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Endocannabinoids in pain modulation

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Summary Five major approaches have been employed to determine the role of endocannabinoids in pain modulation: (1) studies of various markers of endocannabinoid action aimed at determining whether the necessary cannabinoid biochemical machinery is present in those brain areas that control pain sensitivity; (2) administration of exogenous cannabinoids to determine whether endocannabinoid action at appropriate sites would lead to a loss of pain sensitivity; (3) administration of compounds that would affect endocannabinoid action such as antagonists and transport inhibitors to determine whether drug-induced preterbation of cannabinoid action would alter pain sensitivity; (4) studies of genetically altered animals aimed at determining whether pain responses or responses to cannabinergic drugs are altered; and (5) studies that measure the release of endocannabinoids. Converging evidence from each of these research areas indicates that endocannabinods function to control pain in parallel with endogenous opioids but via different mechanisms. © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Cannabinoids have been used to treat pain for many centuries (reviewed in ref. 1). Early uses include surgical anesthesia in China, amelioration of childbirth pain in ancient Israel, as an analgesic in Asia throughout the middle ages, and in the West during the 1800s for a variety of painful ailments, with commercial preparations supplied by Lilly and Squibb. For a variety of reasons, both political and pharmacological (e.g. the instability of cannabis extracts, unpredictable absorption, and insolubility in water), cannabinoids were discontinued as medical agents in the early 20th century. With the discovery of cannabinoid receptors[2] and the identification of endocannabinoids[3] (Mechoulam, this issue), along with their signalling, biosynthetic, and degradatory pathways (reviewed by Suigura, this volume), came a paradigm shift. These developments led to a move to understand the function of endocannabinoids in the brain and periphery. One likely physiological role of endocannabinods is pain suppression, which is the topic of this review.

Five major approaches have been used to examine the hypothesis that cannabinoids play a role in pain modulation, each addressing a specific criterion for establishing such a role. Thus, if endocannabinoids play a role in pain modulation it would be expected that (1) markers of endocannabinoids should be found in anatomical areas known to mediate pain transmission or pain modulation; (2) administration of endocannabinoids, either systemically or directed at appropriate pain relay or modulatory sites, should alter pain sensitivity and change the processing of nociceptive information within discrete spinal and brain pathways; (3) administration of pharmacological agents that alter endocannabinoid availability at the cannabinoid receptor should alter pain sensitivity; (4) genetically altered animals would display expected changes in sensitivity to pain or experimentally induced changes in pain sensitivity; and (5) under appropriate circumstances particular endocannabinoids would be released in specific anatomical areas related to pain. The remainder of this paper is devoted to a review of the evidence in each of these areas.

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ENDOCANNABINOID MARKERS IN ANATOMICAL AREAS RELATED TO PAIN

The distribution of cannabinoid CB_1 receptors in the brain has been examined by receptor autoradiography, immunohistochemistry, and in situ hybridization histochemistry.[4–6] These papers revealed that cannabinoid receptors are frequently localized to nerve endings, suggesting they function as presynaptic modulators of neurotransmitter release. Presynaptic inhibition is a particularly powerful mechanism of neural modulation,

as it can have the final determinant influence on the output signal of a neuron and its subsequent communication to other neurons. Cannabinoid receptors occur in high density in many areas related to pain. They densely populate the periaqueductal gray (PAG) and the rostral ventrolateral medulla, brain areas involved in descending pain modulation. They are concentrated in the superficial layers of the spinal dorsal horn, and they are found in the dorsal root ganglion, from which they are transported to both central and peripheral terminals of primary afferent neurons.[4,7–10] These areas provide peripheral, spinal and central targets through which cannabinoids modulate pain.

The CB_2 cannabinoid receptor is largely absent from the nervous system, being located in immune tissues: the spleen, tonsils, monocytes, B- and T-cells;[11–13] reviewed in ref. 14. These constituents are of obvious importance to pain processing due to the role of inflammatory mediators in the pain response.

