1.0. Introduction
1.0. INTRODUCTION

1.1. Histamine and the Central Nervous System

It is now well established that histamine (HA) functions as a neurotransmitter in the brain (Wada et al., 1991; Schwartz et al., 1994). There are two major sources of amine in the human brain, mast cells and histaminergic neurons. While the precise functional role of mast cell HA remains unclear, the histaminergic neuronal system is now considered a regulatory center for whole brain activity (Wada et al., 1991). Histaminergic neurons, although relatively few in numbers, constitute a long tract originating from cell bodies of the tuberomammillary nucleus of the posterior hypothalamus with widespread projections to a large number of central nervous system (CNS) areas including hypothalamus, thalamus, cerebral cortex, amygdala and septum (Schwartz et al., 1994). In addition, the branching of histaminergic projections in the brain resembles that of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) systems suggesting, as with these monoamine transmitters, a significant global central coordinating function for HA over a host of physiological and behavioral processes (Witkin and Nelson, 2004). Indeed, neuronal HA has been implicated in a variety of physiological functions in the CNS, for example, arousal, circadian rhythm, stress, nociceptive responses, motor activity, feeding, drinking, and aggression (Schwartz et al., 1991).

HA mediates its actions via different receptor subtypes. Following the Human Genome Project, the family of HA receptors has been extended to include four different G-protein coupled receptors: H₁, H₂, H₃, H₄ and current expectations for the therapeutic potential of drugs that target the H₃ and / or H₄ receptors are high. H₃ receptors are presynaptic autoreceptors controlling release and synthesis of HA. In addition to HA, they also regulate the release of a host of other central transmitters. Thus, H₃ heteroreceptors have been shown to regulate release of DA (Schlicker et al., 1993), NE (Schlicker et al., 1989), 5-HT (Schlicker et al., 1988), acetylcholine (ACh) (Blandina et al., 1996) and gamma amino butyric acid (GABA) (Meguro et al., 1995). The widespread distribution of these receptors in the CNS with an extensive modulatory role is indicative of their vast potential in varied
functions of the mammalian brain. Consequently, their selective ligands hold a lot of promise in a variety of CNS disorders. The first selective ligands for H₃ receptors were described as R-α- methyl histamine (RAMH), a selective agonist and thioperamide (THP), a selective antagonist. Indeed, the availability of specific ligands of H₃ receptors made it possible to assess the physiological role of these receptors and permitted the study of potential therapeutic applications of either stimulating or blocking the functions of these receptors. In fact, a wide variety of central effects following H₃ receptor stimulation or blockade have been described by various experimental studies. Experimental evidence from several studies indicate potential value of H₃ receptor antagonists in a variety of CNS disorders such as epilepsy (Vohora et al., 2000; Vohora et al., 2001; Vohora et al., 2004 b), vigilance deficits including attention deficit hyperactivity disorder (ADHD) (Komater et al., 2003), sleep disorders like hypersomnia or narcolepsy (Passani et al., 2004), promoting cognitive functions (Vohora et al., 2004 b; Witkin and Nelson, 2004), obesity (Esbenshade et al., 2006), neurodegenerative disorders such as Alzheimer’s disease (AD) and Parkinson’s disease (PD), drug abuse and several affective and appetite disorders (Alguacil and Perez-Garcia, 2003). A highly localized CNS distribution of H₃ receptor is likely to minimize peripheral side effects, which is an added advantage with these agents (Wada et al., 1991).

While a lot of effort has been directed in evaluating the role of H₃ receptors in above-mentioned CNS disorders, there is a paucity of data available on the neuropsychiatric disorders and on cerebral ischemia. Although no direct correlation exists between HA and these disorders, there are indirect evidences for the involvement of HA in anxiety, depression, schizophrenia and cerebral ischemia (discussed in detail below). Given the fact that no ideal “drug of choice” exists as far as these disorders are concerned, there is a need to search for novel agents / targets. The present work is an attempt to explore any such target by evaluating the effect of selective ligands of HA receptors on experimental models of anxiety, depression, schizophrenia and cerebral ischemia in rodents. Further, since oxidative stress (OS) constitutes a major predisposing factor in the etiology of some of these disorders, the study also investigates the effects of these ligands on markers of OS.
1.2. Correlation between histamine and some CNS disorders

ANXIETY
Anxiety, the most prevalent psychiatric illness in the general community, is present in 15 to 20% of medical clinic patients (Eva Bender, 2004). Increasing evidence supports a role for HA in anxiety (Bongers et al., 2004; Raber, 2005). In most of the studies, HA and stimulation of H₁ receptors or blockade of H₂ receptors increased the measures of anxiety on various tests. Similar to these observations, H₃ receptor antagonists also increased measures of anxiety in various tasks including the object recognition task in wild-type mice (Bongers et al., 2004) and in light/dark test in rodents (Yuzurihara et al., 2000). The latter was shown to be antagonized by an H₁ receptor antagonist. Recently, H₃ receptor deficient mice showed reduced measures of anxiety in elevated plus maze and zero maze (Rizk et al., 2004). These findings support a role for H₃ receptors in mediating histamine effects on measures of anxiety that needs to be investigated in other models. In contrast to above effects, however, it is also reported that people with low histamine have typical symptoms of anxiety (Jackson et al., 1998). Hence, our study aims to clarify the effect of H₃ receptor ligands on the two most extensively employed paradigms for studying anxiety in mice namely the elevated plus maze and the Vogel’s conflict test.

DEPRESSION
Depression is a debilitating disorder affecting up to 21% of the world’s population (Schechter et al., 2005). Despite the advances in the treatment of depression, there continues to be many unmet clinical needs in terms of both efficacy and adverse effects in addition to its incompletely understood pathophysiological mechanisms (Ebmeier et al., 2006). Although no direct correlation exists between histamine and depression, there are indirect evidences for the same. For example, histamine receptors are considered to be targets for the action of tricyclic antidepressants (TCAs) (Schwartz et al., 1981). Further, histamine-induced catalepsy has been proposed as a model for evaluation of antidepressant drugs (Onodera et al., 1991). In addition, a reduced histamine H₁-receptor binding has recently been reported in the brains of depressed patients (Kano et al., 2004) suggesting the involvement of histaminergic neuronal system in the pathophysiology of depression.
Recently, thioperamide (THP), a selective histamine H3-receptor antagonist, has been demonstrated to have an antidepressant effect in the forced swimming test (FST) in rats (Perez-Garcia et al., 1999). Since H3-receptors act as heteroreceptors and modulate not only histamine but also release of a variety of other neurotransmitters including NE and 5-HT, we studied the effect of selective histamine H3-receptor agonist RAMH and antagonist THP on modified FST, the latter being a method that effectively distinguishes the catecholaminergic behavior with that of 5-HT related compounds (Detke et al., 1997; Lucki, 1997; Cryan and Lucki, 2000; Cryan et al., 2002). Further, since oxidative stress is considered to be an important predisposing factor in the etiology of depressive illness (Anisman et al., 2005; Hayley et al., 2005), we measured the cerebral levels of reduced glutathione (GSH), thiobarbituric acid reactive substance (TBARS) and catalase levels in mice subjected to modified FST paradigm and following H3-receptor ligands.

SCHIZOPHRENIA

Schizophrenia afflicts approximately 1% of the population worldwide (Esbenshade et al., 2005). The last decade has seen a tremendous growth of research activity and introduction of several new generations of antipsychotic drugs. In spite of these advances, a significant number of patients are resistant to treatment and even the second-generation antipsychotics have their own set of limitations and adverse effects. Thus, there is a need to identify novel targets for the disease.

Both experimental and clinical evidence exists implicating the role of central histaminergic system in the pathogenesis of schizophrenia (Rauscher et al., 1977; Rauscher et al., 1980; Pillot et al., 2002; Ito C, 2004). For instance, Novoa and Cacabelos (Novoa et al., 2001) indicated malfunctioning of the histaminergic system with altered histamine levels and number of histamine receptors in brains of schizophrenic patients. Further, the mean levels of tele-methylhistamine, an index of histaminergic activity were found to be significantly elevated in CSF of schizophrenic patients (Prell et al., 1995). In addition to this, differential expression of histaminergic receptors has been reported in schizophrenia e.g. there is H2-up regulation (Martinez-Mir et al., 1993), while H1-down regulation (Nakai et al., 1993) in the brains of such patients. Adding to the scientific evidence is the observation
that antipsychotic drugs especially clozapine and olanzapine target histamine receptors, besides blocking dopamine receptors (Katai et al., 1993; Rodrigues et al., 1995). This indicates that dysfunctioning of histaminergic neuronal system may participate in the extradopaminergic brain dysfunction of schizophrenia and histaminergic agents may improve the refractory schizophrenia. In view of above, it becomes worthwhile to investigate thioperamide (THP), an H<sub>3</sub> receptor antagonist and (R)-α- methylhistamine (RAMH), an agonist for its effects on various behavioral models of schizophrenia viz. catalepsy, climbing behavior and locomotor activities in mice.

CEREBRAL ISCHEMIA
Cerebral ischemia (stroke) is one of the leading causes for severe neurological deficits and deaths in the developed world. Stroke is estimated to be responsible for 9.5% of all deaths. Among the estimated 700,000 people with stroke in the United States each year, 200,000 of them are among persons with a recurrent stroke (Sacco et al., 2006). It is the most common neurological reason for hospitalization. It most often results from a transient or permanent occlusion of the middle cerebral artery (MCA). Although the pathophysiological mechanisms of cerebral ischemia and reperfusion injury remains unclear, several processes such as intracellular calcium accumulation, massive release of excitatory amino acids (EAAs), free radical formation, inhibition of protein synthesis etc. have been implicated (Ghoneim et al., 2002; Chaudhary et al., 2003; Salim et al., 2003; Meenakshisundaram et al., 2004) Several components of reactive oxygen species (ROS) have been found to be generated after ischemia reperfusion injury and play an important role in neuronal loss after cerebral ischemia (Meenakshisundaram et al., 2004; Adachi et al., 2005). However, in spite of drugs developed using these approaches, until now, there is no effective neuroprotective therapy for the treatment of cerebral ischemia.

There is evidence that histamine release from nerve endings is enhanced in ischemia and that this could contribute to neuroprotection against ischemic damage (Adachi et al., 2001; Adachi 2002). The brain content of histamine has been reported to increase during cerebral ischemia in primates (Adachi et al., 1991). It was also reported that neuronal histamine release was increased in striatum in MCA occluded rats (Adachi et al., 1992). On the other
hand, impairment of histaminergic transmission or suppression of histaminergic activity may aggravate ischemic neuronal damage (Sugimoto et al., 1994; Adachi et al., 2001; Adachi 2002). Since thioperamide (THP), a potent H₃-receptor antagonist, is known to increase the extracellular concentration of histamine in the rat hypothalamus (Arrang et al., 1983) and is readily transported to the brain across the blood brain barrier, it is likely that it may protect the brain against ischemia. The present study attempts to evaluate any such potential of THP in MCA occlusion (MCAO) induced focal cerebral ischemia in rats. Further, the study also evaluated the effect of MCA occlusion and thioperamide on locomotor activity, grip strength and spontaneous alternation behavior of rats.

Accumulating evidence exists for the involvement of nitric oxide (NO) in ischemic brain damage. This has been due to the fact that glutamate is an important mediator of ischemic brain injury (Hunter et al., 1995; Nakashima et al., 1999) and that the activation of NMDA receptors is known to generate NO in a Ca²⁺-dependent manner (Nakashima et al., 1999). In view of these findings, the present study also involved estimation of nitrate and nitrite levels following MCAO and THP pretreatment.
1.3. AIMS AND OBJECTIVES

In view of above, the present study was planned with the following aims and objectives:

- To investigate some selective histamine H₃ receptor ligands (THP and RAMH) in rodent models for anxiety, depression, schizophrenia and cerebral ischemia.

- To probe the involvement of oxidative stress in some of these disorders (depression and cerebral ischemia) and in the action of selective H₃ receptor ligands.
2.0. Literature Review
2.0. LITERATURE REVIEW

2.1. HISTAMINE AND CNS

2.1.1. Introduction

The history of histamine (β-aminoethylimidazole) parallels that of acetylcholine (ACh). Both compounds were synthesized as chemical curiosities before their biological significance was recognized. Both were detected as uterine stimulants in extracts of ergot, from which they were subsequently isolated; and both proved to be contaminants of ergot that resulted from bacterial action. When Dale and Laidlaw (1910) subjected histamine to intensive pharmacological study, they discovered that it stimulated a host of smooth muscles and had an intense vasodepressor action. It was not until 1927 that Best, Dale, Dudley, and Thorpe isolated histamine from impeccably fresh samples of liver and lungs, thereby establishing that this amine is a natural constituent of the body. Demonstration of its presence in variety of other tissues soon followed—hence the name *histamine* after the Greek word for tissue, *histos.*

Histamine is formed from the amino acid histidine by histidine decarboxylase.

\[
\text{Histidine} \xrightarrow{\text{Decarboxylase}} \text{Histamine}
\]

It is metabolized to N-methylhistamine by an N-methyl-transferase or to imidazole acetic acid by diamine oxidase. The N-methylhistamine can be further metabolized to N-methylimidazole acetic acid by monoamine oxidase as well.
Histamine is produced by and released from mast cells of the peritoneal cavity and connective tissues. Anaphylaxis and allergic responses due to antibody/antigen interactions are two important responses mediated in part by histamine. Major effects of histamine include arteriolar dilation and increased capillary permeability, a decrease in heart rate (H₂), bronchoconstriction (H₁), and gastric acid secretion (H₂).

2.1.2. Histaminergic neuronal system
Garbarg and co-workers (Garbarg et al., 1974) demonstrated for the first time, the existence of histaminergic neurons in the mammalian brain. The histaminergic neuronal system is known to be a regulatory center for whole brain activity (Wada et al., 1991) and its role as neurotransmitter in the brain has been established beyond doubts (Schwartz et al., 1991).
History

1983 The H₃ receptor is pharmacologically identified (Arrang et al., 1983).

1999 H₃ receptor cloned (Lovenberg et al., 1999).

2002 H₃ knock-out mice identified (mH₃⁻/⁻ mice) (mice that do not have this receptor) (Toyota et al., 2002).

Histamine-releasing neurons are located exclusively in the tuberomammillary (TM) nucleus of the hypothalamus, from where they project to practically all brain regions, with ventral areas (hypothalamus, basal forebrain, amygdala) receiving a particularly strong innervation (Schwartz et al., 1991). The intrinsic electrophysiological properties of TM neurons (slow spontaneous firing, broad action potentials, deep after hyperpolarizations, etc.) are extremely similar to other aminergic neurons. Their firing rate varies across the sleep-wake cycle, being highest during waking and lowest during rapid-eye movement sleep. Histamine release is enhanced under extreme conditions such as dehydration or hypoglycemia or by a variety of stressors.

Histamine exists in at least two cellular stores in the brain i.e. in neurons and in mast cells. Neuronal histamine turns over rapidly, with a half-life of less than 1 hr, while mast cell histamine turns over very slowly, with a half-life of several days. Because of the rapid turnover of neuronal histamine, α-fluoromethylhistidine (α-FMH), an irreversible inhibitor of L-histidine decarboxylase depletes neurons of histamine within several hours (Motoki et al., 2005). Histamine, whether released by neurons or mast cells, produces its effects in tissues by interacting with G-protein-coupled receptors. To date, 4 separate histamine receptors have been cloned and characterized (details are given below). The central histamine system is involved in many central nervous system functions including arousal, anxiety, activation of the sympathetic nervous system, stress-related release of hormones from the pituitary and of central aminergic neurotransmitters, antinociception, water retention and suppression of eating (Brown et al., 2001).
2.1.3. Histamine receptors

Following the Human Genome Project, the family of histamine receptors has been extended to include four different G-protein-coupled receptors (GPCRs): the H₁, H₂, H₃ and H₄ receptors, and current expectations for the therapeutic potential of drugs that target the H₃ and/or H₄ receptor are high. Histamine activates four types of receptors. H₁ receptors are mainly postsynaptically located and are coupled positively to phospholipase C. High densities are found especially in the hypothalamus and other limbic regions. Activation of these receptors causes large depolarizations via blockade of a leak potassium conductance, activation of a non-specific cation channel or activation of a sodium-calcium exchanger. H₂ receptors are also mainly postsynaptically located and are coupled positively to adenylyl cyclase. High densities are found in hippocampus, amygdala and basal ganglia. Activation of these receptors also leads to mainly excitatory effects through blockade of calcium-dependent potassium channels and modulation of the hyperpolarization-activated cation channel. H₃ receptors are exclusively presynaptically located and are negatively coupled to adenylyl cyclase. High densities are found in the basal ganglia. These receptors mediate presynaptic inhibition of histamine release and the release of other neurotransmitters, most likely via inhibition of presynaptic calcium channels. Finally, histamine modulates the glutamate NMDA receptor via an action at the polyamine-binding site. Since the cloning of the histamine H₃ receptor cDNA in 1999 by Lovenberg and co-workers, this histamine receptor has gained the interest of many pharmaceutical companies. Many potent and relatively selective H₃ receptor agonists and inverse agonists have now been developed. For H₃ receptor agonists and H₃ receptor inverse agonists/antagonists, interesting activities in several preclinical models of important human diseases, including obesity, migraine, attention-deficit hyperactivity disorder, Alzheimer's disease, schizophrenia, as well as for myocardial ischaemia, and inflammatory diseases, have been reported (Leurs et al., 2005). Histamine H₄ receptors were discovered by Nakamura et al (2000) and have a role in inflammatory conditions. Table 1 depicts the histamine receptor subtypes along with their post receptor mechanisms. Some of the selective agonists and antagonists of these receptors are given in Tables 2 and 3 respectively.
### Table 1. Histamine receptor subtypes.

