British Journal of Pharmacology (2007) 150, 693–701

0007–1188/07 $30.00
www.brjpharmacol.org

RESEARCH PAPER

The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB_2 receptors

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Background and purpose: 2-arachidonoyl glycerol (2-AG) is an endogenous cannabinoid with central antinociceptive properties. Its degradation is catalysed by monoacylglycerol lipase (MGL) whose activity is inhibited by URB602, a new synthetic compound. The peripheral antinociceptive effects of 2-AG and URB602 in an inflammatory model of pain are not yet determined. We have evaluated these effects with and without the cannabinoid CB_1 (AM251) and CB_2 (AM630) receptor antagonists.

Experimental approach: Inflammation was induced in rat hind paws by intraplantar injection of formalin. Nociception was assessed behaviourally over the next 60 min, in 19 experimental groups: (1) control; (2-6) 2-AG (0.01-100 μg); (7) AM251 (80 μg); (8) AM251 + 2-AG (10 μg); (9) AM630 (25 μg); (10) AM630 + 2-AG (10 μg); (11-16) URB602 (0.1-500 μg); (17) 2-AG + URB602 (ED_{50}); (18) AM251 + URB602 (ED_{50}); (19) AM630 + URB602 (ED_{50}). Drugs were injected s.c. in the dorsal surface of the hind paw (50 μl), 15 min before formalin injection into the same paw.

Key results: 2-AG and URB602 produced dose-dependent antinociceptive effects for the late phases of the formalin test with ED_{50} of 0.65 ± 0.455 μg and 68 ± 14.3 μg, respectively. Their combination at ED_{50} doses produced an additive antinociceptive effect. These effects were inhibited by AM630 but not by AM251 for 2-AG and by the two cannabinoid antagonists for URB602.

Conclusions and implications: Locally injected 2-AG and URB602 decreased pain behaviour in a dose-dependent manner in an inflammatory model of pain. The antinociceptive effect of 2-AG was mediated by the CB_2 receptor.
British Journal of Pharmacology (2007) **150,** 693–701. doi:10.1038/sj.bjp.0706990; published online 18 December 2006

Keywords: 2-arachidonoyl glycerol; URB602; endocannabinoids; cannabinoid receptors; monoacylglycerol lipase; formalin test; inflammatory pain

Abbreviations: 2-AG, 2-arachidonoyl glycerol; AUC, area under the curve; CB, cannabinoid; $CPS\text{-}WST_{0,1,2}$, composite pain score-weighted scores technique; DMSO, dimethylsulphoxide; FAAH, fatty-acid amide hydrolase; MGL, monoacylglycerol lipase

Introduction

Δ^9-Tetrahydrocannabinol, the major psychoactive component of cannabis, activates two distinct G protein-coupled receptors, identified as the cannabinoid CB_1 (Matsuda *et al.*, 1990) and CB_2 receptors (Munro *et al.*, 1993). The CB_1 receptor is primarily expressed throughout the central nervous system including the spinal cord and on sensory neurons in dorsal root ganglia and in the periphery (Hohmann and Herkenham, 1999; Farquhar-Smith *et al.*, 2000; Rice *et al.*, 2002; Walczak *et al.*, 2005) whereas CB_2 expression seems restricted to peripheral tissues with immune functions (Munro *et al.*, 1993; Galiègue *et al.*, 1995). The two major and most studied endogenous cannabinoids are anandamide and 2-arachidonoyl glycerol (2-AG).

For many years, evidence has accumulated suggesting that cannabinoids can produce antinociception through peripheral mechanisms involving CB_1 (Hohmann, 2002 for review) and CB_2 receptors (Malan *et al.*, 2002 for review). In rodents, anandamide attenuated formalin-induced nociceptive behaviours (Calignano *et al.*, 1998; Guindon *et al.*, 2006a, b) when administered locally into the hind paw. Although Richardson *et al.* (1998, 2000) have shown that cannabinoid antihyperalgesic effects are predominantly mediated by CB_1 cannabinoid receptors as most of cannabinoid effects are blocked by CB_1-selective antagonists, there is evidence

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Received 8 May 2006; revised 3 July 2006; accepted 17 August 2006; published online 18 December 2006

that CB_2 mechanisms may also contribute to the antinociceptive effects of cannabinoids (Ibrahim *et al*., 2006). Indeed, Hanus *et al*. (1999) have shown that HU308, a CB_2 selective agonist, could suppress the late-phase response in the formalin test when injected intraperitoneally. Moreover, Calignano *et al*. (1998) and Jaggar *et al*. (1998) both demonstrated that palmitoylethanolamide, a peroxisome proliferator-activated receptor-alpha agonist (Lo Verme *et al*., 2005) whose anti-inflammatory effects were antagonized by a CB_2 receptor antagonist, produced antinociception in the formalin test (late phase). In addition, AM1241, a CB_2 selective agonist, attenuated thermal nociception and hyperalgesia (Malan *et al*., 2001, 2002; Quartilho *et al*., 2003) and suppressed hyperalgesia evoked by intradermal administration of capsaicin (Hohmann *et al*., 2004). These data suggest a potential therapeutic relevance of cannabinoid CB_2 receptors as a target for pain treatment without having unwanted central side effects.

