Unilateral Hypothalamus Inactivation Prevents PTZ Kindling Development through Hippocampal Orexin Receptor 1 Modulation

Nasibe Akbari [1], Mahmoud Elahdadi Salmani [1,3*], Mahdi Goudarzvand [2], Taghi LashkarBoluki [1,3], Iran Goudarzi [1,3], Kataneh Abrari [1,3]

1. School of Biology, Damghan University, Damghan, Iran.
2. Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran.
3. Institute for Biological Sciences, Damghan University, Damghan, Iran.

Article info:
Received: 25 August 2013
First Revision: 8 October 2013
Accepted: 11 November 2013

Key Words:
Pentylenetetrazol,
Kindling Development,
Epileptogenesis,
Orexinergic System,
Convulsion.

ABSTRACT

Introduction: Epilepsy is a neural disorder in which abnormal plastic changes during short and long term periods lead to increased excitability of brain tissue. Kindling is an animal model of epileptogenesis which results in changes of synaptic plasticity due to repetitive electrical or chemical sub-convulsive stimulations of the brain. Lateral hypothalamus, as the main niche of orexin neurons with extensive projections, is involved in sleep and wakefulness and so it affects the excitability of the brain. Therefore, we investigated whether lateral hypothalamic area (LHA) inactivation or orexin-A receptor blocking could change convulsive behavior of acute and kindled PTZ treated animals and if glutamate has a role in this regard.

Methods: Kindling was induced by 40 mg/kg PTZ, every 48 hours up to 13 injections to each rat. Three consecutive stages 4 or 5 of convulsive behavior were used to ensure kindling. Lidocaine was injected stereotaxically to inactivate LHA, unilaterally. SB334867 used for orexin receptor 1 (OX1R) blocking administered in CSF.

Results: We demonstrated that LHA inactivation prevented PTZ kindling and hence, excitability evolution. Hippocampal glutamate content was decreased due to LHA inactivation, OX1R antagonist infusion, lidocaine injection and kindled groups. In accordance, OX1R antagonist (SB334867) and lidocaine injection decreased PTZ single dose induced convulsive behavior. While orexin-A i.c.v. infusion increased hippocampal glutamate content, it did not change PTZ induced convulsive intensity.

Discussion: It is concluded that LHA inactivation prevented kindling development probably through orexin receptor antagonism. CSF orexin probably acts as an inhibitory step on convulsive intensity through another unknown process.

1. Introduction

Epilepsy is a neurological disorder which is characterized by recurrent spontaneous seizures and affects many people worldwide (Blumcke, Beck, Lie, & Wiestler, 1999). In spite of different medicinal treatments for this pathology, about 20-30% of cases are resistant to conventional therapies (Jallon, 1997; Kwan & Brodie, 2006). However, common epileptogenic mechanism for most epilepsies is a net increase of excitability in the neuronal networks (Gupta, Elgammal, Proddutur, Shah, & Santhakumar, 2012; Jacobs et al., 2009; Ruethrich, Grecksch, Becker, & Krug, 1996; Stringer, 1993). Among animal studies, kindling as a model of epileptogenesis, develops gradually the susceptibility of the brain neuronal networks to the epileptic process (Goddard, 1967). Further, PTZ, as a chemical kindling

* Corresponding Author:
Mahmoud Elahdadi Salmani, PhD.
Damghan University, School of Biology, Damghan University, Cheshme Ali Road, Damghan, Iran.
Tel/Fax: +98-2325247146
E-mail: elahdadi@du.ac.ir, elahdadi@gmail.com

agent, increases net brain excitability due to repeated sub-convulsive administrations which enhances animal convulsions (Corda, Giorgi, Longoni, Orlandi, & Biggio, 1990; Ruethrich et al., 1996).