<table>
<thead>
<tr>
<th>Receptor Subtype</th>
<th>Distribution</th>
<th>Post receptor mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₁</td>
<td>Smooth muscles, endothelium, brain</td>
<td>G protein coupled receptors; ↑ IP₃, DAG</td>
</tr>
<tr>
<td>H₂</td>
<td>Gastric mucosa, cardiac muscle, mast cells, brain</td>
<td>G protein coupled receptors; ↑ cAMP; adenylyl cyclase activation</td>
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<tr>
<td>H₃</td>
<td>Presynaptic: brain, myenteric plexus, other neurons</td>
<td>G protein coupled receptors; ↓ cAMP; ↓ Ca²⁺ influx;</td>
</tr>
<tr>
<td>H₄</td>
<td>Leukocytes, bone marrow, colon, liver, lung, small intestine, spleen, testis, thymus, tonsil, and trachea.</td>
<td>G protein coupled receptors; post receptor mechanism not known.</td>
</tr>
</tbody>
</table>

![Structures of some of the selective histamine receptor agonists](image-url)
Table 2. Histamine receptor agonists

<table>
<thead>
<tr>
<th>Effector</th>
<th>$H_1$</th>
<th>$H_2$</th>
<th>$H_3$</th>
<th>$H_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist</td>
<td>2-Methylhistamine</td>
<td>4- Methylhistamine</td>
<td>R- (α)-methyl histamine (RAMH) (Arrang et al., 1987)</td>
<td>4- Methylhistamine (Lim et al., 2005), OUP16 (Hashimoto et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>2-Pyridylethylamine</td>
<td>Dimaprit</td>
<td>S- (α)-methyl histamine (RAMH) (Arrang et al., 1987)</td>
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<tr>
<td></td>
<td>2-Thiozolethylamine</td>
<td>Impromidine</td>
<td>Imetit (Garbarg et al., 1992)</td>
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<td></td>
<td></td>
<td></td>
<td>Immezipip (Vollinga et al., 1994)</td>
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<td></td>
<td></td>
<td></td>
<td>BP 294* (Krause et al., 1995)</td>
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<td></td>
<td></td>
<td></td>
<td>FUB 307* (Krause et al., 1995)</td>
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<td></td>
<td></td>
<td></td>
<td>Immethridine (Kitbunnadaj et al., 2004)</td>
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<td></td>
<td></td>
<td></td>
<td>Methimmepip (Kitbunnadaj et al., 2005)</td>
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<td></td>
<td></td>
<td></td>
<td>VUF 5657 (Kitbunnadaj et al., 2005)</td>
<td></td>
</tr>
</tbody>
</table>

*; Pro - drug of RAMH; +; partial agonists; #; $H_2$-agonists; ‖; partial $H_1$-agonist; @; irreversible antagonist
<table>
<thead>
<tr>
<th>Effector</th>
<th>Antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₁</td>
<td>Astemizole</td>
</tr>
<tr>
<td></td>
<td>Cetirizine</td>
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<tr>
<td></td>
<td>Chlorpheniramine</td>
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<tr>
<td></td>
<td>Cyphephedrine</td>
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<td></td>
<td>Cyclizine</td>
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<td></td>
<td>Diphenhydramine</td>
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<td></td>
<td>Dimenhydrinate</td>
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<td></td>
<td>Loratadine</td>
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<td></td>
<td>Mepyramine</td>
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<td></td>
<td>Promethazine</td>
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<td></td>
<td>Pyrilamine</td>
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</table>

| H₂      | Cimetidine  |
|         | Famotidine  |
|         | Loxatidine  |
|         | Nizatidine  |
|         | Ranitidine  |
|         | Roxatidine  |

| H₃      | Thiopentamide (THP) (MR-12842) |
|         | (Arrang et al., 1987)           |
|         | Clopenopropit (VUF-91153)       |
|         | (Verderi et al., 2003)          |
|         | Ciprofloxin (Lignaeau et al., 1998) |
|         | Idropropil (Schilteck et al., 1996) |
|         | FUB 181 (Onodera et al., 1998)  |
|         | Hill et al., 1997               |
|         | Carbopropamide (Schwartz et al., 1990) |
|         | Impromidine (Schwartz et al., 1990) |
|         | Apromidine (Schwartz et al., 1990) |
|         | Burinamide (Hill et al., 1997)  |
|         | Impentamine (Hill et al., 1997)  |
|         | Bethamine (Alvarez et al., 1993) |

| H₄      | JNJ 7777120, JNJ 10191584 |
|         | (Jablonskawski et al., 2004) |

**Table 3. Histamine receptor antagonists**
<table>
<thead>
<tr>
<th></th>
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<th>GT-2016 (Onodera et al., 1998)</th>
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<tr>
<td></td>
<td></td>
<td>GR-175737 (Leurs et al., 1998)</td>
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<td></td>
<td></td>
<td>UCL-1199 (Leurs et al., 1998)</td>
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<td></td>
<td></td>
<td>GT-2227 (Leurs et al., 1998)</td>
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<tr>
<td></td>
<td></td>
<td>AQ-0145 (Murkami et al., 1995)</td>
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<tr>
<td></td>
<td></td>
<td>EEDQ® (Taylor et al., 1992; Detzner et al., 1994)</td>
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<td></td>
<td></td>
<td>GT-2331 (Fox et al., 2002)</td>
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<tr>
<td></td>
<td></td>
<td>JNJ 6379490 (Shah et al., 2002)</td>
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<tr>
<td></td>
<td></td>
<td>A-349821 (Faghih et al., 2003)</td>
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<td></td>
<td></td>
<td>A-304121 (Fox et al., 2003)</td>
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<tr>
<td></td>
<td></td>
<td>A-317920 (Fox et al., 2003)</td>
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<td></td>
<td></td>
<td>ABT-239 (Esbenshade et al., 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A-331440 (Hancock et al., 2005)</td>
</tr>
</tbody>
</table>

*; Pro - drug of RAMH; +; partial agonists; #; H₂-agonists; †; partial H₁-agonist; @; irreversible antagonist
2.1.4. Clinical potential of histamine receptor antagonists

Histamine is a biogenic amine that plays an important role in inducing anaphylactic responses, such as bronchospasm, an increase in vascular permeability, and hypotensive shock in peripheral organs in various mammalian species. These actions are mediated by histamine H₁ receptor stimulation. Some typical H₁ antagonists include diphenhydramine, chlorpheniramine and tripelennamine.

H₁ antagonists are used clinically in allergic disorders, prurites, common cold, motion sickness, vertigo, preanaesthetic medications, cough, Parkinsonism, acute muscle dystonia, as sedative, hypnotic and anxiolytic.
Literature Review

Histamine stimulates muscle contractions in the atrium and the uterus, as well as gastric acid secretion in the gut. These responses are mediated through H₂ receptors. H₂ antagonists are a widely used class of drugs in peptic ulcer.

H₃ antagonists have been developed and are under clinical trial for a variety of CNS disorders and at present no drug is available for therapeutic use. Thioperamide is a prototypical H₃ antagonist that stimulates the release and synthesis of histamine and reverses the action of R-α-methylhistamine at the autoreceptor.
2.1.5. Histamine $H_3$ receptors

$H_3$ receptors are expressed in the central nervous system and, to a lesser extent, the peripheral nervous system, where they act as autoreceptors in presynaptic histaminergic neurons, and control histamine turnover by feedback inhibition of histamine synthesis and release as well. The histamine $H_3$ receptors also been shown to presynaptically inhibit the release of a number of other neurotransmitters (i.e. it acts as an inhibitory heteroreceptor) including dopamine, GABA, acetylcholine, noradrenaline, and serotonin.

A. Location

- Central nervous system (mainly in the hypothalamus, thalamus, cerebral cortex, amygdala and septum)
- Peripheral nerves
- Heart
- Lungs
- Gastro intestinal tract
- Endothelial cells

B. Histamine $H_3$ receptors gene and $H_3$ receptor Isoforms

The cloning of the human histamine $H_3$ receptor demonstrated that the receptor is a 445 amino acid G-protein coupled receptor expressed almost exclusively in the brain (Lovenberg et al., 1999). Cloning of $H_3$ receptors has been achieved in rats, guinea pig, mouse, rhesus monkey also (Witkin and Nelson, 2004). The $H_3$ receptor is coupled to the $G_i$ G-protein so it leads to inhibition of the formation of cAMP. Also, the $\beta$ and $\gamma$ subunits interact with N-type voltage gated calcium channels, to reduce action potential mediated influx of calcium and hence reduce neurotransmitter release. Soon after the cloning of the $H_3$ receptor gene, several $H_3$ receptor isoforms have been identified including various functional and non-functional isoforms. There are at least six $H_3$ receptor isoforms in the human and up to 12 discovered so far In rats, there has been six $H_3$ receptor subtypes reported so far. Mice also have three reported isoforms (Bakker et al., 2004).
subtypes all have subtle difference in their pharmacology (and presumably distribution, based on studies in rats) but the exact physiological role of these isoforms is still unclear.

The gene sequence for H3 receptors expresses only about 30% homology with both H1 and H2 receptors. The diverse expression of H3 receptors throughout the cortex and subcortex indicates its ability to modulate the release of a large number of neurotransmitters. Because of this, H3 receptor ligands are being investigated for the treatment of numerous neurological conditions, including obesity (because of the histamine/orexinergic system interaction), movement disorders (because of H3 receptor-modulation of dopamine and GABA in the basal ganglia), schizophrenia and ADHD (again because of dopamine modulation) and research is even underway as to whether H3 receptor ligands could be useful in modulating wakefulness (because of effects on noradrenaline, glutamate and histamine).

C. Selective ligands

The first selective ligands for H3 receptors, as previously mentioned, were described by Arrang et al (Arrang et al., 1987). R- (α)-methyl histamine, a chiral agonist and thioperamide, an antagonist derived from imidazolyl piperidine displayed high selectivity and potency at nanomolar concentration in vitro. It was shown that both (R) and (S) α-methyl histamine behaved as full H3 receptor agonists, but the (R) isomer was approximately 100 times more potent than the (S) isomer and 15 times as potent as histamine itself. Its selectivity towards H3 receptors was also high, being 1000 times as high as histamine. Similarly, thioperamide was found to be a potent H3 receptor antagonist exhibiting negligible affinity for H1 and H2 receptors as well as for other receptors such as α or β noradrenergic, muscarinic, opiate, dopamine or serotonin indicating its high selectivity (Arrang et al., 1987). The availability of the potent and selective agents at H3 receptors thus made it possible to assess the physiological role of these receptors in the control of HA synthesis and release in the brain and in the peripheral organ of animals. Imetit is another H3 receptor agonist and is more potent than RAMH (Garbarg et al., 1992). Azomethine derivatives of RAMH were prepared as lipophilic prodrugs to improve the
bioavailability of the hydrophilic drug, particularly its entry into the brain (Kimball et al., 2004; 2005). Among the H3 receptor antagonists, clobenprat developed by Vander Goot et al. (1992) is approximately 10 times and 6 times more potent than thioperamide in its affinity for H3 receptors and in increasing HA release, respectively (Miyazaki et al., 1997). Table 2 and 3 depicts the selective agonists and antagonist of histamine H3 receptors.

D. Physiological actions

**Autoreceptor vs heteroreceptor function**

A large body of experimental data confirms the autoreceptor function of H3 receptors in inhibiting HA release both *in vitro* and *in vivo*. Thus, H3 receptor antagonists enhance while the agonists inhibit neuronal HA release and synthesis (Arrang et al., 1987; Garbarg et al., 1989; Oishi et al., 1989; Mochizuki et al., 1991). Presynaptic H3 receptors occur both on histaminergic neurons of the CNS (autoreceptors) and on non-histaminergic neurons of the central and autonomic nervous system (Schlicker et al., 1996). They regulate not only the release of HA but also of the other transmitters such as NE (Schlicker et al., 1989), DA (Schlicker et al., 1993), 5-HT (Schlicker et al., 1988), ACh (Blandina et al., 1996) and GABA (Meguro et al., 1995). Thioperamide and other H3 receptor antagonists have been demonstrated to enhance while HA and RAMH have been shown to reduce the release of these transmitters via H3 receptor *in vitro* studies. Such effects of H3 heteroreceptors have been most thoroughly investigated in the mouse brain cortex where they cause inhibition of NE release and in the guinea pig small intestine causing inhibition of ACh release. Contrary to these observations, a study demonstrated no inhibition of ACh release from rat entorhinal cortex synaptosomes following H3 receptor activation. Although auto radiographic studies have shown that the presence of H3 receptors is not restricted to histaminergic neurons, the modulation of 5-HT and catecholaminergic tone was not confirmed by *in vivo* studies. H3 receptors were shown to have no important role in the regulation of monoaminergic activity in contrast with their regulatory functions in modulating histaminergic activity in the rat brain. Thus, it seems likely that H3 receptor
mediated control of histamine release is a major regulatory mechanism for most of its physiological actions (Vohora et al., 2004 b).

Peripheral actions

An inhibitory effect of $H_3$ receptor stimulation on peripheral neurotransmission is also well documented. Thus, $H_3$ receptors have been identified regulating the adrenergic neurotransmission in the guinea pig mesentric artery (Ishikawa et al., 1987), guinea pig atria (West et al., 1994) and human heart (Malinowska et al., 1998), cholinergic neurotransmission in the guinea pig ileum (Schlicker et al., 1992) and human airways (Ichinose et al., 1989) and nicotinic responses in the guinea pig enteric ganglia (Tamura et al., 1988). In addition, $H_3$ receptor stimulation has been shown to inhibit the release of neurotransmitters from non-adrenergic noncholinergic nerves in the guinea pig bronchioles and ileum (Taylor et al., 1992; Hill et al., 1997). Other responses to $H_3$ receptor activation include inhibition of gastric acid secretion in conscious dogs (Soldani et al., 1993) and relaxation of rabbit middle cerebral artery via an endothelium dependent mechanism (Hill et al., 1997).

Central actions

A wide range of central effects following $H_3$ receptor stimulation or blockade has been described by various experimental studies. Consequently, selective ligands for $H_3$ receptor find therapeutic indications in a variety of CNS disorders such as epilepsy, sleep disorders, Alzheimer's disease, attention deficit hyperactivity disorders (ADHD), obesity, depression etc. The details of these are dealt in next section.

E. Therapeutic potential

$H_3$ receptor activation has been shown to inhibit gastric acid secretion induced by food and pentagastrin in cats, dogs and rabbits but not in rats. RAMH, however, exhibited a remarkable gastro-protective effect in these species (Morini et al., 2000). It was found to reduce substantially the severity of macroscopically and histologically assessed damage to
gastric mucosa caused by concentrated acid. Further, the protection was shown to be reversed by selective blockers of H₃ receptors such as ciproxifen and clobenpropit (Morini et al., 2000). The beneficial effects of H₃ receptor agonists in neurogenic inflammation and in migraine are also suggested (Leurs et al., 1998). In the CVS, using isolated guinea pig hearts, it was shown that H₃ receptor agonists inhibit NE release in early myocardial ischemia. In addition, reperfusion-induced arrhythmias are shown to be inhibited by H₃ receptor stimulation (Leurs et al., 1998). H₃ receptor blockade, on the other hand, has been demonstrated to improve cardiac function in canine anaphylaxis. Recently, it was suggested that H₃ receptor activation, by inhibiting adrenergic NE release contribute to cardiovascular collapse in anaphylactic shock, which can be reversed by H₃ receptor blockers (Chrusch et al., 1999). Selective H₃ receptor agonists were suggested to represent a novel therapeutic frontier in myocardial ischemia (Levi et al., 2000). McLeod et al (1999) suggested that the decongestant activity of combined H₁/H₃ blockade to be a novel approach for the treatment of allergic nasal congestion without the hypertensive liability of current therapies. However, the data available for peripheral actions are scanty at present and more investigations are needed to confirm such effects.

**Epilepsy**

Histamine is believed to be an anticonvulsant inhibitory neurotransmitter. The first early indication of the role of histamine in seizures was suggested by the fact that several brain penetrating H₁ receptor antagonists occasionally produce convulsions in epileptic patients and healthy young children. Further evidence of the involvement of HA was derived from animal studies in which drugs that deplete brain HA (e.g. α-FMH and brocresin) were found to potentiate convulsions in experimental animals. Conversely, drugs that enhance brain HA (e.g. metoprine and L-histidine) and HA itself exhibited potent anticonvulsant effects (Vohora et al., 2004 a). Recently, an increased density of H₁ receptors in the brains of rat with absence epilepsy was reported (Midzyanovskaya et al., 2003). H₁ receptor knockout and histidine decarboxylase-deficient pentylentetrazole-kindled mice had accelerated seizure development (Chen et al., 2003).
Blockade of H₃ receptors is an important means for enhancing neuronal HA levels in the brain. Thus, thioperamide and clobenpropit have shown to have a protective action against various convulsive models such as MES (Yokoyama et al., 1993; Yokoyama et al., 1994), PTZ (Vohora et al., 2000) and amygdaloid kindling (Kakinoki et al., 1998) in rodents. The protective effects correlated well within the increase in histidine decarboxylase activity in brain. The protection afforded by H₃ receptor antagonists is further shown by various workers, to be reversed on pre-treatment with either H₃ receptor agonists or by H₁ receptor antagonists but not by H₂ receptor antagonists (Yokoyama et al., 1993; Yokoyama et al., 1994; Kakinoki et al., 1998; Vohora et al., 2000). VUF 9153 given icv was shown to delay the progression of seizure kindling induced by PTZ in rats. This effect was prevented by the H₁ receptor agonist immepip and by the histidine decarboxylase inhibitor α-FMS (Zhang et al., 2003). H₃ receptor antagonists were effectively combined with low doses of antiepileptic drugs (AEDs) to produce additive and synergistic effects on efficacy with reduced toxicity (Vohora et al., 2001). The anticonvulsant effects were believed to be mediated by the interaction of released HA from histaminergic nerve terminals with histamine H₁ receptors on the postsynaptic neurons. Their potential in epilepsy is exciting as the arousal and improved cognitive effects reported with H₃ receptor antagonists differentiate them from other AEDs, which can cause sedative, cognitive and vigilance problems, thereby greatly compromising the quality of life (Vohora et al., 2004 b).