NEUROBEHAVIORAL STUDIES OF CANNABINOID ANALGESIA

Preclinical studies in animals revealed that cannabinoids block pain responses in every pain model tested. Perhaps the earliest study of this type was performed by Dixon,[15] one of the fathers of modern pharmacology, who demonstrated that cannabinoids suppress canine reactions to pinpricks. Early studies by Bicher and Mechoulam[16] and Kosersky et al.[17] paved the way for many subsequent studies which verified the ability of cannabinoids to profoundly suppress behavioral reactions to noxious stimuli, inflammation, and nerve injury. In models of acute or physiological pain, cannabinoids are highly effective against thermal,[18–22] mechanical[23] and chemical pain.[23,24] Typically, cannabinoids were comparable with opiates both in potency and efficacy.[19,25] In models of tonic or chronic pain both inflammatory[26] and neuropathic,[27] cannabinoids show even greater potency and efficacy.

Studies demonstrating pain-suppressive effects of cannabinoids produced an impressive array of results, but these results require careful interpretation because cannabinoids also produce profound motor effects that may cloud the interpretation of these studies. Cannabinoid receptors densely populate the terminals of striatal output neurons that innervate the globus pallidus and the substantia nigra. By presynaptic inhibition, activation of these receptors blocks the communication between corticostriatal neurons and the output of the basal ganglia (reviewed in ref. 28). At high doses in animal studies, this produces an odd condition called catalepsy, a state characterized by frozen postures and immobility. Although the effects of cannabinoids on movement are complex (both inhibition and activation can occur depending on dose), this hypoactivity produced at higher doses of cannabinoids raised the concern that even at doses where frank motor disability is not evident, the delayed reactions of animals in pain tests may have resulted from motor dysfunction rather than pain inhibition.

In light of the aforementioned interpretational issues surrounding behavioral assessments of analgesia, neurophysiological studies of the effects of cannabinoids on pain pathways became a matter of some importance. Moreover, such studies offered the possibility of gaining an appreciation of the neurophysiological changes produced by cannabinoids that lead to analgesia. Yet, as of 1990, virtually nothing was known about the effects of cannabinoids on the neural pathways that transmit pain messages from the spinal cord to the brain, and the determination of whether cannabinoids actually affect nociceptive neurotransmission was a crucial missing link in the developing story on cannabinoids and pain. Therefore, we undertook a series of studies that examined the effects of cannabinoids on noxious stimulation-produced activity in spinal and thalamic neurons.[29–34] In these studies, extracellular single-neuron recordings were obtained from anesthetized rats, and the responses of both nociceptive neurons and non-nociceptive neurons to a variety of stimuli were studied. In these experiments (e.g. Fig. 1), cannabinoids produced profound suppression of cellular nociceptive responses. A summary of the findings of a series of experiments indicates that cannabinoids exhibit the following characteristics:

- Suppression of behavioral and neurophysiological responses to all types of nociceptive stimuli tested.
- High potency (effects observed at $\sim 75\,\mu g/kg$, i.v.),
- High efficacy (typically $>80\%$ reduction in response to noxious stimulation)
- Effects CB_1 receptor-mediated.
- Suppression of both wide dynamic range neurons (respond to touch and pain) and nociceptive specific neurons (respond to pain only).
- No suppression of low threshold mechanoreceptive neurons (respond to touch only).
- Spinal and thalamic neurons affected similarly.
- Behavioral analgesic time course and potency highly correlated with neuronal suppression of nociceptive stimulus-evoked activity.
- Suppression of windup (a model of central sensitization which is observed in chronic pain).
- Suppression of increased spontaneous firing following complete Freunds adjuvant (CFA—an inflammatory agent).

