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Interaction of γ Aminobutyric Acid B Receptors and Electromagnetic Field in the Fear Response

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ABSTRACT

One of the most neurotransmission system consistently linked with fear response is GABAergic system. GABA through $GABA_B$ receptor can influence fear response. The aim of the present investigation was to examine the effect of IP injection of baclofen ($GABA_B$ agonist) and CGP35348 ($GABA_B$ antagonist) along with EMF exposure on frequency of fear response. Fifty adult male rats weighing 180-200 g were used. Animals were divided in ten groups, of which five groups were exposed to ELF-EMF for 30 days at 8 h day^{-1} in a solenoid, creating an electromagnetic field of 500 μT by a 50 Hz electrical current. Animals were then treated with various doses of baclofen and CGP35348 before being exposed to electrical shock. Each animal were received 100 electrical shocks every session. After shock induction, the fear response was determined by monitoring the reaction of shocked animals to a normal rat which was placed in the electroshock chamber. Data was analyzed by Mann-Whitney test. Significant level was considered to be $p<0.05$. It was shown that injection of baclofen and CGP35348 did not induce any significant change in fear response in without EMF groups, while both of baclofen and CGP35348 significantly increased frequency of fear response in EMF exposure male rats. Results indicated that $GABA_B$ receptors and EMF exposure possibly involved in the modulation of fear response.

Keywords: Fear Response, EMF, Baclofen, CGP35348, Rat

1. INTRODUCTION

Along with the rapid increase in the applications of electrical power and electrical facilities, exposures to Extremely Low Frequency Electromagnetic Field (ELF EMF) have significantly been enhanced in term of both intensity and duration (Lacy-Hulbert *et al*., 1998). It seems that these fields may cause some kind of discomfort and may influence behavior such as increase in passivity and situational anxiety, but has not verified effect on the social and territorial behavior (Balassa *et al*., 2009).

Santos *et al*. (2008) showed that GABAergic mechanisms regulate the neural systems that mediate conditioned fear. Researchers showed that inhibition of GABA transmission by GABA-synthesizing enzyme glutamic acid decarboxylase inhibitor (semicarbazide) increased fear conditioned response in the anterior hypothalamic nucleus (Santosand and Brandao, 2011). Specifically, in a conditioned fear paradigm mice deficient in the $GABA_{B(1a)}$ showed generalized freezing to both paired and unpaired tones (Shaban *et al*., 2006).

GABAergic antagonists (bicuculline methiodide and 2-oH-saclofen) block the inhibitory effects of serotonin in the lateral amygdala: a mechanism for modulation of sensory inputs related to fear condition (Stutzmann and

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LeDoux, 1999). Baclofen (10 mg kg^{-1}, IV) inhibited the evoked potential in the lateral amygdala with an effect that was most marked on the positive-going component. Baclofen also significantly reduce paired-pulse inhibition of the negative-going component at short inter pulse intervals (<200 ms) (Sokol *et al*., 2005). Blockade of $GABA_B$ receptors in the lateral amygdala nucleus by phaclofen generally enhance the electrically elicited medial geniculate nucleus activity. Thus, the electrically induced inputs to the lateral amygdala from the medial geniculate nucleus or auditory association cortex may be under prolonged suppression mediated by $GABA_B$ receptors in the lateral amygdala. Also, the mediations of auditory fear potentiation of startle are associated with $GABA_B$ transmissions in the lateral amygdale (Shuchang *et al*., 2005). Linkage studies directed to a potential involvement of the $GABA_{B(1)}$ gene in conditions of panic disorders (Sand *et al*., 2000).

Activation of both $GABA_A$ and $GABA_B$ receptors in the nucleus accumbens shell (AcbSh) decrease the level of fear/anxiety and increase food intake in free-feeding animals, without a positive correlation between both behaviors (Lopes *et al*., 2007).

So, the aim of the present study was to examine the interaction of $GABA_B$ receptor agonist (baclofen) and antagonist (CGP35348) along with EMF on the frequency of fear response.

2. MATERIALS AND METHODS

2.1. Animals

All the procedures involving animal subjects were reviewed and approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University.

Fifty adult male Sprague-Dawley rats (180-200 gr) were used. The rats were acclimatized for 1 week before being used for the experiment. After preincubation at the same conditions, animals were randomly divided into 10 groups (n = 5): Sham 1 (received normal saline at the same volume of drugs); Sham 2 (exposed to EMF and received normal saline); Experimental 1 (received baclofen at 1.7 mg kg^{-1}); Experimental 2 (received baclofen at 3 mg kg^{-1}); Experimental 3 (exposed to EMF and received baclofen at 1.7 mg kg^{-1}); Experimental 4 (exposed to EMF and received baclofen at 3 mg kg^{-1}); Experimental 5 (received CGP35348 at 100 mg kg^{-1}); Experimental 6 (received CGP35348 at 200 mg kg^{-1}); Experimental 7 (exposed to EMF and received CGP35348 at 100 mg kg^{-1}) and Experimental 8 (exposed to EMF and received CGP35348 at 200 mg kg^{-1}).

2.2. Electromagnetic Field Exposure System

Five groups of animals were exposed to ELF-EMF for 30 days at 8 h day^{-1} in a solenoid. The solenoid, a 50Hz electrical current, created an electromagnetic field of 500 μT. Humidity, temperature (23-24°C) and photoperiod (12h dark: 12h light) reflected natural conditions and were similar in all groups.

2.3. Drugs

Baclofen and CGP35348 (prepared from Ciba Gigie as a gift) were diluted in isotonic saline (0.9%) and injected intraperitoneally (IP).

