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Presynaptic kainate receptors modulating glutamatergic transmission in the rat hippocampus are inhibited by arachidonic acid

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Abstract

Kainate receptors are ionotropic glutamate receptors located postsynaptically, mediating frequency-dependent transmission, and presynaptically, modulating transmitter release. In contrast to the excitatory postsynaptic kainate receptors, presynaptic kainate receptor can also be inhibitory and their effects may involve a metabotropic action. Arachidonic acid (AA) modulates most ionotropic receptors, in particular postsynaptic kainate receptor-mediated currents. To further explore differences between pre- and postsynaptic kainate receptors, we tested if presynaptic kainate receptors are affected by AA. Kainate (0.3–3 μM) and the kainate receptor agonist, domoate (60–300 nM), inhibited by 19–54% the field excitatory postsynaptic potential (fEPSP) slope in rat CA1 hippocampus, and increased by 12–32% paired-pulse facilitation (PPF). AA (10 μM) attenuated by 37–72% and by 62–66% the domoate (60–300 nM)-induced fEPSP inhibition and paired-pulse facilitation increase, respectively. This inhibition by AA was unaffected by cyclo- and lipo-oxygenase inhibitors, indomethacin (20 μM) and nordihydroguaiaretic acid (NDGA, 50 μM) or by the free radical scavenger, *N*-acetyl-L-cysteine (0.5 mM). The K^+ (20 mM)-evoked release of [^{3}H]glutamate from superfused hippocampal synaptosomes was inhibited by 18–39% by domoate (1–10 μM), an effect attenuated by 35–63% by AA (10 μM). Finally, the K_D (40–55 nM) of the kainate receptor agonist [^{3}H]-(2*S*,4*R*)-4-methylglutamate ([^{3}H]MGA) (0.3–120 nM) binding to hippocampal synaptosomal membranes was increased by 151–329% by AA (1–10 μM). These results indicate that AA directly inhibits presynaptic kainate receptor controlling glutamate release in the CA1 area of the rat hippocampus.
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Keywords: Kainate; Arachidonic acid; Hippocampus; Synaptic transmission; Nerve terminals; Glutamate release; Binding

1. Introduction

Kainate receptors are a subtype of ionotropic glutamate receptors that are abundantly expressed in the hippocampus (for review see, Lerma et al., 2001). Kainate receptors mediate frequency-dependent transmission in mossy fiber/CA3 pyramidal cell synapses and in CA1 interneurons (reviewed in Kamiya, 2002). Kainate receptors also fulfil a neuromodulatory role in the hippocampus, mainly through a presynaptic action (reviewed in Malva et al., 2003). Interestingly in the hippocampal CA1 area, a robust activation of presynaptic kainate receptors leads to inhibition of glutamatergic transmission and glutamate release (e.g. Chittajallu et al., 1996; Kamiya and Ozawa, 1998) as well as GABAergic transmission and GABA release (e.g. Rodríguez-Moreno and Lerma, 1998; Cunha et al., 2000a). These presynaptic inhibitory effects are at odds with the expected depolarizing effect of kainate receptor-gated ionotropic channels permeable to cations (reviewed in Lerma et al., 2001). Interestingly, it was found that the inhibition of GABA and glutamate release by activation of kainate receptors in the CA1 area of the hippocampus involves a metabotropic pathway since it is disrupted by interference with Gi/Go protein (Rodríguez-Moreno and Lerma, 1998; Cunha et al., 2000a; Frerking et al., 2001). Surprisingly, the only pharmacological difference between these presynaptic kainate receptors triggering metabotropic responses and their postsynaptic counterparts ion channels is a different sensitivity to agonists in the case of GABAergic neurons (Rodríguez-Moreno et al., 2000).

It was reported that *cis*-unsaturated fatty acids, like the *trans*-cellular messenger arachidonic acid (AA; see Lynch et al., 1991), inhibit kainate receptor-mediated responses (Wilding et al., 1998). In this work (Wilding et al., 1998), it was reported that AA inhibits the currents triggered by kainate receptor activation but it is not known if AA also affects presynaptic kainate receptors. Indeed, in contrast to the AA modulation of ion channels, which normally results

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from a direct interaction of AA with voltage sensitive ion channels or receptor-operated ion channels (e.g. Kovalchuck et al., 1994; Meves, 1994; Miller et al., 1992; Wilding et al., 1998), the AA modulation of metabotropic receptors involves the activation of protein kinase C (e.g. Cunha and Ribeiro, 1999; Cunha et al., 2000b; Freeman et al., 1990). Thus, as a way to further elucidate the nature of presynaptic kainate receptors controlling hippocampal glutamatergic transmission, we now tested if presynaptic inhibitory responses mediated by kainate receptors in the hippocampus were also sensitive to AA.

2. Methods

2.1. Chemicals and solutions

Domoic acid, 5-nitro-6,7,8,9-tetrahydrobenzo(g)indole-2,3-dione-3-oxime (NS-102) and 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) were from Research Biochemicals International, kainate, bovine serum albumin (BSA, fatty acid free), *N*-acetyl-L-cysteine, indomethacin, nordihydroguaiaretic acid (NDGA), linolenic acid, arachidic acid, ETYA (5,8,11,14-eicosatetraynoic acid) and arachidonic acid were from Sigma, [^{3}H]-(2*S*,4*R*)-4-methylglutamate ([^{3}H]MGA) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were from Tocris Cookson and [^{3}H]glutamate (specific activity 45 Ci/mmol) was from Amersham. Free fatty acids were made up into a 30 mM stock solution in ethanol, aliquoted and stored under nitrogen atmosphere at −20 °C. Indomethacin was made up into a 20 mM stock in methanol and nordihydroguaiaretic acid was made up into a 20 mM stock in ethanol. GYKI 52466 was made up in a 1 mg/ml solution in methanol and NS-102 was made up in a 5 mM stock in dimethylsulfoxide. Aqueous dilutions of these stock solutions were made daily. The maximal concentrations of ethanol, methanol or dimethylsulfoxide used were devoid of effects on synaptic transmission and on [^{3}H]glutamate release.

