Characterization of the Effects of Phosphodiesterases (PDEs) Isozyme Inhibitors in Animal Models of Epilepsy

J Nandhakumar, A Kanikkai Raja, Manoj G Tyagi*
Department of Pharmacology, Christian Medical College, Vellore 632002, Tamil Nadu, India

*Correspondence to: Manoj G Tyagi, tyagi237@indiatimes.com
Published online: March 20, 2010

Abstract

Epilepsy is a neurological disorder. Phosphodiesterase (PDE) enzymes are responsible for the hydrolysis of the cyclic nucleotides and therefore have a critical role in regulating intracellular levels of the second messengers cAMP, cGMP, and hence cell function as well as downstream signaling in the various body systems. This study was conducted to evaluate the effect of phosphodiesterase isozymes 3, 4 and 5 inhibitors on the maximal electroshock and isoniazid induced convulsions. The results of this study suggested that zonisamide and gabapentin are anticonvulsant drugs were able to attenuate both the MES and isoniazid induced chemical convulsions. On the other hand, PDE 4 inhibitor rolipram and PDE 5 inhibitor, sildenafil was actually potentiating the convulsive phenomenon that the onset of epileptic threshold was reduced as tested by MES and isoniazid induced convulsions. These studies ascertain the action of PDE-3, 4 and 5 inhibitors such as cilostazol, rolipram and sildenafil against MES induced seizures in mice. In which sildenafil (5 mg/kg, i.p.) produced a reduction in the tonic limb flexion significantly (p<0.01) when compared to other groups. Like wise, rolipram (2.4 mg/kg, i.p.) treated animals showed significant (p<0.05) reduction in tonic limb flexion. In the similar manner, sildenafil produced a reduction in the tonic extensor, clonus and stupor phases of convulsion significantly (p<0.01) when compared to other groups and the action of PDE-3, 4 and 5 inhibitors on isoniazid (INH) induced seizures in mice. Sildenafil (5 mg/kg, i.p.) produced a gradual reduction in the onset of action, jerky movements and convulsion significantly (p<0.01) when compared to other groups. The PDE-3, 4 and 5 inhibitors against MES induced seizures in rats. In which sildenafil (3.5 mg/kg, i.p.) produced a gradual reduction in the tonic limb flexion significantly (p<0.01) when compared to other groups. Similarly, sildenafil produced a gradual reduction in the tonic extensor, clonus and stupor phases of convulsion significantly (p<0.01) when compared to other groups except cilostazol (7 mg/kg, i.p.) treated rats. This study concludes that PDE-5 inhibitor, sildenafil having strong proconvulsant activity in MES and INH induced animal models of epilepsy.

Keywords: Epilepsy; phosphodiesterase isozymes; maximal electroshock; isoniazid induced convulsions; anticonvulsant drug; inhibitors; tonic limb flexion; tonic extensor; clonus.

