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Effect of Metabotropic Glutamate Receptors II /III Agonists on Spike-Wave Discharge in Primary Somatosensory Perioral Cortex of Male WAG/Rij Rats

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The aim of this study was investigation of metabotropic glutamate receptors II/ III on spike-and-wave discharges in perioral somatosensory cortex of male WAG/Rij rats. Eighteen male WAG/Rij rats was used in this experiment. Rats anesthetized with a combination of ketamine and xilusinip and 2 μl of drugs injected with a 10 μl Hamilton syringe bilaterally. At the end of the experiment, all animals were killed by high dose of ketamine. The brain was removed rapidly and fixed in 10% formalin. Cresyl violet stained coronal sections were viewed with a light microscope to verify cannula placement. In all groups mean numbers and mean duration of Spike-and-wave discharges was decreased, but peak frequency was only in L-AP4 group was reduced. These agonists are able to suppress epileptic discharges in WAG/Rij rats and could be useful for treatment of Absence epilepsy in the future.

Key words: Metabotropic, Glutamate, WAG/Rij, Spike-and-wave, Perioral somatosensory cortex.

Spike-and-wave is the term that describes a particular pattern of the electro encephalogram (EEG) typically observed during epileptic seizures. The spike-and-wave discharge is a regular, symmetrical, generalized EEG pattern seen especially during absence epilepsy, also known as 'petit mal' epilepsy [1]. The basic mechanisms underlying these patterns are complex and involve part of the cerebral cortex, the thalamocortical network, and intrinsic neuronal mechanisms [2]. Some studies suggest that a thalamocortical (TC) loop is involved in the initiation of spike-and-wave oscillations [1,2]. One of the most important theory is the cortical focus theory that infer that the origin of spike-and-wave discharge located in the cortex instead of thalamus or other subcortical areas [3]. Wistar Albino Glaxo Rijswijk (WAG/Rij) rats are described as a genetic model for absence epilepsy. These animals exhibit 7-11 Hz SWD lasting from 1–30 seconds[3]. Based on cortical focus theory and the nonlinear association analysis of SWDs, in these animals perioral somatosensory cortex (S1po) is the initiation site for SWDs [1]. Seizures were produced in this zone and disseminated quickly in other areas of the cortex and thalamus[3]. Glutamate receptors are classified into two types: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are divided into three subtypes: kainate, AMPA, and NMDA receptors. iGluRs are involved in fast synaptic transmission at glutamatergic synapses [4]. mGluRs

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are G-protein coupled receptors of the glutamate receptor family. They are play an important role in glutamatergic transmission [4,5]. Based on signal transduction mechanisms, the pharmacological profile and receptor protein, mGluRs are classified into three groups: group 1 (mGluR1 and 5), 2 (mGluR2 and 3), and 3 (mGluR4, 6, 7, and 8) [6].

Members of group I (mGluR1 and mGluR5) are coupled to the Gq signaling pathway and stimulate PIP2 hydrolysis. Members of group II (mGluR2,3) and III (mGluR4,6,7,8) are coupled to G_i/G_0 signaling pathways and inhibit adenylate cyclase activity in hetrologous expression system [7]. Agonists and antagonists of the mGluRs influence the development and propagation of seizure in some animal models. Group II /III mGluRs have been shown to located on presynaptic terminals at glutamatergic neurons and their activation resulted in decreasing glutamate release[7-10]. Such an action could explain the marked inhibitory effects of group II agonists, such as APDC a highly selective agonist of group II metabotropic glutamate receptros, on epileptiform activity. L-AP4 and L-SOP are classic agonists of group III metabptropic glutamate receptors that inhibit synaptic transition in lateral prefrontal path in a reversible and dose dependent manner [3]. Both of them show acute anticonvulsant activity without proconvulsant effects. Activation of metabotropic glutamate receptors decreases glutamate release [7,9]. The most likely candidate to mediate the action of L-AP4 is the subtype mGluR8 [11].

