A Possible Non-genomic Epileptogenic Properties of Estradiol Attenuated by MK801 and DNQX in Amygdala Kindled Rats

Mehdi Saberi\textsuperscript{a*}, Fatemeh Saberi\textsuperscript{b} and Roshanak Vesali Mahmoud\textsuperscript{c}

\textsuperscript{a}Department of Pharmacology and Toxicology, Applied Neuroscience Research Center, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran. \textsuperscript{b}Department of Pharmacology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran. \textsuperscript{c}Department of Psychology, Faculty of Psychology and Education, Tehran University, Tehran, Iran.

Abstract

Although the epileptogenic properties of estrogens have been widely demonstrated in several models and species, the mechanism(s) by which estrogens can acutely change seizure parameters including after discharge and seizure duration remains to be determined. In the present study, we examined the role of NMDA (N-methyl-D-aspartate), non-NMDA and estrogen receptors in estradiol benzoate (EB) effects on kindled seizure parameters.

Different groups of fully kindled male rats received either EB (30 μg/Kg); EB plus MK801 (2 mg/Kg, as NMDA antagonist); DNQX (7.5 mg/Kg); tamoxifen (TAM, 0.1 mg/Kg, as non-NMDA antagonist) or intra-amygdala injection of anisomycin (30 mmol/mL, a protein synthesis inhibitor). Kindled seizure parameters including after discharge duration (ADD) and stage 5 duration (S₅D) were determined at 0.25 and 3 h post sesame oil (EB solvent) or EB treatment.

While pretreatment with either MK801 or DNQX could block the ADD prolongation induced by EB at 0.25 h, they had no effect on S₅D prolongation at 3 h. Moreover, application of anisomycin or TAM had no effect on estradiol induced ADD and S₅D prolongation. These results indicate that both NMDA and non-NMDA receptors could be involved in EB induced ADD prolongation. The observed short term non-estrogenic receptor or protein synthesis dependent effects of EB may provide a non-genomic mechanism for the stimulatory effects of the steroid on seizure activity.

Keywords: Male rats; Amygdalakindling; Seizure; Estradiol benzoate; MK801; DNQX.

Introduction

The stimulatory and epileptogenic properties of estrogens and their role in the catamenial epilepsy are well established elsewhere (1, 12, 15). Also, rapid stimulatory effects of estradiol (E₂) on kindled seizure parameters have been demonstrated previously in male rats ((14, 19, 20, 21). Although, the mechanisms of these diverse effects are not clear, the amplifying effect of E₂ on excitatory amino acid activity may play an important role in these complex behaviours. In the classical genomic mechanism taking and lasting hours to days, steroids activate intracellular receptors that regulate transcription and protein synthesis (8, 9, 12). In the more novel non-genomic mechanism, steroids induce
very rapid, short-term effects that are more likely due to direct interactions with specific membrane receptors (2, 6, 7, 13, 15, 18, 33). Many of the long-term genomic and short-term membrane effects of E, (estradiol) can influence synaptic excitatory (3, 25, 30, 31) and inhibitory neurotransmissions (4, 13, 23). Specifically, estrogens augment cerebral purkinje cell responsiveness to iontophoretically applied glutamate (24), alter the sensitivity of neurons to glutamate and NMDA (7, 24, 25, 29) and activate group I and II metabotropic glutamate receptor signalling pathways (2). The activation of glutamate receptors mediate processes involved in the synaptic plasticity associated with learning, memory and epileptogenesis (1). In the hippocampal slice preparation, bath application of E increases the extra-cellular CA neuron field potential in response to the stimulation of the Schaffer collaterals, which makes glutaminergic synapses onto CA neurons (2, 27). In addition, super-fusion of E induces a rapid and reversible increment in the amplitude of Schaffer collateral-activated excitatory post synaptic potential (EPSP) in the presence of NMDA antagonist which was blocked by non-NMDA antagonist (31). Also, E potentiates depolarization response to glutamate, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate and quisqualate (32). Foy and co-workers have demonstrated that oestrogen acts rapidly via presumed membrane mechanisms to enhance both NMDA and AMPA receptor/channel processes in response to glutamate released from Schaffer collateral terminals (2). These in-vitro observations are correlated with the role of both NMDA and non-NMDA receptors in epileptogenic and stimulatory properties of E.2.