There is also evidence that 2-AG, but not anandamide, acts as a potent and full-efficacy agonist of the cannabinoid CB_2 receptor (Gonsiorek *et al*., 2000; Sugiura *et al*., 2000; Maresz *et al*., 2005). 2-AG is hydrolysed to arachidonic acid and glycerol by an enzyme, monoacylglycerol lipase (MGL) (Dinh *et al*., 2002, 2004; Saario *et al*., 2004), although some data suggested that 2-AG might also be metabolized by anandamide amidohydrolase/fatty-acid amide hydrolase (FAAH) (Di Marzo *et al*., 1998; Goparaju *et al*., 1998; Lang *et al*., 1999). However, Kathuria *et al*. (2003) demonstrated that URB597, a selective FAAH inhibitor that greatly reduced anandamide degradation in the rat brain, had no effect on 2-AG levels. Moreover, Lichtman *et al*. (2002) have shown that 2-AG breakdown is preserved in mutant $FAAH^{-/-}$ mice although anandamide hydrolysis was almost completely absent. Thus, URB602, a non-competitive MGL inhibitor that blocks 2-AG hydrolysis was developed (Hohmann *et al*., 2005; Makara *et al*., 2005) and found to be more potent than URB597 in enhancing 2-AG levels and antinociception in stress models.

Therefore, the present study was designed to investigate the peripherally mediated antinociceptive effects of 2-AG, URB602, separately and in combination, after intraplantar injection, in a model of inflammatory pain. The mechanism by which 2-AG might produce its antinociceptive effect was also investigated by using specific antagonists for the cannabinoid CB_1 and CB_2 receptors.

Methods

Animals

This research protocol was approved by the Animal Ethics Committee of the University of Montreal and all procedures related to the use of animals conformed to the guidelines of the Canadian Council for Animal Care. One hundred and twenty-five male Wistar rats (Charles River, St Constant, Quebec, Canada) weighing 180–220 g, at the time of testing, were housed two per cage in standard plastic cages with sawdust bedding in a climate-controlled room, under a 12 h light/dark cycle. They were allowed free access to food pellets and water.

Formalin test

Rats were acclimatized to the testing environment (a clear Plexiglas box $29 \times 29 \times 25$ cm) for 15 min or until cessation of explorative behaviour. 2-AG (0.01–0.1–1–10 or 100 μg in 50 μl), URB602 (0.1, 1, 10, 30, 100 or 500 μg in 50 μl), AM251 (80 μg in 50 μl) and AM630 (25 μg in 50 μl) were injected subcutaneously (s.c.) in the dorsal surface of the right hind paw 15 min before the injection of 2.5% formalin (50 μl) next to the previous injection. Following each injection, the rat was immediately put back in the observation chamber. Nociceptive behaviour was observed with the help of a mirror angled at 45° below the observation chamber. Observation of the animal's behaviour was made every 5 min and for 60 min, starting after formalin administration (Tjölsen *et al*., 1992). In each 5-min period, the total time the animal spent in three following different behavioural categories was recorded: (1) the injected paw has little or no weight placed on it; (2) the injected paw is raised; (3) the injected paw is licked, shaken or bitten. Nociceptive behaviour was quantified using the composite pain score-weighted scores technique ($CPS\text{-}WST_{0,1,2}$) calculated for the first (0–15 min) and second (15–50 min) phase of the behavioural response (Watson *et al*., 1997). The area under the curve (AUC) which corresponds to $CPS\text{-}WST_{0,1,2} \times$ time (min) was calculated for the early (first) phase (0–15 min) and the late (second) phase (15–50 min) using the trapezoidal rule.

Protocol

The experiments were conducted in a randomized manner by the same experimenter, without knowledge of the treatments. In a first study, the dose–response curve for 2-AG was determined using the data from the late phase. In a second study, the antinociceptive effects of 2-AG (at 10 μg, an analgesic dose, dissolved in 2% acetonitrile) were studied in the absence or in presence of cannabinoid antagonists to determine whether these effects were mediated through the CB_1 or the CB_2 receptor: AM251 (80 μg; Malan *et al*., 2001; Guindon and Beaulieu, 2006; Guindon *et al*., 2006a, b) and AM630 (25 μg; Malan *et al*., 2001; Guindon and Beaulieu, 2006; Guindon *et al*., 2006a, b) were co-injected with 2-AG. In a third study, the dose–response curve for URB602 was determined using the data from the late inflammatory phase. In a fourth study, the effects of 2-AG, URB602 and their combination were observed to note any additive or synergic effect of the drugs. In a fifth study, the antinociceptive effects of URB602 (at ED_{50} dose, 70 μg in 6.6% dimethylsulphoxide (DMSO)) were studied in the absence or in presence of cannabinoid antagonists to determine whether these effects were mediated through the CB_1 or the CB_2 receptor: AM251 and AM630 were co-injected with URB602. For the second study ($n = 7$ per group) and for all of the other studies ($n = 4$ per group), the tested drugs were dissolved in the same total volume (50 μl) and administered in the right hind paw. Previous experiments in the formalin test have already shown that there was no difference in pain behaviour between 0.9% NaCl and 8% DMSO in normal saline injected in the paw of rats (Guindon *et al*., 2006a, b).

Finally, in order to exclude any possible systemic effect of the drugs, 100 μg of 2-AG and 500 μg of URB602 were administered s.c. on the dorsal surface of the contralateral (left hind paw) or ipsilateral paws ($n=4$ and $n=3$ per group, respectively).