Sleep and wakefulness

Narcolepsy is a disorder in which electrophysiological and behavioral sleep occurs during normal waking hours. Modafmil, due to its indirect activation of histaminergic system, was shown to increase extracellular levels of histamine in hypothalamus of anaesthetized rats (Ishizuka et al., 2003). This provide validation that H₃ receptor antagonists will promote states of wakefulness in humans. Pharmacological studies using H₁ receptor agonists and histamine synthesis inhibitors (exhibiting an enhanced and reduced wakefulness respectively), and the well-known sedative effect of antihistaminics observed clinically support the hypothesis that HA may be a “waking amine”. The critical role of histaminergic neurons in arousal mechanisms is consistent with their anatomic disposition.
Literature Review

HA containing cell bodies are present in the tuberomammillary nucleus of the posterior hypothalamus, an area involved in the maintenance of wakefulness. Furthermore, both neurochemical and electrophysiological studies indicate that the activity of histaminergic neurons is maximum during periods of wakefulness. $H_3$ receptor antagonist ciproxifan and thioperamide enhanced wakefulness in cats and rats while RAMH, a selective agonist, reduced it (Ligneau et al., 1998; Vohora et al., 2001). Furthermore, ciproxifan reportedly increased the discharge rate of ‘waking selective neurons’ in the posterior hypothalamus in freely moving cat, an effect reversed by histamine $H_3$ receptor agonists such as imetit and RAMH (Vanni-Mercier et al., 2003), providing evidence for the histaminergic nature of ‘waking selective neurons’ and their role in cortical desynchronization during waking. $H_3$ receptor stimulation or blockade may become a novel approach to the treatment of sleep disorders such as hypersomnia or narcolepsy and may promote wakefulness in vigilance deficits (Vohora et al., 2004 b).

Attention deficit hyperactivity disorders (ADHD)

$H_3$ receptor antagonists are suggested to have beneficial effects in attention deficit hyperactivity disorders (ADHD) as they not only improve vigilance and cognitive deficits but also normalize motor disturbances. GT-2016 (Gilatech Inc) was studied on an immature rat model (cognitive impairment and motor patterns similar to those observed in ADHD) and improved acquisition in passive avoidance task. Recently, THP was reported to enhance performance in a five-trial inhibitory avoidance response without any effect on locomotor activity Thus, $H_3$ receptor blockade may provide a safe alternative to psychomotor stimulant for the treatment of ADHD (Vohora et al., 2004 b). Esbenshade et al (2005) have reported that ABT-239 (selective potent $H_3$ receptor antagonist) has high affinity and selectivity for human $H_3$ receptors. Fox et al (2005) reported the activity of ABT-239 in various rodent models and found to be suitable for ADHD.

Obesity

A role for endogenous histamine in the control of food intake was suggested by the observation that $H_3$ receptor antagonists when infused into the ventromedial hypothalamus
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(VMH) induced feeding in rats. ICV administration of HA, on other hand, depresses feeding (Vohora et al., 2004 b). Recently dysfunction in HA-mediated neuro-transmission has been identified in the obese Zucker rat, a genetic model for obesity. H₃ receptors were identified with moderate density in the VMH, an area responsible for controlling food intake by histaminergic neurons and administration of thioperamide is demonstrated to suppress food intake (Itoh et al., 1998). H₃ receptor antagonists/inverse agonists are currently under investigation for the control of feeding, appetite and body mass and may have clinical utility as anti-obesity agents (Hencock AA, 2003). Recent work has shown that a novel H₃ receptor antagonist A-331440 reduced body weight comparably with dexfenfluramine in mice, reduced body fat and produced a normalization in the insulin tolerance test (Hencock et al., 2004). More recently histamine H₃ receptor antagonists are going under preclinical trials for treating obesity and cognitive disorders (Esbenshade et al., 2006; Ito et al., 2006).

Stress and Depression

A role for histamine in response to stress has been suggested. For example, amitriptyline partially prevented the increase in plasma corticosterone and prevented the decrease in [³H] histamine binding sites engendered by foot shock in rats (Ghi et al., 1995 a, b). The release of histamine in prefrontal cortex elicited by handling was antagonized by intracortical administration of RAMH and potentiated by THP (Westernik et al., 2002). Effects of other stress hormones (ACTH and prolactin) were also dampened by administration of H₃ receptor agonists, an effect prevented by THP (Knigge et al., 1999). The fact that brain histamine levels are increased under some conditions of stress and that H₃ receptor antagonists augment brain histamine levels and enhance performance in a host of models of cognition are congruent with the data that a certain level of stress or arousal is a positive modulator of behavioral performance. However, histamine is augmented in brain with stress, and the benzodiazepine anxiolytics and the anxiolytic 5-HT₁A receptor agonist buspirone decrease histamine turnover in rodents (Oishi et al., 1986, 1992; Chikai et al., 1993), suggesting that increases in central histamine with H₃ receptor antagonists could push the system too far and exacerbate existing anxiety.
Preliminary reports on the effect of \( H_1 \) receptor antagonists on the forced swimming test suggest that they have antidepressant effects (Perez-Garcia et al., 1999; Akhtar et al., 2005). They were, however, found to be devoid of anxiolytic effects (Perez-Garcia et al., 1999). In this context, it is interesting to note that these types of compounds inhibit brain monoamine oxidase enzyme in various mammalian species (Sakurai et al., 2001).

**Schizophrenia**

Please see section 2.2.3.1. for details.

**Pain**

There is also some evidence to suggest a role for \( H_3 \) receptors in pain perception. \( H_3 \) receptor antagonists have been reported to elicit antinociceptive effects (Malmberg-Aiello et al., 1994, 1997). In this context, a role for histamine in pain elicited by mechanical, chemical and thermal stimuli was demonstrated in mice and rats. THP produced small but significant antinociceptive effects by both parenteral and icv routes. Further, the selective \( H_3 \) receptor agonist RAMH was hyperalgesic and also prevented the effects of THP. Additional studies with the \( H_3 \) receptor antagonist impromidine and burimamide showed limited antinociceptive efficacy that was prevented by RAMH (Lamberti et al., 1996).

**Cognitive functions**

Both experimental and clinical evidence indicate that the central histaminergic system plays an important role in learning and memory. While icv administration of histamine and \( H_1 \) receptor agonists are reported to facilitate various learning and memory task in animal models, histamine depletors such as \( \alpha \)-FMH and \( H_1 \) blockers impair them. In addition, the possibility of the use of histamine receptor ligands in Alzheimer’s disease (AD) was suggested, as the histamine levels in the hypothalamus, hippocampus and temporal cortex were significantly lower in the brains from such patients. Recently, \( H_3 \) receptor antagonist such as thioperamide, clobenpropit and GT-2016 as part of programme to develop these compounds by Glaxo Wellcome, UK and Gliatech, USA, were subjected to a battery of
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to a battery of preclinical tests and were found to be effective in the treatment of cognitive deficit disorders. A number of experimental studies are also available indicating the pro-cognitive effects of $H_3$ receptor antagonists. Thioperamide, the prototype of these compounds, has been demonstrated to improve learning and memory in various experimental models in rodents including step-through passive avoidance response, elevated plus maze, social memory tests, the senescence-accelerated model of mice, open field test, object recognition task and foot shock avoidance in T-maze. Another $H_3$ receptor antagonist ciproxifan was reported to improve cognitive functions in a five-choice reaction task in rodents and on repeated acquisition avoidance response in spontaneously hypertensive rat (SHR) pups.

Although HA might affect cognition on its own, neurochemical studies suggest that HA also modulates the activity of cholinergic neurons (Vohora et al., 2004 b). The pro-cognitive effects of THP may be due to an action on the cholinergic basal forebrain (Orsetti et al., 2002). In addition to THP, clobenpropit and ciproxifan inhibited ACh release in basolateral amygdala (Passani et al., 2001) the latter being an important nucleus in learning and memory processes. The interaction of histamine with $H_3$ receptors was also reported to inhibit the cholinergic tone in frontal cortex and hippocampus (Passani et al., 1998). Interestingly, tacrine, an acetylcholinesterase inhibitor was found to inhibit histamine N-methyltransferase activity (Morisset et al., 1996) and also have synergistic effect with THP on a spatial memory task in mice (Vohora et al., 2005).

Miscellaneous

(i). Recently it was shown that the histaminergic signaling system exerted a neuroprotective role against neurodegenerative-induced processes in the hamster. The major protective role against neurodegenerative events appears to be strongly related to the expression activity of $H_1, 3$ R subtypes of amygdalar neurons (Canonaco et al., 2005).

(ii). The effectiveness of histamine-related drugs in the treatment of peripheral and central vestibular disorders was reported due to their action on the vestibular nuclei, it has also
such as thioperamide, clobenpropit, and betahistine decreased the electrical discharge of afferent neurons by interfering with the postsynaptic response to excitatory amino acid agonists. The antivertigo action of histamine-related drugs may be caused, at least in part, by a decrease in the sensory input from the vestibular endorgans (Chavez et al., 2005).

(iii). Reactive hyperemia (RH) is an abrupt blood flow increase following release from mechanical occlusion of an artery, with restoration of intra-arterial pressure. Evidence exists that histamine is an important endogenous mediator of various functions of the gut, including blood flow. The vascular effects of histamine in the intestinal circulation are due to its agonistic action on histamine H1, H2 and H3 receptors. It was shown that histamine H3 receptors do not play any role in the control of intestinal vasculature at basal conditions but these receptors participate in the intestinal hyperemic reaction in response to complete temporal intestinal ischemia (Pawlik et al., 2004).

(iv). L-dopa-induced dyskinesia (LID) remains a major complication of the treatment of Parkinson's disease. The neural mechanisms underlying LID are thought to involve over activity of striatal glutamatergic neurotransmission, with resultant under activation of the output regions of the basal ganglia. Histamine H3 heteroreceptors can reduce glutamate and gamma-amino butyric acid (GABA) transmission in the striatum and substantia nigra reticulata, respectively. Histamine H3 receptors may be involved in the neural mechanisms underlying L-dopa-induced dyskinesia in Parkinson's disease (Gomez-Romerez et al., 2006).

(v). Histamine H3 receptors are involved in regulating the release of NE in both central and peripheral nervous systems. Both in blood vessels and the heart, H3 receptors are located on noradrenergic nerve endings and upon stimulation mediate an inhibition of noradrenaline release. Yamamoto et al (Yamamoto et al., 2004) studied the effect of RAMH and THP on ischemia/reperfusion-induced changes in carrier-mediated NE release and cardiac function in isolated rat heart. Ischemia/reperfusion evoked massive NE release, which was markedly suppressed by the treatment with desipramine, a neuronal NE transporter blocker. It was reported that ischemia/reperfusion-induced carrier mediated NE
release in rat heart was negatively regulated by the activation of histamine H$_3$ receptors, probably located on cardiac noradrenergic nerve endings (Yamamoto et al., 2004).

(vi). Using a cyanide model to induce neurotoxic effects in rat brain homogenates, neuroprotective properties of three H$_3$ antagonists, namely clobenpropit, thioperamide and impentamine, and compared them to aspirin, a known neuroprotective agent were studied by Badenhorst et al (2005). Superoxide anion levels and malondialdehyde concentration were assessed using the nitro blue tetrazolium and lipid peroxidation assays. Clobenpropit and thioperamide significantly reduced superoxide anion generation and lipid peroxidation. Impentamine reduced lipid peroxidation at all concentrations used, but only reduced superoxide anion generation at a concentration of 1 mM. In the lipid peroxidation assay, all the drugs compared favourably to aspirin. This study demonstrates the potential of these agents to be neuroprotective by exerting antioxidant effects (Badenhorst et al., 2005).
2.2. HISTAMINE AND NEUROPSYCHIATRIC DISORDERS

2.2.1. Anxiety

A. Definition
Anxiety is defined as a complex feeling of apprehension, fear, and worry often accompanied by pulmonary, cardiac, and other physical sensations. It is a ubiquitous condition that varies from the physiologic to the pathologic in its presentation. When pathologic, it can exist as a primary disorder, or it can be associated with medical illness, neurologic syndromes, or other primary psychiatric illnesses (eg, depression, psychosis) (Brawman et al., 1997).

B. Prevalence
Anxiety disorders are highly prevalent worldwide, is present in 15 to 20% of medical clinic patients (Eva Bender, 2004), although most people contend with anxiety as a normal and periodic component of daily life, the emotional responses of some people are greatly exaggerated. Panic disorder has a lifetime prevalence of 1.5 - 3.5%. In contrast, phobic disorders have lifetime prevalence as high as 10-13%, but they encompass several subcategories of anxiety conditions (Hales et al., 1997).

C. Types
Various anxiety disorders are as follows:

Generalized anxiety disorders (GAD)
GAD is defined as persistent fear, worry, or tension in the absence of panic attacks. The disabling persistent worry usually is out of proportion to the impact of the feared event and typically revolves around routine life circumstances. GAD is clinically associated with tremulousness, shaking, insomnia, irritability, and restlessness. Patients often complain of
somatic symptoms (eg, muscle tension, cold clammy hands, dry mouth, diaphoresis, nausea, diarrhea, urinary frequency).

Panic disorders

Panic disorder is defined by the presence of recurrent and unpredictable panic-attacks, which are distinct episodes of intense fear and discomfort associated with a variety of physical symptoms, including palpitations, sweating, trembling, shortness of breath, chest pain, dizziness and a fear of death. Panic attacks have a sudden onset, developing within ten minutes and usually resolving over the course of an hour.

Phobic disorders

Phobic disorders (e.g, social phobia, specific phobia, agoraphobia without panic disorder) are persistent fears that are usually excessive or unreasonable. They occur in the presence of or in anticipation of an encounter with a specific object or situation. Adults, unlike children, recognize that their fear is excessive or unreasonable. Phobic disorders often lead to exaggerated avoidance behaviors and leave their victims significantly disabled from a functional standpoint. The two current clinical types, social and specific, have several subtypes. Social phobias are fears of situations involving possible scrutiny (eg, public speaking, performing in public). Specific phobias are fears of a circumscribed stimulus (ie, object, situation) that provoke an intense anxiety response (eg, insects, spiders, situations involving heights).

Stress disorders

Patients may develop anxiety after exposure to extreme traumatic events such as the threat of personal death or injury or the death of a loved one. The reaction may occur shortly after the trauma or be delayed and subject to occurrence. In both syndromes, individuals experience associated symptoms of detachment and loss of emotional responsivity. The patient may be depersonalized and unable to recall specific aspects of the trauma. It is
hypothesized that stress may be resulted due to the excessive release of NE in response to stress.

Obsessive-compulsive disorders

Obsessive-compulsive disorder consists of an anxiety-producing obsession comprised of persistent thoughts or ideas that are intrusive and inappropriate combined with a compulsion of repetitive behaviors that reduce the anxiety of the obsession. This disorder causes significant distress to the patient and seldom produces any pleasure or gratification.

D. Etiology

Many anxiety disorders demonstrate a familial pattern. First-degree biological relatives of patients with panic disorders have up to a 7-fold increased probability, as compared to the general population, of presenting with the same illness. In addition many abused drugs (e.g. alcohol, amphetamine, narcotics) raise anxiety levels. Panic attacks in patients who were susceptible to them can be precipitated by caffeine, carbon monoxide or glue inhalation, sodium lactate infusion, isoproterenol and benzodiazepine receptor antagonists (e.g. flumazenil).

E. Pathophysiology

The neurotransmitters primarily associated with anxiety disorders are norepinephrine, gamma-aminobutyric acid (GABA), and serotonin. In experimental models of anxiety, anxiogenic agents share in common the property of altering the binding of benzodiazepines to the gamma amino butyric acid A (GABA_A) receptor / chloride ion channel complex. Benzodiazepines are thought to bind two separate GABA_A receptor sites: type I, which has a broad neuroanatomic distribution, and type II, which is connected in the hippocampus, striatum and neocortex. The antianxiety effect of various benzodiazepines and side effects such as sedation and memory impairment are influenced by their relative binding to type I and type II receptor sites. Drugs that affect norepinephrine (eg, tricyclic antidepressants, monoamine oxidase inhibitors [MAOIs]) are also efficacious in the treatment of several anxiety disorders. Serotonin (5 HT) also appears to play a role in anxiety. Buspirone, a
partial 5HT\textsubscript{1A} receptor agonist, and certain 5HT\textsubscript{2A} and 5HT\textsubscript{2C} receptor antagonists may also have beneficial effects.

F. Treatment

Short-acting benzodiazepines in parenteral form are most useful in this disease. Benzodiazepines should only be prescribed in motivated and cooperative individuals who have reliable follow-up arrangements. Barbiturates are not recommended because of their high addictive potential, marked side effects, slow onset of action, and low therapeutic indices. Tricyclic antidepressants and MAOIs also are of limited use and should not be prescribed in the acute setting. Beta-blockers do not reduce intrinsic anxiety, although they do reduce anatomic components (eg, tachycardia, diaphoresis). They are helpful for cardiac, endocrine, and other medical conditions commonly associated with stress and anxiety. Antidepressants have well-known pharmacological profiles and could be useful in patients with concomitant depression and anxiety. They have demonstrated benefit as adjunct agents in the treatment of GAD. Antidepressants have been related to long-term outpatient use for other chronic anxiety disorders (Sussman, 1994). Serotonin reuptake inhibitors (eg, paroxetine) generally are preferred as the drug of choice for long-term treatment of panic disorders, although traditional tricyclic antidepressants also have been utilized. Serotonin receptor agonists stimulate 5-HT\textsubscript{1}-receptors, producing anxiolytic effects. Buspirone is a nonsedating antipsychotic drug unrelated to benzodiazepines, barbiturates, and other sedative-hypnotics. It has been found to be comparable with benzodiazepines in reducing symptoms of anxiety in double-blind placebo-controlled clinical trials and has fewer sedative or withdrawal adverse effects than benzodiazepines. Also have fewer cognitive and psychomotor adverse effects, which makes its use preferable in elderly patients. Major limitations include lack of antipanic activity and reduced anxiolytic effects in patients recently withdrawn from benzodiazepines. Also has a longer onset of action and, thus, is of fairly limited use as a sole agent in the treatment of acute anxiety.
Table: 4. Pharmacological agents for the treatment of anxiety disorders.

