Research Article

The Role of GLU\textsubscript{K5}-Containing Kainate Receptors in Entorhinal Cortex Gamma Frequency Oscillations

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Using in vitro brain slices of hippocampus and cortex, neuronal oscillations in the frequency range of 30–80 Hz (gamma frequency oscillations) can be induced by a number of pharmacological manipulations. The most routinely used is the bath application of the broad-spectrum glutamate receptor agonist, kainic acid. In the hippocampus, work using transgenic kainate receptor knockout mice have revealed information about the specific subunit composition of the kainate receptor implicated in the generation and maintenance of the gamma frequency oscillation. However, there is a paucity of such detail regarding gamma frequency oscillation in the cortex. Using specific pharmacological agonists and antagonists for the kainate receptor, we have set out to examine the contribution of kainate receptor subtypes to gamma frequency oscillation in the entorhinal cortex. The findings presented demonstrate that in contrast to the hippocampus, kainate receptors containing the GLUK5 subunit are critically important for the generation and maintenance of gamma frequency oscillation in the entorhinal cortex. Future work will concentrate on determining the exact nature of the cellular expression of kainate receptors in the entorhinal cortex.

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1. INTRODUCTION

KARs are made up of various combinations of five subunits: GLU\textsubscript{K5}, GLU\textsubscript{K6}, GLU\textsubscript{K7}, KA1, and KA2 \cite{1, 2} which are abundantly expressed in the neocortex \cite{3}. These subunits make up tetramers of either homomeric or heteromeric assemblies, with GLU\textsubscript{K5-7} being able to form functional homomeric receptors \cite{1, 4}. KA1 and KA2 cannot form functional receptors when expressed alone \cite{5, 6}, yet are able to form functional KARs when expressed heteromerically with other subunits \cite{1, 7, 8}. Differential patterns of expression of KARs in the CNS coupled with the existence of splice variants and mRNA editing suggest complex neurophysiological roles for the various subunits, and different roles in neuronal networks depending on their localization \cite{9, 10}.

Of particular interest is the role of KARs in the generation and maintenance of network neuronal oscillatory activity in cortical regions \cite{9, 10}. Gamma frequency oscillations occur between 30–80 Hz and have been observed in many areas of the brain, including the hippocampus \cite{11–13} and cortical regions \cite{14–16}. Cortical gamma oscillations are important in higher brain functions, such as learning, memory, and cognition \cite{17–19}, as well as processing of sensorimotor information \cite{15, 16, 20}. In carrying out these functions, cortical gamma oscillations are implicated in various central processes, including long-term potentiation (LTP) and synaptic plasticity \cite{21}, with important roles in temporal regulation of neuronal activity.

Gamma frequency oscillations are recordable from the MEC during wakefulness in humans \cite{22, 23}, as well as in vivo in rodents \cite{12}, in vitro from perfused guinea pig brains \cite{24, 25}, and isolated rat brain slices \cite{26, 27}. These gamma oscillations in the MEC play a role in the formation, processing, storage, and retrieval of memories \cite{17, 18}. Previously it has been demonstrated in an in vitro preparation of the MEC that application of nanomolar
concentrations of kainate (200–400 nM) can induce persistent gamma frequency oscillations [26–28]. Using this in vitro model of MEC gamma frequency oscillations it has been elucidated that this activity is primarily generated by inhibitory-based neuronal networks [29–31]. A similar has been elucidated that this activity is primarily generated in vitro model of MEC gamma frequency oscillations it has been demonstrated in both the hippocampus and neocortex [32, 33].

