THE EFFECT OF PRESYNAPTIC GROUP II METABOTROPIC GLUTAMATE RECEPTORS ON THE NETWORK OSCILLATORY ACTIVITY IN THE RAT MEDIAL ENTORHINAL CORTEX

Mr. Salahuddin Mohammed1*, Mr. Demissew Berihun Haile2 and Mrs. Aleem Unissa3

1Associate Professor of Pharmacology, College of Health Sciences, Department of Pharmacy, Mizan Tepi University, Mizan Teferi, Ethiopia.
2Lecturer of Clinical Pharmacy, College of Health Sciences, Department of Pharmacy, Mizan Tepi University, Mizan Teferi, Ethiopia.
3Nizam Institute of Pharmacy, Hyderabad, India.

KEYWORDS:
Metabotropic Glutamate receptors, Beta and Gamma oscillations,

ABSTRACT
The metabotropic glutamate receptors (mGluRs) modify the excitatory glutamate transmission in the brain. Kainic acid (KA, 250 nM) was functional to induce oscillatory activity. The concentrations were applied in two different groups of animals (100nM, P18-22) and (300nM, P44-49). 50nM LY354740 (group II mGluR agonist) and 50nM LY341495 (group II mGluR antagonist) were bath applied. In the adults mEC the selective Group II mGluR agonist LY354740 (50nM) increases the power of oscillatory activity in the band between 10-70 Hz whilst the antagonist LY341495 (40 nM) both slows and reduces the peak power of oscillatory activity. We expected that the group II and group III mGluR have complex age and cell dependent effects on the synaptic transmission in the mEC, so we recorded the oscillations from the P18-22 slices. In P18-22 mEC the mGlu2/3 agonist LY354740 (50 nM) increases the power of oscillatory activity in the band between 10-50 Hz whilst the antagonist LY341495 (40 nM) both slows and reduces the peak power of oscillatory activity. Activity is, in general, slower than that in adult slices. The data thus indicate that pharmacological manipulations of the presynaptic receptors will affect the network activity and synchrony in the medial entorhinal cortex (mEC).
1. INTRODUCTION:
The past three decades has highlighted the evolution of glutamate as an important excitatory neurotransmitter in the vertebral nervous system. Besides being involved in various intermediary metabolic functions it also influences the synaptic transmission and the neuronal plasticity [1, 2]. The glutamate plays a critical role in interacting with the diverse amount of receptors present in the brain to generate its activity. Also glutamate has been involved in various cognitive and pathological conditions.

The involvement of glutamate in various conditions like amnesia, stroke, and schizophrenia has evolved the need to develop strategies which would modulate the activity of the glutamate via glutamate receptor [3, 4]. Since the pervasive nature of the glutamate receptor function, drugs that intervene with activity of glutamate receptor function are expected to affect the glutamate functioning thereby causing disturbance to brain functioning.

The physiological function of the body is mediated by glutamate activating various ligand gated ion channels, NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) and the Kainate receptor (KA) [5, 6]. They interact with various subclasses of receptors instigate the opening the Na+ and Ca2+ channels by the receptor itself or by the presence of the sensitive voltage ion channels [7, 8].

The past two decades has emphasized the evidence of the metabotropic glutamate receptors (mGluRs) in a way to modulate the network activity via new class of receptors, the G-Protein coupled receptors (GPCRs) [3]. The mGluRs has been divided into eight subtypes and three groups viz Group I comprising of (mGluR1 and mGluR5); Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, mGluR8).

The Group I mGluR are associated with the G-Proteins, coupled with Phospholipase C (PLC) would cleave the Phosphatidyl inositol bis-phosphate (PIP2) into Inositol triphosphate (IP3) and Diacylglycerol (DAG). The IP3 would interact with IP3 receptors and increases intercellular calcium levels and DAG on other hand activates PKC (Protein Kinase C) [3, 9].

