Inhibition of Endocannabinoid Metabolism Attenuates Enhanced Hippocampal Neuronal Activity Induced by Kainic Acid

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ABSTRACT The endogenous cannabinoid system regulates neuronal excitability. The effects of inhibiting fatty acid amide hydrolase (FAAH), the enzyme responsible for metabolism of the endocannabinoid anandamide, on kainic acid (KA)-induced neuronal activity were investigated in the rat in vivo, using the selective FAAH inhibitor URB597. Hippocampal neuronal ensemble unit activity was recorded in isoflurane-anesthetized rats using 16-wire microelectrode arrays. Separate groups of rats were administered with single doses of KA alone, KA and URB597 (0.3 or 1 mg kg^{-1}, i.p.), or URB597 (1 mg kg^{-1}) alone. The role of the cannabinoid CB1 receptor in mediating the effects of URB597 was explored using the CB1 selective antagonists AM251, either alone or prior to KA and URB597 (1 mg kg^{-1}) administration, and SR141716A, administered prior to KA and URB597 (1 mg kg^{-1}). Neuronal firing and burst firing rates were examined in animals with confirmed dorsal hippocampal placements. KA induced an increase in both firing and burst firing rates, effects which were attenuated by URB597 in a dose-related manner. Pretreatment with AM251 or SR141716A partly attenuated the URB597-mediated effects on firing and burst firing rate. Rats treated with AM251 or URB597 alone did not exhibit any significant change in either firing or burst firing rates compared with basal activity. These results suggest that the inhibition of endocannabinoid metabolism can suppress hyperexcitability in the rat hippocampus, partly via a CB1 receptor-mediated mechanism. **Synapse 62:746–755, 2008.**

INTRODUCTION

Cannabinoid compounds have been reported to exhibit both antiepileptic (Blair et al., 2006; Wallace et al., 2001; 2003) and neuroprotective properties (Marsicano et al., 2003) via cannabinoid receptors. The endogenous cannabinoid (endocannabinoid) system is integral in controlling neuronal excitability and synaptic plasticity. This system comprises two G-protein coupled receptors (CB1 and CB2) and endogenous ligands such as anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) (reviewed in Freund et al., 2003). The cannabinoid CB1 receptor is highly expressed in the hippocampus (Herkenham et al., 1991) and located presynaptically on terminals of cholecystokinin-containing perisomatic basket cells, a subgroup of GABAergic interneurones (Katona et al., 1999; Tsou et al., 1998; 1999), and on glutamatergic terminals (Domenici et al., 2006; Monory et al., 2006; Takahashi and Castillo, 2006). AEA and 2-AG are synthesized upon demand and released from postsynaptic sites to act at presynaptic CB1 receptors, dampening inhibitory input from basket cells to glutamatergic pyramidal cells (Wilson and Nicoll, 2001). AEA and 2-AG are removed from the extracellular synaptic space by a putative reuptake system (Deutsch and Chin, 1993; Hillard et al., 1997; Maccarrone et al., 2000; Piomelli et al., 1999) and are metabolized by fatty acid amide hydrolase (FAAH) and monoacylglycerol (MAG) lipase, respectively (Cravatt et al., 1996; Cravatt and Lichtman, 2003; Hillard et al., 1995).

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In this study, we investigated the role of the endocannabinoid system in regulating in vivo hippocampal neuronal activity under an acute kainic acid (KA)-induced hyperexcitable condition. KA is a well-established in vivo model of *status epilepticus* (Ben-Ari, 1985; Buckmaster and Dudek, 1997a; 1997b; 1999; Lothman et al., 1981; Nadler, 1981). The potent inhibitor of FAAH enzyme activity, cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597) (Kathuria et al., 2003), was used to block the metabolism of AEA, and was coadministered with KA. The role of CB1 receptors in mediating these effects was assessed using the selective CB1 antagonists, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251) and *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716A) (Howlett et al., 2002).

MATERIALS AND METHODS

Animals

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986, UK. Experiments were performed on male Lister hooded rats (University of Nottingham Biomedical Sciences Services Unit in-house colony) weighing 200–350 g (n = total of 48). Rats were group housed on a 12:12 h light:dark cycle, and food and water were available ad libitum. Rats were anesthetized with a 3.5% isoflurane/O_2:N_2O (50%:50%) mixture. The isoflurane level was reduced progressively and maintained at 1.5–2% throughout the experiment to maintain a constant state of areflexia. Core body temperature was monitored throughout and maintained at 37°C using a homeothermic heating pad (Harvard Apparatus Ltd., Edenbridge, UK).

Surgery

Rats were placed in a stereotaxic frame and a 5-mm-diameter craniotomy was performed over the dorsal hippocampus. The dura mater was excised and a 16 microwire Teflon-coated stainless steel electrode array (50-μm-diameter wire; NB Labs, Denison, Texas) was slowly lowered into the right dorsal hippocampus (3.5 mm posterior and 3.0 mm lateral to bregma, measured from the center of the array; 3.0 to 3.5 mm ventral to the cortical surface; Paxinos and Watson, 1998) and the exposed cortex kept moist with 0.9% sodium chloride. Electrodes had an impedance of ~100 KΩ measured at 1 KHz (Robinson, 1968). Multiple extracellular single units (filtered at 250 Hz to 8 kHz) were recorded from the 16-electrode array using a Plexon Multichannel Acquisition Processor system (Plexon, Dallas, Texas).

Recording procedure

All drugs were administered intraperitoneally (i.p.) resulting in a period of ~20–30 min to time of initial drug effect. The electrode array was allowed to settle for ~30 min after being lowered into dorsal hippocampus, before recordings were made in epochs of 3 min every 20 min, over a total period of 123 min. Basal recordings were obtained for 30 min prior to drug administration.

