

# Chapter 44

## Endocannabinoid Mechanisms of Pain Modulation

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**Abstract** Cannabinoids are antinociceptive in animal models of acute, tissue injury–, and nerve injury–induced nociception. This review examines the biology of endogenous cannabinoids (endocannabinoids) and behavioral, neurophysiological, and neuroanatomical evidence supporting the notion that cannabinoids play a role in pain modulation. Behavioral pharmacological approaches, in conjunction with the identification and quantification of endocannabinoids through the use of liquid and gas chromatography mass spectrometry, have provided insight into the functional roles of endocannabinoids in pain modulation. Here we examine the distribution of cannabinoid receptors and endocannabinoid-hydrolyzing enzymes within pain modulatory circuits together with behavioral, neurochemical, and neurophysiological studies that suggest a role for endocannabinoid signaling in pain modulation. This review will provide a comprehensive evaluation of the roles of the endocannabinoids 2-arachidonoylglycerol and anandamide in stress-induced analgesia. These findings provide a functional framework with which to understand the roles of endocannabinoids in nociceptive processing at the supraspinal level.

**Keywords** 2-arachidonoylglycerol, anandamide, CB1, fatty acid amide hydrolase, monoacylglycerol lipase, periaqueductal gray, rostral ventromedial medulla

### Introduction

The discovery, cloning, and characterization of cannabinoid receptors,<sup>1–3</sup> along with the isolation of endogenous ligands for these receptors, such as anandamide<sup>4</sup> and 2-arachidonoylglycerol (2-AG),<sup>5,6</sup> established the existence of an endocannabinoid

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neuromodulatory system. Cannabinoid receptors occur in high densities in the rodent brain ( $>1$  pmol/mg protein).<sup>2</sup> The heterogeneous distribution of cannabinoid receptors in the central nervous system<sup>2,7</sup> suggests a neuroanatomical basis for the profound behavioral effects induced by exogenous cannabinoids. The cannabinoid system is thus a major neurochemical system whose functional significance has only recently been explored. Cannabinoid receptors are localized in neuroanatomical regions subserving transmission and modulation of pain signals, such as the periaqueductal gray (PAG), the rostral ventromedial medulla (RVM),<sup>2,7</sup> and the dorsal horn of the spinal cord.<sup>7</sup> These findings suggest that endocannabinoids play a key role in central nervous system modulation of pain signaling. This review will focus on elucidating the pain modulatory functions of cannabinoids and endocannabinoids mediated primarily at the supraspinal level.

## Cannabinoid Receptor Subtypes

Two subtypes of cannabinoid receptors—CB<sub>1</sub> and CB<sub>2</sub>—have been identified. CB<sub>1</sub> is enriched in the brain.<sup>3,8,9</sup> By contrast, CB<sub>2</sub> is mainly expressed in immune tissues, including the spleen, tonsils, monocytes, and B and T cells<sup>10-12</sup> and is found only at low levels in neurons of the central nervous system.<sup>8,9,11</sup> In pathological pain states, CB<sub>2</sub> messenger RNA (mRNA) is also detected in the lumbar dorsal horn concurrently with the appearance of activated microglia.<sup>13</sup> CB<sub>1</sub> is negatively coupled to adenylate cyclase through Gi/o proteins.<sup>14,15</sup> Activation of these receptors inhibits N- and P/Q-type calcium channels<sup>16,17</sup> and activates inward rectifying potassium<sup>18</sup> and potassium A<sup>19</sup> channels. CB<sub>2</sub> is also negatively coupled to adenylate cyclase but is not coupled to calcium channels.<sup>14</sup> These signal transduction properties suggest that activation of CB<sub>1</sub> suppresses neuronal excitability and neurotransmitter release by modulating calcium and potassium conductances.

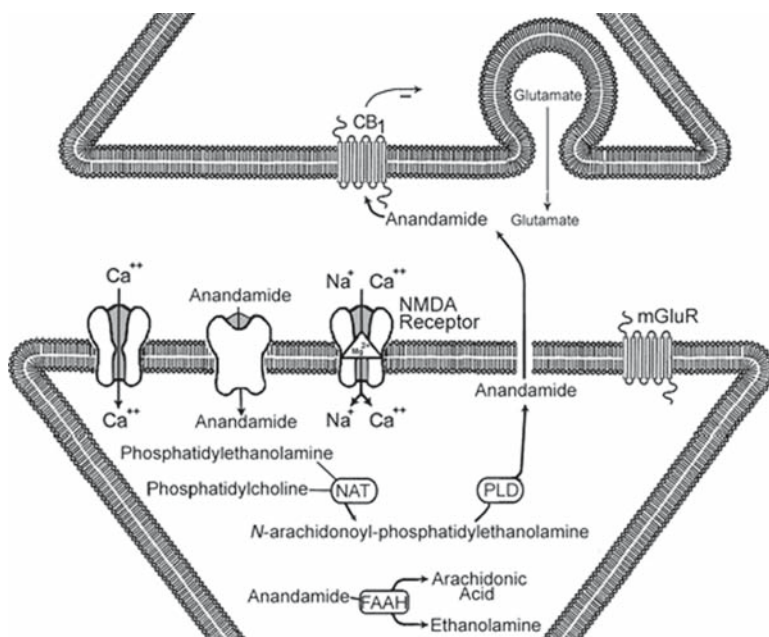
This review will examine evidence suggesting that endocannabinoids act at CB<sub>1</sub> receptors in the central nervous system to modulate pain processing. A more extensive review of the role of CB<sub>1</sub> receptor activation in modulating acute and sustained nociception at spinal and peripheral levels is available elsewhere.<sup>20,21</sup> A role for peripheral CB<sub>1</sub> and CB<sub>2</sub> receptors in modulating acute,<sup>22</sup> tissue injury–,<sup>23-29</sup> and nerve injury–induced<sup>30</sup> nociception has recently been demonstrated following systemic and local hind paw injections of CB<sub>2</sub>-selective agonists. Interested readers are referred to recent reviews of peripheral cannabinoid antinociceptive mechanisms.<sup>20,31-34</sup>

## Endocannabinoids

Several putative endocannabinoids have been isolated in the brain, including anandamide, 2-AG, noladin ether, virodhamine, and *N*-arachidonoyldopamine (NADA). Other endogenous cannabinergic compounds include the related fatty acid derivatives

oleamide, palmitoylethanolamide, and a novel family of arachidonoyl amino acids. These substances lack affinity for cannabinoid receptors but appear to facilitate endocannabinoid function. The functional roles of these latter compounds remain poorly understood and are beyond the scope of this review. Because anandamide and 2-AG are the best characterized of the endocannabinoids isolated thus far, this review will focus on understanding the role of these endocannabinoids in pain modulation.

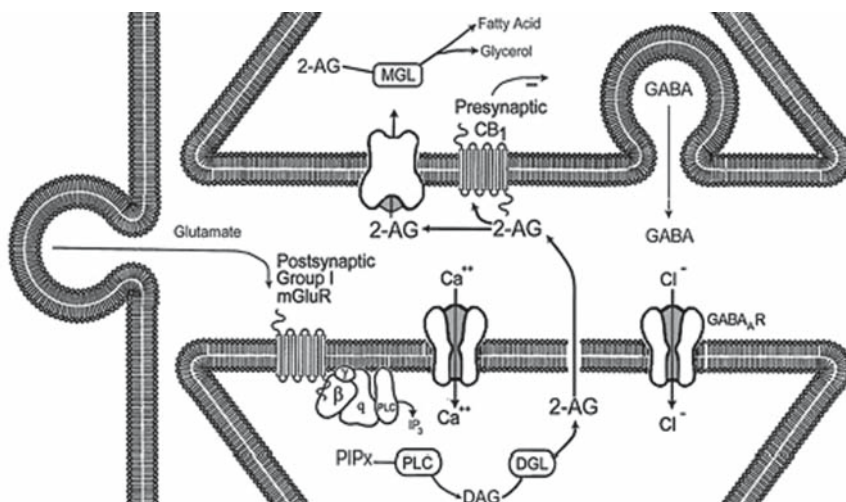
Anandamide<sup>4</sup> and 2-AG<sup>5,6,35</sup> are thought to be produced upon demand (ie, by activity-dependent or receptor-stimulated cleavage of membrane lipid precursors) and to be released from cells immediately after their production (for review, see Piomelli<sup>36</sup>). Anandamide is synthesized *in vitro* in a 2-step process (Fig. 44.1; for review, see Piomelli<sup>36</sup>). First, the phospholipid precursor *N*-arachidonoyl-phosphatidylethanolamine (NAPE) is formed from phosphatidylethanolamine through a mechanism that is both  $\text{Ca}^{2+}$  and cyclic AMP dependent, and catalyzed by the enzyme *N*-acyltransferase. Second, NAPE is believed to be hydrolyzed by a NAPE-specific phospholipase D—an enzyme that remains molecularly uncharacterized—to generate anandamide and the metabolic intermediate phosphatidic acid. Anandamide shows preferential affinity for  $\text{CB}_1$  ( $K_i [\text{CB}_1 \text{ vs } \text{CB}_2] = 89 \text{ vs } 371 \text{ nM}$ )