2.2. Electrophysiological recordings of hippocampal synaptic transmission

The handling and use of the animals was according with the EU guidelines for use of experimental animals (86/609/EEC), the rats being anesthetized under halothane atmosphere before being sacrificed by decapitation to minimize suffering. One 400 μm hippocampal slice, obtained as previously described (Cunha et al., 2000b) from 6 to 8 weeks old male Wistar rats, was transferred to a 1 ml recording chamber for submerged slices and continuously superfused, at a flow rate of 3 ml/min, with gassed (95% O_2 and 5% CO_2) Krebs solution, kept at 30 °C, of the following composition: NaCl 125 mM, KCl 3 mM, NaH_2PO_4 1.25 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2 mM, $MgSO_4$ 1 mM and glucose 10 mM. Drugs were added to this superfusion solution. Electrophysiological recordings of field excitatory postsynaptic potentials (fEPSP) were obtained as previously described (Cunha et al., 2000b). Stimulation (rectangular pulses of 0.1 ms applied once every 15 s) was delivered through a bipolar concentric electrode placed on the Schaffer fibres, in the stratum radiatum near the CA3/CA1 border. Orthodromically evoked fEPSPs were recorded through an extracellular microelectrode (4 M NaCl, 2–5 MΩ resistance) placed in the stratum radiatum of the CA1 area. The intensity of the stimulus was adjusted to evoke a fEPSP with an amplitude of 0.7–1 mV without appreciable population spike contamination. Recordings were obtained with an Axoclamp 2B amplifier coupled to a DigiData 1200 interface (Axon Instruments) and averages of eight consecutive responses were continuously monitored on a personal computer with the LTP 1.01 software (Anderson and Collingridge, 1997). Responses were quantified as the initial slope of the averaged fEPSPs. The amplitude of the afferent presynaptic volley was quantified as a peak-to-peak measurement between the negative peak of the presynaptic volley and the positivity that immediately preceded this negative peak (Dunwiddie and Miller, 1993). To elicit paired-pulse facilitation (PPF), the Schaffer pathway was stimulated twice with 50 ms inter-pulse interval and the synaptic facilitation was quantified as the ratio (P_2/P_1) between the slopes of the fEPSP elicited by the second (P_2) and the first (P_1) stimuli.

2.3. [^{3}H]Glutamate release from hippocampal nerve terminals

A synaptosomal fraction from the CA1 area of the rat hippocampus was prepared as previously described (Lopes et al., 2002) from male Wistar rats (6–8 weeks old; 150–210 g) and resuspended in 1 ml of gassed Krebs solution. The evoked release of [^{3}H]glutamate was as previously described (Lopes et al., 2002). Basically, the synaptosomes were diluted 1:1 in gassed Krebs solution, equilibrated for 10 min at 37 °C, and labeled with [^{3}H]glutamate (0.2 μM) during 5 min at 37 °C under direct but gentle bubbling with 95% O_2 and 5% CO_2. The synaptosomal suspension was then diluted 1:6 with gassed Krebs solution and layered over Whatman GF/C filters into four parallel 90 μl superfusion chamber (adapted from Swinny filter holders, Millipore) through the aid of a roller pump (flow rate: 0.8 ml/min, which was kept constant through the experiment). A series of four parallel superfusion chambers was used to enable both control and test conditions to be performed in duplicate from the same batch of synaptosomes. After setting up the synaptosomes, a 20 min equilibration period was performed before starting sample collection. The effluent was then collected in 1 min fractions for scintillation counting (500 μl to 5 ml Scintran Cocktail T, Wallac). Synaptosomal disruption during the experiments was small since only 3 ± 2% of the total lactate dehydrogenase

(EC 1.1.1.27) was released (see Cunha and Ribeiro, 1999).

The synaptosomes were stimulated with 20 mM KCl (isomolar substitution of Na^+ by K^+ in the Krebs superfusion solution) at 3 and 9 min after starting sample collection (S_1 and S_2). At the end of the experiments, the filters were removed from the superfusion chambers and analyzed by scintillation counting for determination of tritium retained by the synaptosomes. The amount of radioactivity released by each pulse (S_1 and S_2) of potassium (evoked release) was calculated by integration of the area of the peak upon subtraction of the estimated basal tritium outflow from the total outflow of tritium obtained upon K^+ stimulation. The basal outflow was assumed to decline linearly from the 2 min interval before onset of stimulation to the fifth minute after onset of stimulation. The amount of radioactivity recovered in the effluent of the evoked release peak was mostly glutamate, as assessed by HPLC, and was essentially Ca^{2+}-dependent (see Lopes et al., 2002). Thus, we considered that the evoked release of tritium in the present experimental conditions corresponds to a Ca^{2+}-dependent [^{3}H]glutamate release.

