Analgesic and Reinforcing Properties of ^{9}-THC-Hemisuccinate in Adjuvant-Arthritic Rats

Susan L. Broom
Kenneth J. Sufka
Mahmoud A. ElSohly
Samir A. Ross

SUMMARY. The use of ^{9}-THC hemisuccinate (HS) in a suppository formulation is an attempt to develop a cannabinoid possessing possible therapeutic effects with a minimal side effect profile. The purpose of this study was to investigate the antinociceptive and reinforcing effects of rectally administered ^{9}-THC-HS in rats. Tests were conducted in two groups of animals: Complete Freund's adjuvant-inflamed animals (CFA)

Susan L. Broom, MA, is Psychology Graduate Research Assistant, University of Mississippi, University, MS 38677.

Kenneth J. Sufka, PhD, is Associate Professor of Psychology and Pharmacology, University of Mississippi, University, MS 38677.

Mahmoud A. ElSohly, PhD, is Professor of Pharmaceutics, Research Professor, National Center for Natural Products Research, University of Mississippi, University, MS 38677, and is President, ElSohly Laboratories, Oxford, MS 38655.

Samir A. Ross, PhD, is Associate Professor of Pharmacognosy, Associate Research Professor, National Center for Natural Products Research, University of Mississippi, University, MS 38677.

Address correspondence to: Kenneth J. Sufka, PhD, Associate Professor of Psychology and Pharmacology, Peabody Building, University of Mississippi, University, MS 38677 (E-mail: pysufka@olemiss.edu).

[Haworth co-indexing entry note]: "Analgesic and Reinforcing Properties of ^{9}-THC-Hemisuccinate in Adjuvant-Arthritic Rats." Broom, Susan L. et al. Co-published simultaneously in *Journal of Cannabis Therapeutics* (The Haworth Integrative Healing Press, an imprint of The Haworth Press, Inc.) Vol. 1, No. 3/4, 2001, pp. 171-182; and: *Cannabis Therapeutics in HIV/AIDS* (ed: Ethan Russo) The Haworth Integrative Healing Press, an imprint of The Haworth Press, Inc., 2001, pp. 171-182. Single or multiple copies of this article are available for a fee from The Haworth Document Delivery Service [1-800-342-9678, 9:00 a.m. - 5:00 p.m. (EST). E-mail address: getinfo@haworthpressinc.com].

and non-inflamed controls. A hotplate test was administered to index hyperalgesia and possible analgesic effects of 9-THC-HS on thermal nociception. CFA animals demonstrated shorter latencies than non-inflamed animals. The highest dose of 9-THC-HS produced longer hotplate latencies. Additionally, the reinforcing properties of 9-THC-HS were evaluated using the Conditioned Place Preference (CPP) paradigm. 9-THC-HS produced an increase in preference scores in non-inflamed animals (positive reinforcement), but did not affect preference scores in CFA animals. These data suggest that 9-THC-HS has therapeutic potential and is unlikely to possess an abuse liability when used in the context of chronic pain.

KEYWORDS. 9-THC, adjuvant-inflamed, rat, hotplate, conditioned place preference

INTRODUCTION

The role of cannabinoids (CB) in pain modulation is well documented (Fuentes et al. 1999). Administration of anandamide, 9-tetrahydrocannabinoid (9-THC), and various selective CB receptor agonists, have shown antinociceptive effects in a variety of acute (Buxbaum 1972; Welch and Stevens 1992) and chronic (Sofia et al. 1973; Smith et al. 1998) models of nociception (for review see Pertwee 2001). These antinociceptive effects are mediated by CB1 receptors located at spinal (Yaksh 1981; Lichtman and Martin 1991; Welsh and Stevens 1992) and supraspinal sites (Lichtman and Martin 1991; Martin et al. 1993) as well as CB1 (Richardson et al. 1998) and CB2 receptors (Jagger et al. 1998) located in peripheral tissues (for review see Pertwee 2001). Although numerous studies suggest otherwise (Onaivi et al. 1990; McGregor et al. 1996; Sanudo-Pena et al. 1997; Tzschentke 1998), several experiments implicate cannabinoid systems in reward. For example, 9-THC is self administered in humans (Chait and Burke 1994) and squirrel monkeys (Tanda et al. 2000), lowers intracranial self-stimulation thresholds in rats (Gardner et al. 1988) and produces place preference in rats (Lepore et al. 1995). 9-THC has been shown to increase firing of dopamine neurons in the nucleus accumbens (Gessa et al. 1998), as well as increase dopamine levels in the shell of the nucleus accumbens (Tanda et al. 1997). Collectively, these studies suggest that CB reinforcement is likely mediated through the same