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzodiazepines (BZDs) e.g. alprazolam, lorazepam, diazepam, oxazepam</td>
<td>Activate a specific benzodiazepine receptor that facilitates inhibitory GABAergic transmission</td>
</tr>
<tr>
<td>Tricyclic antidepressants (TCAs) e.g. imipramine, amitriptyline, clomipramine</td>
<td>Enhance the functional activity of noradrenaline and serotonin by blocking the reuptake of both neurotransmitters</td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors (SSRIs) e.g. fluoxetine, citalopram, paroxetine</td>
<td>Block the reuptake of serotonin to enhance its functional activity</td>
</tr>
<tr>
<td>Monoamine oxidase inhibitors (MAOls) e.g. phenelzine</td>
<td>Enhance the functional activity of noradrenaline and serotonin by inhibiting the degradation of both neurotransmitters by monoamine oxidase</td>
</tr>
<tr>
<td>Beta-blockers e.g. oxprenolol, propranolol</td>
<td>Block beta-adrenergic receptors to prevent the functional activity of adrenaline and noradrenaline</td>
</tr>
<tr>
<td>Antihistamines e.g. hydroxyzine</td>
<td>Block histamine receptors to prevent its functional activity</td>
</tr>
<tr>
<td>Azaspirones e.g. buspirone</td>
<td>Enhances some noradrenaline and dopamine neurotransmission while reducing serotonin and acetylcholine neurotransmission in the brain</td>
</tr>
</tbody>
</table>
G. Animal models

Vogel's conflict test (Vogel Test)

The Vogel conflict test (Vogel et al., 1971) is a well-known method to examine the anti-anxiety like action of various psychoactive drugs. This test is extensively used for the preclinical evaluation of putative anxiolytics, and the methodology of the test has been investigated in detail. However, because rats are usually used in this test, only few studies tried to apply the test to other species, of potential interest are mice. One of the important advantages of mice over rats are subject of behavioral pharmacology is that a great deal more information about mouse genetics and mouse strains are currently available. In addition, recent advances in molecular biology have enabled the production of various kinds of transgenic and knockout mice, which in turn have enabled investigation of the genetic factors associated with the response to psychoactive drugs.

The anxiolytic activity of compounds is assessed by determining their ability to study drinking behavior that has been suppressed by punishment (Vogel et al., 1971). The procedure was modified by Umezu (1999) using mice and Millan (2001) using rats. Animals were deprived of water for 24 h and were deprived of food for at least 16 h before testing. After the first 24 h of water deprivation, they were placed in a sound-attenuating chamber for a training period, in which they were allowed 200 licks from a bottle containing tap water. The experiment was performed the next day. Vehicle or compounds were administered, and at specified times after dosing, animals were placed in the chamber and allowed access to tap water. The first lick at the stainless steel sipper tube of a water bottle initiated a 3-min test session in which every 20th lick was punished by a 0.2 sec, 0.1 mA shock delivered via the sipper tube. If animals failed to drink within 5 min, the experiment was terminated, and they were evaluated for signs of CNS depression.

Elevated plus maze test

The elevated plus maze test (EPM) is a rodent model of anxiety that is used extensively in the discovery of novel anxiolytic agents and to investigate the psychological and neurochemical basis of anxiety. The elevated plus maze used today is in the shape of a
cross or plus with two elevated closed arms running east west. The test has a widespread appeal because it is a simple and less time consuming procedure, does not involve any sophisticated equipment, or prior training or noxious stimuli. Based on the spontaneous behavior of the animal, the test provides a valid and reliable measure of anxiety in animals and for testing of antianxiety drugs. However, the predictive value of the test remains unclear; although anxiolytics, such as benzodiazepine receptor agonist chlordiapoxide, produce reliable and reproducible effects, other anxiolytics, such as the partial $5$-$\text{HT}_{1A}$ receptor agonists do not. This suggests that the use of the EPM as a model of anxiety has some limitations.

EPS as developed by Pellow et al (1985), consists of two open and two closed arms, facing each other, to measure anxiety phenomenon. The rationale for using the plus maze is that the open arms are more fear provoking and that the ratio of either time spent on open: closed arms or entries into the open-closed arms reflect the relative safety of closed arms compared with the relative fearfulness of open arms. Anxiolytics would be expected to increase the proportion of entries into and time spent on open arms. The elevated plus maze test is a sensitive behavioral test that reveals animals neophobia or anxiety and can be used to unveil antineophobic and anxiolytic actions of drugs.

Light-dark paradigm
This test is used as a measure of the animals's anxiety level (Hascoet and Bourin, 1998). Mice are normally nocturnal but are also exploratory. When placed in a novel environment in which they can choose to be in the dark place or brightly lit place, they spent most of their time in the dark place. However, habituation to the novel environment or treatment with anxiolytic drugs increases the percentage time of mice spend in the brightly lit half of the chamber.

H. Role of histamine in anxiety
Increasing evidence supports a role for HA in anxiety (Bongers et al., 2004; Raber, 2005). In most of the studies, HA and stimulation of $H_1$ receptors or blockade of $H_2$ receptors increased the measures of anxiety on various tests. Similar to these observations, $H_3$
receptor antagonists also increased measures of anxiety in various tasks including the object recognition task in wild-type mice (Bongers et al., 2004) and in light/dark test in rodents (Yuzurihara et al., 2000). The latter was shown to be antagonized by an H₁ receptor antagonist. Recently, H₃ receptor deficient mice showed reduced measures of anxiety in elevated plus maze and zero maze (Rizk et al., 2004). Histamine metabolism is associated with anxiety. People with low histamine have been found with typical symptoms of anxiety (Jackson et al., 1998). Anxiolytic drugs are known to decrease brain histamine turnover. Histamine H₁ receptor antagonists and H₃ receptor agonists decrease the anxiety state. These findings show that acute stresses increase brain histamine turnover, especially in the diencephalon, which would be partly related to the pathology of anxiety (Ito, 2000).

2.2.2. Depression

A. Definition
A depressive disorder is a syndrome (group of symptoms) that reflects a sad mood exceeding normal sadness or grief. More specifically, the sadness of depression is characterized by a greater intensity and duration and by more severe symptoms and functional disabilities than is normal. Depression symptoms are characterized not only by negative thoughts, moods, and behaviors, but also by specific changes in bodily functions (e.g., eating, sleeping, and sexual activity).

B. Prevalence
Depression is a serious disorder with estimates of lifetime prevalence as high as 21% of the general population in some developed countries (Schechter et al., 2005). Depressive disorders are a huge public health problem, can increase the risks for developing coronary artery disease, HIV, asthma, and some other medical illnesses. Furthermore, it can increase the morbidity (illness) and mortality (death) from these conditions. It is a potentially life-threatening disorder that affects millions of people all over the world. It can occur at any age from childhood to late life and is a tremendous cost to society as this disorder causes severe distress and disruption of life and, if left untreated, can be fatal. Depression is not a
homogeneous disorder, but a complex phenomenon, which has many subtypes and probably more than one etiology.

C. Types
Three of the most common types of depressive disorders are:

Major Depression
Major depression is characterized by a combination of symptoms, including sad mood that interfere with the ability to work, sleep, eat, and enjoy once-pleasurable activities. Disabling episodes of depression can occur once, twice, or several times in a lifetime.

Dysthymia
Dysthymia is a less severe type of depression. It involves long-term (chronic) symptoms that do not disable, but yet prevent the affected person from functioning at "full steam" or from feeling good. Sometimes, people with dysthymia also experience episodes of major depression. This combination of the two types of depression is referred to as double-depression.

Bipolar Disorder (Manic Depression)
Another type of depression is bipolar disorder, which was formerly called manic-depressive illness or manic depression. This condition shows a particular pattern of inheritance. Not nearly as common as the other types of depressive disorders, bipolar disorder involves cycles of depression and mania, or elation. Bipolar disorder is often a chronic, recurring condition. Sometimes, the mood switches are dramatic and rapid, but most often they are gradual. A significant variant of bipolar disorder is designated as bipolar II. (The usual form of bipolar disorder is referred to as bipolar I.) Bipolar II is a syndrome in which the affected person has repeated depressive episodes punctuated by what is called hypomania (mini-highs). These euphoric states in bipolar II do not fully meet the criteria for the complete manic episodes that occur in bipolar I.
D. Symptoms (Briggita, 2002)

Not everyone who is depressed or manic experiences every symptom. Some people experience a few symptoms and some many symptoms. The severity of symptoms also varies with individuals.

**Depression Symptoms of manic depression**

- Persistently sad, anxious, or "empty" mood.
- Feelings of hopelessness.
- Feelings of guilt, worthlessness, helplessness.
- Loss of interest or pleasure in hobbies and activities that were once enjoyed.
- Insomnia, early-morning awakening, or oversleeping.
- Decreased appetite and/or weight loss, or overeating and weight gain.
- Fatigue, decreased energy, being "slowed down."
- Thoughts of death or suicide, suicide attempts.
- Restlessness, irritability.
- Difficulty concentrating, remembering, making decisions.
- Persistent physical symptoms that do not respond to treatment, such as headaches, digestive disorders, and chronic pain.

**Mania symptoms of manic depression**

- Inappropriate elation.
- Inappropriate irritability.
- Severe insomnia.
- Increased talking speed and/or volume.
- Disconnected and racing thoughts.
- Increased sexual desire.
- Markedly increased energy.
- Poor judgment.
- Inappropriate social behavior.
E. Etiology

There is abundant evidence from family twin and adoption studies that genetic factors play an important role in the etiology of depressive disorder. There is strong epidemiological evidence for a genetic contribution, especially for bipolar disorders, and heritability is estimated to be as high as 80% (Berrettini, 1999). There are various other causes that are responsible for the development of the depressive condition including diet, environmental toxins and biochemical individuality. Thousands of chemicals are found in our food, water and air. Many of these substances are known, or suspected, to cause damage to the brain or to interfere with neurotransmitter synthesis even in minuscule concentrations. Deficiencies of certain vitamins or minerals can also reduce the body’s ability to excrete toxic elements, thus amplifying their deleterious effects on the brain. In certain people, histamine builds up to abnormally high levels because a process called methylation is impaired. This process is needed not only to break down histamine, but also to synthesize neurotransmitters and to clear environmental toxins from the body. As a result, people with high histamine cannot synthesize enough neurotransmitters and are also more vulnerable to the deleterious effects of environmental chemicals. Certain nutrients, primarily calcium and the amino acid methionine are used to activate methylation, and people with this trait can expect to see major improvements in how they feel within two months of starting these supplements. At the opposite end of the spectrum, there are people who break down histamine too quickly that these individuals are primarily deficient in folic acid. Since folic acid is also needed to make neurotransmitters, they frequently become depressed as well and, in fact, they may be the most severe cases, with a tendency towards despair. They also improve quickly when taking folic acid with associated nutrients and avoiding the supplements recommended for the previous group.

F. Pathophysiology

The depression is one of the more commonly encountered psychiatric disorders of worldwide significance. However, the underlying pathophysiology has not been clearly defined. The first major hypothesis of depression monoamine hypothesis was formulated about 30 years ago and proposed that the main symptoms of depression are due to a functional deficiency of the brain monoaminergic transmitters norepinephrine (NE), 5-
HT, and/or DA, whereas mania is caused by functional excess of monoamines at critical synapses in the brain (Schildkraut, 1965; Copper, 1967; Matussek, 1972). Evidence for this hypothesis came from clinical observations and animal experiments, which showed that the antihypertensive drug reserpine, which causes a depletion of presynaptic stores of NE, 5-HT, and DA, induced a syndrome resembling depression. Considering the origin of the noradrenergic, serotonergic, and dopaminergic neurones in the brain and their projections into many areas of the brain, it is clear that monoaminergic systems are responsible for many behavioral symptoms, such as mood, vigilance, motivation, fatigue, and psychomotor agitation or retardation. Abnormal function and the behavioral consequences of either depression or the manic state may arise from altered synthesis, storage, or release of the neurotransmitters, as well as from disturbed sensitivity of their receptors or subcellular messenger functions (Stahl, 1998).

Many attempts have been made to prove the hypothesis of reduced monoamine availability by measurement of neurotransmitters and/or their metabolites in post-mortem brain tissues and body fluids, such as cerebrospinal fluid (CSF), blood, and urine (Potter et al., 1985). Although repeated data showing decreased levels of the NE metabolite methoxy-4-hydroxyphenylglycol (MHPG), which indicates NE turnover in brain, support the hypothesis of a deficient noradrenergic system (Schatzberg et al., 1995). Similar to the noradrenergic system the data on determinations of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) could not prove the hypothesis of exclusively reduced serotonergic transmission. Many studies reported decreased central serotonergic turnover in major depression (Cheetham et al., 1991; Maes et al., 1994) but findings also suggested that reduced 5-HT function might not be present in all depressed patients (Leonard, 2000). Tyrosine hydroxylase and tryptophan hydroxylase are essential for NE and 5-HT synthesis, respectively, and were found to be up- or down-regulated in postmortem brain samples, suggesting a minor importance for transmitter synthesis (Leonard, 2000).
G. Treatment

The first-generation antidepressants, the tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs), increase the concentrations of 5-HT and/or NE and are effective in alleviating the symptoms of depression. Although both types of drugs have been used with great success for many years, there are several undesirable side effects that limit their application. TCAs acts on many other transmitter systems in the CNS and periphery, e.g., the histaminergic or cholinergic systems (Feighner, 1999) leading to sedation, hypotension, blurred vision, dry mouth, and other unwanted effects. In addition, TCAs may be life threatening and fatal in overdose, especially due to their effects on the cardiovascular system (Glassman et al., 1998). Also, the irreversible MAOIs have their own problems, such as an interaction with tyramine (cheese effect), which causes potentially lethal hypertension (Feighner, 1999). The development of newer antidepressants has aimed to improve the safety and tolerability of the TCAs and reuptake inhibitors, and selectivity for a single monoamine seemed to be the key to this goal. Since the introduction of fluoxetine as the first selective serotonin-reuptake inhibitor (SSRI), a great number of similarly acting drugs have followed and SSRIs are now applied in the treatment of several psychiatric disturbances, such as anxiety, panic, or obsessive compulsive disorder, where altered serotonergic transmission is assumed (Feighner, 1999). Because preclinical and clinical studies have shown that chronic stimulation of the 5-HT system also affects the NE system and vice versa (Murphy et al., 1998), there has been renewed interest in the role of neurotransmitters other than serotonin. The development of the newest generation of antidepressants, including reboxetine (a selective NE-reuptake inhibitor), venlafaxine (a dual reuptake inhibitor), or the multiple receptor acting substances mirtazapine, nefazodone, bupropion, and trazodone, may positively influence therapeutic potentials with reduced incidence of side effects due to reduced affinities for other systems (Feighner, 1999). Interestingly, one drug, tianeptine, shows a quite atypical mechanism, namely an increase in 5-HT uptake, but most probably this substance predominantly counteracts stress effects in the hippocampus (McEwen et al., 1997). The effect of antidepressants on the NE and 5-HT receptor systems has been known for a long time, and the decreased sensitivities of \( \beta \) -adrenoceptors and cortical 5-HT\(_{2A} \) receptors have often been suggested to be a prerequisite.
for antidepressant action (Peroutka et al., 2001). The delay in antidepressant response makes it clear that the immediate effects of these drugs are not the main explanation of their antidepressant action, but gradual adaptive changes in neuronal responses might be ultimately responsible for the therapeutic benefits (Blier et al., 2001). Recent research with SSRIs and dual uptake inhibitors has shifted the research focus beyond the levels of receptors to those of protein kinase-mediated phosphorylation of transcription factors, which ultimately leads to changes in programs of gene expression (Manier et al., 2001). Considering the currently available drugs for antidepressant treatment, there is now no doubt that the NE and 5-HT system are important in the pathophysiology.

H. Animal models
Despite the advances made in recent years, we are still limited in the nature of studies that can be performed in the clinic to examine the neuronal state of depressed patients. Preclinical studies on animals can provide crucial information on the whole range of neuronal function not accessible in humans and potentially allow us to identify novel targets for antidepressant drug development.

Reserpine
Reserpine treatment induces a syndrome of locomotor hypomotility and reduced body temperature. Reserpine nonselectively depletes synaptic stores of brain monoamines NE, DA, and 5-HT. Iproniazid by preventing the breakdown of monoamine neurotransmitters is able to reverse the effects of reserpine on motility in rats. However, not only iproniazid, but virtually all monoamine oxidase inhibitor and tricyclic antidepressants can reverse the effects of reserpine on motility in rats and mice (Danysz et al., 1991). The validity of the reserpine model is also not entirely based on the capacity of antidepressants to restore activity in reserpinized animals. There are a number of reports of patients who have taken reserpine as a treatment for hypertension reporting dysphoric reactions to the drug (Bein, 1978). This suggested that the syndrome observed in animals might indeed be analogous to the human condition. It is not clear, however, whether what is modeled in this case is acute dysphoria or true depression. This model has some limitations. Firstly, it is not entirely clear
precisely how reserpine induces its mood-lowering effect in people. Secondly, the corollary is also true, that is the precise mechanism by which the monoamine oxidase inhibitors exert their antidepressant effect in people is not known.