Introduction

Epilepsy is a neurological disorder that consists of recurrent seizures. It is a disorder of brain characterized by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, abnormal, hypersynchronous and rhythmic firing of populations of brain cortical neurons [1]. Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000 [2]. While research into the mechanisms of epilepsy has centered on electrophysiology over the last couple of decades, progress has recently been made in the fields of biochemical and molecular biology aspects of this neurological disorder. For instance, the functions of biological membranes include the actions of receptors, enzymes, ion channels, etc. Therefore, it is proposed that the disturbances of the membrane functions are possibly associated with the provocation of epilepsy. Many receptors, when stimulated by various neurotransmitters and hormones, may stimulate second messengers to elicit biological reactions. In cell physiology, a second messenger system is a method of cellular signaling whereby a diffusible signaling molecule is rapidly produced, which can then go on to activate effector proteins within the cell to exert a cellular response. Secondary messengers are a component of signal transduction cascades. Secondary messenger
systems can be activated by diverse means, either by activation of enzymes that synthesize them, as is the case with the activation of cyclases that synthesize cyclic nucleotides, or by opening of ion channels to allow influx of metal ions, such as in Ca$^{2+}$ signaling. These small molecules may then go on to exert their effect by binding to and activating effector molecules such as protein kinases, ion channels, and a variety of other proteins, thus continuing the signaling cascade [3]. The messengers first reported are cyclic nucleotides, e.g. cAMP and cGMP, and those recently discovered are the reaction products of inositol (PI) response [4], i.e. inositol triphosphate and diacylglycerol. The products of the former release stored intracellular Ca$^{2+}$ and the latter activate protein kinase C. Phosphodiesterase (PDE) enzymes are responsible for the hydrolysis of the cyclic nucleotides, and therefore, have a critical role in regulating intracellular levels of the second messengers cAMP, cGMP, and hence, cell function as well as downstream signalling in the various body systems [5]. Recent evidence that the cyclic nucleotide phosphodiesterases exist in several molecular forms and that these isozymes are unequally distributed in various tissues [6]. Twelve members of the PDE family have been identified and these can be further divided into a number of subtypes and splice variants. The PDE types differ in their amino acid sequence, substrate specificities, kinetic properties, allosteric regulators, inhibitor sensitivities and in their organ, tissue and sub cellular distribution [7, 8]. Through the selective inhibition of the major phosphodiesterase isozyme of a diseased tissue, it may then be possible to alter the course of diseases characterized by an abnormal metabolism of cyclic nucleotides. Out of the twelve PDE gene families PDE-3, PDE-4 and PDE-5 are belongs to cGMP-inhibited [9-11], cAMP-specific [12-16] and cGMP-specific [17-22] types of affinity to cyclic nucleotides respectively. PDE-3 and PDE-4 enzyme are expressed in the hippocampus, striatum and other discrete sites of the brain and may affect of calcium ions and electroshock may modify their activity. Therefore, the present study examined the influence of cyclic nucleotide PDE inhibitors, especially PDE-3, 4 and 5, in order to prove the effective role in the induction of convulsive seizures. We used pharmacological tools like cilostazol, rolipram and sildenafil to block the PDE 3, 4 and 5 isozymes respectively to evaluate the effect on maximal electroshock and chemical convulsant induced seizures in mice and rats.

Methods

Animals Used: Male Swiss Albino mice weighing between 23-26 g and male Wistar strain rats weighing between 160-220 g were utilized for this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 24 ± 2 Degrees Celcius and relative humidity of 30-70%. A 12:12 dark: light cycle was followed during the experiments. All the animals were allowed free access to water ad libitum and fed standard commercial pellet rat chaw (M/s Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee and were in accordance with the guidelines of the CPCSEA.

Drugs and Chemicals: The following drugs and chemicals were used for conducting this study. 10% w/v of dimethyl sulfoxide (DMSO) Sigma, USA, Cilostazol (Sigma, USA), Rolipram (Sigma, USA), Sildenafil (Sun Pharma, Mumbai, India), Zonisamide (Sun Pharma, Mumbai, India) and Gabapentin (Micro labs Ltd., Bangalore, India). Except rolipram and cilostazol, other drugs are soluble in sterile water for injection, rolipram and cilostazol is soluble in DMSO.

A. Maximal electroshock (MES) method: The mice were divided into five groups with six animals (n=6) in each. Group I served as solvent control, received 10 % w/v of dimethyl sulfoxide [DMSO] (5 ml/kg, i.p.), group II received zonisamide (50 mg/kg, i.p.), treated as positive control and Group III, IV and V received PDE- 3 inhibitor such as cilostazol (10 mg/kg, i.p.), PDE-4 inhibitor such as rolipram (2.4 mg/kg, i.p.) and PDE-5 inhibitor such as sildenafil (5 mg/kg, i.p.) respectively. All the drugs were administered intraperitoneally 25 min prior to the electroshock. The electroshock was induced in animal by passing a current of 45 mA for 0.2 sec duration through electroconvulsometer (Techno India) using corneal electrodes [23]. The incidence of seizures, tonic limb flexion, tonic extensor, clonus, stupor of the animals were observed and noted as per earlier described method [24].

