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Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models

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1 While cannabinoid receptor agonists have analgesic activity in chronic pain states, they produce a spectrum of central CB_1 receptor-mediated motor and psychotropic side effects. The actions of endocannabinoids, such as anandamide are terminated by removal from the extracellular space, then subsequent enzymatic degradation by fatty-acid amide hydrolase (FAAH). In the present study, we compared the effect of a selective FAAH inhibitor, URB597, to that of a pan-cannabinoid receptor agonist HU210 in rat models of chronic inflammatory and neuropathic pain.

2 Systemic administration of URB597 ($0.3\,mg\,kg^{-1}$) and HU210 ($0.03\,mg\,kg^{-1}$) both reduced the mechanical allodynia and thermal hyperalgesia in the CFA model of inflammatory pain. In contrast, HU210, but not URB597, reduced mechanical allodynia in the partial sciatic nerve-ligation model of neuropathic pain. HU210, but not URB597, produced a reduction in motor performance in unoperated rats.

3 The effects of URB597 in the CFA model were dose dependent and were reduced by coadministration with the cannabinoid CB_1 antagonist AM251 ($1\,mg\,kg^{-1}$), or the CB_2 and SR144528 ($1\,mg\,kg^{-1}$). Coadministration with AM251 plus SR144528 completely reversed the effects of URB597.

4 These findings suggest that the FAAH inhibitor URB597 produces cannabinoid CB_1 and CB_2 receptor-mediated analgesia in inflammatory pain states, without causing the undesirable side effects associated with cannabinoid receptor activation.

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Abbreviations: 2-AG, 2-arachidonoyl glycerol; AUC, area under the curve; CFA, complete Freund's adjuvant; DMSO, dimethyl sulphoxide; FAAH, fatty-acid amide hydrolase; PNL, partial sciatic nerve ligation; PWT, paw withdrawal threshold; PWL, paw withdrawal latency; THC, Δ^9-tetrahydrocannabinol

Introduction

The psychoactive ingredient of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (THC), is known to produce its physiological actions *via* an endogenous cannabinoid neurotransmitter system, specifically cannabinoid G-protein-coupled CB_1 and CB_2 receptors (Pertwee, 2005). There is now considerable evidence demonstrating that THC and a number of synthetic cannabinoid receptor agonists have analgesic activity in acute and chronic pain models. In particular, cannabinoid agonists reduce the allodynia (pain due to normally non-noxious stimuli) and hyperalgesia (increased pain sensitivity to normally noxious stimuli) associated with nerve injury-induced models of neuropathic pain (Herzberg *et al*., 1997; Bridges *et al*., 2001; Fox *et al*., 2001; Scott *et al*., 2004) and with inflammatory pain models (Smith *et al*., 1998; Hanus *et al*., 1999; Clayton *et al*., 2002; Kehl *et al*., 2003; De Vry *et al*., 2004). The antiallodynic, antihyperalgesic and anti-inflammatory actions of cannabinoid agonists in these chronic pain models are mediated *via* both cannabinoid CB_1 and CB_2 receptors (Hanus *et al*., 1999; Bridges *et al*., 2001; Fox *et al*., 2001; Clayton *et al*., 2002; Ibrahim *et al*., 2003; Kehl *et al*., 2003; De Vry *et al*., 2004). However, non-selective cannabinoid agonists produce a spectrum of motor and psychotropic side effects, which are mediated by central cannabinoid CB_1 receptors (Compton *et al*., 1993; Herzberg *et al*., 1997; Fox *et al*., 2001; Malan *et al*., 2001; Scott *et al*., 2004).

Like other neurotransmitter systems, the components of the cannabinoid signalling system also include endogenous cannabinoids (endocannabinoids), such as arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG), as well as mechanisms for their synthesis, membrane transport and metabolism. The actions of endocannabinoids are terminated by removal from the extracellular space (anandamide *via* an anandamide membrane transporter), then subsequent enzymatic degradation (Hillard & Jarrahian, 2003; Lambert & Fowler, 2005). To date, two enzymes have been identified that metabolise endocannabinoids, namely fatty-acid amide hydrolase (FAAH) and monoglyceride lipase (MGL), which preferentially degrade anandamide and 2-AG, respectively (Sugiura *et al*., 1995; Cravatt *et al*., 1996;

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Goparaju *et al*., 1998; 1999; Beltramo & Piomelli, 2000; Dinh *et al*., 2002; Saario *et al*., 2004). It has been demonstrated that systemic application of anandamide produces analgesia in a number of acute and inflammatory pain models, albeit with reduced efficacy compared to synthetic cannabinoid receptor agonists (Devane *et al*., 1992; Fride & Mechoulam, 1993; Smith *et al*., 1994; Compton & Martin, 1997; Calignano *et al*., 1998; Jaggar *et al*., 1998; Richardson *et al*., 1998b). The reduced efficacy of systemically administered endocannabinoids is likely to be due to their rapid degradation, because metabolically stable anandamide analogues have increased analgesic efficacy and nonselective enzyme inhibitors enhance anandamide induced analgesia *via* cannabinoid CB_1 receptor-dependent mechanisms (Compton & Martin, 1997; Adams *et al*., 1998).

