Deletion of CRTC1 is associated with strong neuroenergetic dysfunctions in a mouse model of mood disorders.

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Introduction

CRTC1 (CREB-regulated transcription coactivator 1) is a coactivator of the transcription factor CREB, and is predominantly expressed in the brain. CRTC1 plays an important role in brain plasticity, and its dysfunction has been implicated in many neurological disorders, such as neurodegenerative and mood disorders. Mice lacking the *Crtc1* gene, show a depressive-like phenotype and have been proposed as a model for preclinical mood disorder studies[1,2]. The goal of this project was to define ^{1}H-MRS visible biomarkers related to this behaviour and understand the underlying molecular mechanism.

Methods

Crtc1$^{-/-}$ animals (KO) and their countertypes (WT) were scanned in a horizontal 14.T/26cm Varian magnet (Agilent Inc., USA), under isoflurane anesthesia (1-2%). T_2-weighted FSE images (25×0.6mm slices, TE_{eff} /TR=50/4000ms, nt=8) were acquired to localize the VOI for spectroscopy in the dorsal hippocampus (DH) and cingulate prefrontal cortex (cPFC). Indirect carbon spectroscopy (^{1}H[^{13}C]-MRS) was performed in the bilateral dorsal hippocampus using a SPECIAL-BISEP sequence upon infusion of 70% enriched 20% (w/v) [U-$^{13}C_6$] glucose for 4 hours followed by metabolic flux analysis[3,4]. *Ex vivo* metabolic analysis was performed on microwaved-fixed brains of 6 weeks animals after chloroform-methanol extraction using ^{1}H-NMR (flip angle 30°, TR=3s) as well as ^{31}P-NMR (flip angle 90°, TR=5s) on a DRX-600 spectrometer (Bruker BioSpin). Longitudinal scans were performed in animals of 6, 12 and 24 weeks following animal isolation after the first scan. Before each scan, locomotor activity as well as depressive-like behaviour was assessed using an openfield (OF), forced swim test (FST) and tail suspension test (TST). Non-invasive, dynamic ^{18}FDG PET was performed as previously described[5]. Gene expression and mitochondrial mass measurement was performed using qPCR of brain tissue punches in naïve animals of 6 weeks.

Results

^{1}H-MRS indicated neuroenergetic alterations in DH (reduced phosphocreatine/creatine ratio and lactate) as well as inflammatory process in cPFC (increased choline containing compounds (CCC) and tissue size) of KO animals. ^{1}H[^{13}C]-MRS, PET and molecular analysis indicated that the neuroenergetic alterations is due to a significant reduced glucose uptake and glycolytic rate with no alteration of mitochondrial neuroenergetics (in vivo TCA, TCA gene expression). Furthermore, *ex vivo* analysis of the neurochemical profile assessed with ^{1}H-NMR and ^{31}P-NMR indicated no difference in ATP or the NADH/NAD^{+} ratio but confirmed the drop in phosphocreatine. The increased expression level of creatine kinases (CKB and mtCK) suggest that mitochondria are under pressure to compensate for the lack of ATP produced by glycolysis. Finally, the depressive-like behaviour correlated with the amount of brain lactate and followed a similar evolution after social isolation of the mice, while the increased CCC in cPFC remained constant.

Discussion

In this preclinical study we have identified a set of potential biomarkers translatable in a clinical setting. By linking a gene to a pathophysiological mechanisms, we have opened up opportunities for targeted treatments. By using *in vivo* metabolic imaging techniques we have made this alteration clinically translatable and defined some biomarkers, which predict behaviour in mice.

Conclusion

This study defined a set of biomarkers measurable with ^{1}H-MRS in a mouse model of mood disorders, described the associated underlying mechanism of impaired neuroenergetics in DH and linked these alterations with the animal's depressive-like behaviour. This preclinical model of mood disorders will provide a useful basis to investigate for potential human MRS biomarkers in mood disorder and help defining a subpopulation with potential responsiveness to a targeted treatment.

References

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