Following basal recording, rats were administered KA (10 mg kg^{-1}; Sigma-Aldrich, Gillingham, UK) dissolved in saline to induce increased excitatory activity (Kunz and Oliw, 2001; Westbrook and Lothman, 1983). Separate groups of rats were injected with the FAAH inhibitor URB597 (Alexis Biochemicals, Lausen, Switzerland) at doses of 0.3 mg kg^{-1} (n = 5 rats) or 1 mg kg^{-1} [n = 5 rats; dissolved in 5% EtOH/5% Cremophor EL (Fluka Biochemika, Buchs, Switzerland)/90% saline, (v/v %)], or with vehicle (n = 5 rats), coadministered with KA.

In other groups, rats were either administered with URB597 (1 mg kg^{-1}) alone (n = 6 rats) following basal recording, or no drugs were administered (n = 5 rats), and basal neuronal activity in anesthetized rats was recorded for 123 min.

Further experiments were conducted using the selective CB1 receptor antagonists AM251 and SR141716A. AM251 (3 mg kg^{-1}; Tocris, Bristol, UK) dissolved in 5% EtOH/5% Cremophor EL/90% saline (v/v %) was administered after 20 min of basal recording, either alone (n = 6 rats), or 10 min prior to either KA (n = 6 rats), or KA and URB597 (1 mg kg^{-1}; n = 6 rats) administration. Recordings were then made for a further 100 min in rats treated with AM251 alone, and 90 min in rats treated with KA or KA and URB597 after AM251, following basal recordings. In another group, SR141716A (3 mg kg^{-1}; supplied by the National Institute of Mental Health Chemical Synthesis and Drug Supply Program, contract N01-MH-32005; dissolved in 5% EtOH/5% Cremophor EL/90% saline, v/v %) was administered after 20 min of basal recording, 10 min prior to coadministration of KA and URB597 (1 mg kg^{-1}; n = 4 rats); recordings were then performed for a further 90 min in this group.

Histology

At the end of each experiment, animals were deeply anesthetized and current (0.1 mA for 5–10 s) passed through medial, central, and lateral pairs of electrodes to mark placements. Brains were removed and stored in 4% paraformaldehyde/4% potassium ferrocyanide solution for 48 h. Histological sections (200 μm) were taken with a vibratome (Camden Instruments, Loughborough, UK) and electrode placements were revealed by Prussian Blue reaction (Hong et al., 2000).

Data analysis

Data from animals with confirmed hippocampal electrode placements were sorted into individual neuronal units using both automatic and manual sorting techniques in Offline Sorter (Plexon). Briefly, principle component analysis was used to display the recorded waveforms in three-dimensional space. Each electrode was manually checked for artifacts (e.g., noise). Automatic sorting (T-Dist E-M) methods were then used to separate the waveforms into individual units. The resulting clusters were inspected and the units were considered to be separate only if the cluster borders did not overlap (see Stevenson et al., 2007). Sorted spike trains were then analyzed with NeuroEXplorer v3.2 (NeuroExplorer, Littleton, Massachusetts). Changes in firing rate (spikes per second) were examined in neurones with basal firing rates in the range of 0.1–10 spikes s^{-1}, selected to represent a population of putative pyramidal neurones (Frank et al., 2001), and expressed as a percentage of basal firing. Burst rate (bursts min^{-1}) was calculated for neurones with basal burst firing >0.1 burst min^{-1}, and expressed as a percentage of basal burst firing. Only neurones included in analysis of firing rate were considered for evaluation of burst firing rate. A burst was defined as an event containing a minimum of three spikes, and an interspike interval of <10 ms (Tropp Sneider et al., 2006).

Statistical analysis of the percentage change compared with both basal firing rate and burst firing rate data was assessed. Comparisons were made between populations of neurones, using a two-way analysis of variance (ANOVA). Firing and burst firing rate data were divided into four groups: First, data from rats treated with KA and vehicle, KA and low dose URB597 (0.3 mg kg^{-1}), and KA and high dose URB597 (1 mg kg^{-1}) were compared. Second, rats administered with AM251, KA, and URB597 (1 mg kg^{-1}) were compared with KA and vehicle, and KA and high dose URB597 (1 mg kg^{-1}) groups. Third, rats treated with SR141716A, KA, and URB597 (1 mg kg^{-1}) were compared with KA and vehicle, and KA and high dose URB597 (1 mg kg^{-1}) groups. Finally, groups of rats administered with either AM251 or URB597 alone, and a no-drug control group were compared. Post hoc comparisons were made with Tukey's HSD test.