Paw oedema

At the end of the formalin test, paw oedema was measured at the base of the right hind paw using a digital micrometer (Mitutoyo Corporation, Aurora, IL, USA) with an instrumental error of $\pm$(maximum measuring length/75 μm) and a resolution of 0.001 mm (Petricevic *et al*., 1978; Nackley *et al*., 2003b; Ghilardi *et al*., 2004; Guindon *et al*., 2006a, b).

Statistical analysis

Pain behaviour for each treatment group was expressed as mean$\pm$s.e.m. The dose–response curve for 2-AG and URB602 were determined using ALLFIT software (De Léan *et al*., 1978). In the second study, the antinociceptive effects of 2-AG in absence or presence of cannabinoid antagonists were assessed for significance using factorial experimental design (Winer, 1971). In the next study, the effects of 2-AG, URB602 and their combination at ED_{50} dose were assessed for significance using factorial experimental design. In the last study, the antinociceptive effects of URB602 in absence or presence of cannabinoid antagonists were assessed for significance using factorial experimental design. To compare ipsi vs contralateral paw injections of the drugs (2-AG and URB602), an analysis of variance adapted for factorial experimental design was used. The different components of the total variation were settled *a priori* using multiple regression analysis (Draper and Smith, 1998). The critical level of significance was set at 5% ($P<0.05$).

Drug sources and preparation

2-AG, an endogenous cannabinoid, is an agonist with a threefold selectivity for the CB_1 receptor ($K_i=0.47$ mM) over the CB_2 receptor ($K_i=1.4$ mM) (Lee *et al*., 1995; Mechoulam *et al*., 1995; Sugiura *et al*., 1996). 2-AG was purchased from Sigma (St Louis, MO, USA) in acetonitrile solution and further dissolved in normal saline (0.9% NaCl in water) to a final concentration of 0.002–20% acetonitrile. AM251 and AM630 are 306-fold (Gatley *et al*., 1996, 1997) and 70–165-fold (Pertwee *et al*., 1995; Hosohata *et al*., 1997; Ross *et al*., 1999; Malan *et al*., 2001) selective for CB_1 and CB_2 receptor antagonists, respectively. AM251 and AM630 were dissolved in normal saline containing 8 and 2.5% DMSO, respectively. AM251 and AM630 were purchased from Tocris (Ellisville, MO, USA). URB602, which is a selective inhibitor of MGL, was purchased from Cayman Chemical (Ann Arbor, MI, USA) and further dissolved in normal saline containing between 0.01 and 33% DMSO. Both 2-AG and URB602 were stored at -20°C, protected from light and were prepared freshly on the day of the experiment.

Results

Antinociceptive effects of 2-AG and involvement of cannabinoid receptors

2-AG decreased pain behaviour in the formalin test with an ED_{50} of 0.65 ± 0.455 μg for the late phase (Figure 1). Pain behaviour following injection of 2-AG in the ipsilateral hind paw was statistically different when compared with the control (NaCl 0.9%) group only for the late phase of the formalin test (Figure 2a). Pain behaviour following injection of 2-AG in the contralateral hind paw was not statistically different when compared with the control (NaCl 0.9%) group (Figure 2b).

When given locally (dorsal surface of the paw) at 10 μg, 2-AG produced an antinociceptive effect when compared with the control group reaching significance only for the late phase of the formalin test (Figure 3a and c). This antinociceptive effect was inhibited only by AM630 as there was no statistical difference between this antagonist given alone compared with its combination with 2-AG for the early and late phases, respectively (Figure 3a–c). Indeed, pain behaviour was significantly greater in the AM630/2-AG co-administration group compared with the group receiving 2-AG alone.

Paw oedema

Oedema of the injected paw did not differ among the groups; thus neither 2-AG nor AM251 nor AM630 influenced paw oedema (Figure 3d).

Antinociceptive effects of URB602, 2-AG and their combination at ED_{50} doses

URB602 decreased pain behaviour in the formalin test with an ED_{50} of 68 ± 14.3 μg for the late phase (Figure 4). Pain behaviour following injection of URB602 in the ipsilateral hind paw was statistically different when compared with the

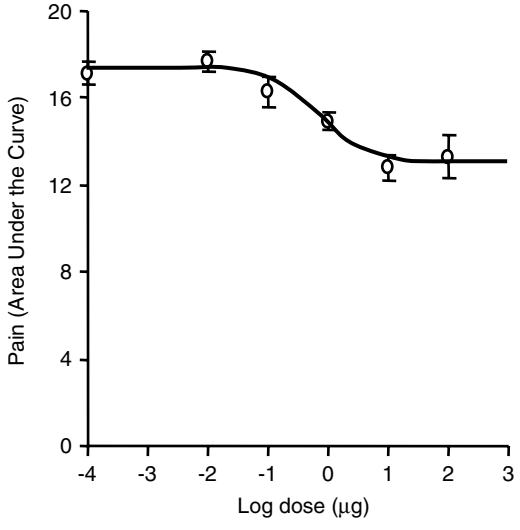

Figure 1 Dose–response curve for 2-AG in the late inflammatory phase of the formalin test. Data is expressed as mean$\pm$s.e.m. ($n=4$ per group).

