Running head: KAINATE RECEPTORS IN THE DENTATE GYRUS

Kainate receptors containing GluR5 subunits contribute to synaptic transmission and long-term potentiation in the hippocampal dentate gyrus

by

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Abstract

It has recently been shown that presynaptic kainate receptors can play a role in synaptic plasticity in the CA3 region of the hippocampus (Bortolotto et al., 1999); however, a role for these receptors in synaptic transmission and synaptic plasticity in other regions of the brain has been difficult to establish. In the present experiments we show that kainate receptors can contribute significantly to synaptic transmission in the dentate gyrus (DG) of the hippocampus, and that furthermore, they appear to play a role in synaptic plasticity in this region. Kainate receptors in the DG appear to be located post-synaptically at excitatory synapses where they can influence synaptic plasticity by alleviating the Mg^{2+} blockade of NMDA receptors. These results demonstrate that kainate receptors in the DG play a vital post-synaptic role at excitatory synapses, and may contribute to learning and memory processes in this region.

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Abbreviations

ACSF: Artificial cerebral spinal fluid

AMPA: Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

CA1: Cornu ammonis 1

CA3: Cornu ammonis 3

DG: Dentate gyrus

EC: Entorhinal cortex

EPSC: Excitatory post-synaptic current

EPSP: Excitatory post-synaptic potential

GluR5: Glutamate receptor subunit 5

KA: Kainate

LPP: Lateral perforant path

mA: Milliamp

ml: Milliliter

MPP: Medial perforant path

mV: Millivolt

NMDA: N-methyl-D-aspartate

μM: Micromolar

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1. INTRODUCTION

1.1 Glutamate receptor as the basis of excitatory synaptic transmission

In the central nervous system, the fast excitatory effects of glutamate are mediated primarily by the NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-5-methyl-4-isoxazole propionic acid), and KA (kainate) subtypes of ionotropic receptors (Dingledine et al., 1999). While the physiological roles of NMDA and AMPA receptors have been studied extensively, especially in the hippocampal formation, a lack of specific pharmacological agonists and antagonists for the KA receptor have hindered our understanding of how KA receptors contribute to neuronal signaling (Lerma, 1997; Wilding & Huettner, 1997). Recently, the development of specific receptor blockers for AMPA receptors and certain specific blockers for KA receptor subunits has provided progress to the understanding of the physiological contribution of KA receptors to excitatory synaptic transmission (for summary of glutamate receptor pharmacology, see Table 1).

Table 1. Selective pharmacological agents for glutamate receptors

Drug	Functions
APV	Competitive antagonist of NMDA receptors
ATPA	Selective GluR5 agonist
CNQX	Competitive AMPA/KA receptor antagonist
Domoate	Kainate receptor agonist
Glutamate	Endogenous glutamate receptor ligand; agonist for NMDA, AMPA, kainate, and metabotropic glutamate receptor agonist
GYKI52466	Non-competitive AMPA receptor antagonist; less potent than GYKI53655
GYKI53655	Non-competitive AMPA receptor antagonist
Kainate	Kainate receptor agonist; also AMPA receptor agonist at high concentration
LY382884	Competitive GluR5 subunit antagonist

1.2 Subunit properties of kainate receptors

Structurally, KA receptors are tetrameric protein complexes whose composition includes at least one of the five different subunits that are unique to KA receptors: GluR5, GluR6, GluR7, KA1, and KA2 (also known as Glu_{K5}, Glu_{K6}, Glu_{K7}, Glu_{K1}, and Glu_{K2} repectively; Hollmann and Heinemann, 1994). These subunits are not equal however, and only the GluR5-7 subunits have been shown to form functional homomeric channels by themselves; these subunits also show a high affinity of binding to kainate and glutamate and, receptors with GluR5-7 show low conductance to Na^+ (Sommer et al., 1992; Egebjerg and Heinemann, 1993; Schiffer et al., 1997). These subunits can also form distinct heteromeric receptors when they coassemble either with KA1 or KA2 subunits, or each other (Werner et al., 1991; Herb et al., 1992; Schiffer et al., 1997; Paternain et al., 2000). Unlike GluR5-7, KA1 and KA2 have a low binding affinity to kainate and glutamate, but exhibit high channel conductance to Na^+. A subunit editing site on GluR5 and GluR6, located at the channel pore of the receptor (Q621R) also determines the channel conductance of the receptor to Ca^{2+}. A schematic illustration of the membrane topology and subunit composition of a kainate receptor is shown on Figure 1 and 2 respectively.

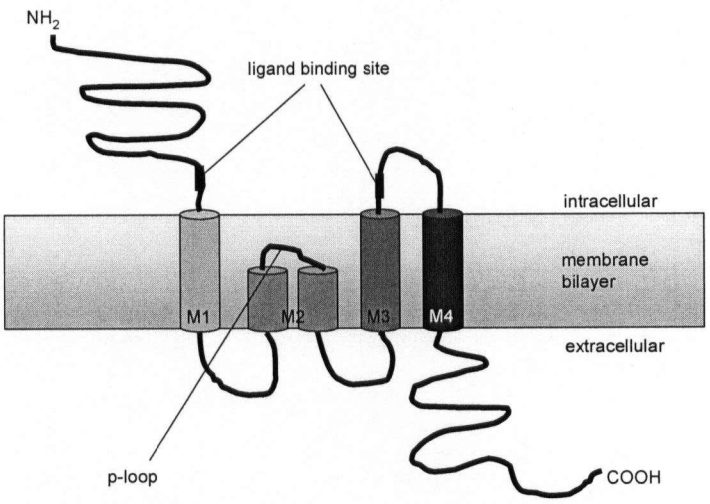

Figure 1. Membrane topology of a kainate receptor subunit. Each kainate receptor subunit contains three membrane spanning domains (M1, M3, and M4), and a *p-loop* (M2), which constitutes the pore of the channel. Ligand binding sites are at the extracellular space next to the M1 and M3 domains (adapted from Dingledine et al., 1999).

