INTRODUCTION

The pathophysiology of various neuronal disorders is characterized by a complex sequence of events, including an alteration in gross behavior, neurochemical, electrophysiological and hemodynamic factors. The neurodegenerative changes also induce several molecular alterations which include disturbed ion homeostasis, increase in intracellular Na⁺ and Ca²⁺ concentrations. Apart from this, the extracellular levels of the excitotoxic amino acids such as glutamate and aspartate increase dramatically, further precipitating membrane depolarization and an additional accumulation of intracellular Ca²⁺ (Choi, 1988). Elevated intracellular Ca²⁺ levels appear to be critically involved in the series of excitotoxic events leading to neuronal injury and death, which include free radical formation, membrane lipid peroxidation, mitochondrial dysfunction, activation of proteolytic enzymes and transcription of pathological genes.

The excitotoxicity, neuronal degeneration and death are some critical factors in the clinical manifestations of several neurological disorders, including cerebral stroke, Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, epilepsy, and during withdrawal from addictive drugs. All these conditions have been projected to be effective targets for therapeutic interventions involving the manipulations of adenosine receptor functions. Several evidences have been accumulated regarding the neuroprotective effects of nucleoside adenosine in many neuronal injuries. Adenosine was reported to potentially interfere with many of the steps in the pathological sequence of events occurring in neuronal damages and excitotoxicity (Cunha, 2005). Various experimental studies have emphasized that activation of adenosine receptors can provide an effective neuroprotective strategy for minimizing pathological outcomes in neurological disorders (Phillis, 2002).

Adenosine is formed within the cells as a result of hydrolysis of adenosine monophosphate (AMP) through the action of ecto-5'-nucleotidase, hence its formation depended upon the breakdown and synthesis of
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Adenosine triphosphate (ATP), an energy molecule. Extra cellular adenosine concentrations are kept in equilibrium by specific reuptake mechanisms working via specialized bidirectional transporters. Adenosine is demonstrated to exert its biological actions through four cell surface G protein-coupled receptor subtypes namely A₁, A₂A, A₂B and A₃ adenosine receptors. These receptors are widely distributed in the central nervous system (CNS), cardiovascular system (CVS), renal and respiratory systems, gastrointestinal and urogenital tract (Table 1).

Table 1. Characteristics of adenosine receptors, their distribution and implications in the CNS

<table>
<thead>
<tr>
<th>RECEPTOR SUBTYPE</th>
<th>SECOND MESSINGERS</th>
<th>DISTRIBUTION IN THE CNS</th>
<th>MAJOR THERAPEUTIC POTENTIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>Gi, Go</td>
<td>cerebral cortex, hippocampus, striatum, thalamus, cerebellum</td>
<td>Activation: seizure suppression, neuroprotection, spinal analgesia</td>
</tr>
<tr>
<td></td>
<td>AC, PLC, PLD, K⁺, Ca²⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₂A</td>
<td>Gₛ, Gₒf</td>
<td>striatum, nucleus accumbens, olfactory tubercle, globus pallidus, cerebral cortex, hippocampus</td>
<td>Activation: anti-inflammatory action. Inhibition: Parkinson’s disease</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₂B</td>
<td>Gₛ</td>
<td>low level in the brain</td>
<td>Inhibition: antiasthmatic</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>hippocampus, thalamus, hypothalamus, striatum</td>
<td></td>
</tr>
<tr>
<td>A₃</td>
<td>Gₛ, Gₒ</td>
<td>low level in the brain</td>
<td>Inhibition: anti-inflammatory action</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>hippocampus, thalamus, cerebral cortex, cerebellum</td>
<td></td>
</tr>
</tbody>
</table>

AC, adenylyl cyclase; Ca²⁺, calcium channel; G-protein inhibiting AC (Gᵢ, Gₒ, Gₗ) and stimulating AC (Gₛ, Gₒf); K⁺, potassium channel; PLC, phospholipase C; PLD, phospholipase D.

Adenosine acts as an important messenger molecule for extracellular signaling and as a homeostatic modulator at the synaptic level (Cunha, 2001). Adenosine is known to modify the release of neurotransmitters such as acetylcholine, noradrenalin, dopamine, serotonin, γ-amino butyric acid (GABA), glutamate and also the post-synaptic responsiveness and the action
of a number of other neurotransmitter systems (Mehta and Kulkarni, 1984; Kulkarni and Mehta, 1985; Ribeiro et al., 2003) (Fig. 1), respectively.

