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EFFECT OF DIHYDROPYRIDINE COMPOUNDS AMRINONE AND MILRINONE IN EPILEPSY: AS PHOSPHODIESTERASE-III INHIBITORS

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ABSTRACT

Pyridine compounds are the important structural feature of many biologically active compounds and shown diverse pharmacological properties. However, some pyridine compounds have been reported to effective in epilepsy. In this study, the role of pyridine compounds, amrinone and milrinone as specific phosphodiesterase-III (PDE-III) inhibitors in the generation of seizures in mice against chemically induced, isoniazid (INH) at the dose of 500mg/kg, s.c and maximal electroshock (MES) at 60 mA for 0.2 sec. PDE-3 inhibitors significantly enhanced the onset of seizures induced by INH and MES. In particular, milrinone potentiated the convulsive phenomenon more significantly when compared with amrinone. Pyridine compounds further focus our attention because of their easy functionalization at various ring positions, which makes them attractive synthetic compound for designing and development of novel pyridine compounds as phosphodiesterase III inhibitors or other biologically active compounds in future.

Keywords: **PDE-III inhibitors, amrinone, milrinone, seizures.**

INTRODUCTION

Epilepsy is one of the most common afflictions of human beings with a prevalence rate of approximately 1 % of the total population (1). An epileptic seizure is a transient paroxysm of uncontrolled discharges of neurons causing an event that is discernible by the person experiencing the seizure and/or by the observer (2, 3). Epilepsy is the second most common neurologic disorder after stroke. The incidence is highest in the first 10 years of life and declines thereafter through the age of 50 until the elderly years when the incidence increases again. Epilepsy begins before the age of 18 in over 75% of patients. (4).

Recently, much attention has been focused on pyridine derivatives for their broad-spectrum of biological activities. These compounds exhibited wide range of pharmacological activities like anti-tubercular, anti-inflammatory, antibacterial, anticonvulsant and antifungal. In particular, a large number of pyridine derivatives are well known as antiepileptic agents (5-7). Some pyridine derivatives have been reported as antiepileptic and PDE-III inhibitor (8). Various structural modifications were carried out in pyridine ring system. These structural changes resulted in some fruitful biological activities. This review aimed at utilizing pyridines as antiepileptic and PDE-III inhibitor activities, to examine the role of cyclic phosphodiesterase-III in the generation of seizures. Pyridine compounds, amrinone and milrinone were study to block the PDE-III and evaluate the effect on maximal electroshock MES and chemical induced seizures in mice.

Epilepsy and cyclic nucleotide phosphodiesterase isozyme: Seizure is a characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain. The pathophysiological basis for epileptic disorders is both complex and intricate. The search for newer antiepileptic drugs have focused the research on cell signaling elements like the cytoskeletal structures, transmembrane enzymes and ion channel modulators. There is recent evidence that the cyclic nucleotide PDEs exist in several molecular forms and that these isozymes are unequally distributed in various tissue makes the PDEs particularly suitable targets for pharmacologic manipulation, for it suggests that by finding selective inhibitors of the different PDE isozymes, one may be able to raise the concentration of cyclic nucleotides in discrete cell types (9). Through the selective inhibition of the major PDE isozyme of a diseased tissue, it may then be possible to alter the course of diseases characterized

by an abnormal metabolism of cyclic nucleotides.

Twelve members of the 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 (10,11). PDE-III is characterized by its high affinity for cAMP and cGMP. cAMP is postulated to be anticonvulsant while cGMP is considered to be proconvulsant (12). PDE-3 enzyme is highly expressed in the hippocampus, striatum and other discrete sites of the brain and may affect the influx of Ca^{2+} ions (13,14). In mammals, PDE are encoded by at least 19 different genes and PDE isoforms are expressed differently in different tissues (15). Electroshock has been reported to increase the expression of PDE-IV isoform in rat brains suggesting ECS regulates the activity of cAMP system by modifying PDE isoform expression (16,17). However these studies were limited to PDE-IV family in the cerebral cortex and the hippocampus.

MATERIAL AND METHODS

Isoniazid (INH) and Maximal Electroshock (MES) induced seizures: Seizures were induced in the animals by using chemical convulsant, Isoniazid (INH). INH is a GABA synthesis inhibitor, (500 mg/kg, s.c). 15 mins prior to the injection of INH the animals were pretreated with varying doses of amrinone (0.5 mg/kg, 0.6 mg/kg and 0.7 mg/kg, i.p) and milrinone (50µg/kg, 100 µg/kg, 200 µg/kg and 300 µg/kg, i.p). The animals were subjected to electroshock (60mA/0.2 secs) via the corneal electrodes. After induction of seizures, tonic limb flexion, tonic extensor, clonus, stupor and recovery/mortality of the animals were observed.