There is conflicting evidence as to whether endogenously released cannabinoids have a pain modulatory role. In support of this proposition, it has been demonstrated that painful stimuli increase anandamide release within pain modulatory brain structures (Walker *et al*., 1999). In addition, the selective cannabinoid CB_1 receptor antagonist, SR141716 increases allodynia and hyperalgesia in inflammatory and neuropathic pain models, produces hyperalgesia in acute pain models and enhances pain responsiveness to the formalin test (Herzberg *et al*., 1997; Calignano *et al*., 1998; Richardson *et al*., 1998a; Strangman *et al*., 1998). In contrast, other studies have been unable to demonstrate an 'endogenous cannabinoid tone' in these pain models (Beaulieu *et al*., 2000; Fox *et al*., 2001). The differences between these studies might be due to variations in stress levels and to a reduction in endogenous cannabinoid levels *via* metabolism. Thus, mice with a deletion of FAAH are hypoalgesic and display an increase in anandamide-induced analgesia (Cravatt *et al*., 2001; Lichtman *et al*., 2004b). Recently, a number of potent and selective FAAH inhibitors have been identified, including URB597, OL-53 and OL-135 (Boger *et al*., 2000; Kathuria *et al*., 2003; Lichtman *et al*., 2004a). In the present study, we examined the effects of the selective FAAH inhibitor, URB597, on allodynia and hyperalgesia in animal models of neuropathic and inflammatory pain.

Methods

Male Sprague–Dawley rats, initially weighing between 160 and 200 g, were used for all experiments. Animals were housed individually, under a 12 : 12 h light/dark cycle, with environmental enrichment and free access to food and water. All animals were allowed to acclimatize to their holding cages for 3–4 days before any behavioural, or surgical procedures were carried out. All experiments were carried out in the light cycle. Experiments were carried out following the guidelines of the NH&MRC 'Code of Practice for the Care and Use of Animals in Research in Australia' and with the approval of the Royal North Shore Hospital/University of Technology Sydney Animal Care and Ethics Committee.

For the inflammatory pain model, 0.15 ml of Complete Freund's Adjuvant (CFA, Sigma, Sydney, Australia) was injected subcutaneously into the plantar surface of the rear left hand paw under brief halothane (1–3% in O_2) anaesthesia. For the neuropathic pain model, rats underwent partial ligation of the sciatic nerve (PNL) under halothane anaesthesia (Seltzer *et al*., 1990). Briefly, the left sciatic nerve was exposed at mid-thigh level and freed from the surrounding connective tissue at a site near the trochanter just distal to the posterior biceps semitendinosus nerve branches off the common sciatic nerve. A 4–0 silk suture was inserted into the nerve to tightly ligate the dorsal 1/3–1/2 of the nerve trunk approximately 3 mm proximal to the trifurcation of the sciatic nerve at the popliteal fossa. The muscle (4–0) and then the skin (3–0) were closed with silk sutures. In sham-operated animals, the left sciatic nerve was exposed as above, but was left intact.

To assess mechanical allodynia, mechanical paw withdrawal thresholds (PWTs) were measured with a series of von Frey hairs (range 0.4–15 g). Rats were placed in elevated perspex enclosures (28 × 15 × 18 cm) with wire mesh bases and given 15–20 min to acclimatise to the testing environment. Each von Frey hair was tested six times at random locations on the plantar surface of the left hindpaw. Von Frey hairs were pressed perpendicularly against the hindpaw and held for approximately 2 s. Testing began with the 2.0 g von Frey hair. A positive withdrawal response was noted if the paw was sharply withdrawn, if any paw licking took place, or if the animal flinched upon removal of the von Frey filaments. If the animal responded, then the next heavier hair was tested. If the animal did not respond, then the next lighter hair was tested. Once there was a change in response, four more hairs were tested and the mechanical PWT was calculated using the up–down paradigm (Chaplan *et al*., 1994). If the animals did, or did not respond to any hairs, then the mechanical PWT was assigned as 0.2 g, or 15 g, respectively. To measure thermal paw withdrawal latency (PWL) rats were placed in perspex enclosures (15 × 15 × 18 cm) and given 10–15 min to acclimatise. The testing was conducted using a plantar tester (Ugo Basile, Italy) according to the method of Hargreaves *et al*. (1988). Focal infrared heat was applied through the plastic bottom of the cage to the rear left hand paw and the latency for the rat to respond by moving its paw away from the noxious heat source was recorded. To measure motor performance, ambulation was tested using a rotarod device (Ugo Basile, Italy), with a maximal cutoff time of 300 s (e.g. Fox *et al*., 2001; Malan *et al*., 2001). Animals were tested for mechanical PWT, thermal PWL and trained on the rotarod at least three times, on consecutive days to allow accommodation to the testing apparatus before performing any procedures.