The Group II and III mGluRs inhibit Adenylate cyclase (AC) activity thus decreases the secondary messenger cyclic adenosine monophosphate (cAMP) activity [3, 9]. The activation of Group II and III mGluR would thus produce decrease in the intercellular calcium whereas the activation of Group I mGluR may produce increase in the calcium [8].

The mGluRs modulate the neuronal network activity and other functions such as neuronal plasticity and the transmitter release [2]. They have been also involved in maintaining the dynamic balance between the inhibition and excitation of the neurons in the central nervous system (CNS) [8].
There has been evidence of the generation of gamma oscillatory activity by the activation of kainate receptors [10]. On the insertion of electrodes onto the brain slices, we were able to see the oscillatory activity which is due to the tonically active mGluR in the entorhinal cortex. This point out the differentiated control of the synchrony in neurons providing afferent input to hippocampus.

1.1. THE PERFORANT PATH

The perforant path provides input into the hippocampus. The axons arises evidently from layers II/III and less prominently from layers IV/V. Axons arising from layers II/IV project into the granule cells of the dentate gyrus and the pyramidal cells of CA3 region[11].

Axons arising from layers III/V project into CA1 pyramidal cells and the subiculum[11]. The perforant path can thus be segregated into two distinct medial perforant pathway (MPP) and lateral perforant pathway (LPP)[11]. The concept of LTP was first generated in this pathway. The input provided in the form of unidirectional network by hippocampus forms connections with dentate gyrus and CA3 via perforate path[11]. The CA3 pyramidal neurons receive input from the Dentate gyrus via Mossy fibre network. They further send axons to CA1 pyramidal cells via Schaffer collateral pathway (SCP)[11]. The CA1 pyramidal receive input directly from perforant path and send axons to the subiculum[11].

The entorhinal cortex receives high process sensory input from every sensory modality as well as input relating to ongoing cognitive processes [11]. The layers II/IV provides input of carry axons projecting into Dental gyrus and CA3 pyramidal neurons and layers III/V will provide input to that of subiculum and CA1 pyramidal cells [11].

There have been studies to demonstrate the modulator activity of the pre-synaptic mGluRs. The suppression of the P/Q Ca^{2+} channels and the activation of K^{+} channels is the important pharmacological mechanism for the reduction of glutamate release [2, 3].

---

**Figure 1. The Perforant Path**
There have been studies carried by Billups and colleagues that the activation of mGluRs will change the state of presynaptic terminal and will not produce depression of the synaptic current[12].

There has been evidence of the decrease in the glutamate release in layer II and increase in layer V of mGluR activation[13].

There has also been indication of an increase in synaptic activity with redistribution of synaptic efficacy[14]. These studies has emphasized on the importance of synaptic depression as an essential set point in generation of network synchrony between the neuronal cells[15]. This synaptic depression influences the mGluR activity. Thus the critical role highlighting the importance of presynaptic mGluR in setting the rate of synaptic depression within the pyramidal neurons is an important set point to determine network synchrony[15]. The group III mGluR has been found to have time and cell dependent effects influencing the functional mechanism of synaptic mechanism.

As part of the temporal lobe, the entorhinal cortex (EC) processes information from polymodal and unimodal cortices and interacts strongly with the hippocampus. Recently, the medial EC (mEC) has been shown to exhibit pharmacologically induced neuronal network oscillations in vitro, using the excitant kainic acid [10].

In recent years, modelling studies have highlighted the critical role of short-term synaptic depression between principal cells in the generation of neuronal network synchrony [15]. There has been evidence regarding role of acetyl choline in modulating the memory function in the entorhinal cortex region [16]. One of the mechanisms mediating activity-dependent short-term synaptic depression at glutamatergic synapses involves presynaptic metabotropic glutamate receptors (mGluR), and over the last ten years we have shown that group II and III mGluR have complex age and cell-type dependent effects on synaptic transmission in the mEC.
We hypothesised that activation of group II mGluR, which are thought to be almost exclusively presynaptic, would act to maintain synaptic transmission and hence increase coherence in pharmacologically evoked oscillations. To investigate this possibility we evoked network oscillatory activity using kainic acid, and then test the effects of the selective group II agonist LY354740 (50nM) and the antagonist LY341495 (40nM).