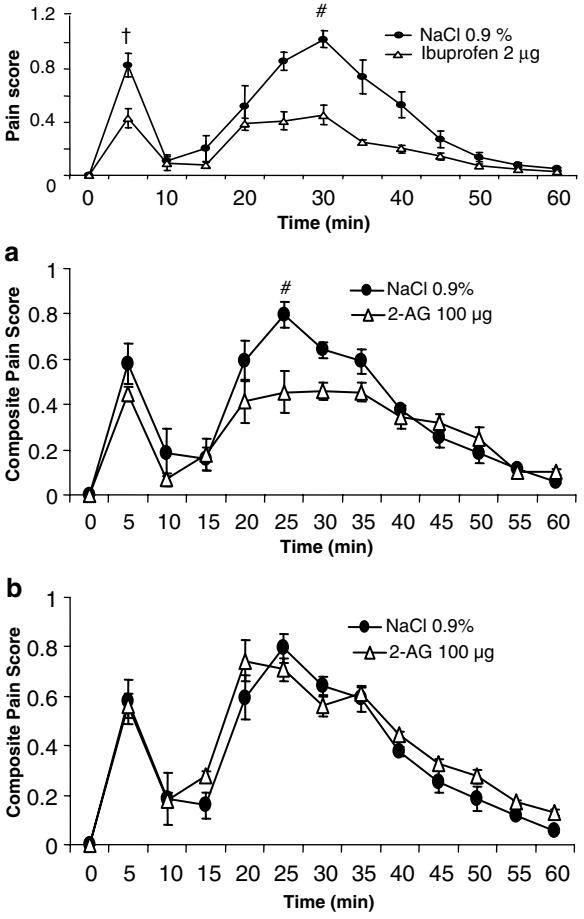

Figure 2 Antinociceptive effects of 2-AG after injection in the dorsal surface of the ipsilateral (**a**) and contralateral (**b**) hind paws 15 min before 2.5% formalin (50 μl). (**a**) Upper shows the antinociceptive effects of an active comparator in the formalin test, ibuprofen a non-steroidal anti-inflammatory drug administered in the dorsal surface of the paw 15 min before formalin (data from Guindon *et al.*, 2006b; † and # indicate a significant difference compared with controls for the first and second phases of the formalin test, respectively). Data is expressed as mean $\pm$ s.e.m. ($n=4$ per group). AUC for the inflammatory phase, # ($F(1,6)=11.78$) $P<0.025$ for 2-AG 100 μg vs control (NaCl 0.9%) group. In (**b**), there was no effect of 2-AG ($F(1,6)=0.05$ and 4.50 for the early and late phases, respectively) given contralaterally in the formalin test.

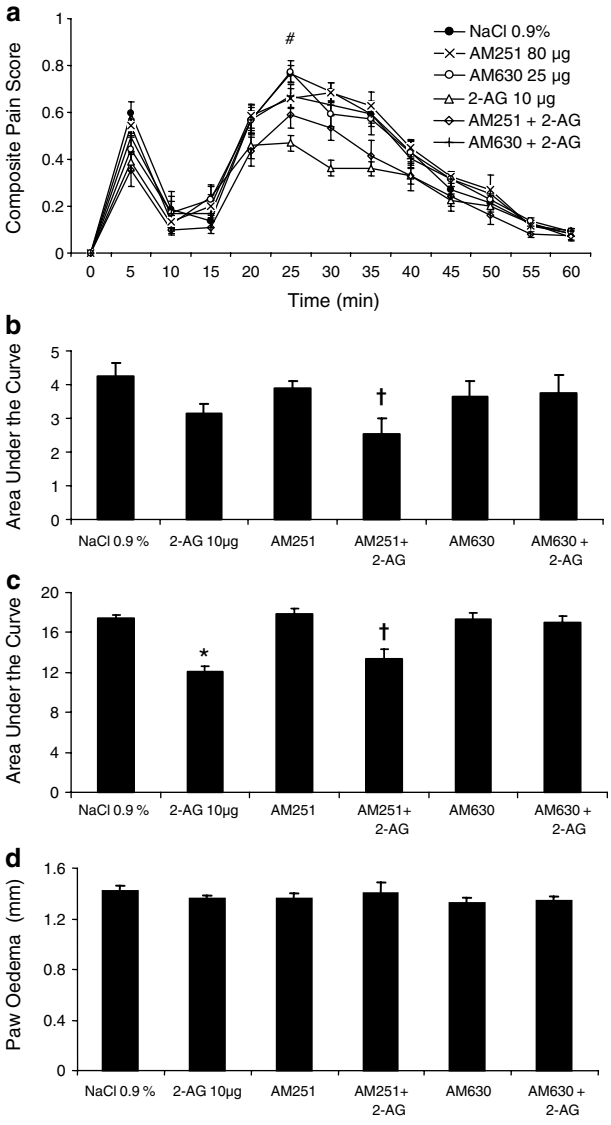

Figure 3 (**a**) Antinociceptive effect of 2-AG when administered at a dose of 10 μg into the hind paws 15 min before 2.5% formalin (50 μl). Effects of cannabinoid antagonists on the antinociceptive effects of 2-AG, for the acute (**b**) and inflammatory (**c**) phases of the test. Data is expressed as mean $\pm$ s.e.m. ($n=7$ per group). AUC for the inflammatory phase, # ($F(1,36)=35.61$) $P<0.001$ for 2-AG vs control (NaCl 0.9%) group; *$P<0.001$ for 2-AG vs control (NaCl 0.9%) group; † $P<0.05$ (early phase), $P<0.001$ (late phase) for AM251 + 2-AG vs AM251 group. Note that the effects of 2-AG were reversed by AM630 ($F(1,36)=0.04$ and 0.18; AM630 alone vs 2-AG + AM630). In (**d**) the corresponding values of paw oedema are shown. None of the treatments, 2-AG ($F(1,36)=0.79$) or AM251 ($F(1,36)=0.38$) or AM630 ($F(1,36)=0.10$) modified the oedema. For all groups ($F(5,36)=0.58$).

control (NaCl 0.9%) group either for the early or the late phase of the formalin test (Figure 5a). Pain behaviour following injection of URB602 in the contralateral hind paw was not statistically different when compared with the control (NaCl 0.9%) group (Figure 5b).