**Apomorphine-induced hypothermia**

Hypothermia induced by high doses of dopamine agonist apomorphine is reversed by antidepressant compounds (Puech et al., 1981). It is likely that this effect is due to the agonist action of the compound on dopaminergic receptors on noradrenergic terminals, preventing noradrenaline release (Redrobe et al., 1998). The test does not attempt to mimic any of the behavioral symptoms of depression per se, but simply reveals activity of compounds in elevating the synaptic concentration of NE. As a simple in vivo indicator of elevated NE levels this test has proved to be useful in the identification of compounds with that activity but it does not increase our knowledge or understanding of the mechanisms underlying depression.

**5-Hydroxytryptophan-induced behavioral syndrome**

A similar mechanism-based approach has been used to identify agents that can enhance synaptic concentrations of serotonin. In this case, the simple in vivo test, originally developed in rats but used more routinely in mice, detects elevated serotonin levels by measuring the potency of a compound in potentiating the behavioral syndrome induced by administration of 5-hydroxytryptophan (5-HTP) the metabolic precursor to 5-HT (Grahame-Smith, 1971 a, b). This test provides a relatively rapid and accurate index of SSRI potency in vivo. These tests offer predictive validity for antidepressant action based on the hypothesis that elevation of 5-HT or NE levels are involved in the relief from depressive symptoms. The tests themselves cannot be said to model depressive signs or symptoms. They are selective for the particular mechanism of action to the extent that compounds that show activity, in the reversal of reserpine or apomorphine-induced hypothermia will not necessarily enhance 5-HTP syndrome or vice versa. The behavioral tests have the advantage of being relatively quick, they can use mice and thus use less test compound and do not involve use of invasive surgery.
Olfactory bulbectomy

The bilateral removal of the olfactory bulbs of a rat (hamsters and mice have also been used) results in a complex constellation of behavioral, neurochemical, neuroendocrine and neuroimmune alterations, many of which are correlated with changes observed in major depression (Kelly et al., 1997). To many investigators the bulbectomy model seems to be an obscure test because the rationale for its use as an animal model has been often questioned based on construct and etiological validity arguments. However, this model has one of the best portfolios for the prediction of known antidepressant compounds following repeated administration and is reliable between laboratories (Kelly et al., 1997). The most consistent behavioral change caused by bulbectomy is hyperactive response in a novel, brightly lit open field apparatus, which is reversed almost exclusively by chronic, but not acute, antidepressant treatment (Kelly et al., 1997). Reasons for this time-dependent reversal are not fully understood but have generated much interest. Recent studies have shown that the hyperactive response might be related to increases in defensive behaviors (Stock et al., 2001) or alterations in aversively motivated behavior (Primeaux et al., 1999). Furthermore, it has been shown that antidepressant compounds prefrentially enhance habituation to novelty in the bulbetomized rat, and that these effects are not secondary to anosmia (loss of sense of smell) (Mar et al., 2000). Furthermore, increased striatal glutamate release during novelty exposure-induced hyperactivity has been demonstrated that might have a modulatory role on the antidepressant-sensitive response (Ho et al., 2000). Comparing the behavioral and biochemical effects of bulbectomy in young versus aged rats, Slothkin and colleagues (Slothkin et al., 1999) suggested that this test might provide a useful animal model to test therapeutic interventions for geriatric depression.

Forced swimming test (FST)

The forced swimming test (FST) was developed by Porsolt and colleagues (Porsolt et al 1977) in the rats and subsequently in the mice (Porsolt 2000). This test is the most widely used tool for assessing the antidepressant activity pre-clinically. The widespread use of this model is largely a result of its ease of use, reliability across laboratories and ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rats, following initial escape-oriented movements, develop an immobile posture when
placed in an inescapable cylinder of water. If they are replaced in the testing apparatus 24 hours later, they resume this posture quickly. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. If antidepressants treatments are given between the two exposures the subjects will actively persist engaging in escape directed behaviors for longer periods of time than after vehicle treatment. However, the major drawback of the traditional FST is that it is unreliable in the detection of the effects of selective 5-HT reuptake inhibitors (SSRIs), which are the most widely prescribed antidepressant drugs of today (Cryan et al 2002)

The modified forced swimming test
In an effort to enhance the sensitivity of the traditional FST in the rat so that it can be SSRI responsive, several simple procedural modifications have been made (Lucki, 1997). These developments include increasing the water depth to 30 cm from traditional depth of 15-18 cm. These alterations enabled investigators to distinguish specific behavioral component of active behaviors, namely (1) climbing behavior (also known as thrashing), which is defined as upward-directed movements of the forepaws along the side of the swim chamber; (2) swimming behavior, the movement (usually horizontal) throughout the swim chamber that also includes crossing into the other quadrant; and (3) immobility, which is defined, as in the traditional Porsolt test. As a result of the increase in water depth, there is considerable less immobility than in the traditional test because the animals cannot have contacts with the cylinder bottom. The major advantages of the modified FST over its traditional counterpart is that it reveals that catecholaminergic agents decrease immobility with a corresponding increase in climbing behavior, whereas 5-HT related compound such as SSRIs also decrease the immobility but increase the swimming behavior. Recent studies have shown that 5-HT$_{2c}$ receptors play an instrumental role in mediating the effects of the SSRI fluoxetine in the test (Cryan et al 2000). One major drawback of the FST (as with many antidepressants-sensitive paradigm) is the fact that short-term antidepressant treatments reverse the immobility whereas in the clinic it can take weeks for the same antidepressant to elevate mood. However, it has been demonstrated that doses of
antidepressant drugs that are inactive acutely elicit antidepressant-like effects when administered chronically, which further validates the modified paradigm.

**Learned helplessness**

The learned helplessness paradigm is based on the fact that following repeated uncontrollable shocks; animals demonstrate escape deficits that are reversible by antidepressant agents (Weiss et al., 1998). The behavioral deficits are sensitive to a broad spectrum of antidepressant compounds usually following short-term treatment. The major drawback of the model is that most of the depression-like symptomatology does not persist beyond 2-3 days following cessation of the uncontrollable shock (Weiss et al., 1998). A recent modification of the rat learned helplessness procedure incorporates aspects of chronic mild stress paradigm (Gambarana et al., 2001). By chronic exposure to mild stressors the effective uncontrollable shock can be maintained for a prolonged period, and chronic treatment with the SSRI fluoxetine and the nonselective monoamine uptake inhibitor imipramine reversed these changes. The chronic stress procedure involves restraint and novel housing, and avoids the problems associated with food deprivation used in the traditional chronic mild stress procedure (Reid et al., 1997). Vollmayer and Henn (2001) have recently proposed key factors that can be manipulated to enhance both the usability and the reliability of the rat learned helplessness paradigm. These include using a larger testing apparatus, a mild shock presentation and a relatively difficult shock avoidance task (Porsolt, 2000). Furthermore, they point out that animals can be artifactually avoid shock as a result of their position in the apparatus, which should also be taken into account.
Table 5. Widely used rodent models sensitive to effects of antidepressant agents.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Ease of use</th>
<th>Reliability</th>
<th>Specificity</th>
<th>Application to mice</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forced swimming test</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Yes</td>
<td>Sensitive to acute antidepressant treatments, does not reliably detect SSRIs.</td>
<td>Porsolt, 1977; 2000</td>
</tr>
<tr>
<td>Modified forced swimming test</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Yes</td>
<td>Sensitive to acute antidepressant treatments; differentiates antidepressants from different classes including SSRIs.</td>
<td>Lucki I, 1997</td>
</tr>
<tr>
<td>Tail suspension test</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Yes</td>
<td>Sensitive to acute antidepressant treatments; certain strains climb their tail.</td>
<td>Porsolt, 2000</td>
</tr>
<tr>
<td>Olfactory bulbectomy</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
<td>Yes</td>
<td>Behavioral effects evident only following chronic treatment; mechanism of action poorly understood.</td>
<td>Kelly et al., 1997</td>
</tr>
<tr>
<td>Learned helplessness</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>Yes</td>
<td>Sensitive to short-term antidepressant treatments; ethical restrictions in some countries.</td>
<td>Weiss et al., 1998; Porsolt, 2000</td>
</tr>
</tbody>
</table>
I. Role of histamine in depression

Recent experimental studies show the possible therapeutic potential of selective histamine \( H_3 \) receptors in a variety of neuropsychiatric disorders including depression (Onodera et al., 1994, 1998; Perez-Garcia et al., 1999; Robio et al., 2001; Fox et al., 2002). Although no direct correlation exists between histamine and depression, there are indirect evidences for the same. For example, histamine receptors are considered to be targets for the action of tricyclic antidepressants (TCAs) (Schwartz et al., 1981). In addition, a reduced histamine \( H_1 \)-receptor binding has recently been reported in the brains of depressed patients (Kano et al., 2004) suggesting the involvement of histaminergic neuronal system in the pathophysiology of depression. It has been reported that people with obsessive-compulsive tendencies, seasonal depressions, and oppositional defiant behavior have high whole blood histamine levels (Walsh, 1992). People with high histamine have been found with typical symptoms of high intelligence, thought blanking, perfectionism, competitiveness, obsessions, compulsions, suicidal and seasonal depression, defiance, and phobia (Jackson et al., 1998). People with low histamine have been found with typical symptoms of under-achievement, cyclic or suicidal depression, and anxiety (Jackson et al., 1998). Recently, we have shown an antidepressant effect of THP in the modified forced swimming FST in mice (Akhtar et al., 2005).

2.2.3. Schizophrenia

A. Definition

Schizophrenia is a chronic, severe, disabling brain disease, characterized by perturbation of language, perception, thinking, and social activity (Braunwald et al., 2001). The onset of this disorder is generally in adolescence or in early adulthood. The illness is chronic, generally lifelong and is characterized by periodic psychotic exacerbations and incomplete remissions. Negative symptoms and cognitive impairments are particularly prominent during these incomplete remissions. Some degree of deterioration occurs in the first 3-5 years after the onset of psychotic symptoms and degree of uncontrolled psychosis appears to be related to the extent of such deterioration.
B. Prevalence

Schizophrenia is present in 1% of individuals worldwide (Esbenshade et al., 2005). Overall lifetime prevalence is approximately 1-1.5%. Social costs of schizophrenia are substantial. An estimated 300,000 episodes of acute schizophrenia occur annually resulting direct and indirect costs that have been estimated at more than $33 billion.

C. Symptoms

The main clinical features of the disease are as follows:

Positive symptoms:
- Delusions
- Hallucinations usually in the form of voices and often exhortatory in their message.
- Thoughts disorders, comprising wild trains of thoughts and irrational conclusions, often associated with the feeling that thoughts are inserted or withdrawn by an outside agency.

Negative symptoms:
- Withdrawal from social contacts
- Flattening of emotional responses
- Anhedonia, poverty of speech and thoughts, asociality, lack of motivation, apathy and ambivalence.

A domain of cognitive/neuropsychological dysfunction.

D. Types

Schizophrenia can be classified according to the specific symptomatology present, although such distinctions do not correlate with either course of illness or response to treatment and many individuals have symptoms of more than one type (Braunwald et al., 2001).
The four main symptom subtypes are:

1. Catatonic: Describes patient whose clinical presentations are dominated by profound changes in motor activity, negativism and echolalia or echopraxia.
2. Paranoid: Describe patients who have a prominent pre-occupation with a specific delusional system.
3. Disorganized type disease: in which disorganized speech and behavior are accompanied by superficial or silly affect.
4. Residual type: Negative symptomatology exits in the absence of delusions, hallucinations or motor disturbances.

E. Etiology
Both genetic and environmental factors have been implicated. Psychosocial stressors (such as an emotionally stressful family setting) are one of the most common precipitating factors of significance. Genetic factors are most strongly supported by the evidence, but the fact that half of the patients with schizophrenia have no family history of disease and half of the identical twins of schizophrenia patients do not have schizophrenia suggest that environmental factors play a role as well. Family twins and adoption studies show that genetic factors are involved in at least a subset of individuals who develop schizophrenia. There is a substantial evidence suggesting that schizophrenia is associated with a neurodevelopmental disorder affecting the cerebral cortex and occurring in the first few months of prenatal development. In the first-degree relatives, the risk is 10% even monozygotic twins, one of who has schizophrenia; the probability of other being affected is only about 50%. Prenatal obstetric complications and prenatal second trimester viral infection are the two environmental factors most strongly associated with an increased risk of schizophrenic illness.
F. Pathophysiology

Schizophrenia is clearly a brain disease and many abnormalities in structure, function and neurochemistry have been observed in the several thousands of studies of schizophrenia.

Neurochemical abnormalities in schizophrenia:

Dopamine theory

This hypothesis states that schizophrenia is related to relative excess of dopamine dependent neuronal activity. The major support of this hypothesis derives from the efficacy of the dopamine blocking antipsychotics in treating schizophrenia and the ability of dopamine enhancing agents such as amphetamine to exacerbate the symptoms of schizophrenia (Snyder et al., 1974). No drugs without dopamine blocking activity have any proven efficacy in the treatment of schizophrenia. In fact, the clinical potency of various antipsychotic drugs is directly related to their in vitro ability to bind to dopamine D₂ receptors (Seeman et al., 1976; Creesal et al., 1979). A functional hyperactivity of the dopamine system can theoretically occur in one of two ways: (i) too much neurotransmitter because of excessive production by the presynaptic neurons, inadequate break down, or deficient uptake. (ii) Excessive response of the postsynaptic neurons to normal amounts of dopamine because of increased number or affinity of receptors. Dopamine is a catecholamine synthesized from tyrosine. The enzyme tyrosine hydroxylase converts tyrosine to dihydroxyphenylanaline (DOPA) and enzyme DOPA decarboxylase converts DOPA to dopamine. Two enzymes. Monoamine oxidase (MAO) and catechol-o methyl transferase (COMT) are involved in the catabolism of dopamine with homovanillic acid (HVA) and to some extent dihydroxy phenylacetic acid (DOPAC) being its major metabolites. Thus, dopamine has a significant role in schizophrenic pathophysiology, an imbalance between dopamine and one (or more) of these other neurotransmitters systems may be the principal neurochemical abnormality related to the production of schizophrenic symptoms. The fundamental pathological processes associated with schizophrenia remain uncertain, but multiple lines of evidence suggest that this condition is associated with (i) excessive stimulation of striatal dopamine (DA) D₂ receptors, (ii) deficient stimulation of prefrontal DA D₁ receptors. The evidence for hyper stimulation of striatal D₂ receptors rests on strong pharmacological evidence and has recently received support from brain
studies. The hypothesis of deficient prefrontal cortex (PFC) D₁ receptor stimulation is almost entirely derived from preclinical studies. Preliminary imaging data compatible with this hypothesis have recently emerged (Laruelle et al., 2003).

**Serotonin**
There was substantial interest in the serotonin system and schizophrenia pathophysiology in the 1960s. Serotonergic mechanisms also play a role in schizophrenia, principally because clozapine and other antipsychotics distinguish themselves from the conventional neuroleptics by their potent antagonism of the 5-HT₂A receptors. Potent 5-HT₂A antagonists appears to be the only property common to atypical antipsychotics currently available, which produce fewer extra pyramidal side effects than earlier dopamine-selective compounds. Atypical antipsychotics retain efficacy against positive symptoms, clearly have lesser propensity to cause extra pyramidal side effects and perhaps tardive dyskinesia, and may have greater efficacy against negative and cognitive symptoms. Serotonin’s role in schizophrenia is now related to its modulation of dopamine neurotransmission (Meltzer 1991).

**Norepinephrine**
Alteration in norepinephrine (NE) neurotransmission has also been implicated in schizophrenia. A diminution of NE tone has been related to deficit symptoms in schizophrenia, whereas increase in NE activity (increase in metabolites in the cerebrospinal fluid and plasma) have been documented during stress induced exacerbation of psychosis (VanKammen et al., 1990). NE modulates DA functions, an interaction that may be the mechanism whereby the NE system plays role in schizophrenia.

**Glutamate**
Evidence from multiple fields of study suggests a role of glutamergic mechanisms (particularly glutamate hypofunction) in the pathophysiology of schizophrenia. Antagonism between the glutamatergic and DA systems, reduced activity at NMDA receptors in schizophrenia could explain functional hyperactivity of dopamine system. Nonetheless, glutamatergic mechanisms and glutamate-dopamine interactions appear
relevant and also offer the potential for novel therapeutic strategies in the schizophrenia (Goff et al., 2001).

**Acetylcholine**

Data from various sources implicate muscarinic cholinergic (ACh) hyperactivity in the production of negative symptoms and increase in positive symptom may result from a relative decrease in ACh activity (Tandon et al., 1989; Tandon et al., 1993 a, b).

**Other neurotransmitter systems**

Several other neurotransmitters (such as GABA, neurotensin, cholecystokinin, various opioids) have been implicated at different times in schizophrenia. It is likely that multiple neurotransmitter systems are relevant to the pathophysiology of schizophrenia. While the role of dopamine appears prominent, interactions between other neurotransmitter systems (serotonin, glutamate and acetylcholine) on the one hand and dopamine on the other are likely to be important in the pathophysiology of the illness. A better understanding of the role of these systems and their interactions with the dopaminergic neurotransmission in schizophrenia is likely to lead to the development of more efficacious treatments without side effects.

