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Effects of pentoxifylline and H-89 on epileptogenic activity of bucladesine in pentylenetetrazol-treated mice

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ABSTRACT

The present study shows interactive effects of pentoxifylline (PTX) as a phosphodiesterase (PDE) inhibitor, H-89 as a protein kinase A (PKA) inhibitor and bucladesine (db-cAMP) as a cAMP agonist on pentylenetetrazol (PTZ)-induced seizure in mice. Different doses of pentoxifylline (25, 50, 100 mg/kg), bucladesine (50, 100, 300 nM/mouse), and H-89 (0.05, 0.1, 0.2 mg/100 g) were administered intraperitoneally (i.p.), 30 min before intravenous (i.v.) infusion of PTZ (0.5% w/v). In combination groups, the first and second components were injected 45 and 30 min before PTZ infusion. In all groups, the control animals received an appropriate volume of vehicle. Single administration of PTX had no significant effect on both seizure latency and threshold. Bucladesine significantly decreased seizure latency and threshold only at a high concentration (300 nM/mouse). Intraperitoneal administration of H-89 (0.2 mg/100 g) significantly increased seizure latency and threshold in PTZ-treated animals. All applied doses of bucladesine in combination with PTX (50 mg/kg) caused a significant reduction in seizure latency. Pretreatment of animals with PTX (50 and 100 mg/kg) attenuated the anticonvulsant effect of H-89 (0.2 mg/100 g) in PTZ-exposed animals. H-89 (0.05, 0.2 mg/100 g) prevented the epileptogenic activity of bucladesine (300 nM) with significant increase of seizure latency and seizure threshold. In conclusion, we showed that seizure activities were affected by pentoxifylline, H-89 and bucladesine via interactions with intracellular cAMP and cGMP signaling pathways, cyclic nucleotide-dependent protein kinases, and related neurotransmitters.

1. Introduction

Epilepsy represents a chronic neurological disorder with a high incidence rate in human and is characterized by recurrent unprovoked seizures (Sander and Shorvon, 1996). Epileptic seizures are classified by their pattern of activities in the brain and their behavioral effects. Seizures can be described as either focal or generalized affecting the entire cortex. Although different animal species were used in the past to assess the mechanisms underlying seizure (Bradford, 1995; Fisher, 1989), the physiological basis of epileptic seizure is still vague.

In vivo and *in vitro* studies show that cyclic adenosine monophosphate (cAMP) plays an important role in the pathophysiology of epileptic disorders (Ludvig and Moshe, 1987). It has been demonstrated that cAMP levels change during seizure in the brain (Ferrendelli and Gross, 1980). In addition, cAMP levels are elevated in the cerebral cortex (Ferrendelli and Gross, 1980) and in spinal fluid (Myllyla et al., 1975) after an epileptic attack. It was recently reported that intracellular cAMP signaling pathways in cerebral cortical and hippocampal neurons are involved in seizure disorders (Naseer et al., 2010). Cyclic AMP causes a decrease in the spontaneous discharge rate of mammalian neurons (Boulton et al., 1993).

Nevertheless, the role of cAMP during seizure is still controversially discussed. Some studies showed that cAMP exhibits anticonvulsant effects by scavenging free radicals and suppressing inflammatory mechanisms (Ray et al., 2005; Whitcomb et al., 1990). On the other hand, cAMP appears to be a proconvulsant second messenger that exerts its effects particularly by activating cAMP-dependent protein kinase pathways. Cyclic AMP-induced protein kinase activation results in the phosphorylation of the cAMP response element binding protein (CREB), γ-aminobutyric acid$_A$ (GABA$_A$), and N-methyl-D-aspartic acid (NMDA) receptors (Jancic et al., 2009; Lan et al., 2001; Poisbeau et al., 1999; Westphal et al., 1999), which play important roles for seizure-mediated processes (Bracey et al., 2009; Esteban et al., 2003; Lopez et al., 2007; Poisbeau et al., 1999).

PDE enzymes consist of several subtypes that are distributed in different tissues. These enzymes play critical roles in cyclic nucleotide

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catalysis which make them predisposed as tools for pharmacological manipulation. PDE inhibitors affect different seizure-mediated pathways by increasing intracellular levels of cyclic nucleotides (Bracey et al., 2009; Jeon et al., 2005).

PTX as a methyl xanthine agent inhibits various types of PDE enzymes and elevates intracellular cyclic nucleotides levels (Cunha et al., 2002; Maurice et al., 2003). Adenosine that possesses a clear anticonvulsant potential during pentylenetetrazol-induced seizures (Berman et al., 2000) acts as a neuro-protective agent for hypoxic stress, status epilepticus and catatonia with mainly inhibitory effects on neural activity (Dunwiddie, 1999; Singh and Kulkarn, 2002; Thorat and Kulkarn, 1990). The effects of adenosine were pharmacologically antagonized by PTX (Fredholm and Persson, 1982). PTX effects on seizure activity appear to be dependent on different controversially discussed mechanisms (Tariq et al., 2008).

Bucladesine is a cell membrane permeable cyclic nucleotide analogue which mimics the action of endogenous cAMP and acts as a phosphodiesterase inhibitor (Azami et al., 2010; Campos-Toimil et al., 2008; Sharifzadeh et al., 2007b). This component is widely used to increase intracellular cAMP level (Neumann et al., 2002; Qiu et al., 2002). Besides provoking epileptic seizure, the injection of bucladesine into the amygdala (AM) causes neuronal damage at the injection site and in the CA1–3 hippocampal regions (Tojo et al., 1990). Increased excitability and enhancement of epileptiform activity by cAMP analogues was also previously shown (Boulton et al., 1993). Moreover, the administration of bucladesine caused long latency limbic type behavioral seizure with epileptiform EEG events (Ludvig et al., 1992).

