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NEURONAL VERSUS GLIAL CB2 RECEPTOR: INSIGHTS FROM A NEW STRAIN OF CB2-KO-EGFP REPORTER MICE

Introduction: The cannabinoid CB2 receptor (CB2R) has emerged as a promising therapeutic target for pain and central nervous system disorders, yet its brain expression has remained controversial due to low basal levels and the lack of reliable antibodies. Previous green fluorescent protein (GFP) reporter mouse lines have produced conflicting findings, possibly because GFP was either randomly inserted into the genome or placed in the 3'-untranslated region of the CB2R gene (*Cnr2*), complicating interpretation.

Methods: We generated a new strain of CB2-KO-eGFP mice, in which the endogenous CB2-coding sequence was replaced by eGFP while preserving the remaining CB2R gene structure. To examine GFP expression in the brain, we performed fluorescence-based flow cytometry and immunohistochemistry (IHC) using a GFP antibody. We then used RT-PCR and RNAscope in situ hybridization (ISH) to confirm CB2R deletion at the mRNA level. Lastly, we compared behavioral phenotypes between wild-type (WT) and CB2-KO-eGFP mice.

Results: Loss of CB2R expression was confirmed by qRT-PCR, RNAscope in situ hybridization, and cannabinoid pharmacological assays. GFP-immunostaining was detected across multiple brain regions, including cingulate cortex, hippocampus, red nucleus, and cerebellum, and in several cell types such as microglia, astrocytes, and neurons. Flow cytometry revealed strong GFP signals in spleen and blood cells and quantifiable GFP expression in brain tissue. Notably, ~70% of microglia and ~4% of neurons in cortex and hippocampus expressed GFP under normal physiological conditions. Functionally, CB2R deletion reduced cannabinoid-induced analgesia, hypothermia, and catalepsy, confirming the receptor's physiological relevance.

Conclusion: These findings demonstrate that CB2R is indeed expressed in healthy brain tissue and across multiple neural and glial cell types, resolving long-standing uncertainty regarding CB2R localization. This new mouse line provides a reliable and highly informative tool for studying CB2R expression and function in both the brain and peripheral immune system.

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