2.4. Behavioral Testing

Fear response was induced by application of 0.2 mA electrical current stimulation applied every 3 sec for 5 min, i.e., each animal was received 100 electrical shocks every session. After shock induction, the fear response was determined by monitoring the reaction of shocked animals to a normal rat which was placed in the electroshock chamber. The number of freezing behavior as the sign of fear response was appropriately recorded for 20 min.

2.5. Statistical analysis

Data were analyzed using SPSS (version 18). Non parametric test Mann-Whitney to assess the effects of baclofen and CGP35348 on groups of rats subjected to fear response was carried out. Data were presented as Mean ± SEM. The significant value were considered $p<0.05$.

3. RESULTS

Baclofen at both doses of 1.7 and 3 mg kg^{-1} showed no significant difference with sham 1 in the frequency of fear response (**Table 1**). Baclofen at both doses in animals exposed to EMF, significantly ($p<0.05$) increased frequency of fear response in comparison to that of sham 2 (**Table 1**).

CGP35348 at both doses of 100 and 200 mg kg^{-1} in comparison with sham 1 showed no significant difference in frequency of fear response (**Table 2**). CGP35348 at both doses in animal exposed to EMF significantly ($p<0.05$) increased the frequency of fear response in comparison to that of sham 2 (**Table 2**). Results showed that the frequency of fear response in sham 2 in comparison to that of sham 1 was significantly ($p<0.05$) decreased.

Table 1. Interaction of EMF and baclofen on frequency of fear response

Experiment type	Groups		Frequency of fear response
Without exposure to EMF	Sham 1	0.96±0.14[a]	
	Experimental 1	Baclofen 1.7 mg kg^{-1}	0.89±0.24[a]
	Experimental 2	Baclofen 3 mg kg^{-1}	1.02±0.26[a]
With exposure to EMF	Sham 2		0.66±0.23[b]
	Experimental 3	Baclofen 1.7 mg kg^{-1}	1.27±0.19[a]
	Experimental 4	Baclofen 3 mg kg^{-1}	1.19±0.04[a]

Dissimilar characters showed significant level at $p<0.05$

Table 2. Interaction of EMF and CGP35348 on frequency of fear response

Experiment type	Groups		Frequency of fear response
Without exposure to EMF	Sham 1	0.96±0.14[a]	
	Experimental 5	CGP35348 100 mg kg^{-1}	0.97±0.09[a]
	Experimental 6	CGP35348 200 mg kg^{-1}	1.07±0.15[a]
With exposure to EMF	Sham 2		0.66±0.23[b]
	Experimental 7	CGP35348 100 mg kg^{-1}	1.03±0.15[a]
	Experimental 8	CGP35348 200 mg kg^{-1}	0.95±0.13[a]

Dissimilar characters showed significant level at $p<0.05$

4. DISCUSSION

In the present study, baclofen had no effect on the frequency of fear response in rats without exposure to EMF. It has been shown that baclofen, 30 mg day^{-1} for 4 weeks, was significantly more effective than placebo in reducing the number of panic attacks and scores on the Hamilton anxiety scale, Zung scale and Katz-R nervousness subscale (Todd and Baker, 1995). Activation of $GABA_B$ receptor in the nucleus accumbens shell decreased the level of fear/anxiety, without a positive correlation between these behaviors (Lopes *et al*., 2007). Lopes *et al*. (2012) reported that the activation of both $GABA_A$ and $GABA_B$ receptors within the AcbSh can cause fear/anxiety behavior in 24 h food-deprived rats as demonstrated by this fact that the injections of both doses of the $GABA_A$ receptor agonist (muscimul) and $GABA_B$ receptor agonist (baclofen) decreased the total number of risk assessment exhibition in 24 h food-deprived rats (Lopes *et al*., 2012). In the present study, CGP35348 had no effect on the frequency of fear response in rats without exposure to EMF. Mombereau *et al*. (2004) reported that the freezing behavior increases in $GABA_{B1}$ receptor deficit mice and administration of CGP56433 a $GABA_B$ receptor antagonist decreases the freezing behavior (Mombereau *et al*., 2004). The varied results of the present study from those of other studies may be related to the procedure of study. Saclofen, as $GABA_B$ antagonist, injection in nucleus accumbens shell did not affect the fear/anxiety behaviors (Lopes *et al*., 2012). 2-OH saclofen blocked the inhibitory effects of serotonin in the lateral amygdala: as a mechanism for modulation of sensory inputs related to fear conditioning (Stutzmann and LeDoux, 1999). In the present study EMF led to diminished frequency of fear response. McKay *et al*. (2000) reported that magnetic fields display evidence of forgetting, as inferred by their marked attenuation of freezing behavior, during contextual extinction 24 h later (McKay *et al*., 2000). On the other hand in the present study EMF and administration of $GABA_B$ agonist and antagonist increased frequency of fear response. In the literature there was not any study related to interaction of EMF and GABAergic system on fear response; so it seems that clarification of possible mechanisms behind the observed effect needs further studies. The reson that $GABA_B$ agonist and antagonist had similar effects may be as follow: $GABA_B$ receptor mainly is presynaptic receptor or auto receptor, but in some synapses it is postsynaptic receptor or both presynaptic and postsynaptic (Kaneda and Kita, 2005; Moldavan *et al*., 2006). (1) In the present study agonist and antagonist of $GABA_B$ receptor may act through different receptors, meaning that one of them may act through presynaptic receptor while another one act through postsynaptic receptors. (2) In the present study the lack of the effect of $GABA_B$ receptor agonist and antagonist may be due to their very small doses on freezing behavior.

5. CONCLUSION

In the present study agonist and antagonist of $GABA_B$ receptor after exposure to EMF and without exposure to EMF, both similarly increased frequency of fear response.

6. ACKNOWLEDGEMENT

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6.1. Conflict of interest

All the authors can confirm that there is no financial or other relationship that would cause a conflict of interest.

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