ACTIVATION OF GROUP III METABOTROPIC GLUTAMATE RECEPTORS REGULATES STRESS-INDUCED ANALGESIA THROUGH AN ENDOCANNABINOID MECHANISM

by

LAURA CORRINE GREGG

(Under the direction of Andrea G. Hohmann)

ABSTRACT

The endocannabinoid 2-arachidonoylglycerol (2-AG) produces adaptive changes in pain responses following exposure to environmental stressors. This phenomenon, termed stress-induced analgesia (SIA), is dependent upon mobilization and accumulation of 2-AG in the periaqueductal gray (PAG). 2-AG within the PAG may activate CB_1 receptors on GABAergic neurons to reduce inhibition of output neurons that form part of a descending antinociceptive pathway. Alternatively, 2-AG may activate CB_1 receptors on glutamatergic neurons to inhibit pro-nociceptive pathways that facilitate pain. 2-AG acts as a retrograde signal that binds to CB_1 receptors to produce antinociception. However, the mechanisms contributing to the mobilization of 2-AG are only beginning to be discovered. We examined the role of presynaptic group III metabotropic glutamate receptors (mGluRs), which are negatively coupled to adenylyl cyclase and reduce GABAergic inhibition, on the phenomenon of SIA in the PAG. Microinjection into the dorsolateral PAG (dlPAG) of the group III mGluR agonst L-AP4 produced a dose-dependent enhancement of SIA through a CB_1-dependent mechanism. By contrast, off-site injections of L-AP4 failed to enhance SIA. The L-AP4-induced enhancement of SIA was blocked by the group

III mGluR antagonist UBP1112 at a dose that was found to be ineffective in modulating SIA when administered alone. Microinjection of the group III mGluR antagonist UBP1112 into the dlPAG produced a dose-dependent suppression of SIA and also blocked the enhancement of SIA induced by L-AP4. This effect involved the dorsolateral PAG because off-site injections failed to alter SIA. Our findings suggest a previously unrecognized role for group III mGluRs in controlling endocannabinoid-dependent stress-induced analgesia, presumably by controlling the mobilization of endocannabinoids, likely 2-AG, in the PAG.

KEYWORDS: endocannabinoid, antinociception, metabotropic glutamate receptor

ACTIVATION OF GROUP III METABOTROPIC GLUTAMATE RECEPTORS REGULATES STRESS-INDUCED ANALGESIA THROUGH AN ENDOCANNABINOID MECHANISM

by

LAURA CORRINE GREGG

B.S., Georgia College & State University, 2005

M.S., The University of Georgia, 2008

A Dissertation Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2010

ACTIVATION OF GROUP III METABOTROPIC GLUTAMATE RECEPTORS REGULATES STRESS-INDUCED ANALGESIA THROUGH AN ENDOCANNABINOID MECHANISM

by

LAURA CORRINE GREGG

Major Professor: Andrea Hohmann

Committee: Philip Holmes
Jonathon Crystal

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2010

DEDICATION

This dissertation is dedicated to Brian, Oz, Harley and my family.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Andrea Hohmann, and my dissertation committee for all their help and advice. Without them, this truly would not be possible.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

CHAPTER 1: INTRODUCTION

1.1. Statement of Purpose

The purpose of this dissertation is to illustrate the role of metabotropic glutamate receptors and endocannabinoids in an animal model of stress antinociception. Exposure to environmental stressors activates neural pathways that suppress pain, a phenomenon known as stress-induced analgesia. Mobilization of endocannabinoids such as 2-arachidonylglycerol (2-AG) and anandamide mediate an opioid-independent form of stress-induced analgesia. Endocannabinoids, the brain's own cannabis-like compounds, bind to cannabinoid receptors and can produce centrally-mediated pharmacological effects, such as antinociception. Uncovering the mechanisms controlling endocannabinoid mobilization in the brain would potentially lead to the development of therapeutic interventions.

An important site of action in stress antinociception is the periaqueductal gray (PAG). A functional link between 2-AG accumulation in the PAG and stress-induced analgesia has been suggested. Metabotropic glutamate receptors have been implicated in mediating 2-AG accumulation in this model, yet the mechanisms for biosynthesis are only beginning to be discovered. The research revealed in this dissertation will further examine the role of metabotropic glutamate receptors in endocannabinoid-mediated stress-induced analgesia.

1.2. Overview

Chapter 2 will introduce and discuss the recent literature regarding endocannabinoids and their receptors, metabotropic glutamate receptors, the model of stress-induced analgesia, and the possible connections between all three. This review will look at important sites of action for endocannabinoids and their receptors, metabotropic glutamate receptors and the model of stress-induced analgesia. Chapter 3, following the review, will present a manuscript containing original data obtained for the purpose of identifying the role of group III metabotropic glutamate receptors in endocannabinoid-mediated stress antinociception. Lastly, a discussion will follow to summarize the main points of this dissertation.

CHAPTER 2: LITERATURE REVIEW

2.1. Endocannabinoids

The medical benefits, notably pain relief, of marijuana have been recognized for centuries. The active component of marijuana, Δ^9-tetrahydrocannabinol (Gaoni and Mechoulam, 1964) and binds mostly to CB_1 G protein-coupled receptors to exert its psychotropic effects (Howlett et al., 1990; Matsuda et al., 1990; Zimmer et al., 1999). Two types of cannabinoid receptors, CB_1 and CB_2, have been identified and are implicated in mediating antinociception (Walker and Hohmann, 2005). The brain produces its own cannabis-like compounds, referred to as endocannabinoids that bind to these cannabinoid receptors. The major endocannabinoids that have been most studied to date are 2-arachidonylglycerol (2-AG) (Mechoulam et al., 1995; Stella et al., 1997) and ananadamide (AEA) (Devane et al., 1992). Endocannabinoids are synthesized and released on demand and produce centrally-mediated pharmacological effects, such as antinociception, through binding to cannabinoid receptors (Piomelli, 2003). 2-AG biosynthesis occurs through the consecutive activation of phospholipase C (PLC) and diacylglycerol lipase-α (DGL-α) (Bisogno et al., 2003; Jung et al., 2007; Uchigashima et al., 2007). Some theories concerning what mediates 2-AG mobilization will be discussed later in this review. Deactivation of 2-AG occurs through the hydrolytic enzyme MGL (Hohmann et al., 2005), which metabolizes 2-AG into fatty acid and glycerol (Dinh et al., 2002). It has been suggested that AEA is synthesized in a two-step process –first by cleavage of the phospholipid precursor *N*-arachidonyl-phosphatidylethanolamine by the enzyme phospholipase D (PLD) and then

production of anandamide and phosphatidic acid (Di Marzo et al., 1994). However, the observation that NAPE-PLD-/- mice show now changes in AEA accumulation, suggests that this mechanism is unlikely to control 2-AG formation *in vivo* (Liu et al., 2008). The enzyme fatty acid amide hydrolase (FAAH) deactivates AEA, through hydrolysis of AEA into arachidonic acid and ethanolamine (Cravatt et al., 1996). Under some conditions, 2-AG may also be hydrolyzed by FAAH (Maione et al., 2006), although this mechanism is unlikely to be a major mechanism controlling 2-AG degradation *in vivo*.

2.2. Cannabinoid Receptors

Cannabinoid CB_1 receptors have been found in both the central nervous system and certain peripheral tissues (Pertwee, 1993, 1997). Among other areas, such as the hippocampus, cerebellum and cortex, CB_1 receptors are expressed in brain regions implicated in descending pain modulatory systems including the periaqueductal gray (PAG) (Herkenham et al., 1991). In the periphery, CB_1 receptors have been located in such tissues as the adrenal gland, heart, lung and bone marrow, while CB_2 receptors are located mainly in immune tissues, spleen and tonsils (Galiegue et al., 1995; Lynn and Herkenham, 1994). It was thought that CB_2 receptors were only expressed in the periphery, although research is beginning to show CB_2 receptor expression and functionality in the brain (Morgan et al., 2009; Onaivi et al., 2006).

2.3. Endocannabinoid Signaling

Endocannabinoid release can be triggered in multiple ways. Endocannabinoid release can follow a strong depolarization of postsynaptic neurons that causes an elevation of Ca^{2+} concentration (Wilson and Nicoll, 2001). Strong activation of certain metabotropic glutamate receptors (mGluRs) at basal Ca^{2+} levels can also trigger the release of endocannabinoids (Maejima et al., 2001). Simultaneous elevation of Ca^{2+} concentration and stimulation of the mGluR will also trigger endocannabinoid release (Varma et al., 2001). Following the trigger, endocannabinoids are synthesized and released on demand (Piomelli, 2003). The endocannabinoid 2-AG acts as a retrograde messenger at central nervous system synapses; that is, 2-AG is produced in the postsynaptic cell and when released into the synapse, binds to a presynaptic receptor (Kano et al., 2009). It has been found that 2-AG acts as a retrograde signal specifically through production by DGL-α (Tanimura et al., 2010). Cannabinoid receptors are G –protein coupled receptors that require coupling through a pertussis toxin-sensitive G protein ($G_{i/o}$) that then inhibits adenylate cyclase (Howlett et al., 1988; Matsuda et al., 1990). Cortical neurons express CB_1 receptors that are negatively coupled to adenylyl cyclase activity through a $G_{i/o}$ protein (Jung et al., 1997). CB_1 receptors are localized to presynaptic sites on GABAergic and glutamatergic neurons (Katona 1999, 2006). Cannabinoid receptors located on GABAergic and glutamatergic nerve terminals, when activated, inhibit GABAergic and glutamatergic transmission through a presynaptic mechanism (Chan et al., 1998; Shen et al., 1996). This cannabinoid-mediated neurotransmitter inhibition has been shown to be CB_1 -dependent (Vaughan et al., 2000).