**Fig. 44.1** Hypothetical model showing pathways of anandamide formation and deactivation. FAAH indicates fatty acid amide hydrolase; mGluR, metabotropic glutamate receptor; NAPE, *N*-arachidonoyl-phosphatidylethanolamine; NAT, *N*-acyltransferase; NMDA, *N*-methyl-D-aspartic acid; and PLD, phospholipase D.

in vitro and acts as a low-affinity agonist at vanilloid TRPV1 receptors.<sup>37-39</sup> Systemic administration of exogenous anandamide produces antinociception, suggesting that the endocannabinoid may also suppress pain under physiological conditions. This effect, however, is not reliably blocked by the selective CB<sub>1</sub> antagonist SR141716A (rimonabant),<sup>40,41</sup> likely owing to the fact that anandamide is readily metabolized in vivo by fatty acid amide hydrolase (FAAH) into ethanolamine and arachidonic acid.

In vitro experiments suggest that 2-AG formation (for review, see Piomelli<sup>36</sup>) occurs via successive activation of 2 enzymes (Fig. 44.2). First, the 2-AG precursor 1,2-diacylglycerol (DAG) is formed from phospholipase C-mediated hydrolysis of membrane phosphoinositides. Newly formed DAG may subsequently be hydrolyzed by DAG-lipase (DGL) to yield 2-AG. DAG can alternatively be phosphorylated by DAG kinase to yield phosphatidic acid. Therefore, DGL-mediated hydrolysis of DAG is likely the first committed step in 2-AG biosynthesis (for review, see Piomelli<sup>36</sup>). In brain slices and cultured cells, 2-AG formation may be stimulated by neural activity,<sup>35</sup> membrane depolarization,<sup>42</sup> or pharmacological activation of G protein-coupled receptors such as group I metabotropic glutamate receptors.<sup>43</sup> 2-AG is a naturally occurring 2-monoacylglycerol that activates both CB<sub>1</sub> and CB<sub>2</sub> receptors.<sup>5,6</sup> Although brain concentrations of 2-AG are 170-fold higher than those of anandamide,<sup>35</sup> the role of endogenous 2-AG in pain modulation is just beginning to be appreciated. 2-AG has been postulated to be the true natural ligand for cannabinoid receptors, with cannabinoid receptors serving primarily as



**Fig. 44.2** Hypothetical model showing pathways of 2-AG formation and deactivation. 2-AG indicates 2-arachidonoylglycerol; DAG, diacylglycerol; DGL, diacylglycerol lipase; GABA, MGL, monoacylglycerol lipase; mGluR, metabotropic glutamate receptor; PLC, phospholipase C, and PIPx, phospholipid precursors.

2-AG receptors.<sup>44,45</sup> Exogenous 2-AG, administered systemically, suppresses noxious stimulus-induced responding in the tail-flick assay,<sup>5</sup> suggesting that endogenous 2-AG may suppress pain responding under physiological conditions. Behavioral responses to 2-AG are also enhanced by related, endogenous 2-acylglycerols, which fail to show significant activity in any of the tests employed when administered alone.<sup>46</sup> This “entourage effect” is likely to help regulate the activity of endocannabinoids in the nervous system<sup>46</sup>; competing for the same enzyme for hydrolysis may potentiate endocannabinoid actions.

## Endocannabinoid Degrading Enzymes

Three of the 5 putative endocannabinoids—anandamide, 2-AG, and NADA—are susceptible to degradation by FAAH,<sup>47-50</sup> although a second enzyme, monoacylglycerol lipase (MGL),<sup>51</sup> catalyzes hydrolysis of 2-AG *in vivo*.<sup>52</sup> Immunocytochemical methods have been employed to map the distribution of FAAH in the brain.<sup>53-55</sup> The anatomical correspondence of FAAH and CB<sub>1</sub> mRNA also supports the hypothesis that endocannabinoids act as retrograde messengers.<sup>54</sup> Recent electrophysiological studies have provided confirmation of this hypothesis.<sup>56,57</sup> Significantly, immunocytochemical studies have demonstrated FAAH expression in the ventral posterior lateral nucleus of the thalamus,<sup>53-55</sup> which is the termination zone of the spinothalamic tract. This pathway is the major source of ascending nociceptive information to the brain. Furthermore, FAAH has been identified in Lissauer’s tract and in neurons of the superficial spinal cord dorsal horn (ie, in close proximity to the termination zone of nociceptive primary afferents). These observations confirm that a mechanism for endocannabinoid deactivation is present in regions of the central nervous system implicated in nociceptive processing and further support the notion that endocannabinoids play a role in pain modulation.

Although FAAH reportedly metabolizes 2-AG *in vitro*,<sup>58</sup> MGL is likely to play the predominant role in 2-AG deactivation.<sup>51</sup> MGL is a serine hydrolase that converts monoglycerides to fatty acids and glycerol. Northern blot, immunocytochemical, and *in situ* hybridization studies reveal that MGL is heterogeneously distributed in the rat brain, with the highest levels observed in the cortex, the thalamus, the hippocampus, and the cerebellum.<sup>51</sup> Ultrastructural studies suggest that MGL is localized predominantly if not exclusively on axon terminals.<sup>59</sup> The recent development of pharmacological inhibitors of MGL such as URB602 has provided pharmacological tools for studying the functions of endogenous 2-AG in pain modulation, as described later in this review.<sup>52</sup> *In vitro* studies suggest that overexpression of MGL attenuates 2-AG accumulation in rat cortical neurons without altering anandamide accumulation.<sup>51</sup> Moreover, virally mediated RNA silencing of MGL is associated with marked enhancements of both basal and Ca<sup>2+</sup>-stimulated 2-AG levels in HeLa cells.<sup>60</sup> Activation of mGlu5 receptors stimulates the formation of 2-AG (but not anandamide) in cultured cells derived from rat corticostriatal and hippocampal slices.<sup>43</sup> This formation of 2-AG is calcium-dependent and

catalyzed by phospholipase C and 1,2-diacylglycerol lipase.<sup>43</sup> Also, the metabotropic glutamate 5 (mGlu5) receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) prevents 2-AG formation induced by the group I mGlu receptor agonist 3,5-dihydroxyphenylglycine (DHPG).<sup>43</sup> More work, however, is necessary to determine whether the same processes control 2-AG formation *in vivo*.