As a consequence, adenosine is reported to be a neuromodulator in a variety of behavioral paradigms such as catatonia, tardive dyskinesia, pain perception and inflammation (Mendonça et al., 2000; Ribeiro et al., 2003; Bishnoi et al., 2006). It also modulated affective mood disorders and associated cognitive states (Kulkarni and Mehta, 1985; Kaster et al., 2004).

**Estimation of endogenous adenosine and its metabolites**

The concentration of adenosine in tissues is maintained in the nano molar range, which is determined by the influence of highly complex pathways regulating its formation, reuptake and action in the cells (Deussen et al., 2006). Adenosine kinase is one of the critical adenosine metabolizing enzymes that catalyzes rapid phosphorylation of adenosine to adenosine-5'-monophosphate. Adenosine acts as an important messenger molecule for extracellular signaling and is an inhibitory neuromodulator in several neuronal...
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disorders. Inosine and hypoxanthine are the metabolic products of extracellular adenosine deamination. In accordance to the significant role played by adenosine either as a physiological autacoid or a pharmacological tool, there has been a great interest to develop quantitative analytical methods to determine the concentration of adenosine and its metabolites in tissues, biological fluids, or even in in vitro enzymatic reactions. Several procedures based on distinct methods of high performance liquid chromatography (HPLC) or radiochemical detection have been reported for the analysis of adenosine in biological samples which are sensitive (Gamberini et al., 1992; Sottofattori et al., 2001; Farthing et al., 2007). However, most of the available techniques depend on several analytical steps and are time-consuming. Moreover, certain reported chromatographic methods possess larger run times and gradient elution methods with poor resolution (Sottofattori et al., 2001; Farthing et al., 2007). In this context, development of an efficient, simple and sensitive method for the estimation of endogenous adenosine and related purines may assist the evaluation of adenosinergic mechanism in several neuronal disorders.

Recent microdialysis studies demonstrated the release of adenosine during epileptogenesis (Berman et al., 2000) which needs to be correlated with drug action. HPLC or radiochemical detections have been used to detect adenosine in biological samples (Marin et al., 2007). However, estimation of adenosine in different brain areas in normal and under disease conditions has not been studied extensively in biological samples.

GABA is recognized as the principal inhibitory neurotransmitter distributed in the central neural system of mammals. The monitoring of GABA is an essential tool in elucidating normal and pathological neural system functions in several neurological disorders (Kulkarni, 2006). Trace level measurements in the brain are especially important in studying the role of GABA in neurophysiology, behavioral effects, pathology, disease diagnosis and control since its changes have been associated with various diseases and disorders such as epilepsy, anxiety, sedation, schizophrenia, addiction, etc. (George and Kulkarni, 1996). Reverse-phase high performance liquid
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chromatography (RP-HPLC) system with electrochemical detection (ECD) has been commonly used to determine amino acids concentration obtained from brain samples. Since GABA is electroactive, the HPLC system complemented with a derivatizing technique has been used to study this neurotransmitter (Clarke et al., 2007; Canevari et al., 1992; Donzanti and Yamamoto, 1988; Murai et al., 1989). Derivatization of amino acids has been carried out with o-phthalaldehyde (OPA) in the presence of a thiol group such as β-mercaptoethanol and tert-butylthiol which transform this amino acid into detectable forms (Boyd et al., 2000; Canevari et al., 1992; Murai et al., 1989; Donzanti and Yamamoto, 1988; Lasley et al., 1984). Despite the great sensitivity of these labeling techniques, electroactive derivatives are relatively unstable participate in auxiliary reactions in presence of excess OPA in the derivatization matrix (Clarke et al., 2007; Rowley et al., 1995; Donzanti and Yamamoto, 1988). Further adjustments of the chromatographic parameters are necessary for efficient measurement of this amino acid without interferences.

Studies correlating the adenosine levels during the ictal stages with that of other inhibitory mediators like GABA in the brain are very scarce. Immunological and binding experiments have localized the adenosine A\textsubscript{2A} receptors to the presynaptic nerve terminals of GABA in limbic and neocortical regions (Cunha and Ribeiro, 2000). However, the role of these presynaptic receptors in neuromodulatory action of adenosine has not been understood. The scanty research data relating the role of the presynaptic A\textsubscript{2A} adenosine receptors in modulating the endogenous levels of GABA is inconclusive.