When given locally (dorsal surface of the paw) at doses corresponding to their ED_{50}, 2-AG, URB602 and their combination produced a significant antinociceptive effect when compared with the control group either for the first (Figure 6a and b) or the second phase (Figure 6a and c) of the formalin test. The combination of 2-AG with URB602 was significantly different when compared with each drug given alone for the late phase (Figure 6a and c), revealing an additive antinociceptive effect of this combination.

Paw oedema

Oedema of the injected paw did not differ among the groups; 2-AG and URB602 did not influence paw oedema when given alone, neither did their combination (Figure 6d).

Antinociceptive effects of URB602 and involvement of cannabinoid receptors

When given locally (dorsal surface of the paw) at 70 μg, URB602 produced an antinociceptive effect when compared

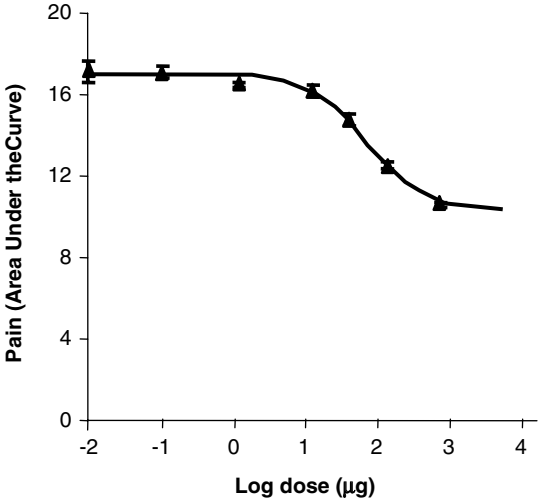

Figure 4 Dose–response curve for URB602 in the inflammatory phase of the formalin test. Data is expressed as mean ± s.e.m. ($n=4$ per group).

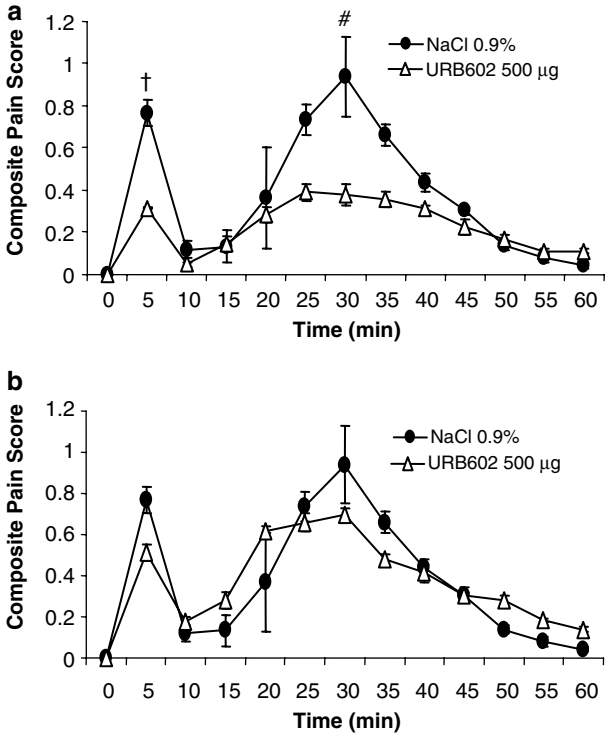

Figure 5 Antinociceptive effects of URB602 after injection in the dorsal surface of the ipsilateral (**a**) and contralateral (**b**) hind paws 15 min before 2.5% formalin (50 μl). Data is expressed as mean ± s.e.m. ($n=3$ per group). AUC for the acute phase, † ($F(1,4)=98.19$) $P<0.001$ for URB602 500 μg vs control (NaCl 0.9%); AUC for the inflammatory phase, # ($F(1,4)=257.99$) $P<0.001$ for URB602 500 μg vs control (NaCl 0.9%) group. In (**b**), there was no effect of URB602 given in the contralteral paw ($F(1,4)=5.25$ and 1.96 for the early and late phases, respectively).