Orexin is a neuropeptide secreted from a small group of neurons located in LHA (Bonnavion & de Lecea, 2010; Boutrel, Cannella, & de Lecea, 2010; Sakurai, 2007). They project to different parts of the brain acting as a neuromodulatory system (Bonnavion & de Lecea, 2010; Sakurai, 2007). There are two transcripts of orexin, orexin-A and orexin-B, and orexin receptor is a member of GPCR family with two receptor subtypes; orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R). Orexin-A binds to both orexin receptors with equal affinity (Kilduff & Peyron, 2000). Because, LHA localization of orexin neurons is correlated to the feeding center, it was assumed that orexin is involved only in feeding, appetite and energy homeostasis (Bonnavion & de Lecea, 2010; Sakurai, 2007). Interestingly, diversity of destinations and functions of this system has revealed that arousal and sleep are also attributable to orexin system function (Bonnavion & de Lecea, 2010; Sakurai, 2007), for any kind of dysfunction leads to sleep disorders like narcolepsy and cataplexy (Baier et al., 2011; Diniz Behn, Klerman, Mochizuki, Lin, & Scammell, 2010). Lagrange et al reported a clinical case of Rasmussen`s syndrome with comorbid narcolepsy and cataplexy who developed complex partial seizures later. This patient had no detectable orexin-A in his CSF (Lagrange, Blaivas, Gomez-Hassan, & Malow, 2003). Further, many epileptic patients become sleepy due to generalized convulsions and many others experience sleep disorders (Manni & Terzaghi, 2010; Nunes & da Costa, 2010). On the other hand, the CSF orexin is deceased after tonic clonic convulsions in any of epileptic patients (Rejdak, Papuc, Grieb, & Stelmasiak, 2009). Therefore, alterations in behaviorand also orexin amount points out the involvement of orexinergic system in epileptogenesis.

Hippocampus, as the site of plastic changes in learning and memory processes, is susceptible to seizures and epilepsy. In addition, glutamate is the main excitatory neurotransmitter in the three circuit loop of the hippocampus and also whole brain. Any increase or decrease of glutamate content will alter excitability of the related circuits (Stanley & Fadel, 2011). Therefore, orexin may control hippocampal neurotransmission through direct as well as trans-synaptic modulation of various pathways, including the septo-hippocampal pathway (Stanley & Fadel, 2011).

Altogether, this work is going to investigate the effect of LHA inactivation on PTZ kindling development and if glutamate content changes of hippocampus might be involved in this probable effect.

2. Methods

2.1. Animals

Male Sprague Dawley rats weighing 170-200 (young; two months old) at the beginning of kindling procedure were used. All experiments were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 23-80, revised 1996) and conformed to the research ethical standards for the care and use of animals in Damghan University. Rats were kept 5 per cage, in a 12 h light, dark periods starting at 7 am with free access to food and water.

2.2. Kindling Procedure

PTZ (40 mg/kg, i.p.) dissolved in 1 ml distilled water for all PTZ kindling groups. Every PTZ receiving rat was behaviorally monitored for 20 min in a blind manner and this time was extended only if convulsions has not ended. When PTZ injections were used in kindling paradigm, each rat received 13 injections every 48 hours and considered kindled after 3 consecutive 4-5 convulsive stages. Convulsions were scored as follows; 0, no response; 1, ear and facial twitching; 2, convulsive waves through the body; 3, myoclonic jerks; 4, tonic-clonic convulsions, rearing; 5, generalized tonic-clonic seizures, turnover into side position, loss of postural control (modified from Racine et al. (Corda et al., 1990; Racine, Okujava, & Chipashvili, 1972)).

2.3. Experimental Groups

Animals used for kindling development divided into two experimental groups; PTZ kindled (kindled) and lidocaine treated PTZ kindled (lidocaine). As the effect of lidocaine on convulsion and glutamate content was reproduced also by single PTZ induced convulsion, we employed single dose PTZ injection to compare the effect of agonist and antagonists on convulsive behavior. Single dose PTZ (45 mg/kg; i.p.) groups were divided to; 1. PTZ single dose (PTZ), 2. Orexin treated single dose PTZ (ORX+PTZ), 3. Orexin antagonist treated (SB334867) single dose PTZ (SB+PTZ), 4. Control. Control group was used to compare glutamate content data from all groups. Convulsive behavior of kindling

development groups was compared to kindled group while the behavior of single dose PTZ groups was compared to PTZ group.

2.4. Surgical Preparation

Cannulae were surgically implanted to infuse lidocaine (in PTZ kindling) or saline (in control animals), intranuclear, into right LHA. Rats were anesthetized with a mixture of ketamine (90 mg/kg, i.p.) and xylazine (6.0 mg/kg i.p.) and placed in a stereotaxic apparatus (Stoelting Instruments). Body temperature was maintained using a towel pad. Stainless steel guide cannulae (23 gauges) fitted with infusion cannulae (30 gauges) with 1 mm longer tip were positioned unilaterally above the right LHA upon coordinates from atlas (Paxinos & Watson, 2007) of rat brain (AP= -2.8 mm caudal to bregma, Lat.= +1.3 mm and DV= -8.6 mm ventral from skull surface). The guide cannulae were fixed and armed on the skull using acrylic dental cement with surgical screws. Infusion cannulae were replaced with dummy cannulae and cut to extend 0.5 mm beyond the guide cannulae to prevent clogging. Tetracycline antibiotic ointment was applied on skull skin incision to prevent infections.