**Interaction of adenosine with neurotransmitter signaling pathways**

Adenosine has been considered as an important mediator of intercellular communication in both the peripheral and central nervous systems (Boison, 2007). At the central level, adenosine has potent depressant effects on neuronal activity. These include the ability of adenosine and its metabolically stable analogues to inhibit both spontaneous firing of neurons and evoked electrical potentials in discrete brain regions.
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The adenosine-mediated control of neurotransmitter release appears to result from a balanced activation of inhibitory adenosine A₁ and facilitatory adenosine A₂A receptors. However, the behavioral studies correlating the effects of adenosine with other neuronal signaling pathways in relation to epilepsy and related disorders are scarce.

Adenosine and GABA

A number of evidences have indicated the possible involvement of adenosine in the post-synaptic hyperpolarizing action of GABA, a major inhibitory neurotransmitter in the central nervous system (Thorat and Kulkarni, 1990a). The location of adenosine A₂A receptors on the presynaptic nerve terminals of GABA has been speculated (Cunha and Ribeiro, 2000). Evidences indicate that, besides acting on potassium currents, adenosine may also affect chloride movements in hippocampal neurons (Akhondzadeh and Stone, 1994). Therefore, it may be speculated that adenosine along with GABA may mediate the inhibitory neurotransmission in the central nervous system (Mehta and Kulkarni, 1984). Any disturbance in the balance between the two transmitters may lead to pathophysiological conditions of the brain. Drugs modulating the actions of these substances will have beneficial clinical application. However, the dependence liability of GABAergic drugs following chronic consumption warrants the search for alternative pathways which may become more reliable in combination with the adenosine-related therapies.

NO signaling pathway

Nitric oxide (NO), a gaseous molecule formed enzymatically by nitric oxide synthase (NOS) from L-arginine (L-Arg), functions as a neurotransmitter/neuromodulator in the brain. Nitric oxide synthesis in the CNS, induced mainly by N-methyl-D-Aspartate (NMDA) receptor stimulation has recently been implicated in the regulation of various behavioral, cognitive and neuropsychiatric disorders (Denninger and Marletta, 1999). Nitric oxide is regarded as an important pathogenic factor involved in the mechanisms underlying seizure initiation and/or propagation. Several in vivo and in vitro studies examining the role of NO in epileptogenesis provide controversial
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evidences with either anticonvulsant (Yahyavi-Firouz-Abadi et al., 2006) or proconvulsant (Riazi et al., 2006) effect in different seizure paradigms.

Literature reports point to the relationship between L-arginine-NO-cGMP signaling pathway and adenosine-mediated transmission in the CNS. NO modulated the basal adenosine release in vivo in striatum and hippocampal slices (Fallahi et al., 1996). It is shown that NO formation is regulated by the NMDA receptors which in turn are subject to the modulation by adenosine A₁ receptors. This interplay of NO signaling pathway and adenosine could be important in the excitotoxic or stimulatory insults of the brain.

5-HT signaling pathway
The serotonergic neurotransmission has been widely recognized in the mood disorders and in the action of antidepressant drugs. Approximately 30% to 70% epileptic patients have the incidence of depressive disorders in their lifetime (Prueter and Norra, 2005). The available data suggest that antidepressants could have both proconvulsant and anticonvulsant effects and that drug dosage is the most important factor in determining the direction of their action (Pisani et al., 1999).

Evidence is accumulating for the critical involvement of serotonergic receptors in the treatment of epilepsy and related neuropsychiatric disorders. Selective serotonin reuptake inhibitors (SSRIs) are currently the most commonly prescribed antidepressants. SSRIs restore the levels of 5-HT in synaptic clefts of neurons by binding at 5-HT reuptake transporters. It is probable that drugs increasing serotonergic transmission have lower convulsant liability than other antidepressants (Pacher and Kecskemeti, 2004).