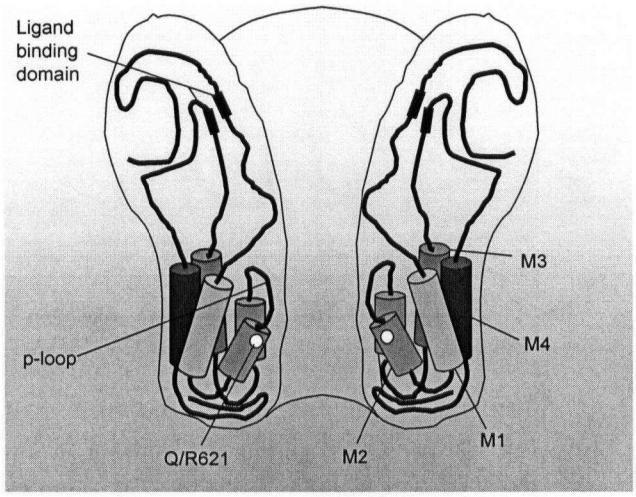

Figure 2. Subunit composition of a kainate receptor. Each kainate receptor is composed of four subunits. The diagram illustrates the site of the *p-loop* forming the channel pore, and the ligand binding sites at the extracellular space. A Q/R editing site is present close to the channel pore (Q/R621, represented by white circle); unedited receptors contain a high channel conductance to Ca^{2+}, and display a linear gating property to monovalent cations (adopted from Dingledine et al., 1999).

1.3. Kainate receptors play a role in synaptic transmission in the hippocampus

Radioactive *in situ* hybridization has shown that both hippocampal pyramidal neurons and granule cells express the GluR5-7 subunits, with the GluR6 subunit being most prevalent (Wisden and Seeburg, 1993; Bahn et al., 1994; Bureau et al., 1999; Paternain et al., 2000; Kohama and Urbanski, 1997), when compared to the GluR5 and GluR7 subunits (Petralia et al., 1994; Porter et al., 1997; Bureau et al., 1999; Bailey et al., 2001).

In the hippocampal CA3 subfield, it has become apparent that kainate receptors can play an important neurophysiological role at the mossy fiber synapse. Presynaptic kainate receptors appear to act to depress both mossy fiber and associational-commissural inputs (Vignes et al., 1998; Contractor et al., 2000; Karniya and Ozawa, 2000; Frerking et al., 2001; Ji and Staubli, 2002), while post-synaptic KA receptors appear to contribute to mossy fiber evoked EPSCs (Castillo et al., 1997; Vignes and Collingridge, 1997; Mulle et al., 1998), and frequency-dependent facilitation (Schmitz et al., 2001; Contractor et al., 2001; Lauri et al., 2001) and act also at excitatory synapses onto interneurons (Cossart et al., 1998; Frerking et al., 1998). It has been reported that KA receptors in the CA3 can modulate the release of transmitter in the mossy-fiber-CA3 synapse. Schmitz et al. (2000) reported that incubation of low dose of kainate (200nM) enhanced presynaptic fiber volley, as well as increased cell excitability. When kainate is added to the synapse at high dose (10μM), on the other hand, presynaptic fiber volley amplitude became suppressed. Post-synaptic KA receptors in the CA3 synapse can be activated in a frequency-dependent manner. Post-synaptic EPSC was seen to be enhanced after repetitive stimulation of the afferent fiber (at 25 or 100Hz). The frequency-dependent nature of the KA receptors can also be seen in kainate-dependent LTP at the mossy fiber synapse, which was also subjective to block by the selective GluR5 antagonist,

LY382884 (Bortolotto et al., 1999).

In the CA1 region, KA receptors also modulate both excitatory and inhibitory synaptic transmission (Chittajallu et al., 1996; Clark et al., 1997; Rodriguez-Moreno et al. 1997; Frerking et al., 1998). Chittajallu and colleagues (1996) provided first evidence that KA receptors play a role on transmitter release in the CA1 region of the hippocampus. It has been observed that bath perfusion of kainic acid produced a dose-dependent reduction of glutamate current in the CA1 pyramidal neurons. This effect was inhibited when slices were added with an AMPA/KA receptor blocker, CNQX. Fluorescent imaging also revealed that bath application of kainic acid induces presynaptic Ca^{2+} signals (Kamiya & Ozawa, 1998), and paired-pulse facilitation was also enhanced when slices were exposed to kainate, further suggesting the existence of presynaptic KA receptors. Although it is evident that KA receptors function in an ionotropic manner, pharmacological studies also reported that KA receptors can exhibit metabotropic actions. The application of metabotropic enzyme inhibitors for protein kinase C and phospholipase C can suppress the release of GABA from the CA1 interneurons (Rodriguez-Moreno et al., 1997), indicating that KA receptors are capable of initiating G-protein coupled cascades for cell signaling.

