Bridging the Gaps: Special Commentary

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It's what's on the inside that counts: evidence for intracellular G-protein–coupled receptor signaling in inflammatory pain

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The importance of G-protein–coupled receptor (GPCR) signaling in the context of pain therapy is well established.[5] However, an exciting new report this month by Vincent et al.[8] challenges the assumption that the main pool of nociceptive GPCRs is located in the plasma membrane. This report demonstrates the presence of unique subcellular pools of GPCRs, which are activated by endogenous ligands and are upregulated after inflammatory injury. These data have important implications for those involved in drug development and for those interested in injury-induced protein changes.

During drug design, the location of a ligand-binding domain influences the development of agonists and antagonists for that receptor. For example, agonists to TRPV1 typically bind to intracellular domains of the receptor.[2,9] Clearly, if one developed a compound to activate TRPV1, the membrane permeability of the agent would need to be taken into account. In contrast, it has largely been presumed that development of compounds for classic extracellularly activated receptors such as GPCRs could be completed without regard for cellular permeability. That is, for a GPCR with an extracellular ligand-binding domain, the membrane permeability of a new drug would be less likely to impact the efficacy of the compound.

However, the work by Vincent et al.[8] highlights the fact that this assumption for GPCRs may be incorrect. These new data demonstrate that distinct intracellular pools of GPCRs influence the development of hypersensitivity and pain after injury. This report implicates activation of nuclear membrane–bound metabotropic glutamate receptor 5 (mGluR5) in inflammatory pain.[8] Nuclear membrane mGluR5 expression and cellular functionality have previously been described,[3,4] but the consequences of this system in disease or normal physiology have been unclear. These intracellular mGluR5 receptors bind glutamate with the same affinity as plasma membrane–bound mGluR5, but initiate unique signaling cascades. Nuclear membrane mGluR5 leads to Fos expression, whereas plasma membrane mGluR5 leads to Jun expression.[1] Following up on their 2016 article in Nature Communications,[7] the current report illustrates the consequence of normal and abnormal intracellular spinal cord mGluR5 signaling in the context of the complete Freund's adjuvant (CFA) model of inflammatory pain.

After CFA paw injection, rats show increased tactile sensitivity to intrathecal glutamate. Inhibitors that block glutamate uptake, thereby increasing extracellular glutamate, reduce this glutamate-induced tactile hypersensitivity. This result implies that the pronociceptive effects of glutamate after CFA injury are driven by intracellular glutamate binding. Coupled to this finding are the seemingly paradoxical results that control rats show potentiation of the glutamate-induced behavior with uptake inhibition. Taken together, these data suggest that under normal circumstances, extracellular glutamate is the primary driver of nocifensive behavior, but after inflammatory injury (or neuropathic injury[7]), glutamate effects are driven by intracellular ligand binding and intracellular receptor activation.

Vincent et al. demonstrate that a key mediator of the intracellular glutamate response after injury is mGluR5. The authors tested the ability of cell membrane permeable and impermeable antagonists of mGluR5 to reduce glutamate-induced tactile hypersensitivity. They found that membrane permeable antagonists had a greater analgesic effect. On the molecular level, glutamate injection after CFA caused an increase in Fos expression that was blocked with glutamate uptake inhibitors and permeable mGluR5 antagonists. This is a clear example of a single receptor using different signaling cascades depending on the receptor location in the cell.[6] The fact that plasma membrane and nuclear membrane mGluR5 activate distinct signaling cascades suggests that spatial constraints in the different compartments may dictate downstream signaling of this GPCR. Clearly, this study should give drug developers a pause because the possibility of nonplasma membrane–bound GPCRs with a role in pain could dramatically impact the efficacy of a drug, depending on its membrane permeability.

Another important implication of Vincent et al's work is on research describing changes in protein expression after injury. Typically, because it is expected that GPCRs are primarily active on the plasma membrane, many researchers use whole cell protein analysis to detect injury and pain-associated protein expression changes. Here, the authors show that CFA injection increases the spinal cord nuclear membrane expression of mGluR5 with no observable change in plasma membrane–bound mGluR5. The mechanism of increased nuclear membrane mGluR5 expression after CFA injection is unknown, but the fact that expression of plasma membrane–bound mGluR5 did not change suggests that de novo synthesis with altered trafficking at the translational or posttranslational level is involved. If these authors had looked at the total protein only, they might have assumed that the plasma membrane–bound pool of the mGluR5 was increased after CFA.

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Overall, this report shows how receptor compartmentalization can actually lead to heightened responses after injury. The dual localization of mGluR5 has interesting implications for development of long-term hypersensitivity and possibly chronic pain. An abhorrent cellular process that increased nuclear membrane–bound mGluR5 could cause chronic pain in the absence of peripheral injury. The findings in this report demonstrate that we need to reconsider how receptor localization alters nociceptor signaling in the spinal cord and elsewhere in the nervous system. It is likely that this phenomenon of distinct pools of dormant GPCRs may extend beyond mGluR5 to other receptors involved in pain. This additional complexity in pain biology provides exciting new avenues for scientific inquiry and has significant implications for therapeutic development.

Conflict of interest statement

The author has no conflict of interest to declare.

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