However, the exact mechanism through which E2 exerts its stimulatory effects in in-vivo models of epilepsy such as kindling have not been established yet. The electrical kindling model is regarded as an excellent experimental animal model which is very similar to human complex partial seizures (17). Based on above evidences, in the present study we investigated the role of protein synthesis, NMDA, non-NMDA and estrogenic receptors in observed effects of E2 on kindled seizure parameters in male rats.
any drugs.

Drug treatments
The aim of the study was assessment of the role of NMDA, non-NMDA and estrogenic receptors in EB (estradiol benzoate) effects on ADD (after discharge duration) and S,D (stage 5 duration). So to evaluate the effects of MK 801, DNQX) 6, 7- dinitro - quinoxaline - 2, 3-dione( and TAM individually kindled seizure parameters, fully kindled male rats (6-8 animals per each group) were treated with either P.B.S (Phosphate-buffered saline, as drug solvent), MK801 (0.2 mg/Kg, i.p.), DNQX (7.5 mg/Kg, i.p., pH=7.4) or TAM (1 mg/Kg) followed by sesame oil (EB solvent, 0.5 mL/Kg) injection after 5 min interval. Kindled seizure parameters were determined at 0.25 h and 3 h post sesame oil injection. Accordingly, the stimulation and recording times were included 0.25 and 3 h post final injection.

After one day recovery, EB (30 μg/Kg, i.p.) was applied instead of sesame oil in the above protocol of treatment and kindled seizure parameters were recorded as mentioned above. The doses of drugs were selected as previously described for EB (19, 20), TAM (5), MK801 and DNQX (6).

Intra-amygdala injection
Two groups of animals with implanted intra-amygdala electrodes and cannula, received intra-amygdala anisomycine (1 μL of 30 mmol/mL in normal saline solution) through the cannula (29) followed by either sesame oil (control) or EB (test) injection (i.p.) after a 5 min interval. Kindled seizure parameters were recorded at 0.25 h and 3 h post the latter injection. All animals were euthanized by diethyl ether anaesthesia at the end of experimental procedure and their brains were removed, sectioned and examined under microscope for electrode tip placement verification.

Statistical analysis
The results are expressed as mean ± S.E.M. and statistical significance was evaluated by one way ANOVA.Data expressed as percent of control, were compared within and between groups by Wilcoxon and Mann–Whitney U-test, respectively. P < 0.05 was taken as significant.

The expressed data as percent of stimulation (AD) threshold were compared within and between groups by non-parametric Wilcoxon and Mann–Whitney U-test, respectively.

Results
Treatment of animals with either MK801 (0.2 mg/Kg, i.p.), DNQX (7.5 mg/Kg, i.p.) or TAM (1 mg/Kg) alone had no behavioral changes.

MK801 or DNQX treatment alone
EB treatment (30 μg/Kg, i.p.) alone was associated with significant increase in ADD (P=0.027) and S,D (P=0.043) at 0.25 and 3 h post injection, respectively, when compared to the control group. Administration of either MK801 (0.2 mg/Kg, i.p.) or DNQX (7.5 mg/ Kg, i.p.) alone had no significant effect on kindled seizure parameters in comparison to the respective control values (Figures 1a and 1b).

Drug pretreatments
While EB (30 μg/Kg, i.p.) application resulted in a significant increase in ADD, pretreatment of the animals with either MK801 or DNQX could inhibit ADD prolongation (P = 0.042 and P = 0.046, respectively) induced by EB injection at 0.25 h when compared to the EB alone treated group (Figure 1a). However, pretreatment of the animals with DNQX or MK801 could not prevent EB induced S,D increment when compared to the EB alone treatment group. On the other hand, the S,D increased significantly at 0.25 h (P=0.021) after DNQX pretreatment in comparison to the EB group (Figure 1b). Moreover, pretreatment of animals with TAM had no effect on EB induced ADD and S,D prolongation (Figure 2a and 2b).

