Targeting Chronic Pain with Epigenetic Drugs: Focus on mGlu2 Receptors

Santina Chiechio1, Magda Zammataro1, Robert W. Gereau IV2, Agata Copani1,3 and Ferdinando Nicoletti4,5

1Department of Pharmaceutical Sciences, University of Catania, Italy; 2Washington University Pain Center and Department of Anesthesiology, Washington University School of Medicine, St. Louis, Missouri; 3I.B.B., CNR, Catania, Italy; 4Department of Human Physiology/Pharmacology, University of Rome La Sapienza, Rome, Italy; and 5I.N.M. Neuromed, Pozzilli, Italy.

Abstract

Histone deacetylase (HDAC) enzymes regulate gene expression by affecting chromatin structure and/or the activity of transcription factors. We have recently demonstrated that histone deacetylase inhibitors (HDACIs) behave as epigenetic agents capable of inducing analgesia by up-regulating metabotropic glutamate type 2 (mGlu2) receptors. Specifically, the regulation of mGlu2 receptor expression appears to involve the acetylation of the NF-κB transcription factor. mGlu2 and mGlu3 receptors belong to class II metabotropic glutamate receptors. These receptors are coupled to G i/o proteins and play an important role in mediating antinociception in a variety of inflammatory and chronic pain models. We have shown that the HDACI-mediated mGlu2 receptor up-regulation occurs in the dorsal horn of the spinal cord and in the dorsal root ganglia, supporting a predominant role for mGlu2 receptors as mediators of analgesia in experimental animal models of chronic pain. We suggest that drugs that increase the expression of mGlu2 receptors, such as HDACIs or acetylating drugs (e.g. L-acetylcarnitine), may be effective in patients with chronic pain that are refractory to conventional analgesics.

Keywords: Chronic Pain; Epigenetic drugs; Histone deacetylase; mGlu2 receptors

Established chronic pain reflects pathological functioning of the pain pathways along the entire pain neuraxis, from peripheral nociceptors to the higher pain centers in the CNS. Numerous mechanisms at the molecular and cellular level contribute to the development of chronic pain. These include changes in gene expression, loss of spinal interneurons, rearrangements of central connections, and tissue remodeling (1). Many of these modifications are common to different forms of persistent pain and are not targeted by current analgesic drugs. Sometimes, neuronal plasticity associated with chronic pain underlies the development of resistance to the most effective analgesics (as occurs in patients experiencing breakthrough pain during treatment with opioids) (2-6).

Neuropathic pain (i.e., pain originating from structural or functional lesions of the pain pathways) is a remarkable example of a relatively common form of chronic pain that is difficult to treat. Neuropathic pain is less sensitive to opioids than other types of pain, and is treated with drugs that have not been specifically developed for this indication (for example, antidepressant or antiepileptic drugs) (7-13). The current treatment of neuropathic pain is limited by the occurrence of side effects (e.g., sedation and cerebellar effects with antiepileptic drugs or anticholinergic effects with amitriptyline), which reflect the low selectivity of these drugs for pathological vs. physiological mechanisms. Pregabalin and gabapentin show some degree of selectivity because their target, the alpha2/delta subunit of voltage-sensitive calcium channels, is up-regulated under conditions of chronic...
pain (14-16). However, there are patients that are resistant to both drugs for unknown reasons. Thus, there is an unmet need for additional analgesic drugs targeting “disease-dependent” mechanisms of nociceptive sensitization.

Disease process-specific analgesics could potentially come from epigenetic approaches that are able to induce a long lasting suppression of disease-related enhancement of pain transmission. Epigenetic-modulating agents such as histone deacetylase inhibitors (HDACi) have recently received increased attention because of their anticancer activity (23, 24). In addition to cancer, epigenomic alterations have been associated with a number of other diseases, including neurodegenerative, neurodevelopmental and mood disorders (25).