Based on above mentioned information, we used L-AP4, a highly selective agonist of group III mGluRs, and APDC for group II mGluRs. Spike-and-wave discharge is in common in human absence epilepsy and in EEG recorded from WAG/Rij rats. In 2o % of patients antiepileptic drugs are unable to decrease or stop absence seizure, necessitating the development of new protocols to treat epileptic patients [12,13]. This study performed to understand effects of mGluRs agonists (APDC, L-AP4) on spike-and-wave discharges in perioral somatosensory cortex of WAG/Rij rats.

MATERIALS AND METHODS

For investigation of mGluRs agonisteffects on SWD in rats we assessed three parameters of SWDs, mean numbers, mean duration and peak frequency of SWDs. The adaptation time to lab condition for rats was 30 minutes. After adaptation we begin to recording for one hour for pre injection period. Drugs injected to S1po area of rats. After injection of drugs, post injection recording started and continued for one hour.

Animals

Eighteen male WAG/Rij rats of 6-7 months age and 250-300g body weight were used. Rats were purchased from Shefa Neuroscience Research Center in Tehran-Iran. Animal were kept in standard condition (temperature was 22±2 0C, 12 h light/ dark cycle with 08:00 AM lights on). The food and water were freely available throughout the study. Every effort was made to reduce animal suffering and minimize the number of used animals. Before surgery, animals were housed in small groups at one cage and after surgery each animal was housed individually. All experiments performed at the same time (08:00 AM to 04:00 PM).

Cannulae and electrodes implantation

Rats were anesthetized with injection of ketamine and xylazine (i.p 80 and 5 mg/kg) respectively[3,14]. Surgical procedure was done using a stereotaxic instrument and Paxinous and Watson atlas[14]. The incisor bar set 3.3 mm below the interaural line. Three groups were considered in this expriment, including: L-AP4 (L-(+)-2-Amino-4-phosphonobutyric acid), APDC ((2R,4R)-4-Aminopyrrolidine-2,4-dicarboxylate) and control group. Cannulae were implanted bilateraly in S1po cortex of rats. The coordinates of the cannula tip were the following: 2.1 mm posterior, 5.5 mm lateral to the bregma, and 4.0 mm vertical from the skull surface. Two electrodes were used: A monopole recording electrode in the frontal region (coordinates: AP: 0.22mm, L: 0.24mm, V: 0.26mm) in the right hemisphere and the ground electrode was implanted in occipital cortex (coordinates: AP: -11.04mm, L: 4mm, V: 0.26mm). Coordinates were taken with bregma zero-zero and skull flat. All electrodes were fixed in the socket by means of their pins and the socket was fixed to the skull with dental cement. In the control group cannula implanted in S1po cortex for injection of ACSF(Na 150; K 3.0; Ca 1.4; Mg 0.8; P 1.0; Cl 155) instead of drugs.

Recording

After one week of recovery, the animals were put in Faraday cage for electro

encephalography (EEG) in freely moving way. The socket of the rats was connected to a flexible, shielded wire for recording EEG. Signals were amplified by DAM 80 AC preamplifier (WPI Inc, USA) and processed with a power lab running chart software (ver. 05, AD Instrument, Australia). EEG was recorded with the sampling rate of 1 kHz. Before recording, the animals were kept in Faraday cage for 30 minutes to acclimate. SWD mainly occur during drowsiness and light sleep, so in order to prevent the animal from sleeping it was stimulated with sound or touch (Figure 1).

Drug injection

The solubility of L-AP4 in NaOH is more than its solubility in water. It dissolved in 0.1 N NaOH as stock solution and diluted in ACSF but APDC dissolved in water based on safetry data sheet of drugs adopted from Tocris Bioscience webpage. All drugs produced by Tocris Bioscience (UK). At the end of surgery we solved Acetaminophen in water to alleviate the pain (6 mg/ml) [8]. All drugs are water solvable. A 10µl Hamilton syringe was used to inject 2 µl of drug in S1po of rats. Dosage of drugs were selected based on similar studies; 20 nmol for L-AP4 and 40 nmol for APDC [15].