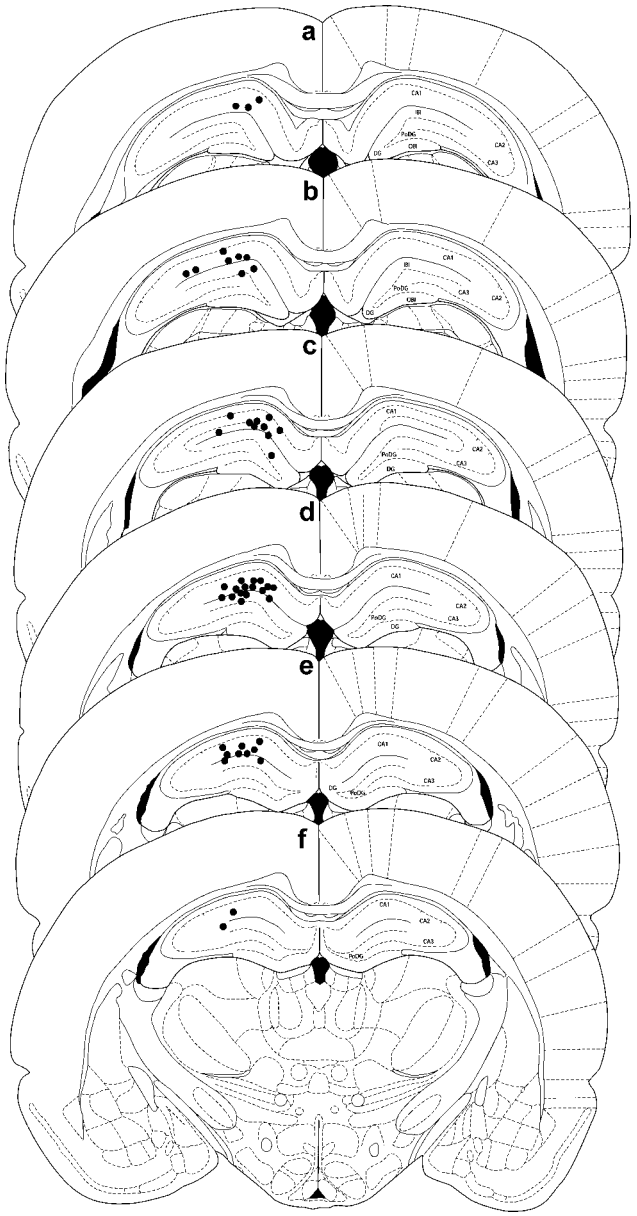

Fig. 1. Schematic representation of histological verification of dorsal hippocampal recording sites. The most medial electrode placements of the array are shown (●) in coronal brain images (Paxinos and Watson, 1998) for each experiment included in this study (n = 48 rats). The distance posterior to bregma in each image is as follows: (**a**) −4.3 mm; (**b**) −4.16 mm; (**c**) −3.8 mm; (**d**) −3.6 mm; (**e**) −3.3 mm; (**f**) −3.14 mm.

RESULTS

KA increased firing rate in hippocampal neurones

Histological confirmation of dorsal hippocampal recording sites is shown in Figure 1. KA-evoked neuronal firing changes and the effect of URB597 on KA-induced firing are illustrated in Figure 2. KA (10 mg kg^{-1}, i.p.) administration caused a progressive increase in firing rate of hippocampal neurones over the time course (123 min) of these experiments. This effect occurred after ∼20 min. The largest increases in firing rate were consistently observed between 100 and 120 min. For example, firing rate increased from 0.2 spikes s^{-1} in Unit 1 and 0.8 spikes s^{-1} in Unit 10 during basal recording, to 1.5 spikes s^{-1} and 3.4 spikes s^{-1}, respectively, at 100 min, following the administration of KA (Fig. 2).

First, groups of rats treated with KA and vehicle, or coadministered KA and either low or high dose