2.4. Endocannabinoids and Antinociception

Antinociceptive effects of cannabinoids have been widely reported for centuries. The stimulation of cannabinoid receptor in the brain has been found to produce analgesia (Lichtman et al., 2006). Systemic administration of exogenous AEA produced antinociception, but this effect could not be blocked with the CB_1 antagonist rimonabant (Adams et al., 1998). An explanation for this could be that AEA is quickly degraded by FAAH. Systemic administration of exogenous 2-AG also produces antinociception (Mechoulam et al., 1995). Local injections of cannabinoid agonists have been used to uncover supraspinal sites of action in cannabinoid-mediated antinociception. Brain regions implicated include the dorsolateral PAG (dlPAG), dorsal raphe nucleus, rostral ventromedial medulla (RVM), amygdala, and thalamus (for review, see Hohmann and Suplita, 2006). Exposure to formalin has been found to modify RVM neuronal activities and this effect is prevented by PAG cannabinoid receptor stimulation (de Novellis et al., 2005). Reynolds (1969) found that analgesia is produced from electrical stimulation of the PAG. Later studies demonstrated that PAG neurons innervate midbrain nuclei that make up a descending antinociceptive pathway (Liebeskind et al., 1973; Duggan and Griermith, 1979). These findings are supported by the fact that microinjection of cannabinoids into the PAG produces analgesia in a variety of pain models (Fields and Basbaum, 1999). Stimulation of the dlPAG, specifically, produces an opioid-independent analgesia (Cannon et al., 1982). Endocannabinoids are also involved in a response to stress that produces an opioid-independent antinociception; a phenomenon termed stress-induced analgesia (SIA; Hohmann et al., 2005). This phenomenon involves brain pathways from the amygdala to the PAG, RVM and dorsal horn of the spinal cord (Hohmann and Suplita, 2006). Although the release of endocannabinoids in the RVM and spinal cord contribute to non-opioid SIA, the PAG plays a major role in mediating

pain through descending pathways (Hohmann and Suplita, 2006). Recent research has shown that 2-AG and anandamide levels are elevated in dorsal midbrain fragments containing the entire PAG (Hohmann et al., 2005). Moreover, 2-AG accumulation at this site correlates with SIA expression: this endocannabinoid accumulation is also CB_1 dependent (Hohmann and Suplita, 2006). Therefore, exposure to an environmental stressor activates endocannabinoid mobilization to induce CB_1-dependent antinociception through a descending pain pathway that includes the PAG. The changes in glutamate or GABA levels in the PAG may greatly affect nociceptive perception, as this midbrain area is part of the endogenous antinociceptive system (Gebhart et al., 1984). Analgesia is produced by cannabinoids at least in part by activating a descending PAG-RVM antinociceptive pathway that project to the dorsal horn of the spinal cord (Vaughan et al., 2000). This analgesia is produced within the PAG by reducing GABAergic inhibition of output neurons that form part of the descending pain pathway.

2.5. Metabotropic Glutamate Receptors

Glutamate receptors can be found throughout the brain and are mandatory for excitatory neurotransmission, development, and synaptic plasticity. There are two major classes of glutamate receptors: ionotropic receptors and metabotropic receptors. Metabotropic glutamate receptors are a heterogenous family of G-protein coupled receptors that are subdivided into three groups based on biochemical and pharmacological properties and their sequence homology (Pin and Duvoisin, 1995). Group I mGluRs, which include types 1 and 5 mGluRs (referred to here as mGluR1 and mGluR5), activate phospholipase C to generate diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) through coupling to $G_{q/11}$ (Conn and Pin, 1997). Group I mGluRs are

predominately expressed at postsynaptic synapses (Conn and Pin, 1997). Group II mGluRs, which include type 2 and 3 mGluRs (referred to here as mGluR2 and 3), are linked to $G_{i/o}$ and inhibit cAMP formation (Pin and Archer, 2002). Group II mGluRs are localized to both pre- and postsynaptic sites (Pin and Archer, 2002). Group III mGluRs, which include mGluR4, 6, 7, and 8, are also coupled to $G_{i/o}$ and inhibit adenylyl cyclase (Schoepp, 2001). However, unlike group II mGluRs, group III mGluRs are believed to be exclusively expressed at presynaptic sites to regulate neurotransmitter release (Schoepp, 2001). Metabotropic glutamate receptors are also found in glial cells where they play an important role in glial-neuronal communication and neuroprotection (Winder and Conn, 1996; Yao et al., 2005). mGluRs mediate a wide range of effects such as the release and postsynaptic response of glutamate and regulation of calcium, potassium, and non-selective cation channels (Anwyl, 1999). Following glutamate spillover, mGluRs also modulate the activity of GABAergic synapses. Activation of mGluRs will trigger G-protein activity and downstream signal transduction pathways. This activation can either increase or decrease various protein kinase activities.

mGluRs are present in many areas of the brain that are involved in the transmission and modulation of nociceptive information, including the midbrain PAG (Ohishi et al., 1995; Shigemoto et al., 1992; Tamaru et al., 2001). Recent studies demonstrate that activation of all groups of mGluRs in the PAG inhibits GABAergic transmission through a presynaptic reduction in the probability of transmitter release from nerve terminals (Drew and Vaughan, 2004). It has also been found that various mGluR agonists, following microinjection into the PAG and RVM, can regulate antinociceptive pathways in different pain models (Kim et al., 2002, Maione et al., 1998, 2000).

2.6. Group I Metabotropic Glutamate Receptors

Activation of group I metabotropic glutamate receptors can modulate additional signaling pathways including other cascades downstream of G_q, pathways stemming from $G_{i/o}$, G_s, and other molecules independent of G proteins (Hermans and Challiss, 2001). Subtype 1 and 5 mGluRs are mainly located on postsynaptic terminals and this seems to be regulated by a small family of "Homer" proteins (Brakeman et al., 1997). The group I mGluRs are positively linked to phospholipase C, therefore their activation results in an increase in phosphoinositide (PI) turnover and activation of protein kinase C (PKC). Most PKC isotypes require diacylglycerol (DAG) for their activation and are Ca^{2+}-dependent (Nikishizuka, 1988). This way, subtype 1 and 5 mGluRs can stimulate PI turnover, which then releases Ca^{2+} and generates DAG. Some studies have found that activation of group I mGluRs enhances the release of glutamate and GABA (Cartmell and Schoepp, 2000). Other, more recent studies determined that activation of group I mGluRs in the PAG suppresses GABAergic transmission through a presynaptic mechanism, yet they can also enhance GABAergic synaptic transmission through an action potential dependent mechanism (Drew and Vaughan, 2004). This inhibition of GABA occurs through a presynaptic reduction in the probability of transmitter release from nerve terminals. Inhibition of a postsynaptic glutamate transporter suppresses GABAergic transmission through activation of mGluR5 and presynaptic cannabinoid CB_1 receptors (Drew et al., 2008). Therefore, group I mGluR activation exhibits complex effects on GABAergic transmission, showing opposing inhibitory and excitatory influences within the PAG.

2.7. Group I mGluRs and Antinociception

Group I metabotropic glutamate receptors have been observed in brain regions implicated in mediating pain, such as the PAG (Azkue et al., 1997; Shigemoto et al., 1992). Activation of group I metabotropic glutamate receptors in the PAG has been shown to have antinociceptive effects in rodents in the hot-plate and formalin test (Maione et al., 1998, 2000). Group I mGluR activation also causes the production and release of endocannabinoids that subsequently inhibit neurotransmitter release through presynaptic cannabinoid G-protein-coupled receptors (Jung et al., 2005; Ohno-Shosaku et al., 2002). Research has found a functional link between the antinociceptive effects of activation of group I mGluR and cannabinoid receptors within the PAG and that these effects are mediated specifically by type 5 mGluRs (Palazzo et al., 2001). This coincides with recent work determining that the antinociceptive cannabinoid-mediated effects of stress-induced analgesia occur through activation of group I mGluR5, which then induces the mobilization of 2-AG through the PLC/DGL pathway at spinal and supraspinal levels (Gregg et al., 2007; Nyilas et al., 2009). These observations suggest that activation of group I mGluRs, specifically mGluR5, triggers mobilization of endocannabinoids, likely 2-AG, through hydrolysis of diacylglycerol (DAG) to produce antinociception through a CB_1 – dependent mechanism.

2.8. Group III Metabotropic Glutamate Receptors

Group III metabotropic glutamate receptors are negatively coupled to adenylyl cyclase, meaning that following activation they inhibit forskolin-stimulated cyclic AMP (cAMP) formation. Group III mGluRs are also predominately localized to presynaptic sites. Subtype 7

mGluRs seem to be localized mostly at glutamatergic terminals, whereas type 4 mGluRs are found presynaptically at both glutamatergic and nonglutamatergic terminals (Bradley et al., 1996). Many biochemical studies indicate that activation of group III mGluRs lead to a suppression of the release of excitatory and inhibitory amino acids (Cartmell and Schoepp, 2000). Activation of group III mGluRs specifically inhibits GABAergic transmission in the PAG through a presynaptic mechanism and these effects are blocked by a group III mGluR antagonist (Drew and Vaughan, 2004). This inhibition occurs through a presynaptic reduction in the probability of transmitter release from nerve terminals. Presynaptic type 8 mGluRs are expressed in the PAG on GABAergic and glutamatergic neurons and stimulation leads to a facilitation of glutamate and an inhibition of GABA release (Marabese et al., 2005). This modulation of neurotransmitter release requires the participation of coupling to adenylate cyclase. This receptor has been implicated in responses to novel stress environments (Linden et al., 2002) and is also implicated in modifying neuronal activation in stress-related brain regions (Linden et al., 2003). Activation of astroglial group III mGluRs exerts neuroprotective effects and the underlying mechanism is thought to be at least partially related to the enhancement of glutamate uptake (Yao et al., 2005).

2.9. Group III mGluRs and Antinociception

Recent research demonstrates the presence of group III metabotropic glutamate receptors in the PAG, a structure that plays a role in mediating antinociception (Ohishi et al., 1995). Microinjection into the PAG of group III mGluR agonists are pro-nociceptive in the hot-plate and formalin tests (Maione et al., 1998, 2000). Other studies conclude that administrations of

group III mGluRs are antinociceptive in animal models of persistent pain (Fisher and Coderre, 1996). Marabese et al. (2007) determined that stimulation of mGluR8 could have an antinociceptive effect in the tailflick test, while stimulation of mGluR7 could worsen nociception. A group III mGluR antagonist blocked both of these effects. These findings are not surprising, considering that presynaptic modulation of the amino acids glutamate and GABA in the PAG may be effective in alleviating pain (Marabese et al., 2006; Thomas et al., 2001) and that group III mGluRs modulate both glutamate and GABAergic transmission presynaptically in the PAG (Drew and Vaughan, 2004). Also, GABAergic interneurons inhibit the PAG antinociceptive pathway (Moreau and Fields, 1986), therefore the increase in glutamate and decrease in GABA caused by mGluR8 stimulation may be an important part in analgesia production. Also, the differing localization of mGluR7 on only glutamatergic synapses may explain the opposing effects of mGluR7 and mGluR8 on nociceptive responses. A link has been determined between the antinociceptive effects of activation of cannabinoid receptors within the PAG and group III mGluRs (Palazzo et al., 2001). A group III mGluR antagonist blocked the antinociceptive effect caused by activation of cannabinoid receptors. These findings coincide with findings from our lab concluding that activation of group III mGluRs enhances endocannabinoid-mediated stress antinociception through a CB_1-dependent mechanism (Gregg et al., manuscript currently in preparation).