mesolimbic dopaminergic systems involved in opioid and psychostimulant reward (Koob and Bloom 1988). While one literature clearly suggests CB receptors present a viable target for analgesic drugs (for review see Pertwee 2001), a second literature suggests these putative analgesic compounds are likely to possess an abuse liability (Gessa et al. 1998).

Recent research indicates that the use of certain analgesics (e.g., morphine) that possess several liabilities (tolerance, dependence, etc.) may not be as controversial as previously suggested. Rats given repeated administration of morphine in the context of formalin-induced inflammatory nociception displayed less tolerance to morphine (Vaccarino et al. 1993; Bardin et al. 2000) and less severe withdrawal symptoms in response to a naloxone challenge (Vaccarino and Couret 1993; Bardin et al. 2000). In addition, rats given repeated administration of morphine in the context of chronic inflammatory pain induced by complete Freund's adjuvant (CFA) developed tolerance at slower rates and showed lower incidences of naloxone precipitated withdrawal symptoms (Lerida et al. 1987). Similar findings on tolerance and dependence have been observed clinically with opioid therapies (for review see Melzak 1991). Long-term use of codeine and oxycodone for chronic rheumatic conditions significantly reduced pain without requiring increased dosing except in cases where a worsening of the painful condition existed (Ytterberg et al. 1998). Collectively, these data suggest the liabilities of analgesics may be greatly reduced when these compounds are used in the context of pain management.

The medical use of cannabis for the treatment of chronic pain remains highly controversial. However, this controversy may be obviated by the development of the pro-drug ^{9}-THC hemisuccinate (^{9}-THC-HS) and its formulation as a suppository (Mattes et al. 1993). This formulation is but one solution to the undesirable inhalation route of cannabis administration. Moreover, rectal administration of ^{9}-THC-HS has been shown to produce a pharmacokinetic profile that is highly desirable for putative therapeutic agents. First, blood levels of ^{9}-THC and other CB metabolites do not show the rapid elevation typical of the inhalation route, which is commonly associated with euphoric effects. Second, blood levels of these constituents remain relatively stable for up to 6-8 hrs post administration (for review, see Walker et al. 1999). These pharmacokinetic factors, along with the notion that context is important in drug responses, suggest that ^{9}-THC-HS may not possess the reinforcing properties when administered in the context of chronic pain. To explore this possibility, the present study examined the antinociceptive as well as the rewarding properties of ^{9}-THC-HS in the complete Freund's adjuvant (CFA) model of chronic inflammatory pain.

METHOD

Place Preference Test

The conditioned place preference (CPP) paradigm (for review see Carr et al. 1989) is a procedure that is commonly used to evaluate the reinforcing and aversive properties of drugs (van der Kooy 1987). This paradigm is based on traditional learning principles and involves the pairing of a drug state with environments having distinctive stimuli (i.e., place). Following several drug-place pairings, an animal's preference is ascertained by examining approach responses to and maintenance of contact with the drug-paired environment. The CPP paradigm has become a frequently used method in behavioral pharmacology for examination of the positively reinforcing properties of abused drugs.