EEG Analysis

Data of mean peak frequency, mean number and mean duration of SWDs that were obtained from EEG analysis were averaged and expressed as mean±standard errors. Frequency refers to the rate of SWD per second. The amplitude of SWDs was 2.5 time higher than other brain signals and it was the feature that we used for detection of SWDs. Pvalues smaller than 0.05 were considered significant. In all experiments, the mean number, mean duration and mean peak frequencies of SWDs were scored over than 30 minutes before and after injection. For comparing before and after injection paired t-test was used.

Histological verification

At the end of the experiment, all animals were killed by high dose of ketamine. The brain was removed rapidly and fixed in 10% formalin. Cresyl violet stained coronal sections were viewed with a light microscope to verify cannula placement. Cannula penetration in cortex was used for detection of cannula placements. Only histological verified recording sites were included in the analysis (Figure 2).

RESULT

Effects of drugson mean number of SWDs

The intrusion criteria for SWDs in this study were that its duration should be more than 3 seconds. At the end of recording we calculated numbers of SWDs that were more than 3 seconds. Numbers of SWDs in all groups are presented in Fig.1. There was a noticeable decrease in mean number of SWDs in all groups but L-AP4 was completely suppressed SWDs in rats ($P<0.01$). In APDC group mean number of SWDs was found to decrease after injection (100±14 vs. 53.1±16.7). In L-AP4 group SWDs, are completely suppressed and displayed a greater reduction in mean number when compared to the other groups (100±14 vs. 4±.7) (Figure 3)

Effects of drugs on mean duration of SWDs

In all groups, except for the control, the mean duration of spike-wave discharges were revealed to reduce. The reduction in APDC and L-AP4 was significant before and after of drug injection. In APDC group difference before and after of injection was significant and change in mean number was with reduction in mean duration of SWDs (100±14% vs. 63±13.6%). As mentioned in previous section L-AP4 was completely suppressed SWDs and mean duration of SWDs coordinately decreased with mean number of

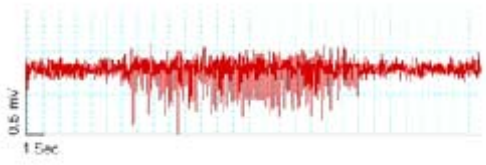

Fig. 1. SWD recorded during this experiment. The amplitude of SWDs is 2.5 time more than baseline and Its duration is more than5 seconds

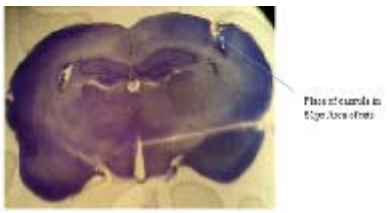

Fig. 2. Cresyl violet staining of brain slicesfor detection of Cannula placement. Cannula is in exact place

SWDs in this group (100±14% vs. 3±.7%) (Figure 4)

Effects of drugs on Peak frequency of SWDs

Effect of drugs on peak frequency of SWDs was examined in all experimental groups before and after drugs injection or stimulation. In APDC (100±9.2% vs. 98.4±17%), Significant change in peak frequency was found only in L-AP4 group (10s0±9.6% vs. 44±5.4%). For measurement of peak frequency in L-AP4 group, some points of EEG, recorded from rats selected randomly and change of this parameter measured (Figure 5).