Here, we focus on the potential use of epigenetic drugs for the treatment of chronic pain states based on our recent observation that analgesic effects can be achieved by inducing up-regulation of receptors that mediate analgesia such as metabotropic glutamate type 2 (mGlu2) receptors (26, 27). mGlu2 receptors belong to group II mGluRs. mGlu2 receptors are G-protein coupled receptors, and based on their pharmacology, sequence similarities and intracellular signaling mechanisms these receptors have been divided into three groups (28-30). Group I mGlu receptors include Gq protein-coupled mGlu1 and mGlu5, whose activation induces the increase of intracellular levels of inositol-3-phosphate, diacylglycerol, and calcium. Group II mGlu (mGlu2 and mGlu3) receptors and group III (mGlu4, mGlu6-8) receptors are coupled to Gi/o proteins; thus, their activation reduces neuronal excitability by inhibiting cAMP production, and mediates the inhibition of voltage-gated calcium entry with the ensuing reduction of glutamate release (31). With the exception of mGlu6, all mGlu receptors are expressed in the pain neuraxis, and can thus modulate pain transmission (32). In particular, activation of group I has been shown to increase pain sensitivity and to mediate central sensitization mechanisms. By contrast, group II mGlu receptors are mostly expressed in presynaptic terminals, where they control the release of glutamate and other neurotransmitters. In presynaptic terminals, mGlu2 receptors are found in extrasynaptic sites remote from the active zone of the synaptic cleft (33). This particular localization makes these receptors to be activated only when excessive glutamate is released, as occurs in exaggerated pain transmission, thus not interfering with normal glutamate transmission. Group II mGlu receptors are expressed throughout the pain neuraxis (32), including in the primary sensory neurons of DRG and in the dorsal horn of the spinal cord (34-36).

We have shown that transcription of group II mGlu receptors is regulated by the NF-κB pathway (37), and that acetylation mechanisms are instrumental for mGlu2-overexpression induced by LAC or HDAC inhibitors (27, 37).

Post-translational acetylation affects a variety of proteins, including histones and transcription factors. Acetylation mechanisms are controlled by two opposing classes of enzymes, histone acetyl transferases (HATs) and HDACs, which control the reversible acetylation within the aminotermi of
lysine residues leading to chromatin remodeling and transcription regulation (38). Drugs can affect protein acetylation levels by interfering with these specific enzyme systems. For example, blocking HDACs with HDAC inhibitors results in an increased level of acetylation (39). Also, drugs can affect acetylation behaving as donors of acetyl groups. This is the case of L-acetylcarnitine, which is able to increase protein acetylation levels in cultured DRG neurons (Figure 1), an effect that is not shared by the non acetylated derivative L-carnitine (37).

Among transcription factors, members of the NF-κB family are regulated by reversible acetylation (40, 41). The NF-κB family comprises five members, c-Rel, RelB, p65 (RelA), p105/p50 (NF-κB1) and p100/p52 (NF-κB2) that can form homo and heterodimers (42). Reversible acetylation of p65 and p50 by p300 or PCAF has been shown to either stimulate NF-κB transcriptional activity or terminate its response. Several acetylation sites have been identified, resulting in different NF-κB-mediated responses depending on the specific acetylated lysine, as summarized in Figure 2. For example, acetylation at lysine residue K310 of the p65 subunit has been demonstrated to be required for a full NF-κB transcriptional activity. On the contrary, acetylation at K122 and K123 reduce the binding affinity of the transcription factor to DNA, facilitating the removal of the p50/p65 heterodimer from the nucleus and the export by the inhibitory protein IκBα. Thus, acetylation at K122 and K123 results in a faster termination of the NF-κB transcriptional activity, reestablishing the latent form of the transcription factor bound to the inhibitory protein IκBα (43).

In vivo and in vitro studies have shown that inhibition of HDAC enzymes results in increased NF-κB acetylation (38, 44, 45). HDAC enzymes form a family of at least 11 isoforms that have been classified into four classes according to their sequence similarities and cellular localization. (46, 47). Many drugs belonging to different chemical classes are able to inhibit HDACs (23, 39). We have recently reported that two different HDAC inhibitors, the hydroxamate derivative suberoylanilide hydroxamic acid (SAHA), and the benzamide derivative MS-275, are able to induce...
analgesia by selectively up-regulating the expression of mGlu2 receptor protein.

In our experiments both drugs were able to increase the expression of mGlu2 receptors in the mouse spinal cord and in the DRGs, together with an increased acetylation level of p65 at lysine 310 (27). Along with this increased expression of mGlu2 receptors, mice exhibited an analgesic response to the persistent inflammatory pain induced by formalin injection in the paw. Interestingly, the analgesic effects of both SAHA and MS-275 were antagonized by a single injection of LY 341495, a selective antagonist of group II mGlu receptors, suggesting that mGlu2 over-expression is instrumental for the analgesic effect of SAHA and MS-275 (27). Our studies suggest that the LAC and HDAC inhibitors induce analgesia by up-regulating mGlu2 receptors through a mechanism involving the acetylation of the NF-κB transcription factor (Figure 3).

Overall, our results indicate that mGlu2 receptors are important mediators of endogenous analgesia especially in models of persistent pain. At this time, we cannot exclude a role for mGlu3 receptors in pain modulation. More information about the specific contribution of these receptors in chronic pain conditions may come from the use of positive and negative allosteric modulators of mGlu2 or mGlu3 receptors, or from mGlu2 and mGlu3 knock-out mice.

References
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