Male Lewis strain rats (100-125 g; Harlan, Indianapolis, IN) were housed in suspended steel cages (360 cm^2), maintained under a 12 hour light/dark cycle in a temperature controlled vivarium (22 1°C). Food and water were available *ad lib*. After a one-week acclimation period, animals received one week of handling exposure to reduce experimenter-related stress. Research protocols were approved by the Institutional Animal Care and Use Committee and were conducted under the ethical guidelines of the International Association for the Study of Pain (Zimmerman 1983).

The groups in this study formed a 2 × 3 factorial design that combined 2 levels of inflammation (CFA inflamed vs. non-inflamed) with 3 levels of drug (0.0, 2.5, or 5.0 mg/kg ^{9}-THC-HS). Sample sizes were n = 7 per group. Persistent unilateral inflammation was produced by injections of 0.1 ml of complete Freund's adjuvant (CFA: Sigma; St. Louis, MO) into the left hind paw (Butler et al. 1992). This model of arthritis produces long-lasting inflammation leading to hyperalgesia and joint destruction and bony proliferation of the metatarsal, tarsal, and ankle regions. Non-inflamed control rats did not receive this explicit manipulation. CFA was administered several hours after acclimation to the place preference apparatus.

^{9}-THC-HS (5 mg/kg/ml) or vehicle (Wacbee W) was administered immediately before the start of each conditioning trial (see below). Compounds were melted (45°C) prior to rectal administration to obviate the possibility of the animal evacuating a solid suppository. Pilot data examining plasma levels at various time points of rectal administration of melted ^{9}-THC-HS show that peak plasma ^{9}-THC levels were detected at 15 min post administration (approximately 110 ng/ml). This was followed by a gradual decline in plasma ^{9}-THC levels at 30 min (40 ng/ml); relatively stable ^{9}-THC levels were detected from between 60-360 min post-administration (15-25 ng/ml).

Six T-shaped place preference chambers were used in this study (see Sufka and Roach 1996 for details). The place preference procedure involved three phases consisting of one apparatus acclimation trial, eight drug/vehicle conditioning trials, and six discrete choice trials. The acclimation trial allowed animal access to the entire apparatus for a 15 min period one day before conditioning trials. The eight conditioning trials (1 per day of 60 minutes) consisted of counter-balanced, alternate-day pairings of drug (0.0, 2.5 and 5.0 mg/kg/ml) with the white compartment and vehicle (0.0) with the black compartment for a total of four pairings each. Drug preference was determined by the animal's choice behavior (i.e., first entry) to the drug-paired (white) vs. vehicle-paired (black) compartment on six discrete preference trials conducted two per day over a three day period and were conducted under drug-free states. From these choice measures, a single preference score was derived using the following formula: Preference Score = number of white compartment entries/6 (Sufka 1994).

Hotplate Test

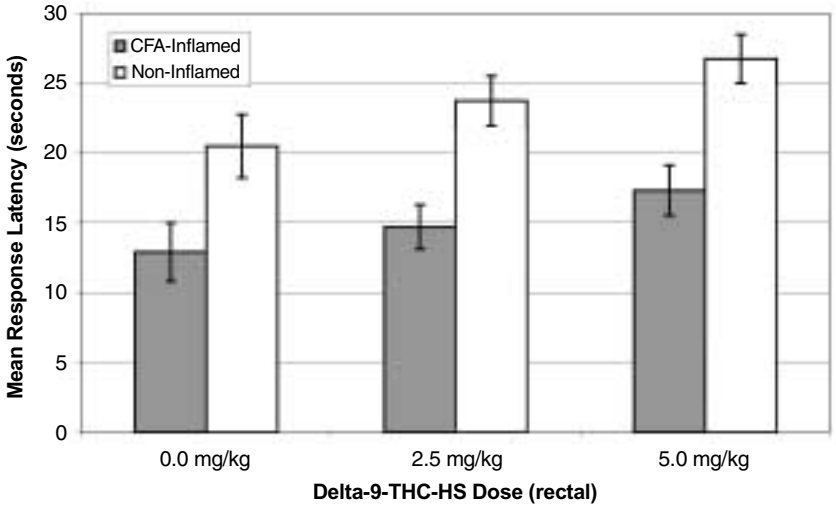
Tests of thermal nociception were conducted on the fourth day of drug exposure (Day 7 or 8 of conditioning trials). Rats were removed from the conditioning apparatus and placed on a hotplate apparatus with a surface temperature maintained at 50°C. Latency to lick a hind-paw served as the dependent measure. Animals that failed to exhibit a lick response in 30 seconds were removed from the hotplate and assigned a latency score of 30 seconds. Animals were euthanized at the conclusion of the experiment.