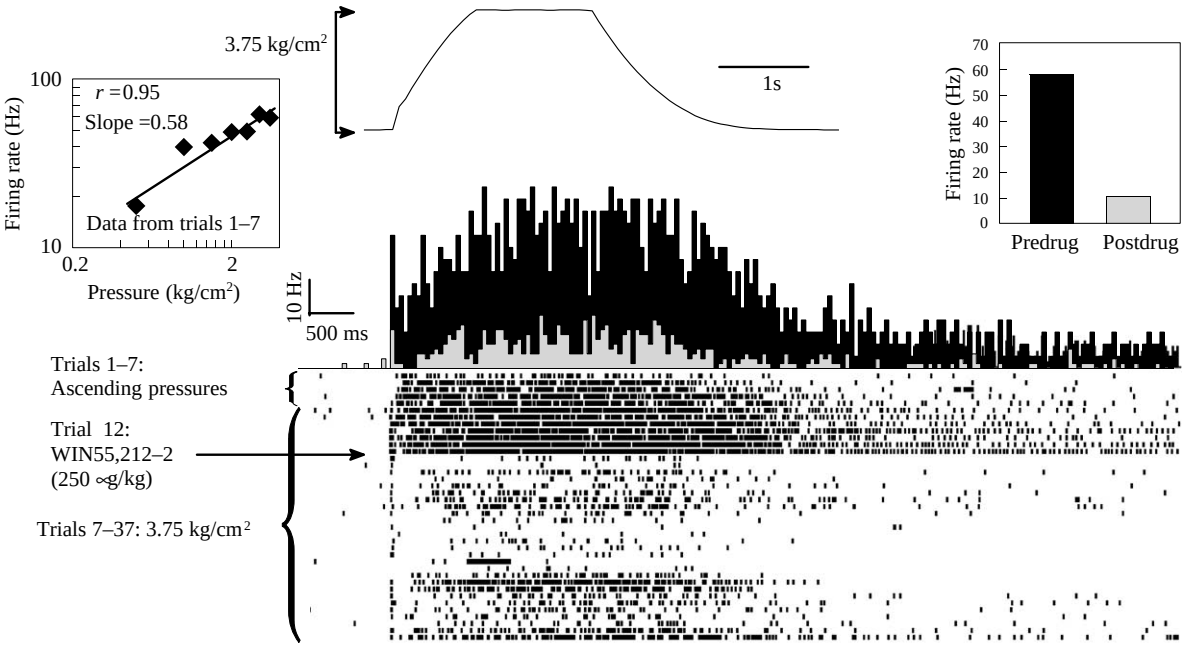

Fig. 1 Example of inhibition of evoked activity in a WDR neuron by the cannabinoid WIN55,212-2. The responses of the neuron to mechanical pressure were examined during 37 trials corresponding to each row of dots in the raster plot (top row=trial 1); each dot represents the time of occurrence of a single action potential relative to the stimulus onset. Trials 1–7 consisted of applications of increasingly strong mechanical stimulation ranging from non-noxious to noxious levels (0.5, 1, 1.5, 2, 2.5, 3, 3.75 kg/cm^2). The concomitant increases in density of dots under the stimulus in the first seven rows are indicative of the increasingly strong response of the neuron. Left (inset): the mean firing rates of the neuron during a graded series of stimulations are plotted (log–log coordinates) against the applied pressure. The neuron's systematic change in responsiveness was the basis for classifying this cell as a WDR neuron. Center: the noxious stimulus illustrated by the pressure waveform (top center) was administered every 2 min for trials 7–37. Trials 8–12 constituted baseline trials; after trial 12 (arrow), WIN55,212-2 (250 mg/kg, i.v.) was administered. A marked decrease in the responsiveness of the neuron is indicated by the sharply decreased density of dots in subsequent rows of the raster plot. Right (inset): comparison of the mean firing rate during the stimulus for the five baseline trials to the firing rate during the stimulus for the first 10 postinjection trials illustrating, approximately, an 82% decrease in responsiveness. The black peristimulus time histogram between the raster plot and the pressure waveform represents the baseline firing rate prior to injection, whereas the gray peristimulus time histogram represents the firing rate for the first 10 postinjection trials (from Hohmann et al. (29)).

The reader will note that the first eight bullets reflect effects on nociceptive or physiological pain, whereas the last two employed chronic pain models. In windup, we studied the increasing response of neurons to trains of C-fiber strength electrical stimulation,[34] and the CFA experiments examined responses to an inflammatory stimulus. The results of these experiments are consistent with behavioral tests discussed above which showed that cannabinoids are active in models of inflammatory and neuropathic pain. This is important because these models are better indicators of the therapeutic potential of cannabinoids than tests of physiological (acute) pain.