2.10. Summary

There are many potential therapeutic uses for the endocannabinoids AEA and 2-AG and their cannabinoid receptors, the one most discussed here is for relief of pain. A review of the

literature regarding cannabinoid-like compounds, both exogenous and endogenous, in specific models of pain identifies the importance and value of their use as analgesics. The underlying mechanisms for endocannabinoid mobilization, and specifically 2-AG mobilization, under certain physiological paradigms are only beginning to be uncovered. The mechanism by which 2-AG is synthesized is becoming better understood, but more research is needed to pinpoint the triggers for 2-AG mobilization in the brain. The role of metabotropic glutamate receptors in endocannabinoid-mediated antinociception is a hot debate for this topic. The role of group I mGluRs, specifically type 5, in endocannabinoid mobilization and the effects activation of these receptors have on nociceptive responses has recently been evaluated (Nyilas et al., 2009). Less research has been done to determine the role of group III mGluRs in controlling nociceptive responses and whether these effects are dependent upon cannabinoid receptors activation. The more that is known about the pathways and triggers of endocannabinoid-mediated antinociception, the closer we will be to uncovering novel therapeutic agents with minimal side effects. Thus, the following original research will attempt to uncover the role of group III metabotropic glutamate receptors in endocannabinoid-mediated stress-induced analgesia in the midbrain periaqueductal gray.

2.11. References

Adams IB, Compton DR, Martin BR. 1998. Assessment of anandamide interaction with the cannabinoid receptor: SR141716A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. J Pharmacol Exp Ther 284 (3): 1209-17.

Anwyl R. 1999. Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. Brain Res Rev 29: 83-120.

Azkue JJ, Knopfel T, Kuhn R, Mateos JM, Grandes P. 1997. Distribution of the metabotropic glutamate receptor subtype mGluR5 in rat periaqueductal grey and relationship with ascending spinofugal afferents. Neurosci Lett 228: 1-4.

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di Marzo V, Doherty P. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163: 463-8.

Bradley SR, Levey AI, Hersch SM, Conn PJ. 1996. Immunocyctochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype-specific antibodies. J Neurosci 16: 2044-56.

Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL, Worley PF. 1997. Homer: a protein that selectively binds metabotropic glutamate receptors. Nature 386 (6622): 284-8.

Cannon JT, Prieto GJ, Lee A, Liebeskind JC. 1982. Evidence for opioid and non-opioid forms of stimulation-produced analgesia in the rat. Brain Res 243 (2): 315-21.

Cartmell J, Schoepp DD. 2000. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75 (3): 889-907.

Chan PK, Chan SC, Yung WH. 1998. Presynaptic inhibition of GABAergic inputs to rat substantia nigra pars reticulata neurons by a cannabinoid agonist. Neuroreport 9: 671-5.

Conn PJ, Pin JP. 1997. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37: 205-37.

Cravatt BF, Giang DK, Mayfield SP, Bogert DL, Lerner RA, Gilula NB. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384 (6604): 83-7.

De Novellis V, Mariani L, Palazzo E, Vita D, Marabese I, Scafuro M, Rossi F, Maione S. 2005. Periaqueductal grey CB1 cannabinoid and metabotropic glutamate subtype 5 receptors modulate changes in rostral ventromedial medulla neuronal activities induced by subcutaneous formalin in the rat. Neurosci 134: 269-81.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258 (5090): 1946-9.

Di Marzo V, Fonatan A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D. 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372 (6507): 686-91

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D. 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 99: 10819-24.

Drew GM, Vaughan CW. 2004. Multiple metabotropic glutamate receptor subtypes modulate GABAergic neurotransmission in rat periaqueductal grey neurons in vitro. Neuropharmacol 46: 927-34.

Drew GM, Mitchell VA, Vaughan CW. 2008. Glutamate spillover modulates GABAergic synaptic transmission in the rat midbrain periaqueductal grey via metabotropic glutamate receptors and endocannabinoid signaling. J Neurosci 28 (4): 808-15.

Duggan AW, Griermith BT. 1979. Inhibition of the spinal transmission of nociceptive information by supraspinal stimulation in the cat. Pain 6 (2): 149-61.

Fields HL, Basbaum AI. 1999. Central nervous system mechanisms of pain modulation. In: Wall, PD, Melzack R. (Eds), Textbook of Pain. Churchill Livingston, Edinburgh, pp. 309-29.

Fisher K, Coderre TJ. 1996. The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. Pain 68: 255-63.

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P. 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232: 54-61.

Gaoni Y, Mechoulam R. 1964. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86: 1646-7.

Gebhart GF, Sandkuhler J, Thalhammer JG, Zimmermann M. 1984. Inhibition in spinal cord of nociceptive information by electrical stimulation and morphine microinjection at identical sites in midbrain of the cat. J Neurophysiol 51: 75-89.

Gregg LC, Bolton NM, Moise AM, Suplita II RL, Hohmann AG. November 2007. Group I Metabotropic Glutamate Receptors Regulates Endocannabinoid-mediated Stress-induced Analgesia Through the Phospholipase C/Diacylglycerol Lipase Pathway. Poster session presented at the meeting of *Society for Neuroscience,* San Diego, CA.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. 1991.

Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11: 563-83.

Hermans E, Challiss RA. 2001. Structural, signaling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. J Biochem 359: 465-84.

Hohmann AG, Suplita RL 2nd, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. 2005. An endocannabinoid mechanism for stress-induced analgesia. Nature 435: 1108-12.

Hohmann AG, Suplita RL 2nd. 2006. Endocannabinoid mechanisms of pain modulation. Aaps J 8 (4): E693-708.

Howlett AC, Johnson MR, Melvin LS, and Milne GM. 1988. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. Mol Pharmacol 33: 297-302.

Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M. 1990. The cannabinoid receptor: biochemical, anatomical and behavioral characterization. Trends Neurosci 13 (10): 420-3.

Jung M, Calassi R, Rinaldi-Carmona M, Chardenot P, Le Fur G, Soubrie P, Oury-Donat, F. 1997. Characterization of CB1 receptors on rat neuronal cell cultures: binding and functional studies using the selective receptor agonist SR141716A. J Neurochem 68: 402-9.

Jung KM, Astarita G, Zhu C, Wallace M, Mackie K, Piomelli D. 2007. A key role for diacylglycerol lipase-alpha in metabotropic glutamate receptor-dependent endocannabinoid mobilization. Mol Pharmacol 72 (3): 612-21.

Kano M, Ohno-Shasaku T, Hashimotodani Y, Uchigashmima M, Watanabe M. 2009. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89: 309-80.

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF. 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19: 4544-58.

Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF. 2006. Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci 26: 5628-37.

Kim SJ, Calejesan AA, Zhuo M. 2002. Activation of brainstem metabotropic glutamate receptors inhibits spinal nociception in adult rats. Pharmacol, Biochem, and Behav 73: 429-37.

Lichtman AH, Cook SA, Martin BR. 1996. Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J Pharmacol Exp Ther 276: 585-93.

Liebeskind JC, Guilbaud G, Besson JM, Oliveras JL. 1973. Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioural observations and inhibitory effects on spinal cord interneurons. Brain Res 50 (2): 441-6.

Linden AM, Johnson BG, Peters SC, Shannon HE, Tian M, Wang Y, Yu JL, Koster A, Baez M, Schoepp DD. 2002. Increased anxiety-related behavior in mice deficient for metabotropic glutamate 8 (mGlu8) receptor. Neuropharmacol 43: 251-9.

Linden AM, Bergeron M, Baez M, Schoepp DD. 2003. Systemic administration of the potent mGlu8 receptor agonist (*S*)-3,4-DCPG induces c-Fos in stress-related brain regions in wild-type, but not mGlu8 receptor knockout mice. Neuropharmacol 45: 473-83.

Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, Palmiter RD, Krystal G, Rai R,

Mahadevan A, Razdan RK, Kunos G. 2008. Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacol 54: 1-7.

Lynn AB, Herkenham M. 1994. Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. J Pharmacol Exp Ther 268 (3): 1612-23.

Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. 2001. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate receptors. Neuron 31: 463-75.

Maoine S, Marabese I, Leyva J, Palazzo E, de Novellis V, Rossi F. 1998. Characterization of mGluRs which modulate nociception in the PAG of the mouse. Neuropharmacol 37: 1475-83.

Maione S, Oliva P, Marabese I, Palazzo E, Rossi F, Berrino L, Rossi F, Filippelli A. 2000. Periaqueductal gray matter metabotropic glutamate receptors modulate formalin-induced nociception. Pain 85: 183-9.

Maione S, Bisogno T, de Novellis V, Palazzo E, Cristino L, Valenti M, Petrosino S, Guglielmotti V, Rossi F, Di Marzo V. 2006. Elevation of endocannabinoid levels in the ventrolateral periaqueductal grey through inhibition of fatty acid amide hydrolase affects descending nociceptive pathways via both cannabinoid receptor type 1 and transient receptor potential vanilloid type-1 receptors. J Pharmacol Exp Ther 316: 969- 82.

Marabese I, de Novellis V, Palazzo E, Mariani L, Siniscalco D, Rodella L, Rossi F, Maione S. 2005. Differential roles of mGlu8 receptors in the regulation of glutamate and γ-aminobutyric acid release at periaqueductal grey level. Neuropharm 49: 157-166.

Marabese I, de Novellis V, Palazzo E, Scafuro MA, Vita D, Rossi F, Maione S. 2006. Effects of

(*S*)-3,4-DCPG, an mGlu8 receptor agonist, on inflammatory and neuropathic pain in mice. Neuropharmacol 52: 253-62.

Marabese, I. Rossi F, Palazzo E, de Novellis V, Starowicz K, Cristino L, Vita D, Gatta L, Guida F, Di Marzo V, Rossi F, Maione S. 2007. Periaqueductal gray metabotropic glutamate receptor subtype 7 and 8 mediate opposite effects on amino acid release, rostral ventromedial medulla cell activities, and thermal nociception. J Neurophysiol 98:43-53.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346 (6284): 561-4.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50 (1): 83-90.

Moreau J, Fields HL. 1986. Evidence for GABA involvement in midbrain control of medullary neurons that modulate nociceptive transmission. Brain Res 397: 37-46.

Morgan NH, Stanford IM, Woodhall GL. 2009. Functional CB2 type cannabinoid receptors at CNS synapses. Neuropharmacol 57 (4): 356-68.

Nishizuka Y. 1988. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334 (6184): 661-5.

Nyilas R, Gregg LC, Mackie K, Watanabe M, Zimmer A, Hohmann AG, Katona I. 2009. Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia. Eur J Neurosci 29 (10): 1964-78.

