Endocannabinoids rein in pain outside the brain

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Enhancers of endocannabinoid signaling are potential analgesics, but they cause unacceptable psychiatric side effects. A new study reports an inhibitor of endocannabinoid breakdown that has analgesic activity and cannot enter the CNS.

Medical marijuana is a popular topic among scientists, clinicians, activists and politicians. Indeed, preparations of the *Cannabis sativa* plant are now being used in medicinal settings to stimulate appetite and for symptomatic relief in multiple sclerosis. The major psychoactive ingredient of these preparations activates cannabinoid type 1 (CB1) receptors in the brain, the same receptors that recognize endogenous lipid-like mediators known as endocannabinoids. Although potential medicinal uses of cannabis preparations have garnered the lion's share of public attention, steady progress has been made in determining the potential therapeutic uses of a variety of drugs that target the components of the endocannabinoid system, which consists of the endocannabinoids, their receptors and the enzymes involved in their biosynthesis and degradation. Pain relief is a potential indication for drugs targeting the endocannabinoid system. CB1 receptors mediate analgesic actions and endocannabinoids modulate neural nociceptive mechanisms[1,2]. Receptors present in both the peripheral nervous system (PNS) and CNS participate in these pain-relieving effects[3]. However, separating the roles of PNS and CNS endocannabinoid actions has been difficult given that most drugs targeting cannabinoidergic transmission readily penetrate the blood-brain barrier, acting on receptors in all nervous system areas. Endocannabinoid-targeted drugs that act only in the periphery have the potential to provide anti-nociceptive benefit without producing intoxication or other CNS impairing effects. Such drugs would not only be safer for users, but would also avoid the legal problems that have bedeviled some cannabinoidergic drugs proposed for medicinal use.

Clapper *et al.*[4] applied a new, peripherally acting inhibitor of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) to mouse models of visceral, neuropathic and inflammatory pain. The effects of the drug, URB937, were lost in mice lacking FAAH or the CB1 receptor. Compounds that block CB1 receptor function also blocked the URB937 analgesic actions, whereas drugs that interfere with the function of other endocannabinoid targets did not prevent analgesia. These findings indicate that peripheral cannabinoidergic transmission is indeed important in pain control. Clapper *et al.*[4] also highlight the newly developed peripheral FAAH antagonist as a representative of a potential new class of anti-nociceptive drugs.

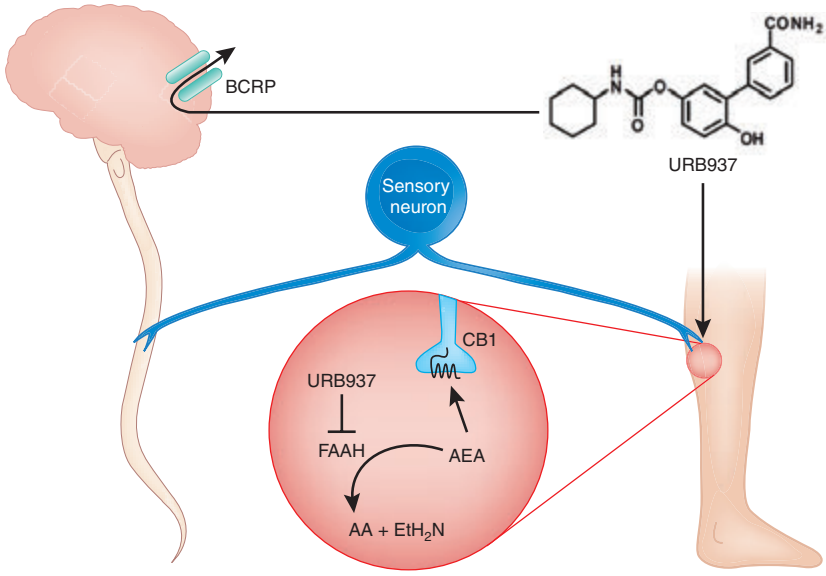

Figure 1 Mechanism of analgesic action of the peripherally targeted FAAH inhibitor URB937. The drug is excluded from the brain by a transporter with a pharmacology resembling BCRP, but is free to act on peripheral FAAH. The site of analgesic action is most likely peripheral pain-sensing nerve endings (red circle). Expanded red circle shows the suspected molecular mechanism of drug action, inhibition of FAAH-catalyzed conversion of anandamide (arachidonoyl ethanolamide or AEA) to arachidonic acid (AA) and ethanolamide (EtH_2N). This allows for persistent AEA activation of peripheral cannabinoid type 1 (CB1) receptors on peripheral nerve endings. Activation of CB1 receptors ultimately reduces transmission of pain signals to the spinal cord, preventing pain sensation.

The new FAAH inhibitor was found to be excluded from the CNS largely via a transporter present in cells of the blood/brain barrier, with pharmacological properties similar to the breast cancer resistance protein (BCRP). Compounds recognized by BCRP, such as URB937, are extruded from the brain by active transport from the cerebrospinal fluid to the peripheral circulation. Thus, peripheral administration of URB937 elevated anandamide in peripheral organs, but not in the CNS (**Fig. 1**). Drug administration reduced nociception in rat and mouse tests involving peripheral nerve injury and inflammation, including peripheral weak acid injection, sciatic nerve ligation, and injection of peripheral nerve inflammatory agents and irritants. However, URB937 did not have generalized anti-nociceptive effects (for example, it did not alter responses on the hot plate test) and the peripheral effects were restricted to limbs affected by the nerve injury and inflammation models. Furthermore, the drug did not affect feeding or locomotor activity, unlike CNS-penetrant FAAH inhibitors. The peripheral actions of URB937 led to alterations in spinal processing of nociceptive information, as indicated by the observation that the drug reduced pain-induced activation of spinal neurons, as indicated by c-Fos staining.