SITES OF ACTION OF CANNABINOIDS

Although the studies discussed above demonstrated the ability of cannabinoids to suppress nociceptive neurotransmission, one must ask about the site of action of the drugs. Ostensibly, the cannabinoids could produce analgesia by an action in the brain via descending modulation, by a direct spinal action and/or by an action on the peripheral nerve. The consensus from studies conducted in a number of different laboratories is that cannabinoids exert effects at all three locations.

Central nervous system actions

Early studies in our laboratory indicated that cannabinoids, administered intracerebroventricularly, suppress tail-flick responses[35] and spinal nociceptive responses in rats.[32] This effect occurs at low doses, and further studies using direct brain injections indicated that the antinociceptive effects can be elicited from at least seven different brain areas, which include the dorsal periaqueductal gray (PAG) in the mid brain, the rostral ventrolateral medulla (RVM) and the noradrenergic nucleus A5 in the medulla.[36–38] All of these areas participate in descending inhibition of spinal nociceptive projection pathways.[39] The important role of the PAG in cannabinoid analgesia

finds support in the findings of Christie's group demonstrating modulation of postsynaptic currents in the RVM and the PAG in brain slices.[40,41] Work from Fields' group[42] confirmed previous demonstrations of behavioral analgesia produced by microinjections of cannabinoids in the RVM by demonstrating that cannabinoids also exert marked effects on neurons in the RVM, a site that plays a major role in descending suppression of spinal nociceptive neurons. Thus, work in several laboratories supports the idea that cannabinoids produce antinociceptive effects by actions in the PAG and the RVM, whose circuits inhibit spinal nociceptive neurotransmission. These targets of cannabinoid action evidently form a major component of the antinociceptive effects of cannabinoids.

Spinal actions

Yaksh[22] and later Welch's group and others[43,20,21,30,44–48] have shown that cannabinoids inhibit pain in part by a direct spinal action. These observations are consistent with the dense labelling by CB_1 receptor antibodies or CB_1 receptor radioligands of cannabinoid receptors in the superficial and deep layers of the dorsal horn.

Direct spinal application of cannabinoids inhibits the nociceptive responses of spinal nociceptive neurons.[30] This inhibitory action of cannabinoids in the spinal cord appears to occur in part due to presynaptic inhibition of glutamatergic neurons in the substantia gelatinosa.[49] This work, carried out in slices of rat spinal cord, revealed the reduced frequency and amplitude of strychnine- or bicuculline-induced spontaneous excitatory postsynaptic currents (sEPSC) produced by the cannabinoid agonist WIN55,212-2. Moreover, the frequency but not the amplitude of miniature EPSCs was reduced by the cannabinoid. These findings provide a mechanism by which cannabinoids could produce analgesic effects through actions in the spinal cord.

Peripheral actions

Recent work also indicates an action of cannabinoids in the periphery. Injections of low doses of anandamide into an area of inflammation in the paw produced by carageenan reduced the ensuing hyperalgesia.[50] This finding is consistent with the presence of cannabinoid receptors in the peripheral nerve and their transport to the distal endings.[9] Subsequent work by Calignano et al.[51] showed that endocannabinoids acting in the periphery may modulate pain responses.

PHARMACOLOGICAL ACTIONS OF CANNABINOID ANTAGONISTS AND TRANSPORT INHIBITORS

Studies of the effects of SR141716A, a specific cannabinoid CB1 receptor antagonist (reviewed by Walker et al.[52]) suggest that endocannabinoids participate in endogenous pain modulation and that this action involves the periaqueductal gray matter (PAG) (Fig. 2). Work from