Under the mentioned anesthesia, stereotaxic injection of lidocaine 2% was performed into LHA (13 times, each 30 min before PTZ treatment) to inactivate the region, transiently. Orexin-A (10 nM (Aou et al., 2003)) and SB334867 (20 nM (Huang, Dai, Lee, Chiou, & Hwang, 2010)) or saline were injected into right lateral ventricle (LV; i.c.v) in single dose PTZ groups using coordinates of right LV (AP= -0.72 mm caudal to bregma, Lat. = +1.8 mm and DV= -4 mm ventral from skull surface) in a 5 µl of volume. Every drug infusion or injection was achieved using a 30 gauge cannula connecting to a 5 µl Hamilton syringe through a polyethylene tubing (PE-20). Infusion took 4 minutes for intra LHA and 5 minutes for i.c.v injections, while the pump infusion rate was set to 0.5 and 1 µl/min, respectively. Cannula remained about 3 minutes in the site for the drug to diffuse in the region. All drug solutions were freshly prepared on the day of experiment. All animals were sacrificed quickly after behavioral tests and the brain cut in 40 µm slices to ensure the placement of injection cannula in LHA and right LV.

2.5. Determination of glutamate levels

Concentration of glutamate in the hippocampus was measured using a glutamate assay kit (abcam; cat, 83389). Briefly, animals were sacrificed (quickly after behavioral monitoring) after (last) PTZ treatment, right hippocampus excised quickly from the brain and stored in -80˚C freezer. On the day of ELISA test, right hippocampus was homogenized in 100 µl of kit assay buffer and then centrifuged at 15000 g for 20 min. Supernatants (10 µl from 100 µl) were incubated with reaction mix solution for 30 min at 37˚C. Absorption determined at 450 nm using a spectrophotometer and amounts of glutamate determined based on the standard curve. Samples were used as duplicate and the data was averaged into one glutamate amount number for each duplicate.

2.6. Drugs

Orexin-A (cat, 1455; OX1R and OX2R agonist) and SB334867 (cat, 1960; OX1R antagonist) from Tocris Bioscience and PTZ (cat, P6500) from Sigma Aldrich was used in this research. Lidocaine 2% was purchased from local drug market in IRAN.

2.7. Statistical Analysis

ANOVA was used to compare averages between groups, which was followed by post hoc comparisons using the tukey test when appropriate. In this regard, total glutamate content was analyzed employing one way ANOVA. Whole kindling period average values were compared using non-parametric Mann Whitney U test and single dose PTZ induced acute seizure behavioral scores were compared using non-parametric Kruskal Wallis which was followed by Dunn`s test. All of statistical tests were performed by spss v.16 software. Minimum level of significance was $P<0.05$.

3. Results

3.1. Lateral Hypothalamus Inactivation Prevented kindling Development

Lidocaine was used to inactivate right LHA, 30 min before every PTZ (40 mg/kg) injection. Each PTZ induced convulsion of kindling paradigm was weaker (and below stage 4 or 5) in lidocaine compared to kindled group (Mann Whitney U test, $U=11.500$, $P<0.001$, Fig. 1-A1, $n=16$). Therefore, PTZ kindling was prevented in lidocaine treated animals.