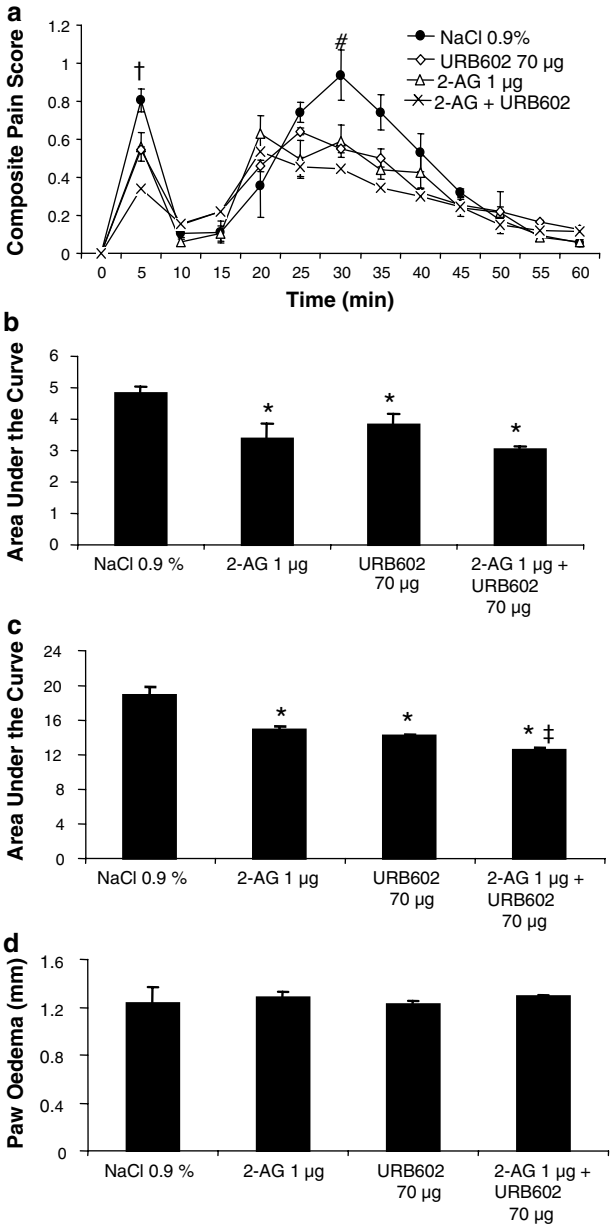

Figure 6 (**a**) Antinociceptive effects of 2-AG, URB602 and their combination at ED_{50} doses when administered into the hind paws 15 min before 2.5% formalin (50 μl). Antinociceptive effects of the combination of 2-AG with URB602 for the acute (**b**) and the inflammatory (**c**) phases of the test. Data is expressed as mean ± s.e.m. ($n=4$ per group). AUC for the acute phase, † ($F(1,12)=16.15$) $P<0.005$ for all the drugs vs control (NaCl 0.9%) group; AUC for the inflammatory phase, # ($F(1,12)=56.46$) $P<0.001$ for all the drugs vs control (NaCl 0.9%) group. *$P<0.005$ for all the drugs vs control (NaCl 0.9%) group; ‡ ($F(1,12)=7.91$) $P<0.025$ for URB602 + 2-AG vs 2-AG or URB602 given alone. In (**d**), values for the corresponding paw oedema are shown. No treatment affected oedema ($F(3,12)=0.17$): neither 2-AG nor URB602 ($F(1,12)=0.22$) nor their combination ($F(1,12)=0.15$) modified the oedema.

with the control group, reaching significance only for the late phase of the formalin test (Figure 7a and c). This antinociceptive effect was inhibited by AM251 and AM630 as there was no statistical difference between those antagonists given alone compared with their combination with URB602 for the early and late phases, respectively (Figure 7a–c).