The antinociceptive effects of exogenous 2-AG are preserved in FAAH(−/−) mice,<sup>61</sup> suggesting that FAAH does not catalyze 2-AG deactivation *in vivo*. Unlike anandamide and oleamide, monoacylglycerol lipids such as 2-AG exhibited equivalent hydrolytic activity in FAAH(+/+) and (−/−) mice.<sup>61</sup> These observations formed the basis for the conclusion that FAAH is an important regulator but not mediator of fatty acid amide activity *in vivo*.<sup>61</sup>

Transgenic approaches involving FAAH and CB<sub>1</sub> knockouts have recently been used in conjunction with pharmacological approaches to better evaluate the role of endocannabinoids in pain modulation. Mutant mice lacking the CB<sub>1</sub> gene fail to show typical antinociceptive responses to prototypical cannabinoid agonists.<sup>9,62</sup> It should be acknowledged, however, that high doses of Δ<sup>9</sup>-tetrahydrocannabinol do exhibit a CB<sub>1</sub>-independent antinociception,<sup>9</sup> although the receptor mechanism underlying this effect has not been evaluated. Cravatt et al developed mice lacking the FAAH gene and observed that these animals exhibited enhanced antinociceptive behavior following exogenous administration of anandamide.<sup>63</sup> Importantly, these enhancements of antinociception were blocked by the selective CB<sub>1</sub> antagonist rimonabant, providing compelling evidence that a CB<sub>1</sub>-dependent process is responsible for FAAH-mediated anandamide hydrolysis. Furthermore, Cravatt et al<sup>63</sup> observed tonic centrally mediated CB<sub>1</sub>-dependent analgesia in FAAH(−/−) mice, an effect likely due to the absence of this key enzyme, which catalyzes hydrolysis of fatty acid amides such as anandamide.<sup>63,64</sup> The behavioral phenotype was associated with a 15-fold increase in endogenous brain levels of anandamide in the FAAH(−/−) mice relative to FAAH(+/+) mice.<sup>63</sup> When mice lacking FAAH were treated with exogenous anandamide, they exhibited profound CB<sub>1</sub>-dependent behavioral responses, including hypomotility, analgesia, catalepsy, and hypothermia. The generation of mutant mice that are incapable of synthesizing or inactivating 2-AG should further elucidate roles of this endocannabinoid in pain modulation.

## Cannabinoid Receptor Pharmacology and Exogenous Cannabinoid Ligands

The development of competitive antagonists<sup>65</sup> and selective agonists for CB<sub>1</sub> has provided important pharmacological tools for investigating the biological functions of cannabinoids in the nervous system. SR141716A (rimonabant) shows high affinity for cannabinoid receptors in the brain ( $K_d = 0.23$  nM)<sup>65</sup> but displays negligible affinity for CB<sub>2</sub> ( $K_i$  [CB<sub>1</sub> vs CB<sub>2</sub>] = 5.6 nM vs >1 μM).<sup>66</sup> At high concentrations, rimonabant has been shown to inhibit vanilloid TRPV1 (formerly VR1) receptors. AM251 is a selective, competitive CB<sub>1</sub> antagonist ( $K_i$  [CB<sub>1</sub> vs CB<sub>2</sub>] = 7.5 nM vs >2 μM)<sup>67</sup> devoid

of vanilloid activity. Potent cannabinoid agonists CP55940 ( $K_i = 0.6$  nM at  $CB_1$  and  $CB_2$ ), HU210 ( $K_i$  [ $CB_1$  vs  $CB_2$ ] = 0.73 vs 0.22 nM), and WIN55212-2 ( $K_i$  [ $CB_1$  vs  $CB_2$ ] = 1.9 vs 0.3 nM) show high affinity for  $CB_1$  and  $CB_2$  and show marked improvements in potency compared with  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the prototypic classical cannabinoid. Selective competitive antagonists and high-affinity agonists have been used both to characterize the roles of cannabinoids in pain signaling and to map the sites of endocannabinoid action within the nervous system.

Studies relying upon the delivery of exogenous compounds that directly activate or block cannabinoid receptors have been indispensable for the initial assessment of the functional role of cannabinoid receptor activation in pain modulation. However, these studies do not provide direct evidence that endocannabinoids mediate these same functions under physiological conditions. More recently, the development of drugs that inhibit the enzymatic degradation of endocannabinoids (ie, through inhibition of FAAH or MGL) has facilitated research examining the functional consequences of activation of the body's endogenous system. URB597<sup>68</sup> is a well-characterized irreversible inhibitor of FAAH ( $IC_{50} = 4.6$  nM) that lacks significant affinity for  $CB_1$  and  $CB_2$  receptors and does not affect MGL, acetyl-cholinesterase, butyryl-cholinesterase, or the anandamide membrane transporter at concentrations up to 300  $\mu$ M. Arachidonoylserotonin<sup>69</sup> is a novel FAAH inhibitor that inhibits anandamide hydrolysis ( $IC_{50} = 5.6$   $\mu$ M), lacks affinity for  $CB_1$ , and does not significantly affect the cellular uptake of anandamide at 25  $\mu$ M. MGL can be inhibited by a variety of nonselective serine hydrolase inhibitors (eg, methyl arachidonoyl fluorophosphate). More recently, 2 selective inhibitors of MGL, URB602<sup>52</sup> and URB754,<sup>70</sup> have been described. URB602 inhibits rat brain MGL ( $IC_{50} = 28 \pm 4$   $\mu$ M) through a non-competitive mechanism, does not affect FAAH activity or anandamide levels, does not affect the activity of lipid-metabolizing enzymes such as diacylglycerol lipase<sup>35</sup> and cyclooxygenase-2,<sup>71</sup> and does not influence the binding of [ $^3$ H]-WIN55212-2 to  $CB_1$  or  $CB_2$  receptors ( $IC_{50} \geq 5$   $\mu$ M) or [ $^{35}$ S]-GTP- $\gamma$ -S to rat cerebellar membranes.<sup>52</sup> The effects of URB754 on pain modulation have not been examined.

Cellular uptake of anandamide reportedly involves facilitated diffusion,<sup>72</sup> although a specific transporter has yet to be cloned. Kinetics studies suggest the presence of an anandamide membrane transporter,<sup>72</sup> and pharmacological studies using inhibitors of anandamide transport<sup>52,72</sup> have supported the notion that anandamide transport inhibition has a role in modulating endocannabinoid tone. Among the most commonly employed drugs of this class are AM404, which also inhibits FAAH activity,<sup>73</sup> and VDM11. While AM404<sup>74</sup> activates TRPV1 receptors at low concentrations, VDM11<sup>75</sup> does not. VDM11 inhibits the cellular uptake of anandamide ( $IC_{50} = 1$ –11  $\mu$ M), does not affect FAAH, and does not bind cannabinoid receptors at biologically relevant concentrations. Recently, a potent new competitive inhibitor of anandamide uptake, LY2318912,<sup>72</sup> was used to radiolabel the anandamide transporter binding site in rat cerebellum. Systemic administration of LY2318912 also induced a 5-fold elevation in brain anandamide levels. Moreover, LY2318912 diminished nociceptive behavior in the formalin test, with no concomitant expression of gross motor deficits typical of administration of direct cannabinoid agonists.<sup>72</sup>

## Antinociceptive Effects of Exogenous Cannabinoids

Preclinical behavioral studies using different types of noxious stimulation (ie, thermal, mechanical, and chemical; for review, see Walker and Hohmann<sup>21</sup>) have demonstrated that cannabinoids effectively induce antinociception. In 1899, Dixon<sup>76</sup> demonstrated that delivery of cannabis smoke to dogs produced a failure to respond to pin pricks. Seminal studies on cannabinoid-induced antinociception by Bicher and Mechoulam<sup>77</sup> and Kosersky et al<sup>78</sup> provided a foundation for subsequent work that verified the ability of cannabinoids to profoundly suppress behavioral reactions to acute noxious stimuli and inflammatory and nerve injury-induced pain. The potency and efficacy of cannabinoids in producing antinociception is comparable to that of morphine.<sup>79,80</sup> However, cannabinoids induce profound motor deficits, including immobility and catalepsy,<sup>81</sup> which are a confound for behavioral studies that assess motor responses to noxious stimuli. Many recent studies of cannabinoid antinociception compensate for this limitation by additionally assessing behavioral measures of immobility and catalepsy to provide intrinsic controls for cannabinoid-induced changes in motor responding. Nonetheless, behavioral studies alone are not sufficient to demonstrate that cannabinoids suppress the processing of nociceptive information. An extensive literature now demonstrates that cannabinoids suppress nociceptive transmission, thus providing a compelling argument for the existence of endocannabinoid mechanisms of pain modulation.