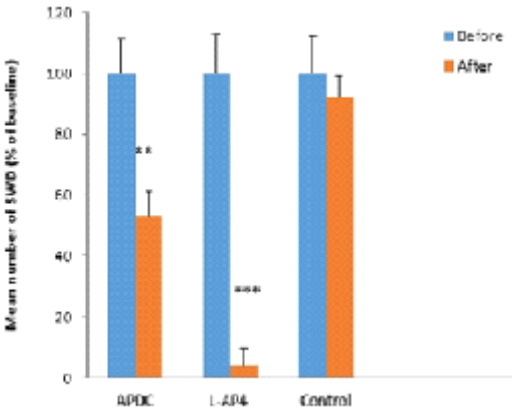

Fig. 3. Effect of drugs on mean numbers of SWDs. differences before and after injection are in all groupssignificant by paired t-test. In L-AP4 group reduction of SWDs number is significantly greater than other groups. Values are mean±SE, *p<0.05, **p<0.01, ***p<0.001.

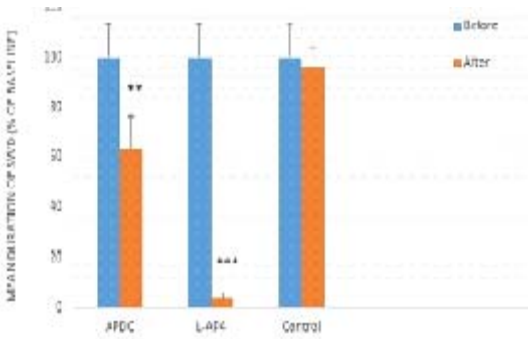

Fig. 4. Effect of drugs on mean duration of SWDs. Reduction of mean duration before and after drug injection by paired t-test indicate that APDC and L-AP4 were significantly changed these parameters. L-AP4 reduce mean duration more than APDC group. Values are mean±SE, **p<0.01, ***p<0.001.

DISCUSSION

There is growing evidence of usefulness of mGluRs agonists in absence epilepsy treatment. Over the last years it has become clear that glutamate also serves a regulatory function through activation of receptors coupled to second messengers [7]. Activation of these receptors with chemical agent or synthetic agonist can change seizure susceptibility. Many studies reported long term depression in glutamatergic transmission and depression of evoked EPSCs after application of group II/III agonists in many regions of brain [16 8,10,17]. The mGluRs play an important role in the initiation of ictal discharges by participating in the interictal-ictal transition, and may play a crucial role in recruiting normal brain tissue into synchronized discharges, thereby facilitating propagation of seizure activity [7]. The present study shows that III mGluRs agonist (L-AP4) and II mGluRs agonist (APDC), significantly decrease the SWDs. Both of drugs have a dose dependent manner in CNS. Group II mGluRs may be a promising target due to their selectivity and inhibitory action on cortical and thalamic neuron [4,18]. Group III mGluRs agonists have shown mixed effects; some studies have demonstrated it as a pro-convulsive while in other studies found it to have a protective role in absence seizure and proconvulsant in generalized seizure [15]. This shows involvement of multiple pathways in Group III mGluRs action. Further studies required before

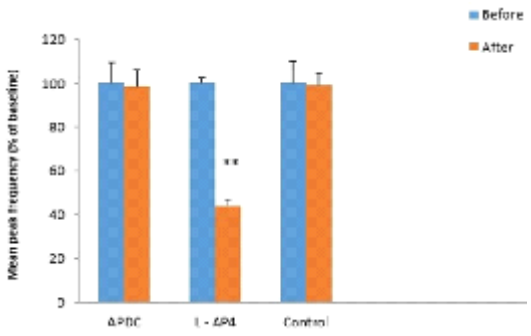

Fig. 5. Effect of drugs on Mean peak frequency of SWDs.Reduction of mean Peak frequency before and after drug injection by paired t-test indicate that only L-AP4 was significant. L-AP4 reduce mean peak frequency more than other groups. Values are mean±SE, **p<0.01.