To date our understanding of the role of the KARs in neuronal network activity has been hampered by a paucity of selective pharmacological agents. The competitive AMPA/KAR antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoline (NBQX), shows little selectivity between AMPA receptors and KARs at high concentrations, yet at low concentrations (1 μM) can be used to block AMPA receptors, and isolate KAR responses [34, 35]. However, NBQX shows no selectivity between different KAR subunits. The role of GLUK5 and GLUk7 subunits in neuronal oscillatory activity in the hippocampus has been previously investigated using receptor knockout mice [9, 10]. However, interpretation of work using transgenic models should be viewed in the light of the knowledge that compensatory factors may play a role. The recent development of pharmacological agents with specificity for distinct subunits has led to the possibility of a detailed pharmacological investigation of the role of specific KARs in cortical gamma frequency oscillations. (S)-3-(2-Carboxybenzyl)willardine (UBP302) is a novel selective GLUK5-containing KAR antagonist, with activity at both homomeric and heteromeric GLUK5-containing receptors [36, 37]. The activity of UBP302 on GLUk7 is controversial, Dolman et al. [37] showed that UBP296 (racemic form of UBP302) only weakly inhibited [3H]kainate binding to human GLUk7 (Ki value of 374 ± 122 μM). However, in an electrophysiological assay UBP302 was found to block rat homomeric GLUk7 receptors with an IC50 value of 4 μM but at a concentration of 100 μM only very weakly blocked rat GLUk5/GLUk7 receptors [38]. 5-Carboxy-2,4-di-benzamido-benzoic acid (NS3763) is another novel glutamate antagonist, which is selective and noncompetitive for homomeric GLUK5-containing KARs [39, 40]. (RS)-2-amino-3-(3-hydroxy-5-tert-butyl-isoxazol-4-yl)propanoic acid (ATPA) is a selective GLUk5-containing receptor agonist [41]. ATPA has been shown to depress excitatory and GABAAergic synaptic transmission in the hippocampus [42, 43]. However, Cossart et al. [35] demonstrated that lower concentrations of ATPA could directly depolarise hippocampal GABAAergic interneurons leading to increases in the levels of tonic inhibition onto pyramidal neurons. More recently, similar concentrations of ATPA to that used in the Cossart et al. [35] study have been shown to facilitate both evoked and action potential-independent glutamate release in the neocortex [44].

The data presented here demonstrates a role of GLUK5-containing KARs in the MEC by examining the contribution of these receptor subtypes to gamma frequency oscillations. Using a pharmacological approach, we have demonstrated that GLUK5-containing KARs are important for the maintenance of gamma frequency oscillations in the MEC. Moreover, the selective activation of GLUK5-containing KARs can induce persistent gamma frequency oscillations in the MEC. We also demonstrate that it is the specific activation of homomeric GLUK5-containing KARs that is important for the generation of gamma frequency oscillations in the MEC.

2. MATERIAL AND METHODS

2.1. Preparation of EC-hippocampal slices

All procedures involving animals were carried out in accordance with UK Home Office Legislation. Male Wistar rats, weighing >150 grammes, were first anaesthetised by inhalation of the volatile anaesthetic isoflurane. This was immediately followed by intramuscular injection of a terminal dose of ≥100 mg/kg ketamine and ≥10 mg/kg xylazine. After confirmation of deep anaesthesia, rats were intracardially perfused with ~50 mL sucrose-modified artificial cerebral spinal fluid (aCSF), composed of (in millimolar (mM)): 252 sucrose, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 2 CaCl2, 2H2O, 10 glucose, and 24 NaHCO3. All salts were obtained from BDH Laboratory Supplies (Poole, UK), except MgSO4 which was obtained from Sigma Chemical Co (Mo, USA).

The whole brain was rapidly removed and maintained in a bath of cold sucrose-modified aCSF (4–5°C) during the dissection procedure. Horizontal slices (450 μm thick) were cut using a vibrosection (Leica VT1000S). Transverse EC-hippocampal slices were then transferred either to a holding chamber or directly to the recording chamber. They were maintained at 32 ± 1°C, at the interface between a continuous perfusion (~2–3 mL/min) of NaCl-based aCSF (containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH2PO4, 1 MgSO4, 1.2 CaCl2·2H2O, 10 glucose, 24 NaHCO3) and humidified carbogen gas (95% O2/5% CO2). Slices were allowed to equilibrate for 60 minutes before any recordings were taken.

2.2. Electrophysiological recording and drug application

Extracellular recordings were taken using glass electrodes pulled from borosilicate glass capillaries (GC129 TF-10, 1.2 mm OD/0.94 mm ID) (Harvard Apparatus, Kent, UK) using a Flaming/Brown micropipette puller, model P-97 (Sutter Instrument Co., Calif, USA). This created electrodes with resistances of 2–4 MΩ. Electrodes were filled with NaCl-based aCSF and positioned in Layer III of the MEC. Control recordings were taken from slices before drug application to confirm that any network activity seen following treatment was due to the presence of drugs.