2. AIMS
1. The project is expected to find the effect of mGluR agonists and mGluR antagonists on the network oscillatory activity in the rat medial entorhinal cortex (mEC).
2. The project is also expected to record the oscillations in the gamma (30-80 Hz) and theta (4-12 Hz) and beta (15-30Hz) frequency range induced electro physiologically by kainic acid (100 nM P18-22; 300nM P44-49).
3. The project is expected to find the role of presynaptic receptors on the network activity of whole neurons.

3. OBJECTIVES
The main objective underlying the project is to find the role of metabotropic glutamate receptors (mGluRs) in synaptic facilitation and depression in controlling synchrony of the oscillatory activities produced by the neurons by the pharmacological manipulation of the presynaptic receptors in the theta, beta and the gamma frequency ranges.

4. MATERIALS AND METHODS:
Slices of combined EC-hippocampal from adult Wistar rats (50-70 g) were used to perform the extracellular recording experiments. All the experiments were performed in accordance with the UK animals (Scientific Procedures act 1986) and European Communities Council Directive 1986 (86/609/EEC) and the Bath, Bristol and Aston university ethical review documents. The rats were anaesthetized with isoflurane and N₂/O₂ and then decapitated to remove the brain, which was immediately placed in an oxygenated artificial cerebrospinal fluid (ACSF) apparatus. The brain slices of 450 μm were prepared using Vibroslice (Campden instruments, Loughborough, UK) and stored in ACSF continuously bubbled with 95% O₂/5% CO₂ at room temperature. After an hour of recovery period the slices were placed on small square lens tissue and the neurons were visualized using differential interference contrast optics and an infrared video camera.

4.1. EXTRACELLULAR RECORDING
After an hour of recovery the slices were transferred to a recording chamber mounted on the stage of Olympus. The chamber was well perfused with oxygenated ACSF warmed at 32°C with a flow rate of 2ml/min. The ACSF is comprised of NaCl (126),KCl (3.25), NaH₂PO₄ (1.25),
NaHCO$_3$ (24), MgSO$_4$ (2), CaCl$_2$ (2.5), and D-glucose (10) and the solution is maintained at a pH of 7.4. All the salts used in the preparation of the ACSF and Sucrose cutting solution were of the Analar grade and purchased from Fischer Scientific apart from sodium chloride which was obtained from Sigma Aldrich. The other compounds such as Indomethacin, Uric acid and Ascorbic acid were obtained from Sigma. The standard drugs LY354740 and LY341495 were purchased from Tocris.

In the recording chamber, the slices were left to equilibrate for 45-60 minutes to induce oscillatory activity before any recordings were started. To induce the oscillations, kainic acid at 200-400nM was added to the ACSF bath. The drugs, group II agonist LY354740 and group II antagonist LY341495 were applied to the slices at 50nM and 40nM respectively. Glass microelectrodes made from filament 1.2mm O.D borosilicate glass (sutter) had open tip resistance of 2-4mΩ and were filled with ACSF. The whole cell extracellular recordings were made from the medial entorhinal cortex region and oscillations were recorded using an NPI EXT-01 extracellular amplifier and filtered using an NPI LHBF48X signal conditioner and Clampex 10.2 software (Molecular devices, USA).

Data was analysed off-line using Clampfit, sigma plot and DaDisp. The changes in the power of oscillations were studied at 60s epoch of time. A clearer idea of changes in specific frequency bands was obtained at 30-90Hz for gamma oscillations and 15-29Hz for beta oscillations. T-tests were used to analyze the significant changes in the power of oscillations in the different drug periods.

