TRPC3c (high activity) transient receptor potential channel expression in the mouse brain

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Introduction: Canonical transient receptor potential type C3 (TRPC3) is a nonselective cation channel in the TRPC subfamily. The TRPC3 subunit assembles into a channel that can be activated either by negative cytosolic Ca^{2+} coupling, or by activation through the G protein-coupled receptor (GPCR) / phospholipase C (PLC) - diacylglycerol (DAG) pathway. TRPC3 channels have a role in Ca^{2+} homeostasis, with a broad representation in neurons and glia throughout the central and peripheral nervous systems (Riccio *et al*., 2002). In cerebellar Purkinje cells, TRPC3 channels are the main effectors of the metabotropic glutamate receptor (mGluR)-mediated slow EPSC (Hartmann *et al.*, 2008). TRPC3 channels also provide negative feedback regulation of cytosolic Ca^{2+}, mediated by calmodulin and inositol trisphosphate receptor binding (CIRB) domain at the C-terminus (Zhang *et al.*, 2001). We previously identified a splice variant (designated TRPC3c) as the dominant TRPC3 mRNA transcript in cerebellar tissue,with functional characterisation demonstrating significantly higher conductance, including Ca^{2+} entry (Kim *et al.*, 2012). The TRPC3c transcript lacks exon 9, which codes for approximately half of the CIRB domain. Given the coupling to glutamatergic transmission, high channel kinetics and Ca^{2+} conductance, the TRPC3c channel is likely to be a significant contributor to ischaemic brain injury. In this study, we have determined the expression of TRPC3c in various regions of the mouse brain by quantifying mRNA levels using qRT-PCR.

Methods: Total RNA was extracted from various regions of the mouse brain (n=16), including general cerebral cortex, hippocampus, midbrain, pons, medulla and cerebellum. Following the reverse transcription, the resulting cDNA was amplified using SYBR-green PCR reagent and StepOne real-time PCR systems (Applied Biosystems) to quantify TRPC3c and TRPC3b mRNA. In order to selectively quantify each of the isoforms, we designed primers which bound to the cDNA transcript of the specific TRPC3 splice variant. This was achieved by targeting of the exon 9 (for TRPC3b) or exon 8-10 junction (for TRPC3c). The specificity of the primers for the respective isoform was verified using PCR amplification of control templates that consisted of pIRES-DsRed2 plasmids with TRPC3b or TRPC3c inserts. The cDNA transcripts of each TRPC3 isoform resulting from qRT-PCR were quantified using standard dilution-series curves generated from amplification of cDNA from TRPC3(b or c)-pIRES-DsRed2 clones.

Results: The TRPC3c mRNA transcript was detected in all regions of the brain tested. However, the level of the expression was significantly variable. In the cerebellum, the TRPC3c isoform was shown to be the dominant isoform, suggesting that this mediates the mGluR-sEPSC in Purkinje neurons. In all other parts of the brain, the TRPC3c expression was found to be less prominent, but still accounting for ~10 - 20% of the total TRPC3 expression. The order of TRPC3c abundance (highest to lowest) with regard to brain region was: cerebellum >> pons > medulla > midbrain > hippocampus > general cerebral cortex.

Conclusion: This study reports quantification of TRPC3c isoform in the brain. This splice variant is highly expressed in the cerebellum, where the TRPC3 channels have been shown to have a prominent functional role. While it is expressed to a lesser extent to other parts of the brain, the high activity of the TRPC3c isoform suggests that it may still have significant functional implications as a mediator of cation entry, and contribute to neuronal signalling, homeostasis and growth as well as affecting neuronal survival in ischaemic episodes.

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