The type 2 cannabinoid receptor as a new PET reporter gene for the brain

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Introduction: Reporter genes play an important role in the understanding of gene expression and function in living subjects. However, for the brain no successful PET reporter systems are available with low endogenous background gene expression and good blood-brain-barrier (BBB) penetration of the PET probe. The aim of this study was to develop a new PET reporter gene system which can be applied to the brain. The type 2 cannabinoid receptor (CB_2) has a very low brain expression in physiological conditions and CB_2 PET radioligands crossing the BBB were recently developed.

Methods: We constructed lentiviral (LV) and adeno-associated viral vector (AAV) transfer plasmids encoding human CB_2, harboring a point mutation at position 80 (D80N) referred to as CB_2(D80N), as such or in combination with enhanced green fluorescent protein (eGFP) or firefly luciferase (FLuc). Rats were stereotactically injected with either 5 µl of AAV-eGFP-T2A-CB_2(D80N) in the right striatum and 5 µl of AAV control vector in the left striatum or 5 µl of AAV-fLuc-T2A-CB_2(D80N) in the right striatum. At different time points (6, 13, 18, 73, 96 and 252 days) after stereotactic injection of the AAV, a CB_2 selective carbon-11 labeled radioligand [^{11}C]GW405833 was injected intravenously and dynamic µPET images (Focus 220, Siemens) were acquired. BLI scans were performed at 16, 58 and 281 days after surgery. Time-activity curves (TAC) and parametric binding potential maps were determined. The animals were sacrificed and perfused and double immunohistochemical staining against CB_2 and eGFP of the brain slices was performed.

Results: The observed CB_2 binding potential increased over time and reached a maximum in right striatum between 18 and 58 days after vector injection in the brain. The time-activity curves persistently expressed an increased uptake in right striatum compared to control left striatum and cerebellum. Immunohistochemical analysis showed colocalization of both CB_2 and eGFP in right striatum. In contrast, only eGFP expression was seen in the contralateral hemisphere.

Conclusions: We have successfully developed a new PET reporter gene system consisting of a lentiviral or an adeno-associated viral vector expressing the CB_2 receptor as the reporter gene which can be quantified for several months.

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