Decrease in Efficacy and Potency of Nonsteroidal Anti-Inflammatory Drugs by Chronic \(\Delta^9\)-Tetrahydrocannabinol Administration

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ABSTRACT

Cannabinoids have been shown to increase the release of arachidonic acid, whereas nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to decrease the analgesic effects of cannabinoids. We evaluated the antinociceptive effects of chronic administration of \(\Delta^9\)-tetrahydrocannabinol (\(9\)-THC), anandamide (an endogenous cannabinoid), arachadonic acid, ethanolamine, and methanandamide on several NSAIDs via p.o. and/or i.p. routes of administration using the mouse p-phenylquinone (PPQ) test, a test for visceral nociception. Our studies with a cannabinoid receptor (CB1) antagonist [\(N\)-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride (SR141716A), a CB2 antagonist [\(N\)-((1S)-endo-1,3,3-trimethyl-bicyclo-heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528)], and an another CB2 agonist [1,1-dimethylbutyl-1-deoxy-$\Delta^9$-THC (JWH-133)] were performed to better characterize PPQ interactions with cannabinoid receptors. The acute affects of \(9\)-THC were blocked by SR141716A (i.p.) and partially blocked by SR144528 (i.p.). When NSAIDs (p.o.) were administered, the ED_{50} values were as follows: 23 mg/kg aspirin, 3 mg/kg indomethacin, 5 mg/kg celecoxib, 3 mg/kg ketorolac, 57 mg/kg acemetaphen (32.3–99.8), and 0.8 mg/kg diclofenac (0.1–4.9). In animals given chronic \(9\)-THC, only diclofenac and acemetaphen were active. Conversely, chronic methanandamide (i.p.) did not alter the antinociceptive effects of the NSAIDs. Neither the CB1 or CB2 antagonist blocked the effects of the NSAIDs. The effects of chronic arachadonic acid, ethanolamine, and anandamide could not be evaluated. In summary, our data indicate that chronic \(9\)-THC alters the cyclooxygenase system. Alternatively, the data suggest that this alteration is not due to chronic endogenous cannabinoid release. Based upon these data, we hypothesize that human subjects who are chronic users of \(9\)-THC may not respond to analgesic treatment with the above NSAIDs.

The marijuana plant is the source of more than sixty oxygen-containing aromatic hydrocarbons called cannabinoids. Among these is the primary active constituent \(9\)-THC. To date, two distinct G-protein-coupled cannabinoid receptors have been identified. The CB1 receptor, which is predominantly located in the central nervous system (Matsuda et al., 1990), has been shown to inhibit adenylate cyclase, and hence reduce cAMP in a dose-dependent, stereoselective manner (Matsuda et al., 1990). The receptor has been reported to regulate Ca^2+ currents (Mackie and Hille, 1992), to activate inward rectifying K^+ channels (Felder et al., 1995), and to activate mitogen-activated-protein kinases (Bouaboula et al., 1995). SR141716A has been shown to be a high-affinity antagonist of the CB1 receptor (Rinaldi-Carmona et al., 1994). The CB2 receptor has been found on splenic macrophages (Munro et al., 1993). Activation of the CB2 receptor also inhibits adenylyl cyclase (Slipetz et al., 1995) and activates mitogen-activated-protein kinase (Munro et al., 1993). SR144528 is the receptor antagonist (Rinaldi-Carmona et al., 1998). CB1 and CB2 receptor ligands now available include the CB1-selective agonist methanandamide (Pertwee, 1999) and the CB2-selective agonist 1,1-dimethylbutyl-1-deoxy-$\Delta^9$-THC (JWH-133) (Huffman et al., 2001).

The recent discovery of cannabinoid receptors has led to the identification of endogenous ligands that bind and activate cannabinoid receptors. To date, several endogenous cannabinoids have been identified in mammalian tissues, the most important being anandamide (Devane et al., 1992) and 2-arachidonyl glycerol (Mechoulam et al., 1994).