The effect of all drugs on pain behaviours was measured at 13–15 days post-PNL surgery and 24–48 h post-CFA injection, as in prior studies (Martin *et al*., 1999; Fox *et al*., 2001). The effect of all drugs on rotarod latency was measured in unoperated animals. On the day of the experiment, behavioural testing was carried out over 30 min before, then over a 6 h period following drug injection. Experimenters were not blind to the drugs injected. URB597 (Kathuria *et al*., 2003), HU210, AM251 (Tocris Cookson, Bristol, U.K.) and SR144528 (gift of Sanofi-Synthelabo, France) were made up in a vehicle solution comprising (v/v%) 18% dimethyl sulphoxide (DMSO), 1% ethanol, 1% Tween-80 and 80% saline on the day of the experiment, and were injected intraperitoneally in a total volume of $1\,\mathrm{ml\,kg^{-1}}$. Each animal underwent only one experiment.

Plots of mechanical PWT, thermal PWL and rotarod latency are presented as mean ± s.e.m. Mean changes in behavioural scores produced by drug injection were calculated as the integral of postinjection values relative to preinjection mean baseline (area-under-the-curve, AUC). Statistical comparisons of behavioural scores were made using a one-way analysis of variance (ANOVA), with time as a within-subjects factor where appropriate. When one-way ANOVAs were significant, *post hoc* comparisons were made against the time 0 point at 24 h post-CFA, or 14 days post-PNL (time effects), or against the vehicle-injected group; using Dunnett's adjustment for multiple comparisons.

Results

URB597 does not affect allodynia in a neuropathic pain model

Prior to PNL surgery and CFA injection, mechanical PWTs were at, or near the cutoff threshold of 15.0 g (Figure 1a and b). Following PNL surgery, the mechanical PWT decreased within 1–2 days and remained stable for 14 days postsurgery (data not shown). The mean mechanical PWT was 14.6 ± 0.4 g prior to PNL surgery and 0.9 ± 0.2 g 14 days after PNL surgery (Figure 1a, $P<0.05$, $n=21$). Following surgery, there was also a transient and variable decrease in thermal PWL ($P>0.05$ one-way ANOVA, $n=18$). The mean thermal PWL was 8.0 ± 0.7 s before PNL surgery, and 4.4 ± 0.5 and 6.1 ± 0.4 s at 7 and 14 days postsurgery. We subsequently examined the effect of cannabinoids only on mechanical PWT at 14 days post-nerve ligation. The mechanical PWT and thermal PWL of matched sham-operated animals did not change over the 14-day postsurgery period ($P>0.05$ one-way ANOVA, $n=8$).

In PNL animals, intraperitoneal administration of the selective FAAH inhibitor URB597 (0.3 mg kg^{-1}) produced no significant change in mechanical PWT over the 6-h time course following injection (Figure 1a, $P=0.3$ one-way ANOVA, $n=6$). In contrast, the pan-cannabinoid agonist HU210 (30 μg kg^{-1}) produced an increase in mechanical PWT which was significant between 1 and 6 h following injection (Figure 1a, $P<0.0005$, $n=8$). A matched group of animals showed no significant change in mechanical PWT after injection of vehicle alone (Figure 1a; $P=0.2$ one-way ANOVA, $n=15$). HU210, but not URB597, produced an increase in the AUC for mechanical PWT which was greater than that produced by vehicle alone ($P<0.0001$ and $=1.0$, respectively).

URB597 reduces allodynia and hyperalgesia in an inflammatory pain model

Intraplantar injection of CFA produced a significant decrease in mechanical PWT and thermal PWL at 24 h postinjection (Figure 1b and c, $P<0.001$). which was maintained for at least 5 days postinjection ($n=6$). The mean mechanical PWT was 14.3 ± 0.2 g before and 1.4 ± 0.2 g at 24–48 h after CFA injection ($n=95$). The mean thermal PWL was 12.8 ± 0.6 s before and 4.3 ± 0.3 s at 24–48 h after CFA injection. We subsequently examined the effect of cannabinoids on both mechanical PWT and thermal PWL at 24–48 h post-CFA injection.