Although the expression of KA receptors in the dentate gyrus was reported previously, their physiological roles in the dentate gyrus have not yet been established. Despite the intense concentration of KA receptors in this region of the hippocampus, Lerma et al. (1997) reported that all responses were blocked by the selective AMPA antagonist GYKI53655 (150 μM, administered in the presence of NMDA and GABA antagonists). Later, Behr and colleagues (2002), showed that the activation of KA receptors at presynaptic terminals in the DG can reduce GABA release in slices obtained from kindled animals, and to a lesser extent in tissue obtained from control animals. This may indicate that the role of kainate receptors in this region is dependent

upon their activation by high frequency activity like that exhibited during kindling, and that their role in the DG may differ from the role they play in other hippocampal subregions. To test this hypothesis, we used the selective GluR5 antagonist, LY382884, to examine the role of KA receptors in synaptic transmission and plasticity in the DG. LY382884 has been used by previous experiments, and have shown to be selective on KA receptor channels containing a GluR5 subunit (Bortolotto et al., 1999).

2. METHODS

Slice preparation. Male Spraque-Dawley rats (2 to 4 weeks old), obtained from the UBC animal colony, were used in the present experiment (UBC Animal Care Protocol A01-0088). All surgical and handling procedures were in accordance with the guidelines of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia. Hippocampal slices were cut as described previously (Christie et al., 2000; Froc et al., 2003) and individual slices were transferred to the recording chamber as needed. Experiments were performed at room temperature in ACSF composed of (in mM): 125 NaCl; 2.5 KCl; 1.25 NaH_2PO_4; 28.0 $NaHCO_3$; 2.0 $CaCl_2$; 1.0 $MgCl_2$; 25.0 Dextrose. Bicuculline (1μM) was routinely added to the bathing medium during recordings to block $GABA_A$ (γ-amino-butyric acid) inhibitory synaptic activity. To isolate or block specific receptor subtypes, the following compounds were used: LY382884 (GluR5 antagonist, 10 μM, Lilly, IA); GYKI53655 (AMPA antagonist, 5 – 50 μM, Sigma); D-APV (NMDA antagonist, 200 μM, Sigma) and CNQX (AMPA/KA antagonist, 20 μM).

Electrophysiological responses were obtained using 1-3 MΩ recording electrodes filled with ACSF and a Dagan BVC-700 amplifier. Responses from the dentate gyrus were elicited with a sharpened tungsten electrode using biphasic current pulses (120 μs, 10-400 μA) and a digital stimulus isolation unit (Getting Instruments, CA) as described

previously. An Olympus BX50wi microscope (10x objective) was used to visually position both the recording and stimulating electrodes for each experiment. Electrodes were positioned in the middle third of the molecular layer for MPP (Medial Perforant Path) recordings, and in the outer third (adjacent to the fissure, distal from the granule cell layer) for LPP (Lateral Perforant Path) recordings (Figure 3). All evoked responses were continuously elicited at 15s intervals, except during the application of the conditioning stimulation. A stable 15 min baseline period was required before any of the receptor antagonists were utilized in an experiment. For LTP experiments, four bursts of 50 pulses at 100Hz (30s between bursts) were used as the conditioning stimuli. Following any manipulation, single pulse stimulation was resumed at 15s intervals for a minimum of 30 min. All data were acquired at 5-10 kHz, and the initial slope of the negative going waveform was used to assess changes in synaptic efficacy (Christie et al., 2000; Froc et al., 2003). Paired-pulse stimuli (50ms interpulse interval; 6-8 stimulations) were applied in all experiments. The normalized difference between the slopes of the two responses was calculated and is presented as a percentage change. In all figures, each data point represents the mean ± SEM of one minute of data (i.e., average of 4 responses).

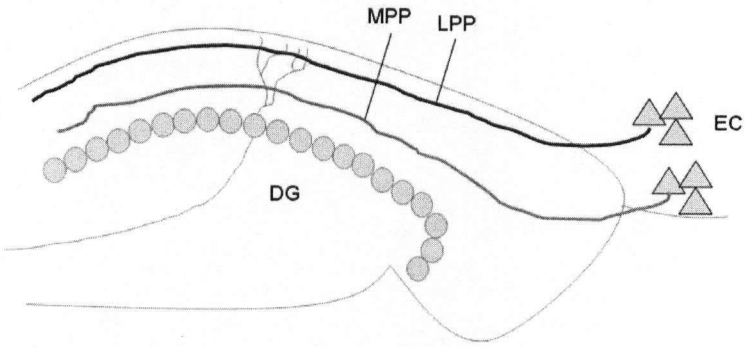

Figure 3. A schematic illustration of the medial and lateral perforant path projections. The medial perforant path (MPP) is positioned at the middle third of the dentate molecular layer, whereas the lateral perforant path (LPP) is located at the outer third of the molecular layer. Both of the perforant paths originate from the pyramidal cells from the entorhinal cortex (EC).

3. RESULTS

In our initial experiments, we placed our electrodes to selectively stimulate and record from the medial aspects of the DG as shown in Figure 4A. These electrode placements routinely resulted in paired-pulse depression when two stimuli were administered at a short interpulse interval (50 ms). To determine whether the selective GluR5 antagonist itself had any effects on synaptic transmission, ACSF containing LY382884 (10 μM) was used following the establishment of stable baseline responses in normal ACSF. To our surprise, ACSF containing the selective GluR5 antagonist reduced the field EPSP by MPP stimulation by 38.1 ± 1.8% (n=5; Figure 4). Paired-pulse depression in these slices remained unchanged (Baseline: 19.97 ± 4.10%, LY382884: 16.44 ± 8.34% of paired-pulse depression, $p>.05$), even after the response had stabilized with LY382884 present in the bath, indicating that LY382884 was likely acting at post-synaptic receptors.