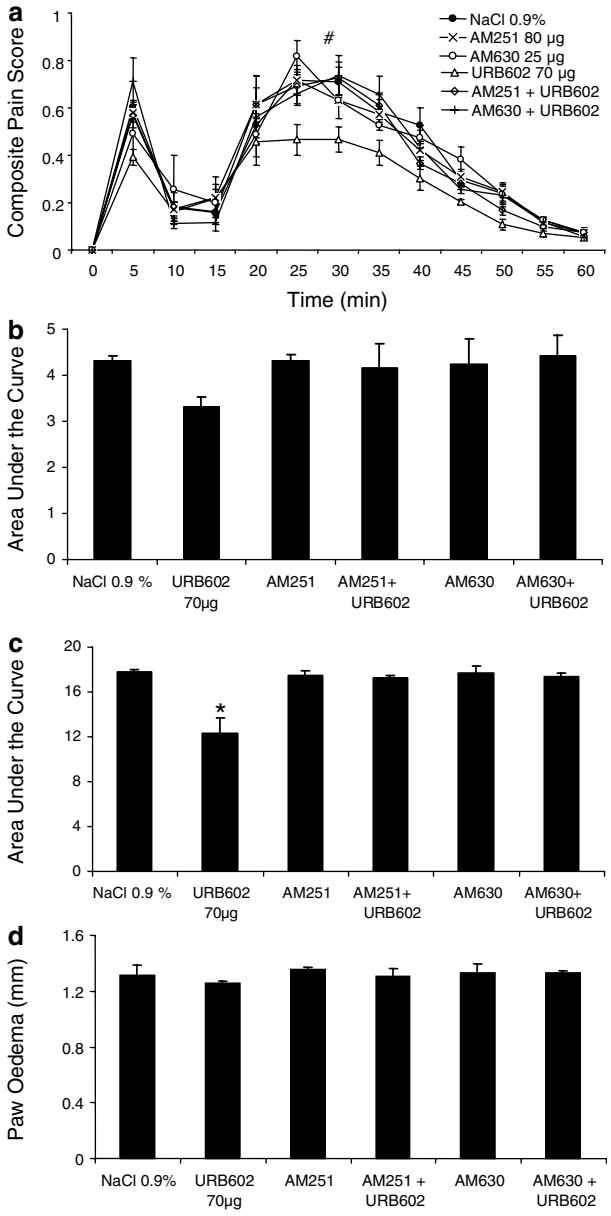

Figure 7 (**a**) Antinociceptive effects of URB602 when administered at a dose of 70 μg into the hind paw, 15 min before 2.5% formalin (50 μl). Effects of cannabinoid antagonists on the antinociceptive effects of URB602 for the early (**b**) and late (**c**) phases of the test. Data is expressed as mean$\pm$s.e.m. ($n=4$ per group). AUC, # ($F(1,18)=33.94$) $P<0.001$ for URB602 vs control (NaCl 0.9%) group; *$P<0.001$ for URB602 vs control (NaCl 0.9%) group. Note that the effect of URB602 was reversed by either AM251 (AM251 vs URB602 + AM251, ($F(1,18)=0.07$ and 0.11) or AM630 (AM630 vs URB602 + AM630, ($F(1,18)=0.05$ and 0.10), early and late phases respectively. In (**d**), values for paw oedema are shown; this did not differ among the groups ($F(5,18)=0.70$); neither URB602 ($F(1,18)=0.10$), AM251 ($F(1,18)=1.92$) nor AM630 ($F(1,18)=0.01$) affected oedema.

Paw oedema

Oedema of the injected paw did not differ among the groups: neither URB602 nor AM251 nor AM630 influenced paw oedema (Figure 7d).

Discussion

In this study, we have shown that local administration of 2-AG, an endocannabinoid and URB602, a selective MGL inhibitor, induced a dose-dependent antinociceptive effect in an inflammatory pain model. These effects were locally mediated and not systemic as 2-AG and URB602 given in the contralateral paw were not antinociceptive at doses higher than the ED_{50} doses used in the ipsilateral paw. Moreover, the antinociceptive effects of 2-AG were prevented by AM630, a selective CB_2 receptor antagonist, but not by AM251, a selective CB_1 receptor antagonist. It is important to note that the antinociceptive effects of non-selective (ibuprofen) or selective cyclooxygenase-2 inhibitors (rofecoxib) in this model were not affected by these CB_1 or CB_2 antagonists (Guindon *et al*., 2006a, b). Thus, these results suggest that 2-AG produces its local anti-inflammatory effects by activating CB_2 receptors in the periphery. Furthermore, local administration of 2-AG with URB602 produced an additive antinociceptive effect.

Although controversial (Coderre *et al*., 1990), there is evidence for the contribution of peripheral nociceptive activity to phase 2 of the behavioural response to formalin. Indeed, primary afferent activity is required for the expression of phase 2 nociceptive behaviours, and central sensitization alone does not underlie phase 2 (McCall *et al*., 1996; Puig and Sorkin, 1996). These authors report that C-fibres exhibit increased activity following the injection of formalin and that this activity occurs during the time periods corresponding to both phase 1 and phase 2, but not interphase, of the behavioural response. Furthermore, Pitcher and Henry (2002) have provided evidence for an essential role of afferent input during the second phase of the formalin test in generating and maintaining the elevated discharge of spinal nociceptive dorsal horn neurones in the second phase. Taken together, these findings on the second phase of the formalin test may favour a wider pharmacological approach to antinociception. Thus along with inhibitors of synaptic transmission from nociceptive afferents, antinociceptive treatments targeted at inhibiting primary afferent activity at a peripheral site could also be effective (Pitcher and Henry, 2002). These observations are important because in the presence of carrageenan inflammation, AM1241 (a CB_2 receptor agonist) following both systemic (i.v.) and intraplantar injections, specifically suppressed C-fibre-mediated activity and wind-up in wide dynamic range neurones (Nackley *et al*., 2004). Similar results were observed following activation of spinal cannabinoid receptors by HU210 that suppressed C-fibre-mediated post-discharge responses (a measure of neuronal hyperexcitability) but through a CB_1 mechanism (Drew *et al*., 2000).

Moreover, CB_2-selective agonists have recently been shown to produce antinociception in models of acute, inflammatory and nerve injury-induced nociception (Hanus *et al*., 1999; Clayton *et al*., 2002; Malan *et al*., 2002; Ibrahim *et al*., 2003, 2006; Nackley *et al*., 2003a; Quartilho *et al*., 2003). There is evidence that selective activation of peripheral CB_2 receptors is sufficient to display antinociceptive effects even though their exact distribution pattern in pain pathways and their precise role remain to be clarified. CB_2

receptors are mostly expressed in cells of the immune system such as B cells, T cells and macrophages (Munro *et al*., 1993; Galiègue *et al*., 1995). However, Munro *et al*. (1993) and Griffin *et al*. (1997) both demonstrated that CB_2 receptor messenger RNA (mRNA) was not detected in the brain, supporting the evidence that CB_2 receptors are absent in neurons of the central nervous system (Munro *et al*., 1993; Zimmer *et al*., 1999; Buckley *et al*., 2000) although recent data may have proved differently (Van Sickle *et al*., 2005). Nevertheless, such data predicts that CB_2 receptor-selective agonists will not produce the unwanted central nervous system effects, as caused by a CB_1-selective cannabinoid agonist. Moreover, there are also reports on the presence of CB_2 receptor mRNA in inflammatory and immune cells at levels 10–100 times those of CB_1 receptor mRNA (Facci *et al*., 1995; Galiègue *et al*., 1995) and on peripheral nerve terminals (Pertwee *et al*., 1995; Griffin *et al*., 1997). Indeed, Clayton *et al*. (2002) have shown that activation of CB_2 receptors by HU210 is sufficient to induce a substantial anti-inflammatory and analgesic effect in the carrageenan model of inflammation.