In CFA animals, URB597 (0.3 mg kg^{-1}) produced an increase in mechanical PWT, which was significant between 1 and 6 h following injection (Figure 1b; $P<0.05$, $n=8$). HU210 (0.03 mg kg^{-1}) produced an increase in mechanical PWT, which was significant between 0.5 and 6 h following injection (Figure 1b; $P<0.01$, $n=13$). A matched group of animals showed no significant change in mechanical PWT following injection of vehicle alone (Figure 1b; $P=0.6$ one-way ANOVA, $n=13$). In these animals, URB597 (0.3 mg kg^{-1}) also produced an increase in thermal PWL, which was significant between 1 and 6 h following injection (Figure 1c; $P<0.05$, $n=8$). HU210 (0.03 mg kg^{-1}) produced an increase in thermal PWL, which was significant between 1 and 6 h following injection (Figure 1c; $P<0.005$ *post hoc* test, $n=12$).

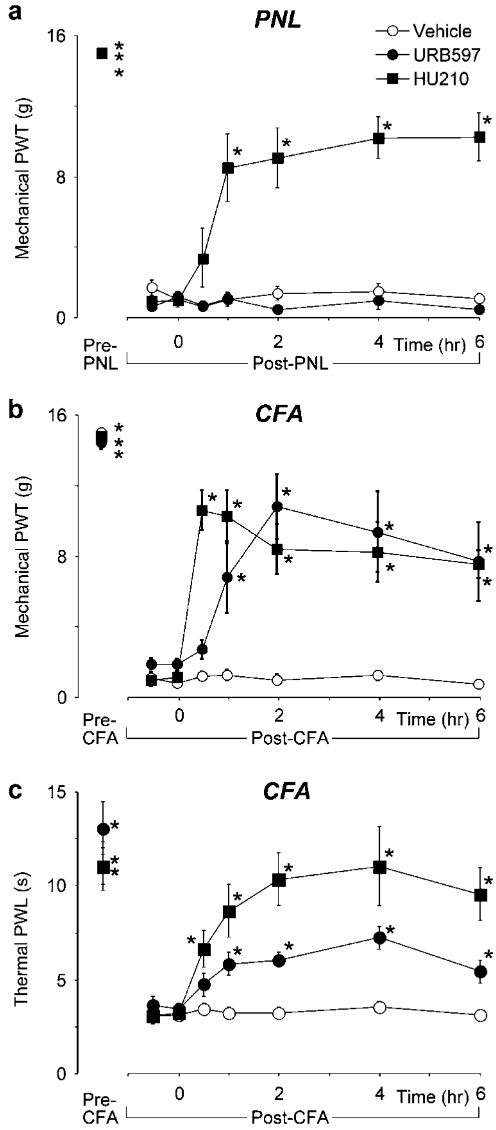

Figure 1 URB597 reduces allodynia in an inflammatory, but not a neuropathic pain model. Time plots of the effect of URB597 (0.3 mg kg^{-1}, filled circles), HU210 (0.03 mg kg^{-1}, filled squares) or vehicle alone (open circles) on (a) mechanical paw withdrawal threshold (PWT) in nerve-injured animals, (b) mechanical PWT in complete Freund's adjuvant (CFA)-injected animals and (c) thermal paw withdrawal latency (PWL) in CFA-injected animals. Animals received an intraperitoneal injection of URB597, HU210 or a matched vehicle at time 0 h (a) 14 days after partial ligation of the sciatic nerve (post-PNL), or (b, c) 24 h after CFA injection (post-CFA) into the plantar surface of the hindpaw. The mechanical PWT and thermal PWT are also shown prior to nerve injury (Pre-PNL) and CFA injection (Pre-CFA). Data are shown as the mean ± s.e.m. *Denotes $P<0.05$ compared to time 0 post-PNL, or post-CFA.

A matched group of animals showed no significant change in thermal PWL following injection of vehicle alone (Figure 1c; $P=0.9$, $n=13$).

The effects of URB597 are mediated by cannabinoid CB_1 and CB_2 receptors

We next examined the dose dependence and the involvement of cannabinoid receptors in the URB597-induced antiallodynia and antihyperalgesia in CFA-inflammatory animals. For mechanical PWT, the AUC for URB597 at $0.1\,\mathrm{mg\,kg^{-1}}$ ($P=0.02$, $n=9$) and $0.3\,\mathrm{mg\,kg^{-1}}$ ($P<0.0001$, $n=8$), but not at $0.03\,\mathrm{mg\,kg^{-1}}$ ($P=0.9$, $n=6$) was significantly greater than that for vehicle alone (Figure 2a). The AUC for HU210 was significantly greater than for vehicle alone (Figure 2a, $P<0.0001$, $n=9$). The AUC for URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) in combination with the cannabinoid CB_1 antagonist AM251 ($1\,\mathrm{mg\,kg^{-1}}$), or the cannabinoid CB_2 antagonist SR144528 ($1\,\mathrm{mg\,kg^{-1}}$) was not significantly greater than for vehicle alone (Figure 3a, $P=0.3$ and 0.6, $n=6$ and 7). The AUC for URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) in combination with both AM251 and SR144528 was not significantly greater than for vehicle alone (Figure 3a, $P=1.0$, $n=6$). The AUC for URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) in combination with AM251 ($P=0.01$), SR144528 ($P=0.003$) or AM251 plus SR144528 ($P=0.0003$) was less than that for URB597 alone. The AUC for AM251 in combination with SR144528 alone was not significantly different to that for vehicle alone (Figure 3a, $P=1.0$, $n=4$).