When the effect of domoate on the release of glutamate was investigated, domoate was added to the superfusion medium 2 min before S_2 and remained in the bath up to the end of the sample collection period. The effect of domoate was calculated as the percentage variation of the amount of tritium released in S_2/amount of tritium released in S_1 in the presence of domoate versus the S_2/S_1 ratio in control conditions, in the same experiment using the same batch of synaptosomes. When we evaluated the changes of the effect of domoate by a modifier, this modifier was applied 15 min before the beginning of sample collection period and was present during S_1 and S_2. When present during S_1 and S_2, arachidonic acid (10 μM), CNQX (10 μM) or GYKI 52466 (100 μM) did not significantly ($P > 0.05$) alter the S_2/S_1 ratio as compared with the S_2/S_1 ratio obtained in control conditions (no added drug).

2.4. Binding assays

Binding of [^{3}H]-(2*S*,4*R*)-4-methylglutamate ([^{3}H]MGA) was for 90 min at 4 °C with 116–235 μg of synaptosomal membrane protein in a final volume of 300 μl in the incubation buffer, essentially as previously described (Cunha et al., 1999). Specific binding was determined by subtraction of the non-specific binding, which was measured in the presence of 100 μM kainate. The binding reactions were stopped by vacuum filtration through Whatman GF/C glass fiber filters, followed by washing of the filters and reaction tubes with 8 ml of incubation buffer, kept at 4 °C. The filters were then placed in scintillation vials with 5 ml of scintillation liquid (Scintran T). Radioactivity bound to the filters was determined after 12 h with an efficiency of 55–60% for 2 min. Saturation curves were performed in triplicate with 10 different [^{3}H]MGA concentrations ranging from 0.3 to 120 nM. The amount of membrane protein was determined by the Lowry method (Cunha et al., 1999). The data were initially processed in Microsoft Excel software to determine the average specific binding, then fitted by non-linear regression using the Raphson–Newton method, performed with the GraphPAD InPlot Software package. An *F*-test ($P > 0.05$) was used to determine whether the curves were fitted best by one or two independent binding sites.

2.5. Statistics

The values presented are mean ± S.E.M. of *n* experiments, except K_D values which are presented as mean (95% confidence interval). To test the significance of the effect of a drug versus control, a paired Student's *t*-test was used. When making comparisons from different set of experiments with control, a one-way variance analysis (ANOVA) was used, followed by a Dunnett's test. $P \leq 0.05$ was considered to represent a significant difference.

3. Results

3.1. Synaptic transmission in CA1 area of hippocampal slices

The superfusion of rat hippocampal slices with increasing concentrations of domoic acid (60–300 nM) caused a concentration-dependent inhibition of synaptic transmission in Schaffer fiber/CA1 pyramid synapses, as measured by the decrease in the initial slope of the field excitatory postsynaptic potentials recorded in the stratum radiatum of CA1 area (Fig. 1). Kainate (0.3–3 μM) also inhibited the fEPSP slope (data not shown). The depression of fEPSP caused by domoate or kainate fully recovered upon washout of the drugs and three series of cumulative application of three concentrations of domoate (60, 100 and 300 nM) produced essentially similar depressions of fEPSP slope ($n = 4$). In parallel with the inhibition of fEPSP slope, domoate (60–100 nM) and kainate (0.3–1 μM) induced a response with an epileptiform pattern (see Fig. 1B), which also disappeared upon washout of the drugs. This domoate-induced epileptogenic-like pattern was attenuated but did not disappear by cutting the connection between CA3/CA2 areas to CA1 area. The amplitude of the inhibition of fEPSP slope by domoate (60–100 nM) and kainate (0.3–1 μM) was similar irrespective of cutting CA3/CA1 connections. The kainate GluR6 receptor antagonist, NS-102 (10 μM), which by itself was devoid of effects on fEPSP slope ($n = 4$), attenuated (47–66%, $n = 2$ for each agonist) the inhibitory effect of both domoate (60–300 nM) and of kainate (0.6–3 μM) on fEPSP slope. Thus, as previously concluded by others (Chittajallu et al., 1996; Kamiya and Ozawa, 1998; Vignes et al., 1998; Frerking et al., 2001), the effect of domoate and kainate on excitatory synaptic transmission in hippocampal CA1 area is likely mediated by kainate receptors.

When arachidonic acid (1–30 μM) was applied to rat hippocampal slices, it was essentially devoid of effects on