Ohishi H, Akazawa C, Shigemoto R, Nakanishi S, Mizuno N. 1995. Distributions of the mRNAs for L-2amino-4-phosphono-butyrate-sensitive metabotropic glutamate receptors, mGluR4

and mGluR7, in the rat brain. J Comp Neurol 360: 555-70.

Ohno-Shosaku T, Shosaku J, Tsubokawa H, Kano M. 2002. Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. Euro J Neurosci 15, 953-61.

Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA, Meyers L, Mora Z, Tagliaferro P, Gardener E, Brusho A, Akinshola BE, Liu QR, Hope B, Iwasaki S, Arinami T, Teasenfitz L, Uhl GR. 2006. Discovery of the present and functional expression of cannabinoid CB_2 receptors in brain. Ann NY Acad Sci 1074: 514-36.

Palazzo E, Marabese I, de Novellis V, Oliva P, Rossi F, Berrino L, Rossi F, Maione S. 2001. Metabotropic and NMDA glutamate receptors participate in the cannabinoid-induced antinociception. Neuropharmacol 40: 319-26.

Pertwee RG. 1993. The evidence for the existence of cannabinoid receptors. Gen Pharmacol 24 (4): 811-24.

Pertwee RG. 1997. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther 74 (2): 129-80.

Pin JP, Duvoisin R. 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacol 34: 1-26.

Pin JP, Acher F. 2002. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. Curr Drug Targes CNS Neurol Disord 1 (3): 297-317.

Piomelli D. 2003. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4: 873-84.

Reynolds DV. 1969. Surgery in the electrical analgesia induced by focal brain stimulation. Science 164: 444-5.

Schoepp DD. 2001. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther 299: 12-20.

Shen M, Piser TM, Seybold VS, Thayer SA. 1996. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 16: 4322-34.

Shigemoto R, Nakanishi S, Mizuno N. 1992. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat. J Comp Neurol 322: 121-135.

Stella N, Schweitzer P, Piomelli D. 1997. A second endogenous cannabinoid that modulates long-term potentiation. Nature 388: 773-8.

Tamaru Y, Nomura S, Mizuno N, Shigemoto R. 2001. Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites. Neurosci 106 (3): 481-503.

Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, Sakinura K, Kano M. 2010. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. Neuron 65: 320-7.

Thomas NK, Wright RA, Howson PA, Kingston AE, Schoepp DD, Jane DE. 2001. (*S*)-3,4-DCPG, a potent and selective mGlu8a receptor agonist, activates metabotropic glutamate receptors on primary afferent terminals in the neonatal rat spinal cord. Neuropharmacol 40: 311-8.

Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M. 2007. Subcellular arrangement of molecules for 2-arachidonoylglycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27 (14):

3663-76.

Varma N, Carlson G, Ledent C, Alger BE. 2001. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J Neurosci 21: 1-5.

Vaughan CW, Connor M, Bagley EE, Christie MJ. 2000. Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. Mol Pharmacol 57: 288-95.

Walker JM, Hohmann AG. 2005. Cannabinoid mechanisms of pain suppression. Handb Exp Pharmacol 168: 509-54.

Wilson RI, Nicoll RA. 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410: 588-92.

Winder DG, Conn PJ. 1996. Roles of metabotropic glutamate receptors in glial functions and glial-neuronal communication. J Neurosci Res 46: 131-7.

Yao HH, Ding JH, Zhou F, Wang F, Hu LF, Sun T, Hu G. 2005. Enhancement of glutamate uptake mediates the neuroprotection exerted by activating group II or III metabotropic glutamate receptors on astrocytes. J Neurochem 92:948-61.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. 1999. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci USA 96 (10): 5780-5.

CHAPTER 3: ACTIVATION OF GROUP III METABOTROPIC GLUTAMATE RECEPTORS REGULATES STRESS-INDUCED ANALGESIA THROUGH AN ENDOCANNABINOID MECHANISM[1]

[1]Gregg, L.C. and A.G. Hohmann.

3.1. Abstract

The endocannabinoid 2-arachidonoylglycerol (2-AG) produces adaptive changes in pain responses following exposure to environmental stressors. This phenomenon, termed stress-induced analgesia (SIA), is dependent upon mobilization and accumulation of 2-AG in the periaqueductal gray (PAG). 2-AG within the PAG may activate CB_1 receptors on GABAergic neurons to reduce inhibition of output neurons that form part of a descending antinociceptive pathway. Alternatively, 2-AG may activate CB_1 receptors on glutamatergic neurons to inhibit pro-nociceptive pathways that facilitate pain. 2-AG acts as a retrograde signal that binds to CB_1 receptors to produce antinociception. However, the mechanisms contributing to the mobilization of 2-AG are only beginning to be discovered. Coupling to the CB_1 receptor requires the pertussis toxin-sensitive G protein ($G_{i/o}$) and inhibits adenylyl cylcase. 2-AG acts as a full agonist at cannabinoid CB_1 receptors, and inhibits adenylyl cyclase through coupling to $G_{i/o}$. We examined the role of presynaptic group III metabotropic glutamate receptors (mGluRs), which are negatively coupled to adenylyl cyclase and reduce GABAergic inhibition, on the phenomenon of SIA in the PAG. Microinjection into the dorsolateral PAG (dlPAG) of the group III mGluR agonst L-AP4 produced a dose-dependent enhancement of SIA through a CB_1-dependent mechanism. By contrast, off-site injections of L-AP4 failed to enhance SIA. The L-AP4-induced enhancement of SIA was blocked by the group III mGluR antagonist UBP1112 at a dose that was found to be ineffective in modulating SIA when administered alone. Microinjection of the group III mGluR antagonist UBP1112 into the dlPAG produced a dose-dependent suppression of SIA and also blocked the enhancement of SIA induced by L-AP4. This effect involved the dorsolateral PAG because off-site injections of the active compounds failed to alter SIA. Our findings suggest a previously unrecognized role for group III mGluRs in controlling

endocannabinoid-dependent stress-induced analgesia, presumably by controlling the mobilization of endocannabinoids, likely 2-AG, in the PAG.

KEYWORDS: endocannabinoid, antinociception, metabotropic glutamate receptor

3.2. Introduction

Previous work from our laboratories suggests that endocannabinoid mobilization mediates stress-induced analgesia (SIA) through accumulation of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the midbrain periaqueductal gray (PAG) (Gregg et al., 2007; Hohmann et al., 2005; Suplita et al., 2006). Endocannabinoids, the brains own cannabis-like compounds, produce pharmacological effects through binding to cannabinoid receptors (for review, see Piomelli, 2003). Cannabinoid CB_1 receptors are located in brain regions specifically implicated in descending pain systems, such as the PAG (Herkenham et al., 1991), Here, CB_1 receptor activation regulates exogenous (Lichtman et al., 1996; Maione et al., 2006; Martin et al., 1995) and endogenous (Gregg et al., 2007; Hohmann et al., 2005; Maione et al., 2006; Suplita et al., 2005) cannabinoid-mediated antinociception. Endocannabinoid-mediated stress antinociception is blocked by CB_1 antagonists and is attenuated in rats rendered tolerant to cannabinoids (Hohmann et al., 2005). Accumulation of 2-AG in dorsal midbrain fragments containing the intact PAG is highly correlated with SIA (Hohmann and Suplita, 2006). Both accumulation of 2-AG in the PAG and SIA can be regulated by activation of group I metabotropic glutamate receptors (mGluRs). These studies have specifically identified a role for the mGluR5 subtype in controlling SIA through a CB_1-dependent mechanism (Gregg et al., 2007; Nyilas et al., 2009). 2-AG accumulation and stress antinociception can also be enhanced

through a CB_1 –dependent mechanism through pharmacological inhibition of 2-AG degrading enzymes (Hohmann et al., 2005; Suplita et al., 2006). These findings suggest a functional link between 2-AG and endocannabinoid-mediated stress antinociception. However, the mechanisms by which 2-AG is mobilized in the brain under physiological conditions remains incompletely understood.

Following receptor activation and cleavage of membrane phospholipid precursors, endocannabinoids are synthesized and released on demand (for review, see Piomelli, 2003). Endocannabinoids such as 2-AG act as retrograde signals (Chevaleyre and Castillo, 2003; Gerdeman et al., 2002; Riegal and Lupica, 2004; Wilson and Nicoll, 2001), binding to CB_1 receptors localized to presynaptic sites on GABAergic and glutamatergic neurons (Katona et al., 1999, 2006). Specifically, it has been shown that 2-AG functions as a retrograde messenger (Kano, et al., 2009; Tanimura et al., 2010). CB_1 receptor activation inhibits GABAergic and glutamatergic synaptic transmission through a presynaptic mechanism in the PAG (Drew et al., 2008; Vaughan et al., 2000). Cannabinoid-mediated analgesia may therefore be produced in the PAG through reduction of GABAergic inhibition of output neurons and/or reduction of glutamatergic excitation of a descending pro-nociceptive pathway. However, the mechanisms controlling 2-AG formation under physiological conditions remain incompletely understood.

Group I mGluRs are positively coupled to phospholipase C (PLC), whereas group III mGluRs are negatively coupled with adenylate cyclase (Pin and Duvoisin, 1995). Following exposure to footshock, activation of the type 5 group I mGluR enhances SIA through activation of diacylglycerol lipase (DGL: Gregg et al., 2007; Nyilas et al., 2009). The consecutive activation of these two enzymes, PLC and DGL, controls 2-AG formation *in vitro* (Bisogno et al., 2003; Jung et al., 2007). Functional coupling of the CB_1 receptor occurs through a pertussis

sensitive G-protein ($G_{i/o}$) (Howlett et al., 1988). Coupling of cannabinoid receptors to $G_{i/o}$ inhibits adenylyl cyclase in cells expressing CB_1 receptors (Felder et al., 1995; Jung et al., 1997). The release of the endocannabinoid 2-AG also acts as a full agonist at CB_1 receptors to inhibit adenylyl cyclase (Stella et al., 1997). Activation of group III mGluRs are also associated with $G_{i/o}$ coupling and inhibition of adenylate cyclase (for review, see Schoepp, 2001).