3.2. Orexin receptor 1 Antagonist Intraventricular Injection Reduced Single Dose PTZ Induced Convulsions

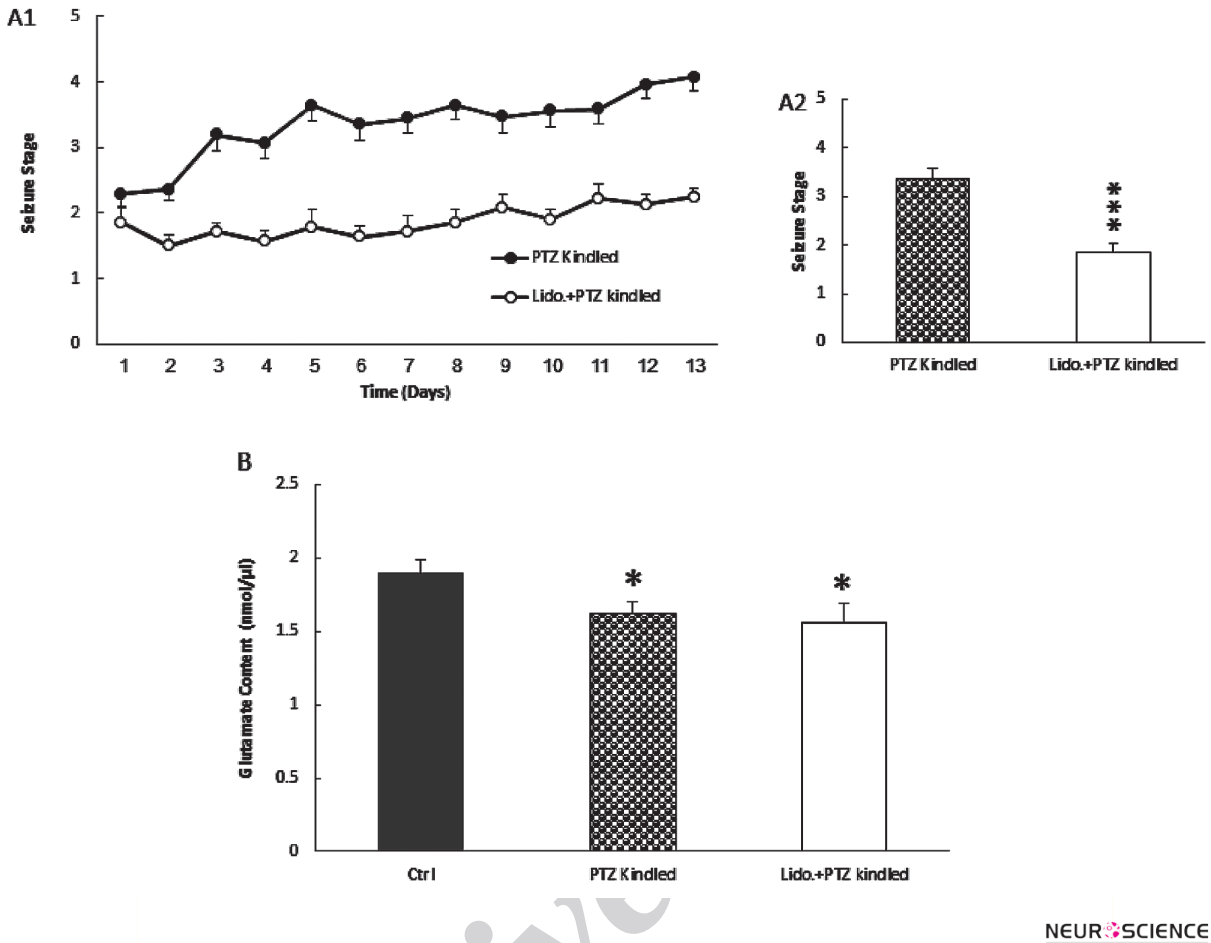


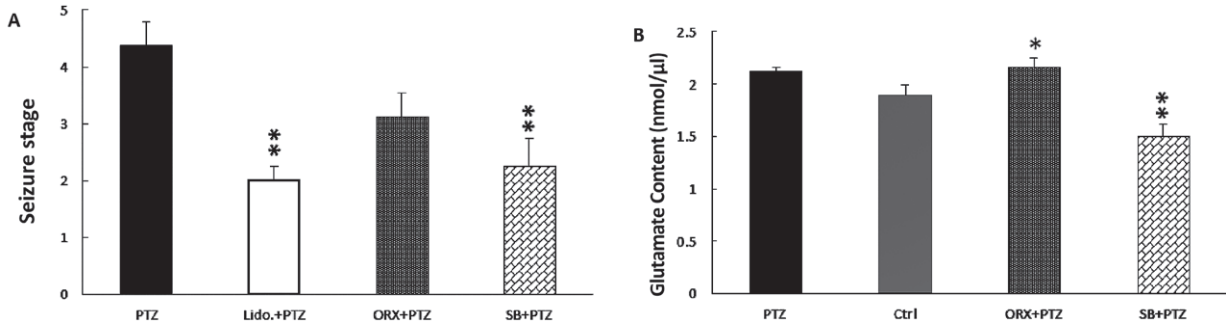
Figure 1. Lateral hypothalamus inactivation decreased kindling development and hippocampal glutamate content. Lidocaine injection before each PTZ administration reduced convulsive intensity and hence kindling development (A1). Total time average of PTZ kindling (inset; A2) showed a decrease of averaged seizure intensity in lidocaine treated animals (P<0.001). Lidocaine intra LHA injection prior to (Lido.+PTZ kindled) or PTZ kindling alone decreased hippocampal glutamate content (P<0.05) compared to control.

* P<0.05; *** P<0.001; PTZ, Pentylenetetrazol; Lido, lidocaine

PTZ (45 mg/kg) induced tonic clonic convulsions in over 90% of naïve animals. Comparing single dose PTZ induced convulsion scores using Kruskal Wallis one way analysis of variance showed a significant difference between groups (H(3) = 11.110, P<0.01). Administration of orexin-A (i.c.v.) before PTZ injection, did not change seizure intensity and convulsive stage, significantly. Conversely, administration of OX1R antagonist, SB334867, reduced (Dunn`s test, P<0.05, Fig. 2-A, n=8) seizure intensity prominently. Similar to lidocaine induced LHA inactivation in kindling paradigm, single dose lidocaine treatment of LHA decreased seizure intensity, too (Dunn`s test, P<0.01, Fig. 2-A, n=8). This data showed that convulsive intensity is altered by orexinergic system modulation.