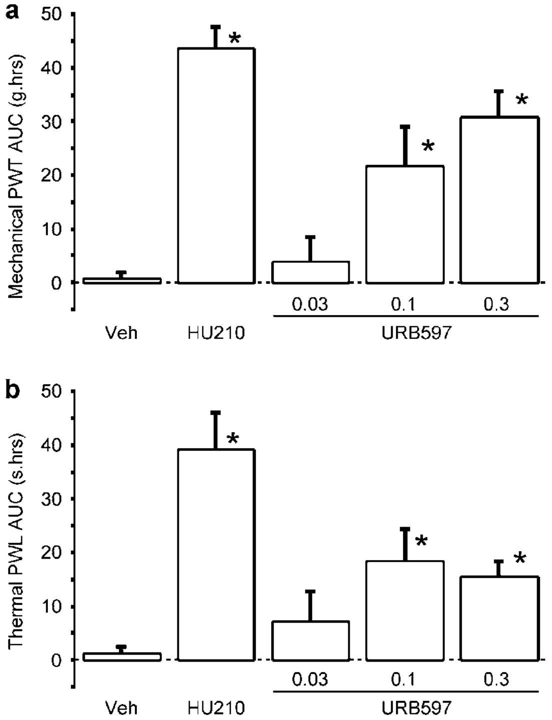

Figure 2 The effect of URB597 is dose dependent. Bar charts depicting the mean effect of intraperitoneal injection of cannabinoids on (a) mechanical paw withdrawal threshold (PWT) and (b) thermal paw withdrawal latency (PWL) in animals 24 h after CFA injection (post-CFA) into the plantar surface of the hindpaw. The mean effect of URB597 (0.03–$0.3\,\mathrm{mg\,kg^{-1}}$), HU210 ($0.03\,\mathrm{mg\,kg^{-1}}$) and vehicle were calculated as the area under the curve (AUC) postinjection compared to the preinjection baseline value. Data are shown as the mean ± s.e.m. *Denotes $P<0.05$, compared to vehicle.

For thermal PWL, the AUC for URB597 at the 0.1 and $0.3\,\mathrm{mg\,kg^{-1}}$ doses ($P=0.005$ and 0.03, $n=9$ and 8), but not at the $0.03\,\mathrm{mg\,kg^{-1}}$ dose ($P=0.6$, $n=6$) was significantly greater than that for vehicle alone (Figure 2b). The AUC for HU210 was significantly greater than that for vehicle alone (Figure 2b, $P<0.0001$). The AUC for URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) in combination with the cannabinoid CB_1 antagonist AM251 ($1\,\mathrm{mg\,kg^{-1}}$, $P=0.2$, $n=6$), or the cannabinoid CB_2 antagonist SR144528 ($1\,\mathrm{mg\,kg^{-1}}$, $P=0.8$, $n=7$) was not significantly greater than for vehicle alone (Figure 2b). The AUC for URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) in combination with both AM251 and SR144528 was not significantly greater than for vehicle alone (Figure 3b, $P=1.0$, $n=6$). The AUC for URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) in combination with SR144528 ($P=0.03$), or AM251 plus SR144528 ($P=0.005$), but not AM251 ($P=0.3$), was less than that for URB597 alone. The AUC for AM251 in combination with SR144528 alone was not significantly different to that for vehicle alone (Figure 3b, $P=1.0$, $n=4$).

URB597 does not affect motor activity

We next examined the effect URB597 on motor ambulation using the rotarod test. In unoperated animals, URB597 produced no significant change in rotarod latency over the