Whereas group I mGluRs are localized to postsynaptic sites, group III mGluRs are believed to reside exclusively on presynaptic sites (for reviews, see Conn and Pin, 1997; Schoepp, 2001). Biochemical studies indicate that activation of group III mGluRs lead to a suppression of the release of excitatory and inhibitory amino acids (Cartmell and Schoepp, 2000). It has been previously suggested that activation of group III mGluRs in the PAG inhibits GABAergic release through a presynaptic mechanism (Drew and Vaughan, 2004) as demonstrated previously for CB_1 receptors (Vaughan et al., 2000). A link has also been demonstrated between the antinociceptive effects of activation of group III mGluRs and cannabinoid receptors within the PAG (Palazzo et al., 2001). Presynaptic group III mGluRs, specifically type 8, are expressed in the PAG on both glutamatergic and GABAergic synapses (Marabese et al., 2005). Activation of these receptors facilitates glutamate and inhibits GABA release (Marabese et al., 2005). Subtype 4 mGluRs are also found presynaptically at both glutamatergic and nonglutamatergic terminals, whereas type 7 mGluRs seem to be localized predominately at glutamatergic terminals (Bradley et al., 1996). Throughout the brain, group III mGluRs may act as autoreceptors on glutamatergic terminals to regulate the release of glutamate (Schoepp, 2001). Considering that presynaptic modulation of the amino acids glutamate and GABA in the PAG may affect nociceptive responses, we asked whether group III mGluRs are implicated in endocannabinoid-mediated SIA.

In the present study, we investigated the role of group III metabotropic glutamate receptors on endocannabinoid-mediated stress-induced analgesia. We hypothesized that activation of group III mGluRs, which are negatively coupled to adenylyl cylclase (Pin and Duvoisin, 1995), with L-AP4 would enhance stress antinociception through a CB_1-dependent mechanism in the PAG. The CB_1 antagonist rimonabant was co-administered with the group III mGluR agonist L-AP4 to verify that group III mGluR activation regulates endocannabinoid-mediated SIA through a mechanism that requires CB_1 receptor activation. We also hypothesized that inhibition of group III mGluRs with UBP1112 would suppress endocannabinoid-mediated stress antinociception in the PAG. The group III mGluR antagonist UBP1112 was co-administered with the agonist to demonstrate pharmacological specificity. The active compounds were microinjected deliberately off-site to verify that the dorsolateral PAG mediated the effects of group III mGluR modulation on cannabinoid receptor activation. The present studies are the first to suggest a role for group III mGluRs in the PAG in controlling endocannabinoid-mediated analgesia *in vivo*.

3.3. Materials and Methods

3.3.1. Subjects and Surgical Procedures

Ninety-eight male adult Sprague-Dawley rats weighing approximately 275-325 g (Harlan, Indianapolis, IN) were used in these experiments. All procedures followed the guidelines set forth by the International Association for the Study of Pain (Zimmermann, 1983) on the ethical treatment of animals. The University of Georgia Animal Care and Use Committee also approved all procedures. Rats were individually housed in a temperature-controlled facility

and allowed food and water *ad libitum*. For surgical procedures, rats were anesthetized using isoflurane. The rat brain atlas of Paxinos and Watson (1998) was used to calculate stereotaxic coordinates for cannulae implantation. Stainless steel guide cannulae (24 g; Small Parts, Inc. Miami, FL) were implanted above the dlPAG so that the needle was inserted 2 mm beyond the guide cannulae (-5.35 mm DV, +1.6 mm AP, +0.67 mm LM) or deliberately off-site (miss by 1.5 mm, ventral) using the skull surface, lambda and the midline suture as the zero point. Cannulae were affixed to the skull with dental acrylic and stainless steel screws. Stainless steel insect pins were used to prevent occlusion of the cannulae before and after microinjections. Animals were allowed to recover five to seven days prior to testing.

3.3.2. Drugs and Chemicals

UBP1112 (a-Methyl-3-methyl-4-phosphonophenylglycine) and L-AP4 (L-(+)-2-Amino-4-phosphonobutyric acid) were purchased from Tocris Bioscience (Ellisville, MO). Rimonabant (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide) was a gift from NIDA. As described in our previous studies (Hohmann et al., 2005) drugs were dissolved in a vehicle containing 100% DMSO and delivered intracranially using a microinfusion pump (UltraMicroPump II,World Precision Instruments, Sarasota, FL). All animals received a single intracranial injection of 1 μl total volume (for drugs administered either alone or co-administered with the relevant antagonists) administered at a speed of 30 nl/sec. All control animals received a vehicle of 100% DMSO.

3.3.3. Behavioral Testing

The tailflick test (D'Amour, 1941) was used to behaviorally quantify stress antinociception. This test measures the latency for a rat to remove its tail from a radiant heat source (IITC Inc., Model 33A, Woodland Hills, CA). Prior to testing, animals were habituated to the testing room and restraining tubes for 15 minutes consecutively. Prior to each intracranial injection of drug or vehicle, stable baseline tailflick withdrawal responses were established 6 times at 2-minute intervals. A cut-off time of 10 seconds was issued to prevent tissue damage. Following each intracranial injection, tailflick latencies were measured to assess changes caused by pharmacological manipulations prior to footshock. These post injection latencies were assessed three times at 2-minute intervals. Intracranial injections were performed 5 minutes prior to exposure to brief continuous footshock for 3 min (3 mA, AC current), the stressor used to induce endocannabinoid-mediated stress antinociception, similar to our previously described methods (Connell et al., 2006; Hohmann et al., 2005; Suplita et al., 2005, 2006, 2008; Gregg et al., 2007). Following footshock administration, tailflick latencies were measured at 2-minute intervals over 60 minutes. The experimenter was blinded to the experimental condition in all studies.

To assess the role of group III mGluRs in the dlPAG in regulating stress antinociception, rats received intracranial injections of either the group III mGluR agonist L-AP4 (1 mM, 100 uM, or 100 nM) or vehicle prior to footshock administration. Separate groups received intracranial injections of either the group III mGluR antagonist UBP1112 (100 μM, 100 nM, or 10 nM) or vehicle prior to footshock administration. To determine whether the effects of L-AP4 were dependent on CB_1 receptor activation and to assess pharmacological specificity, separate groups received microinjections of L-AP4 (100 μM), L-AP4 co-administered with rimonabant

(o.1 μg), L-AP4 co-administered with UBP1112 (10nM), or vehicle. Rimonabant was microinjected at a dose that was previously shown to be inactive in the same behavioral assay (Gregg et al., 2007).

3.3.4. Histology

Rats were sacrificed using CO_2 asphyxiation. Brains were then removed and fixed in Zamboni's fixative, containing 4% paraformaldehyde, for 48 hours. Using a cryostat, coronal sections (40 μm) through the PAG were cut and mounted onto gelatin-subbed slides. Slides were stained with cresyl violet and cover-slipped using Permount. Injection sites were confirmed under a light microscope by an experimenter blinded to the experimental conditions.

3.3.5. Statistical Analysis

Data were analyzed by repeated measures Analysis of Variance (ANOVA). In all studies, the Greenhouse-Geisser correction was applied to the interaction term of all repeated factors. Post hoc comparisons were evaluated using Fisher's Protected Least-Significant Difference (LSD) test to correct for inflated alpha error, with $P < 0.05$ considered statistically significant. SPSS (version 17.0, SPSS Incorporated, Chicago, IL, USA) statistical software was employed for all analyses. Every two consecutive tail-flick latencies were averaged for each animal before (baseline) and after (post-shock) exposure to the environmental stressor to form two-point blocks. These two-point blocks were subjected to statistical analyses with drug treatment serving as the between subjects factor and time serving as the within subjects factor. Post-injection tail-flick latencies, determined immediately prior to footshock, were averaged into a single block for each animal and averaged across animals for each drug treatment. This measure was calculated

to facilitate statistical comparisons of drug-induced changes in the basal nociceptive threshold observed prior to footshock (post-injection tail-flick latency) with basal tail-flick latencies.

3.4. Results

3.4.1. Stress antinociception assessment

Baseline tailflick latencies assessed prior to administration of drug or vehicle and prior to exposure to footshock did not differ between groups in any study. Moreover, post-injection tailflick latencies measured immediately following administration of drug or vehicle and prior to exposure to footshock did not differ between groups. Thus, the present pharmacological manipulations only showed effects following exposure to footshock stress, under circumstances in which the endocannabinoid system is known to be activated. In all studies, exposure to footshock stress produced time-dependent changes in tailflick latencies ($P < 0.0001$), confirming the presence of stress antinociception.

3.4.2. Assessment of group III mGluR activation on stress antinociception

Microinjection of the group III mGluR agonist L-AP4 into the dlPAG increased the magnitude ($F_{3,26} = 5.131$, $P < 0.007$; Fig. 3.1.) and duration ($F_{45,390} = 2.070$, $P < 0.03$; Fig. 3.1.) of post-shock tailflick latencies compared to vehicle. Post hoc analysis showed that stress antinociception was greater in groups receiving the low (100 nM; $P < 0.03$) or the middle (100 μM; $P < 0.002$) dose of L-AP4 relative to vehicle control. Groups receiving the high dose (1 mM) of L-AP4 did not differ from groups receiving either vehicle ($P < 0.2$), the low ($P < 0.5$) or the middle ($P < 0.07$) doses of L-AP4.

3.4.3. Assessment of group III mGluR inhibition on stress antinociception

Microinjection of the group III mGluR inhibitor UBP1112 into the dlPAG decreased the magnitude ($F_{3,26} = 4.146$, $P < 0.02$; Fig. 3.2.) and the time-course ($F_{45,390} = 2.883$, $P < 0.003$; Fig. 3.2.) of stress antinociception induced in the tailflick test. Stress antinociception was greater in groups receiving the middle (100 nM; $P < 0.03$) or high (100 μM; $P < 0.004$) doses of UBP1112 relative to groups receiving the vehicle. The low dose of UBP1112 was insufficient to reliably alter stress antinociception (10 nM; $P < 0.07$).

3.4.4. Site specificity

Microinjections of the active doses of L-AP4 (100 μM) or UBP1112 (100 μM) deliberately off-site of the PAG did not alter SIA ($F_{2,15} = 0.400$; $P < 0.3$; Fig. 3.3.) relative to the vehicle controls.

3.4.5. Pharmacological specificity

The enhancement of SIA produced by the group III mGluR agonist L-AP4 was completely blocked by both the group III mGluR antagonist UBP1112 as well as the CB_1 receptor antagonist rimonabant ($F_{3,26} = 9.556$, $P < 0.0001$; Fig. 3.4). This blockade was also time-dependent ($F_{45,390} = 6.068$, $P < 0.0001$; Fig. 3.4). The dose of UBP1112 used here failed to alter stress antinociception relative to vehicle (10nM; $P < 0.07$; Fig. 3.2.). Similarly, the dose of rimonabant used here has been previously shown to be inactive in altering SIA in this model (Gregg et al., 2007). Stress antinociception was greater in animals receiving L-AP4 compared to

those receiving vehicle ($P < 0.0001$), rimonabant co-administered with L-AP4 ($P < 0.002$), or UBP1112 co-administered with L-AP4 ($P < 0.0001$).

