membranes *(45, 46)*. The exact mechanism(s) by which insulin could affect learning and memory is unclear. However, several pathways have been suggested, like those related to the regulation of brain glucose metabolism *(47, 48)* and involvement in neuromodulation by promoting N-methyl-D-aspartate receptor conductance *(49),* reversing the effects of cholinergic blockade *(50),* and reducing the neuronal norepinephrine reuptake *(51)*.

Although the majority of insulin in the brain originates from the periphery and is transported into the brain by a regionally specifically distributed saturable carriers *(52)*, a smaller proportion of insulin is produced within the particular brain regions with the highest density in the pyramidal cells of the hippocampus and in medial prefrontal cortex, the entorhinal and perirhinal cortices, the thalamus and the granule layer of the olfactory bulb, as well as in the hypothalamus *(38, 53, 54)*. IRs are also regionally specifically distributed predominantly in the olfactory bulb, hypothalamus, cerebral cortex, cerebellum and hippocampus *(55–58)*. The neuronal IR differs from the peripheral IR *(59–60)* in that both 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 (Figure 1). Binding of insulin induces autophosphorylation of the IRβ-subunit thus triggering its tyrosine kinase activity *(61)* and activating 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 *(62)*. The activation of the PI3K pathway, in turn activates protein kinase B (Akt/PKB) involved in glucose metabolism but also in inactivation of glycogen synthase kinase-3 (GSK-3) *(63)*. When activated, alpha isoform of GSK-3 regulates the production of Aß peptides (64) and insulin signaling via activation of PI3K also regulates APP release into the extracellular space *(65)*. Activated GSK-3β isoform is involved in tau-protein phosphorylation *(66)*. Therefore, dysfunction in IR-PI3K signalling cascade could lead to AD hallmarks, Aβ overproduction and tau phosphorylation.

A growing body of evidence implicates impairments in brain insulin signaling in early sporadic AD pathology *(as reviewed elsewhere 1, 67, 68)*. Data from the human post mortem studies have demonstrated decreased insulin and IR mRNA as well as IR protein expression (Figure 1) in cerebro-cortical and hippocampal tissue *(69),* followed by increased density of IR in radioligand binding study *(70),* decreased IR-TK activity and decreased insulin receptor substrate (IRS) mRNA and p-IRS expression *(69),* unchanged or decreased Akt/PKB expression *(69, 71)* and altered p-Akt/PKB to Akt/PKB ratio *(72),* as well as changes of alpha and beta GSK-3 isoforms *(69, 73, 74)* and decreased IDE expression *(75)*. Interestingly, the correlation between Akt/PKB activity/protein level and Braak staging in human AD post mortem analysis has been observed *(74)* suggesting time-dependent and IR-PI3K signaling dependent pattern of changes. However, these post mortem human studies do not provide a clue

TABLE 1

Similarities between the pathological features in human sporadic Alzheimer's disease and its representative animal model, streptozotocin-intracerebroventricularly treated rats.

BRAIN PATHOLOGY	STZ-ICV RAT MODEL (*up to 3 months post treatment*)	HUMAN SPORADIC AD
BEHAVIOURAL		
cognitive deficits	decreased memory and learning	dementia
MORPHOLOGICAL		
gliosis	+	+
synaptic loss	+	+
METABOLIC		
glucose/energy	decreased metabolism	decreased metabolism
NEUROCHEMICAL		
oxidative stress	+	+
Ach transmission	decreased	decreased
insulin receptor signaling	brain insulin resistant state	brain insulin resistant state
NEUROPATHOLOGICAL AD HALLMARKS		
tau protein	hyperphosphorylated	neurofibrillary tangles
amyloid beta	congophyllic amyloid angiopathy	congophyllic amyloid angiopathy plaques

General changes of brain insulin and IR signaling cascade reported in human sAD and its animal model, STZ-icv rats are presented as reviewed by Hoyer S, 2004 (1) and Salkovic-Petrisic & Hoyer, 2007 *(20)* in the reference list. **AD**, Alzheimer's disease; **STZ,** streptozotocin; **icv**, intracererbroventricular; **Ach,** cholinergic.

to a cause-consequence relationship in the amyloid pathology – IR signaling interplay.