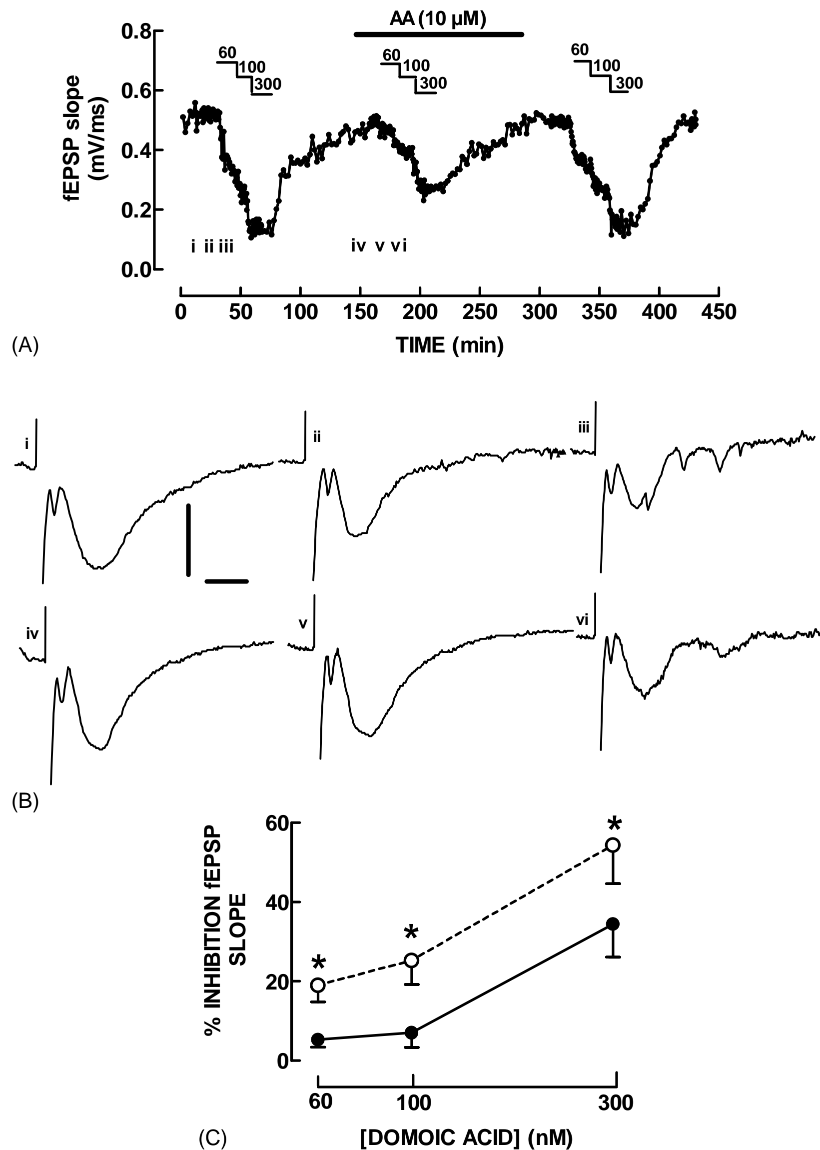


Fig. 1. Activation of kainate receptors inhibits synaptic transmission in Schaffer fibres/CA1 pyramid synapses of rat hippocampal slices and this effect is attenuated by arachidonic acid. Panel A shows an experiment monitoring the time course of field excitatory postsynaptic potential responses and the effect thereupon of the addition of three successive concentrations (60, 100 and 300 nM) of the kainate receptor agonist, domoic acid first in the absence, then in the presence, then again in the absence of AA (10 μM), as indicated in the upper bars. Each point corresponds to the averaged fEPSP slope of two consecutive fEPSP responses. Panel B shown averaged fEPSP responses at the time points indicated by the symbols ((i)–(vi)) shown above the abscissa in panel A, i.e. in control conditions (i); in the presence of 60 nM (ii); and 100 nM of domoic acid (iii). In the presence of AA (10 μM) (iv); together with 60 nM (v); and 100 nM domoic acid (vi); a significant attenuation of the fEPSP slope induced by domoate was observed. The calibration bars correspond to 1 mV and 5 ms. Panel C represented the averaged percentage inhibition of fEPSP slope caused by domoic acid in the absence (○) and in the presence of 10 μM AA (●). Zero percent corresponds to the fEPSP slope in control conditions (i.e. in the absence of any added drugs; 458 ± 31 μV/ms, a value not modified by 10 μM AA) and 100% corresponds to the blockade of fEPSP response. Each value is the mean ± S.E.M. of four experiments. $^{*}P < 0.05$ comparing the effect of each concentration of domoic acid in the absence and in the presence of AA (10 μM).

fEPSP slope, as previously reported (Cunha et al., 2000b and references therein). However, in the presence of AA (10 μM), the inhibitory effect of domoate (60–300 nM) on fEPSP slope was significantly ($P < 0.05$) attenuated (Fig. 1). As illustrated in Fig. 1C, the inhibitory effect of AA was more intense at lower concentrations of domoate and tended to decrease with increasing concentrations of domoate (Fig. 1C). AA (10 μM) also depressed the domoate-induced hyperexcitability in CA1 area as illustrated in Fig. 1 by the decreased appearance of secondary population spikes. AA attenuated in a concentration-dependent manner the domoate inhibition of fEPSP slope, with a maximal effect at 10 μM AA (Fig. 2A). Thus, this concentration of AA was used to probe the effect of AA on hippocampal kainate receptors in most of the remaining experiments.