The more lateral aspects of the perforant path in the DG represent a physically and pharmacologically separate input independent of the MPP (Hjorth-Simonsen,

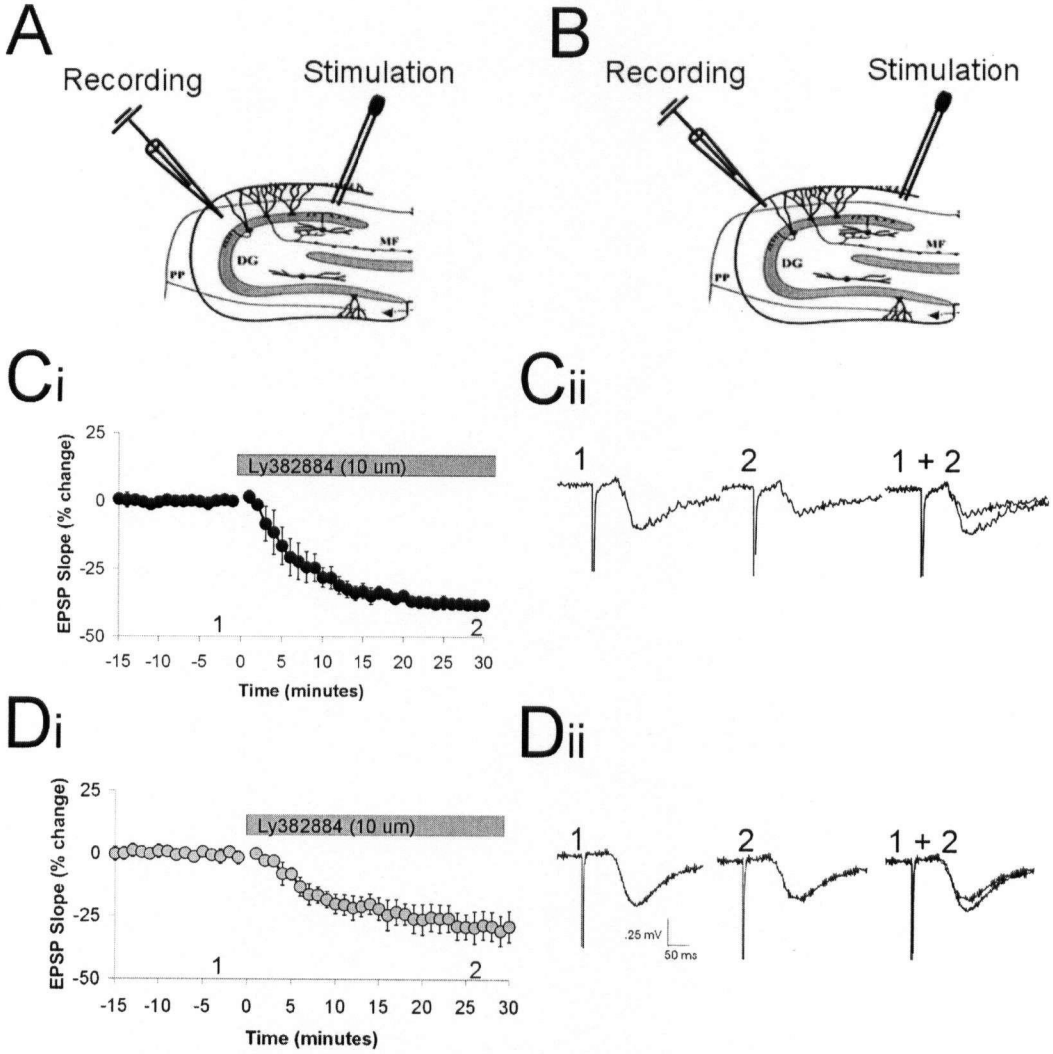

Figure 4. The GluR5 antagonist LY382884 attenuates synaptically evoked responses in the DG. Schematic diagram showing the position of electrodes used when stimulating and recording from the medial aspects of the DG (**A**) versus those used when recording from the terminal regions of the granule cell dendrites for lateral perforant path synapses (**B**). Graph of medial (**C**) and lateral (**D**) perforant path evoked responses in the dentate gyrus prior to and following inclusion of the competitive GluR5 antagonist LY382884 (10μM) in the ACSF. (**Cii, Dii**) Representative traces taken just prior to (1), and 25 minutes following (2), the application of the drug. Scale bars: 0.25mv; 50 ms.

1972). With electrodes positioned as shown in Figure 4B, we reliably obtained paired-pulse facilitation with stimuli administered at 50 ms interpulse intervals, indicating that we were activating LPP fibers (McNaughton and Barnes, 1978; Christie and Abraham, 1992; Froc et al., 2003). In these experiments, the addition of LY382884 produced a 29 ± 6.2% reduction in the LPP evoked EPSP (Figure 1D). The difference between the MPP and LPP in the degree of response suppression by LY382884 was not significantly different ($p>0.05$). Paired-pulse facilitation in the LPP was not affected by LY382884, again indicating a post-synaptic locus for any GluR5 mediated contribution to synaptic transmission in this pathway (Baseline: 15.67 ± 8.31%, LY382884: 22.58 ± 6.35% of paired-pulse facilitation, $p>.05$).