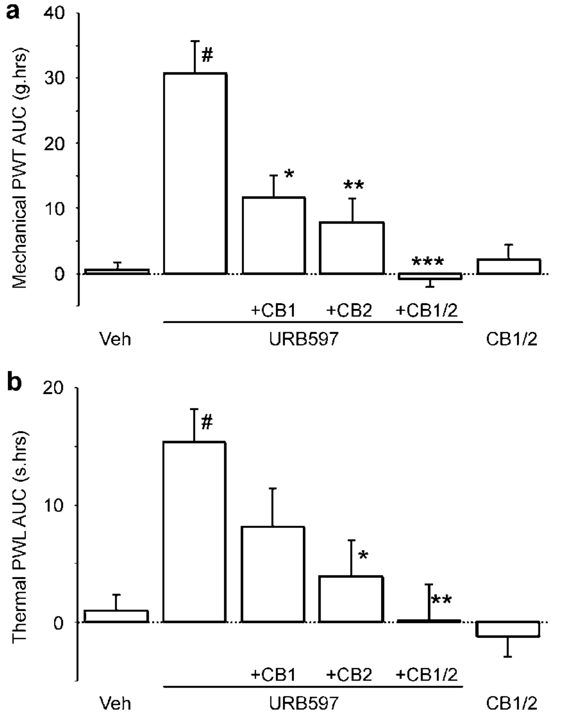

Figure 3 The effect of URB597 is mediated by cannabinoid CB1 and CB2 receptors. Bar charts depicting the mean effect of intraperitoneal injection of cannabinoids on (a) mechanical paw withdrawal threshold (PWT) and (b) thermal paw withdrawal latency (PWL) in animals 24 h after CFA injection (post-CFA) into the plantar surface of the hindpaw. The mean effects of URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$) alone; in combination with the cannabinoid CB_1 receptor antagonist AM251 ($1.0\,\mathrm{mg\,kg^{-1}}$) and/or the cannabinoid CB_2 receptor antagonist SR144528 ($1.0\,\mathrm{mg\,kg^{-1}}$); AM251 and SR144528 alone; and vehicle were calculated as the area under the curve (AUC) postinjection compared to the preinjection baseline value. Data are shown as the mean ± s.e.m. $^{\#}P<0.001$, compared to vehicle. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared to URB597 alone.

6-h time course following injection (Figure 4a, $P = 0.5$ one-way ANOVA, $n = 6$). In contrast, HU210 produced a decrease in rotarod latency, which was significant between 2 and 6 h following injection (Figure 4a; $P < 0.0005$, $n = 6$). A matched group of animals showed no significant change in rotarod latency following injection of vehicle alone (Figure 3a, $P = 0.4$ one-way ANOVA, $n = 6$). Rotarod latency, the AUC for HU210 ($P = 0.0002$), but not URB597 ($P = 0.9$), was significantly greater than that for vehicle alone (Figure 4b).

Discussion

In the present study, it has been demonstrated that acute systemic administration of the selective FAAH inhibitor URB597, like the pan-cannabinoid receptor agonist HU210, reduces the mechanical allodynia and thermal hyperalgesia associated with an inflammatory pain model. In contrast, HU210, but not URB597, reduced allodynia in a chronic neuropathic pain model and reduced motor performance. The effects of URB597 in the CFA-induced model of inflammation were mediated by both CB_1 and CB_2 cannabinoid receptors. These findings suggest that FAAH inhibitors may produce analgesia, at least in inflammatory pain states, without producing the side effects generally associated with cannabinoid receptor agonist administration.