3.3. Lateral Hypothalamus Inactivation Decreased Total Hippocampal Glutamate Content

Hippocampal glutamate measurement was accomplished in PTZ kindling development groups, kindled and lidocaine, and also single dose PTZ injection induced convulsion groups. Hippocampal glutamate content (one way ANOVA, F5,30 = 9.78, P=0.001) was decreased in kindled (P<0.05, n=6) and lidocaine treated PTZ kindled (P<0.05, n=6) compared to control animals (Fig. 1-B). Although PTZ induced convulsions did not change glutamate content significantly, applying orexin-A, before single dose PTZ injection, increased hippocampal glutamate content (P<0.05, Fig. 2-B, n=6). In contrast, blocking OX1R decreased hippocampal glutamate content (P<0.01, Fig. 2-B, n=6). Even, single dose



NEUROSCIENCE

Figure 2. Orexin receptor (OX1R) modulation reduced convulsive intensity and altered hippocampal glutamate content. Single dose i.c.v infusion of OX1R antagonist (SB334867) (P<0.01) and also single dose lidocaine administration (in LHA) (P<0.05) reduced convulsive intensity (A). Orexin-A i.c.v infusion, increased (P<0.05) hippocampal glutamate content, while blocking OX1R (SB334867) (P<0.01) decreased that content compared to control.

* P<0.05; ** P<0.01; PTZ, Pentylenetetrazol; Ctrl, Control; SB, SB334867; ORX, Orexin-A; Lido., lidocaine

lidocaine injection into LHA reduced (P<0.05, n=6, data not shown) the glutamate content. Therefore, glutamate neurotransmission is likely involved in orexin convulsion modulation.

4. Discussion

The present study investigated the effect of LHA inactivation or orexin i.c.v infusion on kindling development and convulsion intensity as well as hippocampal glutamate content measurement following seizures. We demonstrated that: 1. LHA inactivation prevented kindling development in PTZ induced kindling model of epilepsy 2. SB334867, but not orexin reduced PTZ induced convulsions intensity. 3. Orexin receptor 1 blocking reduced while orexin infusion increased total hippocampal glutamate content in PTZ treated animals.

Orexin neuronal system projects to other neuromodulatory centers located in brain stem. They control limbic and neocortical structures (Bonnavion & de Lecea, 2010; Sakurai, 2007), like hippocampus, involved in seizure and epilepsy. We demonstrated that repetitive transient inactivation of LHA before each PTZ administration in kindling paradigm prevented kindling development. This result can be explained simply as removing orexin excitatory effect which might be necessary for kindling process. A more detailed explanation might be the role of orexin on the hippocampal excitation and theta rhythm generation, originates from medial septum in interplay of cholinergic and GABAergic circuits activates hippocampus through orexin induced activation of septohippocampal neurons (Wu, Zaborszky, Hajszan, van den Pol, & Alreja, 2004; Wu et al., 2002). Therefore it is likely that inactivation of LHA resulted in deactivation of that pathway and hence suppression of convulsions. Even, brain stem nuclei, as the main relay center of hypothalamus projection to hippocampus, respond to orexinergic neurons. Microinjection of orexin-A showed an OX1R dependent antinociception in PAG (Azhdari Zarmehri et al., 2011).

Orexin receptors are distributed in different brain areas and their stimulation results in vigilance and wakefulness (Bonnavion & de Lecea, 2010; Boutrel et al., 2010; Sakurai, 2007). Our data revealed that antagonizing OX1R reduced seizure intensity and convulsive stage, while stimulation of orexin receptors could not enhance seizure intensity as compared to PTZ group. Conversely, orexin induced behavioral effect points out a disagreement of our data with others. For example, Kortunay et al showed that orexin increased spike number, amplitude and neuronal excitability in penicillin induced epileptic activity (Kortunay et al., 2012). Clarifying this controversy, it is shown that orexin activates tuberomammillary nucleus (TMN) in posterior hypothalamus which release histamine (Ishizuka, Murotani, & Yamatodani, 2010; Nishida et al., 2007) and histamine in turn has exerted antiepileptic effect in PTZ model following deep brain stimulation in posterior hypothalamus (Nishida et al., 2007). As the main orexinergic receptor in TMN is OX2R and its activation leads to histamine release, then, investigating the involvement of OX2R, which is likely to attenuate orexin-A stimulatory effect on seizure intensity induced by OX1R stimulation, might eliminate this discrepancy. On the other hand, the effect of OX1R antagonism indicates the probable role of OX1R in seizure generation. This will be strengthened by the data

