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Oxytocin is involved in the proconvulsant effects of Sildenafil: Possible role of CREB

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HIGHLIGHTS

- Sildenafil could increase the seizure susceptibility depending on doses in PTZ mice model.
- Oxytocine possesses proconvulsant activity dose dependently.
- The proconvulsant effect of Sildenafil is mediated through Oxytocin pathway.
- CREB phosphorylation is probably involved in this phenomenon.

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ABSTRACT

Sildenafil is a phosphodiesterase type 5 inhibitor mainly used for male erectile dysfunction. One of rare yet serious adverse effects of Sildenafil is its potential to decrease seizure threshold. Ample evidence suggests that Sildenafil exerts central effects through induction of Oxytocin (OT) secretion and CREB phosphorylation. The aim of the present study is to evaluate potential roles of OT and CREB in the proconvulsant effects of Sildenafil.

The Pentylenetetrazole-induced seizure was used as a standard convulsion model in this study. OT release and pCREB expression were evaluated in the hippocampus of mice using ELISA and western blot assays, respectively.

Our results showed that Sildenafil at the dose of 10 mg kg^{-1} or higher, significantly decreased seizure threshold. Pretreatment with a non-effective dose of OT, potentiated while OT receptor antagonist, Atosiban, reversed fully the proconvulsant effects of Sildenafil (5 mg kg^{-1}). At biochemical inspection, Sildenafil markedly increased CREB which was attenuated by coadministration of Atosiban.

The present study shows for the first time that OT release and the subsequent CREB phosphorylation are involved in the proconvulsant effects of acute Sildenafil treatment in an experimental model of seizure.

1. Introduction

Sildenafil is a selective phosphodiesterase 5 (PDE5) inhibitor commonly used for treatment of erectile dysfunction (Moreira et al., 2000). Recent studies, however, report central effects for

Abbreviations: CRE, Cyclic AMP response element; CREB, Cyclic element–binding protein; NFAT, Nuclear factor of activated T-cells; OT, Oxytocin; OTR, Oxytocin receptor; PDE5, Phosphodiesterase type 5; PTZ, Pentylentetrazole.

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Sildenafil in different conditions such as stroke (Wang et al., 2013) and neurodegenerative disorders (Cuadrado-Tejedor et al., 2011).

A part of Sildenafil pharmacological activity is associated with nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway. Inhibition of PDE5 causes an increase in intracellular cGMP level, which leads to smooth muscle relaxation and improves blood flow to the penis (Nieoczym et al., 2012). One of the rare but very serious adverse effects of Sildenafil is convulsion. The proconvulsant effect of Sildenafil was first reported in two patients upon taking the medication (Gilad et al., 2002). Later, an experimental study conducted by *Riazi* et al. demonstrated that Sildenafil increases the seizure susceptibility in both Pentylenetetrazol (PTZ) and Bicuculline models (Riazi et al., 2006) with NO/cGMP pathway suggested as a plausible mechanism (Riazi et al., 2006). Thereafter, different studies emphasized on proconvulsive properties of Sildenafil in various animal settings of epilepsy (Gholipour et al., 2009; Montaser-Kouhsari et al., 2011). However, the exact mechanisms that Sildenafil exerts its central effects remain unknown.

Some evidence point at NO as a key player in Sildenafil central effects. In Conditioned Place Preference (CPP) paradigm, Sildenafil displays rewarding properties that are mediated by NO-cGMP pathway (Tahsili-Fahadan et al., 2006). Moreover, Sildenafil was found to enhance the electrically evoked release of Oxytocin (OT) from the posterior pituitary by two mechanisms, first through cGMP mediated modulation of K^+ channels in the neurohypophysis (Zhang et al., 2007) and second, via secretion of OT through the activation of NO-cGMP signaling (Matsushita et al., 2012).

OT is a cyclic nanopeptide synthesized in neurons of the supraoptic and paraventricular nuclei of the hypothalamus (Bale et al., 2001). It has important roles in lactation and parturition and acts as a neuromodulator in learning, memory (Burbach et al., 1983), social recognition (Ferguson et al., 2001), stress, anxiety responses (Windle et al., 1997) and also depression (Nowakowska et al., 2002; Matsushita et al., 2012). Over the past years there has been increasing interest in the effects of OT on seizure. Some experiments indicate that OT could exert proconvulsant effects in rat Pilocarpine (Croiset and De Wied, 1997) and PTZ models (Sala et al., 2011; Loyens et al., 2012). In contrast, another investigation has reported anti-convulsant properties for OT in PTZ-induced clonic seizure in rats (Erbas et al., 2013).