The cyclooxygenase (COX) enzyme catalyzes the conversion of arachidonic acid to prostaglandins (Vane and Botting, 1987). To date, COX exists in two distinct isomers, COX-1 is
constitutively expressed in a variety of cell types. COX-1 produces prostaglandins that maintain a variety of homeostatic functions such as gastric protection, sodium and water resorption, and vascular homeostasis. COX-2 (COX-2) is constitutively present in the brain and kidney and is induced in most tissues under inflammatory conditions.

The NSAIDs are a heterogeneous group of compounds that are often used clinically for their antiinflammatory, analgesic, antipyretic, and antithrombotic effects (Vane and Botting, 1987; Mitchell et al., 1993). Most currently used NSAIDs inhibit both COX-1 and COX-2 activity (de Leval et al., 2000). The last decade, however, has seen an enormous expansion in research relating to selective COX-2 inhibitors (Urban, 2000). Celecoxib is also not believed to reversibly inhibit COX-1 at therapeutic concentrations. Although not an NSAID, anandamide is often included with the NSAIDs because it has analgesic and antipyretic properties.

Previous studies have shown that NSAIDs such as diclofenac and indomethacin antagonize the effects of ∆9-THC (Ellis et al., 1995; Perez-Reyes et al., 1991; Bhattacharya and Bhattacharya, 1983). In addition, other studies have shown that cannabinoids stimulate the release of endogenous arachadonic acid and alter the synthesis of prostaglandins (Hunter et al., 1991; Pestonjamasp and Burstein, 1998). Based on these studies, our hypothesis is that chronic cannabinoid exposure will alter the activity of the NSAIDs. We evaluated the effects of chronic cannabinoid exposure on COX-1 and COX-2-mediated NSAID activity via the p-phenylquinone (PPQ) test for antinociception. The cannabinoids evaluated included ∆9-THC, anandamide, and methanandamide (k, for CB1 ~ 20 nM; k, for CB2 ~ 815 nM) (Pertwee, 1999). Nevertheless, animals given chronic anandamide failed to generate a writhing response in the PPQ test. Thus, ethanolamine and arachadonic acid were also evaluated because they are products of anandamide metabolism. In addition, we evaluated the effects of NSAIDs at the level of cannabinoid receptors.

Our studies showed that the CB2-specific antagonist SR144528 partially blocked ∆9-THC-induced antinociception in the PPQ test, which previous studies have linked to the CB1 receptor (Walker et al., 1999; Zimmer et al., 1999). Because of the partial blockade, we evaluated JWH-133 (k, for CB1 ~ 680 nM; k, for CB2 ~ 3 nM), which has been shown to have a high selectivity for the CB2 receptor (Huffman, 1999; Pertwee, 1999), to determine whether the PPQ test interacts with the CB2 receptor in ∆9-THC-induced antinociception.

Methods and Materials

Animals. All studies were performed on male ICR mice (Harlan Laboratories, Indianapolis, IN) weighing 20 to 25 g. The mice were housed five per cage and kept on a 12-h light/dark cycle. The facility was maintained at 22 ± 2°C. Food and water were available ad libitum. The mice were brought to the test room and allowed to acclimate for 24 h to recover from transportation and handling. Following use, the mice were killed in a CO2 chamber. All experiments conformed with The Virginia Commonwealth University Division of Animal Resources.

Experimental Design. Chronic THC (10 mg/kg) and vehicle treatment (1:1:18) lasted 6.5 days, during which time the mice received 13 injections (i.p.). These injections were done every 12 h. Injection volumes of 0.3 ml were administered. On the 7th day, mice were tested with various doses of NSAIDs. This protocol was chosen because previous studies determined tolerance to THC developed following 6.5 days of THC administration. Chronic anandamide (10 mg/kg) and methanandamide (10 mg/kg) treatments lasted 3 days, during which time the mice received 12 injections (i.p.) every 4 h. On the 4th day, mice were tested with various doses of NSAIDs. Previous studies determined that mice in the 3-day protocol developed tolerance to anandamide and methanandamide. Ethanolamine and arachadonic acid were also given chronically due to a failure of anandamide to generate a writhing response. Drugs were given for 3 days every 4 h. Injection volumes of 0.3 ml were administered.