In a second series of experiments we attempted to reveal any putative kainate mediated component of synaptic transmission in the MPP and LPP by blocking AMPA receptors with GYKI 53655 (5 or 50 μM) and NMDA receptors with D-APV (200 μM). As in the previous experiments, inhibition was blocked in these experiments. When a concentration of 5 μM was used, GYKI 53655 reduced MPP elicited EPSPs by 62.8 ± 1.6% (n=6), and LPP elicited responses by 86.1 ± 3.7% (n=6). Attempts to wash-out the block were generally ineffective, with responses only recovering approximately 50% of the amount they were blocked over a 45 minute period (data not shown). Despite the fact that this would indicate that this concentration of GYKI 53655 provides an effective block of all AMPA receptors in a slow perfusion system like that used here, we also performed these experiments using a higher concentration of GYKI53655 (50 μM). At this concentration, MPP responses were reduced 90.1% ± 3.1% (n = 7), while LPP responses were reduced 83.3 ± 6.0% (n = 6; Figure 5). Thus, in neither case were evoked responses in the DG completely blocked, despite being exposed to 50 μM GYKI53655 for prolonged periods (up to 45 minutes). The residual responses could be antagonized with the non-specific KA/AMPA antagonist CNQX,

indicating that these were indeed glutamate-mediated responses (Figure 6). Together, the preceding experiments indicate that GluR5 subunits play a functional role at both MPP and LPP synapses in an area known to be involved in learning and memory processes. This led us to speculate that kainate receptors might play an active role in LTP in this region. To control for the fact that LY382884 reduces the size of EPSPs in the DG, we used EPSPs of a similar magnitude in control slices when performing these experiments (LY382884 = 0.09 $\pm$ 0.03 mV; Con. = 0.13 $\pm$ 0.08 mV; $t_{(5)}$=0.719; p=0.5). Under the LY382884 conditions, evoked responses in the MPP exhibited a significant degree of STP (7.01 $\pm$ 1.17%, $p < 0.05$), although this was significantly smaller than that normally observed in control slices ($p<0.05$). Moreover, LTP was completely abolished in the MPP in the presence of LY382884 (-5.84 $\pm$ 2.49%, $p > 0.05$%), as measured 25-30 minutes post-conditioning (Figure 7). This is in sharp contrast to the robust STP (51.10 $\pm$ 8.80%, $t_{(4)} = 7.42$, $p < 0.05$) and LTP (39.27 $\pm$ 5.58%, $t_{(4)} = 10.72$, $p < 0.05$) we obtained in control slices. Similarly, when conditioning stimuli were administered to equivalent sized EPSPs in the LPP, we only induced LTP in normal ACSF (44.16 $\pm$ 11.72%) and not in the presence of LY382884 (6.69 $\pm$ 7.68%, $t_{(4)} = 1.43$, $p > 0.05$). Interestingly, STP (50.72 $\pm$ 8.34%, $t_{(4)} = 5.25$, $p < 0.05$) was not affected by the addition of LY382884 in this path and was similar to that obtained in control slices (61.41 $\pm$ 11.87%; Figure 7). These experiments indicate that kainate receptors play a role in LTP induction in both the medial and lateral perforant path inputs to the dentate gyrus; however, the nature of their role remained unclear.