The anandamide-elevating effects of URB937 were lost in gene-targeted mice lacking FAAH. CB1 endocannabinoid receptors were necessary for the analgesic effects of the compound. Notably, URB937 did not alter tissue levels of the other major endocannabinoid, 2-arachidonoyl glycerol (2-AG). Other FAAH metabolites such as palmitoyl ethanolamide

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did not appear to mediate the analgesic effects of this drug. Thus, the mechanism of drug action is via anandamide actions on peripheral CB1 receptors. This raises questions regarding the relative roles of anandamide and 2-AG in peripheral analgesic actions that will no doubt be addressed in future experiments, possibly through the use of peripherally restricted inhibitors of the 2-AG degrading enzymes MAGL or ABDH6 (refs. 5,6). It is interesting that elimination of the FAAH enzyme in gene-targeted mice did not produce an analgesic effect similar to FAAH inhibitors, whereas the antinociceptive actions of URB937 did not diminish after 7 d of drug treatment. These findings indicate that compensation for loss of FAAH activity can occur, but perhaps only when the enzyme is out of commission for prolonged periods or early in development. This bodes well for use of peripheral FAAH inhibitors in pain management, at least with relatively short-term treatment. However, more extensive testing will be necessary to determine whether the inhibitor is effective with prolonged exposure.

Additional studies will also be needed to identify the site and mechanisms of the peripheral URB937 analgesic actions. Although the authors suggest that spinal mechanisms contribute to the antinociceptive effects, the initial site of drug action is likely on peripheral nerves and may be at nerve endings in peripheral organs (**Fig. 1**). Indeed, it was previously found that CB1 receptors on the peripheral endings of nociceptive sensory neurons mediate analgesia produced by local or systemic treatment with systemic CB1 agonists[3]. Activation of CB1 receptors generally inhibits neuronal excitability and neurotransmitter release. It will be interesting to see whether the CB1 receptors responsible for anandamide control of pain act at peripheral nerve endings and, if so, what molecular targets and other neurotransmitters are implicated in these analgesic actions.

Other recent reports have also highlighted the potential therapeutic usefulness of drugs targeting the peripheral endocannabinoid system[7]. For example, CB1 antagonists are known to reduce weight via effects on eating behavior and peripheral metabolism[8,9]. Indeed, the CB1 antagonist rimonabant that acts both peripherally and centrally was developed for treatment of obesity and metabolic syndrome, but its use was quickly discontinued as a result of side effects likely arising from CNS drug actions[10], highlighting the need for peripherally restricted endocannabinoid-targeted drugs. A recent study[11] found that a peripherally restricted CB1 antagonist reduced untoward metabolic effects of obesity, suggesting another use for peripherally restricted drugs targeting the endocannabinoid system. Particularly relevant to Clapper *et al.*[4] is a recent preliminary report that peripherally active cannabinoid receptor agonists do not reduce acute pain in humans with chronic lower back pain, while some weight gain and metabolic side effects were observed[12]. These findings suggest that widespread activation of peripheral cannabinoid receptors may not be efficacious for pain treatment and may have undesirable consequences. Caution must therefore be exercised in judging the potential safety and efficacy of peripherally targeted FAAH inhibitors, as prolonged anandamide activation of cannabinoid receptors could still produce unwanted side effects. However, targeting the anandamide degrading enzyme may provide more specificity and fewer side effects in comparison to cannabinoid receptor agonists. Previously, it was noted that endocannabinoids were elevated locally by inflammatory/painful stimuli[3]. Thus, inhibiting FAAH may predominantly affect endocannabinoid/CB1 signaling in affected regions, avoiding widespread effects produced by activation of all peripheral cannabinoid receptors.

Ultimately, clinical studies of safety and efficacy will be needed to assess the usefulness of the peripherally targeted FAAH inhibitor. For now, Clapper *et al.*[4] have found that anandamide participates in important antinociceptive actions in the PNS.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Prime time for stress

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Stress primes the hypothalamic-pituitary-adrenal axis response to subsequent stressors. A new study finds that acute stress modifies the properties of excitatory synapses impinging on parvocellular neurons of the paraventricular nucleus.

When faced with a perilous or unexpected situation, such as a close encounter with a hungry *Ursus spelaeus* or *Panthera spelea*, our ancestors responded to that stress with either fight or flight. Responses to stress are mediated, in part, by the hypothalamic-pituitary-adrenal (HPA) axis and a single close encounter with a cave bear or lion would have had protracted consequences on our Flintstone HPA axes, resulting in altered sensitivity to future stressors. Although cave bears and cave lions are now long extinct, in the modern world, the HPA axis kicks in when we face challenges in everyday life, as well as more extraordinary circumstances. Versatility in the stress response of the HPA axis is necessary because both the immediate response to the stressor and the adaptation of future behaviors must be managed. Indeed, chronic, as well as acute, stress is known to induce long-term decreases (habituation) or increases (sensitization) in the HPA axis response to the subsequent exposure to a stressor[1–3]. Despite its importance in understanding the physiopathology of the stress system, the cellular mechanism underlying the ability of a previous stressor to alter the responsiveness to further stressors remains mostly unresolved.

A study by Kuzmiski *et al.*[4] sheds new light on this important question and reveals how

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