To evoke gamma frequency oscillations, 400 nM kainic acid ((2S,3S,4S)-3-carboxymethyl-4-(prop-1-en-2-yl)pyrrolidine-2-carboxylic acid; Tocris Cookson, Bristol, UK) was bath applied to EC-hippocampal slices and left to equilibrate for 2–3 hours or until gamma oscillations had stabilised. All other drugs were bath applied to slices...
at known concentrations: UBP302 (S)-3-(2-carboxybenzyl)willardine; gift from Dr. David Jane, Department of Pharmacology, University of Bristol, UK) at 10 μM; ATPA ((RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid; Tocris Cookson, Bristol, UK) at 1–5 μM; NS3763 (5-carboxy-2,4-di-benzamido-benzoic acid; Tocris, Bristol, UK) at 10–15 μM; NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline; Tocris, Bristol, UK) at 1–10 μM; and Carbachol (Sigma, UK) at 10–20 μM.

2.3. Data acquisition

An AppleMac computer with the Axograph OSX software package (AxographX, Dr. John Clements, Australia) was used for all data acquisition. Signals were analogue filtered at 0.01–0.3 kHz and then digitized at a frequency of 10 kHz. Power spectra were constructed, where power at a given gamma frequency was defined as the area under the peak between 20 and 80 Hz. Power spectra were generated from digitized data, using 60 second epochs of recorded activity, and it was from these spectra that values for gamma oscillation peak frequency, peak amplitude, and spectral area power in the gamma frequency band were obtained.

2.4. Data analysis

Data analysis was carried out using Excel and Kaleidagraph software packages. Kaleidagraph software was used to generate pooled power spectra, and the Excel package was used to calculate the mean and standard error of mean (SEM) of results, and to draw up histograms and line graphs. All data is presented as mean ± SEM. SigmaStat (Systat software, USA) was used for all statistical tests. Normality tests were carried out, and if data was found to be normally distributed, two-tailed paired t-tests were run. However, if data failed the normality test, the Wilcoxon signed rank test was carried out. This provided us with P-values for all data sets, and the significance level was set at 95%; values less than P = .05 were deemed to be statistically significant.

3. RESULTS

3.1. Induction of kainate-driven gamma oscillations in the MEC

Previously, it has been demonstrated that low concentrations of kainic acid (kainate) evoke gamma frequency activity in the rat MEC in vitro [26, 27]. In this investigation, we produced persistent gamma oscillations in the MEC by bath application of kainate (400 nM) (Figure 1). Robust gamma frequency oscillations (39.4 ± 1.6 Hz; n = 17) were evoked in layer III of the MEC in all slices to which kainate had been applied (n = 17). This activity was generated within 15 minutes of kainate superfusion, a stable baseline was observed after 60–90 minutes. As previously reported [19], application of the competitive AMPA/KAR antagonist NBQX (10 μM) effectively abolished these kainate-induced gamma oscillations (n = 3) (Figure 1).

3.2. A role for GLU K5-containing KARs in the maintenance of kainate-driven gamma oscillations

A possible role for GLU K5-containing KARs in the maintenance of kainate-driven gamma frequency oscillations in the MEC was investigated by testing the ability of the GLU K5 selective antagonist, UBP302, to inhibit preestablished kainate-induced gamma activity. Gamma oscillations were generated in the MEC by bath application of kainate (400 nM) and allowed to stabilise (n = 9) (Figure 2(a)). In the presence of UBP302 (10 μM), the amplitude of kainate-induced gamma oscillations was significantly reduced (control, 116.7 ± 44.1 μV²/Hz; v. UBP302, 70.0 ± 30.3 μV²/Hz; P < .05; n = 9), and area power of oscillations was also significantly decreased (control, 1586.0 ± 503.3 μV²/Hz; v. UBP302, 1155.1 ± 441.4 μV²/Hz; P < .05; n = 9) (Figures 2(a), 2(b)). However, following UBP302 application, the frequency of oscillations remained unchanged (control, 40.4 ± 2.1 Hz; v. UBP302, 38.9 ± 2.6 Hz; P > .1; n = 9). Washout of the effects of UBP302 on gamma frequency oscillations could not be achieved (n = 9) (Figures 2(a), 2(b)).

3.3. A role for GLU K5-containing KARs in the generation of gamma oscillations in the MEC

To investigate the role that GLU K5-containing KARs may play in the induction of kainate-driven gamma oscillations,
we carried out two experiments, using the selective GLU$_K5$-containing KAR agonist, ATPA, and antagonist, UBP302.