Drug Administration Protocol. For generation of dose-response curve to various NSAIDs, all NSAIDs were given p.o. Injections volumes of 0.3 ml were administered. In animals given chronic vehicle, the following doses of NSAIDs were given: 20, 25, and 30 mg/kg aspirin; 0.1, 0.5, and 1 mg/kg diclofenac; 1, 2, and 10 mg/kg indomethacin; 30, 100, and 110 mg/kg acetaminophen; 1, 3, 10, 15, and 30 mg/kg ketorolac; and 1, 5, 10, and 15 mg/kg celecoxib. In animals given chronic THC (i.p.) for 6.5 days every 12 h, the doses were: 30, 60, and 100 mg/kg aspirin; 20, 60, and 80 mg/kg diclofenac; 10, 15, and 300 mg/kg indomethacin; 100, 110, 200, and 500 mg/kg acetaminophen; and 1 mg/kg, 10, 100, and 300 mg/kg ketorolac. All drugs except for celecoxib were prepared in a solution of Emulphor, ethanol, and saline at a 1:1:18 ratio. The 1:1:18 vehicle has a long history of use by many labs and is devoid of antinociceptive effects in our test protocol. Celecoxib was prepared in 5% carboxymethyl cellulose (5 g of carboxymethylcellulose/100 ml of saline). NSAIDs were administered by p.o. gavage and tested either 30 min (aspirin, celecoxib, naproxen, and acetaminophen) or 60 min (diclofenac and indomethacin) later using the PPQ test for antinociception. JWH-133 had a 15 min time point. Previous time-course studies determined the times of peak effects of the drugs. In each group of experiments, 16 mice are used (eight experimental and eight vehicle).

In antagonist studies designed to determine whether the NSAIDs were activating the cannabinoid receptor, antagonists to the CB1 and CB2 receptor were used. Both drugs were prepared in 1:1:18 for i.p. administration. The peak time point for these drugs was 1 h. Both cannabinoid antagonists had no intrinsic effects in our test system.

The PPQ Protocol. Mice were tested for antinociception by the p-phenylquinone test. Eight mice were housed two per cage and injected i.p with PPQ solution. Seven minutes after PPQ injections stretches were counted. Stretches were counted one cage at a time for 1 min each and then repeated (8 min total). PPQ solution was prepared in an ethanol/saline (3:47) vehicle and heated gently to facilitate solubility. Antinociception was quantified as the percent inhibition of the stretch response determined by the formula: % inhibition = 1 – (total test stretches/total control stretches) × 100.

We calculated the percentage of inhibition for a given dose using eight mice per dose. Dose response curves were generated using at least three doses of drug. ED50 values were determined by log-probit analysis, and 95% confidence intervals were determined using the method of Tallarida and Murray (1987) for graded dose-response curves, omitting doses that produced 0 or 100% effect. Each experiment was replicated in animal given vehicle.

Drugs. The following drugs were obtained from Sigma-Aldrich (St. Louis, MO): aspirin, diclofenac, indomethacin, ketorolac, acetaminophen, naproxen, arachadonic acid, and ethanolamine. Anandamide and methanandamide were obtained from Cayman Chemicals (Ann Arbor, MI). Celecoxib was obtained from Donna D. Gale (Purdue Pharma, Ardsley, NY). ∆9-THC was obtained from the National...
Results

Several NSAIDs that are commonly used for analgesia were tested for antinociception in animals given chronic Δ^9-THC or chronic vehicle via p.o. administration. Results of the dose-response analyses, as determined by the PPQ test for antinociception, are presented in Figs. 1 to 3, with lines determined by linear regression analysis.

Figure 1A shows dose response curves for aspirin in animals given chronic THC versus chronic vehicle (1:1:18). The ED\textsubscript{50} value for aspirin was 23 mg/kg (20–27) in vehicle animals. In animals given chronic THC, however, an ED\textsubscript{50} value could not be obtained, and an increased dose up to 100 mg/kg failed to generate an ED\textsubscript{50} value.

Figure 1B shows the dose response curves generated for diclofenac in animals given chronic THC and chronic vehicle. The ED\textsubscript{50} in vehicle animals was 0.8 mg/kg (0.1–5) and 39 mg/kg (6.7–220) in animals given chronic THC. The 49-fold shift is statistically significant.

