

The contribution of CNS inflammation and Glycogen Synthase Kinase-3 (GSK-3)-cascades on adverse memory learning on mouse models of emotional stress

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SUMMARY

In my thesis I aimed to study the role of the glycogen-synthase kinase 3 (GSK-3) expression, inflammatory mechanisms and associated changes in the brain of mice, using two distinct models of depression. These new models were the ultrasound stress model of “emotional stress” and the model of enhanced contextual learning of adverse memories. In the modified forced swim model (modFST), the classic two-day forced swim in mice is followed by an additional delayed session on Day 5, where increased “despair” behaviour and upregulated GSK-3 are context-dependent. In the ultrasound stress model of “emotional stress”, mice are

exposed to unpredictably presented ultrasound mimicking signals of anxiety and distress that are naturally emitted by small rodents. In **Chapter 4** I studied hippocampal gene and protein expression of both GSK-3 β and GSK-3 α , as well as the associated molecules forkhead transcription factor O subfamily member 3a (FOXO3a), phosphatase and tensin homolog (PTEN) and protein kinase B phosphorylated at serine 473 (AktpSer473) in mice exposed to the ultrasound stress model of “emotional stress”. While the upregulation of GSK-3 β activity in stressed animals was accompanied by similar over-expression of hippocampal GSK-3 α , no correlation between the latter and scores of depressive-like behavior in the forced swim test and other signs of altered emotionality were found suggesting different functional roles of two GSK-3 isoforms in stress-induced depressive syndrome. We also found decreased densities of Ki67-positive and doublecortin-positive cells and downregulated expression of neurotrophins, including brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin receptor kinase B (TrkB) in the hippocampus, suggesting decreased plasticity in the CNS of stressed mice. Stressed mice also displayed increased plasma levels of cytokines tumor necrosis factor (TNF), interleukin (IL)-1 β and IL-6 and hippocampal expression of IL-1 β and IL-6. Additionally, signs of microglia activation, increased density of

ionized calcium binding adaptor molecule 1 (Iba1)-positive cells and concentrations of oxidative stress markers 3-nitrotyrosine and malondialdehyde were found.

In **Chapter 2** and **Chapter 3** we studied gene expression of GSK3 isoforms and markers of neuroinflammation in the hippocampus and prefrontal cortex of mice exposed to the modFST. We found brain over-expression of GSK3 α , a poorly studied molecule in animal models, with distinct expression dynamics from GSK3 β after the modFST (**Chapter 2**). Our findings provide the first evidence for the involvement of GSK-3 α in a depressive-like phenotype in an animal model of depression. In **Chapter 3** we report messenger ribonucleic acid (mRNA) overproduction of pro-inflammatory cytokines IL-1 β and TNF, as well as cyclooxygenase-1 (COX-1) in the hippocampus and prefrontal cortex of mice subjected to the modFST. Overproduction of both IL-1 β and TNF positively correlated with expression of GSK-3 β , but not GSK-3 α , and total duration of floating correlated with expression of both GSK-3 isoforms in the examined brain regions. In addition, it has been found that mice exposed to the modFST demonstrate increased plasma corticosterone concentrations, elevated concentrations of protein carbonyl, a marker of oxidative stress and over-expression of c-Fos in the brain. Most of changes induced by the modFST

were reversed by treatments with a low dose of imipramine or thiamine (Vitamin B1) that were shown to exert anti-stress and antidepressant properties and normalize GSK-3 β expression. Together, our results obtained in studies with two depression models suggest overlapping molecular mechanisms of over-expression of GSK-3 and proinflammatory mechanisms along with oxidative stress to underlie distinct aspects of depressive syndrome. In addition, we identified GSK-3 α as one of potential targets of depression treatment and further demonstrated that thiamine drugs can have a potential in reducing depressive-like changes associated with stress.

SAMENVATTING

De bijdragen van CNS-ontstekingen en glycogen synthase kinase-3 (GSK-3)-cascades op het negatieve herinneringen geheugen in muismodellen van emotionele stress

In mijn proefschrift bestudeerde ik de expressie van de glycogeen-synthase kinase 3 (GSK-3), een moleculaire substraat van stress, evenals de ontstekingsmechanismen en de bijbehorende veranderingen op moleculair en cellulair niveau in de hersenen van muizen, met behulp van twee depressiemodellen : het door ultrasonoor geluid geïnduceerd stressmodel van "emotionele stress" en de gemodificeerde gedwongen zwemtest (modFST). In het model van "emotionele stress" werd het ultrasone geluid willekeurig afgewisseld tussen 20 en 25 kHz wat overeenkomt met het natuurlijk geluid van knaagdieren in een angst en vrees situatie , en frequenties tussen 25-45 kHz wat overeenkomt met het geluid dat muizen produceren in een "neutrale" emotionele toestand. De muizen, 21-dagen blootgesteld aan deze afwisselende frequenties van ultrageluid, vertoonden een depressief beeld. Ten tweede hebben we een aangepaste Porsolt's test voor muizen gebruikt, een model van hulpeloos gedrag bij kleine knaagdieren, waarbij een extra vertraagde sessie op dag 5 na de initiële blootstelling resulteert in een verdere toename van hulpeloos gedrag bij de gevoelige dieren maar niet in veerkrachtige individuele muizen. Verhoogde hulpeloosheid in deze test wordt beschouwd als een teken van verbeterde contextuele conditionering van nadelige herinneringen, een belangrijk mechanisme van depressie. De upregulatie van beide isovormen van GSK-

3 te weten GSK-3 β en GSK-3 α , werd gevonden in beide muismodellen van depressie. Echter, de veranderingen in de expressie van GSK-3 β en niet de veranderingen in de expressie van GSK-3 α in de hersenen, was significant gecorreleerd met kernen van depressief gedrag in deze twee modellen. Voor het eerst hebben wij de verschillende functionele rollen van de twee GSK-3 isovormen in het stress-geïnduceerd syndroom aangetoond. De ontstekingsbevorderende veranderingen in beide diermodellen waren verhoogde plasmaconcentraties en hersenexpressie van tumornecrosefactor (TNF), interleukine (IL) -1 β en IL-6, evenals cyclooxygenase-1 (COX-1). Ultrasonore stress laat tekenen van verlaagde neuronale plasticiteit zien: de densiteit aan Ki67-positieve en dubbelcortine-positieve cellen en een neerwaarts gereguleerde expressie van neurotrofines, en markers van microglia-activering, evenals verhoogde concentraties van oxidatieve stressmarkers 3-nitrotyrosine en malondialdehyde. De laatste veranderingen werden ook in de modFST gevonden. Dezelfde moleculaire en cellulaire veranderingen werden gezien bij de ontwikkeling van depressie achtige syndromen van verschillende oorsprong. De meeste depressieve uitingen waargenomen in deze twee modellen werden tegengegaan door de toediening van antioxidant thiamine (vitamine B1) welke ook de expressie van GSK-3 β normaliseerde. De overlappende veranderingen in de GSK-3-activiteiten en de gerelateerde mechanismen dragen beide bij tot het aanleren van negatieve herinneringen en "emotionele stress" in de gebruikte modellen van depressie.