4.2. THE RECORDING INSTRUMENT
It consists of oscilloscope. Also two humbugs which could absorb the high frequency noise of 50Hz, thereby eliminating it to interfere with the oscillatory activity. We have two amplifiers to amplify the slow and fast oscillations thus making a good recording data to maximize the minuscule and miniature potentials. Every action potential generated will produce an oscillatory event and the activity is recorded.

Figure 3. The Recording Chamber
4.3. DETAILED METHODOLOGICAL PROCEDURE

Initially the medial entorhinal cortex (mEC) slices were cut and placed in an interface chamber with bubbled ACSF prepared at 300-310 milliosmolar concentration. Sucrose cutting solution was used initially to get the brain slices and later ACSF prepared and is bubbled with O₂. The rat is sacrificed by euthanasia (EE/Animal Scientific Act). The slices are placed in the interface chamber for stabilization for 30-45 minutes with ACSF bubbled in. The forceps are thus used to place the slices on the recording chamber with the bubbled ACSF that flows onto the slice getting collected in a small beaker for recirculation. The important component is the heater which provides the heating of the ACSF that flows onto the slice creating moist and humid environment as in vivo. The slice was then kept for stabilization for an hour. Later Kainic acid (KA) was added to activate the Kainate receptor facilitating oscillations. The setup was then kept for half an hour for stabilization. After a stable mode of oscillations was seen drugs have been added. The Clampfit software was used for analyzing the data and Clampex software used for recording of the oscillatory activity. The drugs used were LY354740 and LY341495 as agonist and antagonist which were prepared of 50 nM and 40 nM concentrations respectively. The electrodes were prepared from Sutter instrument and pulled with 2-4mΩ resistance between the electrodes for the extracellular recording. The electrodes were then filled with ACSF. It was fixed in the holders with silver wire inserted. The fine and coarse adjustments of the electron microscope was then used and penetrated enough to touch the extracellular surface of the slice. As soon as it touched the cell we could see the oscillations on the monitor. We then record the oscillations and give it 20-30 mins for stabilization. Then the tags of control, LY354740 or LY341495 were inserted. The set recording was then analysed to check for its stability. The initial time period with the final time period of 1 min was taken and filtered at 50 Hz to eliminate all the noise created with oscillatory activity and the recording system. A power spectrum was then drawn for the initial clump of data and the final clump in layer II and seen whether they were overlapping with no significant change seen in the spectrum with bell shaped curve. A peak power v/s log frequency spectrum was then obtained and for better analysing we take the log linear form of the spectrum.

When we see a stabilized curve we proceed further with the drug LY354740 which is selective Group II mGluR agonist. A 50 nM concentration of 100 nL is pipetted out and poured down into running ACSF bubbled with O₂. It is then allowed to run for some time with the tag on. The agonist LY354740 mimics the Group II mGluR effect viz it boosts or enhances the power of oscillations in a pronounced manner. The effect can be demonstrated by building a power spectrum of the initial LY354740 period and the final LY354740 period.
The curve then shows a significant increase in the power of oscillations when compared to the initial LY354740 period or the final control (Kainic Acid) period. A Bar chart was drawn in an excel worksheet to demonstrate the increase in power (power v/s log frequency) and peak frequency.

The drug LY341495 was added after the addition of LY354740. This drug acts as an antagonist at the group II mGluR. This drug tends to reverse the stimulatory effect. The bath of ACSF is washed with LY341495. The output pipe is allowed to flow out into empty cylinder. Output of LY341495 (20µM) & 300 nM of Kainic acid is added in cylinder of 100ml ACSF and supported with the previous cylinder. The recording is started and set for a period of 20-30 mins to stabilize. The recording is then stopped and clump of recording of first one minute and last minute is taken and filtered by notch filter at 50Hz between cursors one and two or three and four. Either way band pass filter can also be performed on that clump. A power spectrum is generated for LY341495 putting cursors 1 and 2 as initial and 3 and 4 as final. The peak power spectrum of Control, LY354740 and LY341495 also compared with the control as 100%. LY354740 enhances the power and LY341495 brings it back either to control, kainic acid or also more than that of control.

