Effects of Type 2 Diabetes Mellitus on GluN2B subunit distribution and cognitive performance in Goto-Kakizaki Rats

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Introduction

It is estimated that 50 million people are currently living with dementia worldwide, making this disease a major global health issue. This debilitating cognitive decline is both progressive and incurable, although specific risk factors have been identified [1].

Extensive literature has argued for an increased prevalence of dementia in type 2 diabetes mellitus (T2DM) sufferers. They are estimated to have a 73% increased risk for all type dementia and 127% increased risk for vascular dementia [2]. Although the mechanistic link between these two diseases remains unknown, studies highlight the importance of the GluN2B and GluN2A subunits of NMDARs during normal cognition and changes in these proteins have been implicated in diabetes-related cognitive decline [3,4,5].

Current knowledge

Insulin not only regulates blood glucose levels but has also been implicated in several integral mechanisms within the brain. During normal cognition insulin receptors initiate the phosphoinositide-3-kinase and protein kinase B (PI3K/Akt) signalling pathway to promote cell survival and synaptic plasticity. This pathway encourages neuronal coupling and facilitates insertion of NMDARs in the plasma membrane [6].
Therefore, during T2DM insulin dysregulation disrupts synaptic plasticity which ultimately leads to neurodegeneration and cognitive decline. The early hyperinsulinaemic phase of T2DM is associated with increased NMDAR expression and glutamate-induced excitotoxicity. Whilst, the final hypoinsulinaemic stage demonstrates reduced NMDAR cell surface expression and impaired executive functions.

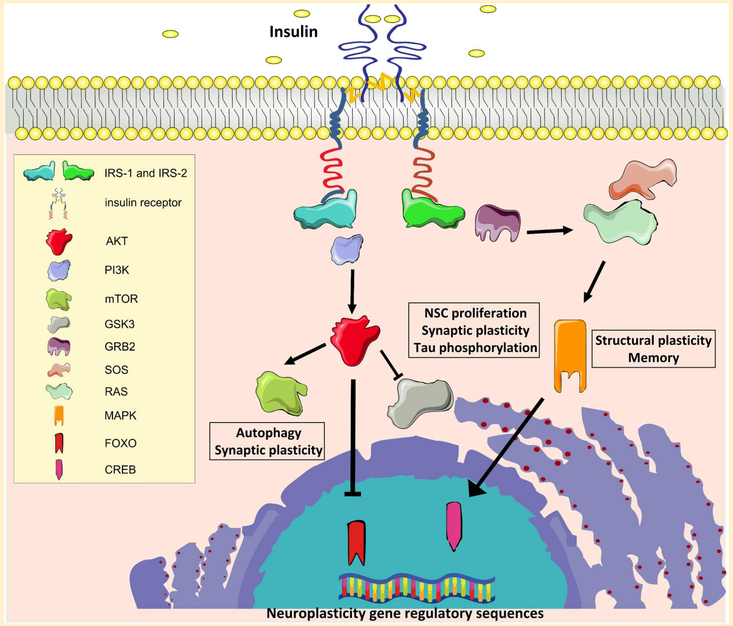
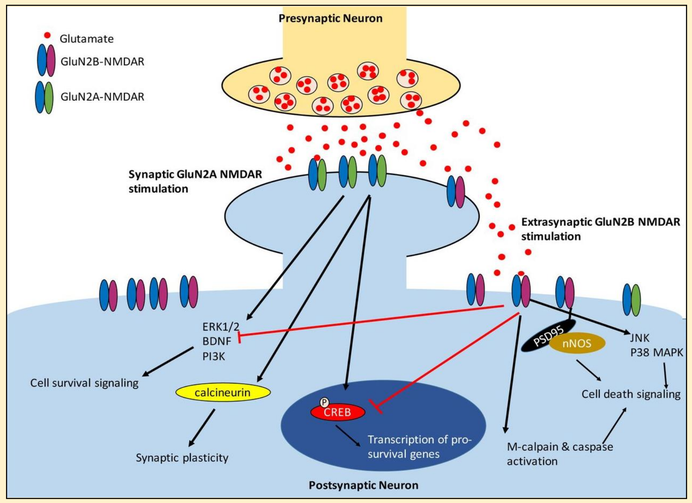


Diagram outlining downstream insulin signalling involving the PI3K/Akt pathway [6]
IRS-1/2, insulin receptor substrate 1 and 2; Akt, protein kinase B; PI3K, phosphoinositide-3-kinase; mTOR, mechanistic target of rapamycin; GSK3, glycogen synthase kinase 3; GRB2, growth factor receptor-bound protein 2; SOS, Son of Sevenless; RAS, rat sarcoma GTPase protein; MAPK mitogen activated protein kinase; FOXO, forkhead box O; CREB, cAMP response element-binding protein; NSC, neural stem cell.

