

Quantitative Metabolic Changes and G Protein-Coupled Receptor Activation Using Autoradiography

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BACKGROUND

Autoradiography (ARG) is a powerful technique that can be applied to study e.g. metabolic changes, receptor binding and activation of G protein-coupled receptors (GPCRs) by novel compounds in brain. These methodologies are easily applicable to various disease models, and combining them with behavioral readouts allows versatile evaluation of the pathophysiology of the CNS disorders and mechanisms of action of novel treatments.

Metabolic activity in brain

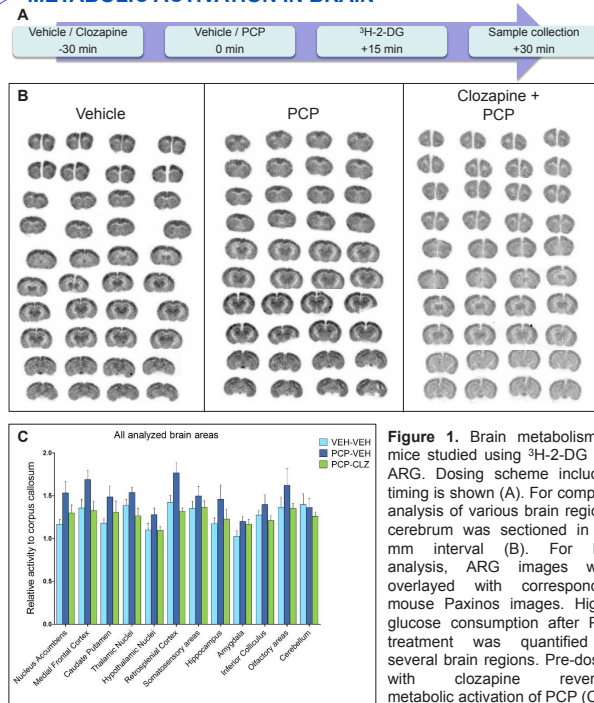
Tissue metabolism is often studied using the radiolabeled glucose analogs, like 2-deoxy-D-glucose (³H-2-DG) or ¹⁸F-fluodeoxyglucose (FDG), which is taken up by high-glucose-using cells in brain, kidney, and tumors. After glucose is absorbed into a cell, phosphorylation prevents it from being released; therefore metabolic activity in different brain regions can be quantified *ex vivo* through the use of autoradiography. Phencyclidine (PCP) is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist that is commonly used to induce schizophrenic-like symptoms in preclinical studies. In schizophrenic patients and PCP treated rodents, hypometabolism is seen in prefrontal cortex, thalamus, and temporal lobes. Currently, clozapine (CLZ) is used in the treatment of schizophrenia.

The objective of the current experiment was to quantify metabolic changes in various brain regions after PCP treatment and to determine whether pre-dosing with CLZ blocks the effect of PCP treatment.

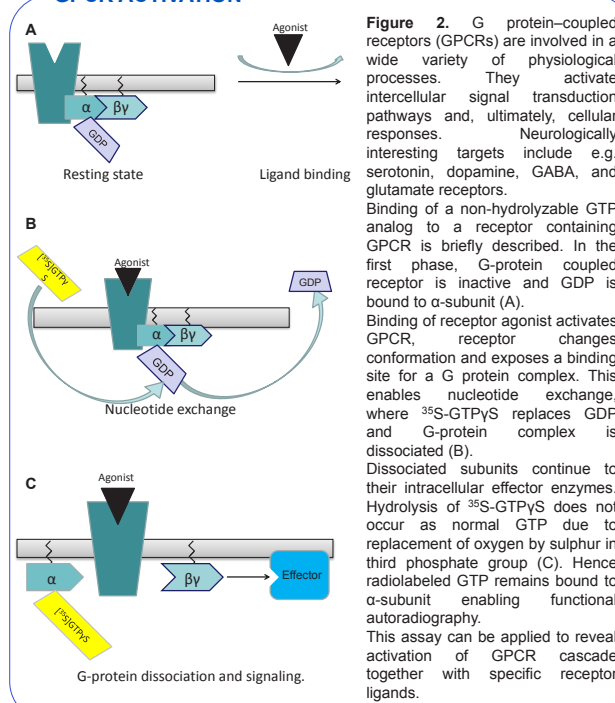
Activation of GPCRs

G protein-coupled receptors (GPCRs) are involved in a wide variety of physiological processes, like sight, taste, smell, behavioral and mood regulation. They activate intercellular signal transduction pathways, and ultimately, cellular responses. Ligand binding to the GPCRs induces an interaction of the receptor with G protein stimulating the release of GDP simultaneously with the exchange of GTP. The use of ³⁵S-GTPγS autoradiography has been applied to study receptor activation after ligand binding to Gi and Gs type GPCRs.