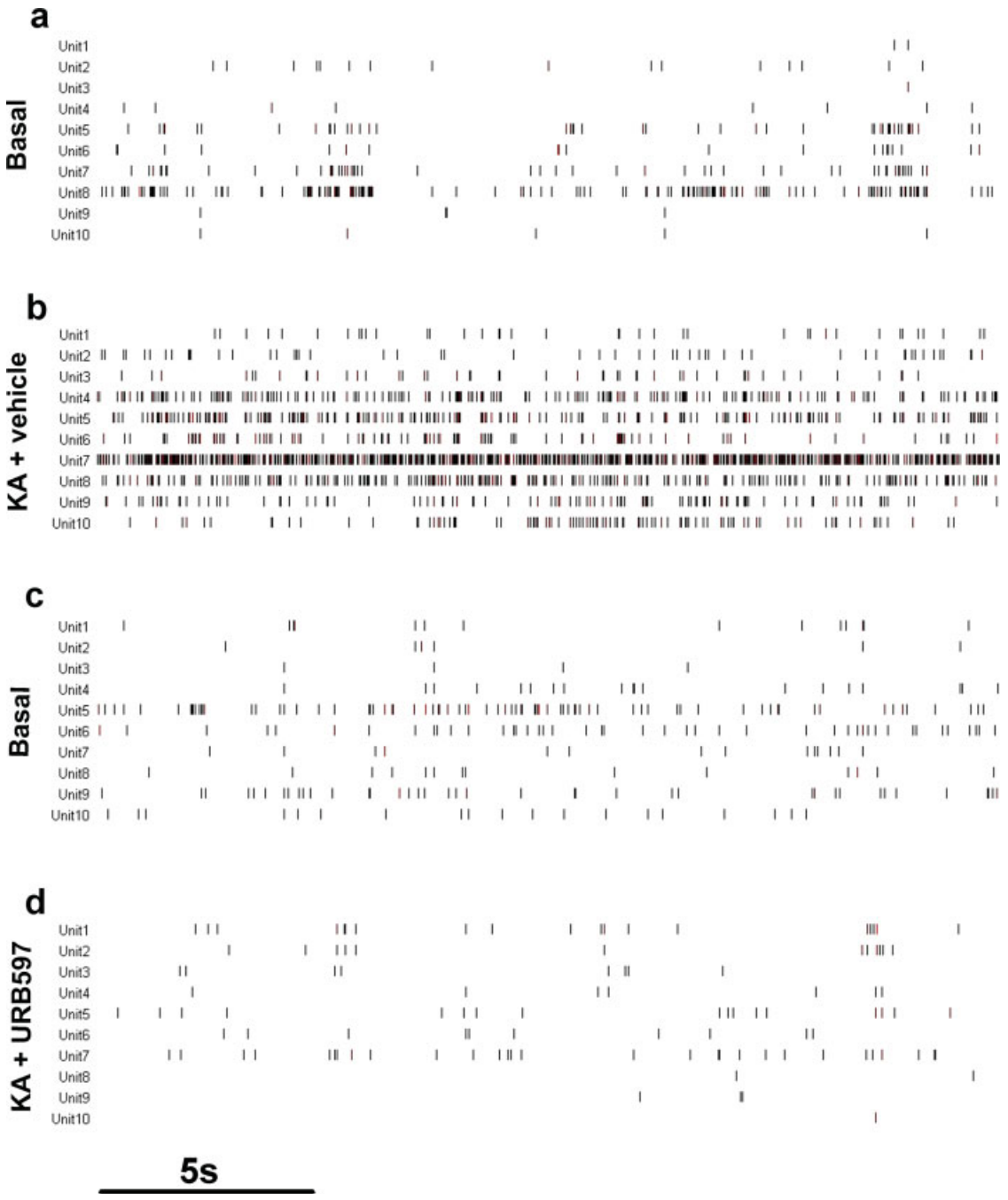

Fig. 2. Simultaneous recordings of multiple hippocampal neuronal activity. (**a**) A representative unit spike raster trace showing basal activity in 10 discriminated individual hippocampal neurones (Unit 1 to Unit 10), recorded medio-laterally (most medial: Unit 1; most lateral: Unit 10) from Ammon's horn in the dorsal hippocampus. This trace shows a sample representation of spontaneous hippocampal unit firing over a 20-s period. (**b**) The activity of the same 10 discriminated hippocampal neurones shown in (a), 60 min after i.p. administration of KA (10 mg kg^{-1}) showing a KA-induced increase in firing. (**c**) The recorded basal activity of 10 hippocampal neurones from a different experimental animal. (**d**) Activity in the same 10 neurones as (c), 60 min after coadministration of KA and URB597 (1 mg kg^{-1}). This trace illustrates the URB597-mediated attenuation of KA-evoked neuronal firing.

URB597 (0.3 or 1 mg kg^{-1}) were assessed. The change in hippocampal firing rate, in neurones exhibiting a basal firing rate in the range of 0.1–10 spikes s^{-1}, revealed a significant effect of the drug groups on hippocampal neuronal firing rate ($F_{(2,12)} = 10.19$, $P < 0.0001$), the time course of experiments ($F_{(6,12)} = 15.20$, $P < 0.0001$), and the drug × time interaction ($F_{(12,109)} = 5.94$, $P < 0.0001$). The increase in firing rate did not differ between KA, and KA with vehicle treated rats (data not shown), and the change in neuronal firing

rate mediated by KA coadministered with vehicle (n = 39 cells, five rats; mean basal firing rate ± SEM = 2.3 ± 0.2 spikes s^{-1}), expressed as a % of basal firing, is illustrated in Figure 3a. KA, coadministered with vehicle, caused an elevation in hippocampal firing rate that was significantly higher than basal firing (during the basal epochs of 0 and 20 min) at 60 min ($P < 0.01$; mean firing rate ± SEM = 7.3 ± 1.8 spikes s^{-1}) and in subsequent epochs ($P < 0.0001$; mean firing rate ± SEM = 9.4 ± 2.4 to 12.2 ± 2.7 spikes s^{-1}).

URB597 coadministration reduced the magnitude of KA-evoked firing

Coadministration of the FAAH inhibitor URB597 with KA attenuated KA-evoked increases in firing rate (Fig. 3a) in a dose-related manner, compared with KA with vehicle. URB597 (1 mg kg^{-1}, i.p.; mean basal firing rate ± SEM = 2.8 ± 0.3 spikes s^{-1}) reduced KA-evoked firing during the epoch at 60 min ($P < 0.01$; mean firing rate ± SEM = 1.9 ± 0.5 spikes s^{-1}) and in all subsequent epochs ($P < 0.0001$; mean firing rate ± SEM = 1.9 ± 0.6 to 3.1 ± 0.7 spikes s^{-1}; n = 39 cells; five rats). The lower dose of URB597 (0.3 mg kg^{-1}; mean basal firing rate ± SEM = 3.1 ± 0.1 spikes s^{-1}) significantly reduced KA-evoked firing during the recording epoch at 80 min ($P < 0.05$; mean firing rate ± SEM = 7.5 ± 2.4 spikes s^{-1}), 100 min ($P < 0.0001$; mean firing rate ± SEM = 8.2 ± 2.4 spikes s^{-1}), and 120 min ($P < 0.01$; mean firing rate ± SEM = 5.5 ± 1.7 spikes s^{-1}; n = 34 cells; five rats).

Further analyses of the KA with high dose URB597 group were performed to establish whether postdrug firing rate differed from basal firing; no significant differences in firing rate were observed between basal and postdrug time points ($P = 0.98$).

AM251 and SR141716A partly blocked URB597-mediated effects on firing rate

To establish whether the effects of FAAH inhibition by URB597 on KA-evoked firing were mediated via CB1 receptors, AM251 (3 mg kg^{-1}, i.p.) or SR141716A (3 mg kg^{-1}, i.p.) were administered 10 min prior to the injection of KA and URB597 (1 mg kg^{-1}). Firing rate in the AM251-pretreatment group was compared with groups of animals coadministered either KA and vehicle, or KA and high dose URB597 (1 mg kg^{-1}). Significant effects on firing rate induced by drug group ($F_{(2,12)} = 12.26$, $P < 0.0001$), time ($F_{(6,12)} = 12.79$, $P < 0.0001$), and drug × time interaction ($F_{(12,192)} = 7.31$, $P < 0.0001$) were observed.

