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Adenosine has been recognized as a potent neuromodulator that functions through the most abundant inhibitory adenosine A₁ receptors and less abundant, but widespread, facilitatory A₂A receptors, respectively. Therefore, adenosine exerts a presynaptic control over the release of several neurotransmitters via inhibitory adenosine A₁ receptors and also inhibits neuronal excitability and synaptic transmission. Adenosine A₂A receptors have been shown to facilitate the release of most neurotransmitter types (glutamate, GABA, glycine, acetylcholine, noradrenalin, serotonin) in different extra-striatal brain regions (Cunha, 2005). Both these receptors demonstrate opposite effects on the release of excitatory neurotransmitters in the brain. In particular, in glutamatergic nerve terminals, it has been shown that adenosine A₁ and A₂A receptors are co-located in a subset of these terminals in the hippocampus and there is a functional interaction between these two adenosine receptors with opposite effects on glutamate release (Lopes et al., 1999). Apart from these presynaptic effects, adenosine A₁ receptors also inhibit neuronal activity by acting post-synaptically, both in distal dendrites as well as in proximal dendrites (Cunha, 2005).

The potent inhibitory mechanism of adenosine and its analogs acting through adenosine A₁ receptors has been documented in several neuronal disorders including ischemia, status epilepticus, kindling, and tardive dyskinesia (Kulkarni and Mehta, 1985; Cunha, 2001; Ribeiro et al., 2003; Bishnoi et al., 2006; Akula et al., 2007a). However, these investigations mainly used indirect approach to establish the role of adenosine in various brain functions. The measurement of brain adenosine and its major metabolites would perhaps give valuable information both in the pathophysiology and in the process of disease modification by pharmacological agents. The availability of a simple and sensitive chromatographic method could solve the purpose of determining the alterations of the purine analogs for better assessment of disease condition. At the same time a chromatographic method for estimation of GABA, another
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potent inhibitory neurotransmitter could provide valuable information regarding the neuronal activities and possible interaction between these two effective inhibitory molecules in the pretext of disease progression and treatment.

The neuroprotective role of adenosine and its analogues have been acknowledged in several neuronal disorders like epilepsy and tardive dyskinesia. The neuromodulatory role of adenosine has been a matter of conjuncture wherein the tendency of adenosine to modulate the release profile of several neurotransmitter mechanisms of brain has taken a leap in the search for alternative therapies for several neuronal disorders including epilepsy. Based on the recent reports regarding the seizure suppressing activities of serotonergic system, nitric oxide modulators, and other mechanisms involving cyclooxygenase inhibitors, a putative interaction of these systems with adenosinergic mechanism can be conceived which could ultimately result in better treatment options in case of refractory seizures.

The most important step in the discovery of a new antiepileptic drug is the choice of an appropriate animal model for the initial screening for anticonvulsant activity. Pentylenetetrazol-seizure threshold is one such valuable paradigm where the animal model effectively evaluates the antiepileptic drugs useful in preventing the seizure generation and propagation with minimum inter-animal variability.

Addiction to alcohol, opioids and/or benzodiazepines (diazepam) represents a serious health and social issue in the community. Manifestation of withdrawal syndrome includes emergence of negative emotional state (eg. dysphoria, anxiety, irritability) and sometimes seizures when access to the drug is prevented. The withdrawal phase was also estimated to inflict oxidative neuronal damage with free radical generation (Vallett et al., 1997; Musavi and Kakkar, 2003). Treatment of withdrawal phenomenon has progressed from empirical or purely social and behavioral approaches to the pharmacotherapeutic attempts to disrupt the mechanisms underlying these disorders. Excess activity of excitatory neurotransmitter, glutamate and activation of NMDA receptors is proposed to be one of the main causes of withdrawal syndrome (Hack and Christie, 2003). Despite these advances, many forms of addiction lack effective therapeutics and the prevalence of this
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disorder remains high. As a result, a significant effort is being dictated within the research community to the identification of novel targets for the development of therapeutics based upon the pathological processes underlying addiction. Adenosine is proposed to modulate noxious events in nervous system since it is able to decrease the excitatory amino acid release, inhibits potassium conductances at the postsynaptic level leading to neuronal hyperpolarization, forbid the activity of NMDA receptors, limit calcium influx, inhibit free radical formation and exert modulatory effects at astrocytic and microglial cells (Fredholm, 1997). In this perspective, adenosine and its analogs acting at A₁ and A₂A receptors could exert neuroprotection and therefore, a possible therapeutic role of adenosine analogs need to be investigated in the pretext of reversing withdrawal symptoms of hyperactivity, oxidative damage and other possible molecular changes such as mitochondrial enzyme activities.

With this background, the present work was aimed to explore the neuroprotective effect of adenosine and its analogs in convulsions and drug withdrawal syndrome by targeting behavioral alterations (anxiety, hyperactivity, seizures) and oxidative stress.

Chapter 1 deals with the development and validation of a simple and sensitive reverse phase high performance liquid chromatography method for the estimation of adenosine and its metabolites in brain samples. The chapter also emphasizes the sample preparation protocol for the preparation of biological sample for chromatographic analysis of purines.

The second part of this chapter deals with the development and validation of an improved chromatographic method for the estimation of GABA in isolated brain tissues.

Chapter 2 describes the standardization of PTZ-seizure threshold model where, the relative potency of several anticonvulsant drugs against intravenously administered PTZ was determined in mice. Barbiturates (pentobarbitone and phenobarbitone), sodium channel blockers (phenytoin and carbamazepine), benzodiazepines (diazepam, chlordiazepoxide, triazolam and clonazepam), GABA, ethanol, ashwagandha, novel
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Antiepileptics (tiagabine, gabapentin, pergabalin), progesterone, and rofecoxib were screened for their possible antiepileptic profile. Effect of these drugs on PTZ-seizure threshold for the onset of myoclonic jerks, generalized clonus, and tonic extensor was noted. TI50 i.e., the dose of an antiepileptic drug required to raise the PTZ-seizure threshold for tonic extensor by 50% was calculated and from these values, the relative potency of the standard anticonvulsant drugs against PTZ-seizure threshold was determined.

Chapter 3 explores the neuroprotective effect of exogenous adenosine against the convulsions-induced by PTZ infusion in mice. Further, this chapter delves on the functional interaction of adenosinergic system with other signaling pathways against PTZ-seizure threshold paradigm in mice. This chapter has been divided into three parts wherein the first part deals with the interaction of adenosine with nitric oxide signaling pathway. Second part describes the interaction between adenosine and serotonergic system. Third part brings into account the possible involvement of adenosinergic system in the anticonvulsant effect of novel targets like cyclooxygenase inhibitors. These signaling pathways have been chosen for these interaction studies with adenosine based on the recent reports regarding their putative role in reducing seizures.

Chapter 4 illustrates the neuroprotective effect of adenosine and adenosine receptor ligands, especially the differential effects of adenosine A₁ and A₂A receptors in the context of alcohol or morphine or diazepam withdrawal syndrome in mice. The chapter explores the beneficial effect of these agents in mitigating the behavioral symptoms of drug withdrawal. In addition, mitochondrial enzyme activities, and oxidative/nitrosative stress markers generated in drug withdrawn mice and their modification by adenosinergic ligands were explored.

The extensive studies reported in the above chapters is expected to enhance our understanding of the neuroprotective functions of endogenous and systemically administered adenosine and adenosine related drugs in convulsions, drug withdrawal behaviors and associated biochemical and neurochemical alterations in the brain, respectively.