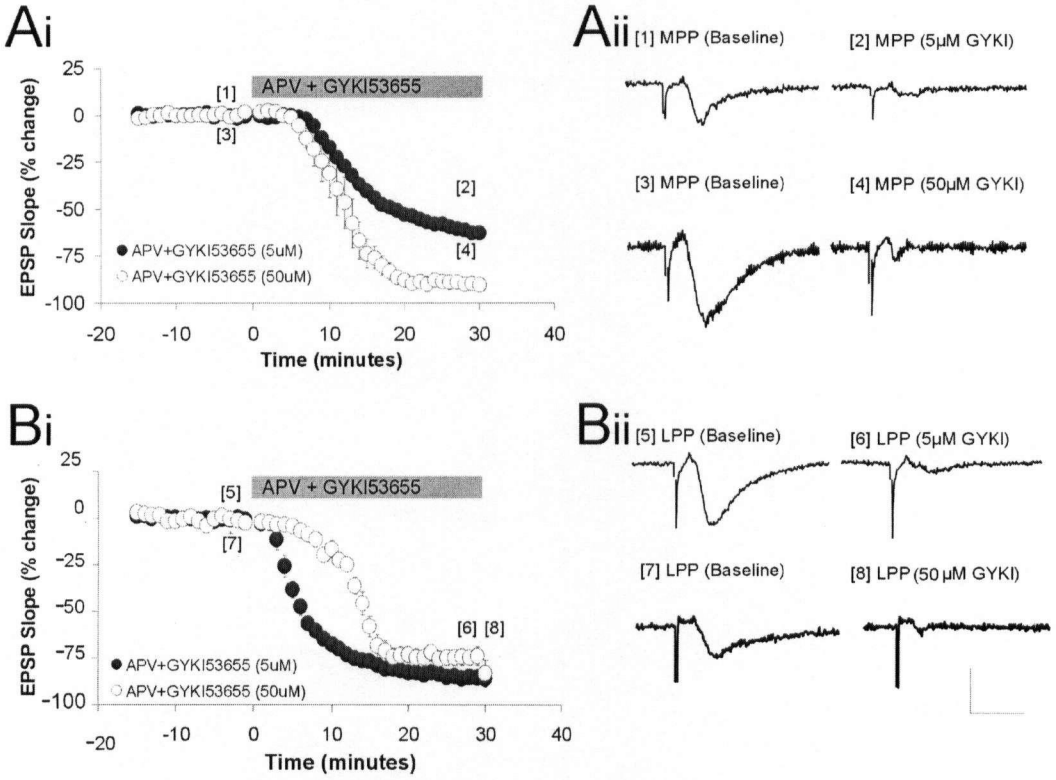

Figure 5. NMDA and AMPA receptor antagonists failed to completely block evoked responses in the DG. The time course for the blockade of both medial (**Ai**) and lateral (**Bi**) evoked responses is shown both prior to and following the wash-in of ACSF containing APV (200 μM) and GYKI53655 (5 or 50μM). Representative traces for the medial (Aii) and the lateral (Bii) perforant path evoked responses are illustrated on the right. Note the residual response left in all instances.

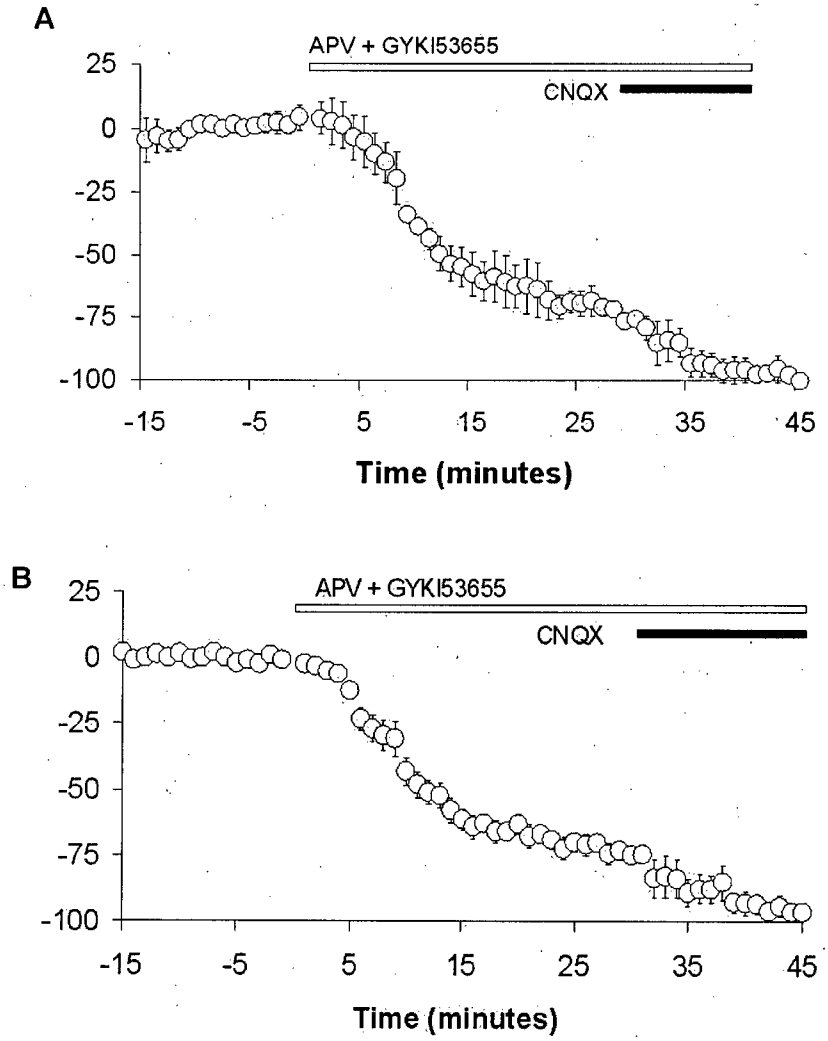

Figure 6. Evoked responses in the medial (A) and lateral (B) perforant paths were challenged by an AMPA/ kainate receptor antagonist (CNQX, 20μM). CNQX abolished all evoked response followed by the NMDA and AMPA receptor antagonists, suggesting residual responses obtained in the presence of APV and GYKI53655 is kainate receptor-mediated.