Figure 2A shows the dose response curves generated for indomethacin in animals given chronic THC and chronic vehicle. The ED\textsubscript{50} of indomethacin was 2.8 mg/kg (0.9–8.9) in vehicle animals. In animals given chronic THC, however, an ED\textsubscript{50} value could not be generated, and an increased dose of 300 mg/kg failed to generate an ED\textsubscript{50} value.

Figure 2B shows the dose response curves generated for ketorolac in animals given chronic THC and chronic vehicle. The ED\textsubscript{50} of ketorolac was 3 mg/kg (1.8–4.6) in vehicle animals. In animals given chronic THC, an ED\textsubscript{50} could not be
generated, and an increased dose of 300 mg/kg failed to produce an ED₅₀ value.

Figure 3A shows dose-response curve generated for naproxen in animals given chronic THC and chronic vehicle. The ED₅₀ for naproxen was 11 mg/kg (2.3–51.5) in vehicle animals. In animals given chronic THC, an ED₅₀ could not be generated, and an increased dose of 60 mg/kg failed to produce an ED₅₀ value.

Unlike the other NSAIDs, celecoxib is a selective COX-2 inhibitor at normal therapeutic doses (Urban, 2000). Figure 3B shows the dose-response curves generated for celecoxib in animals given chronic THC and chronic vehicle. The ED₅₀ of celecoxib is 5 mg/kg (3–16). In animals given chronic THC, an ED₅₀ could not be generated, and an increased dose of 60 mg/kg failed to produce an ED₅₀ value.

Figure 3C shows the dose response curves generated for acetaminophen in animals given chronic THC and chronic vehicle. The ED₅₀ was 57 mg/kg (32–100) in vehicle animals. In animals given chronic THC, an ED₅₀ could be generated. The ED₅₀, however, shifted to 260 mg/kg (145–466), which is a statistically significant shift.

Since a decrease in efficacy or potency of the NSAIDs was seen in animals given chronic THC, we wanted to determine whether the NSAIDs acted at the level of cannabinoid receptors. We challenged aspirin and indomethacin with the receptor antagonists SR141716A and SR144528 to determine whether antinociception could be inhibited. Both SR141716A and SR144528 failed to attenuate the activity of any NSAID in naive animals. Aspirin (30 mg/kg p.o.) produced 75% of the writhing response in naive animals. Pretreatment with 20 mg/kg (i.p.) SR141716A and SR144528 given 10 min before aspirin (30 mg/kg) produced 73 and 79% inhibition of PPQ writhing, respectively. A dose of 10 mg/kg indomethacin (p.o.) produced 80% inhibition of PPQ writhing in naive animals. A dose of 20 mg/kg (i.p.) SR141716A and SR144528 given 10 min before indomethacin (10 mg/kg) produced 84 and 82% inhibition of the PPQ stretch, respectively. Therefore, the
NSAIDs do not appear to be working at the level of cannabinoid receptors.

As expected, SR141716A blocked THC-induced antinociception (Fig. 4) and, unexpectedly, SR144528 produced a partial blockade of THC-induced antinociception (Fig. 4). Because of this unexpected blockade by a CB2 antagonist, we used JWH-133 to determine whether the PPQ test is CB2-sensitive. Surprisingly, JWH-133, a CB2-selective ligand, produced antinociception in a dose-dependent manner in vehicle mice (Fig. 5). The ED<sub>50</sub> of JWH-133 (p.o.) was 100 mg/kg in vehicle animals. Lack of drug prevented generation of complete dose-response curves. As expected, pretreatment with SR144528 (20 mg/kg) i.p. 10 min before JWH-133 (100 mg/kg) blocked JWH-133-induced antinociception. Furthermore, pretreatment with SR141716A (20 mg/kg) i.p. 10 min before JWH-133 also blocked JWH-133-induced antinociception.