H-89 is a PKA inhibitor which competitively blocks the phosphorylation of serine and threonine residues of this enzyme (Hidaka et al., 1984; Sharifzadeh et al., 2005, 2007a). This chemical component has been frequently used to study PKA signaling pathways in neuronal tissues (Kaneishi et al., 2002; Kim et al., 2005). Previous studies have shown the possible involvement of protein kinases in apoptotic cell death induced by neurotoxic compounds (Koriyama et al., 2003; Ueda et al., 1996). In addition, the effect of PKA inhibitors on attenuation of apoptotic cell death has been shown *in vitro* (Ueda et al., 1996). Therefore, PKA inhibitors may represent prime candidates to attenuate PTZ-mediated seizure.

Considering the controversial roles of cyclic nucleotides in the occurrence of seizure, this study aimed to clarify the effects of cyclic nucleotide-related compounds on PTZ-induced seizure in mice. For this reason, we used pharmacological tools such as PTX and db-cAMP to block PDE isozymes and increase intracellular levels of cAMP, respectively, and H-89 for inhibiting cAMP-dependent protein kinases.

2. Materials and methods

2.1. Animals

Male albino mice weighing 20–25 g were obtained from the Pasteur Institute. Animals were kept under a controlled light and dark cycle (12/12 h) and allowed free access to food and water *ad libitum*. All animal manipulations were performed according to the Ethical Committee for the use and care of laboratory animals of Faculty of Sciences of Tehran University (357; 8 November 2000). All efforts were made to minimize animal suffering.

2.2. Chemicals

All chemicals used in this study were purchased from Sigma (St Louis, MO, USA). The following chemical agents were used: bucladesine (50, 100 and 300 nM/mouse), pentoxifylline (25, 50, and 100 mg/kg), H-89 (0.05, 0.1 and 0.2 mg/100 g) and pentylenetetrazol (0.5% w/v). Bucladesine (db-cAMP) and H-89 were dissolved in a solution of DMSO (dimethyl sulfoxide) and distilled water (10%). The solvent for PTX was saline. PTZ was prepared in normal saline to obtain a concentration of 0.5% w/v for intravenous (i.v.) administration.

2.3. Routs of administration

Pentoxifylline (25, 50, 100 mg/kg), bucladesine (50, 100, 300 nM/mouse) and H-89 (0.05, 0.1, 0.2 mg/100 g) were administered intraperitoneally (i.p.) 30 min before intravenous (i.v.) infusion of PTZ. In combination groups, the first and second components were injected 45 and 30 min before PTZ infusion. In all groups, the respective control animals received an appropriate volume of vehicle. For the i.v. infusion, the needle was inserted into the lateral tail vein, fixed to the tail vein by a narrow piece of adhesive tape, and the animal was allowed to move freely (Gholipour et al., 2008, 2009). PTZ solution was infused at a concentration rate of 1 ml/min.

2.4. Seizure threshold determination

Infusion was stopped at the onset of seizure exhibition. The threshold for the appearance of colonic seizures was calculated by the following formula (Dhir et al., 2011; Gholipour et al., 2009):

$$R(\mathrm{ml/s}) \times T(\mathrm{s}) \times C(\mathrm{mg/ml}) \times 1000/B.W.(\mathrm{g})$$

R: infusion rate of 0.5% (w/v) PTZ solution, *i.e.* 1 ml/min. *T*: time for onset of seizure (s), *C*: concentration of infused PTZ (mg/ml), *B.W.*: body weight of the animal (g).

Seizure threshold was measured by the following end points: 1. Initial myoclonic jerk. 2. Onset of generalized clonus with loss of righting reflex. 3. Onset of tonic extensor phase. The minimum time for the occurrence of these stages of convulsion was also recorded. The values of latency, weigh of the animal, and rate of PTZ infusion were substituted in the above mentioned formula. The amount of PTZ in mg/kg for induction of the first signs of each convulsion stage was measured as seizure threshold. In control groups, the latency and value of administered PTZ to any seizure sign manifestation were recorded at the start of each convulsion phase.

2.5. Statistical analysis

All values were shown as means ± S.E.M. of 7 animals per group. Data were analyzed by using one way analysis of variance (ANOVA) followed by Newman Keuls post-hoc test. A P value of 0.05 or less was considered statistically significant.

3. Results

3.1. Effects of PTZ on the onset and threshold of seizure

The administration of PTZ in mice tail vein was associated with a convulsion episode such as sudden fore- and hind limb constriction, hyperextension of tail, postural and myoclonic jerk losing. These signs of epilepsy were observed in PTZ-treated animals. The latency time for the onset of seizure and the value of seizure threshold were 21.24 ± 0.48 min and 72.07 ± 2.19 mg/kg respectively.

3.2. Effect of pentoxifylline pre-treatment on PTZ-induced seizures

Animals belonged to the PTX-treated group received PTX (25, 50 and 100 mg/kg, i.p) 30 min before PTZ (0.5% w/v, i.v.) infusion. Similarly, animals of the control group received saline (0.9%) as a PTX solvent 30 min before PTZ administration. None of the applied PTX doses had any significant effect on seizure threshold and seizure latency compared to control group (Table 1).