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No DREAM, No Pain: Closing the Spinal Gate

Minireview

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Pain transmission in the spinal cord is regulated by a balance of facilitatory and inhibitory influences operating on the neural circuits of the somatosensory system. The transcriptional repressor DREAM acts constitutively to suppress prodynorphin expression in spinal cord neurons. Knocking out DREAM results in sufficient dynorphin expression to produce a strong reduction in generalized pain behavior, highlighting the role that intracellular molecules play in modulating pain gating in the spinal cord.

Dynorphin is an endogenous ligand for the κ opiate receptor, which, along with the μ and δ receptors, is a member of the opiate G protein-coupled receptor family. Agonists of these receptors are major candidate drugs for pain control. Morphine, the gold standard for analgesics, acts via the μ receptor to both activate potassium channels and block calcium currents, reducing membrane excitability and transmitter release (Pasternak, 2001). However, μ receptor activation also affects cognition, perception, mood, respiration, and gastro-intestinal function, and clinical use of μ agonists leads to tolerance, withdrawal, and addiction. Would δ and κ agonists be better analgesic drugs than μ agonists? This is not clear: δ agonists have not been tested in patients yet, and the analgesia produced by κ agonists can be complicated by dysphoria and diuresis.

What about activating the opiate receptors via their endogenous ligands to produce analgesia? β endorphin and the enkephalins, the endogenous ligands for μ and δ receptors, have been repeatedly proposed to act as humoral and synaptic modulators of pain. However, other than in specific circumstances related to stress and placebo, their mechanisms of release are poorly understood. Now, Cheng and coworkers (2002) have found that mice with a null mutation in the repressor DREAM possess a marked reduction in generalized pain behavior, effectively displaying a phenotype of ongoing analgesia. This finding is attributed to the removal of a constitutive block by DREAM of dynorphin expression in spinal cord neurons. DREAM, by suppressing dynorphin levels in the spinal cord, appears to regulate pain transmission by controlling the level of κ receptor activation, offering a new possibility for managing pain. Dynorphin derepression, by blocking DREAM's action, would appear to be an attractive therapeutic opportunity because animals with a null mutation for DREAM show persistent analgesia without any evidence of κ receptor desensitization, or substantial motor or behavioral abnormalities. The specific effect of the DREAM null mutation, apparently limited to a reduction in pain, is rather surprising, given the multiple functions attributed to this protein. DREAM has also been christened calsenilin and KChIP3, and has been found to modulate potassium channels, proteolytic processing (references in Cheng et al., 2002), and apoptosis (Sanz et al., 2001). These diverse roles, coupled with multiple potential target genes for transcriptional repression, might have suggested that the knockout would have a more widespread effect than observed by Cheng and colleagues. Future analysis may reveal additional more subtle phenotypes.

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The Pain Gate

Until the 1960s, the prevailing model for the neural generation of pain was essentially Cartesian: a fixed and dedicated pain pathway was thought to link the site of a peripheral noxious stimulus to the brain site where the perception of pain occurs. In this model, the nature, intensity, and duration of the pain sensation were considered a passive reflection of the qualities of the peripheral stimulus. The nervous system was believed to essentially act as a hardwired telephone link, with the spinal cord merely a switchboard operator dialing the appropriate connection between a peripheral noxious stimulus and the central pain response.

The first major challenge to this view was that of the Spinal Gate Control Theory proposed by Pat Wall and Ron Melzack in 1965 (Melzack and Wall, 1965). Based on a mixture of clinical observation and insights from systems neurophysiology and neuropsychology, they proposed that input signals from primary sensory neurons were actively modulated in the spinal cord by a "gate." Which signals were let through, and therefore relayed to the brain, depended on a balance between inhibitory and facilitatory influences both arising locally and descending from the brain. Although specific elements of this theory have been shown to be invalid or too simplistic, the fundamental model remains. Namely, pain does not arise solely from the faithful relay of signals from primary somatosensory neurons to the brain, but is subject to other modulatory signals and is dependent on prior experience. At one extreme, pain modulation is sufficiently powerful that under certain circumstances massive tissue injury may be sustained without producing pain. Recruitment of the body's multiple endogenous inhibitory control mechanisms to shut down pain when necessary for survival purposes has been observed repeatedly in war casualties. At the opposite extreme, previously innocuous stimuli, such as lightly touching or blowing on the skin, can become excruciatingly painful under certain pathological situations. Moreover, following peripheral inflammation, a lesion to a peripheral nerve, or damage to the central nervous system, pain can arise in the absence of any detectable peripheral stimulus.

