Insulin receptor sensitization improves affective pathology in various mouse models

Chapter 7

Discussion and impact

Affective pathology is a complex construct which encompasses a pathological disturbance in primary emotions, rapidly shifting from neutral to intense perception, associated to dysfunctional coping. In rodents, assessing affective pathology, translated mostly by anxiety- and depressive-like states, is challenging and requires a combined analysis of behavioural, physiological and molecular data in elaborate paradigm constructs. In this regard, we aimed to establish new, reliable and robust models, sensitive to the action of pharmacological compounds such as insulin receptor sensitizers or antidepressants.

Knowing the role of NMDA receptor-mediated neurotransmission in the aetiology of stress-related cognitive deficits and behavioural abnormalities, as well as the hypothesis of different roles of NR2A/B receptors regulating the mechanisms of learning and emotion processing, we have employed ethological stressors and

assessed their effect on building an affective pathology phenotype in mice (Chapter 2).

We found stress-induced increases in hippocampal expression of NR2A and NR2A/NR2B ratio, previously linked to elevated anxiety, impulsivity and aggression, home cage hyperactivity and increases in circulating corticosterone, also present in our behavioural and biochemical data. Moreover, studies have reported a link between these molecular changes and a disruption of long-term memory, but not short-term learning. In summary, our newly validated mouse model of affective pathology accurately resembles the consequences of experiencing chronic stress in humans, and proves useful for translational studies on the effect of putative pharmaca which may compensate or revert this condition.

As diverse etiological factors may contribute to a state of affective pathology, we aimed to characterize the effects of a neuronal insulin receptor sensitizer on a plurality of already established rodent models, which included chronic stress, elderly depression and naïve mice, evaluating several behavioural, molecular and sleep variables, characterized as biological correlates of a depressive state and adaptive response in mice (Chapter 3).

Firstly, in the model of chronic stress, pre-treatment with our compound prevented stress-induced memory impairment, possibly through augmented choline acetyltransferase activity, which increases neurogenesis and is considered to be neuroprotective; extended the duration of REM sleep, but not SWS, a mechanism described as an adaptive response to stress, that loses efficacy with ageing and in neuropsychiatric disorders; and increases hippocampal gene expression of NMDA receptor subunit NR2A, as well as NR2A/B ratio.

Secondly, the delivery of this insulin sensitizer in a model of elderly depression has resulted in the restoration of the hedonic deficit similarly to a classical tricyclic

antidepressant and gene expression profiling demonstrates an upregulation of genes encoding for factors of synaptic plasticity, regulation of sleep and circadian rhythm, known to be implicated in the pathology of depression.

Thirdly, the administration of dicholine succinate to naïve mice has reduced parameters of learned helplessness and decreased anxiety scores in behavioural tests in a similar way to established antidepressants and other thiazolidinediones, without inespecific locomotory side-effects. Furthermore, as insulin regulates GSK-3β activity, treatment precluded the reduction of its inactive form similarly to antidepressants, possibly establishing a link to the effects of insulin receptor sensitizers and hippocampal NMDAr expression.

All of the abovementioned effects support the hypothesis of an antidepressant-like role for insulin receptor sensitizers, at different levels and in different contexts for each model. Together with the lack of signs of toxicity in mammals at effective dosage ranges, the enhancement of insulin receptor signalling may be regarded as a potential pharmacotherapeutic target.

A growing body of clinical and experimental data suggests that excessive nutrition conveyed by the so-called Western diet (*e.g.* high-cholesterol, high-fat, high-salt, high-sugar), and ensuing hypercholesterolemia, obesity and insulin resistance contribute to the pathogenesis of affective disorders and interfere with pharmacotherapy. We considered the use of this type of dietary challenge to investigate the effects of increased cholesterol intake on behaviour and molecular markers, comparing the changes observed in the periphery to those occurring in the central nervous system, and attempting to establish a link to the development affective pathology (Chapter 4).

For the first time, profound behavioural modifications and molecular changes were observed in the brain and liver in association with this diet, along with depression

and anxiety-like behaviour, and similar to stress-induced depression. A significant increase in *Tlr4* gene expression was found in both the hippocampus and the prefrontal cortex, albeit increased protein levels were only detectable in the prefrontal cortex. Expression levels returned to baseline values after reversion to control diet, accompanied by a normalization of anxiety- and depression-related behaviours. Unexpectedly, body weight of mice on the high-cholesterol diet remained unchanged, excluding the contribution of obesity or excessive amounts of fat. This methodology was shown to be effective in inducing dietary-associated affective changes, with prospected value in translational pharmacological and pre-clinical studies.

As a translational approximation to the human clinic and potential use of various compounds as a food supplement, we aimed to evaluate whether it would be effective to deliver compounds by an oral route in mice (Chapter 5). We demonstrated that the delivery of an insulin receptor sensitizer by oral route is effective to elicit antidepressant-like effects similarly to those of the antidepressant imipramine that was used as a reference drug.

Together, we have supported pharmacological delivery of dicholine succinate with food as an effective method of dosing that opens possibilities of the application of this compound with food. Apart from that, our study showed that drug delivery with pellets can be a desirable alternative to invasive dosing, which may greatly improve animal welfare, particularly with the need for repeated or chronic drug administration to physically vulnerable laboratory animals.

Lastly, we investigated the effect of oral delivery of an insulin receptor sensitizer in a non-alcoholic fat liver disease mode induced by a high-cholesterol diet, characterized by hepatic lipid dystrophy, inflammation, depressive- and anxiety-like behaviours and increased expression of TLR-4 (Chapter 6). Treatment with the selected compound

ameliorated diet-induced anxiety- and depressive-like behaviours, likely linked to the normalized expression of pro-inflammatory factor TLR4 and mitochondrial activity marker PPARGC1b, which were expressed abnormally high and low, respectively.

Our results suggest that the seen improvement is associated with the underlying normalization of the secondary mechanisms of NAFLD syndrome, such as inflammation and mitochondrial functions, rather than of its primary features of liver steatosis and dystrophy, since the use of this insulin sensitizer did not elicit changes on the latter. These evidences support a potential use of insulin receptor sensitizers for the treatment of affective symptoms during NAFLD syndrome.

Concluding remarks

This work pioneered to robustly use ethological stressors and high amounts of dietary cholesterol as accurate methodologies to reliably mimic cognitive and emotional abnormalities associated with human phenotypes. Combined with these models, age-induced and naïve learned helplessness were successful tools to study the effects of insulin receptor sensitization, gathering evidence which supports the potential to generate effective antidepressant-like effects in various conditions, restoring baseline levels of brain plasticity and function, memory, gene expression and normal behaviours, albeit the underlying mechanisms are not yet fully understood.

Altogether, insulin receptor sensitization has shown a sound potential in rodent models of depression of various etiologies. In line with existing literature, our data supports the vision that enhancement of insulin receptor mediated signallimg may be a new promising strategy of pharmacotehrapy of affective disturbances in human clinic. Thus, the use of compounds of a similar nature to the insulin receptor sensitizer dicholine succinate can be beneficial for future clinical use in a plurality of metabolic and neuropsychiatric disorders.