labeling it as an important target [4].Group II-III metabotropic receptors have been shown to be localized on presynaptic terminals of glutamatergic neurons and their activation generally results in decreased glutamate release [19]. Such an action could explain the marked inhibitory effects of group II agonists on epileptiform activity [20]. In the present study APDC reduced mean number and mean duration of SWD but mean frequency of SWDs not changed after injection of drugs. As described previously, the group III mGluRs specific agonist L-AP4 inhibited synaptic transition in the LPP of rats in a reversible and dose depended manner. The most likely candidate to mediate the action of L-AP4 in the lateral prefrontal path (LPP) is the subtype mGluR8 [16]. (S)-PPG, L-AP4, L-SOP, and related compounds show very little agonist or antagonist activity at group-I and -II mGluRs or iGluRs, which qualifies them as useful tools to address the overall roles of group-III receptors in brain physiology and in animal correlates of CNS disease. However, their lack of receptor subtype-selectivity within group-III is a major limitation in terms of interpretation of physiological results with such drugs [11]. The mGlutRs are distributed in all part of central nervous system and provide a platform for pre and post synaptic control of glutamate release [21]. Suppression of SWDs in this group is in consistency with findings of Christopher and colleagues that explain that activation of group II-III mGluRs reduces EPSCs in the perforant pathway-CA1stratum lacunosum molecular interneuron [20]. L-AP4 is the most potent and selective agonist for group III mGluR, including mGluR4, 6, 7 and 8, but, unfortunately, it is not selective among individual group III mGluR subtypes [22]. That's why the exact mechanisms underlying its effects are not clear. Group III mGluRs have a lower affinity for glutamate than the group II mGluRs and thus they require higher glutamate concentrations for their activation [23]. But in this study Group III agonist showed a very potent effect than group II and it could be investigated in future.

Some patients have resistance to anti-epileptic drugs (AEDs), which mainly target ion channels on postsynaptic membrane. This drug resistance makes it important to investigate new target for epilepsy treatment. Targets that control glutamatergic neurotransmission are of special interest.

Seizure is the resultof elevation in glutamate level in CNS and if a drug has the ability to decrease or completely suppress glutamate release it resulted in suppressing of epilepsy. As discussed earlier, targeting mGluRs for seizure treatment with specific pharmacological tools provides new avenues to develop new therapeutic approaches for the various forms of epilepsy [7]. The findings of this study are in agreement with other studies that tested antiepileptic effects of mGluRs agonists in hippocampal and amygdale kindling. As mentioned earlier in introduction, L-AP4 has mixed effects, in high doses it can be proconvulsant, but in low doses it can suppress epileptic seizure [15].

Our study adds further evidence to the effect of mGluRs agonists applied on the epileptogenic focus in animal models of epilepsy especially in absence seizure. Our findings are in consistent with this idea that metabotropic glutamate receptors agonists are able to suppress epileptogenesis in absence seizure. L-AP4 completely suppressed SWDs in this study and it revealed that it is more effective than APDC in this study.

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REFERENCES

1. Pinault, D., Vergnes, M., Marescaux, C. Medium-voltage 5–9-Hz oscillations give rise to spike-and-wave discharges in a genetic model of absence epilepsy: in vivo dual extracellular recording of thalamic relay and reticular neurons. *Neuroscience.*, 2001; **105**(1):181-201.
2. Snead, O.C. Basic mechanisms of generalized absence seizures. *Annals of neurology* .,1995; **37**(2):146-157.
3. Sadighi, M., Shahabi, P., Gorji ,A., Pakdel, F.G., Nejad, G.G., Ghorbanzade, A. Role of L-and T-Type Calcium Channels in Regulation of Absence Seizures in Wag/Rij Rats. *Neurophysiology.*, 2013; **45**(4):312-318.
4. Anovadiya, A.P., Sanmukhani, J.J., Tripathi, C. Epilepsy: Novel therapeutic targets. *Journal of*

pharmacology & pharmacotherapeutics., 2012; **3**(2):112.