RESULTS

^{9}-THC-HS effects on thermal nociception are summarized in Figure 1.

Adjuvant-inflamed rats exhibited shorter response latencies than non-inflamed rats under the drug vehicle condition (i.e., hyperalgesia). In general, ^{9}-THC-HS increased latency scores for both groups compared to respective controls. A 2-way ANOVA revealed a significant main effect of inflammation $F(1,36) = 31.44$, $p = 0.0001$ and a significant drug effect $F(2,36) = 3.980$, $p = 0.03$. The inflammation $\times$ drug interaction term was not significant. Post hoc comparisons using Fisher's PLSD detected significantly shorter latency scores for CFA-inflamed animals compared to non-inflamed animals under vehicle condition ($p < 0.05$) (hyperalgesia). Further analyses revealed a significant increase in latency scores for the 5.0 mg/kg non-inflamed group compared to

FIGURE 1. Mean hotplate latency (SEM) as a function of ^{9}-THC-HS dose for CFA-inflamed and non-inflamed animals.



HOTPLATE DATA

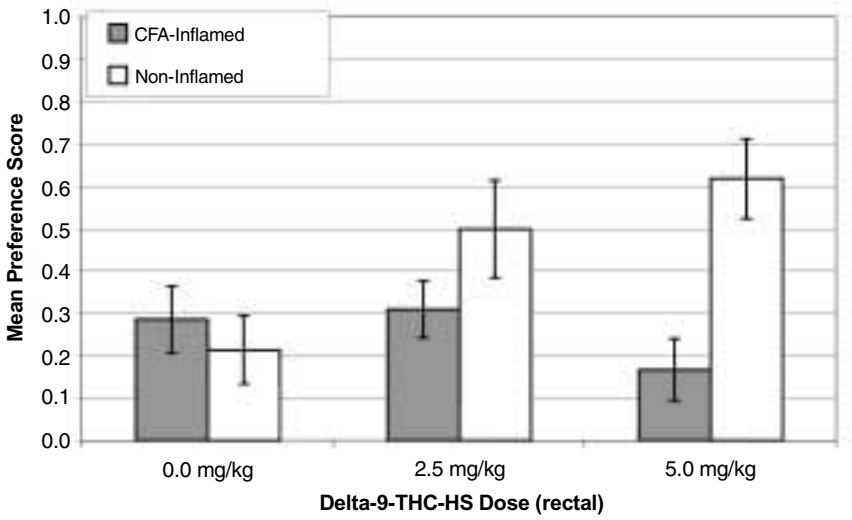
	Inflamed	**Non-Inflamed**
	Mean	Mean
0.0 mg/kg	12.90	20.49
2.5 mg/kg	14.71	23.74
5.0 mg/kg	17.32	26.77
	SE	SE
0.0 mg/kg	2.11	2.27
2.5 mg/kg	1.59	1.78
5.0 mg/kg	1.80	1.75

non-inflamed controls ($p < 0.05$). Although there was a trend in CFA-inflamed latency scores, no significant differences were observed. No further analyses were conducted on these data.

^{9}-THC-HS effects on preference scores for CFA-inflamed and non-inflamed animals are summarized in Figure 2.