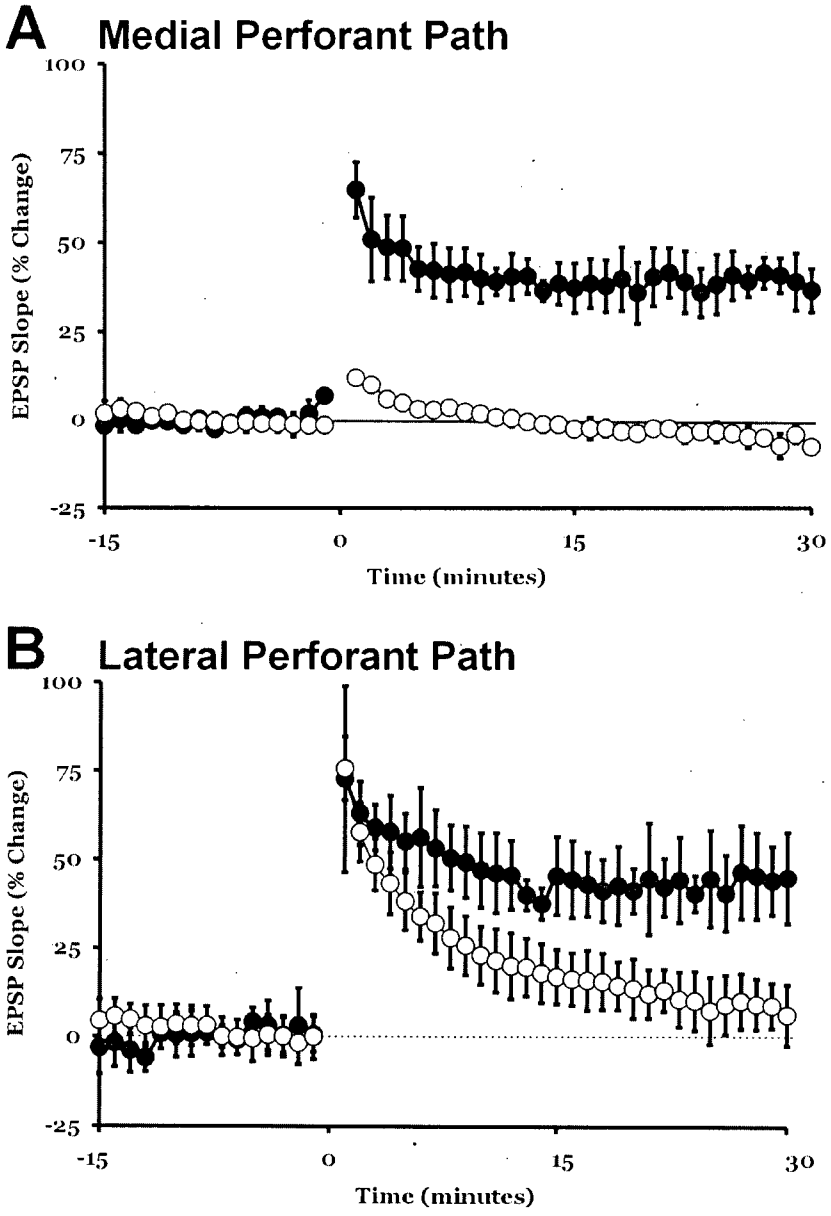

Figure 7. Kainate receptor activation is required for the induction of LTP in the DG. Evoked responses in the medial (**A**) and lateral (**B**) perforant path fail to exhibit LTP when the GluR5 antagonist, LY382884, is present during the application of the conditioning stimulation (open circles). LTP is readily induced with these same conditioning stimuli in both pathways in normal ACSF (filled circles).

One role kainate receptors may play in the DG is to provide either a source of depolarization or a conformational change that results in the alleviation of the NMDA receptor blockade by Mg^{2+}. To determine whether this is in fact the case, we attempted to induce LTP in the presence of LY382884 in a Mg^{2+}-free solution. As is shown in Figure 8, application of conditioning stimuli in this situation induced a significant degree of LTP in the medial path ($33.2 \pm 2.4\%$; $t_{(4)}=2.77$, $p<0.05$). Thus, removal of the Mg^{2+} blockade of the NMDA receptor circumvented the requirement for kainate receptor activation that we observed in normal ACSF. Similar results were also obtained for recordings where LTP was induced in recordings using lateral perforant path stimulation sites (32.1 ± 5.3; $t_{(5)}=6.02$, $p<0.05$).

4. DISCUSSION

Our finding that LY382884 resulted in a substantial decrease in the size of evoked EPSPs in both the medial and lateral perforant path inputs to the dentate gyrus molecular layer comes as quite a surprise given that most immunohistochemical work does not indicate that a high concentration of GluR5 receptors are localized in this region (Paternain et al., 2000). Never-the-less, the receptors that are located in this region appear to play a role in synaptic transmission in the DG. Furthermore, the paired-pulse data would indicate that these receptors are located at post-synaptic sites on dentate granule cell dendrites, rather than at presynaptic locations like those seen in the CA1 and CA3 regions (for review, see Huettner, 2003). In contrast to GluR5, GluR6 subunits have been shown to be evident in the DG in high concentrations (Petralia et al., 1994). In addition, there is also some evidence for mixed GluR5/GluR6 subunit receptors (Paternain et al., 2000) and it may be that LY382884