Studies employing the systemic administration of cannabinoids have been useful in characterizing the antinociceptive effects of cannabinoids in animal models of acute and persistent nociception. The antinociceptive effects elicited by natural, synthetic, and exogenously administered endocannabinoids, along with the blockade of these effects by pharmacological and genetic disruptions of CB<sub>1</sub> activity, strongly suggest that cannabinoids have a specific physiological role in modulating pain sensitivity. Limitations of these approaches include the inability to localize the sites of action of cannabinoids and the failure to identify which endocannabinoids are involved in pain modulation. To address the first limitation, several important studies have used site-specific microinjections of cannabinoids into brain regions implicated in the processing and regulation of nociceptive signals. The second limitation has been addressed directly by identifying and quantifying endogenous mediators by microdialysis and liquid and/or gas chromatography mass spectrometry and indirectly by site-specific administration of pharmacological agents that regulate endocannabinoid uptake or degradation.

## Cannabinoid-Induced Suppression of Nociceptive Transmission

Electrophysiological and neurochemical studies provide convincing evidence that cannabinoids suppress nociceptive transmission *in vivo*.<sup>82-90</sup> Walker's laboratory first demonstrated that cannabinoids suppress noxious stimulus-evoked neuronal activity in nociceptive neurons in the spinal cord and thalamus.<sup>84,85,88,91,92</sup> This suppression is

observed in nociceptive neurons, generalizes to different modalities of noxious stimulation (mechanical, thermal, chemical), is mediated by cannabinoid receptors, and correlates with the antinociceptive effects of cannabinoids.<sup>84-86,88,91</sup> Cannabinoids also suppress C-fiber-evoked responses in spinal dorsal horn neurons recorded in normal, inflamed, and nerve-injured rats.<sup>82,87,90,93</sup> In addition, cannabinoids suppress spinal Fos protein expression, a neurochemical marker of sustained neuronal activation,<sup>94</sup> in a variety of animal models of persistent pain<sup>28,91,92,95-98</sup> through CB<sub>1</sub>- and CB<sub>2</sub>-selective mechanisms. Most electrophysiological studies have focused on wide-dynamic-range and nociceptive-specific cells recorded at the level of the spinal dorsal horn and have provided convincing evidence that cannabinoids suppress the transmission of nociceptive information. In vivo electrophysiological studies of brainstem neurons implicated in the descending control of pain have also provided insight into the role of cannabinoids in pain modulation and will be discussed below.<sup>89,99</sup>

## **Cannabinoid Antinociceptive Efficacy in Tissue Injury Models of Persistent Pain**

Studies using systemic administration of cannabinoids have demonstrated antinociception in multiple models of inflammatory nociception. Kosersky et al.<sup>78</sup> showed that systemic  $\Delta^9$ -THC increases the threshold for paw pressure-induced vocalization following the induction of inflammation in the hind paw. Tsou et al.<sup>92</sup> used the formalin test to show that systemic cannabinoids suppress noxious stimulus-evoked Fos protein expression and pain-related behaviors. The formalin test assesses supraspinally organized pain behavior. Our laboratory demonstrated that neurotoxic destruction of descending noradrenergic projections to the spinal cord reduces the suppression of formalin-evoked Fos protein expression induced by WIN55212-2.<sup>100</sup> The contribution of peripheral and spinal sites of action to cannabinoid antinociception in tissue and nerve injury models of persistent pain is now well documented (for review, see Hohmann<sup>20</sup>). By contrast, the contribution of supraspinal sites to cannabinoid analgesic action in models of persistent pain has received less attention.

## **Cannabinoid Antinociceptive Efficacy in Nerve Injury Models of Persistent Pain**

Antihyperalgesic and antiallodynic efficacy of cannabinoids has been demonstrated in several rodent models of experimental neuropathy. Bennett's group demonstrated antihyperalgesic and antiallodynic efficacy of a cannabinoid following a chronic constriction injury of the sciatic nerve.<sup>101</sup> The changes were blocked by systemic administration of a CB<sub>1</sub> antagonist.<sup>101</sup> Hyperalgesia and allodynia induced by tight

ligation of the L5 spinal nerve is also attenuated by systemic administration of WIN55212-2; these effects were reversed by a CB<sub>1</sub> but not by a CB<sub>2</sub> antagonist.<sup>102</sup> Cannabinoid-induced antinociception remains effective in nerve-injured rats following repeated administration, suggesting that cannabinoids are superior to opioids in alleviating neuropathic pain.<sup>103</sup> The existence of a substantial population of spinal cannabinoid receptors that remain intact following rhizotomy<sup>86,104</sup> may have clinical relevance, especially for deafferentation pain that is refractory to treatment with conventional narcotic analgesics.<sup>105</sup> The experimental studies thereby support the idea that the cannabinoids have a novel therapeutic target in treating neuropathic pain.

One possible mechanism for the antihyperalgesic actions of cannabinoids in neuropathic pain is suggested by cannabinoid-induced suppression of windup and noxious stimulus-induced central sensitization.<sup>90,106</sup> Support for the idea that there are both central and peripheral sites of cannabinoid antihyperalgesic efficacy has recently been demonstrated in a rat model of neuropathy using intrathecal and intraplantar administration of cannabinoid agonists and antagonists.<sup>107</sup> Electrophysiological studies also provide evidence for plasticity of the spinal cannabinoid system following tight ligation of the L5/L6 spinal nerve. Plasticity of cannabinoid systems may contribute to cannabinoid therapeutic efficacy in neuropathic pain states.<sup>106</sup> However, less is known about the possible contribution of supraspinal sites of cannabinoid analgesic action to the control of neuropathic pain.

The nucleus reticularis gigantocellularis pars alpha is implicated in cannabinoid modulation of neuropathic pain.<sup>108</sup> In rats subjected to partial sciatic nerve ligation (Seltzer model), unilateral hind paw injections of formalin contralateral to the site of nerve damage showed a reduced behavioral response to formalin compared with control conditions in the absence of nerve injury.<sup>108</sup> Administration of rimonabant to the nucleus reticularis gigantocellularis pars alpha of nerve-injured rats increased behavioral responses to formalin.<sup>108</sup> Although these data are consistent with the hypothesis that nerve injury activates CB<sub>1</sub>-mediated endogenous antinociceptive mechanisms from the nucleus reticularis gigantocellularis pars alpha in the formalin test, inverse agonist effects<sup>109</sup> can complicate interpretation of studies employing rimonabant to evaluate endogenous cannabinoid tone.<sup>90,110</sup> Further work is necessary to determine whether endocannabinoids mediate the observed effects and to identify a physiological role for a specific endocannabinoid in this effect.

## **Supraspinal Sites Implicated in Cannabinoid Modulation of Pain**

Direct support for the notion that there are supraspinal sites of cannabinoid antinociception was initially revealed in studies assessing acute withdrawal responses to thermal stimulation. The antinociceptive<sup>111</sup> effects of  $\Delta^9$ -THC in the tail-flick test are attenuated following spinal transection, providing indirect evidence that

supraspinal sites play an important role in cannabinoid antinociceptive action. Electrophysiological studies<sup>86</sup> similarly suggest that the suppressive effects of systemically administered cannabinoids on noxious stimulus-evoked responses in spinal nociceptive neurons are attenuated following spinal transection. Direct evidence for supraspinal sites of cannabinoid analgesic action was derived from the observation that intraventricular administration of cannabinoids WIN55212-2, CP55940, and  $\Delta^9$ -THC induces antinociception.<sup>112,113</sup> Consistent with these behavioral studies, intraventricular administration of WIN55212-2 also suppresses noxious stimulus-evoked responding in wide-dynamic-range neurons recorded in the spinal dorsal horn.<sup>86</sup> Using autoradiographic methods, a study employing intraventricular administration of [<sup>3</sup>H]WIN55212-2 confirmed that the radiolabeled drug was confined to periventricular sites throughout the brain. These studies underscore the importance of periventricular structures in contributing to cannabinoid-mediated pain modulation.