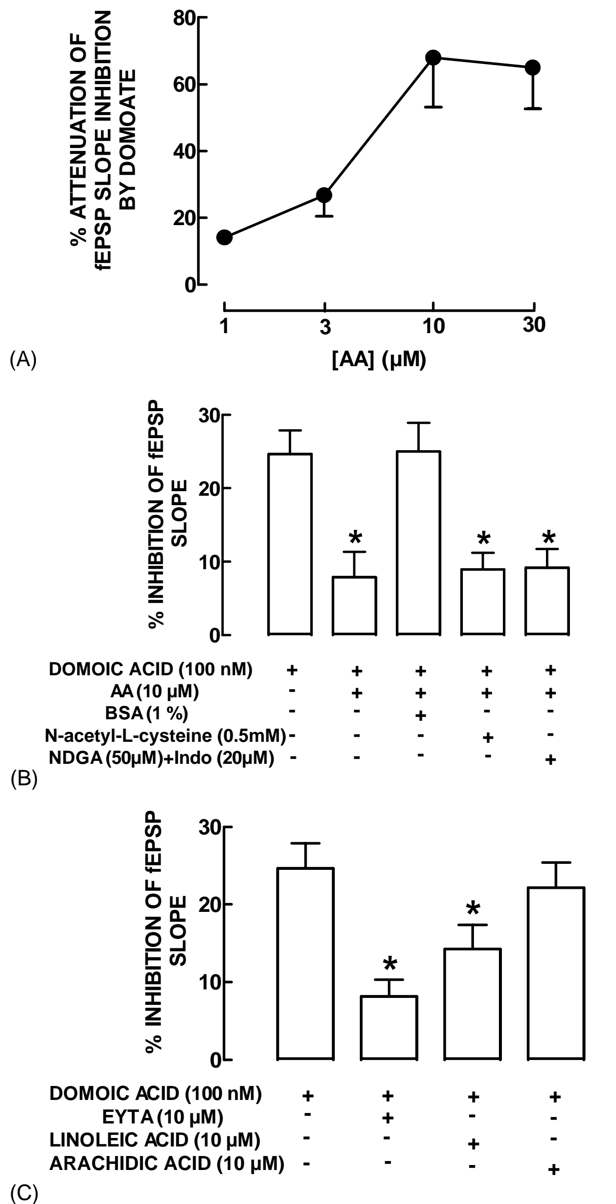


Fig. 2. Arachidonic acid attenuates the domoic acid-induced inhibition of field excitatory postsynaptic potential responses in a concentration-dependent manner and this AA effect is not due to lipoxygenase or cyclooxygenase metabolites and is mimicked by other *cis*-unsaturated, but not saturated, free fatty acids. Panel A shows the percentage attenuation by different concentrations of AA (1–30 μM) of the inhibitory effect of domoic acid (100 nM) on fEPSP slope. Zero percent corresponds to the inhibition of fEPSP slope caused by 100 nM domoic acid (24 ± 4% nhibition) and 100% corresponds to blockade of the inhibitory effect of domoic acid, i.e. to the fEPSP slope value in control conditions in the absence of any drug (529 ± 36 μV/ms). Each point corresponds to the mean ± S.E.M. of three to four experiments. Panels B and C are also shown the percentage attenuation of fEPSP slope caused by 100 nM domoic acid alone (first bar from the left in panels B and C), together with 10 μM AA, together with bovine serum albumin (1%, that quenches free fatty acids), with the free radical scavenger, *N*-acetyl-L-cysteine (0.5 mM) with the simultaneous presence of the lypoxygenase inhibitor, nordihydroguaiaretic acid (50 μM) and the cyclooxygenase inhibitor, indomethacin (Indo, 20 μM), and of 100 nM domoic acid with the non-hydrolysable AA analogue, EYTA (10 μM) or with the *cis*-unsaturated free fatty acid, linoleic acid (10 μM) or with the saturated free fatty acid, arachidic acid (10 μM). Each bar is the mean ± S.E.M. of three experiments, except the bar corresponding to the lack of effect of arachidic acid on 100 nM domoic acid inhibition on fEPSP slope in panel C, which is mean ± S.D. of two experiments. $^*P < 0.05$ compared to the effect of 100 nM domoic acid alone (first bar from the left in panels B and C).

The effect of AA (10 μM) on domoate (100 nM)-induced inhibition of fEPSP slope was prevented by bovine serum albumin (1%, $n = 3$), which binds free fatty acids (Fig. 2B), and was unaffected by the oxygen radical scavenger, *N*-acetyl-L-cysteine (0.5 mM, $n = 3$) (Fig. 2B). Neither BSA (1%, $n = 3$) nor *N*-acetyl-L-cysteine (0.5 mM, $n = 3$) affected fEPSP slope. The simultaneous presence of the lipoxygenase inhibitor, nordihydroguaiaretic acid (50 μM), and of the cyclooxygenase inhibitor, indomethacin (20 μM), also failed to modify the effect of AA (10 μM) on domoate (100 nM)-induced inhibition of fEPSP slope ($n = 3$) (Fig. 2B). The lack of involvement of AA metabolites was further strengthen by the observation that ETYA (10 μM), an analogue of AA that mimics the effect of AA but cannot be metabolized, also depressed domoate (100 nM)-induced inhibition of fEPSP slope ($n = 3$), as did another *cis*-unsaturated fatty acid, linoleic acid (10 μM, $n = 3$) (Fig. 2C). In contrast, a C_{20} saturated free fatty acid, arachidic acid (10 μM, $n = 2$), failed to affect the domoate (100 nM)-induced inhibition of fEPSP slope (Fig. 2C).

3.2. Paired-pulse facilitation in CA1 area of hippocampal slices

When two consecutive pulses are applied to the afferent Schaffer fibers with an interval of 50 ms, the fEPSP response to the second pulse showed a larger slope (Fig. 3) as a result of presynaptic calcium accumulation (Wu and Saggau, 1994). This paired-pulse facilitation is enhanced by drugs presynaptically inhibiting glutamate release and is inhibited by drugs presynaptically facilitating glutamate release, provided these modulatory systems control calcium transients (Wu and Saggau, 1994). Consistent with a presynaptic effect of kainate receptors to modulate fEPSP in CA1 area (Chittajallu et al., 1996; Kamiya and Ozawa, 1998; Vignes et al., 1998; Frerking et al., 2001), domoate (60–300 nM) increased the ratio of PPF in a concentration-dependent manner (Fig. 3). Like the inhibitory effect of domoate on fEPSP slope, the PPF ratio rapidly recovered to control value upon washout of domoate (data not shown).