Considering that adenosine is able to modulate neuronal firing and release of 5-HT, especially through adenosine A₁ and A₂A receptors activation, it is worthwhile to explore the involvement of 5-HT system in the CNS depressant action of adenosine.
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Adenosine and epilepsy

Adenosine is one of the endogenous anticonvulsants known to suppress seizure generation and propagation with mostly inhibitory effects on neuronal activity (Thorat and Kulkarni, 1990; George and Kulkarni, 1997; Dunwiddie and Masino, 2001). Several in vitro and in vivo paradigms have reported the neuroprotective and anticonvulsant actions of adenosine and other adenosine receptor ligands (Dunwiddie and Worth, 1982; Lauro et al., 2008). Adenosine was found to reduce hypoxic stress-induced convulsions (Thorat and Kulkarni, 1990), lithium pilocarpine-induced status epilepticus in rats (George and Kulkarni, 1997) and kindled seizures in mice (Akula et al., 2007b). The anticonvulsant action of adenosine and its analogues has been reported to be mediated through membrane bound G-protein coupled adenosine A₁ and also A₂A receptors in the brain. The adenosinergic system is critically involved in regulating proliferation and hypertrophy of astrocytes leading to astrogliosis, a hallmark of epilepsy (Boison, 2007). The level of endogenous adenosine is hypothesized to increase during situations of epileptic seizures and this seizure-induced rise in adenosine was considered to be sufficient to terminate the ongoing seizure activity (Pagonopoulou et al., 2006; Akula et al., 2007a). Recent studies utilizing microdialysis technique have also shown an increase in the adenosine levels in specific regions of brain following epileptogenesis (Berman et al., 2000).

The trend in search for the new antiepileptic drugs has been in persistence since the existing anticonvulsant drugs fail to completely inhibit the occurrence of seizures. The neuromodulatory adenosine was reported to affect the release of several neurotransmitters in the central nervous system and in this context, exploring the interaction of adenosinergic receptor system with several other neuromodulator mechanisms can result in a potent anticonvulsant strategy. This has been evidenced by the reports that adenosine inhibits the release of other excitatory neurotransmitters like glutamate or acetylcholine, which are important in the generation of epilepsy (Ribeiro et al., 1996; Olney, 1985). In accordance to these experimental evidences, the involvement of adenosinergic neuromodulation need to be
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explored in purview of the modulation of serotonergic, nitric oxide and other widespread neurotransmitter pathways in brain which have become the novel targeted pathways for seizure suppression.

Role of adenosine and its receptor system(s) in the anticonvulsant action of drugs

There is promising evidence that COX-2 inhibitors may protect the brain against neurodegenerative diseases. It is expressed in discrete populations of neurons and is abundant in the cortex and hippocampus, the areas that demonstrate a prominent role in the onset of seizures (Hurley et al., 2002). Neuronal induction of COX-2 by electrical stimulus-, kainate- or pilocarpine-induced seizures was also reported (Voutsinos-Porche et al., 2004). The role of COX-2 inhibitors in attenuating the biochemical changes induced by pentylentetrazol (PTZ) and also its effect in reversing the behavioral changes produced by different animal models of epilepsy has been widely studied earlier in our laboratory (Dhir et al., 2006). Though the increased expression COX-2 gene and simultaneous enhancement of PGE2 levels were advocated during seizures, the precise mechanism underlying the seizure suppressing activity of the COX-2 inhibitors is still obscure.

Intravenous pentylentetrazol-seizure threshold as a model for anticonvulsant action

Pentylenetetrazol (PTZ)-induced convulsions is an animal model employed for screening anticonvulsant effects. PTZ infusion with a constant flow rate through the lateral tail vein in mice induces seizure response in a reliable, reproducible and rapid manner. Antiepileptic drugs can elevate seizure threshold induced by PTZ infusion. In comparison to the subcutaneous (sc) or intraperitoneal (ip) injection of a fixed dose of PTZ in laboratory animals, determination of seizure threshold by iv infusion has an advantage because threshold for clonic and tonic seizures can be separately determined in the same animals, thus providing a sensitive test for evaluation of drug effects in different seizure types in rodents (Yahyavi-Firouz-Abadi et al., 2006). Also, the test is based on threshold dose of PTZ to induce seizures in contrast to
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threshold time to induce seizures in fixed dose PTZ sc or ip paradigms. Inter-animal variability in the time to achieve threshold PTZ concentration in the brain might lead to inter-animal variability in the onset of seizures. Thus, the absorption phase detoured during iv administration results in the low inter-animal variability in the onset of different phases of seizures (Akula et al., 2007a). Hence, screening of potential antiepileptic drugs by the PTZ-seizure threshold test may provide more insight into the seizure susceptibility and different phases of seizures in individual animals.