Both groups showed a black compartment bias under the no drug condition. In general, ^{9}-THC-HS produced a dose dependent increase in preference scores in non-inflamed animals. However, this pattern was not seen in CFA-inflamed animals. Consistent with these observations, a 2-way ANOVA revealed a significant main effect for inflammation, $F(1,36) = 7.353$, $p = 0.01$, no

FIGURE 2. Mean preference scores (SEM) as a function of ^{9}-THC-HS dose for CFA-inflamed and non-inflamed animals.



CPP DATA

	Inflamed	**Non-Inflamed**
	Mean	Mean
0.0 mg/kg	0.29	0.21
2.5 mg/kg	0.31	0.50
5.0 mg/kg	0.17	0.62
	SE	SE
0.0 mg/kg	0.08	0.08
2.5 mg/kg	0.07	0.12
5.0 mg/kg	0.07	0.09

main effect for drug, and a significant inflammation × drug interaction, $F(2,36) = 4.634$, $p < 0.02$. Post hoc comparisons using Fisher's PLSD established no significant difference in baseline preference scores, indicating that the bias was present regardless of inflammation condition. Further post hoc analyses revealed a significant increase in preference scores for non-inflamed animals at the 2.5 and 5.0 mg/kg doses compared to vehicle controls ($p < 0.05$), indicative of cannabinoid positive reinforcement in these animals. However, for adjuvant-inflamed groups, preference scores were unaffected by ^{9}-THC-HS, suggesting that ^{9}-THC-HS lacks positively reinforcing properties in the context of persistent inflammatory pain.

DISCUSSION

While CB receptors present a viable target for pain management, the therapeutic use of cannabinoids remains controversial. However, an emerging literature suggests that analgesic drug liabilities can be diminished when these compounds are utilized in the context of pain management. These observations, along with the highly desirable pharmacokinetic profile of the suppository formulation of ^{9}-THC-HS, suggest that certain cannabinoids may provide for pain relief in some settings with little addictive liabilities. The purpose of the present research was to examine the putative antinociceptive and reinforcing properties of ^{9}-THC-HS in the rat adjuvant arthritis model of chronic inflammatory pain.

In the present study, adjuvant arthritic animals displayed shorter response latencies to a noxious thermal stimulus than non-inflamed controls. This hyperalgesic effect is consistent with reports of long lasting changes in nociceptive responses associated with CFA-induced arthritis (Lewis et al. 1985; Butler et al. 1992). While rectal administration of ^{9}-THC-HS tended to produce a dose-dependent increase in response latencies in both inflamed and non-inflamed groups, this antinociceptive effect was significant in only the non-inflamed animals. These findings are consistent with reports of cannabinoid modulation of thermal nociception in acute models (Buxbaum 1972; Yaksh 1981; Welch and Stevens 1992), but inconsistent with reports of cannabinoid modulation of hyperalgesia in chronic inflammatory models (Sofia et al. 1973; Smith et al. 1998). However, recent research suggests that cannabinoid agonists may be more effective in preventing the development of hyperalgesia than attenuating it (Li et al. 1999a). It is also possible that higher doses of ^{9}-THC-HS are required to modulate the thermal hyperalgesia in this CFA model of chronic inflammation. Finally, subsequent power analyses assuming a large effect size indicated that a significant analgesic effect would have been detected at the 5.0 mg dose in inflamed animals with the addition of as few as 7 animals per cell.

In the conditioned place preference test, both non-inflamed and CFA-inflamed animals that received vehicle in both conditioning compartments displayed a black compartment preference (i.e., preference scores under 0.5). This is not an unexpected finding and it is the principle reason for pairing all drug conditioning trials with the white compartment (i.e., condition against a black compartment preference). These baseline preference scores in vehicle-treated animals did not differ significantly between inflammation groups.