By itself, AA (10 μM) slightly inhibited PPF (8 ± 2%, $n = 4$), probably as a consequence of the ability of AA to modulate calcium transients in hippocampal nerve terminals (Damron and Dorman, 1993). As illustrated in Fig. 3, AA (10 μM) attenuated the facilitatory effect of domoate (60–300 nM) on PPF.

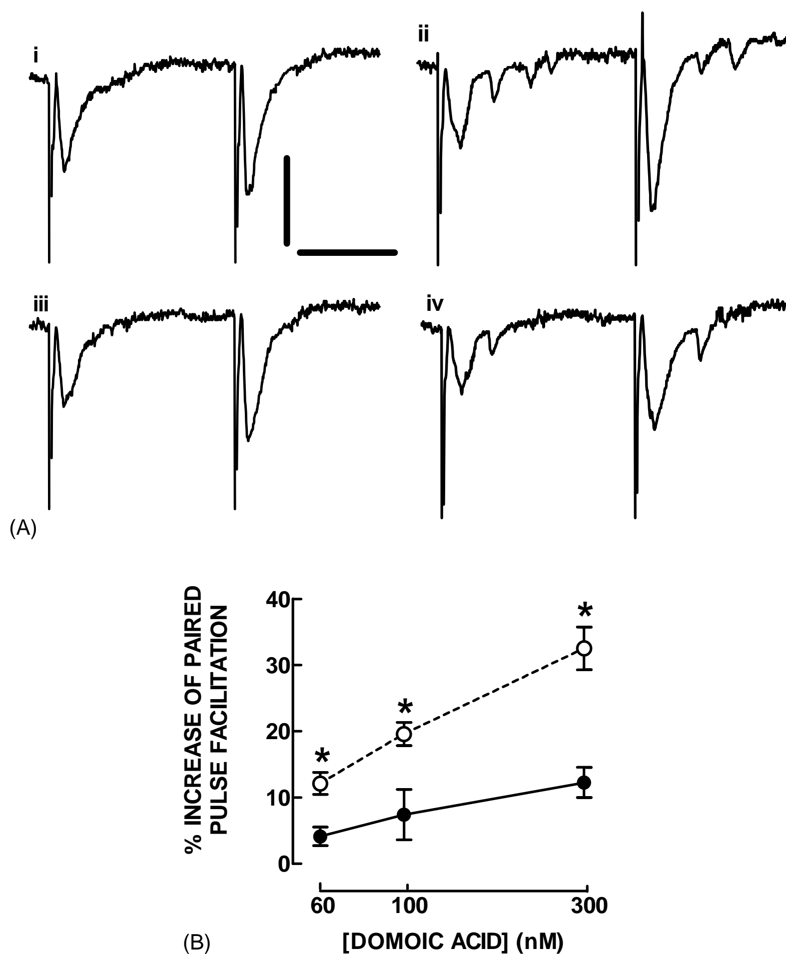
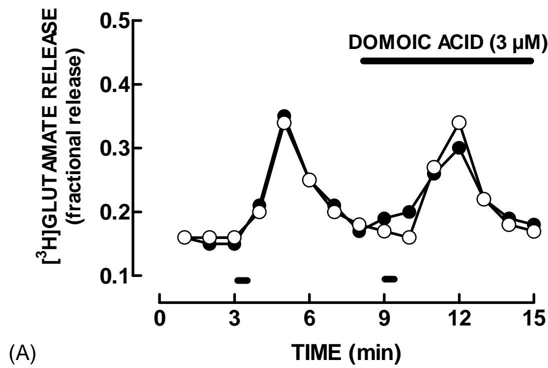
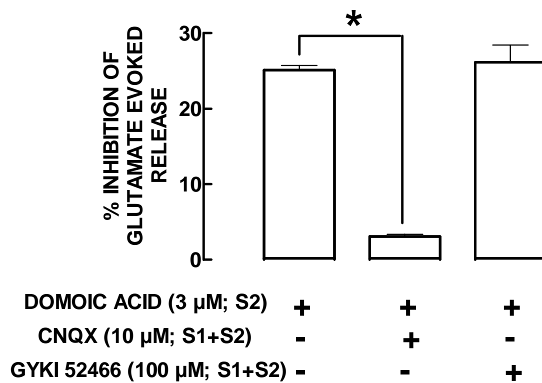


Fig. 3. Activation of kainate receptors increases paired-pulse facilitation in rat hippocampal slices and this effect is attenuated by arachidonic acid. Panel A shows recordings with twin field excitatory postsynaptic potential responses recorded extracellularly in Schaffer fibres/CA1 pyramid synapses upon two consecutive stimulation pulses separated by 50 ms. In the first trace corresponding to control conditions (i), the second fEPSP response is larger than the first (PPF); and 100 nM domoic acid (ii) facilitates PPF. In the second row of recordings is first shown the PPF in the presence of 10 μM AA (iii); and then in the simultaneous presence of 10 μM AA and 100 nM domoic acid (iv). The calibration bars correspond to 1 mV and 25 ms. Note that in this series of recordings, the connection between CA3 and CA1 area was cut and the domoate-induced epileptogenesis (illustrated in the recording in Fig. 1) is drastically reduced. Panel B represented the average percentage facilitation of PPF caused by domoic acid in the absence (○) and in the presence of 10 μM AA (●). Zero percent corresponds to the PPF in control conditions (1.88 ± 0.05 in the absence of any drugs or 1.73 ± 0.05 in the presence of 10 μM AA) and 100% corresponds to the double of these values. Each value is the mean ± S.E.M. of four experiments. $^{*}P < 0.05$ comparing the effect of each concentration of domoic acid in the absence and in the presence of 10 μM AA.

