

Divorce of obligatory partners in pain: disruption of GABA_B receptor heterodimers in neuralgia

Dietmar Benke^{1,*} and Hanns Ulrich Zeilhofer^{1,2}

¹Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland and ²Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland

*Correspondence to: benke@pharma.uzh.ch

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It is now well established that G protein-coupled receptors can exist not only as homodimers, but also as heterodimers or higher order oligomers. However, whether and how dimerization of the receptors is regulated is poorly understood. In this issue of *The EMBO Journal*, the team of Marc Landry provides evidence for an intriguing mechanism by which—under pathological conditions—GABA_B receptor heterodimers at the cell surface are disrupted and thereby inactivated. An impressive set of experiments thus reveals a novel mechanism regulating the number of functional GABA_B receptors in the plasma membrane and shows that the receptor heterodimer may not be as stable as we previously thought.

It is evident that dimerization and oligomerization at least of class A and C G protein-coupled receptors (GPCRs) play important roles in permitting or enhancing their cell surface

trafficking (Milligan, 2010). The assembly process is thought to serve as a quality control mechanism to ensure that only fully mature and functional receptors reach the plasma membrane. The prototype of an obligatory heterodimer among GPCRs is the GABA_B receptor, which controls excitability of neurons by mediating slow inhibitory neurotransmission (Gassmann and Bettler, 2012). Functional GABA_B receptors are built from two related proteins termed as GABA_{B1} and GABA_{B2}. Although both subunits display a similar structural organization—with a large extracellular domain containing a Venus fly-trap structure, seven transmembrane domains and a large intracellular domain located C-terminal—they serve distinct, complementary functions. GABA_{B1} binds the orthosteric ligands whereas GABA_{B2} recruits the G protein and is required for cell surface trafficking of the receptor complex by masking an ER

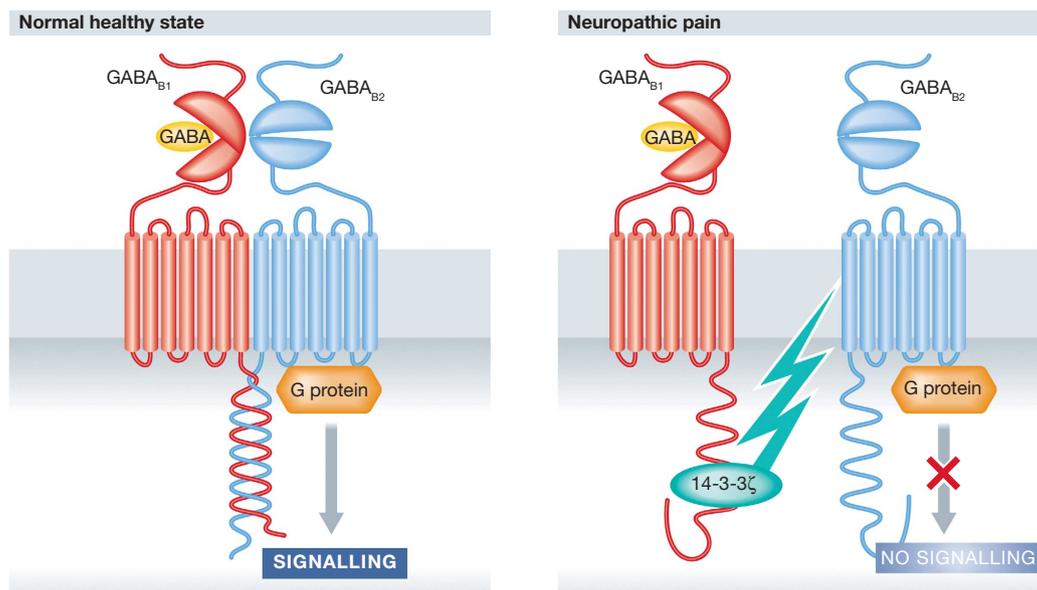


Figure 1 Novel mechanism regulating GABA_B receptor signalling by disrupting the functional receptor heterodimer via interaction with 14-3-3ζ. Functional GABA_B receptors are obligatory heterodimers built from GABA_{B1} and GABA_{B2} subunits. Under normal conditions, binding of GABA to the Venus fly trap-like structure in the N-terminal domain of GABA_{B1} activates Gi/o proteins recruited by GABA_{B2} and thereby modulates distinct effector systems (adenylyl cyclases, potassium channels and voltage-gated Ca²⁺ channels). After induction of neuropathic pain by spinal nerve ligation, 14-3-3ζ is selectively upregulated in the spinal dorsal horn where painful sensory signals are processed and transmitted to the brain. 14-3-3ζ binds to the C-terminal domain of GABA_{B1} and disrupts by a yet-to-be identified mechanism the receptor dimer. This results in non-functional receptors and prevents GABA_B receptor signalling.