What Controls the Pain Gate?

Most efforts until now have examined two general processes that can control the pain gate. First, inhibitory

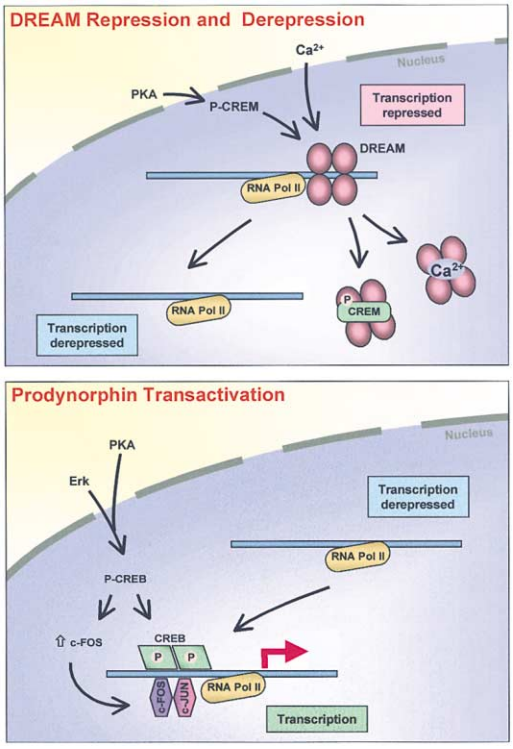

Figure 1. Dynorphin Transcription Is Determined by a Balance between Repression and Transactivation

The transcriptional repressor DREAM, which is constitutively expressed in certain neurons, binds to a downstream response element (DRE) on the prodynorphin gene, blocking RNA polymerase II, and thereby transcription. DREAM binding to the DRE site can be terminated either by calcium entry into the nucleus and binding directly with DREAM or by a PKA-mediated phosphorylation of CREM, both of which are likely to occur after activation of the neuron. Prodynophin transcription is also regulated by transactivators including CREB binding to CRE, and c-*fos* with c-*jun* to an AP-1 site. CREB activation and c-*fos* levels are regulated by activity-dependent activation of PKA and the MAPK ERK.

neuronal circuits synapse onto nociceptive transmission neurons in the spinal cord and act as bouncers at the door, keeping out undesirable inputs (Fields and Basbaum, 1999). Second, excitatory mechanisms can sensitize central neurons, allowing uninvited gatecrashers into the pain party (Woolf and Salter, 2000). While pain needs to be examined and understood at a systems level, it now appears that in addition to extrinsic pain gating mechanisms, a series of intracellular switches contribute to the intrinsic gating of pain. Through regulation of dynorphin levels in spinal cord neurons, Cheng et al. (2002) show that DREAM acts to repress a pain inhibitor. In a Ying and Yang-like balance, opposing forces control molecular switches in the spinal cord. These switches can either allow or, like DREAM, deny expression of pain regulating molecules.

DREAM and Dynorphin

DREAM was discovered in a search for proteins that bind to an element that follows the transcription start site in the promoter region of the dynorphin gene (Carrion et al., 1999). Earlier work had shown that a 110 kDa nuclear complex binds to a downstream regulatory element (DRE) of the prodynorphin gene, and that this binding repressed dynorphin transcription (Figure 1). The fact that the DRE element is functional in both directions downstream, but not upstream, of the TATA box, suggests that it physically impedes the progress of the RNA polymerase II complex (Ledo et al., 2000). A 32 kDa protein was isolated from a human caudate expression library using a double-stranded oligonucleotide encoding the DRE sequence as a probe (Carrion et al., 1999). Coexpression of this protein with a reporter plasmid containing the prodynorphin promoter showed that the protein was capable of repressing basal dynorphin expression, and other reporter assays confirmed that repression is effected through the DRE site. In naming this protein, Carrion and coworkers coined the acronym DREAM because the protein binds to the DRE sequence and acts as a silencer or antagonist modulator (Carrion et al., 1999).

