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Commentary

Association of Mu-opioid and NMDA Receptors in the Periaqueductal Gray: What Does it Mean for Pain Control?

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The brain has an endogenous descending control system to modulate pain. This system is thought to arise from the activation of output neurons in the periaqueductal gray area (PAG) that project to the rostroventral medulla, with a relay to the spinal cord to modulate incoming pain signals (Basbaum and Fields, 1984). Electrical stimulation or microinjection of opioids into the ventral portion of the PAG results in analgesia. Opioids activate the pathway by inhibiting tonic GABA release (or disinhibition). The disinhibition hypothesis predicts that morphine and glutamate stimulate the descending pathway via activation of different cell populations, opioids by inhibiting GABA interneurons or inhibitory projection neurons into the PAG, and glutamate by directly activating PAG output neurons. In this issue of *Neuropsychopharmacology*, Rodriguez-Munoz *et al* provide evidence for a close association of NMDA and mu-opioid (MOR) receptors in the PAG, suggesting that these two receptors are not only colocalized within the same cell population, but that there is bidirectional regulation of the NMDA/MOR interaction during acute morphine tolerance.

One of the key findings of the Rodriguez-Munoz *et al*'s paper is that MOR proteins and NR1 subunits of the NMDA receptor can be immunoprecipitated by antibodies specific for extracellular epitopes on either the MOR or NR1. These results suggest that the two proteins are directly interacting or interact as part of a signaling complex of multiple proteins. Immunoprecipitation studies are supported with surface plasmon resonance and bimolecular fluorescence complementation analyses, demonstrating that the C-terminal tails of the two proteins can directly bind to one another. The studies by Rodriguez-Munoz and colleagues are the first to suggest that protein complexes containing MOR and NR1 subunits may be functionally regulated during morphine tolerance. Colocalization of MOR and NR1 subunits has been previously observed with electron microscopic analyses in dendrites of PAG neurons, but the subcellular compartmentalization within the dendrites was very different for the two proteins (Commons *et al*, 1999). However, the antibodies used in the electron microscopic studies were directed at intracellular C-terminal epitopes, so it is possible that MOR/NR1 complexes were not identified. Rodriguez-Munoz *et al* are appropriately conservative in interpretation of their data by stating that the data support the possibility of a direct physical interaction between MOR and NR1 proteins, but are also consistent with interaction as part of a complex with other binding partners, such as PSD-95. One particularly puzzling result is the fact that the MOR/NR1 complex did not immunoprecipitate with significant numbers of NR2 or NR3 subunits, which may suggest that the MOR binds preferentially to NR1 subunits to limit or control the formation of functional NMDA receptors.

A second key finding in the paper by Rodriguez-Munoz *et al* is that the MOR/NR1 complex may be necessary for morphine-induced antinociception. Intracerebroventricular morphine injections disrupt the MOR/NR1 complex with a time course that parallels decreased morphine antinociception and the emergence of acute (within 24 h of a single injection) behavioral tolerance to morphine. Indeed, mice with a knockdown of NR1 subunits have reduced sensitivity to morphine compared with wild-type mice (Dykstra *et al*, 2011), potentially adding support to the idea that MOR/NR1 complexes may potentiate morphine-induced antinociception with the caveat that these mice would also have severely altered glutamatergic signaling throughout the descending antinociceptive pathway.

Rodriguez-Munoz *et al* also find that disruption of the MOR/NR1 complex by morphine is blocked by protein kinase C (PKC) inhibition with no effect of PKA or G-protein receptor kinase 2 inhibitors. Similarly, PKC inhibition reverses the expression of acute tolerance to morphine. These results are consistent with prior data showing that antinociceptive tolerance to morphine is sensitive to PKC inhibition, but tolerance induced by other opioid agonists, such as DAMGO is not (Hull *et al*, 2011). Thus, it will be interesting to examine whether other opioid agonists disrupt the MOR/NR1 complex. The temporal correlation between the PKC-mediated separation of the MOR/NR1 complex with the development of tolerance is

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interesting, but is not wholly convincing evidence that the MOR/NR1 complex is necessary for antinociception, as injections of morphine and NMDA into the ventricles are likely to affect several brain areas involved in central circuits that regulate pain behaviors. Additional studies looking at changes in synaptic activity would increase the temporal and spatial resolution of MOR/NR1 interactions, and would help to rule out potential circuit effects.

Although the studies by Rodriguez-Munoz and colleagues focus on changes in the MOR/NR1 complex following acute tolerance to morphine, equally intriguing is how the complex may change under conditions of chronic pain. There is strong clinical evidence that opioids are less efficacious under conditions of chronic pain, but NMDA antagonists are clinically useful (Eisenberg *et al*, 2006). NMDA antagonists microinjected into the PAG can reverse behaviors associated with hyperalgesia (Ghelardini *et al*, 2008) and neuropathic pain (Mehta *et al*, 2011). These results provide evidence that acute tolerance may be associated with activation of delayed nociceptive processes in the PAG, following opioid administration or stress. Interestingly, tolerance to clinically used opioids, such as morphine and fentanyl, are dependent on PKC mechanisms, but tolerance to other opioid agonists, such as DAMGO, are not affected by inhibition of PKC (Hull *et al*, 2011), suggesting that other opioid agonists may be more useful in neuropathic pain conditions, because they do not stimulate PKC and potentiate NMDA responses.

Finally, the studies by Rodriguez-Munoz *et al* have outlined a conceptual framework for functional interactions between the opioid and glutamate systems within the PAG that modulate pain. It may be time to revisit the disinhibition hypothesis in light of this novel finding to further delineate processes that contribute to antinociception and the development of morphine tolerance. One key aspect will be to determine what cell type (GABA, glutamate, and so on) these complexes are expressed in. Further studies directed toward understanding MOR/NR1 complexes in chronic pain should help to provide a unified hypothesis for the involvement of the PAG in pain processing.

DISCLOSURE

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