retention signal present in the C-terminal domain of GABA_{B1}. This is a striking example that dimerization is essential for and control of expression of a functional GPCR. The availability of GABA_B receptors at the cell surface is also determined by receptor trafficking, which includes endocytosis, recycling and degradation of the receptors (Benke, 2010). To maintain the required number of cell surface receptors for signalling under a given physiological status, all levels of receptor trafficking need to be precisely balanced. Changing the balance of the different trafficking mechanisms is one means to adjust receptor numbers to changing physiological conditions. An example of such regulation is the recently uncovered mechanism of the glutamate receptor-mediated downregulation of GABA_B receptors where the balance of recycling and degradation of the receptors is shifted towards degradation (Benke *et al.*, 2012). Laffray *et al.* (2012) propose a novel and unexpected mechanism regulating the number of functional receptors at the cell surface. This mechanism is operative *in vivo* under pathological conditions and is based on the disruption of GABA_B receptor heterodimers present in the plasma membrane by the GABA_{B1} interacting protein 14-3-3 ζ .

Seven members of 14-3-3 proteins (14-3-3 β , γ , ϵ , ζ , η , σ and τ) are ubiquitously expressed in mammals. 14-3-3 proteins bind predominantly to phosphoserine and phosphothreonine containing sequences and interact with hundreds of different partners to regulate a variety of cellular processes ranging from protein trafficking, apoptosis, cell cycle, signal transduction, cell adhesion and metabolism. It is therefore not surprising that alterations in the expression levels of 14-3-3 proteins and/or changes in the interaction status with target proteins are increasingly observed in diseases such as cancer, neurodegenerative diseases and epilepsy (Zhao *et al.*, 2011).

Among 14-3-3 proteins, 14-3-3 ζ interacts with the C-terminal domain of GABA_{B1} and has been shown *in vitro* to inhibit the heterodimerization of GABA_{B1} and GABA_{B2} C-terminal domains (Couve *et al.*, 2001). However, the physiological and potential pathological function of this interaction was entirely unresolved. In a rat model of neuropathic pain (spinal nerve ligation), Laffray *et al.* (2012) observed a significant upregulation of 14-3-3 ζ selectively in the ipsilateral dorsal horn of the lumbar spinal cord, the area where nociceptive signal processing in response to the injury takes place. Using several complementary methodologies including coimmunoprecipitation, colocalization immunofluorescence analysis, electron microscopy and two-photon fluorescence lifetime imaging, the authors demonstrated *in vitro* and *in vivo* that upregulation of 14-3-3 ζ results in an increased interaction with GABA_{B1} in the plasma membrane and in a concomitant loss of GABA_{B1}/GABA_{B2} association. This finding suggests that 14-3-3 ζ disrupts existing heterodimers in the plasma membrane (Figure 1). As a consequence, the increased GABA_{B1}/14-3-3 ζ interaction rendered cell surface GABA_B receptors non-functional and impaired GABA_B receptor signalling.

The main unresolved and extremely interesting issue concerns the mechanism of heterodimer disruption by 14-3-3 ζ . The interaction site of 14-3-3 ζ partially overlaps with the coiled-coil domain in the C-terminal domain of GABA_{B1} (Couve *et al.*, 2001). Coiled-coil domains are protein–protein

interaction sites and are one of the domains thought to be involved in the heterodimerization of GABA_{B1} and GABA_{B2}. The most obvious mechanism is a direct competition of 14-3-3 ζ and GABA_{B2} for interaction with GABA_{B1}. 14-3-3 proteins are inherently rigid proteins able to stabilize a given conformation after binding to its partner protein (Obsil and Obsilova, 2011). Thus, binding of 14-3-3 ζ might arrest GABA_{B1} in a conformation that is non-permissive for GABA_{B2} heterodimerization. However, the apparent affinity of the interaction of 14-3-3 ζ with GABA_{B1} is rather low and relatively high concentrations of 14-3-3 ζ are required to prevent heterodimerization of GABA_{B1} and GABA_{B2} C-terminal domains *in vitro* (Couve *et al.*, 2001). Given the relatively moderate increase of 14-3-3 ζ in neuropathic spinal cord, a direct competition mechanism *per se* appears unlikely. On the other hand, 14-3-3 proteins predominantly bind to motifs containing phosphoserine and phosphothreonine. Therefore, phosphorylation of GABA_{B1} within the 14-3-3 ζ binding site might thus foster the GABA_{B1}/14-3-3 ζ interaction. In this regard, it would be important to test whether serine or threonine residues within the 14-3-3 ζ binding site are phosphorylated in chronic pain states and whether phosphorylation is required for 14-3-3 ζ interaction.