First, we tested the ability of UBP302 to inhibit the generation of a kainate-driven gamma frequency oscillation in the MEC. Slices were preincubated in UBP302 (10 μM) for 30 minutes. As expected, UBP302 administration caused no neuronal network activity in slices (n = 11) (Figure 3(a)). However, when kainate was applied to slices following preincubation with UBP302, gamma frequency oscillations were generated in all slices (n = 11) (Figure 3(a)). On washout into kainate alone (400 nM), although the frequency of oscillations did not change significantly (in presence of kainate following preincubation with UBP302, 45.4 ± 2.0 Hz; v. 400 nM kainate alone, 40.3 ± 0.9 Hz; P > .05; n = 11), oscillations were seen to increase significantly in both peak amplitude (in presence of kainate following preincubation with UBP302, 39.2 ± 12.1 μV$^2$/Hz; v. 400 nM kainate alone, 122.9 ± 32.8 μV$^2$/Hz; P < .05; n = 11) and area power (in presence of kainate following preincubation with UBP302, 545.1 ± 159.6 μV$^2$/Hz.Hz; v. 400 nM kainate alone, 1302.5 ± 241.4 μV$^2$/Hz.Hz; P < .05; n = 11) (Figures 3(a), 3(b)).

We next investigated whether gamma frequency oscillations could be generated in the MEC by application of the GLU$_K5$ selective agonist, ATPA. ATPA was bath applied to slices at concentrations of 1 μM, 2 μM, and 5 μM. ATPA induced gamma frequency oscillations in the MEC in the

**Figure 2**: Antagonizing GLU$_K5$-containing KARs with UBP302 inhibits kainate-driven gamma frequency oscillations in the MEC. (a) Extracellular field recordings showing 1 second epochs of activity (i) in the presence of 400 nM kainate, (ii) following 10 μM UBP302 application, and (iii) during a washout period into 400 nM kainate. (b) Pooled power spectra (n = 9) produced from 60 second epochs of extracellular field recorded data, showing a control recording (black), a recording in the presence of 400 nM kainate (blue), application of 10 μM UBP302 (green), and washout back into 400 nM kainate (red). Scale bar represents 200 milliseconds and 100 μV.
majority of slices to which the agonist was applied \((n = 18\) out of a total \(n = 26\)) (Figure 4(a)). Slices showing gamma oscillations upon ATPA application were observed to be dorsal MEC slices. Upon increasing the concentration of ATPA in slices demonstrating gamma oscillations, the mean frequency, peak amplitude, and area power of oscillations increased \((n = 10)\) (Figures 4(a), 4(b)). The frequency of oscillations increased from \(23.0 \pm 1.9\) Hz to \(34.0 \pm 2.4\) Hz, and to \(44.2 \pm 1.5\) Hz \((n = 10)\) (Figure 4(b)), the peak amplitude increased from \(0.9 \pm 0.5\ \mu V^2/Hz\), to \(4.1 \pm 2.3\ \mu V^2/Hz\), and to \(23.3 \pm 8.7\ \mu V^2/Hz\) \((n = 10)\) (Figure 4(b)), and the power increased from \(23.1 \pm 12.6\ \mu V^2/Hz.Hz\), to \(88.4 \pm 38.9\ \mu V^2/Hz.Hz\), and to \(201.8 \pm 71.0\ \mu V^2/Hz.Hz\), at the respective concentrations of ATPA (1 \(\mu M\), 2 \(\mu M\), and 5 \(\mu M\)) \((n = 10)\) (Figure 4(b)). Control readings, taken before ATPA administration, showed that no network activity was spontaneously present in slices \((n = 26)\) (Figure 4(a)). ATPA-induced gamma frequency oscillations were susceptible to the AMPA/KAR antagonist NBQX (10 \(\mu M\)) \((n = 3)\).