5. Tanabe, Y., Masu, M., Ishii, T., Shigemoto, R., Nakanishi, S. A family of metabotropic glutamate receptors. *Neuron.*, 1992; **8**(1):169-179.
6. Miller, S., Kesslak, J.P., Romano, C., Cotman, C.W. Roles of metabotropic glutamate receptors in brain plasticity and pathology. *Annals of the New York Academy of Sciences.*, 1995; **757**(1):460-474.
7. Ure, J., Baudry, M., Perassolo, M. Metabotropic glutamate receptors and epilepsy. *Journal of the neurological sciences.*, 2006; **247**(1):1-9.
8. Bauer, D.J., Christenson, T.J., Clark, K.R., Powell ,S.K., Swain, R.A. Acetaminophen as a postsurgical analgesic in rats: a practical solution to neophobia. *Journal of the American Association for Laboratory Animal Science.*, 2003; **42**(2):20-25.
9. Grueter, B.A., Winder, D.G. Group II and III metabotropic glutamate receptors suppress excitatory synaptic transmission in the dorsolateral bed nucleus of the stria terminalis. *Neuropsychopharmacology.*, 2005; **30**(7):1302-1311.
10. Lorez, M., Humbel, U., Pflimlin, M.C., Kew, J.N. Group III metabotropic glutamate receptors as autoreceptors in the cerebellar cortex. *British journal of pharmacology.*, 2003; **138**(4):614-625.
11. Flor, P.J., Acher, F.C. Orthosteric versus allosteric GPCR activation: the great challenge of group-III mGluRs. *Biochemical pharmacology.*, 2012; **84**(4):414-424.
12. Glauser, T.A., Cnaan, A., Shinnar, S., Hirtz, D.G., Dlugos, D., Masur, D., Clark, P.O., Capparelli, E.V., Adamson, P.C.Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy. *New England Journal of Medicine.*, 2010; **362**(9):790-799.
13. Panayiotopoulos, C. Typical absence seizures and their treatment. *Archives of disease in childhood.*, 1999; **81**(4):351-355.
14. Paxinos, G., Franklin, K.B. The mouse brain in stereotaxic coordinates. *Gulf Professional Publishing*; 2004.
15. Moldrich, R.X., Talebi, A., Beart, P.M., Chapman, A.G., Meldrum, B.S. The mGlu 2/3 agonist 2R, 4R-4-aminopyrrolidine-2, 4-dicarboxylate, is anti-and proconvulsant in DBA/2 mice. *Neuroscience letters* .,2001; **299**(1):125-129.
16. Bushell, T.J., Jane, D.E., Tse, H.W., Watkins, J.C., Garthwaite, J., Collingridge, G.L. Pharmacological antagonism of the actions of group II and III mGluR agonists in the lateral perforant path of rat hippocampal slices. *British journal of pharmacology.*, 1996; **117**(7):1457-1462.
17. Price, C.J., Karayannis, T., Pal, B.Z., Capogna, M. Group II and III mGluRs-mediated presynaptic inhibition of EPSCs recorded from hippocampal interneurons of CA1 stratum lacunosum moleculare. *Neuropharmacology.*, 2005; **49**: 45-56.
18. Alexander, G.M., Godwin, D.W. Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. *Epilepsy research.*, 2006; **71**(1):1-22.
19. Muto, T., Tsuchiya, D., Morikawa, K., Jingami, H. Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. *Proceedings of the National Academy of Sciences.*, 2007; **104**(10):3759-3764.
20. Keele, N.B., Neugebauer, V., Shinnick-Gallagher, P. Differential effects of metabotropic glutamate receptor antagonists on bursting activity in the amygdala. *Journal of neurophysiology.*, 1999; **81**(5): 2056-2065.
21. Dickerson, J.W., Conn, P.J. Therapeutic potential of targeting metabotropic glutamate receptors for Parkinson's disease. *Neurodegenerative disease management.*, 2012; **2**(2): 221-232.
22. Schoepp, D.D., Jane, D.E., Monn, J.A. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology.*, 1999; **38**(10): 1431-1476.
23. Bellone, C., Luescher, C., Mameli ,M. Mechanisms of synaptic depression triggered by metabotropic glutamate receptors. *Cellular and molecular life sciences.*, 2008; **65**(18): 2913-2923.