An alternative mechanism may be based on the scaffolding properties of 14-3-3 proteins. 14-3-3 proteins act as dimers and thus harbour at least two protein interaction sites (Obsil and Obsilova, 2011). Therefore, 14-3-3 ζ may target a second protein or a protein complex to GABA_B receptors that forces the receptor complex to dissociate and prevent reassociation. Proteomic analyses of isolated GABA_{B1}/14-3-3 ζ complexes are needed to address this issue.

Another important aspect of the paper is that it sheds some light on the involvement of GABA_B receptors in neuropathic pain. So far, there is no coherent picture on the contribution of GABA_B receptors to chronic pain states. However, there is increasing evidence that diminished GABA_B receptor activity due to downregulation of the receptors might play a role in at least some models of neuropathic pain (Zeilhofer *et al.*, 2012). Although there might be distinct mechanisms downregulating functional GABA_B receptors in chronic pain conditions, disruption of GABA_B receptor heterodimers via upregulation of 14-3-3 ζ appears to be a contributing factor. In their neuropathic pain model, Laffray *et al.* (2012) observed a diminished analgesic activity of the intrathecally injected GABA_B receptor agonist baclofen. Preventing the binding of 14-3-3 ζ to GABA_B receptors via knocking-down 14-3-3 ζ with siRNA or by using a synthetic peptide disrupting the GABA_{B1}/14-3-3 interaction restored expression of GABA_B receptor heterodimers in the plasma membrane and consequently enhanced the analgesic effect of baclofen. Even more important, disruption of the 14-3-3 ζ /GABA_{B1} interaction by injection of the interfering synthetic peptide alone in the absence of baclofen partially reversed pain in the neuropathic rats. This finding implies that diminished GABA_B receptor signalling contributes to the expression of neuropathic pain. These results might be a starting point for a therapeutic strategy to reduce neuropathic pain based on reversing the GABA_{B1}/14-3-3 ζ interaction. There are already small molecule inhibitors of 14-3-3 protein–protein interactions under development (Zhao *et al.*, 2011), which might be useful for testing the feasibility of such an approach.

References

- Benke D (2010) Mechanisms of GABA_B receptor exocytosis, endocytosis, and degradation. *Adv Pharmacol* **58**: 93–111
- Benke D, Zemoura K, Maier PJ (2012) Modulation of cell surface GABA_B receptors by desensitization, trafficking and regulated degradation. *World J Biol Chem* **3**: 61–72
- Couve A, Kittler JT, Uren JM, Calver AR, Pangalos MN, Walsh FS, Moss SJ (2001) Association of GABA_B receptors and members of the 14-3-3 family of signaling proteins. *Mol Cell Neurosci* **17**: 317–328
- Gassmann M, Bettler B (2012) Regulation of neuronal GABA_B receptor functions by subunit composition. *Nat Rev Neurosci* **13**: 380–394
- Laffray S, Bouali-Benazzouz R, Papon M-A, Favereaux A, Jiang Y, Holm T, Spriet C, Desbarats P, Fossat P, Le Feuvre Y, Decossas M, Héliot L, Langel U, Nagy F, Landry M (2012) Impairment of GABA_B receptor dimer by endogenous 14-3-3 ζ in chronic pain conditions. *EMBO J* (advance online publication, 12 June 2012; doi:10.1038/emboj.2012.161)
- Milligan G (2010) The role of dimerisation in the cellular trafficking of G-protein-coupled receptors. *Curr Opin Pharmacol* **10**: 23–29
- Obsil T, Obsilova V (2011) Structural basis of 14-3-3 protein functions. *Semin Cell Dev Biol* **22**: 663–672
- Zeilhofer HU, Benke D, Yevenes GE (2012) Chronic pain states: pharmacological strategies to restore diminished inhibitory spinal pain control. *Annu Rev Pharmacol Toxicol* **52**: 111–133
- Zhao J, Meyerkord CL, Du Y, Khuri FR, Fu H (2011) 14-3-3 proteins as potential therapeutic targets. *Semin Cell Dev Biol* **22**: 705–712