We next investigated the effect of UBP302 on ATPA-induced gamma frequency oscillations in the MEC. Gamma frequency oscillations were induced in slices by bath application of ATPA \((2–5\ \mu M)\) \((n = 4)\) (Figure 5(a)). UBP302 \((10\ \mu M)\) application caused no significant change in the frequency of gamma oscillations \((control, 42.7 \pm 3.9\) Hz; \(\nu\) UBP302, 33.9 \pm 7.3 Hz; \(P > .1; n = 4\) and yet had significant effects on both the peak amplitude \((control, 20.8 \pm 7.1\ \mu V^2/Hz; \nu\) UBP302, 6.6 \pm 2.8\ \mu V^2/Hz; \(P < .05; n = 4\) and power of oscillations \((control, 359.7 \pm 117.9\ \mu V^2/Hz.Hz; \nu\) UBP302, 141.7 \pm 61.9\ \mu V^2/Hz.Hz; \(P < .05; n = 4\) (Figures 5(a)ii, 5(b))). The effects of UBP302 on an ATPA-induced gamma frequency oscillations were not reversible on washout \((n = 4)\).

### 3.4. A role for homomeric GLU<sub>K5</sub>-containing KARs in gamma frequency oscillations

The GLU<sub>K5</sub> selective KAR antagonist, NS3763, was used to investigate the contribution of homomeric GLU<sub>K5</sub>-containing KARs to gamma activity in the MEC. NS3763 selectively antagonises homomeric GLU<sub>K5</sub> KARs [39] and experiments were carried out to determine the role of these homomeric receptors in both kainate- and ATPA-induced gamma oscillations.

Application of NS3763 \((10–15\ \mu M)\) caused significant decreases in both peak amplitude \((control, 100.2 \pm 48.4\ \mu V^2/Hz; \nu\) NS3763, 46.0 \pm 23.7\ \mu V^2/Hz; \(P < .05; n = 8\) and area power \((control, 822.9 \pm 273.2\ \mu V^2/Hz.Hz; \nu\) NS3763, 449.4 \pm 182.3\ \mu V^2/Hz.Hz; \(P < .05; n = 8)\) of kainate-induced gamma oscillations in the MEC (Figures 6(a), 6(b)). However, no effect was seen on the frequency of kainate-generated oscillations \((control, 38.3 \pm 2.7\) Hz; \(\nu\) NS3763, 36.0 \pm 1.5 Hz; \(P > .1; n = 8\)) (Figure 6(b)).

Application of NS3763 \((10–15\ \mu M)\) to slices demonstrating ATPA-induced gamma oscillations caused no significant change in the frequency of oscillations (control,
Figure 4: Activation of GLU\textsubscript{K5}-containing KARs can induce gamma frequency oscillations in the MEC. (a) Extracellular field recordings showing 1 second epochs of activity in a control setting (i) following application of 1 μM, (ii) 2 μM (iii), and 5 μM ATPA (iv). (b) Pooled line graphs (n = 10) demonstrating the effects of varying ATPA concentration on (i) frequency, (ii) area power, and (iii) peak amplitude of gamma oscillations in the MEC. Scale bar represents 200 milliseconds and 100 μV.

46.7 ± 3.8 Hz; v. NS3763, 38.5 ± 6.2 Hz; P > .1; n = 8) (Figures 7(a), 7(b)). However, the presence of NS3763 caused a significant decrease in both the peak amplitude (control, 191.9 ± 63.1 μV²/Hz; v. NS3763, 28.5 ± 13.6 μV²/Hz; P < .05; n = 8) and area power (control, 1192.9 ± 342.8 μV²/Hz; v. NS3763, 333.6 ± 120.5 μV²/Hz; P < .05; n = 8) of gamma oscillations (Figures 7(a), 7(b)). The effects of NS3763 on either kainate- or ATPA-induced gamma
frequency oscillations were not reversible on washout (n = 12).

3.5. A role for GLU\textsubscript{K5}-containing KARs in carbachol-induced gamma oscillations

Cortical gamma frequency oscillations can also be induced by application of carbachol, an agonist at muscarinic acetylcholine receptors (mAChRs) [24, 25, 45–49]. It is unclear as to the role of GLU\textsubscript{K5}-containing KARs in a cholinergic-mediated gamma frequency oscillation in the MEC. Carbachol will cause an increase in the release of glutamate in the form of rhythmic EPSPs [46]. This, in turn, may lead to activation of KARs [50]. In agreement with previous studies in the MEC [24, 25] bath application of carbachol (10–20 μM) generated persistent gamma frequency oscillations (n = 6) (Figure 8(a)ii). Application of UBP302 (10 μM) had no significant effect on the frequency (control, 41.7 ± 1.6 Hz; v. UBP302, 40.3 ± 0.6 Hz; P > .1; n = 6), peak amplitude (control, 5.9 ± 3.1 μV²/Hz; v. UBP302, 5.5 ± 2.5 μV²/Hz; P > .1; n = 6) or power (control, 155.2 ± 72.7 μV²/Hz.Hz; v. UBP302, 148.7 ± 62.8 μV²/Hz.Hz; P > .1; n = 6) of preestablished carbachol-driven gamma oscillations (Figures 8(a)ii, 8(b)). This lack of effect was further demonstrated by washout back into carbachol causing no significant change in observed gamma frequency oscillations (Figures 8(a)iii, 8(b)).