DREAM binds the prodynorphin promoter as a tetramer to form the 110 kDa complex (Carrion et al., 1998, 1999). DREAM's primary structure contains four calcium chelating EF-hand motifs, which site-directed mutations show bind calcium and then prevent DREAM repression of the prodynorphin promoter (Carrion et al., 1999) (Figure 1). In neuroblastoma cell lines, however, prodynorphin de-repression is PKA mediated, a surprising finding, since DREAM does not contain any PKA phosphorylation consensus sites (Carrion et al., 1998; Carrion et al., 1999). This apparent paradox was solved when it was found that αCREM and DREAM form complexes that are strengthened by PKA mediated αCREM phosphorylation (Ledo et al., 2000). When αCREM interacts with DREAM, the complex is released from the DRE site, enabling prodynorphin transcription (Ledo et al., 2000) (Figure 1).

Neuronal Activation by Noxious Inputs

Noxious stimuli activate high threshold primary sensory neurons in the periphery. This activity is conducted to their central terminals, which synapse on second order nociceptive neurons in the spinal cord. Brief inputs carried by the sensory neurons, generated in response to transient non tissue-damaging noxious stimuli, excite the dorsal horn neurons via the synaptic release of glutamate which activates AMPA/kainate ligand-gated ion channels. The resulting fast synaptic depolarizations encode the duration, intensity, and location of the stimulus (Woolf and Salter, 2000).

The more intense or sustained noxious stimuli associated with tissue damage result in temporal summation of postsynaptic depolarizations. Temporal summation removes the voltage-dependent magnesium block of NMDA glutamate receptors and activates voltage-gated calcium currents, causing a substantial calcium influx. Intense stimulation of nociceptors also activates metabotropic glutamate, neuropeptide GPCR receptors, and tyrosine kinase receptors as a result of the synaptic release of glutamate, substance P, and BDNF. Activation of these receptors, together with the calcium influx, initiate increases in the excitability of spinal neurons, the phenomenon of central sensitization. Central sensitization results in increased synaptic efficacy, and thereby a spread of pain sensitivity beyond the site of injury (secondary hyperalgesia) and the generation of pain in response to low threshold inputs (allodynia) (reviewed in Woolf and Salter, 2000).

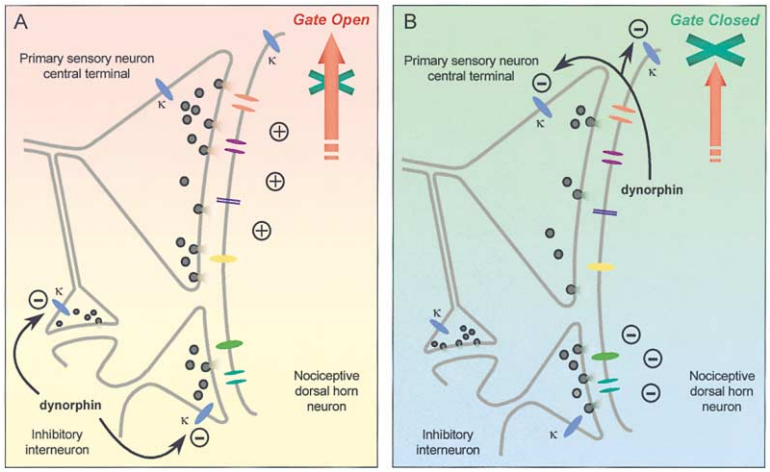

Figure 2. Molecular Switches Controlling Dynorphin Expression in Different Sets of Spinal Cord Neurons Can Potentially Facilitate or Suppress Pain Transmission

Dynorphin expression induced in inhibitory interneurons that innervate pain transmission neurons, by reducing the activity of these neurons, will facilitate pain transmission producing hyperalgesia (A). Dynorphin expression that reduces input to or activity of the pain transmission neurons themselves will result in analgesia (B).