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B. Chemical convulsant method: Isoniazid (INH) induced seizures: Albino mice were divided into five groups each containing six animals (n=6). Seizures were induced in the animals by using chemical convulsant, isoniazid (INH). INH is a GABA synthesis inhibitor, which was injected to induce seizures at the dose of 500 mg/kg, s.c as described earlier [25]. All the drugs were administered intraperitoneally 25 minutes prior to the chemoshock in the same dose as mentioned in MES, except positive control (Gabapentin 2.5mg/kg, i.p.). Onset of action, myoclonic jerks, clonus, and tonic flexion were observed and noted.

C. Maximal electroshock (MES) method for rats: Rats were divided into five groups with six animals (n=6) in each. Group I served as solvent control, received 10 % w/v of dimethylsulfoxide [DMSO] (3.5 ml/kg, i.p.), group II received zonisamide (35 mg/kg, i.p.), treated as positive control and Group III, IV and V received PDE-3 inhibitor such as cilostazol (7 mg/kg, i.p.), PDE-4 inhibitor such as rolipram (1.7 mg/kg, i.p.) and PDE-5 inhibitor such as sildenafil (3.5 mg/kg, i.p.) respectively. All the drugs were administered intraperitoneally 25 min prior to the electroshock. The electroshock was induced in animals by passing a current of 150 mA for 0.2 sec duration through electroconvulsometer (Techno India) using corneal electrodes according to a previously described method [23]. The incidence of seizures, tonic limb flexion, tonic extensor, clonus, stupor of the animals was observed and noted as per earlier described method [24].

Statistical analysis: The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnnett’s test. P values <0.05 were considered significant.

Results

Evaluation of onset of seizures

A. Maximal Electroshock Test: Fig. 1 illustrates the action of PDE-3, 4 and 5 inhibitors such as cilostazol, rolipram and sildenafil against MES induced seizures in mice. In which sildenafil (5 mg/kg, i.p.) produced a reduction in the tonic limb flexion significantly (p<0.01) when compared to other groups. Like wise, rolipram (2.4 mg/kg, i.p.) treated animals showed significant (p<0.05) reduction in tonic limb flexion. In the similar manner, sildenafil produced a reduction in the tonic extensor, clonus and stupor phases of convulsion significantly (p<0.01) when compared to other groups.

B. Chemical convulsant method: Isoniazid (INH) induced seizures: Fig. 2 exhibits the action of PDE-3, 4 and 5 inhibitors on INH induced seizures in mice. Sildenafil (5 mg/kg, i.p.) showed a gradual reduction in the onset of action, jerky movements and convulsion significantly (p<0.01) when compared to other PDE inhibitors such as cilostazol (10 mg/kg, i.p.) and rolipram (2.4 mg/kg, i.p.), DMSO (5 ml/kg, i.p.) and gabapentin (2.5 mg/kg, i.p.).

C. Maximal electroshock (MES) method for rats: Fig. 3 exhibits the action of PDE-3, 4 and 5 inhibitors such as cilostazol, rolipram and sildenafil against MES induced seizures in rats. In which sildenafil (3.5 mg/kg, i.p.) produced a gradual reduction in the tonic limb flexion significantly (p<0.01) when compared to other groups. Similarly, sildenafil produced a gradual reduction in the tonic extensor, clonus and stupor phases of convulsion significantly (p<0.01) when compared to other groups except cilostazol (7 mg/kg, i.p.) treated rats.

Discussion

This study was conducted to evaluate the effect of phosphodiesterase isozyme 3, 4 and 5 inhibitors on the maximal electroshock and isoniazid induced convulsions. The results of this study are depicted in figures 1-3. The results of this
**Fig. 1:** Effect of PDE-3, PDE-4 and PDE-5 inhibitors on maximal electroshock induced convulsions in mice (n=6).