Intra-amygdala anisomycine treatment
Intra-amygdala injection of anisomycine (1 μL of 30 mmol/mL) alone decreased S,D at 0.25 (P=0.029) and 3 h (P=0.046) in comparison to the relative control value. Administration of intra-amygdala anisomycine prior to EB treatment was associated with ADD (at 0.25 h, P = 0.038) and S,D increment (at 0.25 and 3 h, P = 0.044, P=0.049, respectively) when compared to anisomycine treatment group (Figure 3).
Discussion

Epileptic disorders, especially in refractory forms, can interfere with the patient’s performance and active presence in the society (28). In many cases, even multi-drug therapy is not effective and in these states patients have to undergo neurosurgical procedures (16). Regarding to the role of estrogens in induction of epileptic activity, determination of the mechanisms involved in this pathway can influence applied medicinal treatments. It had been shown that systemic administration or local application of E₂ on cerebellum purkinje cell slices could increase significantly the stimulatory response to the glutamate, quisqualate and NMDA rapidly within 5-10 min (6, 11, 33).

In this study, MK801 (a non-competitive NMDA antagonist) pre-treatment inhibited ADD increment induced 0.25 h post EB treatment. However, NMDA antagonist had been more effective against kindling acquisition, but after full kindling the inhibitory effect on stimulation and especially seizure duration was reduced (10). The NMDA receptors are probably more involved in AD propagation and development (10) which is consistent with its involvement in EB effects on ADD increment, as observed in the present study. On the other hand, several reports have stated that E₂ facilitates non-NMDA
prolongation by both receptors antagonists may be an evidence of EB effects on both NMDA and non-NMDA transmissions or their receptor activity. These receptors may play an important role in ADD prolongation probably by acting at different regions of brain. For example, in cerebellum, the NMDA (25) and in hippocampus the non-NMDA (32) receptors participate in EPSP potentiation. The inability of glutamate receptor antagonist to block S,D increment is probably related to the inherent characteristics of S,D. While triggered AD propagates seizure stages, wide spread propagation of AD induces S,D.

Although, attenuation of the GABA inhibitory effect and reduction of the inhibitory post-synaptic potential can cause ADD prolongation, nevertheless the application of GABA has not changed synaptic responsiveness (32). The rapid EB effect on ADD (0.25 h) and its reversible nature is the evidence for direct non-genomic effects of EB on cell membrane.

To rule out the probability of the involvement of genomic effects of EB on kindling parameters, TAM (an estrogenic receptor antagonist) and anisomycine (a protein synthesis inhibitor) were applied ip and intra-amygdala injections respectively. The TAM was applied at low dose (1m/Kg) to inhibit estrogenic receptors without partial effects or antioxidant activity (5). Higher doses of TAM can induce epileptogenic effects as reported previously (20). In addition, TAM pre-treatment could not block ADD and S,D prolongation. Moreover, the inability of anisomycine to inhibit EB induced ADD prolongation confirmed the probable non-genomic rapid effects of EB on ADD.

To facilitate the responsiveness to glutamate, E2 probably either binds directly to a membrane protein, which is partly or totally accompanied by glutamate receptors, or affects glutamate receptors indirectly through disturbing the membrane bilayer lipids (2, 33). This may result from one or a combination of several mechanisms described so far for E2. In addition, the changes in the level or affinity of receptors for NMDA and non-NMDA excitatory amino-acids (3, 30 32) and increased neuronal responsiveness to excitatory amino-acids (25) are probably involved in the late effect of EB. The NMDA
receptor antagonist MK-801 may prevent the hormone-induced changes in spine density, NMDA transmission, and long term potentiation (LTP) magnitude (26).

In conclusion, based on rapid EB effects on ADD (0.25 h), its inhibition by Mk801, and inability of anisomycin (in this study) and tamoxifen to prevent AD prolongation, the acute effect of EB on kindled seizures may be induced more via a non-genomic membrane glutamate receptor rather than intracellular estrogenic receptors. The results of this investigation suggest the possibility of the effectiveness of antiepileptic drugs such as topiramate in catamenial epilepsy.