Contrary to the human post mortem studies, only few literature data could be found on insulin and IR research in transgenic mice AD models, among which only Tg2576 mice expressing human APP were used. Interestingly, these transgenic mice had unaltered basal serum glucose levels but lower basal serum insulin concentrations relative to wild-type mice at the age of 8 month, but had become hyperinsulinemic by 13 months of age *(76)*. Another experiment in this model demonstrated that diet-induced insulin resistance promoted amyloidogenic Aβ1-40 and Aβ1-42 peptide generation in the brain that corresponded with increased γ-secretase activities and decreased IDE activities *(77)*. Further exploration revealed a functional decrease in IR signal transduction in the brain suggested by decreased IRβ autophosphorylation and reduced PI3K-related Akt/PKB phosphorylation *(77)*. Consequent GSK-3 activation positively correlated with β-secretase activity in the brain of insulin-resistant relative to normoglycemic Tg2576 mice. Also, acutely induced reduction in energy production in Tg2576 mice has been demonstrated to cause a long-lasting increase in β-secretase levels and activity, pointing to brain energy deficits as an early potentially amyloidogenic signal *(78)*. Therefore, although modest, data obtained from APP transgenic mice models has suggested a possible link between APP/Aβ pathology and impaired IR signaling cascade in which, in line with the amyloid cascade hypothesis, the APP/Aβ pathology was initiating IR signaling dysfunction.

A very recent research has revealed the alterations of brain insulin system in STZ-icv rat AD model (Figure 1) *(20)*. Changes were regionally specific and pronounced in hippocampus, suggesting time-dependent development of dysfunction in IR signaling cascade in the form of decreased insulin and IR gene/protein expression, increased IR-TK activity (phosphorylation/dephosphorylation imbalance?) *(38),* progressing further downstream the PI3K pathway and leading to decreased Akt/PKB expression and decreased ratio of p-GSK-3/GSK-3 *(37),* finally resulting in amyloid pathology in the form of congophyllic amyloid angiopathy in meningeal capillaries and tau pathology in the form of tau hyperphosphorylation (both found not earlier than 3 months after drug treatment) *(37, 38)*. These data of altered brain IR signaling induced by STZ-icv administration in adult rats have been supported by generally similar results of IR-PI3K signaling cascade dysfunction found in the rat pups treated intra-cortically with low STZ dose *(79, 80)*. Therefore, in addition to cognitive deficits in learning and memory and other neurochemical changes which resemble those found in human sAD, central administration of STZ toxin triggers amyloid and tau pathology without involving APP gene related manipulation (Table 1).

CHALLENGING THE AMYLOID CASCADE HYPOTHESIS

Only post mortem histological and neurochemical examinations of the human brain offers a definitive and reliable diagnosis of AD, and yet these analyses are just