3.5. Discussion

The present study suggests a role for activation of group III metabotropic glutamate receptors in controlling the mobilization of endocannabinoids in the dlPAG under physiological conditions. Manipulations of group III metabotropic glutamate receptors in the dlPAG bi-directionally altered endocannabinoid-mediated stress antinociception, possibly by controlling endocannabinoid mobilization. Intracranial dlPAG injections of the group III mGluR agonist L-AP4 enhanced antinociception following exposure to an environmental stressor. Moreover, this enhancement of antinociception required a CB_1-dependent mechanism. By contrast, intracranial dlPAG injections of the group III mGluR antagonist UBP1112 suppressed endocannabinoid-mediated stress-induced analgesia. The group III mGluR antagonist UBP1112, administered at a dose that was insufficient to reverse SIA, also effectively blocked the L-AP4-induced enhancement of stress antinociception. This activation of group III mGluRs controls stress-induced analgesia through a mechanism that requires group III mGluRs as well as CB_1 receptor activation.

Activation of cannabinoid CB_1 receptors in the PAG produces antinociception (Finn et al. 2003; Lichtman and Martin, 1996; Martin et al. 1995) and inhibits GABAergic synaptic transmission (Vaughan et al., 2000), to control the descending pain pathway. Previous work from our laboratories has documented a role of endocannabinoids in the PAG in SIA (Gregg et al., 2007; Hohmann et al., 2005; Nyilas et al., 2009; Suplita et al., 2005). 2-AG, but not anandamide

levels, in dorsal midbrain fragments are highly correlated with endocannabinoid-mediated stress antinociception (Hohmann et al., 2005; Hohmann and Suplita, 2006), an effect that is dependent upon activation of both cannabinoid CB_1 receptors and type 5 mGluRs (Gregg et al., 2007).

Our results suggest that activation of group III mGluRs control the mobilization of endocannabinoids that induce SIA. Activation of presynaptic group III mGluRs which are negatively coupled to adenylyl cyclase (Pin and Duvoisin, 1995), decreases the probability of neurotransmitter release from GABAergic terminals in the PAG, an effect previously demonstrated for CB_1 receptors (Drew and Vaughan, 2004; Vaughan et al., 2000). Previous studies have also shown that group III mGluRs in the PAG inhibit GABAergic release and facilitate glutamatergic release (Marabese et al., 2005). It is possible that the facilitation of glutamatergic release observed following activation of group III mGluRs is a direct consequence of inhibition of GABA release (Drew and Vaughan, 2004). Our studies suggest that activation of group III mGluRs enhances stress antinociception through an endocannabinoid-mediated mechanism. These observations are consistent with research demonstrating a link between the antinociceptive effects of cannabinoid receptor activation in the PAG and group III mGluRs (Palazzo et al., 2001). Moreover, our findings suggest that mobilization of 2-AG is a likely mechanism responsible for endocannabinoid-mediated analgesia in the PAG. These claims are supported by previous research suggesting that group III mGluRs regulate endocannabinoid mobilization in *in vitro* electrophysiological studies (Reigel and Lupica, 2004). These claims are also supported by the strong correlation observed between 2-AG, but not anandamide, mobilization in the PAG and endocannabinoid-mediated SIA (Gregg et al., 2007; Hohmann et al., 2005; Hohmann and Suplita, 2006).

In our study, activation of group III mGluRs in the dlPAG enhanced endocannabinoid-mediated stress antinociception through a CB_1-dependent mechanism. A possible explanation for this finding is that activation of group III mGluRs stimulated the mobilization of an endocannabinoid mediator to produce stress antinociception. It is possible that decreased GABA release and increased glutamate release produced by group III mGluR activation further augments mGluR5-dependent 2-AG formation. Some support for this hypothesis is that lower doses of L-AP4, which may be more selective for autoreceptors, were more effective than higher doses of L-AP4 in enhancing endocannabinoid-mediated SIA. Future biochemical studies will test this hypothesis directly by determining whether this endocannabinoid mediator is indeed 2-AG or requires mGluR5 activation. The L-AP4-induced enhancement of stress antinociception is consistent with the previously described time-course of post-stress 2-AG accumulation in the PAG (Hohmann et al., 2005). Moreover, microinjection of L-AP4 did not alter basal nociceptive thresholds measured prior to footshock, suggesting that L-AP4-induced endocannabinoid mobilization in the dPAG is also likely to occur in an activity-dependent fashion. Our observations concur with previous findings that show that activation of group III metabotropic glutamate receptors in the PAG produces antinociception (Marabese et al. 2007).

In our study, microinjection into the dlPAG of a pharmacological inhibitor of group III mGluRs, UBP1112, suppressed stress antinociception immediately following termination of the stressor. It is noteworthy that the same group III mGluR antagonist also blocked the enhancement of stress antinociception produced by the group III mGluR agonist L-AP4. These observations confirm the pharmacological specificity of our manipulations. The most striking observation of our studies was that the CB_1 receptor antagonist rimonabant completely blocked the enhancement of endocannabinoid-mediated SIA produced by L-AP4. Importantly,

microinjection of rimonabant blocked the enhancement of stress antinociception induced by activation of group III mGluRs at a dose that was not sufficient to suppress stress antinociception. Considering that rimonabant does not bind to group III mGluRs, this effect must be indirect and dependent upon endocannabinoid mobilization. Our findings indicate that activation of presynaptic group III mGluRs enhances stress antinociception through a mechanism that requires presynaptic CB_1 receptor activation.

In conclusion, our findings demonstrate that activation of group III mGluRs in the PAG suppresses nociceptive responding through a mechanism that requires cannabinoid CB_1 receptors. Moreover, inhibition of group III mGluRs locally in the dlPAG selectively suppresses endocannabinoid-mediated stress-induced analgesia. A parsimonious explanation for our findings is that activation of group III mGluRs in the dlPAG promotes endocannabinoid signaling to produce SIA through a CB_1-receptor dependent pathway. Biochemical and anatomical studies are required to determine whether 2-AG is the endocannabinoid mediator manipulated herein and to identify the subtype of group III mGluRs are responsible for the effects observed herein.

3.6. References

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di Marzo V, Doherty P. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163: 463-8.

Bradley SR, Levey AI, Hersch SM, Conn PJ. 1996. Immunocyctochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype-specific antibodies. J Neurosci 16: 2044-56.

Cartmell J, Schoepp DD. 2000. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75 (3): 889-907.

Chevaleyre V, Castillo PE. 2003. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38: 461-72.

Conn PJ, Pin JP. 1997. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37: 205-37.

Connell K, Bolton N, Olsen D, Piomelli D, Hohmann AG. 2006. Role of the basolateral nucleus of the amygdala in endocannabinoid-mediated stress-induced analgesia. Neurosci Lett 397: 180-4.

D'Amour FE, Smith DL. 1941. A Method for Determining Loss of Pain Sensation. J Pharmacol Exp Ther 72: 74-9.

Drew GM, Vaughan CW. 2004. Multiple metabotropic glutamate receptor subtypes modulate GABAergic neurotransmission in rat periaqueductal grey neurons in vitro. Neuropharmacol 46: 927-34.

Drew GM, Mitchell VA, Vaughan CW. 2008. Glutamate spillover modulates GABAergic

synaptic transmission in the rat midbrain periaqueductal grey via metabotropic glutamate receptors and endocannabinoid signaling. J Neurosci 28 (4): 808-15.

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma LA, Mitchell RL. 1995. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48: 443-50.

Finn DP, Jhaveri MD, Beckett SR, Roe CH, Kendall DA, Marsden CA, Chapman V. 2003. Effects of direct periaqueductal grey administration of a cannabinoid receptor agonist on nociceptive and aversive responses in rats. Neuropharmacol 45: 594-604.

Gerdeman G, Lovinger DM. 2001. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85: 468-71.

Gregg LC, Bolton NM, Moise AM, Suplita II RL, Hohmann AG. November 2007. Group I Metabotropic Glutamate Receptors Regulates Endocannabinoid-mediated Stress-induced Analgesia Through the Phospholipase C/Diacylglycerol Lipase Pathway. Poster session presented at the meeting of *Society for Neuroscience,* San Diego, CA.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11: 563-83.

Hohmann AG, Suplita RL 2nd, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. 2005. An endocannabinoid mechanism for stress-induced analgesia. Nature 435: 1108-12.

Hohmann AG, Suplita RL 2nd. 2006. Endocannabinoid mechanisms of pain modulation. Aaps J 8 (4): E693-708.

Howlett AC, Johnson MR, Melvin LS, and Milne GM. 1988. Nonclassical cannabinoid

analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. Mol Pharmacol 33: 297-302.

Jung M, Calassi R, Rinaldi-Carmona M, Chardenot P, Le Fur G, Soubrie P, Oury-Donat, F. 1997. Characterization of CB1 receptors on rat neuronal cell cultures: binding and functional studies using the selective receptor agonist SR141716A. J Neurochem 68: 402-9.

Jung KM, Astarita G, Zhu C, Wallace M, Mackie K, Piomelli D. 2007. A key role for diacylglycerol lipase-alpha in metabotropic glutamate receptor-dependent endocannabinoid mobilization. Mol Pharmacol 72 (3): 612-21.

Kano M, Ohno-Shasaku T, Hashimotodani Y, Uchigashmima M, Watanabe M. 2009. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89: 309-80.

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF. 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19: 4544-58.

Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF. 2006. Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci 26: 5628-37.

Lichtman AH, Cook SA, Martin BR. 1996. Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J Pharmacol Exp Ther 276: 585-93.

Maione S, Bisogno T, de Novellis V, Palazzo E, Cristino L, Valenti M, Petrosino S, Guglielmotti V, Rossi F, Di Marzo V. 2006. Elevation of endocannabinoid levels in the ventrolateral periaqueductal grey through inhibition of fatty acid amide hydrolase affects descending

nociceptive pathways via both cannabinoid receptor type 1 and transient receptor potential vanilloid type-1 receptors. J Pharmacol Exp Ther 316: 969- 82.

Marabese I, de Novellis V, Palazzo E, Mariani L, Siniscalco D, Rodella L, Rossi F, Maione S. 2005. Differential roles of mGlu8 receptors in the regulation of glutamate and γ-aminobutyric acid release at periaqueductal grey level. Neuropharm 49: 157-166.

Marabese, I. Rossi F, Palazzo E, de Novellis V, Starowicz K, Cristino L, Vita D, Gatta L, Guida F, Di Marzo V, Rossi F, Maione S. 2007. Periaqueductal gray metabotropic glutamate receptor subtype 7 and 8 mediate opposite effects on amino acid release, rostral ventromedial medulla cell activities, and thermal nociception. J Neurophysiol 98:43-53.