Values are mean ± SEM, represent onset time of various phases of convulsion in seconds. Treatments were given 25 mins prior to maximal electroshock (45 mA, 0.2 sec). The data were analyzed by one-way ANOVA followed by Dunnett’s test. * p<0.05, ** p<0.01 and *** p<0.001 vs Sildenafil treated group.

**Fig. 2:** Effect of PDE-3, PDE-4 & PDE-5 inhibitors on chemoshock seizures in mice (n=6).

Values are mean ± SEM, represent onset time of various phases of convulsion in seconds. Treatments were given 25 mins prior to chemical convulsant injection of INH (500 mg/kg, s.c). The data were analyzed by one-way ANOVA followed by Dunnett’s test. * p<0.05, ** p<0.01 and *** p<0.001 vs Sildenafil treated group.

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study suggest that zonisamide and gabapentin are anticonvulsant drugs were able to attenuate both the MES and isoniazid induced chemical convulsion. On the other hand, PDE 4 inhibitor rolipram and PDE 5 inhibitor, sildenafil was actually potentiating the convulsive phenomenon i.e. the onset of epileptic threshold was reduced as tested by MES and isoniazid induced convulsions. The mechanism of its action is through the inhibition of T-type Ca 2+ currents. In addition, zonisamide inhibits sustained repetitive firing of spinal cord neurons, presumably by prolonging the inactivated state of voltage gated sodium channels in a manner similar to actions of phenytoin and carbamazepine. Recent evidence suggests that the cyclic nucleotide PDE exist in several molecular forms and that these isozymes are unequally distributed in various tissues. Phosphodiesterase (PDE) activity is found in every cell in the body, although there is distinct cellular and subcellular distribution of the 12 isoenzymes, which has provided many possibilities for increasingly selective therapeutic targets [27]. In identifying isoenzyme selective targets for specific diseases, a substantial amount of work was undertaken by pharmacologists working in the U.K., particularly in characterizing tissue expression, subcellular distribution and modulation of tissue function by isoenzyme selective inhibitors. The PDE-3, PDE-4 and PDE-5 belong to cGMP inhibitory, c-AMP specific and cGMP specific types of affinity to cyclic nucleotides respectively. According to Riazi et al sildenafil is a proconvulsant drug in the rodents and the role of nitric oxide cGMP pathway is implicated in these actions. Other reports also suggest the proconvulsant action of sildenafil in humans and animals. Studies conducted by Demchenko et al showed that PDE5 blockers oppose the protective vasoconstriction that is the initial response to hyperbaric hyperoxia, decreasing the safety of hyperbaric oxygen and hastening onset of CNS oxygen toxicity. The present study mainly focuses the onset of seizures against the prior administration of PDE-3, 4 and 5 inhibitors such as cilostazol, rolipram and sildenafil. PDE-3 has high affinity for cAMP but can also hydrolyse cGMP. However, since the Km for cGMP has generally been reported to be lower than that for cAMP, and the Vmax is ten times greater for cAMP than for cGMP, cGMP readily inhibits the hydrolysis of cAMP by PDE3 by acting as a potent competitive inhibitor at the catalytic site [28-29]. Thus, expression of PDE3 allows stimuli that elevate cGMP levels to augment cAMP-mediated signalling [29]. There are two PDE3 genes, PDE3A and PDE3B. Cilostazol is a PDE3 inhibitor that is a U.S. FDA - approved therapy for intermittent claudication, owing to its activity on both platelets and endothelium [30]. PDE-4 enzymes are cAMP-specific and play an important role in the biology of haematopoietic cells. These PDEs all hydrolyse cAMP with Km values in the range 1–4 µM [31]. Earlier reported study suggest that G-proteins and PKAs (cAMP-dependent protein kinase), is essential for controlling localized concentrations of cAMP [32-35]. PDE-5, a cGMP-specific PDE family of considerable importance in regulating smooth muscle and endothelial cell function, is also present in platelets. Although having no effect on platelet function when used alone, PDE5 inhibitors augment nitroprusside’s anti-platelet aggregation activity in vitro [36].