Methanandamide (10 mg/kg) was given chronically (i.p.), and several NSAIDs were tested following chronic exposure. Results can be seen Fig. 6. Figure 6 shows the bar graphs generated for aspirin (100 mg/kg), diclofenac (100 mg/kg), acetaminophen (300 mg/kg), and celecoxib (100 mg/kg) following exposure to chronic methanandamide and the 1:1:18 vehicle. All NSAIDs were given p.o. Aspirin (100 mg/kg), which could not produce an ED<sub>50</sub> following chronic THC exposure, produced 84% inhibition of the PPQ stretch in animals following chronic methanandamide and 77% inhibition following chronic vehicle. Celecoxib, which could not generate an ED<sub>50</sub> following chronic THC exposure, was tested at a dose 20× its ED<sub>50</sub> in naive animals. A dose of 100 mg/kg produced 58% inhibition following chronic methanandamide and 52% inhibition following chronic vehicle. For diclofenac and acetaminophen, we used doses that were greater than their ED<sub>50</sub> values following chronic THC exposure. Diclofenac (100 mg/kg) produced 92% inhibition in animals following chronic methanandamide and 82% inhibition in animals following chronic vehicle. Acetaminophen (300 mg/kg) produced 76%

Data are not shown for experiments with chronic anandamide, ethanolamine, and arachidonic acid. The animals would not writhe following chronic exposure and thus could not be tested.

Discussion

The present study is based upon previous work that indicates that acute administration of cannabinoids stimulates the release of arachidonic acid resulting in elevation of prostaglandins (Burstein and Hunter, 1981; Burstein et al., 1983; Laviolette and Belanger, 1986; Bhattacharya, 1986; Hunter et al., 1991). Furthermore, cannabinoids induce COX-2 expression in the central nervous system (Ramer et al., 2001). In addition, NSAIDs antagonize the effects of THC (Fairbairn and Pickens, 1979; Burstein and Hunter, 1982; Hillard and Bloom, 1983; Perez-Reyes et al., 1991; Ellis et al., 1995). The main goal of our work was to determine how chronic administration of Δ<sup>9</sup>-THC affects the antinociceptive effects of the NSAIDs. To evaluate the NSAID activity, we chose an assay system that has been shown to involve prostaglandins and is a standard assay for NSAIDs. Previous studies have linked the CB1 receptor (Walker et al., 1999; Zimmer et al., 1999) to the antinociceptive effects of THC. Thus, to better clarify the role of CB1 and CB2 in the PPQ, we used JWH-133, an agonist with CB2 selectivity.

NSAIDs have been extensively studied in a variety of systems and appear to produce antinociception in a dose-dependent manner due to the inhibitory effect on COX activity (Davies and Skjodt, 2000). Bhattacharya and Bhattacharya (1983), Perez-Reyes et al. (1991), and Ellis et al. (1995) have evaluated antagonism of THCs effects by NSAIDs. They did not, however, evaluate the effects of NSAIDs in animals.
chronically pretreated with THC. ED$_{50}$ values could only be obtained for diclofenac and acetaminophen in animals chronically treated with THC. This profound decrease in efficacy and potency of all the NSAIDs in animals chronically treated with THC clearly indicates some type of plasticity of the arachadonic acid/cyclooxygenase system.

There are several possible explanations for our observed effects. Chronic treatment with THC may continue to increase synthesis of prostaglandins and, thus, decrease the potency and/or efficacy of the NSAIDs. Alternatively, previous studies have indicated that THC has been shown to increase endogenous cannabinoids such as anandamide (Pestonjamasp and Burstein, 1998). This increase in anandamide can rapidly be converted to arachadonic acid (Pratt et al., 1998). Because anandamide can be converted to arachadonic acid, it seems reasonable to speculate that chronic THC exposure results in such high levels of endogenous cannabinoids that arachadonic acid is continually synthesized. This increase in arachadonic acid synthesis could induce COX enzyme and activity. The constant induction of COX could result in decreased NSAID efficacy. Thus, the NSAIDs might not overcome the amount of COX induction.