**G. Treatment**

A group of drugs, which are used in the treatment of psychotic disorders and share in common an antagonist effect on dopamine receptors, referred as antipsychotic drugs, or dopamine antagonist or major tranquillizers. Antipsychotics can be split into two types; typical antipsychotics and atypical antipsychotics. Both types work by altering the level of neurotransmitters in the brain. The neurotransmitter that is affected by typical antipsychotics is dopamine. A new generation of atypical antipsychotics has been introduced over the past decade. Atypical antipsychotics affect both dopamine and other neurotransmitters such as serotonin, histamine, norepinephrine, ACh etc. These atypical antipsychotics have comparable or greater efficacy than traditional antipsychotics in the treatment of the psychotic symptoms of schizophrenia and a much-improved neurologic side effect profile.
## Literature Review

<table>
<thead>
<tr>
<th>Typical Antipsychotics</th>
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<tbody>
<tr>
<td>• Chlorpromazine</td>
<td>• Clozapine</td>
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<td>• Droperidol</td>
<td>• Olanzapine</td>
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<td>• Flupenthixol</td>
<td>• Resperidone</td>
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<td>• Fluphenazine</td>
<td>• Sertindole</td>
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<td>• Haloperidol</td>
<td>• Quetiapine (Seroquel)</td>
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<td>• Penfluridol</td>
<td>• Ziprasidone</td>
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<tr>
<td>• Thioridazine</td>
<td>• Aripiprazole</td>
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<tr>
<td>• Trifluperazine</td>
<td>•</td>
</tr>
<tr>
<td>• Zuclopenthixol</td>
<td>•</td>
</tr>
</tbody>
</table>

### H. Animal models

**Catalepsy test (standard bar test)**

Catalepsy in laboratory is defined as failure to correct an externally imposed posture. When a normal animal is placed in an unusual posture, it will change its posture within seconds. A cataleptic animal, on the other hand will maintain this posture for a prolonged period of time (several minutes or longer). Catalepsy is one of the behavioral tools most used by neuroscientists to study the behavioral mechanisms of neurochemical systems. There are many ways of inducing the catalepsy in the laboratory animals and as many ways of measuring it. The typical catalepsy consists of placing an animal into an unusual posture and recording the time to correct this posture. This time is regarded as an index of the intensity of the catalepsy. The behavioral catalepsy test can employ any of several different types of apparatus, but the most common “standard bar test”, originally described by Kuschinsky and Hornykiewicz (1972). The bar test is by far the most popular method for measuring the catalepsy and “Haloperidol-induced catalepsy” is a widely employed method for screening of neuroleptic drugs in animals. The bar test is most popular method for studying the catalepsy in rodents. The animal usually with its forepaw is placed on the rectangle bar at appropriate height, so that the hind paws should touch the bottom. Time
was recorded when the animal returns to the floor. Catalepsy can also be studied without any apparatus by placing the animal in an unusual position on the flat surface and monitor the time for correcting the posture. The test is simple, does not require sophisticated instrument, non-invasive and does not require training or any painful stimuli.

**Stimulant induced locomotor activity**

Many investigators have used the amphetamine-induced locomotor activity as a reliable method for detecting neuroleptic activity. Amphetamine is an indirect acting sympathomimetic agent, which releases catecholamines from its neuronal storage pools. In animals the drug induces a characteristic hyper locomotor activity. Neuroleptic agents can prevent this activity. Inhibition of locomotor hyper activity induced by dopaminergic drugs has been a frequently used animal model to the study of limbic dopamine functions (Arnt, 1995) and this model can be regarded as a simple method to detect neuroleptic activity in rodents.

**Apomorphine induced climbing test**

Climbing behavior induced by apomorphine in mice is a potential model for the detection of neuroleptic activity. Mice exhibits wall climbing behavior following the subcutaneous (s.c) administration of apomorphine (Costall et al., 1978). An initial 30 minutes period was allowed to animals to get acquainted with the new environment. Untreated animals do not climb, while apomorphine treated animals showed climbing behavior. The range of doses of apomorphine causing the climbing behavior from threshold to maximum was 0.5-2.0 mg/kg s.c. 1.5 mg/kg s.c. was selected as a suitable dose of apomorphine inducing the sub maximal but intense and consistent climbing response, which could be used to analyze the actions of neuroleptic and related agents. Since the onset of climbing occurred within 5-7 minutes of apomorphine administration, and the duration of climbing was only 40-50 minutes. The apomorphine-induced climbing model is a test with a potential to detect antipsychotic compounds not only of classical but also agents with novel activity.
Conditioned Avoidance Response

Atipsychotic activity of a compound is widely studied using conditioned avoidance response (CAR) in experimental animals. This test is more routinely used to screen the compounds possessing the neuroleptic activities. It is carried out in rats using Cook’s pole climbing apparatus, with an electrified grid and a buzzer. A pole with a provision of removing and placing the pole in its position again when the animal climbs on it. The animals are given training to avoid shock after hearing the buzzer. During training buzzer and shock are simultaneously applied. After a week buzzer is applied and shock was given only when the animal did not climb. Rats usually climb the pole within few seconds of hearing buzzer. Blockade of the conditioned response i.e. unable to climb on hearing the buzzer is the main parameter. The conditioned response was taken as completely blockade if the animal fails to climb within 30 seconds on hearing the buzzer. After 30 seconds, a shock was applied. Blockade of conditioned response is regarded as motor paralysis or neurotoxic effect of drug.

I. Role of histamine in schizophrenia

Both experimental and clinical evidence exists implicating the role of central histaminergic system in the pathogenesis of schizophrenia (Rauscher et al., 1977; Rauscher et al., 1980; Pillot et al., 2002; Ito C, 2004). A relation between histamine and schizophrenia was described when a hyporesponsiveness of schizophrenic patients to histamine was observed (Rauscher 1977; 1980). Novoa and Cacabelos (Novoa et al., 2001) indicated malfunctioning of the histaminergic system with altered histamine levels and number of histamine receptors in brains of schizophrenic patients. Further, the mean levels of tele-methylhistamine, an index of histaminergic activity were found to be significantly elevated in CSF of schizophrenic patients (Prell et al., 1995). In addition to this, differential expression of histaminergic receptors has been reported in schizophrenia e.g. there is H_2-up regulation (Martinez-Mir et al., 1993), while H_1-down regulation (Nakai et al., 1993) in the brains of such patients. The dose dependent and complete blockade of amphetamine-induced increases in locomotor activity by THP and by ciproxifan (Morisset et al., 2002) and Alguacil et al (2003), proposed a potential use of H_3 receptor antagonists as therapeutic agents for the treatment of schizophrenia. The locomotor stimulant effects of
methamphetamine are also reduced but not completely absent in mice with deletions of the 
H₃ receptor gene (Toyota et al., 2002). Amphetamine increases tele-methyl histamine (tele-
MeHA) levels apparently as a compensatory response to dopaminergic stimulation (Ito et 
al., 1996). Increases have been reported in caudate putman, nucleus accumbens, 
hypothalamus and cortex (Morisset et al., 2002). Moreover, basal levels of tele-MeHA are 
augmented in methamphetamine-sensitized rats (Morisset et al., 2002), a model of 
schizophrenia. Like wise, tele-MeHA levels in cerebral spinal fluid have also been reported 
to be elevated in schizophrenic patients (Prell et al., 1995). However, the role of increased 
histamine turnover in schizophrenia remains to be clarified. If histamine turn over is 
directly linked to schizophrenic-like symptomatology, then increases in turn over with a H₃ 
receptor antagonist may be contradicted. Further evidence for a potential role of H₃ receptor 
antagonists in the treatment of schizophrenia comes from the data showing a potentiation of 
haloperidol-induced hypolocomotion and catalepsy and associated neurochemical 
alterations (e.g. enhanced up-regulation of enkephalin mRNA) by ciproxifan. A direct H₃ / 
D₂ receptor interaction leading to an enhanced activation of striatopallidal neurons has been 
suggested (Pillot et al., 2002). Furthermore, endogenous histamine and dopamine modulate 
striatal neurons (Pillot et al., 2003), supporting the potential of H₃ receptors as a novel 
targets for the treatment of psychotic disorders and drug abuse. An added advantage is that 
these agents appear to induce few motor adverse effects; the compounds suppressed the 
induction of c-Fos by haloperidol in the dorsolateral aspect of the striatum (Hussain et al., 
2002), an area implicated in the development of extrapyramidal motor symptoms. New data 
have shown that the H₃ receptor antagonists VUF 9153 and THP can potentiate the ability 
of methamphetamine to function as a reinforcer or discriminative stimulus in rats and to 
augment the increases in extracellular levels of dopamine in the shell of the nucleus 
accumbens in rats (Munzar et al., 2004). In this latter study, however, the H₃ receptor 
antagonists were not active when given alone. The data from Munzar et al (2004) indicate 
again that a dopaminergically driven system can be affected by H₃ receptor blockade. Data 
from antipsychotic treatments suggest instead that increase in tele-MeHA turn over may be 
related to their beneficial effects perhaps in the cognitive arena. Thus, only antipsychotics 
with good affinity for 5-HT₂A receptors increase histamine turnover as measured by tele-
MeHA an effect mediated by 5-HT₂A receptor blockade (Morisset et al., 1999). If the
Literature Review

Histamine turnover inducing effects of atypical antipsychotics are related to their efficacy, then H3 receptor antagonists due to their intrinsic ability to increase histamine turnover in brain may also have efficacy in the treatment of some symptoms of schizophrenia (e.g. cognition). More recently Fox et al (2005) and Esbenshade et al (2005) reported that ABT-239 blocked the psychostimulant effects of methamphetamine, a model of the positive symptoms of schizophrenia. Adding to the scientific evidence is the observation that antipsychotic drugs especially clozapine and olanzapine target histamine receptors, besides blocking dopamine receptors (Kata et al., 1993; Rodrigues et al., 1995). This indicates that dysfunctioning of histaminergic neuronal system may participate in the extradopaminergic brain dysfunction of schizophrenia and histaminergic agents may improve the refractory schizophrenia.
2.3. Cerebral ischemia (stroke)

A. Definition
A stroke is defined as a sudden loss of brain function, caused by a blockade or rupture of blood vessel to the brain. An ischemic stroke deprives neurons of oxygen and nourishment resulting in accumulation of noxious metabolites in the brain tissue originating from the injured or dying neurons. Accumulation of noxious metabolites increases with time, and ultimately results in injury to the surrounding healthy neurons. This process can be halted or even reversed in the ischemic brain tissue if restoration of blood flow occurs within a critical time period.

B. Prevalence
Stroke is the second largest cause of mortality and worldwide and most common cause of death in most industrialized countries after cardiovascular disease cancer, and its incidence is expected to rise with the projected increase in the number of ageing population. Stroke is estimated to be responsible for 9.5% of all deaths. It is also the most common neurological reason for hospitalization. More than 70% of stroke survivors remain vocationally impaired, more than 30% require help for activities of daily living, and more than 20% walk only with assistance (Gupta et al., 2004).

C. Types
Two major types of “strokes” cause brain damage: ischemic and hemorrhagic stroke. In ischemic stroke, which represents about 85% of total strokes, lack of circulating blood deprives neurons of oxygen and nourishment.
Ischemic strokes can be grouped into three main categories: thrombotic, embolic and global ischemic (hypotensive) stroke.

Thrombotic Stroke
Atherosclerosis is the most common pathological feature of vascular obstruction resulting in thrombotic stroke (Challa, 1999). Other pathological etiologies of vascular occlusion in
thrombotic stroke are: clot formation due to hypercoagulable state, fibromuscular dysplasia, arteritis, dissection of vessel wall and hemorrhage into a pre-existing plaque leading to an obstruction of the blood flow.

Embolic Stroke
Most emboli lodge in the middle cerebral artery distribution because 80% of the blood carried by the large neck arteries end up in MCA. The two most common sources of emboli are, the left- sided cardiac chambers and “artery to artery” emboli - as in detachment of a thrombus from the internal carotid artery at the site of an ulcerated plaque. Embolic strokes are generally smaller than thrombotic strokes. Many embolic strokes become “hemorrhagic” because ischemic tissue is often reperfused when the embolus lysed spontaneously and blood flow is restored to a previously ischemic area.

Global -Ischemic or Hypotensive Stroke
Profound reduction in systemic blood pressure for any reason is responsible for “hypotensive stroke.” Cerebral gray matter is particularly vulnerable. Global ischemia causes greatest damage to areas between the territories of the major cerebral and cerebellar arteries known as the boundary zone or watershed area. The parietal-temporal-occipital triangle at the junction of the anterior, middle and posterior cerebral arteries is most commonly affected. Watershed infarct in this area causes a clinical syndrome consisting of paralysis and sensory loss predominantly involving the arm. Face is not affected and speech is spared.

Hemorrhagic Stroke
In hemorrhagic stroke, extra vascular release of blood causes damage by cutting off connecting pathways, resulting in local or generalized pressure injury i.e. intracerebral hemorrhage, which is the result of the rupture of a vessel within the brain parenchyma. The primary cause of this rupture is hypertension. Hypertension is a major cause of hemorrhages of the basal ganglia and brainstem. Common locations of hypertensive hemorrhages include the putamen, caudate, thalamus, pons, and cerebellum.
D. Etiology
Stroke occurs when blood flow to the brain is interrupted by either a blockade or burst artery, resulting in a sudden decrease in the blood flow to the area of the brain, depriving brain cells of oxygen and other nutrients. Ischemia develops within minutes, forming two zones around the site of thrombosis or embolism. Brain cells at the center of ischemic region where the cerebral circulation is completely arrested, irreversible cell damage occurs in several minutes. However, cell in the area surrounding the center, the ischemia is incomplete because of the presence of perfusion from the collateral vessels. This region is called penumbra (Astrup et al., 1981), where reduced blood flow falls to the level below the threshold for electrical failure and above the threshold for energy failure. Restoration of cerebral blood flow, even to sub-optimal levels, provides an opportunity for those brain cells to recover and regain functionality.

E. Pathophysiology
Cerebral ischemia leads to a cascade of pathophysiological processes, which contribute to ischemic cell damage. Among the pathophysiological changes that are postulated to occur as a response to stroke are free radical production, excitotoxicity, disruption of sodium and calcium influx, enzymatic changes, stimulation of the inflammatory processes, endothelin release, activation of platelets and leucocytes, delayed coagulation and endothelial dysfunction. All these pathophysiological reactions individually and/or collectively contribute to the brain injury following the onset of stroke (Gupta et al., 2004).

The basic cascade of cerebral ischemia
Cerebral ischemia results from decreased or interrupted blood supply leading to reduced availability of glucose and oxygen in the territory of affected vascular bed and thereby causing cellular energy crisis.

Generation of free radicals during stroke: Role of the mitochondrial respiratory chain
Although free radicals and other oxidants are normally produced by all cell types, their production and elimination rates are equal therefore they are not noxious under normal
physiological circumstances. However, several types of environmental stresses lead to the formation of reactive oxygen and nitrogen species such as superoxide radical anion, hydroxyl radical, singlet oxygen, nitric oxide, and peroxynitrite. All of these molecules have been implicated in neuronal injury. The brain requires a continuous supply of oxygen and glucose to maintain normal function and viability. When this supply is interrupted, a cascade of events takes place, all being triggered by the depletion of energy source in the form of ATP. Thus the loss of ATP alters the cell function by interrupting the ATP-dependent processes, primarily the Na⁺/K⁺ ATPase, whose failure disrupts ionic gradients across the membranes. Glutamate is known to play a predominant role in the pathogenesis of ischemic brain injury. This excitatory amino acid is released at high concentrations within the core of the cerebral infarction and in the penumbral tissue, in these areas, glutamate over activates its receptors leading to a massive influx of calcium that activates a variety of catabolic processes that subsequently produce cell death (excitotoxicity). In addition, the augmented intracellular Ca²⁺ further promotes increase in extracellular glutamate, thus propagating the excitotoxicity.

The main event during cerebral ischemia is the generation of free radicals and other oxidant species; due to their high reactivity, they provoke damage to lipids, DNA, and proteins, leading to neuronal death. They also contribute to the breakdown of blood-brain barrier and brain edema. These reactive species include nitric oxide and super oxide radical anions (O₂⁻), two free radicals with a great tendency to react with each other to form the powerful oxidant peroxynitrite (ONOO⁻). Other important oxidant species are hydrogen peroxide (H₂O₂) and hydroxyl free radical (·OH).
Figure 1. Sources of nitric oxide (NO), superoxide (O$_2^-$) and peroxynitrite (ONOO$^-$) in stroke (Moro et al., 2005).
Vessel occlusion
\[ \downarrow \]
Decreased cerebral blood flow
\[ \downarrow \]
Tissue $O_2$ and glucose
\[ \downarrow \]
Failure of $Na^+ / K^+$ pump
\[ \downarrow \]
thrombolysis
\[ \downarrow \] Tissue ATP
\[ \uparrow \] anaerobic glycolysis
\[ \rightarrow \] Neuronal depolarization

Glutamate release
\[ \rightarrow \] opening of $Ca^{2+}$ channels

AMPA receptors
\[ \downarrow \]
Metabotropic receptors
\[ \downarrow \]
NMDA receptors

$Ca^{2+}$ release from intracellular stores
\[ \rightarrow \]
$Ca^{2+}$ influx
\[ \rightarrow \]
Elevated intracellular $Ca^{2+}$
\[ \rightarrow \]
Free radical formation

Endogenous

**NEURONAL CELL DEATH**

Figure 2: Diagrammatic representation of events that may lead to cell death after the onset of cerebral ischemia (Gupta et al., 2004).
F. Treatment

Present therapeutic approaches to stroke are centered on two distinct approaches; one is primarily vascular (reperfusion) while the other is neuronal (neuroprotection). Reperfusion has conventionally considered as a very attractive approach since perfusion failure underlies all ischemic strokes, and the relief of the initiating event should prevent all consequences of neuronal ischemia. Intravenous tissue plasminogen activator (t-PA) was approved for use in ischemic stroke in 1996. Because it must be given within 3 hours of symptom onset, rapid and efficient evaluation is essential for all patients with stroke-like symptoms who are potential t-PA candidates. Aspirin has been found to be of modest but significant benefit during the acute phase of stroke. According to the combined results of two large trials that enrolled a total of 35,580 patients, aspirin therapy resulted in 9 fewer deaths or nonfatal strokes per 1000 patients during the first few weeks post-stroke among patients who were treated within 48 hours of the onset of their initial stroke (International Stroke Trial C Group 1997, CAST 1997).