that spatial learning and memory was mediated through OX1R in hippocampus (Akbari, Naghdi, & Motamedi, 2006), as the site with high density of orexin receptors (Gotter et al., 2012; Marcus et al., 2001; Trivedi, Yu, MacNeil, Van der Ploeg, & Guan, 1998). Further, antinociception effect of OX2R was more pronounced in VTA than NAc, which is attributable to receptor density in that nucleus (Azhdari-Zarmehri et al., 2013). Thus, orexinergic excitatory system activation, due to PTZ stimulation is likely to increase seizure intensity and kindling rate, which possibly modulate convulsive behavior.

As it is widely known, epilepsy is associated with increase of excitability in brain epilepsy related structures such as hippocampus and glutamate as the main excitatory neurotransmitter plays a pivotal role in this regard (Bradford, 1995; Delorenzo, Sun, & Deshpande, 2005; Rowley, Martin, & Marsden, 1995). As an example, Yang et al demonstrated that increase in neurotransmitter release is involved in epileptogenesis (Yang, Woodhall, & Jones, 2006). Additionally, stimulation of glutamate receptors increases afterdischarges in hippocampal tissue (Miyazaki et al., 1994). Our data showed an increase in hippocampal glutamate content following orexin infusion in CSF, while administration of OX1R antagonist, SB334867, decreased this content. Glutamate is derived from glucose, glutamine and aspartate in the brain (Delorenzo et al., 2005) and it appears that orexin has probably upregulated the mechanisms responsible for glutamate production and hence release. Lungwitz et al. demonstrated that orexin-A has increased anxiety like behavior through glutamate receptor modulation (Lungwitz et al., 2012). In addition, co-administration of orexin A and glutamate showed a synergistic effect on the recorded cells, and orexin A could excite the neurons even if GABA was present (Song, Chen, Xia, Yu, & Hu, 2006). In another study, orexin-A showed antidepressant activity and increased cell proliferation, via OX1R in forced swim stress model (Ito et al., 2008). In addition, high density of OX1R in the hippocampus (Marcus et al., 2001), especially in CA1 area (Trivedi et al., 1998), suggests a modulatory action of orexin on hippocampal function mainly glutamate stimulated receptors activation. On the other hand, glutamate transporters increase the uptake of glutamate (Vandenberg, 1998) which can be converted to glutamine in the glial cells and hence decrease the glutamate content (Pfrieger & Barres, 1996). This transporter or even glial cell might be the likely target of orexin effect. However, further investigation is required to unravel the responsible mechanisms of orexin modulatory function via orexin receptors in hippocampus of epileptic animals. Another data demonstrated that LHA inactivation by lidocaine resulted in a reduction of glutamate content. As these rats did not develop kindling, the reduction might be because of absence of convulsions. Similarly and conversely, glutamate was decreased in kindled animals. Opposing to PTZ group, here the glutamate content reduction was probably due to adaptation of glutamate content following successive convulsions in kindling paradigm.

It is concluded that, orexin neurons localized in LHA are involved in PTZ induced convulsions. In addition, we found that alteration of hippocampal glutamate content is associated with LHA activity and orexin administration induced change in convulsive intensity.

Acknowledgment

This study was supported by Damghan University, Damghan, IRAN, as part of a thesis project. Authors declare no conflict of interests.

References

Akbari, E., Naghdi, N., & Motamedi, F. (2006). Functional inactivation of orexin 1 receptors in CA1 region impairs acquisition, consolidation and retrieval in Morris water maze task. Behav Brain Res, 173(1), 47-52.

Aou, S., Li, X. L., Li, A. J., Oomura, Y., Shiraishi, T., Sasaki, K., et al. (2003). Orexin-A (hypocretin-1) impairs Morris water maze performance and CA1-Schaffer collateral long-term potentiation in rats. Neuroscience, 119(4), 1221-1228.

Azhdari-Zarmehri, H., Reisi, Z., Vaziri, A., Haghparast, A., Shaigani, P., & Haghparast, A. (2013). Involvement of orexin-2 receptors in the ventral tegmental area and nucleus accumbens in the antinociception induced by the lateral hypothalamus stimulation in rats. Peptides, 47, 94-98.