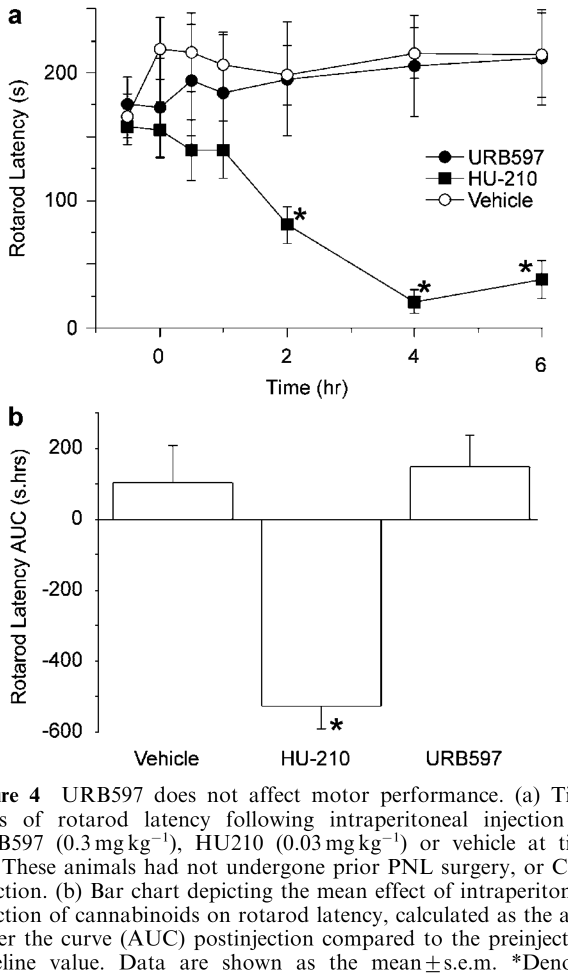

Figure 4 URB597 does not affect motor performance. (a) Time plots of rotarod latency following intraperitoneal injection of URB597 ($0.3\,\mathrm{mg\,kg^{-1}}$), HU210 ($0.03\,\mathrm{mg\,kg^{-1}}$) or vehicle at time 0 h. These animals had not undergone prior PNL surgery, or CFA injection. (b) Bar chart depicting the mean effect of intraperitoneal injection of cannabinoids on rotarod latency, calculated as the area under the curve (AUC) postinjection compared to the preinjection baseline value. Data are shown as the mean ± s.e.m. *Denotes $P < 0.05$, compared to time 0 post-PNL, or post-CFA in (a) and compared to vehicle in (b).

The antiallodynic and antihyperalgesic actions of URB597 in the inflammatory pain model were likely to have been (indirectly) due to activation of cannabinoid CB_1 and CB_2 receptors. In the present study, the effects of URB597 were mimicked by HU210 and were reduced by the selective cannabinoid CB_1 and CB_2 receptor antagonists, AM251 and SR144528. These results are consistent with prior studies which have shown similar CB_1 and CB_2 receptor-mediated effects of cannabinoid agonists in a number of inflammatory pain models (Smith *et al.*, 1998; Hanus *et al.*, 1999; Clayton *et al.*, 2002; Kehl *et al.*, 2003; De Vry *et al.*, 2004). Furthermore, coadministration of AM251 and SR144528 completely reversed the actions of URB597. This reversal was not due to inverse agonism because coadministration of AM251 and SR144528 alone had no significant effect. While these observations suggest that the actions of URB597 were mediated by cannabinoid receptors, a role for other endocannabinoid targets, such as TRPV1, cannot be excluded (see below).

The effects of URB597 were likely to have been due to elevations in endocannabinoid(s), subsequent to inhibition of FAAH, an enzyme which preferentially metabolises fatty acids, including anandamide, palmitoylethanolamine and oleamide, and possibly 2-AG (Cravatt *et al.*, 1996; Goparaju *et al.*, 1999; Beltramo & Piomelli, 2000; Saario *et al.*, 2004; Lambert & Fowler, 2005). URB597 is a selective FAAH inhibitor and does not interact with anandamide transport, or cannabinoid CB_1 and CB_2 receptors (Kathuria *et al.*, 2003; Lichtman *et al.*, 2004a). In the present study, the effect of URB597 was dose dependent, producing maximal effects at doses ($0.3\,\mathrm{mg\,kg^{-1}}$) similar to those which maximally inhibit FAAH activity *in vivo* (Kathuria *et al.*, 2003). However, other unrelated, selective FAAH inhibitors, such as OL-135 (Lichtman *et al.*, 2004a), need to be examined to confirm the involvement of FAAH in the antiallodynic and antihyperalgesic actions observed in the present study. While the identity of the specific endocannabinoid(s) which mediated the effects of URB597 were not directly identified in the present study, anandamide, palmitoylethanolamide and 2-AG have analgesic and anti-inflammatory actions in a variety of pain models (Lambert & Fowler, 2005). However, it might be noted that some of the actions of these endocannabinoids, particularly the anti-inflammatory agent palmitoylethanolamide, are not mediated by cannabinoid CB_1, or CB_2 receptors. It is also possible that the actions of URB597 were mediated by more complex mechanisms. Endocannabinoids, such as anandamide, are degraded by other biosynthetic pathways, such as lipoxygenases and cyclooxygenase-2 (Lambert & Fowler, 2005). Thus, it is possible that FAAH inhibition diverted endocannabinoid degradation through other enzymatic pathways, yielding active metabolites. In addition, other endogenous analgesic agents such as the *N*-arachidonyl amino acids have been shown to interact with FAAH (Huang *et al.*, 2001; Cascio *et al.*, 2004). However, the actions of these, or other endogenous agents must have been mediated by AM251- and SR144528-sensitive mechanisms.