Site-specific injections of cannabinoid agonists to various brainstem regions have been used to identify supraspinal sites of cannabinoid antinociception. Using the tail-flick test, additional studies demonstrated that microinjection of cannabinoids into sites such as the dorsolateral PAG, dorsal raphe nucleus, RVM, amygdala, lateral posterior and submedial regions of the thalamus, superior colliculus, and noradrenergic A5 region produces antinociception.<sup>114-116</sup> Lichtman et al demonstrated that administration of CP55940 in the vicinity of the posterior ventrolateral PAG/dorsal raphe also produced antinociception, catalepsy, and hypothermia that was selective for the active stereoisomer.<sup>113</sup> By contrast, administration of CP55940 to the caudate putamen produced catalepsy but failed to induce antinociception or hypothermia. Microinjection of the cannabinoid HU210 into the dorsal PAG also produces a CB<sub>1</sub>-mediated suppression of formalin-evoked nocifensive behavior and attenuates formalin-evoked Fos protein in the caudal lateral PAG.<sup>98</sup> The intra-PAG injection of the cannabinoid also attenuated aversive defense behavior (ie, locomotor activation) elicited by dorsal PAG injections of the excitatory amino acid D,L-homocysteic acid.<sup>98</sup> Exogenous cannabinoids also modulate ultrasound-induced aversive responses in rats through actions in the dorsal PAG, although these effects were insensitive to blockade by rimonabant.<sup>117</sup> These studies provide support for the hypothesis that endocannabinoids may modulate pain and defense behaviors through actions in the PAG.

While the studies described above identify sites where exogenously administered synthetic cannabinoids induce antinociception, they do not elucidate which endocannabinoids play a role in pain modulation. Investigators commonly hypothesize the role of a particular endocannabinoid from data showing that the compound induces antinociception. This method assumes that appropriate stimulation conditions result in the *in vivo* release of the endocannabinoid and that the compound's net effect is sufficient to suppress pain sensitivity. In other studies, investigators correlate endocannabinoid levels or release, and the observation of antinociception. This method is informative but incapable of establishing causation. With these limitations in mind, the following sections review what is known about the role of particular endocannabinoids in nociceptive responding.

## PAG

The PAG is a common neural substrate underlying both analgesia and aversive responses. Electrical stimulation of the PAG produces analgesia and defensive behavior<sup>118,119</sup> that depends upon the activation of specific subdivisions of the nucleus. Electrical stimulation of the ventrolateral PAG produces analgesia that is blocked by opioid antagonists such as naltrexone,<sup>118</sup> suggesting that there is mediation by endogenous opioid peptides. By contrast, electrical stimulation of the dorsal and lateral PAG produces analgesia that is insensitive to blockade by opioid antagonists,<sup>118</sup> mediated by endocannabinoids, and blocked by cannabinoid antagonists.<sup>120</sup> Walker's group showed that electrical stimulation of the dorsal and lateral PAG resulted in cannabinoid receptor-mediated stimulation-produced analgesia concurrent with the mobilization of anandamide.<sup>120</sup> These actions were blocked by systemic or intra-PAG microinjection of rimonabant, consistent with mediation by CB<sub>1</sub>. We recently demonstrated that 2-AG and anandamide are elevated in dorsal midbrain fragments containing the entire PAG concomitantly with the expression of nonopioid stress-induced analgesia (SIA). We showed that exposure to a 3-minute continuous foot shock induced a CB<sub>1</sub>-mediated SIA independent of endogenous opioids.<sup>52</sup> Moreover, microinjection of FAAH inhibitors such as URB597<sup>52</sup> and arachidonoylserotonin<sup>121</sup> also enhanced SIA in a CB<sub>1</sub>-dependent manner. Microinjection of the MGL inhibitor URB602 into the PAG also induced a CB<sub>1</sub>-mediated enhancement of stress antinociception and selectively elevated levels of 2-AG (but not anandamide) in this region.<sup>52</sup> These data identify a physiological role for endogenous 2-AG in pain modulation at the level of the midbrain PAG.

Not all effects of endocannabinoids are mediated by CB<sub>1</sub> receptors, and therefore, it is important to demonstrate that endocannabinoid actions are blocked by selective cannabinoid antagonists. Microinjection of the FAAH inhibitor URB597 into the ventrolateral PAG has been reported to elevate endocannabinoids (both anandamide and 2-AG) and induce biphasic effects on thermal nociception via activation of CB<sub>1</sub> and TRPV1 receptor mechanisms.<sup>99</sup> In this study, the TRPV1-mediated antinociception and CB<sub>1</sub>-mediated nociception caused by URB597 correlated with enhanced or reduced activity of RVM off-cells, suggesting that these effects occur via stimulation or inhibition of excitatory PAG output neurons, respectively.<sup>99</sup> At the highest dose tested, however, URB597 (4 nmol/rat) and WIN55212-2 (25–100 nmol) caused only CB<sub>1</sub>-mediated analgesia, correlating with stimulation (possibly disinhibition) of RVM off-cells.<sup>99</sup> Thus, anandamide but not 2-AG may affect the descending pathways of pain control by acting at either CB<sub>1</sub> or TRPV1 receptors in select PAG subregions.<sup>99</sup>

In vitro electrophysiological studies indicate that cannabinoids inhibit both gamma-aminobutyric acid-ergic (GABAergic) and glutamatergic synaptic transmission presynaptically in rat PAG through a CB<sub>1</sub>-specific mechanism.<sup>122</sup> The cellular actions of cannabinoids are distinct from those of mu opioids because cannabinoids lack direct postsynaptic action on PAG neurons. Exogenous cannabinoids are likely to reduce the probability of transmitter release from presynaptic

terminals via a  $\text{Ca}^{2+}$ -independent mechanism,<sup>122</sup> suggesting that endocannabinoids behave similarly under physiological conditions.

Metabotropic glutamate and *N*-methyl-D-aspartic acid (NMDA) receptors are required for cannabinoid antinociception at the level of the PAG.<sup>123</sup> Infusion of WIN55212-2 into the PAG produced dose-dependent increases in paw withdrawal latencies in the plantar test.<sup>123</sup> This antinociceptive effect was blocked by pretreatment with rimonabant, which at high doses also produced modest hyperalgesia. Blockade of mGlu5 metabotropic glutamate receptors but not mGlu1 receptors completely blocked the effects of WIN55212-2. Both mGlu5 and mGlu1 receptors belong to the group I class of metabotropic glutamate receptors, which are G-protein-coupled and positively coupled to phospholipase C. Pretreatment with antagonists for group II (which includes mGlu2 and mGlu3) and group III (which includes mGlu4, mGlu6, mGlu7, and mGlu8) metabotropic glutamate receptors, which are negatively coupled to adenylate cyclase and preferentially localized to presynaptic active zones associated with autoreceptors, also suppressed WIN55212-2-induced antinociception. In addition to these metabotropic glutamate receptors, a selective antagonist for ionotropic glutamate (NMDA) receptors also blocked the antinociceptive effects of WIN55212-2. More work is necessary to elucidate the role of metabotropic glutamate receptors in endocannabinoid mechanisms of pain suppression.