3.3. Glutamate release from hippocampal synaptosomes

When CA1 hippocampal synaptosomes, previously loaded with [^{3}H]glutamate, were stimulated for 30 s with 20 mM K^+, they released tritium in a Ca^{2+}-dependent manner that is mostly [^{3}H]glutamate, as assessed by HPLC (see Lopes et al., 2002). Two periods of chemical stimulation (S_1 and S_2), separated by a 6 min interval, produced a similar evoked tritium release (Fig. 4A), with an S_2/S_1 ratio of 0.89 ± 0.02 ($n = 15$). As illustrated in Fig. 4A, domoate (3 μM) enhanced by $14 \pm 4\%$ ($n = 4$) the basal outflow of tritium, in accordance with the ability of kainate receptors to trigger calcium influx and the spontaneous release of glutamate from hippocampal nerve terminals (reviewed in Malva et al., 1998). Consistent with its effect on glutamatergic transmission, application of 3 μM domoate 2 min before the second stimulation period (S_2) caused a decreased K^+-evoked release of tritium (Fig. 4A). As illustrated in Fig. 4B, this inhibitory effect of domoate on the evoked release of glutamate was attenuated by $88 \pm 11\%$ ($n = 4$) by the ionotropic non-NMDA receptor antagonist, CNQX (10 μM), but not by the non-competitive AMPA receptor antagonist, GYKI 52466 (100 μM, $n = 3$). Also, as illustrated in Fig. 4C, this inhibitory effect of domoate (1–10 μM) on the evoked release of glutamate was concentration-dependent. As observed when studying glutamatergic transmission, AA (10 μM) decreased the domoate (1–10 μM)-induced inhibition of evoked glutamate release (Fig. 4C).





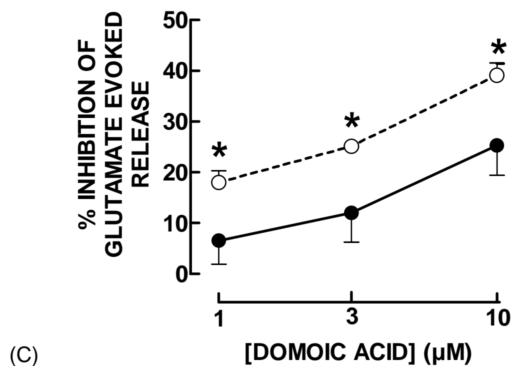
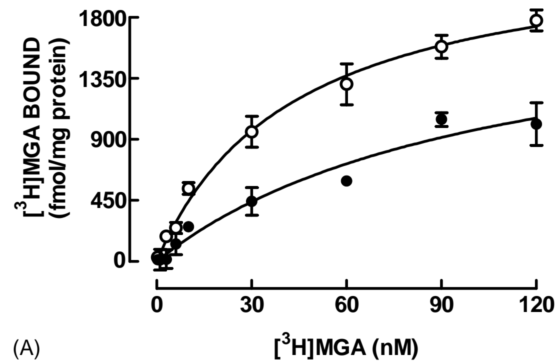


Fig. 4. Activation of presynaptic kainate receptors inhibits the evoked release of glutamate from rat hippocampal synaptosomes and this effect is attenuated by arachidonic acid. Panel A shown the time course of [^{3}H]glutamate release, measured as the fractional release expressed in terms of percentage of total radioactivity present in the synaptosomes at the beginning of the collection of each sample. The synaptosomes were challenged with two periods of stimulation with 20 mM K^+ for 30 s (S1 and S2), as indicated by the bars above the abcissa. The symbols (○) represent tritium release from a control chamber, to which no drug was added, and the symbols (●) represent the tritium release in the test chamber, to which domoic acid (3 μM) was added through the superfusate, as indicated by the upper bar. Note that domoic acid (3 μM) enhanced the basal outflow of glutamate and inhibited the evoked release of glutamate. Panel B shows the ability of the ionotropic non-NMDA receptor antagonist, CNQX (10 μM), but not the AMPA receptor antagonist, GIKY 52466 (100 μM), to prevent the effect of domoic acid on the evoked release of glutamate. The drugs were present in S1 and/or S2 as indicated under the bars. The results are mean ± S.E.M. of three to four experiments. $^*P < 0.05$ vs. the inhibitory effect of domoic acid (first left column). Panel C shows the concentration-dependent inhibitory effect of domoic acid on the evoked release of glutamate in the absence (○) and in the presence of 10 μM AA (●). Zero percent corresponds to the S2/S1 ratio in control conditions (0.96 ± 0.04, not modified by 10 μM AA) and 100% corresponds to the full blockade of the evoked release of tritium (S2/S1 = 0). The results are mean ± S.E.M of three to four experiments. $^*P < 0.05$ comparing the effect of each concentration of domoic acid in the absence and in the presence of 10 μM AA.