Conflicting evidence

Following an extensive literature review it is clear there are several areas of conflicting research, namely:

1. **The downregulation of GluN2B NMDARs during T2DM**
   - Disease models of T2DM have demonstrated a marked reduction in cell surface expression of the GluN2B subunit. The severity of downregulation was correlated to the degree of cognitive impairment [7]
   - However, it has been shown that this downregulation is not always detected and some studies even show upregulation of the GluN2B subunit [8]
2. **The distinctive role of NMDAR subunits during T2DM**
   - Some debate that the GluN2B and GluN2A subunits have distinct functions and are generally identified in opposing locations [9]
   - Others argue that both subunits can be located in close proximity to one another and even within the same synapse [10]. In fact, GluN2A and GluN2B are often co-assembled within the same heterotrimeric NMDAR complex.



Functional localisation of GluN2B and GluN2A NMDARs at synaptic and extrasynaptic locations [5]

- ERK1/2 - extracellular signal-regulated protein kinase 1 and 2
- BDNF - brain -derived neurotrophic factor
- PSD95 - postsynaptic density protein 95
- nNOS - neuronal nitric oxide synthase
- JNK - c-Jun N-terminal kinase
- p38MAPK - p38 mitogen-activated protein kinase

Hypothesis

Whilst there are several explanations for this conflicting data, one of the most interesting is the importance of subunit location. When located within the synapse, GluN2B subunits are functionally similar to GluN2A subunits. Here they ensure cell survival and maintain the synaptic plasticity and long term potentiation involved in learning and memory. However, when located (more frequently) extrasynaptically, the GluN2B subunit is associated with glutamate-induced excitotoxicity and cellular death. Studies have not highlighted how these separate populations of GluN2B subunits may be effected differently during T2DM. My working hypothesis is that there is a T2DM-related relative increase in GluN2B levels on the cell surface, particularly at extrasynaptic sites, which increases the susceptibility of neurones to excitotoxicity.

It is proposed:

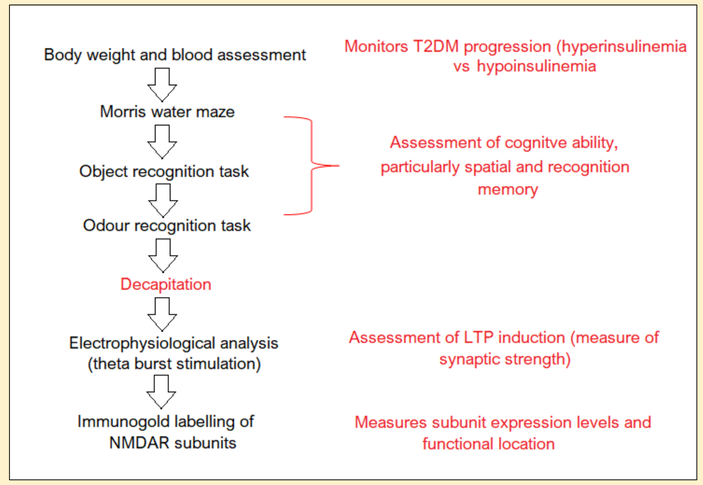
- Synaptic GluN2B NMDARs underly normal cognition and are downregulated during T2DM. However, extrasynaptic GluN2B NMDARs which mediate glutamate-induced excitotoxicity underly cognitive impairment and are maintained/upregulated during T2DM.
- It is expected that the severity of this downregulation will correlate to the extent of T2DM development and the degree of cognitive decline.

Study design

This study will compare Goto-Kakizaki (GK) rats (non-obese models of T2DM) with age and sex-matched Wistar controls. It is expected that GK rats will exhibit cognitive impairment which correlates to the extent of T2DM progression and degree of synaptic GluN2B and GluN2A downregulation.

Both strains of rat will be assessed every 3 months, beginning at 3 months of age until 18 months of age, totalling 6 periods of assessment.

General health and progression of T2DM will be examined by measuring body weight and blood content. Cognitive ability will be assessed via performance in the Morris water maze, object recognition and odour recognition tasks. These assess spatial and recognition memory, both of which show impairment in dementia and T2DM sufferers [11]. Following decapitation, one hemisphere of the rat's brain will be used for electrophysiological analysis (LTP induction) and one hemisphere will be used to assess subunit expression levels/distribution via immunogold labelling.



Flow diagram of key experiments

Relevance and impact

An improved understanding of the signalling mechanisms involved in T2DM could provide a more conclusive mechanism which links the severity of insulin resistance with the degree of cognitive decline. This would have important clinical implications for diabetes sufferers as the disease and its comorbidity with cognitive decline becomes increasingly prevalent yet remains incurable.
Potential drug targets could be unveiled and selectively targeted to prevent or reverse the progression of diabetes-related cognitive decline.

References

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