In non-inflamed animals, rectal administration of ^{9}-THC-HS produced a significant dose-dependent increase in place preference scores, a pattern of effects indicative of reward (for reviews see van der Kooy, 1987; Carr et al. 1989). These findings add to a literature that is considered equivocal at best on

the reinforcing properties of cannabinoids (see Tzschentke 1998 for review). For example, Mallet and Beninger (1998) report that in Wistar rats anandamide failed to produce place preference while ^{9}-THC produced place aversion. Place aversion has also been reported in Lister hooded rats using either CB receptor agonists or ^{9}-THC (Cheer et al. 2000). In contrast, cannabinoids produce place preference (when using a similar procedure and comparable doses) in Long Evans rats (Lepore et al. 1995), are self-administered in squirrel monkeys (Tanda et al. 2000) and produce lower thresholds for intracranial self stimulation in Lewis strain rats (Gardner et al. 1988). While the use of various animal models and paradigms may contribute to such equivocal findings, a growing literature suggests that strain differences in drug sensitivity may be an equally important methodological consideration (for review see Mogil 1999). Lepore et al. (1996) report that in an intracranial self stimulation paradigm, Lewis rats, which we used in the present study, are much more responsive to the rewarding properties of ^{9}-THC compared to Fischer 344 and Sprague-Dawley rats.

In contrast to non-inflamed groups, CFA-inflamed animals given rectal ^{9}-THC-HS did not show significant changes in their place preference scores, a finding that suggests an absence of drug reward in these groups. This finding is somewhat surprising in light of studies that demonstrate analgesic drugs produce place preference though their negative reinforcing properties (i.e., pain reduction) in models of chronic pain (Sufka 1994; Sufka and Roach 1996). However, one requirement for an analgesic drug to possess negative reinforcing properties is that it be sufficiently potent in reducing inflammatory nociception. While rectal administration of ^{9}-THC-HS significantly affected thermal nociception in non-inflamed animals, it was only modestly analgesic in attenuating thermal hyperalgesia in the CFA model of chronic inflammation and, therefore may not possess the necessary negatively reinforcing properties to support place preference. Given that ^{9}-THC-HS does not possess the same reinforcing properties in inflamed groups as it does in non-inflamed controls, we suggest that context can be an important determinant in drug responses.

To our knowledge, this is the first study assessing both the analgesic and reinforcing properties of cannabinoids in the context of chronic pain. While the finding that animals in persistent pain show less analgesia and reward have been interpreted by some to suggest accelerated tolerance (Gutstein et al. 1995; Li et al. 1999b), this interpretation is unlikely. Animals in the present study received low doses of ^{9}-THC-HS and on alternate day exposures, a procedure highly unlikely to produce tolerance. A more likely explanation is that the context in which drugs are employed is an important determinant in drug effects. Bardin et al. (2000, p. 61) have suggested, "that theories of (opiate) tolerance, withdrawal, and reward should incorporate the effects of pain." For example, opioid agonists given repeatedly in the context of persistent or chronic

pain show reduced tolerance, physical dependence and withdrawal while maintaining analgesic efficacy (Lerida et al. 1987; Vaccarino and Couret 1993; Vaccarino et al. 1993). The results of the present study are consistent with these findings and extend the importance of context to include cannabinoid systems and reward behaviors. Further studies evaluating therapeutic compounds should consider the context as an important determinant in drug response.

AUTHOR NOTE

This research was supported in part by The National Center for Natural Products Research and ElSohly Laboratories, Inc. The authors would like to thank Jamie Neal, Brad Sheals, and Sarah Hoffman for assisting in data collection.

REFERENCES

Bardin L., J.A. Kim, S. Siegal, 2000. The role of formalin-induced pain in morphine tolerance, withdrawal, and reward. *Exp Clin Psychopharmacol* 8:61-67.

Butler, S.H., F. Godefroy, J.M. Besson, J. Weil-Fugazza, 1992. A limited arthritic model for chronic pain studies in the rat. *Pain* 48:73-81.

Buxbaum, D.M. 1972. Analgesic activity of ^{9}-tetrahydrocannabinol in the rat and mouse. *Psychopharmacologia* 25:275-280.

Carr, G.D., H.C. Fibiger, A.G. Phillips. 1989. *Conditioned place preference as a measure of drug reward*. In: J.M. Liebman and S.J. Cooper (Eds.), *The Neuropharmacological Basis of Reward*. Clarendon Press: Oxford. pp. 264-319.