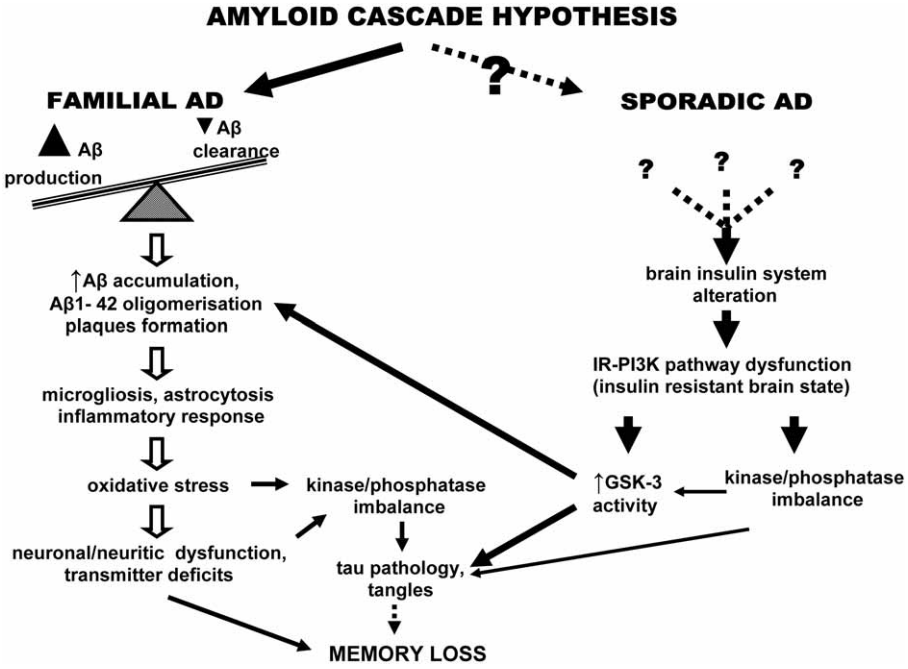

Figure 2. *Challenging of amyloid cascade hypothesis in sporadic Alzheimer's disease. Amyloid cascade hypothesis fits into the explanation of the early-onset familial Alzheimer's disease (AD) pathophysiology, for which transgenic mice are the representative experimental model. In this form pathological mutations of amyloid beta (Aβ)-production related gene cause imbalance in Aβ production and clearance by increasing the production of total Aβ or the Aβ1-42/Aβ1-40 ratio, leading to increased Aβ accumulation, Aβ1-42 oligomerization and senile plaque formation. These processes further initiates a whole range of pathological cascade effects; microgliosis and astrocytosis, inflammatory response, oxidative stress, neuronal/neuritic dysfunction, cell death, neurotransmitter deficits, and finally, memory loss. Additionally, these processes induce kinase/phosphatase activity imbalance which can cause tau protein hyperphosphorylation and formation of neurofibrillary tangles which contribute to memory loss. However, in sporadic AD (sAD), for which streptozotocin-intracerebroventricularly (STZ-icv) treated rats are the proposed model, amyloid cascade hypothesis does not seem likely to truly represent its ethiopathogenesis. In sAD, alterations of brain insulin system lead to insulin receptor (IR) signaling dysfunction down the phosphatydilinositol-3 kinase (PI3K) pathway and induce insulin resistant brain state. This further leads to activation of glycogen synthase kinase-3 (GSK-3) which isoforms alpha and beta consequently induce Aβ accumulation and tau hyperphosphorylation. Dysfunction in IR signaling could also induce kinase/phosphatese imbalance which could additionally contribute to GSK-3 activation and tau hyperphosphorylation.*

static intersections of a long on-going process in AD pathology development. To make a movie of them, one would have difficulties where to start and how to number these intersections in order. Transgenic mice AD models have been designed just for this purpose and by playing with combinations of amyloid-relevant gene mutations, a lot of important details of AD neuropathology and behavioural/cognitive impairments have been revealed. However, disadvantage of transgenic mice AD models is that the amyloid-relevant gene mutation is exclusively the inevitable pathological start point due to they take amyloid cascade hypothesis for granted. Unfortunately, in the real life, mutations of genes, particularly those encoding for APP, PS1 and PS2, are causing only minority of AD cases (less than 5%) being determined as early-onset familial AD while in the prevailing majority of AD cases, determined as late-onset sporadic AD, gene mutations are not a direct cause of a disease *(1)*. Moreover, the real cause in sAD is not known and, instead, more general and common factors like aging, peripheral insulin resistance, even environmental toxins *(81)* have been implicated as possible risk factors. Therefore, transgenic mice AD models should not be taken as a representative model upon which a general conclusion covering ethiopathogenesis of both AD forms would be drawn since, bearing in mind a direct cause of disease, transgenic mice models could be assumed to represent the early-onset familial AD form only. Contrary to that, STZ-icv rat model is based on the regionally selective toxicity of exogenous substance which targets brain IR signaling cascade inducing insulin resistant brain state and resemblance to human sAD in different pathological aspects (Table1), and is therefore representing the other, late-onset sporadic AD form. Such a split in animal AD models regarding their representativeness of AD forms provides the ground for challenging the current amyloid cascade hypothesis *(82)*. Namely, based on STZ-icv rat model data, it is more likely to assume that development of insulin resistant brain state precedes and, after some time, leads to amyloid pathology in the sporadic AD (Figure 2). The factors causing this insulin resistant brain state (selective toxicity of STZ in rats) in humans could possibly be looked for in the peripheral insulin resistance, and the research on epidemiological link between diabetes type 2 and sAD is currently a »hot topic« in neuroscience *(83)*. Additionally, they could be looked for in elevated corticosterone levels *(1)* found frequently in AD and diabetic patients, or maybe among environmental toxins

(81) exposure to which in humans is continuously increasing, just as the prevalence of sAD. In the view of presented data, challenging the amyloid cascade hypothesis opens a new dimension of sAD ethiopathogenesis paving also the way to the new AD therapeutic strategies oriented to disease-modifying drugs.

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