4. DISCUSSION

A number of studies have examined the contribution of various KAR subunits to gamma frequency oscillations in the hippocampus in vitro. Fisahn et al. [10] focused on the roles of GLU\textsubscript{K5} and GLU\textsubscript{K6} subunits in kainate-induced hippocampal gamma oscillations, using brain slices from transgenic GLU\textsubscript{K5} and GLU\textsubscript{K6} receptor knockout mice. Knockout of GLU\textsubscript{K5} increased sensitivity of the hippocampal network to the effects of kainate and higher susceptibility to oscillatory and epileptogenic activity. Slices from GLU\textsubscript{K6}-knockout mice could not support kainate-induced gamma oscillations or epileptiform activity, suggesting distinct roles for GLU\textsubscript{K5} and GLU\textsubscript{K6} subunits in the hippocampus. Fisahn et al. [10] concluded that GLU\textsubscript{K5}-containing receptors may be expressed on axons of hippocampal interneurons and have a function in inhibitory tone, and that GLU\textsubscript{K6}-containing KARs may be found in the somatodendritic region of pyramidal cells and interneurons, and provide excitatory drive. Functional receptors of both subtypes must interact to allow generation of stable gamma oscillations in the hippocampus [9, 10].

Subsequently, Brown et al. [51] used pharmacological approaches to investigate the role of GLU\textsubscript{K5}-containing receptors in hippocampal gamma oscillations. This study used the GLU\textsubscript{K5}-selective agonists ATPA and iodowillardiine but found that neither could induce gamma network activity in area CA3 of rat hippocampal slices. The GLU\textsubscript{K5} selective
Figure 6: Blocking homomeric GLU$_{K5}$-containing KARs significantly reduces kainate-driven gamma frequency oscillations in the MEC. (a) Extracellular field recordings showing 1 second epochs of activity (i) in the presence of 400 nM kainate and (ii) following application of 10 μM NS3763. (b) Pooled power spectra (n = 8) produced from 60 second epochs of extracellular field recorded data, showing a control recording (black), recording in the presence of 400 nM kainate (blue), application of 10 μM NS3763 (green), and a washout back into 400 nM kainate (red). Scale bar represents 200 milliseconds and 100 μV.

Figure 7: Blocking homomeric GLU$_{K5}$-containing KARs effectively reduces power and amplitude of ATPA-generated gamma frequency oscillations in the MEC. (a) Extracellular field recordings showing 1 second epochs of activity (i) in the presence of 5 μM ATPA and (ii) following application of 15 μM NS3763. (b) Pooled power spectra (n = 4) produced from 60 second epochs of extracellular field recorded data, showing a control recording (black), recording in the presence of 5 μM ATPA (blue), and application of 15 μM NS3763 (green). Scale bar represents 200 milliseconds and 100 μV.
It was not surprising then that application of the GLUK5 subunit selective agonist ATPA [41] successfully evoked gamma frequency oscillations in the MEC. However, care must be taken with interpretation of this data as ATPA has recently been shown not to be entirely selective for GLUK5-containing KARs [40]. In fact, ATPA can activate both homomeric and heteromeric KAR complexes containing GLUK5, and also GLUK6/KA2 heteromeric KARs [48]. Thus, it cannot initially be assumed that these gamma oscillations have been generated via GLUK5-containing receptor complexes, since they could have been induced through GLUK6/KA2 heteromeric receptors. UBP302, however, is an antagonist with selectivity for GLUK5-containing KARs [36, 37]. Whilst UBP302 has been shown to block GLUK7 with an IC50 value of 4 μM—this makes it ~10-fold selective for GLUK5 versus GLUK7—it does not have activity on GLUK6 or GLUK6/KA2 up to 100 μM. Indeed, some controversy surrounds the activity of UBP302 on GLUK7 as it has been reported that UBP302 failed to demonstrate any potent activity in a binding assay on GLUK7 (personal communication, D.E. Jane). In any case, as UBP302 only blocks homomeric GLUK7 and activation of GLUK7 requires very high glutamate concentrations (EC50 value 5.9 mM) [52] it may not be relevant to this study. Application of UBP302 onto slices showing ATPA-generated network activity causes reduced peak amplitude and an approximately 60% reduction in area power of gamma frequency oscillations. This inhibition of ATPA-generated gamma oscillations by UBP302 suggests that the observed activity must, at least in part, be due to activation of GLUK5-containing KARs.
Moreover, NS3763 application caused a significant reduction in peak amplitude and spectral power of preestablished kainate-driven gamma oscillations. This demonstrates that homomeric GLU\textsubscript{K5}-containing KARs are at least partially responsible for the maintenance of these kainate-driven gamma frequency oscillations. Application of NS3763 to preestablished ATPA-generated oscillations caused an approximately 80% reduction in area power of gamma frequency oscillations and also a reduction in peak amplitude. This suggests that a large component of an ATPA-driven gamma oscillation is maintained through homomeric GLU\textsubscript{K5} KARs. The activity of the selective homomeric GLU\textsubscript{K5}-containing KAR antagonist, NS3763, on both kainate- and ATPA-generated gamma frequency oscillations, tells us that homomeric GLU\textsubscript{K5}-containing KARs are involved in the observed network activity.