Molecular Switches that Open and Close the Pain Gate

Several activity-dependent events driven by noxious inputs, such as calcium influx, the activation of PKA, and the pERK/pCREB pathway, initiate alterations in transcription in dorsal horn neurons. Calcium binding to DREAM on entry into the nucleus would produce a derepression of genes whose promoter contains a DRE site (Figure 1), including c-*fos* and prodynorphin. PKA would also initiate a derepression of DREAM, though in this case through αCREM. Induction of CREM (Naranjo et al., 1997) and activation of ERK and CREB (Ji et al., 2002) occur in the spinal cord following noxious stimulation, as do increases in c-*fos* and prodynorphin expression (Naranjo et al., 1991). Surprisingly, in the DREAM knockout, no constitutive elevation of c-*fos* in the spinal cord, nor of dynorphin in whole brain preparations was observed, implying that regulation through DRE occurs in a more complex context of multiple tissue specific repressors and transactivators. Changes in spinal cord levels of c-*fos* following nociceptive input, as well as region-specific differential expression of prodynorphin in the brain of the DREAM null mutant animals would be of interest in future studies, as would the functional consequence of the increased dynorphin expression found in ventral horn neurons.

Prodynorphin expression is driven by c-*fos* and c-*jun*, which bind a noncanonical AP-1 element in the dynorphin promoter (Naranjo et al., 1991), and by three CREB binding CRE consensus sites (Cole et al., 1995) (Figure 1). Inhibition of the MAPK cascade leads to a reduction of prodynorphin, and of pain hypersensitivity (Ji et al., 2002). If dynorphin is a negative pain regulator, as the DREAM knockout suggests, why does reducing it by inhibiting ERK activation inhibit pain? There are two possible explanations. The first is that the MAPK signal transduction cascade is permissive, acting on many targets that may exert both positive and negative influences on excitability. While the net effect of MAPK inhibition is to reduce pain hypersensitivity by blocking transcription of pain promoting genes like NK1 (Ji et al., 2002), it also reduces prodynorphin levels, which may represent an intrinsic negative feedback pain suppressor. The second explanation is that dynorphin may have diverse actions, acting both as a pain suppressor and a facilitator depending on how and where it is expressed, and on which neurons it acts.

Preprodynorphin precursor protein is broken up into different peptides; some are highly selective for κ receptors and others are non-opiate in their action, and each may contribute to dynorphin's different roles (reviewed in Laughlin et al., 2001). Pharmacological and prodynorphin gene knockout data suggests that under some circumstances dynorphin can actually act as a hyperalgesic agent—creating pain (Laughlin et al., 2001). At high concentrations, dynorphin activates the NMDA receptor, which would facilitate pain by inducing central sensitization. Moreover, dynorphin acting via κ receptors may not only inhibit activity in nociceptive transmission neurons, producing analgesia, but may also inhibit the many inhibitory interneurons in the spinal cord (Figure 2). Dampening inhibitory neuron activity would lead to an overall increase in excitability of nociceptive neurons (disinhibition), another example of the balance of forces operating in the pain pathway. It is essential, therefore, to identify which cells in the dorsal horn of the spinal cord express dynorphin in response to DREAM derepression and after inflammation, and in addition establish on which cells dynorphin acts. Does dynorphin act via κ receptors on presynaptic primary afferent inputs preventing activation of spinal cord nociceptive neurons, or by a direct postsynaptic inhibition of these neurons? Conversely, is dynorphin's effect directed onto inhibitory interneurons? Action on inhibitory interneurons will open the pain gate, while action on pain transmission neurons will close it (Figure 2).

Given the heterogeneity of neurons and their circuits, the result of opening or closing a single molecular switch is, not surprisingly, very complex. Since millions of individuals suffer from chronic pain, teasing out these mechanisms, although daunting, is essential if we are to stand any hope of controlling the pain gate within patients. The study by Cheng et al. is nevertheless an encouraging first step.

Sweet DREAMs are made of this. Who am I to disagree?

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