References

(1) Beyenburg S, Stoffel WB, Bauer J, Watzka M, Blumcke I, Biblingmaier F and Elger CE. Neuroactive steroids and seizure susceptibility. Epilepsy Res. (2001) 44: 141-153.
(2) Boullere MI, Weick JP, Becklund BR, Kuo SP, Groth RD and Mermelstein PG. Estradiol activates group i and ii metabotropic glutamate receptor signaling, leading to opposing influences on camp response element-binding protein. J. Neurosci.(2005) 8: 5066-5078.
(3) Brann DW and Mahesh VB. Excitatory amino acids: Function and significance in reproduction and neuroendocrine regulation. Front. Neuroendocrinol. (1994) 15: 3-49.
(4) Colzato LS, Hertsg S, Wildenberg WPM and Hommel B. Estrogen modulates inhibitory control in healthy human females: evidence from the stop-signal paradigm. Neurosci. (2010) 167: 709-715.
(5) Fitts JM, Klein RM and Powers CA. Comparison of tamoxifen and testosterone propionate in male rats: differential prevention of orchidectomy effects on sex organs, bone mass, growth, and the growth hormone-IGF-i axis. J. Androl. (2004) 25: 523-534.
(6) Foy MR, Xu J, Xie X, Brinton RD, Thompson RF and Berger TW. 17-b-Estradiol Enhances NMDA Receptor-Mediated EPSPs and Long-Term Potentiation. J. Neurophysiol. (1999) 81: 925-929.
(7) Gingerich S, Kim GL., Chalmers JA, Koletar MM, Wang X and Wang Y and Belsham DD. Estrogen receptor alpha and G-protein coupled receptor 30 mediate the neuroprotective effects of 17b-estradiol in novel murine hippocampal cell models. Neurosci. (2010) 170: 54-66.
(8) Hashemizadeh Bashtian M, Emami S A, Mousavifar N, Esmaily HO, Mahmoudi M and Mohammad Poor H. Evaluation of fenugreek (Trigonella Foenum-graceum L.), effects seeds extract on insulin resistance in women with polycystic ovarian syndrome. Iran. J. Pharm. Res. (2013) 12: 475-481.
(9) Hojo Y, Hattori T, Enami T, FurukawaA, SuzukiK, Ishii H, MukaiH, MorrisonJH, Janssen WGM, KominamiS, Harada N, Kimoto T and Kawato S. Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017 and P450 aromatase localized in neurons. PNAS. (2004) 101: 865-870.
(10) Holmes KH, Bilkey DK and Laverty L. The infusion of an NMDA antagonist into perirhinal cortex suppresses amygdala kindled seizures. Brain Res. (1992) 587: 285-90.
(11) Li CS, Kaba H, Satio H and Seto K. Oestrogen infusions into the amygdala potentiate excitatory transmission from the accessory olfactory bulb to tubero-infundibulocarcinate neurones in the mouse. Neurosci. Lett. (1992) 143: 48-50.
(12) McEwen BS. Neuroendocrine interactions. In: Bloom FE and Kupfer J (eds.) Psycho-pharmacology. New York: Raven press (1995) 1: 705-717.
(13) Mellery V, Lasmoles F and Lieberherr M. G alpha (q/II) and G betagamma proteins and membrane signalling of calcitriol and estradiol. J. Cell Biochem. (1999) 75: 138-146.
(14) Nabekura J, Oomura Y, Minama T, Mizuno Y and Fukuda A. Mechanism of rapid effect of 17b-estradiol on medial amygdala neurons. Sci. (1986) 233: 226-228.
(15) Orchinik M and McEwen B. Novel and classical actions of neuro-active steroids. Neurotransmissions (1993) 9: 1-6.
(16) Ping Z, Wen XJ, Song WG, Hong-Yu Z and Xin T. Evaluation of efficacy and safety of anterior corpus callosotomy with keyhole in refractory seizures. Seizure (2009) 18: 417-419.
(17) Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephal. Clin. Neurophysiol. (1972) 32: 281-294.
(18) Rosner W, Hryb DJ, Khan MS, Nakhla AM and Romas NA. Androgen and estrogen signalling at the cell membrane via G-proteins and cyclic adenosine monophosphate. Steroids (1999) 64: 100-106.
(19) Saberi M, Jorjani M and Pourgholami MH. Effects of chronic estradiol benzoate treatment on amygdala kindled seizures in male rats. Epilepsy Res. (2001) 46: 45-51.
(20) Saberi M, Pourgholami MH and Jorjani M. The acute effects of estradiol benzoate on amygdala kindled seizures in male rats. Brain Res. (2001) 891: 1-6
(21) Saberi M and Pourgholami MH. Estradiol alters afterdischarge threshold and acquisition of amygdala kindled seizures in male rats. Neurosci. Lett. (2003) 340: 41-44.
(22) Saberi M, Razvanizadeh AR and Bakhtiyarian A. The antiepileptic activity of Vitexagnuscastusextract on amygdala kindled seizures in male rats. Neurosci. Lett. (2008) 441: 193-196.
(23) Schumacher M, Coirini H and McEwen BS. Regulation of high-affinity GABAA receptors in the dorsal hippocampus by estradiol and progesterone.
Epileptogenic Properties of Estradiol Attenuated by MK801 and DNQX