The data obtained from this study show that pre-treatment with PDE-3, 4 and 5 inhibitors potentiates the onset of action and various phases of convulsions against INH and maximal electroshock induced convulsions as depicted in Fig 1 to 3. At same time the effect of onset of action, after administration of PDE-3 and 4 were less when compared to PDE-5 inhibitor. Our study results also clearly suggest that rate of onset of convulsive time was significantly (p<0.05 and p<0.01) reduced with sildenafil against INH and MES induced seizures in both mice and rats. It has recently been reported that the elevation of cGMP levels provides a depolarized state at the rod outer segment of retina [37] and a GTP-binding protein (a G-protein, Go) regulates the neuronal Ca2+ channel [38]. This Ca2+ is responsible for the phosphorylation process of intracellular proteins, such as ion channels, receptors, enzymes and transcription factors that exhibit significant neuronal excitability and epileptic seizures [39]. These events were associated with a significant increase in intracerebellar cyclic GMP [40]. Thus, in conclusion the present study finds that PDE-5 inhibitor, sildenafil having strong proconvulsant activity in MES and INH induced animal models of epilepsy and also PDE-3 & 4 inhibitors, such as cilostazol and rolipram possess less proconvulsant action. The probable mode of action may be the cGMP levels elevated by certain excitatory amino acids and may allow one to imply that an excitatable state exists in the neuronal cells through the action of the G-protein as well as the ion channel. Although the findings here seem to indicate divergent features of the excitable neuronal cells, they may provide clues to observing a close association of receptors, enzymes and channels of the membranes in exploring the provocation of seizures.

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Fig. 3: Action of PDE-3, PDE-4 & PDE-5 inhibitors on maximal electroshock induced convulsions in rats (n=6).

Values are mean ± SEM, represent onset time of various phases of convulsion in seconds. Treatments were given 25 mins prior to maximal electroshock (150 mA, 0.2 sec). The data were analyzed by one-way ANOVA followed by Dunnett’s test. * p<0.05, ** p<0.01 and *** p<0.001 vs Sildenafil treated group.

Table 1: Treatment protocol of maximal electroshock (MES) model for mice and rats, and chemoconvulsant (INH) model for mice.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Drugs used</th>
<th>Category</th>
<th>MES for Mice (45 mA for 0.2 sec)</th>
<th>Chemoconvulsant (INH) for Mice (500 mg/kg, s.c)</th>
<th>MES for Rats (150 mA, 0.2 sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group- I</td>
<td>10% DMSO</td>
<td>Solvent Control</td>
<td>5 ml/kg, i.p.</td>
<td>5 ml/kg, i.p.</td>
<td>3.5 ml/kg, i.p.</td>
</tr>
<tr>
<td>Group- II</td>
<td>Zonisamide/Gabapentin</td>
<td>Positive Control</td>
<td>(Zonisamide) 50 mg/kg, i.p.</td>
<td>(Gabapentin) 2.5 mg/kg, i.p.</td>
<td>(Zonisamide) 35 mg/kg, i.p.</td>
</tr>
<tr>
<td>Group- III</td>
<td>Cilostazol</td>
<td>PDE-3 inhibitor (cGMP-inhibited)</td>
<td>10 mg/kg, i.p.</td>
<td>10 mg/kg, i.p.</td>
<td>7 mg/kg, i.p.</td>
</tr>
<tr>
<td>Group- IV</td>
<td>Rolipram</td>
<td>PDE-4 inhibitor (cAMP-specific)</td>
<td>2.4 mg/kg, i.p.</td>
<td>2.4 mg/kg, i.p.</td>
<td>1.7 mg/kg, i.p.</td>
</tr>
<tr>
<td>Group- V</td>
<td>Sildenafil</td>
<td>PDE-5 inhibitor (cGMP-specific)</td>
<td>5 mg/kg, i.p.</td>
<td>5 mg/kg, i.p.</td>
<td>3.5 mg/kg, i.p.</td>
</tr>
</tbody>
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Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

JN: experimental work, manuscript preparation; AK: experimental work; MGT: conception of research work, manuscript preparation.

References


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