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Original article

Intra-hippocampal microinjection of oxytocin produced antiepileptic effect on the pentylenetetrazol-induced epilepsy in rats

Amir Erfanparast*, Esmaeal Tamaddonfard, Farzin Henareh-Chareh

Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

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ABSTRACT

Background: In addition to its role as a circulating hormone, oxytocin can also act as a neurotransmitter and a neuromodulator within the brain. In this study, we investigated the intra-hippocampal effect of oxytocin on an experimental seizure model induced by pentylenetetrazole (PTZ) in rats. We also used atosiban (oxytocin antagonist), diazepam and flumazenil (gamma-aminobutyric acid or GABA-benzodiazepine receptor agonist and antagonist, respectively) to clarify the involved mechanism.
Method: In ketamine-xylazine anesthetized rats, the right and left sides of the dorsal hippocampus (CA1) were implanted with two guide cannulas. Epileptic behaviors were induced by intraperitoneal (*ip*) injection of PTZ (60 mg/kg), and the latency time to onset of first myoclonic jerk, and the duration of epileptic seizures were determined for 30 min.
Results: Intra-hippocampal microinjections of oxytocin at doses of 10 and 20 ng/site, diazepam (100 and 200 ng/site) and co-administration of their ineffective doses significantly ($p < 0.01$) increased the onset of first myoclonic jerk and decreased duration of epileptic seizure. Antiepileptic effects of oxytocin (20 ng/site) were inhibited by atosiban (20 and 40 ng/site) and flumazenil (100 and 200 ng/site) pretreatments. On the other hand, prior administration of flumazenil (100 and 200 ng/site) and atosiban (20 and 40 ng/site) prevented the antiepileptic effects induced by diazepam (100 and 200 ng/site).
Conclusions: The results of the present study showed that at the level of the hippocampus oxytocin suppressed the severity of epileptic behaviors. A hippocampal GABA-benzodiazepine receptor mechanism may be involved in antiepileptic effect of oxytocin.

Introduction

Oxytocin is a neuropeptide which normally is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus [1]. Although oxytocin has an established role as a circulating hormone, it can also act as a neurotransmitter and as a neuromodulator by interacting with its central oxytocin receptor within the brain [2]. The relationship of oxytocin with various physiological and pathophysiological phenomena has been documented well. Oxytocin has prominent effects on social behavior and anxiety, appetite, memory and learning, antinociception, social recognition and stress [3,4]. Taken together, oxytocin may be an important key in some disease and develop a novel treatment for them. Oxytocin receptors in the rat brain have most prominently been distributed in the accessory olfactory bulb, anterior olfactory nucleus islands of Calleja, central and extended amygdala, CA1 of hippocampus, ventral medial hypothalamus, nucleus accumbens, brainstem, and spinal cord [5].

Epilepsy is one of the most serious neurological disorders which caused by partial or generalized discharges in the brain [6]. Clinically, sporadic and stereotyped disturbance of consciousness, behavior, cognition, sensation, and motor function are the characteristics of epileptic seizures [7,8]. Several experimental models in rodents have been developed to investigate the pathophysiology of epileptic seizures and to find out new effective anti-epileptic drugs. Chemical compounds such as pentylenetetrazol (PTZ), pilocarpine, penicillin, bicuculline, picrotoxin, and kainic acid induce generalized seizures after systemic administration [9–11].

Hippocampus, a forebrain structure of the temporal lobe, has been linked to a number of different functions including modulation of aggressive behavior, autonomic and endocrine functions, antinociception, and certain forms of learning and memory [12–14]. In addition, abnormalities in hippocampal synaptic transmission lie at the core of the pathogenesis of

* Corresponding author.
E-mail addresses: a.erfanparast@urmia.ac.ir, erfanparast@gmail.com (A. Erfanparast).

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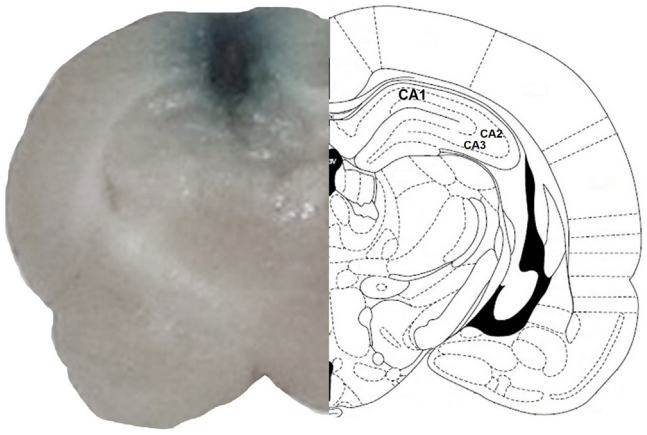

Fig. 1. Schematic illustration of coronal section of the rat brain showing the approximate location of the dorsal hippocampus adapted from Paxinos and Watson, 2007 (right side). Cannulas path into the dorsal hippocampus of rats in this study (left side). The CA1, CA2, CA3 regions of hippocampus were defined.

epilepsy [15,16]. In temporal lobe epilepsy (TLE), loss of neurons in CA1 and CA3 regions of the hippocampus, and damage to mossy cells and inhibitory interneurons in the hilar region of the hippocampus, and granule cells of hippocampal formation were documented well [17].