AM251 pretreatment (n = 117 cells; six rats; mean basal firing rate ± SEM = 2.4 ± 0.1 spikes s^{-1}) attenuated in part the effects of URB597 on KA-evoked activity (Fig. 3b). During the epochs at 60 min ($P <$ 0.0001; mean firing rate ± SEM = 6.4 ± 0.7 spikes

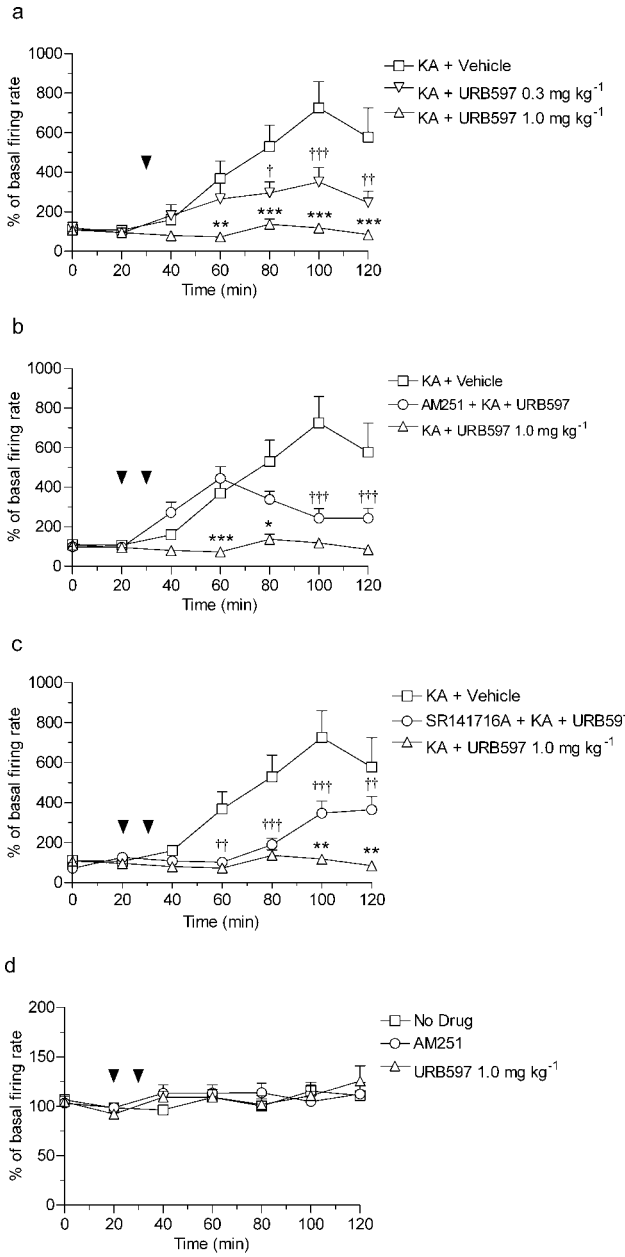

Fig. 3. The effects of URB597 on KA-evoked neuronal firing rate, expressed as a percentage of basal (mean ± SEM), in a population of hippocampal neurones. (**a**) Rats treated at 30 min with either KA and vehicle (□), KA and URB597 (▽; 0.3 mg kg^{-1}), or KA and URB597 (△; 1 mg kg^{-1}); URB597 at both a low dose ($^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.0001$) and high dose ($^{**}P < 0.01$; $^{***}P < 0.0001$) reduced KA-evoked firing. (**b**) Rats administered AM251 at 20 min, prior to coadministration of KA and high dose URB597 at 30 min (○), were compared with KA and vehicle (□; $^{\dagger\dagger\dagger}P < 0.0001$), and KA and high dose URB597 (△; $^{*}P < 0.05$; $^{***}P < 0.0001$) groups. (**c**) Rats receiving SR141716A, prior to KA and high dose URB597 (○), compared with KA and vehicle (□; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.0001$), and KA and high dose URB597 (△; $^{**}P < 0.01$) groups. (**d**) AM251 (○) and URB597 (△) administered alone, as single injections at 20 and 30 min, respectively, were compared with basal activity in a control group of rats receiving no drug treatment (□); no significant differences were observed.

s^{-1}) and 80 min ($P < 0.05$; mean firing rate ± SEM = 5.1 ± 0.5 spikes s^{-1}), AM251-pretreated rats exhibited a significantly greater increase in KA-evoked firing than rats coadministered KA and high dose URB597, but no significant difference to rats treated with KA and vehicle ($P = 0.36$ and 0.02, respectively). The blockade of URB597-mediated effects by AM251 was not present during the later epochs of 100 min ($P = 0.13$; mean firing rate ± SEM = 3.2 ± 0.6 spikes s^{-1}) and 120 min ($P = 0.05$; mean firing rate ± SEM = 3.7 ± 0.8 spikes s^{-1}), where no significant difference between the AM251 pretreatment and high dose URB597 groups was demonstrated. However, at these time points firing in KA and vehicle treated rats was significantly higher than in rats pretreated with AM251 ($P < 0.0001$). In summary, at 60 and 80 min, AM251 antagonized the effect of URB597, but not at 100 and 120 min.

SR141716A treatment ($n = 50$ cells; four rats; mean basal firing rate ± SEM = 3.5 ± 0.6 spikes s^{-1}) also mediated a partial blockade of the effects of URB597 (Fig. 3c). Significant effects on firing rate induced by drug group ($F_{(2,12)} = 14.25$, $P < 0.0001$), time ($F_{(6,12)} = 21.64$, $P < 0.0001$), and drug × time interaction ($F_{(12,125)} = 7.82$, $P < 0.0001$) were observed.