## RVM

Researchers have targeted synthetic cannabinoids at other brainstem nuclei such as the RVM<sup>108,116,124</sup> and the nucleus reticularis gigantocellularis<sup>108</sup> to better characterize sites of cannabinoid-mediated antinociception. Walker's group demonstrated that site-specific administration of cannabinoids (WIN55212-2 and HU210) in the RVM produced antinociception in the tail-flick test.<sup>116</sup> Mediation by  $\text{CB}_1$  receptors was evident because the antinociceptive effects of HU210 were blocked by rimonabant and the receptor-inactive enantiomer WIN55212-3 failed to induce antinociception following microinjection to the same site.<sup>116</sup>

Electrophysiological studies have provided functional insight into the mechanism mediating these antinociceptive effects. In vivo recordings provide direct evidence that cannabinoids modulate on- and off-cells in the RVM,<sup>89,125</sup> thereby demonstrating the ability of these ligands to control descending pain modulatory signaling via a process similar to that of morphine. In lightly anesthetized rats, on-cells exhibit a burst of activity before the tail-flick nociceptive reflex, enhancing nociceptive transmission, whereas off-cells show a suppression of firing before the tail-flick reflex, inhibiting nociceptive transmission. Cannabinoids increased ongoing off-cell activity and reduced both the off-cell pause as well as the on-cell burst that occurs just prior to the tail-flick reflex. These actions were mediated by a  $\text{CB}_1$  mechanism that is not dependent upon endogenous opioids.<sup>89</sup> Pharmacological inactivation of the RVM with site-specific administration of the  $\text{GABA}_A$  receptor

agonist muscimol also blocked the antinociceptive effects but not the motor deficits of systemically administered WIN55212-2.<sup>89</sup> This work identifies a GABAergic link in cannabinoid antinociceptive mechanisms. At the cellular level, cannabinoids exert their physiological effects in the RVM by presynaptic inhibition of GABAergic neurotransmission.<sup>124</sup> Collectively, these results suggest that nociceptive responsiveness is modulated in the RVM by endocannabinoids, although the specific endocannabinoids mediating these actions remain to be identified.

The nucleus reticularis gigantocellularis pars alpha within the RVM represents a major source of descending control induced by cannabinoids and is also directly activated by noxious stimulation. Microinjection of WIN55212-2 to the nucleus gigantocellularis pars alpha produced antinociception in the tail flick and formalin tests in otherwise untreated rats.<sup>108</sup> These effects were blocked by a CB<sub>1</sub> antagonist. Microdialysis studies coupled with high-performance liquid chromatography mass spectrometry, together with site-specific administration of inhibitors of endocannabinoid degradation and synthesis, would be particularly useful in identifying which endocannabinoids mediate these effects.

## Role of the Amygdala

The amygdala consists of a nuclear complex located in the limbic forebrain and plays a key role in the coordination of fear and defensive reactions. The amygdala is optimally positioned anatomically to receive and integrate sensory information from multiple modalities and, in turn, to mediate emotional, autonomic, and somatic motor reactions to salient stimuli (especially threatening stimuli).<sup>126</sup> Within the amygdala, CB<sub>1</sub> immunoreactivity has been detected in a subset of GABAergic interneurons in the basolateral complex,<sup>127</sup> a site implicated in the formation and storage of aversive memories.<sup>128</sup> Anandamide and 2-AG are elevated in the basolateral amygdala in a conditioned fear aversion paradigm,<sup>127</sup> supporting the hypothesis that endocannabinoids serve naturally to inhibit extinction of aversive memories. Endocannabinoids and CB<sub>1</sub> receptors in the basolateral nucleus of the amygdala are implicated in the long-term depression of GABAergic inhibitory currents, suggesting that endocannabinoids regulate aversive memory extinction via selective inhibition of local inhibitory networks in the amygdala.<sup>127</sup>

The amygdala also plays a critical role in modulating antinociception. Microinjection of cannabinoids into the basolateral nucleus of the amygdala produces antinociception in the tail-flick test.<sup>96</sup> Microinjection of  $\mu$  opioid agonists into the basolateral nucleus of the amygdala similarly results in marked antinociceptive responding in the radiant heat tail-flick<sup>129,130</sup> and formalin tests.<sup>131</sup> Moreover, bilateral lesions of the amygdala rendered nonhuman primates less sensitive to the antinociceptive effects of the potent synthetic cannabinoid WIN55212-2.<sup>132</sup> In rodents, microinjection of the GABA<sub>A</sub> agonist muscimol into the central nucleus of the amygdala, but not into the basolateral nucleus of the amygdala, reduced the antinociceptive effects of systemic WIN55212-2.<sup>133</sup> Moreover, FAAH and MGL are

localized to postsynaptic and presynaptic sites, respectively, in the basolateral and lateral amygdala.<sup>53,55,59</sup> These data indicate that mechanisms exist for deactivation of anandamide and 2-AG in the basolateral amygdala. Both conditioned<sup>134,135</sup> and unconditioned<sup>136</sup> SIA depend on intact functioning of the amygdala. These observations, together with the demonstration of cannabinoid-mediated antinociceptive effects following site-specific administration to the basolateral nucleus of the amygdala,<sup>114</sup> suggest that endocannabinoids may serve naturally to suppress environmentally induced pain by actions in the amygdala. Below, we provide evidence that endocannabinoids may specifically mediate antinociceptive effects induced by exposure to environmental stressors, through actions in the PAG and to a lesser extent in the RVM and spinal cord.

## Behavioral Evidence for a Role of Endocannabinoids in SIA

Stress activates neural systems that suppress pain sensation. This adaptive response is known as SIA and depends on the recruitment of brain pathways that project from the amygdala to the midbrain PAG and descend to the brainstem RVM and dorsal horn of the spinal cord (for review, see Walker and Hohmann<sup>21</sup>). For years, it has been recognized that endogenous opioid peptides participate in this process,<sup>137,138</sup> but the inability of opioid antagonists to block stress antinociception elicited by distinct stressor parameters made it clear that other unidentified mechanisms were also involved.

We hypothesized that endocannabinoids might mediate nonopioid SIA induced by brief, continuous foot shock.<sup>52</sup> First, agonists of CB<sub>1</sub> receptors—the predominant cannabinoid receptor subtype present in the brain<sup>2,7</sup>—exert profound antinociceptive effects<sup>21</sup> and suppress activity in nociceptive neurons.<sup>84,86,88,89</sup> Second, CB<sub>1</sub> antagonists increase the activity of nociceptive RVM neurons<sup>89</sup> and enhance sensitivity to noxious stimuli,<sup>23</sup> which suggests that an intrinsic endocannabinoid tone regulates descending antinociceptive pathways.<sup>21</sup>

We quantified the poststress sensitivity to pain in rats using the tail-flick test after exposure to a 3-minute foot shock stressor.<sup>52</sup> As demonstrated previously,<sup>138,139</sup> this stimulation protocol caused a profound antinociceptive effect that was not altered by systemic injection of the opiate antagonist naltrexone but was virtually eliminated by systemic administration of the competitive CB<sub>1</sub> receptor antagonists/inverse agonists rimonabant and AM251. Moreover, in rats rendered tolerant to the antinociceptive effects of cannabinoids (by daily treatment with WIN55212-2 for 14 days) a marked attenuation in stress antinociception was observed.<sup>52</sup> It was unlikely that this change was due to altered opioid tone, because cannabinoid-tolerant rats showed no changes in antinociceptive responsiveness to morphine and rats tolerant to morphine showed no attenuation of nonopioid stress antinociception.<sup>52</sup>

Pharmacological blockade of TRPV1 via systemic administration of capsaizine also failed to alter stress analgesia in our testing paradigm,<sup>121</sup> suggesting that endocannabinoid-mediated stress analgesia was not dependent on TRPV1. The same dose of capsaizine that failed to affect endocannabinoid-mediated

stress antinociception, however, reliably reduced capsaicin-induced antinociception in the tail-flick test.<sup>121</sup>