Chait, L.D., K.A. Burke. 1994. Preference for high-versus low-potency marijuana. *Pharmacol Biochem Behav* 49:643-647.

Cheer, J.F, D.A. Kendall, C.A. Marsden. 2000. Cannabinoid receptors and reward in the rat: a conditioned place preference study. *Psychopharmacology (Berl)* 151(1): 25-30.

Fuentes J.A., M. Ruiz-Gayo, J. Manzanares, G. Vela, I. Reche, J. Corchero. 1999. Cannabinoids as potential new analgesics. *Life Sci* 65:675-685.

Gardner, E.L., W. Paredes, D. Smith, A. Donner, C. Milling, and D. Morrison. 1988. Facilitation of brain stimulation reward by ^{9}-tetrahydrocannabinol. *Psychopharmacol* 96:142-144.

Gardner, E.L., S.R. Vorel. 1998. Cannabinoid transmission and reward-related events. *Neurobiol Dis* 5(6 Pt B):502-533.

Gessa, G.L., M. Melis, A.L. Muntoni, M. Diana. 1998. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB_1 receptors. *Eur J Pharmacol* 341:39-44.

Gutstein, H.B., K.A. Trujillo, H. Akil. 1995. Does chronic nociceptive stimulation alter the development of morphine tolerance? *Brain Res* 680:174-179.

Jagger, S.I., F.S. Hasnie, S. Sellaturay, A.S.C. Rice. 1998. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* 76:189-199.

Koob, G.F., F.E. Bloom. 1988. Cellular and molecular mechanisms of drug dependence. *Science* 242:715-723.

Lepore, M., X. Liu, V. Savage, D. Matalon, E.L. Gardner. 1996. Genetic differences in delta-9-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. *Life Sci* 58(25):PL365-72.

Lepore, M., S.R. Vorel, J. Lowinson, E.L. Gardner. 1995. Conditioned place preference induced by ^{9}-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. *Life Sci* 56(23,24):2073-2080.

Lerida, M., P. Sanchez-Blazquez, J. Garzon. 1987. Incidence of morphine withdrawal and quasi-abstinence syndrome in a model of chronic pain in the rat. *Neurosci Lett* 81:155-158.

Lewis, A.J., R.P. Carlson, J. Chang. 1985. Experimental models of inflammation. In: I.L. Bonta, M.A. Bray, and M.J. Parnham (Eds.). *The Pharmacology of Inflammation* (*Handbook of Inflammation*, vol 5). New York: Elsevier. pp. 371-397.

Li, J.Y., R.S. Daughters, C. Bullis, R. Bengiamin, M.W. Stucky, J. Brennan, D.A. Simone. 1999a. The cannabinoid receptor agonist WIN 55,212-2 mesylate blocks the development of hyperalgesia produced by capsaicin in rats. *Pain* 81:25-33.

Li, J.Y., C.H. Wong, K.S. Huang, K.W. Liang, M. L. Lin, P.P. Tan, J.C. Chen. 1999b. Morphine tolerance in arthritic rats and serotonergic system. *Life Sci* 64(10): PL111-116.

Lichtman, A.H., B.R. Martin. 1991. Spinal and supraspinal components of cannabinoid-induced antinociception. *J Pharmacol Exper Therap* 258:517-523.

Mallet, P.E., R.J. Beninger. 1998. Delta-9-tetrahydrocannabinol, but not the endogenous cannabinoid receptor ligand anandamide, produces conditioned place avoidance. *Life Sci* 62(26):2431-2439.

Martin, B.J., N.K. Lai, K.T. Patrick, J.M. Walker. 1993. Antinociceptive actions of cannabinoids following intraventricular administration in rats. *Brain Res* 629: 300-304.