5. RESULTS

In order to better assess mGluR mediated effects in mEC, which we have previously shown to be age-dependent, combined EC-hippocampal slices were prepared from P18-22 and P44-49 rats. Field recordings were made from an interface chamber at a temperature of 32-34˚C. Kainic acid (100nM P18-22, 300nM P44-49) was applied to induce oscillatory activity. 50nM LY354740 (group II mGluR agonist) and 40nM LY341495 (group II mGluR antagonist) were bath applied and due to the nanomolar potencies of these drugs a wash was carried out between the agonist and antagonist application. Oscillatory activity was measured by calculation of the Fast Fourier Transform (FFT) of 60s epochs of data taken from stable baseline and during drug application periods. Spectrograms were constructed using 7.5s samples of data, processed in MatLab, and statistical analyses performed using a Mann-Whitney test between spectra.
Full Text Available On www.ijupbs.com
Ci

Control

\[ 20 \log_{10}(\text{Source amplitude} \cdot (\text{Hz}^2)) \]

LY354740

50\mu V

0.5s

Cii

LY354740

\[ 20 \log_{10}(\text{Source amplitude} \cdot (\text{Hz}^2)) \]

LY341495

Full Text Available On www.ijupbs.com
Figure 4. In adult mEC the mGlu$_{2/3}$ agonist LY354740 (50 nM) increases the power of oscillatory activity in the band between 10-70 Hz whilst the antagonist LY341495 (40 nM) both slows and reduces the peak power of oscillatory activity. A. Raw data showing activity recorded during different experimental periods. B. Averaged power spectra (n=6) showing the effects of LY354740 and LY341495. Ci. Wavelet average spectrogram showing 7.5s of control activity and a similar period in LY354740. Cii. Wavelet average spectrogram showing 7.5s of...
control activity and a similar period in LY341495. Di. Mann-Whitney wavelet spectrogram analysis showing significant increased oscillatory activity in the 2-20 Hz and 30-60 Hz bands following application of LY354740. Dii. Mann-Whitney wavelet spectrogram analysis showing significant decreased oscillatory activity in the 30-60 Hz band and increased activity in the 2-20 Hz band following application of LY341495.
Full Text Available On www.ijupbs.com
Mann-Whitney Z-statistic

Di
Figure 5. In P18-22 mEC the mGlu$_{2/3}$ agonist LY354740 (50 nM) increases the power of oscillatory activity in the band between 10-50 Hz whilst the antagonist LY341495 (40 nM) both slows and reduces the peak power of oscillatory activity. Activity is, in general, slower than that in adult slices. A. Raw data showing activity recorded during different experimental periods. B. Averaged power spectra (n=6) showing the effects of LY354740 and LY341495. Ci. Wavelet average spectrogram showing 7.5s of control activity and a similar period in LY354740. Cii. Wavelet average spectrogram showing 7.5s of control activity and a similar period in LY341495. Di. Mann-Whitney wavelet spectrogram analysis showing significant increased oscillatory activity in the 30-60 Hz band following application of LY354740. Dii. Mann-Whitney wavelet spectrogram analysis showing significant decreased oscillatory activity in the 2-15 Hz band following application of LY341495.
6. DISCUSSION

The project is expected to discover the role of presynaptic mGluR in synaptic depression and synaptic facilitation with emphasis on measuring the effect on oscillations in the theta, beta and gamma frequency range. The modulation of the network activity is carried out by the action of selective Group II mGluR agonist LY354740 and selective Group II mGluR antagonist LY341495 directly on the network of whole neurons. The EPSPs generated on the addition of LY354740 were generally of greater power and amplitude in the adult slices of the mEC. The selective group II mGluR agonist LY354740 tends to boost the oscillatory activity both in power and amplitude in the adult mEC.