Martin WJ, Patrick SL, Coffin PO, Tsou K, Walker JM. 1995. An examination of the central sites of action of cannabinoid-induced antinociception in the rat. Life Sci 56: 2103-9.

Nyilas R, Gregg LC, Mackie K, Watanabe M, Zimmer A, Hohmann AG, Katona I. 2009. Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia. Eur J Neurosci 29 (10): 1964-78.

Palazzo E, Marabese I, de Novellis V, Oliva P, Rossi F, Berrino L, Rossi F, Maione S. 2001. Metabotropic and NMDA glutamate receptors participate in the cannabinoid-induced antinociception. Neuropharmacol 40: 319-26.

Paxinos G, Watson C. 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pin JP, Duvoisin R. 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacol 34: 1-26.

Piomelli D. 2003. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4: 873-84.

Riegel AC, Lupica CR. 2004. Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. J Neurosci 24: 11070-8.

Schoepp DD. 2001. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther 299: 12-20.

Stella N, Schweitzer P, Piomelli D. 1997. A second endogenous cannabinoid that modulates long-term potentiation. Nature 388: 773-8.

Suplita RL 2nd, Farthing JN, Gutierrez T, Hohmann AG. 2005. Inhibition of fatty-acid amide hydrolase enhances cannabinoid stress-induced analgesia: sites of action in the dorsolateral periaqueductal gray and rostral ventromedial medulla. Neuropharmacol 49: 1201-9.

Suplita RL 2nd, Gutierrez T, Fegley D, Piomelli D, Hohmann AG. 2006. Endocannabinoids at the spinal level regulate, but do not mediate, nonopioid stress- induced analgesia. Neuropharmacol 50: 372-9.

Suplita RL 2nd, Eisenstein SA, Neely MH, Moise AM, Hohmann AG. 2008. Cross-sensitization and cross-tolerance between exogenous cannabinoid antinociception and endocannabinoid-mediated stress-induced analgesia. Neuropharmacol 54 (1): 161-71.

Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, Sakinura K, Kano M. 2010. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. Neuron 65: 320-7.

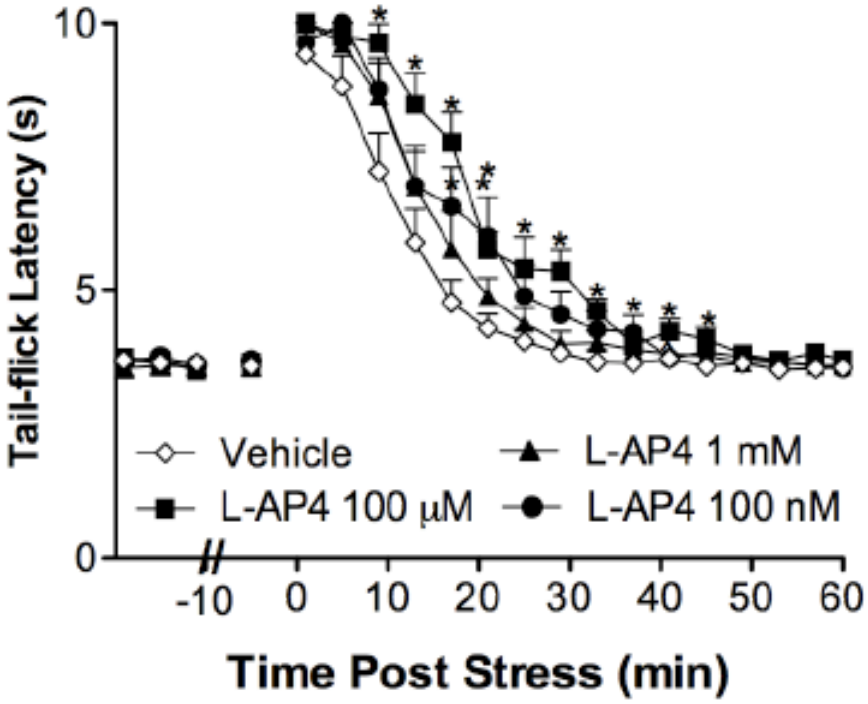
Vaughan CW, Connor M, Bagley EE, Christie MJ. 2000. Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. Mol

Pharmacol 57: 288-95.

Wilson RI, Nicoll RA. 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410: 588-92.

Zimmermann M. 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16: 109-10.

A


10
5
0
Tail-flick Latency (s)
Vehicle
L-AP4 1 mM
L-AP4 100 μM
L-AP4 100 nM
-10
0
10
20
30
40
50
60
Time Post Stress (min)

B

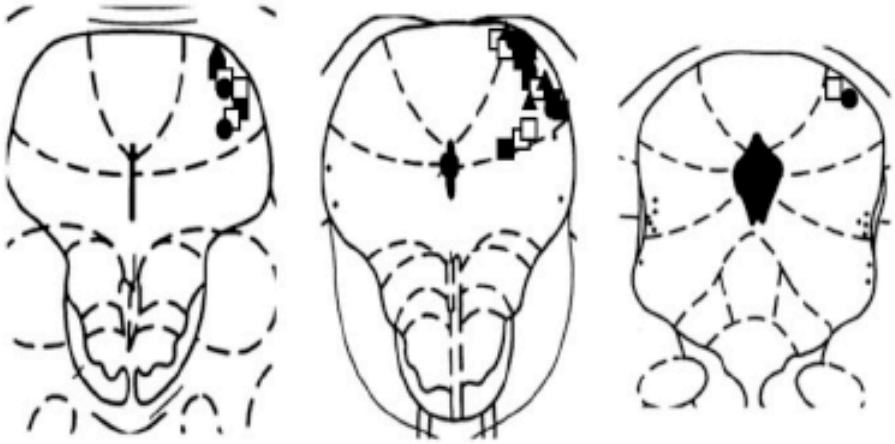
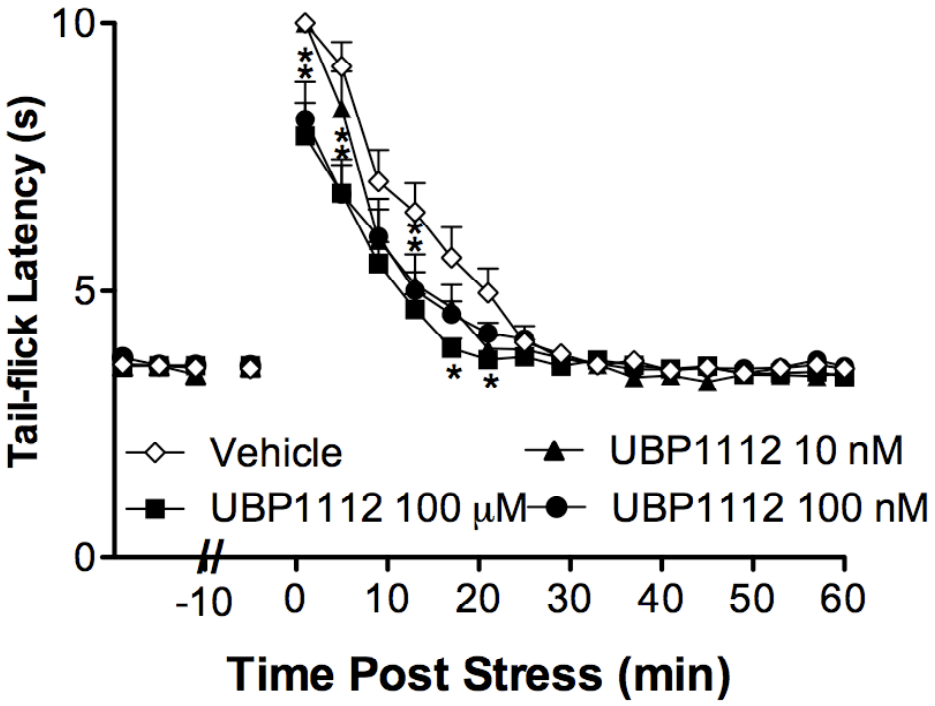


Figure 3.1. (A) Microinjection of the group III mGluR agonist L-AP4 (100 nM or 100μM) into the dlPAG produces a concentration-dependent enhancement of endocannabinoid-mediated stress antinociception. (B) Microinjection sites into the dlPAG for groups receiving L-AP4 1 mM (▲), 100 μM (■), 100 nM (●), or DMSO (□). Data are Mean + S.E.M. **P* < 0.05 versus control (ANOVA, Fisher's PLSD test; N = 6-10 per group).

A


10
5
0
Tail-flick Latency (s)
Vehicle
UBP1112 10 nM
UBP1112 100 μM
UBP1112 100 nM
-10
0
10
20
30
40
50
60
Time Post Stress (min)

B

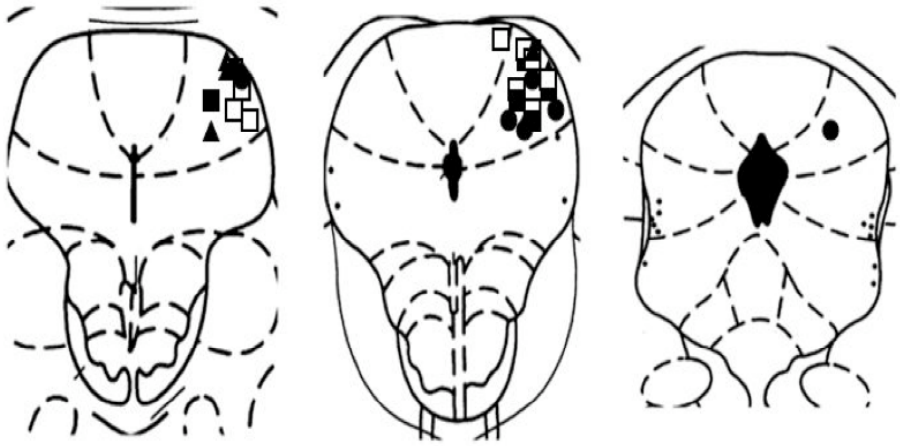
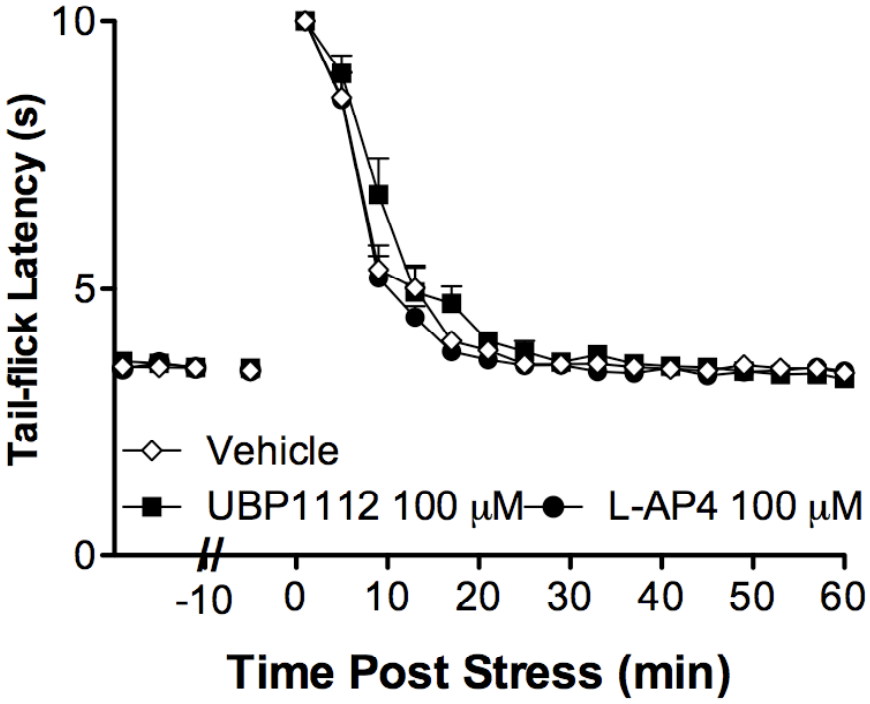