Azhdari Zarmehri, H., Semnanian, S., Fathollahi, Y., Erami, E., Khakpay, R., Azizi, H., et al. (2011). Intra-periaqueductal gray matter microinjection of orexin-A decreases formalin-induced nociceptive behaviors in adult male rats. J Pain, 12(2), 280-287.

Baier, P. C., Hallschmid, M., Seeck-Hirschner, M., Weinhold, S. L., Burkert, S., Diessner, N., et al. (2011). Effects of intranasal hypocretin-1 (orexin A) on sleep in narcolepsy with cataplexy. Sleep Med, 12(10), 941-946.

Blumcke, I., Beck, H., Lie, A. A., & Wiestler, O. D. (1999). Molecular neuropathology of human mesial temporal lobe epilepsy. Epilepsy Res, 36(2-3), 205-223.

Bonnavion, P., & de Lecea, L. (2010). Hypocretins in the control of sleep and wakefulness. Curr Neurol Neurosci Rep, 10(3), 174-179.

Boutrel, B., Cannella, N., & de Lecea, L. (2010). The role of hypocretin in driving arousal and goal-oriented behaviors. Brain Res, 1314, 103-111.

Bradford, H. F. (1995). Glutamate, GABA and epilepsy. Prog Neurobiol, 47(6), 477-511.

Corda, M. G., Giorgi, O., Longoni, B., Orlandi, M., & Biggio, G. (1990). Decrease in the function of the gamma-aminobutyric acid-coupled chloride channel produced by the repeated administration of pentylenetetrazol to rats. J Neurochem, 55(4), 1216-1221.

Delorenzo, R. J., Sun, D. A., & Deshpande, L. S. (2005). Cellular mechanisms underlying acquired epilepsy: the calcium hypothesis of the induction and maintainance of epilepsy. Pharmacol Ther, 105(3), 229-266.

Diniz Behn, C. G., Klerman, E. B., Mochizuki, T., Lin, S. C., & Scammell, T. E. (2010). Abnormal sleep/wake dynamics in orexin knockout mice. Sleep, 33(3), 297-306.

Goddard, G. V. (1967). Development of epileptic seizures through brain stimulation at low intensity. Nature, 214(5092), 1020-1021.

Gotter, A. L., Roecker, A. J., Hargreaves, R., Coleman, P. J., Winrow, C. J., & Renger, J. J. (2012). Orexin receptors as therapeutic drug targets. Prog Brain Res, 198, 163-188.

Gupta, A., Elgammal, F. S., Proddutur, A., Shah, S., & Santhakumar, V. (2012). Decrease in tonic inhibition contributes to increase in dentate semilunar granule cell excitability after brain injury. J Neurosci, 32(7), 2523-2537.

Huang, S. C., Dai, Y. W., Lee, Y. H., Chiou, L. C., & Hwang, L. L. (2010). Orexins depolarize rostral ventrolateral medulla neurons and increase arterial pressure and heart rate in rats mainly via orexin 2 receptors. J Pharmacol Exp Ther, 334(2), 522-529.

Ishizuka, T., Murotani, T., & Yamatodani, A. (2010). Modanifil activates the histaminergic system through the orexinergic neurons. Neurosci Lett, 483(3), 193-196.

Ito, N., Yabe, T., Gamo, Y., Nagai, T., Oikawa, T., Yamada, H., et al. (2008). I.c.v. administration of orexin-A induces an antidepressive-like effect through hippocampal cell proliferation. Neuroscience, 157(4), 720-732.

Jacobs, M. P., Leblanc, G. G., Brooks-Kayal, A., Jensen, F. E., Lowenstein, D. H., Noebels, J. L., et al. (2009). Curing epilepsy: progress and future directions. Epilepsy Behav, 14(3), 438-445.

Jallon, P. (1997). The problem of intractability: the continuing need for new medical therapies in epilepsy. Epilepsia, 38 Suppl 9, S37-42.

Kilduff, T. S., & Peyron, C. (2000). The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. Trends Neurosci, 23(8), 359-365.

Kortunay, S., Erken, H. A., Erken, G., Genc, O., Sahiner, M., Turgut, S., et al. (2012). Orexins increase penicillin-induced epileptic activity. Peptides, 34(2), 419-422.

Kwan, P., & Brodie, M. J. (2006). Refractory epilepsy: mechanisms and solutions. Expert Rev Neurother, 6(3), 397-406.

Lagrange, A. H., Blaivas, M., Gomez-Hassan, D., & Malow, B. A. (2003). Rasmussen's syndrome and new-onset narcolepsy, cataplexy, and epilepsy in an adult. Epilepsy Behav, 4(6), 788-792.