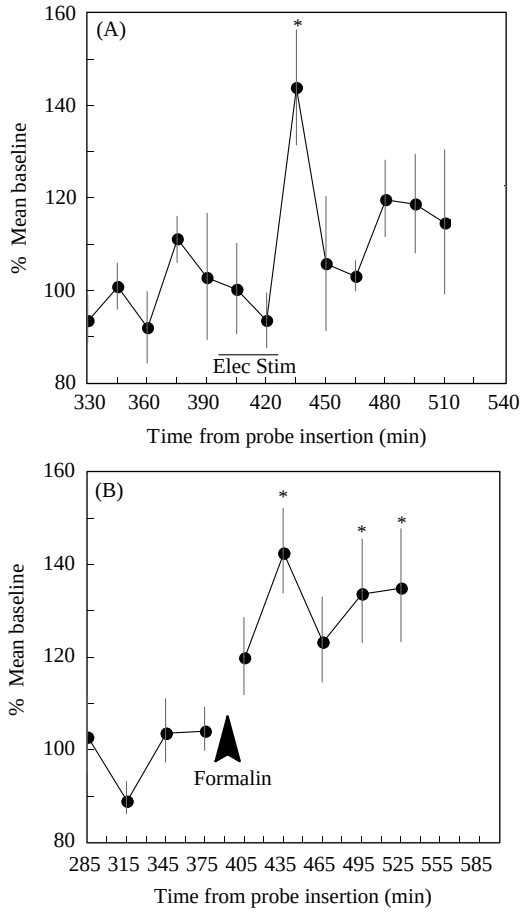

Fig. 2 Stimulation of the release of anandamide in the PAG of the rat using electrical depolarization or pain. (A) Increased extracellular levels of anandamide following electrical stimulation of the periaqueductal gray matter (PAG) in urethane-anesthetized rats. Following the establishment of stable baseline values, electrical stimulation (monopolar 0.1 ms/1 mA, 60 Hz, 5 s trains, 5 s rest) was delivered for 30 min. Microdialysis samples were collected in 15 min intervals and analyzed using HPLC with detection by atmospheric pressure chemical ionization mass spectrometry, selected-ion monitoring mode at molecular weight 348.3 (N=5, $P<0.05$, repeated measures analysis of variance).*Significantly different from baseline average via post hoc test ($P<0.05$). The delay in the measurement presumably reflects the time needed to produce sufficient overflow of anandamide in the extracellular space to achieve recovery by microdialysis. (B) Increased extracellular levels of anandamide in the PAG following induction of prolonged pain in urethane-anesthetized rats. Following establishment of a stable baseline, formalin solution was injected subcutaneously in both hind paws (4%, 150 μl). Samples shown span 30 min intervals (N=6; $P<0.001$, repeated measures analysis of variance).*Significantly different from baseline average via post hoc test ($P<0.05$) (from Walker et al. (58)).

our laboratory showed that blocking the cannabinoid CB_1 receptor with SR141716A produces hyperalgesia in the formalin test and blocks the analgesia produced by electrical stimulation of the dorsal PAG.[53] These findings confirmed those of Richardson et al.,[44–46] who found that this cannabinoid antagonist, injected intrathecally, produced hyperalgesia, and that the same effect occurs with spinal CB_1 receptor knockdown with an antisense oligonucleotide. Chapman[48] found that spinal nociceptive neurons exhibit markedly greater C-fiber-mediated responses following low doses (0.1–1 ng in 50 μl applied to spinal cord) of SR141716A. The pro-nociceptive actions of the antagonist provide evidence that endocannabinoids serve naturally to suppress pain. Presumably, the pain-enhancement by the antagonist occurs due to blockade of a tonic or evoked pain-inhibitory effect of endocannabinoids. However, the conclusions from these and other experiments that use SR141716A in this manner are limited by two factors. First, several reports have suggested that SR141716A acts as an inverse agonist, an effect that would mimic that of blocking endocannabinoids (reviewed in ref. 52). Second, these studies do not identify any particular endocannabinoid that might be involved in the proposed suppression of pain.

Another approach to examining the role of endocannabinoids in pain has been to employ an anandamide transport inhibitor such as AM404. Blocking transport would be expected to block the metabolism of anandamide and cause increased levels to occur in the vicinity of cannabinoid receptors, both processes leading to increased occupation of cannabinoid receptors. Beltramo et al.[54] showed that administration of AM404 caused the accumulation of anandamide in cultures of cortical neurons and enhanced the hot-plate analgesia produced by systemically administered anandamide. AM404 alone did not alter pain sensitivity, suggesting that anandamide does not act tonically to maintain pain thresholds for thermal stimuli, in contrast to the studies discussed above that support this notion. The paper did not address whether environmentally produced analgesia (e.g. stress) was affected by AM404.