Endocannabinoids such as anandamide and 2-AG are present in peripheral tissues and their concentrations in paw tissue have already been measured (Beaulieu *et al*., 2000). Furthermore, the expression of CB_1 and CB_2 receptors has recently been reported in rat and mouse paw tissues (Walczak *et al*., 2005, 2006). Therefore, the antinociceptive effects of 2-AG may be mediated by a direct action on cannabinoid CB_2 receptors located in the injected paw. Indeed, 2-AG given on the contralateral side did not attenuate formalin-evoked pain behaviour, strongly suggesting that the effects of 2-AG were mediated by a local peripheral site of action. Similar results have been reported with anandamide administered locally (Guindon *et al*., 2006a, b). However, it remains to be demonstrated whether the antinociceptive effects of 2-AG are mediated through locally expressed CB_2 receptors and whether 2-AG itself is involved in this analgesic response. Moreover, the present study measured the antinociceptive effects of exogenously administered 2-AG and endogenous 2-AG may behave differently under physiological conditions.

However, other mechanisms by which CB_2 selective agonists induce antinociceptive effects may be involved. One possibility is that they could act on the peripheral terminals of primary afferent neurons (Malan *et al*., 2002), but their presence on peripheral neurons is still a matter of debate. Alternatively, an indirect mechanism may be involved; CB_2 selective receptor agonists could activate the CB_2 receptor on mast or other immune cells, thereby decreasing the inflammation-evoked release from local inflammatory cells of sensitizing molecules acting on the peripheral nociceptor (e.g. nerve growth factor, prostanoids, cytokines, adenosine triphosphate, 5-hydroxytryptamine or histamine) and thus decreasing the sensitivity of primary afferent neurons and inhibiting pain response (Malan *et al*., 2002). Another hypothesis is that CB_2 cannabinoid receptors may inhibit the responsiveness of primary afferent neurones by stimulating local release of the endogenous opioid endorphin from cells such as keratinocytes (Malan *et al*., 2004).

Moreover, a selective inhibition of 2-AG degradation could offer the opportunity to investigate the functions of 2-AG by blocking its deactivation and thus amplify its intrinsic actions and enhance its analgesic effects. Therefore, we have tested the hypothesis that a selective inhibitor of the 2-AG-deactivating enzyme, MGL, may selectively elevate 2-AG concentrations in the periphery and thereby decrease pain behaviour. Thus, Hohmann *et al*. (2005) developed URB602, a non-competitive MGL inhibitor that blocks 2-AG hydrolysis in rat brain slices without affecting FAAH-catalysed anandamide degradation. Interestingly, Makara *et al*. (2005) have shown that URB602 increased 2-AG levels and enhanced retrograde signalling from pyramidal neurons to GABAergic terminals in the hippocampus. Therefore, a possible mechanism for the antinociceptive effects of locally administered URB602 in the hind paw may involve the increase of 2-AG concentrations in the periphery. Moreover, the ability of inhibitors of either MGL or FAAH to magnify endocannabinoid-dependent, stress-induced analgesia also highlights the significance of these enzymes as previously un-exploited targets for the treatment of pain and stress-related disorders (Hohmann *et al*., 2005). However, the specificity of URB602 for inhibiting MGL at the concentrations employed in the present study cannot readily be determined without measuring endocannabinoid levels in the peripheral paw tissue.

The fact that the antinociceptive effects of URB602 were inhibited by both cannabinoid antagonists was unexpected. Indeed, if URB602 inhibited MGL and therefore 2-AG degradation, we would have thought that AM251 would not interfere with the antinociceptive effects of URB602, as it did not when administered together with 2-AG. One explanation may be that 2-AG present locally in a high concentration acted on both CB_1 and CB_2 receptors to produce its antinociceptive effects. Another possibility is that, in the presence of an inhibitor of MGL, there is a shift of 2-AG catabolism towards FAAH, the enzyme responsible for the hydrolysis of anandamide, which is also responsible for the degradation of 2-AG (Di Marzo *et al*., 1998). In that case, an increased concentration of anandamide, which is also present in peripheral tissue, may explain the antinociceptive effects observed.

Furthermore, although URB602 is a selective inhibitor of MGL, it has low potency (Hohmann *et al*., 2005). Therefore, the use of a more potent and selective agent could be considered, such as URB574 which inhibits MGL activity with a IC_{50} of about two orders of magnitude lower than URB602 (Makara *et al*., 2005). In addition, Begg *et al*. (2005) have shown recent evidence for cannabinoid receptors beyond CB_1 and CB_2. Thus, 2-AG may exert its antinociceptive effects at uncharacterized CB_2-like receptors, activated by 2-AG and inhibited by AM630, a selective CB_2 receptor antagonist.

These findings are the first to demonstrate that peripherally injected 2-AG, URB602 and their combination decreased pain behaviour in a model of inflammatory pain. The present work provides evidence that activation of a cannabinoid CB_2 mechanism in the periphery is sufficient to decrease nociception in animal models of inflammatory pain. Finally, as cannabinoid-based drugs could be effective

for the treatment of inflammatory pain responses, this approach is interesting because peripheral administration of drugs could avoid troublesome psychotropic and other systemic effects.

Acknowledgements

This work was supported by a grant from the Association des Anesthésiologistes du Québec.

Conflict of interest

The authors state no conflict of interest.

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