Lungwitz, E. A., Molosh, A., Johnson, P. L., Harvey, B. P., Dirks, R. C., Dietrich, A., et al. (2012). Orexin-A induces anxiety-like behavior through interactions with glutamatergic receptors in the bed nucleus of the stria terminalis of rats. Physiol Behav, 107(5), 726-732.

Manni, R., & Terzaghi, M. (2010). Comorbidity between epilepsy and sleep disorders. Epilepsy Res, 90(3), 171-177.

Marcus, J. N., Aschkenasi, C. J., Lee, C. E., Chemelli, R. M., Saper, C. B., Yanagisawa, M., et al. (2001). Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol, 435(1), 6-25.

Miyazaki, S., Katayama, Y., Furuichi, M., Kano, T., Yoshino, A., & Tsubokawa, T. (1994). N-methyl-D-aspartate receptor-mediated, prolonged afterdischarges of CA1 pyramidal cells following transient cerebral ischemia in the rat hippocampus in vivo. Brain Res, 657(1-2), 325-329.

Nishida, N., Huang, Z. L., Mikuni, N., Miura, Y., Urade, Y., & Hashimoto, N. (2007). Deep brain stimulation of the posterior hypothalamus activates the histaminergic system to exert antiepileptic effect in rat pentylenetetrazol model. Exp Neurol, 205(1), 132-144.

Nunes, M. L., & da Costa, J. C. (2010). Sleep and epilepsy in neonates. Sleep Med, 11(7), 665-673.

Paxinos, George, & Watson, Charles. (2007). The Rat Brain in Stereotaxic Coordinates (6th ed.): Academic Press.

Pfrieger, F. W., & Barres, B. A. (1996). New views on synapse-glia interactions. Curr Opin Neurobiol, 6(5), 615-621.

Racine, R., Okujava, V., & Chipashvili, S. (1972). Modification of seizure activity by electrical stimulation. 3. Mechanisms. Electroencephalogr Clin Neurophysiol, 32(3), 295-299.

Rejdak, K., Papuc, E., Grieb, P., & Stelmasiak, Z. (2009). Decreased cerebrospinal fluid hypocretin-1 (orexin A) in patients after repetitive generalized tonic-clonic seizures. Epilepsia, 50(6), 1641-1644.

Rowley, H. L., Martin, K. F., & Marsden, C. A. (1995). Decreased GABA release following tonic-clonic seizures is associated with an increase in extracellular glutamate in rat hippocampus in vivo. Neuroscience, 68(2), 415-422.

Ruethrich, H., Grecksch, G., Becker, A., & Krug, M. (1996). Potentiation effects in the dentate gyrus of pentylenetetrazol-kindled rats. Physiol Behav, 60(2), 455-462.

Sakurai, T. (2007). The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nat Rev Neurosci, 8(3), 171-181.

Song, C. H., Chen, X. W., Xia, J. X., Yu, Z. P., & Hu, Z. A. (2006). Modulatory effects of hypocretin-1/orexin-A with glutamate and gamma-aminobutyric acid on freshly isolated pyramidal neurons from the rat prefrontal cortex. Neurosci Lett, 399(1-2), 101-105.

Stanley, E. M., & Fadel, J. R. (2011). Aging-related alterations in orexin/hypocretin modulation of septo-hippocampal amino acid neurotransmission. Neuroscience, 195, 70-79.

Stringer, J. L. (1993). Alterations in both excitation and inhibition occur before the onset of seizure activity in the hippocampus. Epilepsy Res, 16(2), 99-109.

Trivedi, P., Yu, H., MacNeil, D. J., Van der Ploeg, L. H., & Guan, X. M. (1998). Distribution of orexin receptor mRNA in the rat brain. FEBS Lett, 438(1-2), 71-75.

Vandenberg, R. J. (1998). Molecular pharmacology and physiology of glutamate transporters in the central nervous system. Clin Exp Pharmacol Physiol, 25(6), 393-400.

Wu, M., Zaborszky, L., Hajszan, T., van den Pol, A. N., & Alreja, M. (2004). Hypocretin/orexin innervation and excitation of identified septohippocampal cholinergic neurons. J Neurosci, 24(14), 3527-3536.

Wu, M., Zhang, Z., Leranth, C., Xu, C., van den Pol, A. N., & Alreja, M. (2002). Hypocretin increases impulse flow in the septohippocampal GABAergic pathway: implications for arousal via a mechanism of hippocampal disinhibition. J Neurosci, 22(17), 7754-7765.

Yang, J., Woodhall, G. L., & Jones, R. S. (2006). Tonic facilitation of glutamate release by presynaptic NR2B-containing NMDA receptors is increased in the entorhinal cortex of chronically epileptic rats. J Neurosci, 26(2), 406-410.