The selective group II mGluR antagonist LY341495 has suppresses the oscillatory activity in adult mEC. The LY341495 has suppressed the oscillatory power with a greater power frequency in the theta (4-12 Hz) frequency range.

The selective group II mGluR agonist LY354740 and antagonist LY341495 also modulate the frequency at which the oscillations are generated. The LY354740 has increased the frequency and LY341495 has decreased with both the frequency changes corresponding to the gamma (10-70 Hz) and theta (2-20) frequency range.

The modulation of the network activity is carried out by the action of selective Group II mGluR agonist LY354740 and selective Group II mGluR antagonist LY341495 directly on the network of whole neurons. The EPSPs generated on the addition of LY354740 were generally of greater power and amplitude in the P18-22 slices of the mEC. The selective group II mGluR agonist LY354740 tends to boost the oscillatory activity both in power and amplitude in the P18-22 slices of the mEC.

The selective group II mGluR antagonist LY341495 reduces the oscillatory activity in P18-22 slices of the mEC. The LY341495 has suppressed the oscillatory power with a greater power frequency in the 10-50 Hz frequency range.

The selective group II mGluR agonist LY354740 and antagonist LY341495 also modulate the frequency at which the oscillations are generated. The LY354740 has increased the frequency and LY341495 has decreased with both the frequency changes corresponding to the 10-50 Hz frequency range.

There has been evidence from the previous literature that the deep layer of the mEC is driven by the action potential dependent evoked release prominently due to the NMDAR mediated EPSPs (excitatory post synaptic potentials) with weak synaptic inhibition [17]. The superficial layer of the mEC on the other hand is driven by the action potential independent release prominently by the inhibitory post synaptic potentials (IPSPs) due to the stimulation of the GABA [18, 19, 20].
Thus superficial layer II of the mEC has dominant inhibition factor over excitation and the deep layer V of the mEC has predominant excitation factor over inhibition. Since the mGluR activation produces decreased glutamate release by decrease in the voltage gated calcium [21,22,23] the superficial layer driven by action potential independent GABA mediated IPSPs are less affected by the decrease in the Calcium release but the deep layer being Action potential dependant is affected much likely due to the changes in the calcium. Thus the deep layers of the mEC are more likely to be affected by the mGluR ligands as it is action potential dependent.

There has been evidence in the previous literature about the dual role of mGluRs having autoreceptor function at the glutamatergic synapses and hetero receptor function at the GABAergic synapses [24,25,26]. The GABA release can not only be modulated by the GABA receptors but also by the mGluRs acting presynaptically to inhibit GABA [27, 28]. This modulatory effect has been attributed by the Group II and Group III mGluR at the pre presynaptic terminal [3,29]. The oscillations measured form the P18-22 slices of the rats demonstrated mostly theta and beta activity. In contrast the activity measured from the adult slices has demonstrated mostly theta and beta activity.

6. CONCLUSIONS:
Oscillatory activity in slices taken from mEC at P18-22 show mostly slow theta/beta activity. Oscillatory activity in both P18-22 and adult mEC is enhanced by activation of group II mGluR, at both low (2-20 Hz) and higher (30-60 Hz) frequencies. In both adult and P18-22 mEC slices, oscillatory power is reduced by antagonism of group II mGluR. Presynaptic mGluR may play a role in controlling coherent activity between cortical neurones during network activity. In particular, gamma activity does not seem to be supported in the absence of functional mGluR.

ACKNOWLEDGEMENTS
I am grateful to my supervisor Dr. Gavin Woodhall, colleagues and friends, for their patience, for being inspirational, and for teaching me the importance of using curiosity as the driving force behind research. I would like to thank him for invaluable lessons about the importance of guidance and for the freedom they granted to me during my work in their laboratory.

7. REFERENCES