Mattes, R.D., L.M. Shaw, J. Edling-Owens, K. Engelman, M.A. ElSohly. 1993. Bypassing the first-pass effect for the therapeutic use of cannabinoids. *Pharmacol Biochem Behav* 44:745-747.

McGregor, I.S., C.N. Issakidis, G. Prior. 1996. Aversive effects of the synthetic cannabinoid CP 55,940 in rats. *Pharmacol Biochem Behav* 53(3):657-664.

Melzack, R. 1991. The tragedy of needless pain. *Sci Am* 262:27-33.

Mogil, J.S. 1999. The genetic mediation of individual differences in sensitivity to pain and its inhibition. *Proc Natl Acad Sci* 96(14):7744-7751.

Onaivi, E.S., M.R. Green, B.R. Martin. 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. *J Pharmacol Exper Therap* 253:1002-1009.

Pertwee, R.G. 2001. Cannabinoid receptors and pain. *Progr Neurobiol* 63:569-611.

Richardson, J.D., S. Kilo, K.M. Hargreaves. 1998. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB_1 receptors. *Pain* 75:111-119.

Sanudo-Pena, M.C., K. Tsou, E.R. Delay, A.G. Hohman, M. Force, J.M. Walker. 1997. Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neurosci Lett* 223(2):125-128.

Smith, L., K. Fujimori, J. Lowe, S. Welch. 1998. Characterization of ^{9}-tetrahydrocannabinal and anandamide antinociception in nonarthritic and arthritic rats. *Pharmacol Biochem Behav* 60:183-191.

Sofia R.D., S.D. Nalepa, J.J. Harakal, H.B. Vassar. 1973. Anti-edema and analgesic properties of ^{9}-tetrahydrocannabinal (THC). *J Pharmacol Exper Therap* 186: 646-655.

Sufka, K.J. 1994. Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. *Pain* 58:355-366.

Sufka, K.J., J.T. Roach. 1996. Stimulus properties and antinociceptive effects of selective bradykinin B_1 and B_2 receptor antagonists in rats. *Pain* 66:99-103.

Tanda, G., P. Munzar, S.R. Goldberg. 2000. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nature Neurosci* 3:1073-1074.

Tanda, G., F.E. Pontieri, G. Di Chiara. 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. *Science* 276:2048-2049.

Tzschentke, T.M. 1998. Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects. Recent progress and new issues. *Progr Neurobiol* 56:613-672.

Vaccarino, A.L., P. Marek, B. Kest, S. Ben-Eliyahu, L.C. Couret Jr, B. Kao, J.C. Liebeskind. 1993. Morphine fails to produce tolerance when administered in the presence of formalin pain in rats. *Brain Res* 627(2):287-290.

Vaccarino, A.L., L.C. Couret Jr. 1993. Formalin-induced pain antagonizes the development of opiate dependence in the rat. *Neurosci Lett* 161(2):195-198.

van der Kooy, D. 1987. Place conditioning: a simple and effective method for assessing the motivational properties of drugs. In: M.A. Bozarth (Ed.), *Method for assessing the Reinforcing properties of Abused drugs*. New York: Spring. pp. 229-240.

Walker, L.A., E.C. Harland, A.M. Best, M.A. ElSohly. 1999. ^{9}-THC hemisuccinate in suppository form as an alternative to oral and smoked THC. In: G.G. Nahas, K.M. Sutin, and D.J. Harvey (eds.). *Marihuana and Medicine*. Totowa, NJ: Humana Press, pp. 123-135.

Welch, S.P., D.L. Stevens. 1992. Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. *J Pharmacol Exper Therap* 262:10-18.

Yaksh, T.L. 1981. The antinociceptive effects of intrathecally administered levonantradol and desacetyllevonantradol in the rat. *J Clin Pharmacol* 21:334s-340s.

Ytterberg, S.R., M.L. Mahowald, S.R. Woods. 1998. Codeine and oxycodone use in patients with chronic rheumatic disease pain. *Arthritis Rheum* 41(9):1603-1612.

Zimmerman, M. 1983. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16:109-110.