It has been suggested that carbachol-driven activity could cause excess glutamate release and that this overspill of glutamate could activate KARs [50]. The lack of effect of the GLU\textsubscript{K5} selective antagonist, UBP302, on carbachol-induced gamma oscillations in the MEC suggests that GLU\textsubscript{K5}-containing KARs are not involved in the generation or maintenance of gamma oscillations induced via activation of muscarinic cholinergic receptor. However, we cannot rule out the possibility that other KAR subtypes may be involved in these mAChR-mediated gamma oscillations.

We have shown that GLU\textsubscript{K5}-containing KARs are implicated in the generation and maintenance of gamma frequency oscillations in the MEC evoked by kainate. However, we can only speculate on the cellular localisation of these GLU\textsubscript{K5}-containing receptors in the MEC. Research performed by Christensen et al. [40] in the hippocampus, suggested likely localisations of KAR subtypes in hippocampal CA1 inhibitory interneurons terminating with pyramidal cells, concluding that heteromeric GLU\textsubscript{K6}/KA2 receptors are expressed in somatodendritic compartments of interneurons, and that GLU\textsubscript{K5} complexes, with either GLU\textsubscript{K6} or KA2, are found at presynaptic terminals. It seems likely from our results that in the MEC, both homomeric and heteromeric GLU\textsubscript{K5}-containing KARs are present.

Presynaptic KARs are involved in regulation and modulation of neurotransmitter release at inhibitory and excitatory synapses in the hippocampus [50, 53, 54]. In contrast, postsynaptic KARs mediate excitatory postsynaptic currents (EPSCs) in many brain regions [35, 55]. KAR activation in the hippocampus modulates GABA release at terminals of inhibitory interneurons and causes an increase in spontaneous IPSCs but a reduction in the amplitude of these IPSCs impinging on to CA1 interneurons [40]. This suggests that KARs may be present in two distinct populations in hippocampal inhibitory interneurons, and the same may be true of KARs in the MEC [2, 40]. However, other reports have demonstrated that kainate can increase the frequency and amplitude of spontaneous IPSCs, but not action potential-independent miniature IPSCs in stratum radiatum interneurons [56]. Moreover, these authors also observed that kainate can directly depolarise the axonal plexus of inhibitory interneurons producing both increased antidromic and presumably orthodromic spiking. This effect would explain the ability of KAR activation to increase spontaneous but not miniature IPSC activity. The presence of KARs at an axonal loci has been well documented in the hippocampus, most notably in mossy fibres [57, 58].

As outlined in the previous paragraph, there is a large corpus of data on the role of KAR in the hippocampus. However, with respect to the MEC there is a paucity of such information. In order to put the current results presented in this paper into context, future work will concentrate on combining intracellular recordings from individual neurones (pyramidal and interneuron), specific pharmacological KAR tools, and transgenic KAR subunit knockout animals [9, 10] to elucidate the exact nature of cellular expression of KARs in the MEC.

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