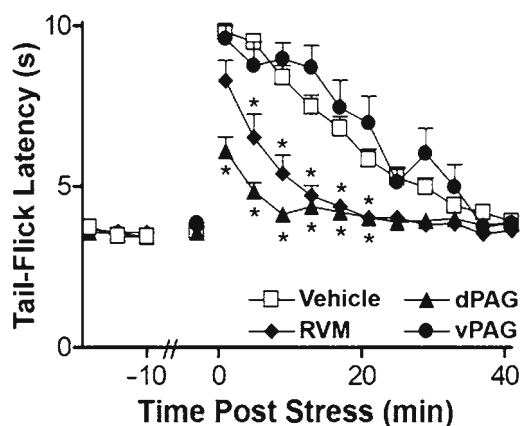
We reasoned that if endocannabinoid activation of CB<sub>1</sub> receptors mediates nonopioid SIA, then inhibition of endocannabinoid deactivation should enhance stress antinociception. To test this hypothesis, we administered FAAH inhibitors (URB597, arachidonoyl serotonin, or palmitoyl trifluoromethyl ketone) to rats and examined the resultant stress-induced antinociception in the tail-flick assay.<sup>52,121</sup> Regardless of the pharmacological method used to inhibit FAAH, postshock SIA was enhanced in animals treated systemically with FAAH inhibitors. In all cases, these effects were blocked by rimonabant, consistent with a CB<sub>1</sub>-dependent mechanism of action.<sup>52,121</sup> Systemic administration of rimonabant also attenuates fear-conditioned antinociceptive responses in the formalin test, together with freezing behavior and defecation, suggesting that CB<sub>1</sub> receptors and endocannabinoids may also contribute to fear-conditioned analgesia.<sup>140</sup>

## Sites of Action of Endocannabinoid-Mediated SIA

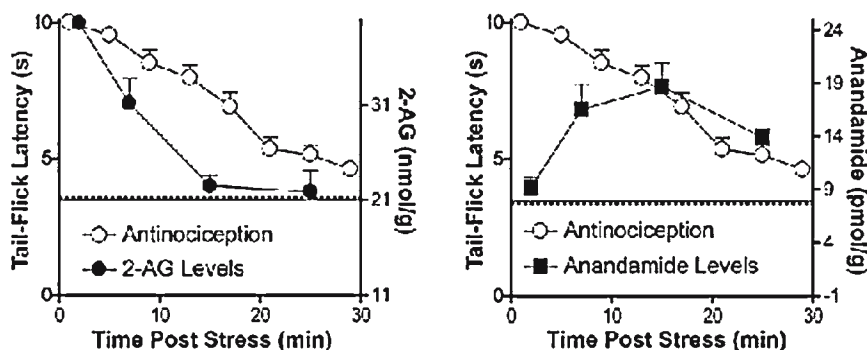
To further investigate the sites of action of endocannabinoids in mediating stress antinociception, we microinjected rimonabant at multiple levels of the neuraxis and quantified poststress sensitivity to pain in rats using the tail-flick test. We targeted brain structures involved in pain and stress responsiveness that contain CB<sub>1</sub> receptors and are implicated in cannabinoid antinociception, including the dorsolateral PAG, ventral PAG, RVM, basolateral nucleus of the amygdala, central nucleus of the amygdala, and lumbar spinal cord.<sup>52,121,141</sup> Rimonabant microinjection into the dorsolateral PAG produced the greatest suppression of SIA relative to all other sites surveyed (Fig. 44.3 and data not shown). These findings are consistent with the presence of CB<sub>1</sub> receptors in the PAG and suggest that this structure plays a pivotal role in nonopioid SIA.

## Stress Mobilizes Endocannabinoids to Suppress Pain

To determine whether endocannabinoid release is involved in SIA, we measured anandamide and 2-AG levels in dorsal midbrain fragments (containing the intact PAG) of rats killed without exposure to or at various times after foot shock.<sup>52</sup> Liquid chromatography/mass spectrometry (LC/MS) analyses revealed that midbrain 2-AG levels were markedly increased 2 minutes after shock and returned to baseline  $\approx$ 15 minutes later. This response preceded a sustained increase in anandamide levels, which peaked 7 to 15 minutes following the shock. No such changes were observed in the occipital cortex, a brain region that contains CB<sub>1</sub> receptors but is not considered part of the SIA circuit. The rapid poststress accumulation of 2-AG in the PAG suggests that endocannabinoid release, rather than intrinsic CB<sub>1</sub> activity, is responsible for SIA.



**Fig. 44.3** The dPAG plays a pivotal role in nonopioid stress-induced analgesia. Rimonabant (2nmol) microinjection into the dPAG induced a maximal suppression of stress antinociception ( $F_{3,48} = 96.42$ ,  $P < .0001$ ) relative to the vPAG, the RVM, or control conditions. SIA was assessed as the postshock (0.9mA for 3 minutes) tail-flick latency by an investigator blinded to the experimental condition. Vehicle groups did not differ from each other and were pooled for all sites.<sup>52,121</sup> dPAG indicates dorsolateral PAG; RVM, rostral ventromedial medulla; SIA, stress-induced analgesia; and vPAG, ventrolateral PAG.



**Fig. 44.4** Stress antinociception shows a temporal correspondence with 2-AG accumulation in midbrain periaqueductal gray. A significant correlation was observed between 2-AG ( $r = 0.943$ ,  $P < .03$ ) but not anandamide ( $r = -0.479$ ,  $P = .26$ ) accumulation and stress antinociception over the same time course. Stress antinociception was assessed as the postshock (0.9mA for 3 minutes) tail-flick latency. (—) basal nociceptive threshold; (—) basal endocannabinoid level.<sup>52</sup> 2-AG indicates 2-arachidonoylglycerol.

We compared the time courses of endocannabinoid mobilization in the PAG with those of SIA. A strong temporal correspondence was found between these parameters ( $r = 0.943$ ,  $P < .03$ ), consistent with mediation by a common mechanism (Fig. 44.4). By contrast, anandamide was released with a strikingly dissimilar time course that does not closely correspond to that of 2-AG mobilization or SIA

over the same interval ( $r = -0.479$ ,  $P = .26$ ). This temporal correlation points to 2-AG as a key mediator of nonopioid SIA.

## Endogenous 2-AG Mediates SIA

If mobilization of 2-AG in the PAG mediates SIA, selective inhibitors of MGL should increase accumulation of 2-AG and enhance SIA.<sup>52</sup> Consistent with this prediction, microinjection of the novel MGL inhibitor URB602 into the dorsolateral PAG or ventrolateral PAG enhanced SIA.<sup>52</sup> Basal nociceptive thresholds in non-shocked rats were unaffected. The effect of URB602 was likely due to the accumulation of 2-AG in the PAG because the URB602-mediated enhancement of SIA was prevented by coadministration of rimonabant and accompanied by an elevation in midbrain 2-AG levels.<sup>52</sup> Microinjection of URB602 into the PAG increased accumulation of 2-AG in brains of rats exposed to the stressor relative to vehicle-treated controls without altering levels of anandamide. These findings indicate that the MGL inhibitor URB602 enhances both 2-AG accumulation and SIA. These studies suggest that endogenous 2-AG plays a physiological role in pain modulation.

## Site-Specific Enhancement of Endocannabinoid Deactivation Enhances Stress Antinociception

Because the PAG serves key functions in both the descending control of pain<sup>21,120</sup> and the antinociceptive actions of cannabinoid agonists,<sup>115</sup> we examined the impact on stress antinociception of pharmacologically manipulating endocannabinoid deactivation using site-specific microinjections. Microinjection of either the FAAH inhibitor URB597<sup>52</sup> or arachidonoyl serotonin<sup>121</sup> to the dorsolateral PAG enhanced the magnitude and duration of endocannabinoid-mediated stress antinociception. These effects were blocked by coadministration of rimonabant, at a dose that was insufficient to reverse stress antinociception. It has recently been reported that site-specific microinjections of URB597 into the ventrolateral PAG enhance nociceptive behavior assessed in the plantar and tail-flick tests in otherwise naive rats,<sup>99</sup> despite producing enhanced anandamide and 2-AG levels. Consistent with our results, however, the highest dose of URB597 tested produced CB<sub>1</sub>-mediated antinociception.