Unlike the other NSAIDs, diclofenac remains active, albeit significantly less active, in animals given chronic THC. When efficacy remains the same, however, there is a decrease in potency. Diclofenac is reported to have a potency greater than all the NSAIDs tested (Skoutakis et al., 1988), which is in agreement with our observations. In fact, diclofenac was so potent that the ED$_{50}$ in animals given chronic THC was lethal in our chronic vehicle (1:1:18) animals. Thus, not only is there tolerance to diclofenac's antinociceptive effects but also to its toxic effects. Therefore, it is likely that diclofenac's potency could allow it to exert antinociceptive effects in animals given chronic THC.

The least potent drug tested, acetaminophen, shows results similar to diclofenac. Efficacy remains the same and potency decreases. The 4-fold shift in the curve for animals given chronic THC is significant and is evidence that the animals become cross-tolerant to acetaminophen. Unlike the other drugs, Acetaminophen is not an NSAID. Thus, it seems reasonable to speculate that it may work via another mechanism, attributable to the fact that acetaminophen does not act on systemic COX (Hanel and Lands, 1982; Marshall et al., 1987). Thus, if chronic THC induced COX, it would not be expected to alter acetaminophen's ability to produce antinociception to the degree of the other NSAIDs. It is also important to note that both COX-1 and COX-2 appear altered by chronic THC, in that celecoxib, a selective COX-2 inhibitor, was decreased in efficacy and potency.

Anandamide, an endocannabinoid, was given chronically and due to the lack of a stretching response in the PPQ could not be evaluated. Arachadonic acid and ethanolamine were also tested since they are products of anandamide metabolism. Failure in the stretching response to the PPQ test, however, was also seen, and the drugs could not be evaluated. These data are interesting because they could indicate some basic disruption of prostaglandins by chronic anandamide or its metabolic products. Thus, methanandamide was used as a representative for the endogenous cannabinoid anandamide to evaluate whether the decrease in NSAID efficacy and activity was due to chronic endogenous cannabinoid release. Because the NSAID-induced antinociception was not attenuated following chronic methanandamide exposure, it does not seem likely that the decrease in NSAID activity is due to endogenous cannabinoid release. Because methanandamide is a synthetic cannabinoid, however, the possibility cannot be ruled out. Thus, the nature of the differential effect remains to be elucidated. Although, one explanation for the difference between methanandamide and THC is that methanandamide is a metabolically more stable chiral analog than anandamide (Jarbe et al., 2001). Thus, unlike anandamide, it fails to degrade to arachadonic acid metabolites and induce the COX system.

SR141716A completely blocks THC-induced antinociception, which is in agreement with previous observation by Rinaldi-Carmona et al. (1994). SR144528 partially attenuated THC-induced antinociception in the PPQ test, which was unexpected since previous studies have linked THC-
induced antinociception to the CB1 receptor (Walker et al., 1999; Zimmer et al., 1999). Our unexpected results with SR144528, however, suggest that the PPQ test is sensitive to CB2 receptors. Thus, studies with JWH-133 were done to determine the role CB1 and CB2 receptors play in antinociception in the PPQ test. Results showed that antinociception could be induced using a CB2-selective ligand. Such results suggest the possibility of CB2 receptor involvement in producing Δ⁹-THC-induced antinociception in the PPQ test and confirms the SR144528 block of THC. SR141716A, however, also blocked JWH-133-induced antinociception. This result indicates that the drug also has activity at the CB1 receptor. Thus, JWH-133 cannot confirm the possibility of CB2 receptor involvement in producing antinociception in the PPQ test. Nevertheless, the NSAIDs are not acting at either the CB1 or CB2 receptors. Thus, cross-tolerance of NSAIDs to THC is not due to a direct NSAID/cannabinoid receptor interaction.

In conclusion, THC given chronically decreases efficacy and potency of the NSAIDs tested. This decrease does not seem to be endogenous-cannabinoid release mediated. Our data indicate that diclofenac and acetaminophen differ from the other NSAIDs tested, possibly because of their potency or activity at a non-COX site. In addition, our studies indicate that the PPQ test is sensitive to both CB1 and CB2 induced activity. Based upon our results, chronic THC users may not respond as well as nonusers to NSAID treatment. Although diclofenac and acetaminophen were active in animals given chronic THC, in a clinical situation, these doses could be very toxic.

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References


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