Figure 3.2. (A) Microinjection of the group III mGluR inhibitor UBP1112 (100nM or 100μM) into the dlPAG produces a dose-dependent suppression of endocannabinoid-mediated stress antinociception. (B) Microinjection sites in the dlPAG for groups receiving UBP1112 100 μM (■), 100 nM (●), 10 nM (▲), or DMSO (□). Data are Mean + S.E.M. **P* < 0.05 versus control (ANOVA, Fisher's PLSD test; N = 6-10 per group).

A


10
5
0
Tail-flick Latency (s)
Vehicle
UBP1112 100 μM
L-AP4 100 μM
-10
0
10
20
30
40
50
60
Time Post Stress (min)

B

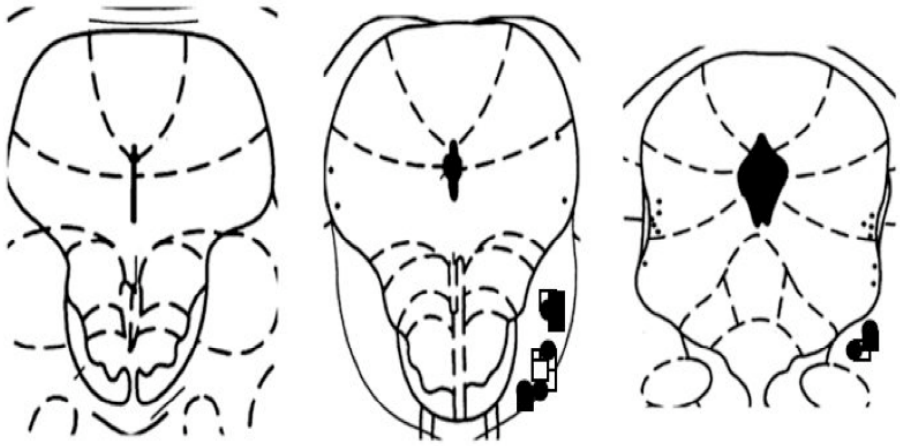
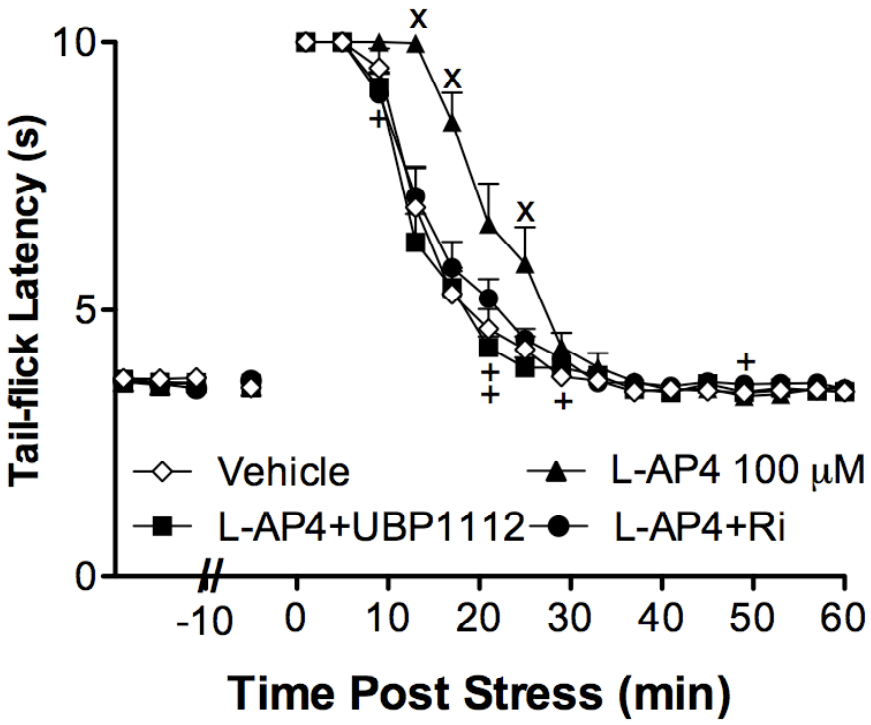


Figure 3.3. (A) Off-site injection of neither L-AP4 (100 μM) nor UBP1112 (100 μM) altered stress antinociception relative to vehicle controls. (B) Sites of microinjections of 100 μM UBP1112 (■), L-AP4 (●), or DMSO (□). Data are Mean + S.E.M. (ANOVA, Fisher's PLSD test; N = 6 per group).

A


Tail-flick Latency (s)
10
5
0
-10
0
10
20
30
40
50
60
Time Post Stress (min)
Vehicle
L-AP4 100 μM
L-AP4+UBP1112
L-AP4+Ri

B

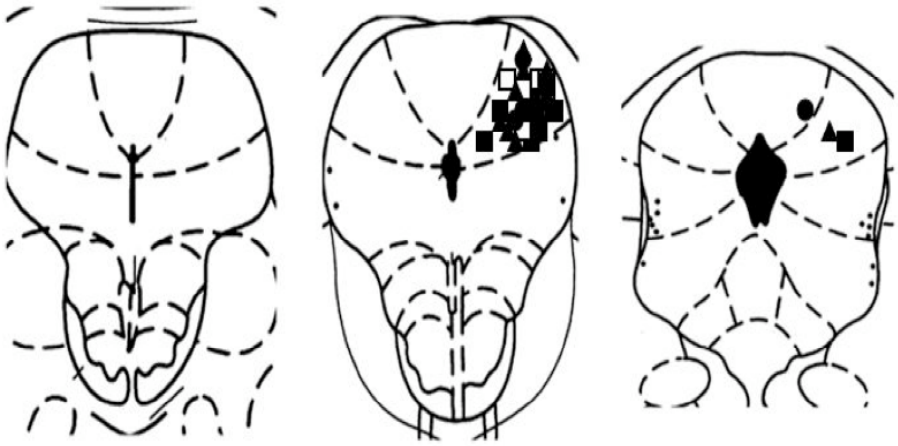


Figure 3.4. (A) Microinjection of the group III mGluR agonist L-AP4 (100μM) enhances endocannabinoid-mediated stress antinociception through a mechanism that requires group III mGluR and CB_1 receptor activation. The CB_1 antagonist rimonabant (0.1 μg) and the group III mGluR antagonist UBP1112 (10 nM) blocked the L-AP4 induced enhancement of stress antinociception. (B) Microinjection sites in the dlPAG for groups receiving L-AP4 + UBP1112 (■), L-AP4 (▲), L-AP4 + Rimonabant (●), or DMSO (□). Data are Mean + S.E.M. $^{X}P < 0.05$ versus all conditions, $^{+}P < 0.05$ versus agonist alone (ANOVA, Fisher's PLSD test; N = 7-8 per group).

CHAPTER 4: DISCUSSION

Much research has been conducted to evaluate the role of endocannabinoids in pain suppression. Understanding the mechanisms of endocannabinoid mobilization and synthesis are key components in uncovering novel therapeutic agents. Considering that the endocannabinoid 2-AG is mobilized under the phenomenon known as stress-induced analgesia, this model is an important research tool for this investigation. Metabotropic glutamate receptors have also been implicated in endocannabinoid mobilization to produce antinociception under this model. Both group I and group III mGluRs have been found to mediate nociceptive responses in a cannabinoid receptor-dependent manner.

The present studies were conducted to evaluate the role of group III metabotropic glutamate receptors in regulating endocannabinoid-mediated stress-induced analgesia in the PAG by performing site-specific pharmacological manipulations. Particularly, pharmacological activation of group III mGluRs with L-AP4 in the dlPAG enhanced stress antinociception and this effect was blocked with both a group III mGluR antagonist and a CB_1 receptor antagonist. Pharmacological inhibition of group III mGluRs with UBP1112 also suppressed stress antinociception in the dlPAG in this study. Antinociception was evaluated using the tailflick model following exposure to an environmental stressor.

As illustrated in this document, endocannabinoid-mediated stress-induced analgesia was enhanced by the group III mGluR agonist L-AP4 and suppressed by the group III mGluR antagonist UBP1112 following microinjection into the dlPAG. CB_1 receptor activation was

necessary for this enhancement of antinociception following exposure to the footshock stressor, suggesting endocannabinoid specificity, possibly attributable to elevated 2-AG levels. Pharmacological specificity was determined by blocking the agonist-induced enhancement of SIA with the group III mGluR antagonist. Off-site injections of each active compound also failed to alter SIA, showing specificity to the dlPAG.

In summary, pharmacologically activating group III mGluRs in the PAG is efficacious in alleviating nociceptive responses following exposure to a stressor. Given that antinociceptive effects occur coincidentally with the time-course of 2-AG accumulation in the PAG, it is possible that group III mGluR activation may induce antinociception by facilitating mobilization of the endocannabinoid 2-AG. The antinociceptive effects induced by activation of group III mGluRs were blocked by the CB_1 antagonist rimonabant, adding further confirmation to the endocannabinoid mobilization hypothesis. Pharmacologically elevating endocannabinoids in the central nervous system may prove to be useful in uncovering novel therapeutic agents with minimal side effects.