A wide variety of drugs which interfere at various points in the ischemic cascade, so called neuroprotective agents have also been studied but with mixed successes. Of these, antagonists and scavenger of free radicals have been most extensively studied. Despite proving effectiveness in animal's models of acute ischemic stroke these drugs have largely failed to fulfill their promise in clinical trials and currently there is no neuroprotective drug, which is being used clinically. Therefore, presently only rt-PA is the drug used clinically for treatment of stroke.

Limitations of current therapy

A large number of drugs with varying mechanism of actions have been studied in humans, but as yet, there is no medical treatment approved for the treatment of stroke beyond tissue plasminogen activator, a thrombolytic agent restricted to administration within 3 hours after stroke. Aspirin, other antiplatelet, and anticoagulants are used as preventive therapy. None of the currently available neuroprotective agents have demonstrated unequivocal efficacy when administered after stroke in humans although a number of compounds are in development stage. Thrombolytic therapy may lead to a decrease in neuronal damage and
improve recovery after acute ischemic stroke. With the higher dose there is reperfusion damage and hemorrhage transformation are suggested to be responsible for the majority of complications. Lower dose of thrombolytic agent may decrease the risk factor but also decrease its effectiveness. Preliminary evidence suggests that combination of a neuroprotective agent may have additive effect.

Table 6: Compounds that have failed recently in clinical evaluation for the treatment of acute ischemic stroke (Green et al., 2003).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism of action</th>
<th>Outcome</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salofotel</td>
<td>NMDA antagonist</td>
<td>Negative (III)</td>
<td>Adverse events</td>
</tr>
<tr>
<td>Carvene</td>
<td>Kappa opioid peptide receptor antagonist</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>Lebeluzole</td>
<td>NOS inhibitor and Na⁺ channel blocker</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>Gavistinol</td>
<td>Antagonist at the glycine site of NMDA receptor</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>Enlimomab</td>
<td>Anti-ICAM antibody</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>Citicoline</td>
<td>Cell membrane stabilizer</td>
<td>Negative (III)</td>
<td>Lack of efficacy and adverse events</td>
</tr>
<tr>
<td>Ca²⁺ antagonists</td>
<td>Ca²⁺ channel antagonists</td>
<td>Negative (meta analysis)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>Aptiganel</td>
<td>NMDA receptor antagonist</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>Clomithiazole</td>
<td>GABA&lt;sub&gt;A&lt;/sub&gt; receptor modulator</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
<tr>
<td>BMS204352</td>
<td>K⁺ channel blocker</td>
<td>Negative (III)</td>
<td>Lack of efficacy</td>
</tr>
</tbody>
</table>
What will be the ideal drug for stroke?

Various agents have been developed for the treatment of stroke, however only a few drugs have shown limited efficacy in the clinical trials. Recently protective effects of antioxidant agents like melatonin (Sinha et al., 2001), adenosine (Gupta et al., 2002), resveratrol (Sinha et al., 2002), \( \alpha \)-tocopherol (Chaudhary et al., 2003) and also the combination of antioxidant like melatonin and meloxicam (Gupta et al., 2002) in experimental model of middle cerebral artery occlusion in rats. TAK-044 has recently been documented to have antioxidant and anti-inflammatory properties (Gupta et al., 2005). However, an agent which can improve the blood flow and also beneficial effect on the biochemical cascade will be more useful in the treatment of cerebral ischemia. Also the agents, which relieve the spasm of the nutrient artery and having an antioxidant property, could be an additional important approach to prevent the future damage immediately after the spasm of the affected artery in the brain. The rationale for the combination therapy is based on the increasing knowledge of the pathophysiological mechanisms of the ischemic brain damage. Each agent affects only one of the several mechanisms in the ischemic cascade whereas the combination therapy will affect various points in the cascade (Gupta et al., 2004). With the associated side effects of the western medicines, traditional medicines are gaining lot of importance and are now being studied to find the scientific basis of their therapeutic actions. However, Indian herbals that can increase blood flow and have antioxidant activity in the brain may have a potential against this disorder.

G. Prevention

Risk factors for stroke are either modifiable or non-modifiable. Among the latter are age, sex, race/ethnicity, and family history. Beginning at age 55 years, the rate of stroke doubles every decade. Men are more likely than women to experience early stroke (prior to age 65 yr) and carotid artery stenosis. With respect to race, blacks have a higher incidence of both ischemic and hemorrhagic stroke than do non-Hispanic whites. This difference has been ascribed to the higher prevalence of hypertension and diabetes among blacks.
Non-modifiable risk factors for stroke

- Age
- Sex
- Race/ethnicity
- Family history

Modifiable risk factors for stroke

- Hypertension,
- Diabetes,
- Cigarette smoking,
- Hyperlipidemia,
- Carotid artery stenosis,
- Atrial fibrillation,
- Excessive alcohol consumption
- Physical inactivity

II. Animal models

Innumerable in vitro and in vivo models of cerebral ischemia have been described over the years. The in vitro models include cultured neurons with or without synaptic formation, glia and cultured brain slice. However, these models can only indicate the levels of cytotoxicity of the therapy. The experimental model of stroke should fulfill the following:

- The production of ischemia should be constant, reliable and reproducible.
- High percentage of infarcts can be produced with the predictable average size.
- The neurological and pathological evaluation should not be too complicated.
- Physiological variations are controllable without much difficulty.
- No barbiturates to be used at the time of arterial occlusion
- Neuroimaging studies such as auto radiography, MRI etc can be performed economically and easily.
- Long-term follow up is possible and resistant to infection.
- Economical and easy availability of animals of same species.
Classification of animal ischemia models.
A good in vivo animal model of stroke must reproduce the etiology, anatomical, functional and metabolic consequences of human pathology and must also permit the study of anti-ischemic drugs in conditions pertinent to the clinical therapeutics (Gupta et al., 2004)

Experimental ischemia models

- Focal Ischemia
  - Middle cerebral artery occlusion
  - Single carotid artery occlusion

- Global Ischemia
  - Total body ischemia
  - Global cerebral ischemia

- Forebrain Ischemia
  - Bilateral common carotid occlusion
  - Four vessels occlusion

Models of global ischemia

In the models of global ischemia, there is total interruption of blood supply to the brain leading to cerebral necrosis. It can be induced broadly by two ways either by total body ischemia or by global cerebral ischemia (Green et al., 2003; Gupta et al., 2004)

Global models

I. Total body ischemia
- Decapitation
- Cardiac arrest and resuscitation
- Profound systemic hypotension

II. Global cerebral ischemia
- Increased intracranial pressure
- Combination of occlusion of the major artery
- Cervical compression
I. **Total body ischemia**: The total body ischemia can be induced by the following methods:

a. **Decapitation and cardiac arrest without resuscitation.** Decapitation of animals is the easiest way to achieve global cerebral ischemia. However, the major disadvantage of this model is that studies involving post ischemic re-circulation is impossible. The use of this model is limited for studying the morphological and biochemical changes during early stages of ischemia.

b. **Cardiac arrest and resuscitation.** This model can be carried out in various animal species like monkeys, dogs, cats, and rats to induce complete global ischemia. Cardiac arrest can be induced by injection of potassium chloride; electric shock and the mechanical obstruction of the ascending aorta whereas resuscitation is carried out using artificial ventilation, closed chest cardiac massage and electrical defibrillation. It has advantages in the term that it resembles to the clinical setting (mimic cardiac arrest in man), however, the exact ischemic duration limits the adoption of this model to specific experiments. As if the time of cardiac arrest increases from 8 minutes the survival rate becomes low and also the attempts of resuscitation becomes difficult because of brainstem ischemia and involves intensive care including use of various drugs which can interfere with the studies (De Garavilla et al., 1984).

c. **Profound systemic hypotension.** It can be induced by pharmacological agents, results in incomplete but almost global cerebral ischemia. The main advantage of this model is the ease of resuscitation of the animals, however, possibly variability in severity and regional distribution of cerebral ischemia limits the use of this model.

II. **Global cerebral ischemia**: Global cerebral ischemia can be induced by the following methods:

a. **Increased intracranial pressure.** Cerebral perfusion pressure is the difference between the arterial blood pressure and the intracranial pressure. When the intracranial pressure is
increased more than the systolic arterial pressure, it results in a zero cerebral blood flow. It can be carried out in dogs, rabbits, and rats. Advantage of this model is the ease of the control of the brain temperature by changing the temperature of the fluid injected. Although in this model, global cerebral ischemia can easily be achieved the primary pathological process is not ischemia but intracranial hypertension (Marshall et al., 1975; Hallenbeck et al., 1977).

b. Combination of occlusion of the major arteries. It can be performed in the large animals like dogs, cats, and rabbits by the surgical occlusion of the major arteries at a time. It can be combination if common carotid, vertebral, restocervical, mammary, omocervical and axillary artery via left thorectomy or clamping of the descending aorta, brachiocephalic and left subclavian artery. The major limitation of this model is the involvement of extended intrathoracic or intracranial surgery. Precautions have to be taken while performing the surgery as this can result in myocardial injury.

c. Cervical compression. Occlusion of blood vessels by tourniquet when combined with systemic hypotension results in ischemic damage in the brain. It seems difficult to completely occlude the vertebral and anterior spinal arteries from anatomical point. Cervical compression model should be considered not a global cerebral ischemia, but a forebrain ischemia. However, the effect of compression of the various cervical nerves and ganglia on cerebral ischemic injury and post ischemic cerebral circulation is unknown.

Models of forebrain ischemia
Models of forebrain ischemia were first reported in early 1980’s and have been extensively used for the studies on various aspects of cerebral ischemia. Since the cerebral ischemia in these models is not completely global but restricted to the forebrain, these models do not accurately represent cerebral ischemia encountered in clinical settings in which bilateral forebrain ischemia rarely occurs.
Models of forebrain ischemia

- Bilateral common carotid occlusion in Mongolian gerbil
- Four vessel occlusion in rats
- Bilateral common carotid occlusion in spontaneously hypertensive rats
- Two vessel occlusion in rats with hypotension

a. **Bilateral common carotid occlusion in Mongolian gerbil.** It is the most popular model because of the relative ease of the surgical technique involved. The absence of posterior communicating arteries in the gerbils, which are necessary to complete the circle of Willis, enables forebrain ischemia to be induced by only occluding both common carotid arteries. (Levine et al., 1955). The major limitation of this model is that inter-animal variability in the severity of ischemia is very high. Moreover gerbils are known to have a high percentage of post ischemic seizures especially when the duration of ischemia is longer than 15 min. Moreover small size of the gerbils lends difficulties in ministering of physiological variables.

b. **Four-vessel occlusion in the rats.** This is also widely used model since reported by Pulsinelli and Brierley, 1979 (Pulsinelli et al., 1979). It involves difficult surgical procedure. On the first experiment day occlusion devices are placed around both common carotid arteries following surgical dissection and then both vertebral arteries are electrocoagulated at the first cervical level following occipital nuchal dissection. Twenty-four hour later both common carotid arteries are occluded in the awaked rats (Pulsinelli et al., 1988). However a drawback may be inconsistency of the ischemia owing to the difficulty in conforming the complete arterocutetization of the vertebral arteries and existence of collateral pathways mainly from the anterior spinal artery.

c. **Bilateral common carotid artery occlusion in spontaneously hypertensive rats.** Bilateral common carotid artery does not consistently produce ischemic changes in the brain of normal rats. However, bilateral common carotid occlusion in spontaneously hypertensive rat is known to produce consistent ischemic change in the brain. The carotid artery occlusion...
in this model is permanent and the histopathological changes are seen in the forebrain area. Moreover this model is very important since hypertension is one of the major risk factors of cerebral ischemia and the occurrence of severe vascular changes secondary to hypertension may be operative in cerebrovascular diseases. The major limitations of this model are the high cost and mortality rate within 24 hr of occlusion (Ogata et al., 1976). Development of hypertension significantly varies depending on the age and sex. Therefore experimental studies must be performed carefully.

d. Two-vessel occlusion in rats with hypotension. It is simple method and permits rapid screening. It produces delayed and selective neuronal death. Monitoring of physiological variables can be carried out easily. It has been reported that there was no change in the energy state in the tissue following ligation of the carotid arteries only, when combined with systemic hypotension there was a severe change in the energy states. In this model when ischemia was longer, damage was also seen in the caudoputamen and pars reticulata of the substantia nigra. There is deleterious effect of hyperglycemia on ischemic brain injury using this model. The major disadvantage of this model is the alteration in the physiological variables as a result of hypotension.

Models of focal ischemia
Focal ischemia is the most commonly encountered type of stroke in humans. Among of many causes of cerebral ischemia, occlusion of a single trunk artery particularly the internal carotid or middle cerebral artery is the most frequent. It is different from the global ischemia that it produces a heterogeneous pathology, which includes a necrotic core and a penumbra. In the necrotic core, the area at the center of the ischemic territory, there is pan necrosis in which both neurons and glia die. In the penumbra neurons are of therapeutic intervention. Focal ischemia can be divided as permanent or transient. In the permanent focal ischemia model dense region of ischemic damage (core) is formed and the degenerative changes are seen spread out in a large area from this region. However, clinically it is very rare that the cessation of the blood flow occurs permanently in the brain region because in the majority of stroke cases thrombus disintegration and endogenous thrombolysis occurs and the damage is the result of both ischemia and the consequent of reperfusion. Therefore reversible model of
focal ischemia is more clinically relevant than the permanent model. Methods for inducing focal ischemia are given below.

I. Photochemical occlusion with craniotomy without dural opening (Photothrombosis model). This is permanent model of focal ischemia. After exposing the artery to be occluded a photosensitizing dye, Rose Bengal is administered intravenously with simultaneous laser irradiation (Argon laser-activated dye laser operating at 562 nm). Advantage of this model is that it is relatively noninvasive (Watson et al., 1985). However it is not clear whether the thrombus permanently occludes the artery and moreover penumbral region is not seen in this model that have a closer clinical correlate. Other model in the category include carbon microsphere injection into the internal carotid artery, injection of platelet aggregates into the common carotid, injection of small blood clots into common carotid (Kudo et al., 1982).

II. Occlusion of middle cerebral artery. Eighty percent of strokes occur in the territory of the anterior circulation and the majority of these effects the territory of the middle cerebral artery (MCA) (Sudlow et al., 1997). Therefore this model is the most widely used to study focal ischemia. There are three different types of MCA occlusion.

a. Mechanical or electrical arterial occlusion with craniotomy and dural opening:
Proximal MCA occlusion: In this method the coronoid process of the mandible and zygoma is removed and burr hole opened lateral to the forebrain ovale. MCA is identified through the burr hole and occluded at the proximal end. The advantage of the above model is that arterial occlusion is confirmed directly through operative microscope, both temporary and permanent MCA occlusion are possible, mortality rate is low and any kind of monitoring system is applicable because the rat is fixed on a stereotaxic frame. The disadvantage of the above model are exposure of the brain to the air during the craniotomy may alter intracranial pressure and blood brain barrier permeability, clipping or cauterization of the proximal MCA may cause damage to the autonomic nerves around the MCA and auto regulation of cerebrospinal fluid may be lost, requires surgical skillfulness under an operating microscope. Moreover occlusion of only the origin of the MCA does not produce consistent infarcts (Tamura et al., 1981).
Distal MCA occlusion with bilateral common carotid arteries occlusion: This model has produced consistent cerebral infarction with relatively non-invasive surgery. The right distal middle cerebral artery and right common carotid artery are ligated and the left common carotid artery is clipped temporarily. A small contraateral infarction is encountered occasionally. This model can be used as a transient focal ischemia model.

Intraluminal arterial occlusion without craniotomy: Focal ischemia is the most commonly encountered type of stroke in humans (Trystman, 2003). However, clinically it is very rare that the cessation of the blood flow occurs permanent in the brain region because in the majority of stroke cases thrombus disintegrates and endogenous thrombolysis occurs. The neuronal damage is the result of both ischemia and consequent reperfusion. Koizumi et al (Koizumi et al., 1986) developed model of MCA occlusion without craniotomy. In this model a midline incision was made and the right common carotid artery was exposed. A 4.0 monofilament nylon thread (Ethicon, Johnson and Johnson) with its tip rounded by heating quickly by bringing it near a flame was used to occlude the middle cerebral artery. The filament was advanced from the external carotid artery into the lumen of the internal carotid artery until a resistance was felt which ensured the occlusion of the origin of middle cerebral artery. The nylon filament was allowed to remain in the place for 2 hr after which it was gently retracted so as to allow the reperfusion of the ischemic region to induce focal ischemia transiently. The major advantages of this model are firstly the method is simple and secondly the MCA can be occluded and reperfused without craniotomy and for these reasons this model has achieved wide popularity (Koizumi et al., 1986). However the major drawback of this model is high mortality in the permanent ischemic models, therefore reversible model of focal cerebral ischemia (MCAO model) is clinically more relevant than the permanent occlusion model.
New stroke models:

Transgenic mice models
Delayed neuronal death is also a major cause of the high morbidity and mortality associated with stroke (focal cerebral ischemia). Attention has recently been shifted to mice. This is because transgenic mice can be developed relatively easy. Using these models one can investigate the efficacy of anti-apoptotic proteins in preventing the delayed neuronal death after focal cerebral ischemia in transgenic mice. The major problems that are being faced in the development of transgenic mice models for cerebral ischemia is the variability in the vascular territories in the different species of mice used to generate transgenic mice.

Neonatal hypoxic model
Cerebral hypoxia-ischemia remains a major contributor to perinatal morbidity and mortality. It is estimated that between 0.2 to 0.4% of full-term infants and up to 60% of premature infants experience asphyxiation at or before birth. An established model of neonatal hypoxia/ischemia is being used recently. Ligation of the right common carotid artery and treatment with 8% oxygen produces ipsilateral brain damage (Arteni et al., 2003). Oxygen sensitive genes, apoptosis and neurological evaluations can investigate using this model.