Unlike the CFA-induced inflammatory model, URB597 had no effect in the partial sciatic nerve ligation model of neuropathic pain. The differential effects of URB597 in the two chronic pain models may have been due changes in endocannabinoid receptor systems. The lack of effect of URB597 in the neuropathic pain model was unlikely to be due to downregulation of cannabinoid CB_1 and/or CB_2 receptors because HU210 reduced allodynia, as demonstrated previously with a number of cannabinoid agonists and neuropathic pain models (Herzberg *et al*., 1997; Bridges *et al*., 2001; Fox *et al*., 2001; De Vry *et al*., 2004; Scott *et al*., 2004). Indeed, an increase in cannabinoid potency/efficacy might be expected because there is an upregulation of cannabinoid CB_1 receptors in pain processing centres following peripheral nerve injury (Siegling *et al*., 2001; Lim *et al*., 2003). As noted above, anandamide might also act *via* TRPV1 (Pertwee, 2005), which is upregulated in both inflammatory and neuropathic pain models (Carlton & Coggeshall, 2001; Fukuoka *et al*., 2002). TRPV1 receptor deletion and antagonists have antihyperalgesic and antiallodynic activity in inflammatory pain models and to a lesser extent in neuropathic pain models (Ossipov *et al*., 1999; Caterina *et al*., 2000; Walker *et al*., 2003; Honore *et al*., 2005). Thus, pronociceptive TRPV1-mediated actions of endocannabinoids might reduce their antiallodynic and antihyperalgesic cannabinoid receptor-mediated effects, even in the neuropathic pain model. However, the role of TRPV1 in the actions of URB597 remains to be determined.

The differential effects of URB597 in the two chronic pain models may have been due to a number of other factors. Firstly, the lack of effect of URB597 in the neuropathic pain model may have been due to the dosing regime used in the present study. URB597 produces analgesia in naïve animals (Kathuria *et al*., 2003) and reduces inflammation in the carrageenan model (Holt *et al*., 2005), although only at doses above those used in the present study. In this regard, repeated administration of URB597 may prove more efficacious in neuropathic pain models, as observed previously for cannabinoid receptor agonists (Costa *et al*., 2004). Secondly, there may have been differential changes in endocannabinoids, or their metabolism. The present results are consistent with the observation that FAAH deletion reduces the development of thermal hyperalgesia in mice in inflammatory (intraplantar carrageenan), but not in neuropathic (chronic constriction of the sciatic nerve) pain models (Lichtman *et al*., 2004b). Thirdly, the differences may have been due to region-specific changes in FAAH activity and/or endocannabinoids. FAAH is widely expressed throughout the nervous system and peripheral tissues (Cravatt *et al*., 1996; 2004; Tsou *et al*., 1998), and cannabinoid CB_1 and CB_2 receptor activation modulates both pain transmission and peripheral inflammation (see Introduction). URB597 might have had a direct anti-inflammatory action *via* local enhancement of endocannabinoids, such as palmitoylethanolamide, although this was not directly measured in the present study. In this regard, carrageenan induced inflammation in mice is reduced by FAAH deletion (Lichtman *et al*., 2004a) and by URB597 pretreatment ($ED_{50} \sim 0.3\,mg\,kg^{-1}$) in an SR144528-sensitive manner (Holt *et al*., 2005). In contrast, the antiallodynic and antihyperalgesic actions of URB597 in the present study were near maximal at $0.3\,mg\,kg^{-1}$ and were mediated by both CB1 and CB2 receptors. While this may reflect species and inflammatory pain model differences, these results suggest that URB597 targets both peripheral inflammatory processes and (central and peripheral) pain pathways. Finally, the differences between inflammatory and neuropathic pain states might also reflect specific endocannabinoid adaptations in pain transmission and modulation. While the central actions of URB597 in chronic pain states are unknown, it has recently been demonstrated that inhibition of FAAH and MGL enhances stress-induced analgesia by elevating endocannabinoids levels within central pain pathways (Hohmann *et al*., 2005). The changes in central and peripheral endocannabinoids, FAAH and MGL in both the neuropathic and inflammatory pain models, however, remain to be determined (Calignano *et al*., 1998).

In the present study, URB597, unlike HU210, lacked motor side effects in unoperated animals. These findings are in agreement with prior studies which have shown that non-selective cannabinoid agonists, such as HU210 (and WIN55,212 and CP55,940), but not URB597 produce a depression of spontaneous locomotor activity, catalepsy and hypothermia at the doses used in the present study (Compton *et al*., 1993; Herzberg *et al*., 1997; Fox *et al*., 2001; Kathuria *et al*., 2003). While the full spectrum of centrally mediated cannabinoid side effects was not examined in the present study, it has previously been shown that the rotarod test is an indicator of central CB_1-mediated side effects (e.g. Fox *et al*., 2001; Malan *et al*., 2001). The lack of motor effects of URB597 suggests that the antiallodynia and antihyperalgesia observed in the present study were unlikely to be due to a reduction in motor function.

The present findings suggest that there is an elevated endocannabinoid 'tone' in inflammatory pain states which is normally curtailed by enzymatic degradation, but is unmasked by the FAAH inhibitor URB597. The lack of motor effects of URB597 suggest that the elevated endocannabinoid tone is restricted to peripheral sites of inflammation and central pain pathways which have been altered by inflammation. Thus, FAAH may represent a useful therapeutic target for inflammatory pain, in addition to anxiety (Kathuria *et al*., 2003), with fewer side effects than that produced by globally acting cannabinoid agonists.

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