Cannabinoids microinjected into neural targets of the PAG in the RVM induce antinociception and suppress nociceptive processing.<sup>98,116,125</sup> Like opioids,<sup>142</sup> cannabinoids modulate on- and off-cells in the RVM,<sup>89</sup> demonstrating the ability of these ligands to control descending pain signaling. Based upon the anatomy of the midbrain-to-brainstem pain modulation circuit and upon the robust effects of blocking CB<sub>1</sub> receptors in the dorsolateral PAG or RVM in attenuating SIA,<sup>52</sup> we further reasoned that inhibition of endocannabinoid deactivation at the level of the RVM would enhance stress antinociception.<sup>121</sup> Pharmacological inhibition of

FAAH via site-specific microinjections of arachidonoyl serotonin into the RVM enhanced stress antinociception via a CB<sub>1</sub>-specific mechanism.<sup>121</sup>

In rats, spinal transection reduces the antinociceptive<sup>111</sup> and electrophysiological<sup>86</sup> effects of cannabinoids. However, an enduring residual antinociception remains in spinally transected mice,<sup>143</sup> suggesting that endocannabinoids exert an analgesic effect at the spinal level as well as supraspinally. The localization of CB<sub>1</sub> receptors in the spinal dorsal horn<sup>7,104</sup> supports this view. Exogenously administered cannabinoids also produce antinociception when applied directly to the spinal cord<sup>96,143-146</sup> and suppress noxious stimulus-evoked neuronal activity in spinal nociceptive neurons,<sup>82,85-87</sup> suggesting that spinal cannabinoid receptors have a functional role in modulating nociceptive processing. Intrathecal administration of either rimonabant or CB<sub>1</sub> antisense oligonucleotides also elicits hyperalgesia,<sup>147</sup> suggesting that endocannabinoids may act tonically to suppress nociceptive responding.

To identify a physiological role for endocannabinoids at the spinal level, we bidirectionally manipulated endocannabinoid tone at CB<sub>1</sub> receptors in the lumbar spinal cord and assessed endocannabinoid mobilization in the lumbar spinal cord following exposure to a 3-minute continuous foot shock.<sup>141</sup> Stress antinociception was associated with the heightened release of endogenous 2-AG, whereas increases in anandamide mobilization were not detected,<sup>141</sup> perhaps because of greater variability and lower absolute levels of anandamide in these samples. Rimonabant failed to suppress endocannabinoid SIA when administered intrathecally to rats at a dose 10 times greater than that delivered to the PAG and RVM.<sup>141</sup> Nonetheless, pharmacological inhibitors of FAAH and MGL markedly enhanced the magnitude and duration of stress antinociception after intrathecal administration via a CB<sub>1</sub>-specific mechanism.<sup>141</sup> Our results show that, at the level of the spinal cord, endocannabinoids regulate but do not mediate nonopioid SIA.

The activity of endocannabinoids in the descending neural pathway projecting from the PAG to the RVM to the spinal cord is implicated in the activation of endogenous pain suppression mechanisms in response to stress. We also examined neuroanatomically “upstream” centers responsible for activating this mechanism following exposure to a stressor. Situated in the limbic forebrain, the amygdala is implicated in both fear conditioning<sup>148</sup> and the affective<sup>133,149</sup> dimensions of pain. CB<sub>1</sub> immunoreactivity is dense in the basolateral nucleus of the amygdala (BLA)<sup>2,150</sup> but is reportedly absent in the central nucleus of the amygdala (CeA).<sup>150</sup> The anatomical localization of CB<sub>1</sub> in the BLA is consistent with electrophysiological data demonstrating that activation of these receptors presynaptically modulates GABAergic transmission.<sup>150</sup> The distribution of FAAH and MGL at this site also correlates well with the distribution of CB<sub>1</sub> receptors.<sup>59</sup> BLA efferents innervate the CeA, the main amygdaloid output nucleus, which sends projections to the PAG and other regions. Unilateral microinjection of cannabinoid agonists into the amygdala also induces antinociception in the tail-flick test,<sup>114</sup> supporting the notion that this structure plays a role in modulation of pain sensitivity.

Microinjections of rimonabant into the BLA, but not the CeA, suppressed nonopioid stress antinociception in our paradigm.<sup>151</sup> Our data are consistent with the observation that CB<sub>1</sub> agonists depress monosynaptic evoked inhibitory postsynaptic

potentials in the BLA but not in the CeA.<sup>150</sup> Our results, therefore, suggest that CB<sub>1</sub> receptors in the BLA modulate local inhibitory networks in the BLA to ultimately regulate expression of SIA. Nonetheless, neither the FAAH inhibitor URB597 nor the MGL inhibitor URB602 enhanced SIA following microinjection into the BLA<sup>151</sup> at doses that markedly potentiated SIA following microinjection into the midbrain dorsolateral PAG.<sup>52</sup> These differences may reflect differential modulatory roles of distinct endocannabinoids in the ascending “affective” pain pathway compared with descending pain modulatory systems, or higher hydrolytic activity of endocannabinoid-degrading enzymes in the BLA relative to the PAG.

In sum, our results suggest that the coordinated release of 2-AG and anandamide in the PAG, RVM, and lumbar spinal cord mediates nonopioid SIA. The 2 endocannabinoids may act on local CB<sub>1</sub> receptors<sup>2,7,122</sup> to regulate glutamatergic and GABAergic transmission, ultimately disinhibiting descending pain control pathways. Three points are worthy of emphasis.<sup>52</sup> First, endocannabinoid-dependent stress antinociception is not affected by opioid antagonists or morphine tolerance, which implies that it may not require opioid activity. However, mutant CB<sub>1</sub> null mice also display reduced opioid-mediated responses to stress,<sup>152</sup> so opioid SIA need not be independent of endocannabinoids. Second, the residual antinociception observed in the presence of CB<sub>1</sub> antagonists leaves open the possibility that additional mediators of nonopioid SIA remain to be discovered. Third, stress mobilizes both 2-AG and anandamide in the dorsal midbrain, but these 2 endocannabinoids are released with distinctly dissimilar time courses. This observation underscores the existence of functional differences between these signaling molecules<sup>36</sup> that may be relevant to understanding endocannabinoid actions in other brain regions. The ability of both MGL and FAAH inhibitors to enhance endocannabinoid-dependent stress antinociception also highlights the significance of these enzymes as novel targets for the treatment of pain and stress- and anxiety-related disorders.<sup>52,68</sup>

## Conclusions

Interest in the behavioral effects of cannabinoids has burgeoned since the cloning of cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors and the isolation of endocannabinoids. The ability of cannabinoids to induce antinociception in virtually every animal model of acute or persistent pain evaluated has encouraged researchers to try to better understand this important nonopioid system of analgesia. Neuroanatomical studies have revealed that cannabinoid CB<sub>1</sub> receptors, endocannabinoids, and endocannabinoid-degrading enzymes are localized in central nervous system regions subserving the transmission and modulation of nociceptive signaling. Behavioral tests of acute nociception and tissue and nerve injury models of nociception have helped confirm the hypothesis that cannabinoids mediate antinociception via activation of CB<sub>1</sub> and CB<sub>2</sub> receptors. Recent studies have clarified the role of peripheral, spinal, and supraspinal sites in CB<sub>1</sub>-dependent analgesia.

Cannabinergic agents may offer promise in clinical pain management both on their own and as adjuncts to conventional therapeutic agents. Cannabinoids may be particularly efficacious for pain syndromes that are intractable to conventional analgesics (eg, neuropathic pain)<sup>153,154</sup> and in patient populations where the emetic effects of opioids are poorly tolerated (eg, cancer patients, AIDS patients). Furthermore, inhibitors of endocannabinoid-degrading enzymes such as FAAH and MGL may function to selectively enhance CB<sub>1</sub>-mediated neurotransmission only in nervous system areas where endocannabinoids are synthesized and released on demand, thereby precluding the induction of side effects associated with global CB<sub>1</sub> activation.<sup>155</sup> Moreover, synergism between cannabinoid and opioid analgesia has been demonstrated.<sup>144,156</sup> Collectively, these findings suggest that activation of cannabinoid receptors and inhibition of endocannabinoid deactivation may be promising targets for the clinical management of pain.

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