Role of adenosine in drug addiction and withdrawal hyperactivity

Addiction to drugs such as opiates, cocaine, and alcohol imposes profound human and financial demands occurring due to tolerance and the prevailing criminal activities under the influence of such addictive agents. According to the World Health Organization (WHO), the extent of worldwide psychoactive substance use is estimated at 2 billion alcohol users, 1.3 billion smokers and 185 million drug users (WHO, 2004). In this context, the drug addiction poses a severe problem both to the government agencies and to the society. The phenomenon of drug addiction or substance dependence is a chronically relapsing disorder primarily characterized by three prominent features which include: (a) compulsion to seek and take the drug, (b) loss of control in limiting intake, and, (c) appearance of a negative emotional state (e.g., dysphoria, anxiety, irritability and craving) when the drug is withheld after a period of its continuous consumption (i.e., withdrawal syndrome). For example, physiological symptoms of alcohol withdrawal begin from 6 to 48 hours after the last drink and include tremors, elevated blood pressure, increased heart rate, and seizures. These negative emotional states including craving motivate renewed drug consumption.

Reinforcement and neuroadaptation are the two main factors which contribute to the development of addictive process. Reinforcement is a theoretical construct by which a stimulus (e.g., an unconditioned stimulus, such as the drug itself or drug withdrawal, or a conditioned stimulus, such as drug taking paraphernalia) increases the probability of a response (e.g.,
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continued use of the drug) (Roberts and Koob, 1997). Neuroadaptation refers to the processes by which initial drug effects are either enhanced (i.e., sensitization) or attenuated (i.e., counteradaptation) by repeated drug exposure. These factors appear to motivate the initial, short-term (i.e., acute) response to a drug and the establishment of the long-term (i.e., chronic) craving for the drug that characterizes addiction.

Withdrawal is one of the important phenomenons following cessation of drug intake and is associated with the onset of negative emotional states such as dysphoria, anxiety and irritability. The emergence of withdrawal is thought to induce a negative reinforcement, whereby drug taking is motivated by the desire to reduce the negative/adverse aspects of withdrawal. Functional changes that likely contribute to these behavioral changes include alteration of neurotransmitter release and changes in receptor expression. For example, studies have found during periods of withdrawal following chronic psychostimulant, opioid or alcohol administration, rats demonstrate a significant decrease in dopamine and serotonin levels in the nucleus accumbens (Diana et al., 1993; Parsons et al., 1995), features which are commonly associated with dysphoria, depression and anxiety disorders (Charney et al., 1990; Fibeger, 1991). Withdrawal has also been associated with alterations in GABA, neuropeptide Y, dynorphin, corticotrophin releasing factor and norepinephrine (Koob and Le Moal, 2008). Significant alterations are also observed in the brain's stress system and hypothalamic pituitary adrenal (HPA) axis during withdrawal (Koob and Le Moal, 2005).

Changes in receptor expression levels have also been observed in withdrawal. For instance, animals in nicotine withdrawal show down regulation of nicotinic acetylcholine receptors (Mugnaini et al., 2006) and up regulation of L-type calcium channels (Katsura and Ohkuma, 2005). Central excitotoxicity resulting from the overstimulation of glutamate NMDA receptors following drug withdrawal has long been implicated in the mechanism underlying alcohol/diazepam/opioid withdrawal syndrome (Tsuda et al., 1999; Hack and Christie, 2003; Jupp and Lawrence, 2010). This enhanced glutamate activity contributes to the decrease in seizure threshold. In addition, increased
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glutamatergic neurotransmission after withdrawal from these addictive agents has been shown to have a positive correlation with lipid hydroperoxide levels in human cerebrospinal fluid (Tsai et al., 1998; Abdel-Zaher et al., 2010). Abrupt termination of ethanol intake after the long-term consumption in experimental animals depicted the withdrawal signs that closely resemble those observed in humans (Majchrowicz, 1981). This implies that drug withdrawal, a manifestation of central glutamate over excitation is linked to underlying oxidative damage.

