LABORATORY OF

MOLECULAR PHARMACOLOGY

Cannabinoid receptor 1, endocannabinoid signalling, nociception, pain, synaptic plasticity

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Studies on the dimeric structure of mGluRs were approached by several laboratories including ours; this research was conducted as a continuing collaboration with the CNRS laboratory in Montpellier. Briefly, we revealed in studies using pharmacological tools combined with functional assays and DNA recombination technology that the mGluRs are obligatory dimers, and that within the dimer only one subunit activates G-proteins. This was recently supported by crystallographic studies conducted by Brian Kobilka.

In another venue we discovered that distinct splice variants of mGluR1 combine in dimers with important functional outcomes. Therefore, a fraction of mGluR1 receptors exist as heterodimers with respect to their splice variants.

More recently, we detected the Src homology 3-domain growth factor receptor-bound 2-like (endophilin) interacting protein 1 (SGIP1) as a novel CB1R interacting partner. SGIP1 is functionally linked to clathrin-mediated endocytosis and its overexpression in the hypothalamus leads to an energy regulation imbalance resulting in obesity in rodents. We reported that SGIP1 prevents the endocytosis of activated CB1R and that it alters signalling via the CB1R in a biased manner. CB1R – β2 arrestin-associated signalling is profoundly changed, most likely as a consequence of the prevention of the receptor's internalization, an inhibition mediated by SGIP1. To study the role of SGIP1 *in vivo*, we developed SGIP1 knockout mice to explore their phenotype. In a recently submitted manuscript, we report alterations in emotionality of SGIP1 knockout mice based on open field, elevated plus maze, and light/dark box tests. We discovered that a mouse coping with despair in an inescapable situation is enhanced by SGIP1 deletion. In the tail immersion test, the antinociceptive effects of CB1R agonists were significantly enhanced in the SGIP1 knockout mice. In evaluating responses to Δ9-tetrahydrocannabinol in cannabinoid tetrad tests, interesting differences were revealed compared to wild-type mice, including modification of responses in several models of pain.

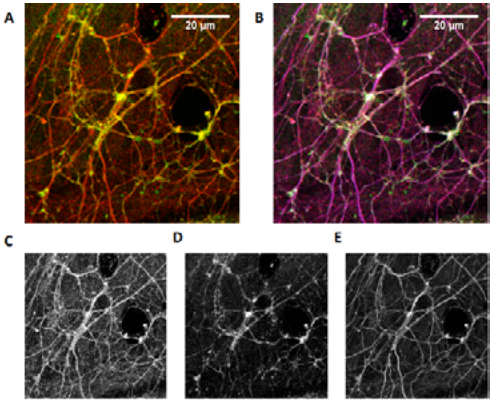

Figure 1. CB1R, SGIP1 and Piccolo are colocalized in autaptic hippocampal neurons.
Autaptic neurons were stained with a-CB1R (red), a-SGIP1 (green) and a-Piccolo (blue) antibodies. The pictures shows the overlay of CB1R and SGIP1 (A) and also and overlay with synaptic marker Piccolo (B). The split channels depict a-CB1R (C), a-SGIP1 (D) and a-Piccolo (E) staining separately. The colocalization of the three proteins is visible in synaptic buttons.

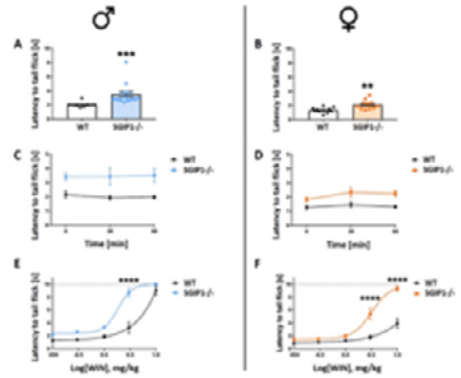

Figure 2. Male and female SGIP1-/- mice have prolonged latencies in the tail immersion test and are more sensitive to cannabinoid agonist WIN55,212-2.
A, B) To assess acute analgesia, we used the tail immersion test. C, D) The tip of the animal's tail was immersed into a water bath of 52°C and the latency to flick the tail was measured in three trials with 30 min inter-trial intervals and averaged. Both sexes of SGIP1-/- had prolonged latencies to flick the tail (E,F). To assess the analgesic effect of cannabinoid receptor agonist WIN 55,212-2 (WIN), the mice were injected with increasing doses of WIN (0, 0.3, 1, 3, 10 mg/kg i. p.) 1 hour apart and the tail flick was measured 1 hour after the application. WIN provides increased analgesia in SGIP1-/- mice of both sexes compared to WT control.

Selected publications:

1. Hlavackova V, Goudet C, Kniazeff J, Zikova A, Maurel D, Vol C, Trojanova J, Prézeau L, Pin JP, Blahos J* (2005) Evidence for a single heptahelical domain being turned on upon activation of a dimeric GPCR. *EMBO J*, **24**:499–509.
2. Hlavackova V, Zabel U, Frankova D, Bätz J, Hoffmann C, Prezeau L, Pin JP *, Blahos J *, Lohse MJ* (2012) Sequential inter- and intrasubunit rearrangements during activation of dimeric metabotropic glutamate receptor 1. *Science Signal*, **5**:ra59.

Shared senior co-authorship:

1. Techlovská S, Chambers JN, Dvořáková M, Petralia RS, Wang YX, Hájková A, Nová A, Franková D, Prezeau L, Blahos J * (2014) Metabotropic glutamate receptor 1 splice variants mGluR1a and mGluR1b combine in mGluR1a/b dimers in vivo. *Neuropharmacology*, **86**:329-336.
2. Hájková A, Techlovská S, Dvořáková M, Chambers JN, Kumpošt J, Hubálková P, Prézeau L, Blahoš J* (2016) SGIP1 alters internalization and modulates signaling of activated cannabinoid receptor 1 in a biased manner. *Neuropharmacology*, **107**:201-214.

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