According to mechanisms of seizures, GABA inhibitory signaling could reduce neuronal excitability and raise the seizure threshold [18]. Several antiepileptic drugs, including benzodiazepines, are thought to inhibit seizures by regulating GABA-mediated synaptic inhibition. Activation of the $GABA_A$ receptors increases the influx of chloride ions leading to hyperpolarization of neuron [19].

Taking these points into consideration, we designed this study to investigate the effect of intra-hippocampal administration of oxytocin, and its antagonist atosiban on PTZ-induced seizures. In addition, the contribution of $GABA_A$ benzodiazepine receptor complex was assessed using diazepam and flumazenil (GABA-benzodiazepine receptor agonist and antagonist, respectively).

Materials and methods

Experimental animals

Experiments were performed on healthy adult Wistar rats (male, 280–320 g). Rats were kept under standard conditions: controlled ambient temperature (22 ± 0.5 °C), a 12 h light–dark cycle (lights on at 07:00 h), and allowed water and food ad libitum. Six rats were used in each experiment. Experiments were conducted between 12:00 p.m. and 16:00 p.m.

Chemicals

Drugs used in the present study included oxytocin, atosiban, diazepam, flumazenil and PTZ were purchased from Sigma-Aldrich Co., St Louis, MO, USA. The drugs were dissolved in normal saline (a sterile solution of 0.9% of NaCl). A drop of Tween 80 was added to diazepam plus normal saline solution.

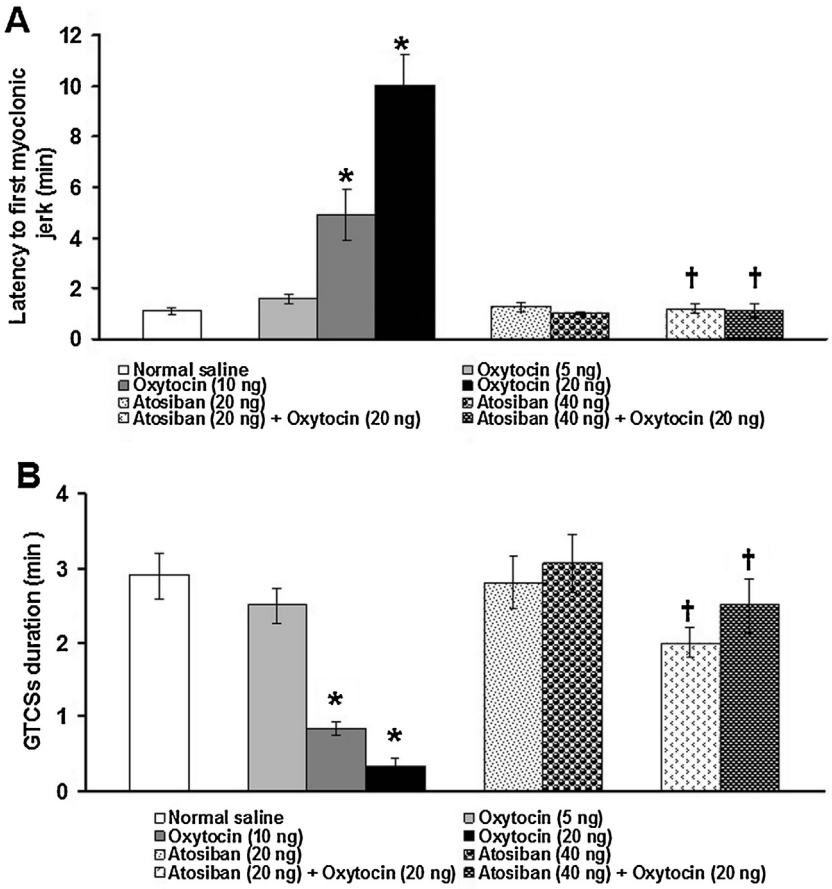

Fig. 2. The effect of intra-hippocampal microinjections of oxytocin and atosiban on the first myoclonic jerk latency (A) and the GTCSs duration (B) induced by *ip* administration of PTZ in rats. Microinjection of oxytocin and atosiban were performed 4 and 6 min before PTZ injection. Values are expressed as the mean ± SEM (n = 6); * $p < 0.01$ compared with normal saline. † $p < 0.01$ compared with oxytocin (20 ng/site).