No differences were seen between SR141716A-pretreated and high dose URB597 groups at 60 min ($P = 0.70$; mean firing rate ± SEM = 3.4 ± 0.8 spikes s^{-1}) or 80 min ($P = 0.48$; mean firing rate ±SEM = 4.3 ± 0.9 spikes s^{-1}), but firing rate was significantly higher in SR141716A-pretreated rats at 100 min ($P < 0.01$; mean firing rate ± SEM = 6.0 ± 1.0 spikes s^{-1}) and 120 min ($P < 0.01$; mean firing rate ± SEM = 5.7 ± 1.0 spikes s^{-1}). Firing rate in SR141716A-pretreated rats was consistently lower than the vehicle group at 60 min ($P < 0.01$), at 80 and 100 min ($P < 0.0001$), and at 120 min ($P < 0.01$). In summary, at 60 and 80 min, SR141716A did not antagonize the effects of URB597, but did so, albeit incompletely, at 100 and 120 min.

AM251 or URB597 administered alone did not alter hippocampal firing

Given the profound reduction in KA-evoked firing mediated by URB597 (1 mg kg^{-1}), further experiments were conducted in which the rats were administered with either AM251 (3 mg kg^{-1}) alone ($n = 133$ cells; six rats) or URB597 (1 mg kg^{-1}) alone ($n = 68$ cells; six rats) to establish whether modulating endocannabinoid metabolism and signaling altered basal hippocampal neuronal activity (Fig. 3d). Comparisons were made between AM251 alone, URB597 alone, and a no-drug control group ($n = 74$ cells; five rats). Mean basal firing rates for rats in these groups were as follows: 3.2 ± 0.1 spikes s^{-1} in rats administered AM251 alone, 2.4 ± 0.1 spikes s^{-1} in rats dosed with URB597 alone, and 2.0 ± 0.1 spikes s^{-1} in no-drug control rats. No significant effect of drug on firing rate ($F_{(2,12)} = 0.06$, $P = 0.94$) was observed, but a significant effect over time was present ($F_{(6,12)} = 2.74$, $P < 0.05$). Further analyses showed a significant difference in firing rate, irrespective of drug treatment, occurring between the 20 and 120 min epochs ($P < 0.0001$), suggesting a change in neuronal excitability over the course of experiments. However, a drug × time interaction was not observed ($F_{(12,272)} = 0.91$, $P = 0.54$). Thus AM251 and URB597, when administered alone, did not significantly alter neuronal firing compared with basal hippocampal activity.

Furthermore, a separate group of rats were administered AM251 (3 mg kg^{-1}) 10 min prior to KA alone ($n = 138$ cells; six rats), to ascertain whether AM251 exacerbated KA-evoked firing (data not shown). When this group was compared with rats administered KA and vehicle, no significant differences were observed ($F_{(1,175)} = 0.69$, $P = 0.41$).

URB597 reduced KA-induced burst firing

The rate of burst firing (burst min^{-1}) was analyzed in putative hippocampal pyramidal neurones that exhibited basal bursting >0.1 burst min^{-1}, and basal firing of 0.1–10 spikes s^{-1}, by normalizing data to a percentage change over basal burst firing.

Comparisons were initially made between KA with vehicle, and KA with URB597 (0.3 or 1 mg kg^{-1}) groups. Significant effects of drug ($F_{(2,12)} = 6.69$, $P < 0.01$), time ($F_{(6,12)} = 11.80$, $P < 0.0001$), and a drug × time interaction ($F_{(12,69)} = 3.61$, $P < 0.0001$) were detected.

Administration of KA alone mediated a progressive increase in burst rate (data not shown) that was similar to the change in burst rate that occurred in rats coadministered with KA and vehicle ($n = 27$ cells; five rats), shown in Figure 4a. URB597 produced a dose-related attenuation of KA-evoked burst firing. High dose URB597 (1 mg kg^{-1}) significantly reduced the magnitude of KA-evoked burst firing at 60 and 80 min ($P < 0.01$), at 100 min ($P < 0.0001$), and during the final epoch at 120 min ($P < 0.01$), compared with burst firing rate in rats treated with KA and vehicle (Fig. 4a; $n = 28$ cells; five rats). However, at the lower URB597 dose, a significant effect on KA-evoked burst firing was only evident later in the recording session during the 100 min epoch ($P < 0.01$; $n = 17$ cells; five rats).

AM251 and SR141716A were associated with a nonsustained blockade of URB597 effects on KA-evoked bursting

The role played by CB1 receptors in the reduction in KA-evoked burst firing was assessed using the