OT is expressed in areas of the brain involved in reward/motivation (Clark-Elford et al., 2014). OT has been shown to induce the activatory phosphorylation of cyclic AMP response element-binding protein (CREB) through activation of MAP kinase signaling and causes neural plasticity in the hippocampus (Tomizawa et al., 2003). CREB is a transcription factor of general importance in both neuronal and non-neuronal cells (Hunter, 1995), the phosphorylation of which on Ser-133 promotes the activation of genes with an upstream cyclic AMP response element (CRE) (Hill and Treisman, 1995). CREB is activated by acute seizures (Pi et al., 2004) and has been implicated in the pathology of epilepsy (Tanis et al., 2008). CREB phosphorylation is increased in rodent epilepsy models, and in the seizure onset region of humans with medically intractable epilepsy (Rakhade et al., 2005). There is strong evidence that Sildenafil has an antidepressant-like effect through the induction of OT secretion and transduction of signaling cascades such as MAP kinase signaling and CREB phosphorylation (Matsushita et al., 2012). Regarding the role of the OT pathway in the central effects of Sildenafil as well as its established roles in seizure, we aimed to examine whether the proconvulsant action of Sildenafil is mediated through OT receptors.

In the current study, we assessed the possible contribution of the OT pathway in the proconvulsant effects of Sildenafil on PTZ-induced clonic seizure in mice. We further investigate whether the subsequent CREB phosphorylation is engaged in this phenomenon.

2. Methods

2.1. Animals

In this study male NMRI mice, weighting 22 ~ 30 g from our breeding center were used. The animals were placed in a temperature-controlled (22 ± 3 °C) colony room on a 12 h light/12 h dark cycle with free access to food and water. All procedures were carried out in accordance with institutional guidelines for animal care and use. The behavioural experiments were conducted between 09:00-15:00 and each mouse underwent treatment once. Each behavioural cohort consisted of 6–10 animals. All efforts were made to reduce animal suffering and to use the minimal number of animals necessary to produce reliable scientific data.

2.2. Drug application

The following compounds were used throughout: Atosiban, an OT receptor antagonist, OT, Ketamine, Xylazine, Pentylenetetrazole (PTZ) and Bicuculline were purchased from (Sigma-Aldrich, UK). Sildenafil citrate a selective inhibitor of PDE5 was a generous gift from the Poursina Pharmaceutical Company (Tehran, Iran). Sildenafil, Atosiban, OT, and PTZ were dissolved in sterile physiological saline solution with appropriate concentrations prior to use. Bicuculline was dissolved in warm 0.1 N HCL (0.2 mg ml^{-1}), and the pH of the solution was adjusted to 5.5 with 1 N NaOH. In all experiments, Sildenafil, OT, Ketamine and Xylazine were administered intraperitoneally (ip), Atosiban was injected intracerebroventricularly (icv), PTZ (0.5%,) and Bicuculline were administered intravenously (iv) for inducing clonic seizure in mice (See Section "Seizure paradigm"). All drugs were reconstituted in a way that the required doses were administered in a volume of 10 $mlkg^{-1}$. The doses were chosen based on our previously published data (Riazi et al., 2006; Montaser-Kouhsari et al., 2011; Payandemehr et al., 2015) and pilot experiments.

2.3. Seizure paradigm

Thirty minutes or one hour after drug or vehicle administration (all details are provided in the experiment section), mice were placed in the restrainer and a 30-gauge dental needle was inserted in the lateral tail vein (Löscher, 2002). The correct needle placement in the tail vein was verified by the appearance of blood in the tubing. The needle was then secured to the tail by a narrow piece of adhesive tape. With mouse moving freely, the PTZ solution (0.5%) or Bicuculline solution was slowly infused into the tail vein at the constant rate of (1 $mlmin^{-1}$) using an infusion pump (NE 1000, New Era Pump System, Inc.), which was connected to the dental needle by polyethylene tubing. Infusion was halted when general clonus (forelimb clonus followed by full clonus of the body) observed. The minimal dose of PTZ or Bicuculline ($mg.kg^{-1}$ of mouse weight) needed to induce general clonus was recorded as an index of clonic seizure threshold. As such, the seizure threshold was time-related and dependent on the PTZ or Bicuculline dose administered.

2.4. Surgery for brain cannula implantation and drug microinfusions

Animals were anesthetized with Ketamine and Xylazine (100 and 10 $mgkg^{-1}$, respectively, ip) before placing in a stereotaxic frame with flat-skull position. The coordinates used for cannula implantation were derived from the atlas of the mouse brain by Paxinos and Franklin (2004), 0.3 mm posterior to the bregma, 1.0 mm lateral to the midline, and 3.1 mm below the skull surface. The cannulas were anchored to the skull with dental cement and a stainless steel stylet was inserted into the guide cannula to