Weiland NG. Estradiol selectively regulates agonist binding sites on the N-Methyl-D-Aspartate receptor complex in the CA1 region of hippocampus. Endocrinol. (1992) 131: 662-668.

Wong M and Moss RL. Electrophysiological evidence for a rapid membrane action of the gonadal steroid, 17β-estradiol on CA1 pyramidal neurones of the rat hippocampus. Brain Res. (1991) 543: 148-152.

Wong M and Moss RL. Long term and short term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurones. J. Neurosci. (1992) 12: 3217-3225.

Yokomaku D, Numakawa T, Numakawa Y, Suzuki S, Matsumoto T, Adachi N, Nishio C and Taguchi H. Estrogen enhances depolarization-induced glutamate release through activation of phosphatidylinositol 3-kinase and mitogen-activated protein kinase in cultured hippocampal neurons. Molec. Endocrinol. (2003) 17: 831-844.

This article is available online at http://www.ijpr.ir

Brain Res. (1989) 487: 178-183.

(24) Smith SS, Waterhouse BD and Woodward DJ. Sex steroid effects on extrahypothalamic CNS. II. Progesterone alone and in combination with estrogen, modulates cerebellar responses to amino acid neurotransmitters. Brain Res. (1987) 422: 52-62.

(25) Smith SS. Estrogen administration increases neuronal responses to excitatory amino acids as a long-term effect. Brain Res. (1989) 503: 354-357.

(26) Smith CC and McMahon LL. Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. J. Neurosci. (2005) 25: 7780-7791.

(27) Teyler TJ, Vardaris RM, Lewis D and Rawitch AB. Gonadal steroid: effects on excitability of hippocampal pyramidal cells. Sci. (1980) 209: 1017-1019.

(28) Vingerhoets G. Cognitive effects of seizures. Seizure (2006) 15: 221-226.

(29) Wang SH, Ostlund SB, Nader K and Balleine BW. Consolidation and reconsolidation of incentive learning in the Amygdala. J. Neurosci. (2005) 25: 830-835.

(30) Weiland NG. Estradiol selectively regulates agonist binding sites on the N-Methyl-D-Aspartate receptor complex in the CA1 region of hippocampus. Endocrinol. (1992) 131: 662-668.

(31) Wong M and Moss RL. Electrophysiological evidence for a rapid membrane action of the gonadal steroid, 17β-estradiol on CA1 pyramidal neurones of the rat hippocampus. Brain Res. (1991) 543: 148-152.

(32) Wong M and Moss RL. Long term and short term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurones. J. Neurosci. (1992) 12: 3217-3225.

(33) Yokomaku D, Numakawa T, Numakawa Y, Suzuki S, Matsumoto T, Adachi N, Nishio C and Taguchi H. Estrogen enhances depolarization-induced glutamate release through activation of phosphatidylinositol 3-kinase and mitogen-activated protein kinase in cultured hippocampal neurons. Molec. Endocrinol. (2003) 17: 831-844.
Journal alert and more ...
Visit http://www.ijpr.ir
or
http://ijpr.sbmui.ac.ir