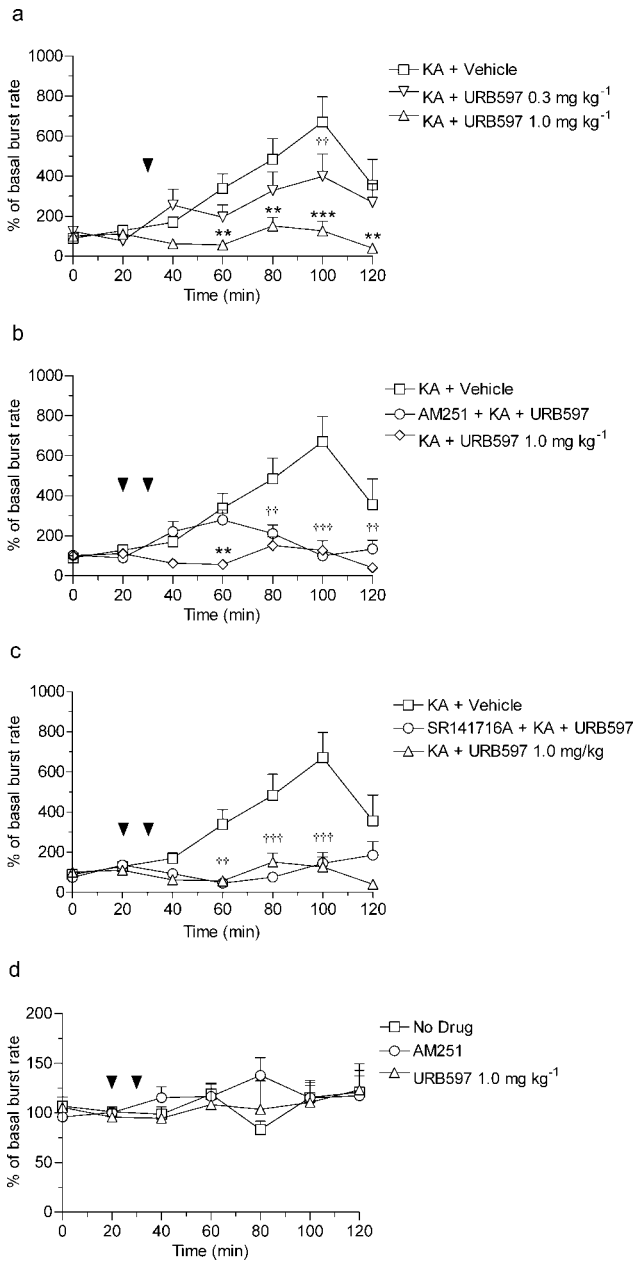

Fig. 4. The effects of URB597 on KA-evoked neuronal burst firing rate (bursts min^{-1}) expressed as a percentage of basal (mean ± SEM). (**a**) Rats coadministered KA and vehicle (□), were compared with groups coadministered KA with low (▽) or high (△) doses of URB597; KA-evoked burst firing was attenuated by both low ($^{\dagger\dagger}P < 0.01$) and high ($^{**}P < 0.01$; $^{***}P < 0.0001$) doses. (**b**) The AM251 pretreatment group (○) was compared with rats administered either KA and high dose URB597 (△; $^{**}P < 0.01$), or KA and vehicle (□; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.0001$). (**c**) The SR141716A pretreatment group (○) was also compared with rats administered either KA and high dose URB597 (△), or KA and vehicle (□; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.0001$). (**d**) Burst rate changes following single administrations of AM251 (○) or URB597 (△), compared with no-drug control rats (□); no significant changes in burst firing rate occurred.

CB1 antagonists, AM251 and SR141716A. Comparisons were initially made between AM251-pretreated, KA and vehicle, and KA and high dose URB597 (1 mg kg^{-1}) groups. These analyses revealed significant effects of drug group ($F_{(2,12)} = 15.54$, $P < 0.0001$), time ($F_{(6,12)} = 7.90$, $P < 0.0001$), and a drug × time interaction ($F_{(12,122)} = 6.61$, $P < 0.0001$).

AM251 attenuated the URB597-mediated reduction in KA-evoked burst firing (Fig. 4b). However, this effect was only evident at earlier stages of the recording. Comparisons made between AM251 pretreated rats, and KA with high dose URB597 revealed significantly higher burst firing in the AM251 pretreated group during the 60 min epoch ($P < 0.01$; $n = 70$ cells; six rats). KA-evoked bursting did not differ between AM251 pretreated, and KA with vehicle groups at this time point ($P = 0.41$).

At 80 min ($P < 0.01$), and in subsequent epochs (100 min, $P < 0.0001$; 120 min, $P < 0.01$), rats pretreated with AM251 exhibited a significantly lower burst firing rate than rats administered with KA and vehicle. Burst firing in AM251 pretreated rats did not differ from KA with URB597 at these time points ($P = 0.40$ at 80 min, $P = 0.70$ at 100 min, and $P = 0.19$ at 120 min).

SR141716A pretreatment did not antagonize URB597-mediated changes in KA-evoked burst firing (Fig. 4c). Comparisons were made between SR141716A-pretreated, KA and vehicle, and KA and high dose URB597 (1 mg kg^{-1}) groups. These analyses revealed significant effects of drug group ($F_{(2,12)} = 14.77$, $P < 0.0001$), time ($F_{(6,12)} = 9.26$, $P < 0.0001$), and a drug × time interaction ($F_{(12,82)} = 6.85$, $P < 0.0001$).

No significant differences were observed between SR141716A-pretreatment ($n = 30$ cells; four rats) and high dose URB597 groups at any time point ($P = 0.89$ at 60 min, $P = 0.31$ at 80 min, $P = 0.81$ at 100 min, $P = 0.06$ at 120 min). A significantly higher firing rate in the vehicle group, compared with SR141716A pretreatment group, was apparent at 60 min ($P < 0.01$), and 80 and 100 min ($P < 0.0001$).

In summary, AM251 antagonized URB597-mediated effects on bursting at 60 min, but not at 80, 100, or 120 min. SR141716A did not however antagonize the URB597-mediated blockade of KA-evoked bursting.

AM251 and URB597 alone did not affect burst rate

The administration of single doses of either URB597, or AM251, did not affect burst firing rate in populations of hippocampal neurones (Fig. 4d). No significant effect of drug groups ($F_{(2,12)} = 0.17$, $P = 0.84$), time ($F_{(6,12)} = 1.12$, $P = 0.35$), or drug × time interaction ($F_{(12,165)} = 0.85$, $P = 0.60$) were observed when rats treated with single doses of URB597 (1 mg kg^{-1}; $n = 33$ cells; six rats), or AM251 (3 mg kg^{-1}; $n = 84$ cells; six rats), were compared with rats that received no-drug treatment ($n = 51$ cells; five rats).

In addition, no significant differences in burst rate were observed when KA and vehicle, and AM251 and KA groups were compared ($F_{(1,101)} = 0.03$, $P = 0.85$; $n = 78$ cells; six rats; data not shown).

DISCUSSION

Inhibiting AEA metabolism by blocking FAAH with URB597 significantly attenuated KA-evoked increases in neuronal firing and burst firing rates in putative pyramidal neurones in the rat hippocampus, in a dose-related fashion. Administered alone, URB597 did not significantly alter firing or burst firing rates.