GENETIC ALTERATIONS OF THE CANNABINOID SIGNALLING SYSTEM

Knockouts of the CB1 receptor and fatty acid amide hydrolase have provided important information about the role of endocannabinoids in pain. Ledent et al.[55] found that CB1 receptor knockout mice failed to exhibit any of the characteristic responses to cannabinoids, including analgesia. No overall change in pain sensitivity was apparent, in contrast to another study published the same year on a different CB1 knockout,[56] in which the investigators observed a higher pain threshold in the −/− mice compared with wild type. The surprising findings of analgesia-like effects of the knockouts in this study are at variance with the other study and are likely due to complex, unexplainable developmental changes that inevitably occur in this type of experiment.

Cravatt et al.[57] produced knockouts of fatty acid amide hydrolase (FAAH), an enzyme believed to be important in the degradation of anandamide and related compounds. They observed a profound enhancement of the analgesic effects of anandamide, which were reversed by the selective CB1 antagonist SR141716A. Moreover, these animals do exhibit tonic analgesia, presumed to be due to the decreased metabolism of endogenous anandamide or a similar substance that is also susceptible to hydrolysis by FAAH. These findings provide solid support to the notion that endocannabinoids serve naturally to downwardly modulate pain sensitivity.

EXAMINATION OF THE RELEASE OF ENDOCANNABINOIDS IN RELATION TO PAIN

Following from the finding that SR141716A blocks the analgesic effect of PAG electrical stimulation, we hypothesized that electrical stimulation would release anandamide in the PAG. To examine this possibility, we set upon the task of measuring endocannabinoids in the PAG using microdialysis.[58] This method permits collection of neurotransmitters/modulators from the extracellular space and is therefore an indicator of the release of these modulators. Using liquid chromatography/mass spectrometry, we were able to establish that analgesia-producing electrical stimulation of the PAG or injections of the chemical irritant formalin into the hindpaws of anesthetized rats, induced the release of anandamide in the PAG (Fig. 2). Thus, it appears that either pain itself, or electrical stimulation (which mimics neuronal excitation) leads to the release of anandamide, which acts on cannabinoid receptors in the PAG to inhibit nociception.

These observations support the notion that pain inhibits pain, i.e. that ascending nociceptive pathways activate the PAG which in turn suppresses nociceptive neurotransmission. A wealth of research shows that indeed the PAG does receive inputs from ascending nociceptive pathways. Information about pain is represented mainly in the caudal 2/3 of dorsolateral and ventrolateral quadrants of the PAG and the juxtaqueductal region.[59–61] The PAG receives a dense spinal input via the spinomesencephalic tract and from collaterals of the spinothalamic tract,[59–66] and it expresses c-fos in response to noxious stimulation[59,67] in regions that overlap with the highest densities of cannabinoid radioligand binding (cf ref. 60 to ref. 4). The spinal-PAG projections tend to follow a rostro-caudal topographic somatotopy representing the rostro-caudal axis of the

body.[61] Lamina I projections to the mesencephalon are far heavier than its projections to the thalamus.[63] Hylden et al.[68] concluded from their anatomical observations that a likely role of these lamina I projections would be to activate descending pain inhibitory mechanisms in the PAG. The literature thus suggests the hypothesis that the formalin-induced increase in the release of anandamide in the PAG is mediated by the projection from spinal lamina I neurons, at least in part.

SUMMARY

This article discussed five approaches to the examination of the role of endocannabinoids pain. While some controversy exists in a few areas, the overwhelmingly positive data from the now hundreds of papers on the subject support the idea that endocannabinoids play a role in pain modulation. Whether the effect is produced tonically remains in question. What is clear is that mobilization of endocannabinoid action by pain itself or by other less natural means such as FAAH inhibition, causes antinociceptive effects. As with opiates, the mechanism by which endocannabinoids produce effects is mediated by multiple sites of action in the nervous system, likely involves peripheral non-neural effects, and is mediated by multiple receptor types. The challenge ahead is to define the conditions and pathways that mediate the pain modulatory effects of endocannabinoids and to exploit the therapeutic potential that they clearly offer.

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