EVALUATION OF ONSET OF SEIZURES

INH induced seizures: In INH induced seizures, result showed that Amrinone in a dose of 0.5 mg/kg significantly potentiated the onset of action, jerky movements and convulsions, whereas the rate of onset of action, jerky movements and convulsions time was reduced more significantly in the doses like 0.6 mg/kg and 0.7 mg/kg of amrinone. Simultaneously the rate of onset of action, jerky movements and convulsion time was reduced at the great extent even in the low doses like (200 µg/mg and 300 µg/mg) of milrinone considerable mortality (67%) was observed while using amrinone (0.6 mg/kg and 0.7 mg/-kg) and milrinone (100 µg/kg, 200 µg/kg and 300 µg/kg).

MES induced seizures: Result illustrates the action of various dose levels of amrinone and milrinone against MES induced seizures. In which 0.6 mg/kg and 0.7mg/kg of amrinone produced a gradual reduction in

tonic limb flexion significantly. Significant was observed in stupor phase of convulsion at the dose of 0.6 mg/kg and 0.7 mg/kg of amrinone. Like-wise milrinone treated animals showed a significant reduction in tonic limb tonic extensor and stupor flexion, phases of convulsion in the 200µg/kg and 300 µg/kg dose levels. Milrinone in the doses like 200 µg/kg and 300µg/kg treated animals produced the significantly reduced the clonus phases of convulsion at the level of $p<0.005$ and $p<0.001$ respectively. Mortality (67%) was observed in both doses like 200µg/kg and 300µg/kg of milrinone (8).

DISCUSSION: The therapeutic use of theophylline/aminophylline is associated with the incidence of intractable seizures and mortality (18,19). The mechanisms involved in these seizures are not well understood and the treatment of the life threatening condition is unsatisfactory. The results of this study suggest that PDE-3 inhibitors potentiate the electro-shock and chemical induced seizures. The bipyridine derivative of selective PDE-3 inhibitors such as amrinone and milrinone is a new class of positive inotropic drugs chemically and pharmacologically distinct from digitalis and catecholamines (20). The mechanism of the positive inotropic effect of PDE inhibitors is similar to that of β-adrenergic agents (21). Milrinone has been the most studied and used extensively as PDE-III inhibitor and it is currently used in the acute treatment of heart failure to diminish long term risk. This study demonstrates the importance of the PDE-III inhibitors such as amrinone and milrinone in the generation of seizure activity with the accumulation of cellular levels of cAMP and cGMP by inhibiting its metabolism. cAMP accumulation is considered to be anticonvulsant and cGMP is considered to be pro-convulsant. The data obtained from this study show that pre-treatment with PDE-III inhibitors potentiates the onset of action and various phases of convulsions against INH and maximal electroshock induced convulsions. Our study results also clearly suggest that rate of onset of convulsive time was significantly reduced with increasing the dose levels of both amrinone and milirinone against INH and MES induced seizures. Earlier studies suggest that the elevated level of cGMP was found in cortical structure in some experimental models of epilepsy (22), and the neuronal excitability was regulated by cGMP and Ca^{2+}/calmodulin dependent protein kinase and its phosphorylation process (23). Apart from these findings, PDE-III inhibitors possess trans-membrane influx of Ca^{2+}. This influx of Ca^{2+} is responsible for the phosphorylation process of intracellular proteins, such as ion channels, receptors, enzymes and transcription factors which

exhibit significant neuronal excitability and epileptic seizures (24).

On the other hand, phosphorylation of variety of substrates regulates the myriad of physiological process, such as immune responses, cardiac and smooth muscle contraction, visual response, glycogenolysis, platelet aggregation, ion channel conductance, apoptosis and growth control. The present study results also early correspond with the generation of seizure activity due to the breakdown of hydrolysis of cGMP which promotes protein kinase phosphorylation process. Thus, in conclusion the study shows a definite relation-ship between the specific PDE-III inhibitors and increase the cellular level of cGMP and Ca^{2+} ions with the generation of seizures. The release of free radicals have been implicated in many drug and chemical induced toxicities. It is possible that increased production of reactive oxygen species could result in oxidant/ antioxidant imbalance and thus, precipitate neurotoxicity. Therefore it appears that non nucleotide mechanism although not well defined could also be contributing significantly to the seizure activity of PDE-III inhibitors (24-26).

Amirinone (R^1= NH_2, R^2= H), Milrinone (R^1= CN, R^2= CH_3)

CONCLUSION: The pyridine moiety is an important structural feature of many biologically active compounds and show diverse pharmacological properties. Pyridines hold considerable interest relative to the preparation of organic intermediates and physiologically active compounds. However, some pyridine compounds have been reported to effective in epilepsy. Pyridines further focus our attention because of their easy functionalization at various ring positions, which makes them attractive synthetic compound for designing and development of novel pyridazinone as PDE-III inhibitors in future.

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