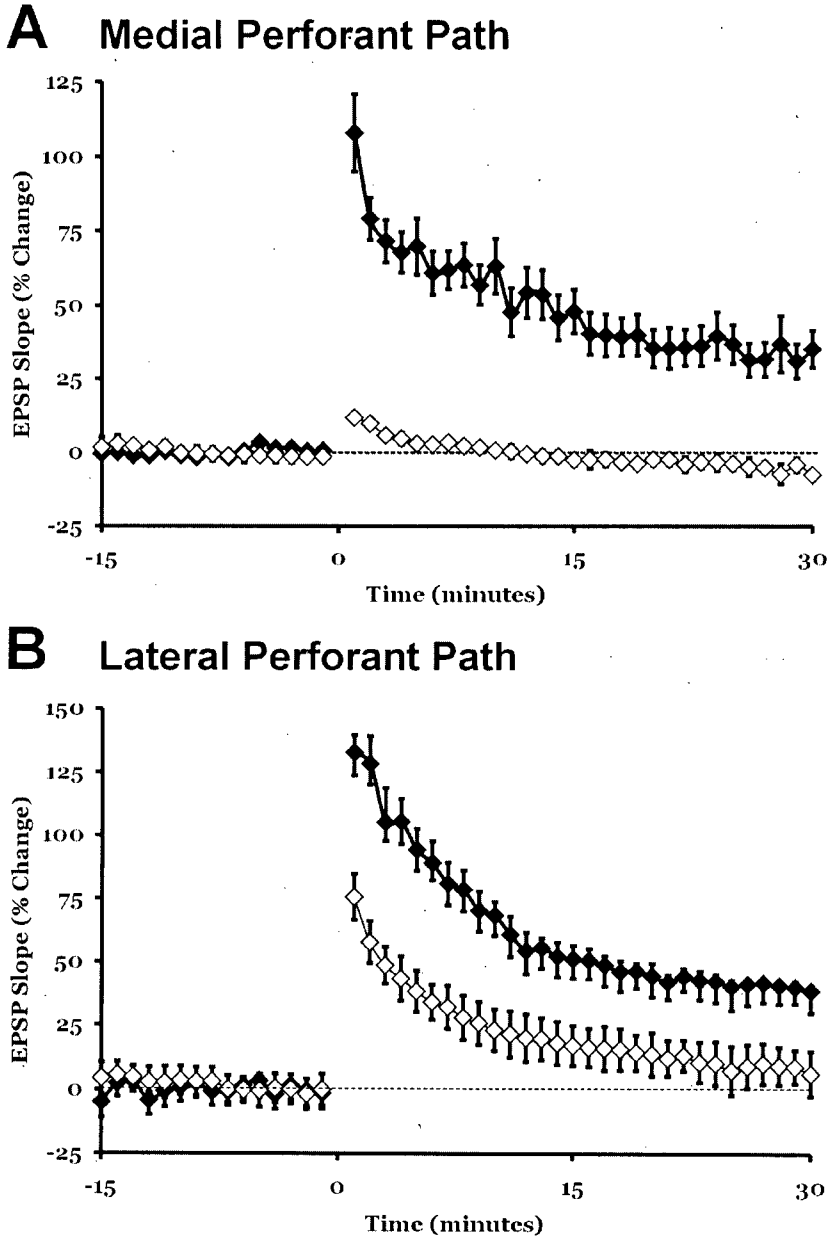

Figure 8. LY382884 failed to block LTP induction in Mg^{2+}-free ACSF. The administration of LTP-inducing conditioning stimuli to the medial (**A**) and lateral (**B**) perforant paths in the presence of LY382884 normally does not result in LTP (open diamonds). These same stimuli, when administered in Mg^{2+}-free ACSF produce robust LTP in both paths in the presence of the Glur5 receptor antagonist LY382884 (filled diamonds).

is acting at these receptors as well. It may also be that receptors with both GluR5 and GluR6 subunits usually immunolabel as only being a GluR6 receptor, and not as GluR5 receptors, or that GluR5 receptors occupy key positions in the dendritic cytoarchitechture. We must also consider the possibility that LY382884 is acting at some site besides the GluR5 subunit, and that the response we are monitoring is in fact not kainate mediated. This seems unlikely given previous work indicating the high degree of specificity LY382884 has for receptors containing the GluR5 subunits (O'Neill et al., 1998). At present, the most parsimonious conclusion we can reach is that GluR5 subunits, located at postsynaptic sites in the DG, play an active role in synaptic transmission in this region.

The other main finding of this study was that LY382884 was effective in blocking LTP at both LPP and MPP synapses. LTP in this region is normally thought of as being dependent upon the activation of NMDA-receptors, and indeed we have previously reported that this is the case in numerous experimental preparations (Christie and Abraham, 1992a,b; Christie et al., 1995; van Praag et al., 1999; Froc et al., 2003; Farmer et al., 2004). Although we have presented evidence for a GluR6 mediated form of NMDA-independent LTP in transgenic mice (Vissel et al., 2001), it should be stressed that this finding reflects a difference found in transgenic animals, rather than a phenomenon present in the normal population. These experiments directly indicate that GluR5 subunits can contribute to the induction of LTP in the DG. In addition, the experiments performed here indicate that one role KA receptors may play in the DG is to act as a source of post-synaptic depolarization, allowing for the subsequent activation of NMDA-receptors by alleviating the Mg^{2+} blockade of this channel.

The role of KA receptors in the DG appears to differ from that they play in the cornu ammonis. It has been shown that LTP induction in GluR6 knockout mice is impaired in the CA1 and CA3 subregions, while being relatively unaffected in the DG.

In contrast, while we have shown an involvement for GluR5 receptors in LTP in the DG, other researchers have failed to find a reduction in LTP in the CA1 and CA3 regions of GluR5 knockout animals (Contractor et al., 2000). It is interesting that Bortolotto et al. (1999) have also shown that LY382884 can antagonize LTP induction in the CA3 region of the hippocampus, another region that does not normally show high GluR5 subunit density in any of the histological experiments to date (Contractor et al., 2001). This may indicate that some compensatory mechanism is engaged when this subunit is knocked out, leading to the discrepancy between the LTP seen in transgenic animals and the lack there of in the pharmacological experiments.

In summary, our results are in agreement with those of Bortolotto and colleagues (1999) on numerous points, despite the fact that our research was carried out in a physiologically, pharmacologically and structurally distinct region of the hippocampus. Together these results indicate that GluR5 subunits have an important role in synaptic plasticity in these regions. Furthermore, our results for the first time indicate that postsynaptically located kainate receptors can play an integral role in synaptic plasticity in the DG, and that they may normally provide the initial depolarization that alleviates the Mg^{2+} blockade of NMDA receptors in the DG.

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