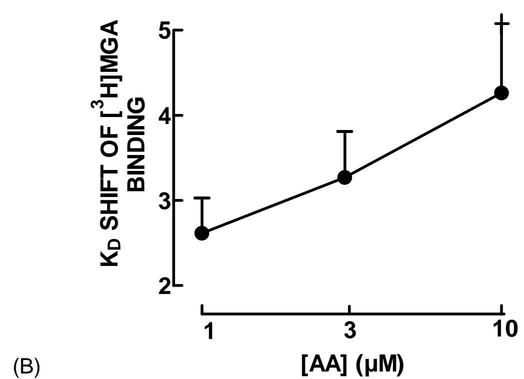


Fig. 5. Inhibition by arachidonic acid of the binding of the selective kainate receptor agonist, [^{3}H]-(2*S*,4*R*)-4-methylglutamate, to synaptosomal membranes of the rat hippocampus. Panel A shows the average saturation curves of [^{3}H]MGA binding in the absence (○) or presence (●) of AA (10 μM). The ordinates represent specific binding of [^{3}H]MGA upon subtraction of the non-specific binding, determined in the presence of 100 μM kainate, from total binding. The curves were generated from the average binding parameters obtained upon fitting by non-linear regression assuming a single binding site. Results are mean ± S.E.M. of three to nine experiments performed in triplicate. Panel B shows the concentration-dependent inhibition by AA of the affinity of [^{3}H]MGA binding, measured as the ratio of the K_D values of [^{3}H]MGA in the absence/presence of AA (K_D shift), estimated from saturation curves similar to these presented in panel A. Each point is the mean ± S.E.M. of three to four experiments.

3.4. Binding to hippocampal synaptosomal membranes

In control conditions, the binding of the kainate receptor agonist, [^{3}H]-(2*S*,4*R*)-4-methylglutamate, to hippocampal synaptosomal membranes displayed a K_D of 48 nM (95% confidence interval: 40–55 nM) and a B_{max} of 1910±71 fmol/mg protein ($n = 9$). As illustrated in Fig. 5A, the presence of AA (10 μM, $n = 3$) in the binding assay

shifted the saturation isotherm of [^{3}H]MGA binding to the right, with a 224 ± 36% increase in K_D value (87–125 nM) and no significant change in B_{max} (1872 ± 58 fmol/mg protein). This inhibition by AA of [^{3}H]MGA binding was concentration-dependent, increasing from 1 to 10 μM (Fig. 5B).

4. Discussion

The main finding of the present work is that AA directly attenuates the inhibitory effect of presynaptic kainate receptors in glutamatergic nerve terminals of the rat hippocampus. Thus, AA attenuated the kainate receptor-induced inhibition of fEPSP slope and facilitation of paired-pulse facilitation in Schaffer fibre/CA1 pyramid synapses. Furthermore, AA also attenuated the kainate receptor-induced inhibition of the evoked release of glutamate from hippocampal nerve terminals. This effect of AA on presynaptic kainate receptors is likely to be a direct effect of AA, rather than involving intracellular transducing systems, since AA directly attenuated the binding to kainate receptors in purified membranes from hippocampal nerve terminals.

Kainate receptors inhibiting glutamatergic transmission in area CA1 of the hippocampus have previously been described (Chittajallu et al., 1996; Kamiya and Ozawa, 1998; Vignes et al., 1998; Frerking et al., 2001). These kainate receptors inhibiting glutamatergic transmission are presynaptic, as previously proposed (reviewed in Kamiya, 2002), since both kainate and domoate increased paired-pulse facilitation (see also Kamiya and Ozawa, 1998) and directly inhibited the evoked release of glutamate from purified nerve terminals in a manner sensitive to the ionotropic non-NMDA receptor antagonist, CNQX, but not to the selective AMPA receptor antagonist, GYKI 52466 (see also Chittajallu et al., 1996). This pharmacological profile indicates the involvement of kainate receptors, which are enriched in membranes from presynaptic nerve terminals in the hippocampus (Cunha et al., 1999).

We now observed that AA inhibits presynaptic kainate receptors, in a manner similar to the previously reported inhibition by AA of the cationic current triggered by postsynaptic kainate receptors in the hippocampus or by heterologously expressed kainate receptors (Wilding et al., 1998). Thus, it appears that AA may interfere with the activation of kainate receptors by agonists, irrespective of whether kainate receptors will trigger a cationic current or a metabotropic action, as is the case of presynaptic kainate receptors in the CA1 area (Rodríguez-Moreno and Lerma, 1998; Cunha et al., 2000a,b; Frerking et al., 2001). This effect of AA is likely to be a direct effect not involving lipoxygenase or cyclooxygenase metabolites, since the effect of AA was unaffected by cyclooxigenase or lipoxygenase inhibitors and was mimicked by the non-hydrolyzable but equi-effective analogue of AA, EYTA. The possible involvement of free radicals was also directly excluded by the observation that a free radical scavenger, *N*-acetyl-L-cysteine, failed to modify the effect of AA on kainate receptor-mediated actions. AA also directly inhibited the binding to kainate receptors in presynaptic hippocampal membranes, a preparation where transducing systems are disrupted since there is no intact cytoplasm, excluding any possible indirect effect of AA on transducing system controlling kainate receptor function (reviewed in Lerma et al., 2001).

In conclusion, the present results further add to previous observations suggesting that pre- and postsynaptic kainate receptors display similar pharmacological properties in spite of the fact that they operate differently (i.e. metabotropic and inhibitory versus ionotropic and excitatory, respectively). Further attempts need to be essayed to find ways of pharmacologically interfering with pre- and postsynaptic kainate receptors to understand their relative impact on different physiological and pathological processes (see Lerma et al., 2001).

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