The selectivity of FAAH for the metabolism of AEA (Cravatt et al., 1996), the abundant expression of FAAH in the hippocampus (Freund et al., 2003), and the lack of a significant inhibition of MAG lipase by URB597 (Kathuria et al., 2003) suggest that the attenuation of KA-evoked activity by URB597 is due to increased concentration of AEA at hippocampal synapses. Previous in vivo characterization of URB597-mediated FAAH inhibition demonstrated an onset of less than 15 min following i.p. administration and effects that persisted for more than 12 h (Fegley et al., 2005). Previous work has also shown URB597 to lack significant effects at a range of receptors, ion channels, and neurotransmitter transporters (reviewed in Piomelli et al., 2006).

Inhibition of FAAH by URB597 does not affect motor function or induce psychotropic effects such as those associated with exogenously applied CB1 agonists (Jayamanne et al., 2006). The lack of a significant effect exhibited by URB597 on baseline neuronal activity, while producing a profound reduction in KA-evoked firing, suggests that inhibition of elevated neuronal activity via FAAH inhibition may have therapeutic relevance for conditions such as epilepsy.

The CB1 receptor antagonists AM251 and SR141716A both mediated, in part, a reduction of the URB597-mediated attenuation of KA-evoked increases in firing rate. AM251, but not SR141716A, partly reduced URB597 effects on burst firing. At earlier time points AM251 blocked URB597-mediated effects; however, this block via CB1 receptors was not sustained throughout the duration of recording. Other studies have demonstrated prolonged effects (>2 h) at CB1 receptors by AM251 in vivo (Gardiner et al., 2002; Xi et al., 2006), suggesting the observations are not explained by a pharmacokinetic effect. SR141716A also blocked the effects of URB597 on firing rate. No blockade of URB597-mediated effects was evident during early time points, but was apparent at later time points. SR141716A did not block URB597-mediated attenuation of KA-evoked burst firing. Structurally, AM251 and SR141716A are similar (Howlett et al., 2002) and the explanation for the differences in time of onset is unclear, although some pharmacological differences are reported, including studies in rat hippocampus (Hajos et al., 2001; Hajos and Freund, 2002). While the differences between antagonists observed in these data remain hard to explain, the presence of a CB1-dependent component is evident.

The partial sensitivity of the URB597-mediated effects to AM251 and SR141716A blockade suggests a role for CB1 receptors. CB1 receptors are located on GABAergic interneurones in the hippocampus (Katona et al., 1999; Tsou et al., 1998, 1999). Inhibition of AEA metabolism would diminish GABAergic inhibitory neurotransmission, predictably augmenting KA-evoked activity. This represents a simplistic view, as manipulation of CB1 receptors in complex in vivo networks may produce varying effects. Recent work demonstrates direct effects of CB1 agonists in suppressing glutamatergic excitatory neurotransmission in the hippocampus under basal conditions (Domenici et al., 2006; Takahashi and Castillo, 2006), and in a KA model of seizure (Monory et al., 2006). This suggests CB1 receptors are involved in the control of excitatory glutamatergic neurotransmission. Blockade of AEA metabolism could therefore confer inhibitory effects via suppression of excitatory synapses under hyperexcitable conditions.

The partial sensitivity to both AM251 and SR141716A observed in these data indicates an additional CB1-independent component to URB597-mediated suppression of KA-evoked neuronal activity. AEA has been shown to directly modulate a number of seven-transmembrane and nuclear receptors, including GPR55 and peroxisome proliferator-activated receptors (reviewed in Alexander and Kendall, 2007), and voltage and ligand-gated ion channels, in some cases at intracellular sites, including T-type calcium channels, voltage-gated, and background potassium channels, α7-nicotinic acetylcholine receptors, TRPV1 receptors, and 5-HT_3 serotonin receptors (reviewed in van der Stelt and Di Marzo, 2005). Given the incomplete nature of CB1 receptor involvement, future studies should examine the identity of a CB1-independent component responsible for mediating the effects of URB597 seen in these data.

Temporal lobe epilepsy (TLE) is a common form of epilepsy that is often difficult to treat (Hauser and Kurland, 1975; Hauser et al., 1991; Semah et al., 1998). Previous in vitro and in vivo studies have shown that exogenously applied CB receptor agonists, such as Δ^9THC and WIN 55, 212-2 are antiepileptic in a number of animal seizure models. This includes in vivo models of maximal electroshock in mice (Wallace et al., 2001) and the pilocarpine model in rats (Wallace et al., 2003), and in vitro, in hippocampal neuronal culture models of acquired epilepsy and status epilepticus (Blair et al., 2006). Moreover, endocannabinoids such as AEA and 2-AG have been shown to exhibit neuroprotective effects (Marsicano

et al., 2003) and antiepileptic effects when applied exogenously (Wallace et al., 2002). In addition, it has been demonstrated that prolonged febrile seizures enhanced endocannabinoid-mediated depolarization-induced suppression of inhibition (DSI) (Chen et al., 2003; 2007). This work found that administration of a CB1 antagonist during febrile seizures blocked seizure-induced potentiation of DSI, eliminated seizure-induced CB1 receptor upregulation, and prevented the development of long-term limbic hyperexcitability. Our data suggest an acute suppressive effect of FAAH inhibition on KA-evoked hyperexcitability. Clement et al. (2003) found that FAAH-KO mice were more susceptible to seizure induced by KA with or without AEA. Direct comparison between our data and the work of Clement et al. is confounded by differences in species used, anesthesia, and possible adaptive changes to FAAH-KO; however, future studies will examine a chronic treatment program with URB597, in a KA model of TLE in the rat. Manipulation of the synthesis, action, or metabolism of endocannabinoids presents an attractive possibility for the treatment of refractory epilepsy.

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