The oxidative load imposed by withdrawal-induced glutamate excitotoxicity accounts for redox regulation involving reactive oxygen species (ROS). ROS reacts with nitric oxide and produces reactive nitrogen species including peroxynitrite which participate in the initiation of lipid peroxidation and the formation of protein adducts, which induces cell damage (Beal, 2000; Musavi and Kakkar, 2003). An increase in the markers of lipid peroxidation and a decrease in the defense mechanism have been proposed in some of the drug withdrawal phenomenon (Musavi and Kakkar, 2003). Free radicals produced due to excessive excitatory burst can damage all cell structures, including lipids, proteins, DNA, and mitochondrial membrane structures (Cadenas and Davies, 2000). As inhibition of mitochondrial respiratory chain results in excess free radical generation, and free radicals themselves are direct inhibitors of the mitochondrial respiratory chain, which thus result in a vicious cycle that leads to oxidative cell damage (Stout et al., 1998; Cadenas and Davies, 2000). Very few studies have attempted to explore the subcellular biochemical changes following drug withdrawal in experimental conditions. Abrupt withdrawal of diazepam was shown to result in oxidative stress and at the same time reduce antioxidant defense mechanisms in rats (Musavi and Kakkar, 2003). In this context, none of the existing studies evaluated whether drug withdrawal leads to dysfunction of key mitochondrial respiratory chain enzymes and/or generates any oxidative/nitrosative stress.

The general reward circuitry of the brain centers on connections between the ventral tegmental area (VTA) and the basal forebrain (which includes the nucleus accumbens (NAc), olfactory tubercle, prefrontal cortex
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(PFC), and amygdala). The putative neurotransmitter, dopamine was proved to be an important component of this system and is implicated in the positive and negative reinforcing effects of all the drugs of abuse. Besides dopamine reward pathway, the glutamate neuronal system innervates and directly influences the mesocorticolimbic dopamine system. Glutamatergic inputs to the VTA and NAc, arising from the PFC, hippocampus and basolateral amygdale, have all been found to be implicated in addiction. In recent years, an emphasis has been laid on serotonin, and norepinephrine, along with endocannabinoids, neuropeptides, opioid system, stress-related hypothalamic-pituitary-adrenal pathways. GABAergic and cholinergic systems were also explored in recent times (Jupp and Lawrence, 2010).

The use of pharmacological agents has become a standard approach to attempt to ameliorate aspects of drug addiction and withdrawal in combination with social and behavioral treatment. Although the drugs of abuse have different initial sites of action, each addiction can result in common neuronal adaptations. In particular, chronic use of most of the major drugs of abuse were reported to causes changes in adenosine-mediated signaling pathways in several brain structures linked to the etiology of addiction. A growing body of evidence indicates that adenosine is a crucial mediator in dependence and withdrawal and that manipulation of adenosine signaling pathways may offer novel therapeutic targets for the management and treatment of withdrawal syndrome. It has also been suggested that the behavioral effects of addictive drugs like alcohol, morphine and diazepam are mediated at least partially via adenosine-dependent mechanisms (Concas et al., 1994; Tao et al., 1995; Listos, 2005).

Though the adenosine A1 receptors have a defined role in the reversal of aversive withdrawal symptoms such as anxiety and seizures, the role of adenosine A2A receptors in this phenomenon is controversial. Mounting literature data proposed the beneficial effect of adenosine A2A receptor antagonist in drug withdrawal based on the receptor knockout studies and other behavioral observations (Ledent et al., 1997; Prediger et al., 2006). Despite these some laboratories have also projected the beneficial effects of
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adenosine A<sub>2A</sub> receptor agonists in case of drug addiction and withdrawal (Kaplan et al., 1999). In this milieu, it has been imminent to elucidate the exact function of the adenosine A<sub>1</sub> and A<sub>2A</sub> receptors in the context of modulating behavioral withdrawal symptoms, and neuroprotection during the oxidative load hoarded by drug withdrawal.

The present research work has been divided primarily into three parts. The first part deals with the development and validation of an effective chromatographic method for the estimation of adenosine and related purinergic molecules in rodent brain samples. This part also includes the development and validation of an additional chromatographic method for the estimation of GABA in discrete areas of the brain, another potent inhibitory neurotransmitter. The second part of the thesis focuses on the neuroprotective effect of adenosine in intravenous PTZ-seizure threshold paradigm, an animal model which has not been extensively evaluated for drug action. Further, this PTZ-seizure threshold paradigm was used for studying the interaction of adenosine with other signaling pathways and neurotransmitter modulators in the brain. The third part of the present thesis deals with the neuroprotective effect of adenosine and adenosine receptor ligands in alcohol, opioid, and benzodiazepine withdrawal syndrome. The effects of adenosine A<sub>1</sub> and A<sub>2A</sub> receptors have been explored in several behavioral, and biochemical alterations with due emphasis to their effect on mitochondrial enzyme activities in brain samples of animals showing withdrawal response to addicting agents.