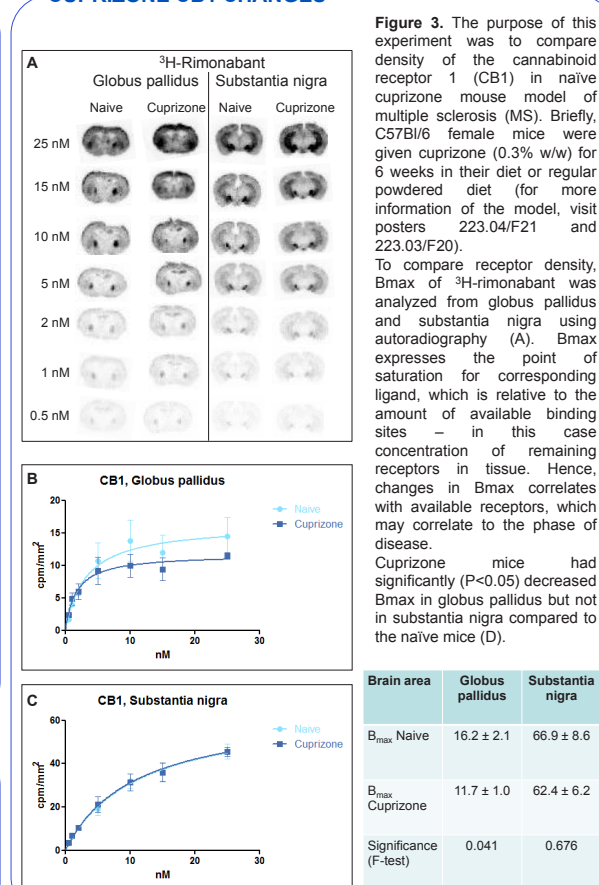
METABOLIC ACTIVATION IN BRAIN



GPCR ACTIVATION



CUPRIZONE CB1 CHANGES



MATERIALS AND METHODS

Animals

All animal experiments were approved by the National Animal Experiment Board, Finland. This study was exploratory in nature and is not within the definition of a nonclinical study subject to compliance with the U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies (21 CFR Part 58). The animal facility at this site is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International.

Metabolic activity in brain

To measure metabolic activity in brain, mice were divided into three groups (vehicle, PCP and CLZ+PCP, n=5/group). Animals were pre-dosed with vehicle or clozapine (6 mg/kg, i.p.) 30 min prior to with vehicle or PCP (5 mg/kg, i.p.) dosing. Finally, mice were dosed with ³H-2-DG (1.4 MBq, i.v.) 15 min post vehicle or PCP administration. Mice were terminated 15 min after ³H-2-DG dosing and the brains were immediately snap frozen with dry ice and isopentane.

³⁵S-GTPγS binding assay

Briefly, 20 μm fresh frozen sagittal cryosections were first preincubated in the assay buffer for 20 min to moisture sections and further 60 min in the assay buffer containing GDP ("GDP loading") prior incubating with ³⁵S-GTPγS for 90 min. ³⁵S-GTPγS incubation included also GDP (basal level) and either unlabeled GTP (nonspecific binding), agonist (HU-210, 1 μM) to measure CB1 receptor activation. The CB1 receptor-mediated activation was confirmed by adding CB1 inverse agonist rimonabant. Finally, sections were briefly washed prior to the imaging. For more detailed information, see dissertation Functional Autoradiography as a Pharmacological Approach for Studying G Protein Coupled Lipid Receptor Signaling, Niina Aaltonen (University of Eastern Finland, 2013).

Autoautoradiography

Quantification of sections was performed using Betalimager (BiospaceLab, France). Scanning time of samples was 6 – 16 h. The counting of emitted beta particles was assessed using MultiVision software (Biospace, France). Specific binding, expressed in cpm/mm², was defined by the difference between total and non-specific binding.

GPCR ACTIVATION IN EAE MOUSE MODEL

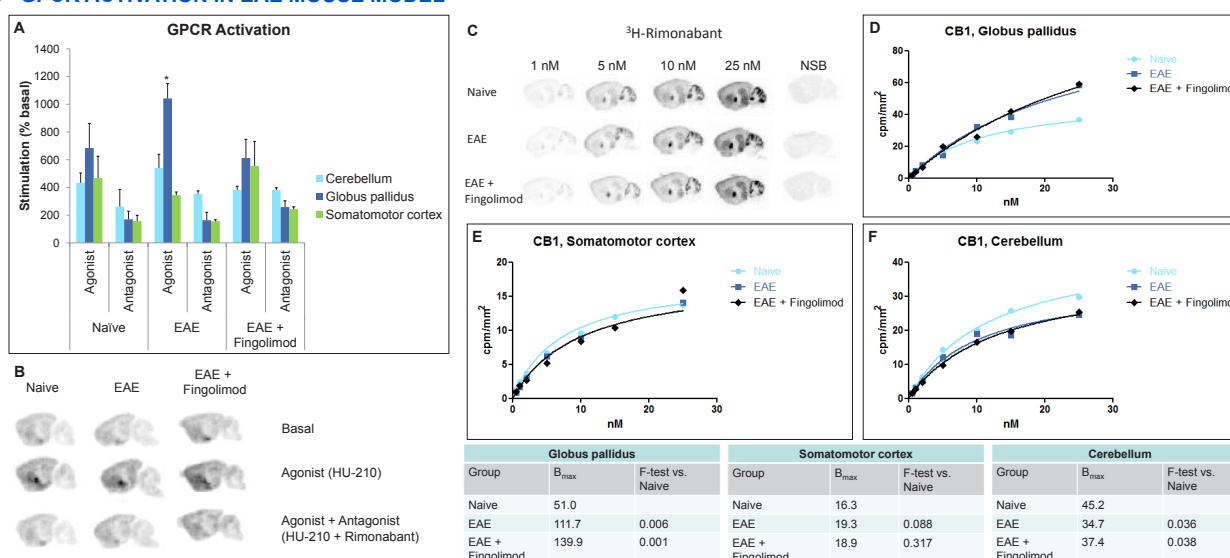


Figure 4. Activation of the cannabinoid receptors (CB1) and available binding sites of the CB1 ligand rimonabant in EAE (MOG₃₅₋₅₅) mouse model of multiple sclerosis (MS). Briefly, injection of pertussis toxin allows immune cells access to the CNS through the blood brain barrier causing T cell related demyelination. Fingolimod (FTY-720), an immune modulating drug approved by FDA for treatment of MS, is commonly used as a positive control in currently used EAE mouse model (for more detailed information about the model, visit posters 223.01/F18 and 223.02/F19). Increased CB1 receptor activity was seen especially in globus pallidus of EAE induced

mice. Interestingly, in the fingolimod treated EAE mice, CB1 receptor activation was reversed to the level of naive mice (A). However, the Bmax analysis of CB1 receptor binding sites revealed up-regulated CB1 level in both EAE and EAE+fingolimod groups compared to naive mice (D – F). This suggests that despite of the elevated CB1 receptor density in the EAE mice, the receptors are not functional in the fingolimod treated mice at studied time point. Sagittal autoradiography images of the CB1 receptor activation and (B) and CB1 receptor binding (D). Autoradiography analysis was performed ca. 14 days post inoculation.

CONCLUSIONS

The current data demonstrate how the combination of assays can be utilized to advance our understanding of disease state and effect of treatment. Digital scintillation autoradiography was selected as methodology for scanning. It enables quantitative and real time imaging of even tritiated samples within hours, compared to minimum of several weeks of exposure time requested to phosphoscreens or films. This accelerates method development substantially. ³H-2-DG autoradiography provides quantitative information of neuronal metabolic activity and changes caused by dosed compound in various regions separately within individual. As an example, we showed increased glucose consumption after PCP exposure which was reversed with clozapine. Changes in the CB1 receptor density were measured in two different animal models of MS. In globus pallidus, we saw increased receptor density when studied in the EAE model as in the cuprizone model the CB1 receptor density was decreased. This difference may be due to different induction of demyelination as well as the phase of disease. EAE mice were studied relatively early phase, ca. 14d as the cuprizone mice showed already severe neuroinflammation after 6 week exposure (see also poster 74.12/R13). Interestingly, increased CB1 receptor density was seen both in the EAE and EAE+fingolimod groups while the functional autoradiography showed that the CB1 receptors in globus pallidus are not activated in the fingolimod treated group. This shows that measuring both receptor density and downstream signaling pathway are vital in understanding both the pathophysiology of the disease and drug mechanism of action. As a summary, the combination of metabolic, receptor and functional autoradiography offers a powerful tool to comprehensively measure changes in disease models or responses to novel molecules.