I. Role of histamine in cerebral ischemia
Since excess release of neurotransmitters exerts influences on the outcome caused by ischemia, there are many reports that studied changes in the brain concentrations of neurotransmitters using animal models of cerebral ischemia. With respect to histamine, the brain content of histamine has been reported to increase during cerebral ischemia in primates. Adachi et al reported that occlusion of middle cerebral artery gradually increased the brain concentration of histamine in the striatum and surrounding cerebral cortex (Adachi et al., 1991). Likewise, a gradual increase in the extra cellular concentration of histamine was observed in the striatum using a microdialysis procedure (Adachi et al., 1992). However, there is a marked difference in ischemic release between histamine and other neurotransmitters. The extra cellular concentration of histamine increases gradually
over several hours, whereas release of glutamate and dopamine occurs immediately after induction of cerebral ischemia, and their values resume to the basal levels after reperfusion of the blood flow. Thus, histamine is released from nerve endings in a manner dissimilar to ischemic release of other neurotransmitters. The increase in the brain histamine level may contribute to the amelioration of ischemic neuronal damage. Augmentation of central histaminergic activity by histidine loading after ischemic events could prevent neuronal injury by stimulating the histamine H$_2$ receptors. Since peripheral administration of histidine is an easy technique in clinical situations, histidine therapy, including combination treatments with other neuroprotective agents, may be a new strategy for brain protection. Augmentation of central histaminergic activity by blockade of histamine H$_3$ receptors or inhibition of histamine inactivation may facilitate the effect of histidine treatments by increasing the histamine concentration in the brain. On the other hand, it was shown that impairment of histaminergic transmission or suppression of histaminergic activity may aggravate ischemic neuronal damage (Sugimoto et al., 1994; Adachi et al., 2001; Adachi 2002).

J. Nitric oxide (NO) and cerebral ischemia

There is an increase in nitric oxide (NO) production during ischemic damage and it has a dual effect that is either toxicity or neuroprotection. The role of NO production during ischemia is currently debated. Some of the proposed mechanisms for toxicity include the reaction of NO with superoxide anion and production of peroxynitrite, a known toxic compound. Other argues that NO might react with thiol groups on the NMDA receptors and reduce toxicity by modifying the NMDA receptor response (Khaldi et al., 2002).

It has been shown that inhibition of NO synthesis attenuates NMDA neurotoxicity in neuronal cultures and reduces the brain damage produced by occlusion of the middle cerebral artery in mice (Nowicki et al., 1991). These initial observations provided evidence that NO is involved in the mechanisms of cerebral ischemia. It is reported that NO plays a crucial role both in the acute phase of ischemic damage and in the delayed events
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contributing to its evaluation (Ladecola, 1997). The dual role of NO is suggested by the fact that NO, a potent vasodilator and an inhibitor of platelet aggregation and leucocyte adhesion improve post ischemic blood flow by enhancing collateral circulation and preventing microvascular plugging by platelets and leucocytes. NO also inhibit Ca\(^{2+}\) influx through the NMDA receptor and limit glutamate neurotoxicity in cerebral ischemia (Khaldi et al., 2002). In addition, NO scavenges reactive oxygen species and partially offset ischemia-induced oxidative damage (Ladecola, 1997). On the other hand, NO, a well known cytotoxin, adversely affect the ischemic brain by several mechanisms. NO promotes oxidative damage by reacting superoxide anion to form peroxynitrite (a strong oxidant) and by perturbing iron metabolism. NO either directly or through its derived species, causes energy failure, produces DNA damage, inhibits DNA synthesis and triggers programmed cell death (Ledecola, 1997). Furthermore, NO might exacerbate damage by enhancing the post-ischemic release of excitatory neurotransmitters. Therefore, the biological properties of NO suggest that this agent can either be protective or destructive in cerebral ischemia.

The impact of NO on ischemic brain injury depends on the stage of evaluation of the tissue damage. Studies using L-arginine and NO donors demonstrate clearly that in the early stages following cerebral ischemia (< 2 hr), the vascular actions of NO are beneficial by promoting collateral circulation and microvascular flow. At the same time, glutamate-induced Ca\(^{2+}\) overload in ischemic neurons leads to a persistent activation of nNOS resulting in continuous NO production. At later times (> 6 hr), iNOS is expressed and large amounts of NO produced by this enzyme contribute to the progression of the tissue damage. Thus, the iNOS inhibitor aminoguanidine, administered more than 6 hr following cerebral ischemia, ameliorates cerebral ischemic damage (Ledecola, 1997).

After middle cerebral artery occlusion (MCAO) in rat, NO levels have been found to be elevated up to the micromolar range within 20 minutes by using electrochemical NO microsensors (Moro et al., 2005). In addition electron paramagnetic resonance spectroscopy with spin-trapping agents has detected NO elevations after bilateral carotid occlusion or focal cerebral ischemia in rat brain. Other indirect methods such as
determination of the NO metabolites nitrate/nitrite also indicate NO production in the ischemic brain, even in humans. In this context, CSF and serum levels of nitrate/nitrite are higher in stroke patients than in control patients (Moro et al., 2005) and also in patients with subarachnoid hemorrhage. Moreover, it has been shown that CSF and plasma levels of l-arginine, the biosynthetic precursor of NO, are decreased after an ischemic event.

In the very early stages of ischemia the beneficial vascular effects of endothelial NO outweigh the neurotoxic potential of neuronal NO. A few hours following ischemia, however the vascular effects of NO are no longer protective and NO becomes predominantly neurotoxic. In the late stages of cerebral ischemia (> 6hr), iNOS is expressed in the setting of post-ischemic inflammation. Large amounts of NO, produced by this enzyme contribute to the delayed progression of the damage. Therefore, the role of NO in ischemic brain injury is protective or destructive depending on the stage of evaluation of the ischemic process and on the cellular compartment producing NO (Ledecola, 1997).
2.4. OXIDATIVE STRESS AND NEUROPSYCHIATRIC/NEUROLOGICAL DISORDERS

2.4.1. Oxidative stress

Oxygen removes electrons from other molecules in the cell to form reactive oxygen species (ROS). These species are major contributing factors in diseases. ROS are controlled by a defense system that depends on the activity of enzymes and other nonenzyme substances. The imbalance between ROS and the body's defense system is called oxidative stress. Scavengers of ROS and antioxidants are important in treating and preventing the damage caused by such stress. (Supinski, 1998; Gutteridge et al., 1999)

Oxygen is necessary in the metabolic production of energy. Mitochondria, through the electron transport chain, use oxygen to oxidize other molecules and generate energy in the form of adenosine triphosphate. (Pugliese, 1995; Gutteridge et al., 1999). During this process, oxygen is reduced to water, producing several intermediary ROS. Reactive Oxygen Species (ROS) is a term collectively describing radicals and other non-radical reactive oxygen derivatives. These intermediates may participate in reactions giving rise to free radicals or that are damaging to organic substrates. ROS in living organisms include the following:

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Non-Radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl</td>
<td>OH⁻</td>
</tr>
<tr>
<td>Superoxide</td>
<td>O₂⁻</td>
</tr>
<tr>
<td>Nitric Oxide</td>
<td>NO⁻</td>
</tr>
<tr>
<td>Thyl</td>
<td>RS⁻</td>
</tr>
<tr>
<td>Peroxyl</td>
<td>RO₂⁻</td>
</tr>
<tr>
<td>Lipid peroxy</td>
<td>LOO⁻</td>
</tr>
<tr>
<td>Peroxy nitrite</td>
<td>ONOO⁻</td>
</tr>
<tr>
<td>Hypochloric acid</td>
<td>HOCI</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H₂O₂</td>
</tr>
<tr>
<td>Singlet Oxygen</td>
<td>¹⁻O₂</td>
</tr>
<tr>
<td>Ozone</td>
<td>O₃</td>
</tr>
<tr>
<td>Lipid peroxide</td>
<td>LOOH</td>
</tr>
</tbody>
</table>

Many other radical species can be formed by biological reactions, for example: phenolic and other aromatic species are often formed during xenobiotic metabolism as part of natural detoxification mechanisms. The production of ROS is one reason the
administration of high concentrations of oxygen may be toxic; an increase in oxygen concentration leads to the formation of these species. (Fuhannan, 1999).

The discovery of superoxide dismutase (SOD), a free-radical scavenger, in 1969 by McCord and Fridovich generated a wave of research on the balance between oxidants and antioxidants. (Pugliese, 1995) An accumulation of oxidants is due to either pathophysiological processes or the inability of antioxidants to counter the accumulation. A lack of production of antioxidants or a destruction of scavengers causes the overproduction of free radicals. An imbalance between production of ROS and production of antioxidants results in oxidative stress (Lawler, 1998).

Formation of ROS

During chemical processes, molecules can be reduced or oxidized. A molecule with an unpaired electron can combine with a molecule capable of donating an electron. The donation of an electron is called oxidation. The gain of an electron is called reduction. During these reactions, the molecule that donates the electron is oxidized and the molecule that accepts the electron is reduced. Reduction and oxidation can leave the reduced molecule unstable and free to react with other molecules to cause damage to cell membranes, proteins, and DNA. These reduced substances are called free radicals (Pugliese, 1995).

In homeostasis, the rates of reduction and oxidation are equal. The reduction-oxidation (redox) balance is maintained by physiological defenses such as specialized enzymes and antioxidants. These antioxidants and enzymes remove or inactivate the free radicals. Some of the most common enzymes and antioxidants are listed in table. The enzymes and antioxidants can become overwhelmed by an increase in reduced molecules or by a decrease in the defense system, allowing the accumulation of free radicals. (Pugliese, 1995; Gutteridge et al., 1999; Espat et al., 2000)

ROS are molecules or atoms formed by reduction of oxygen and can be either free radicals or nonradicals. Within cells, free radicals can be produced by absorption of
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radiant energy, metabolism of drugs, normal metabolic processes, and transition metals. (Cotran et al., 1999) These radicals are designated by using the superscript dot; for example, the hydroxyl radical is designated [OH*]. The oxygen nonradicals can act as oxidizing agents or can be easily converted into radicals. (Halliwell, 1996) The most commonly known ROS are superoxide [O_2*], hydrogen peroxide [H_2O_2]), and [OH*].

A process in which 4 electrons must be added to the structure of oxygen reduces oxygen. In the first step, an electron is added, and [O_2*] is produced. At this point, some [O_2*] can leak out of the mitochondria and set up other chemical reactions, leading to cellular damage. (Supinski, 1998) Superoxide acts as a reducing agent and helps produce other oxidants through chemical reactions. The subsequent steps of adding electrons to oxygen produce other types of ROS (ie, [H_2O_2] and [OH*]) until oxygen is fully reduced.

Many other molecules in the body, such as adrenalin, steroids, and folates, can react with oxygen to produce [O_2*]. Phagocytes are another important producer of [O_2*]. The phagocytes use [O_2*] as a defense against foreign organisms. Defense mechanisms that are a vital part of the immune system can also be destructive. Many inflammatory diseases are characterized by tissue damage produced by ROS (Parkinson, 1995). Although [O_2*] is not considered highly reactive, it is involved in other chemical reactions that produce free radicals.

Hydrogen peroxide, which is produced mainly by the mitochondria, is the most stable of the ROS. SOD, a ROS scavenger enzyme, removes [O_2*] in a reaction that produces [H_2O_2] Hydrogen peroxide can inactivate enzymes, cross cell membranes, and react with both iron and copper ions to produce [OH*] (Pugliese, 1995)

The hydroxyl radical is very damaging and can react with many substances. Hydroxyl radicals are usually produced through the Fenton reaction, in which [O_2*], [H_2O_2], and iron are teamed to form [OH*]. DNA damage is a major injury caused by [OH*]. (Espot 2000). DNA damage due to [OH*] is a contributing factor in cancer and other diseases. (Parkinson, 1995; Halliwell, 1996)

Ischemia-reperfusion injury is also associated with the formation of oxygen free radicals. Endothelial cells respond to ischemia and produce xanthine oxidase. The xanthine oxidase is responsible for the formation of [O_2*] during reperfusion. Endotoxin,
cytokines, neutrophil adherence, and acidosis can all trigger production of xanthine oxidase in endothelial cells. (Espan, 2000)

**ROS and Damage**

The free radicals \([O_2^*], [H_2O_2],\) and \([OH^*]\) are responsible for damage to lipids, proteins, and DNA. Hydroxyl radicals can initiate lipid peroxidation in the cell membrane. Within the lipid part of the membrane, \([OH^*]\) removes hydrogen from unsaturated fatty acids. This reaction produces a lipid radical that removes another hydrogen molecule from the next molecule and thus creates another lipid radical. The entire process is the chain reaction called lipid peroxidation. This reaction can cause cell death and can induce an inflammatory response. As neutrophils invade, they release more ROS and the cycle continues. (Cotran, 1999)

Proteins, particularly enzymes, are damaged by ROS. Hydroxyl radicals oxidize amino acids such as lysine, serine, arginine, and proline. Enzymes modified by oxidation are inactive or may be changed in such a way that the immune system interprets them as foreign and produces autoantibodies to them. (Pugliese, 1995) The human body has a tremendous capacity to repair damage caused by ROS; however, oxidative stress can damage DNA by causing strand breaks, base modification, and cross-linking (Halliwell, 1996). Hydroxyl radicals can attack thymine, change its chemical structure, and remove it as a base. The modified thymine and other changed DNA by-products can be detected in the urine and are an indication of oxidant activity (Pugliese, 1995).

**Enzyme and Nonenzyme Defenses Against ROS**

Oxidants are balanced by the activities of enzymes and nonenzymes called antioxidants. This vitally important defense system controls the production and elimination of oxidants and is essential in controlling the damage that occurs during oxidative stress. Normally, production of ROS is balanced by the activity of antioxidants. However, when the body is overwhelmed by increased production of oxidative agents and defense against these agents is decreased, the ensuing damage contributes to cellular derangements, cell injury,
and death. These pathological changes are manifested through critical illnesses and
diseases (Halliwell, 1996; Gutteridge, 1999).

The human body maintains a balance between the necessary oxidant actions involved in
cell processes and the damage done by oxidants both by preventing formation of free
radicals (through scavenging or removing ROS) and by repairing the damage done by
ROS. (Pugliese, 1995; Halliwell, 1996; Gutteridge, 1999)

The main scavengers responsible for inactivation and termination of free oxygen radicals
are SOD, catalase, and the glutathione system. The intracellular antioxidant SOD
catalyzes the conversion of $[O_2^+]$ to oxygen and $[H_2O_2]$. The $[H_2O_2]$ is then reduced by
either catalase or glutathione peroxidase. Catalase is the oldest known enzyme that
converts $[H_2O_2]$ to water and oxygen within the membrane of the cell. Catalase plays a
major role in the removal of $[H_2O_2]$ from muscle cells in the myocardium (Pugliese,
1998)

Glutathione peroxidase (GPx) is an antioxidant enzyme containing both selenium and
 glutathione. It is found in the cytoplasm and mitochondria. Glutathione consists of
 glutamine, cysteine, and glycine (Pugliese, 1998). N-acetylcysteine is chemically similar
to glutathione and has been studied as a scavenger (Shindoh et al., 1990; Repine, 1997).
 Destruction of $[H_2O_2]$ by glutathione peroxidase produces glutathione disulfide and water
(Gutteridge, 1999). In another reaction, glutathione disulfide is reduced back to
 glutathione. This whole system regenerates glutathione to be used again and again.
 Oxidative stress can be measured by determining the amount of both glutathione and
 glutathione disulfide and by estimating the ratio of one to the other. The ratio of
 glutathione to glutathione disulfide should be high, but if the amount of glutathione is
 less than the amount of glutathione disulfide, a toxic amount of $[H_2O_2]$ can accumulate
(Pugliese, 1998). Selenium levels have also been used to determine oxidative stress.
(Fenech et al., 1998; Metnitz et al., 1999)

Vitamins are scavengers of oxygen free radicals (Gutteridge 1999). Ascorbic acid
decreases lipid peroxidation and levels of $[H_2O_2]$. It is one of the most effective
antioxidants in serum. Vitamin E (alpha-tocopherol) is an important fat-soluble
antioxidant in the prevention of lipid peroxidation. It can protect the polyunsaturated fatty
acids in cell membranes from the autocatalytic chain reaction of lipid peroxidation (Gutteridge, 1999). Outside cell membranes, vitamin E is a poor antioxidant.

Extracellularly, both plasma and red blood cells have scavenger and antioxidant qualities. When $[O_2^+]$ enters red blood cells, catalase can destroy the free radicals. Red blood cells may increase the levels of glutathione peroxidase, thus preventing damage caused by free radicals. (Pugliese, 1995; Espat, 2000)

### 2.4.2. Antioxidant enzymes

Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione Reductase (GR), these enzymes function to protect against toxic oxygen radicals produced during normal metabolism and after oxidative insult (Sun Y, 1990).

1. Superoxide dismutase (SOD) dismutates two molecules of $O_2^-$ to form $H_2O$ and $O_2$

2. Catalase catalyses the reaction $H_2O_2 \rightarrow CAT \rightarrow H_2O + O_2$

Most aerobic cells contain this enzyme. In animals, CAT is present in all major body organs, being especially concentrated in liver and erythrocytes. The inhibitors of CAT include azide, cyanide, GSH and dithiothreitol.
3. Glutathione peroxidase catalyses the oxidation of GSH to GSSH at the expense of $H_2O_2$: $H_2O_2 + 2 \text{GSH} \rightarrow \text{GSSH} + H_2O$

Inhibitors of GPx include iodoacetate, cyanide and superoxide radicals.

4. Glutathione reductase catalyses the reaction

$$\text{GSSH} + \text{NADPH} + H^+ \rightarrow 2\text{GSH} + \text{NADP}^+$$

Inhibitor of GR is 1,3-bis-2-chloroethyl-1-nitrosourea.

5. Glucose 6 phosphate dehydrogenase catalyses the reaction

$$\text{Glucose 6 phosphate} + \text{NADP}^+ \rightarrow \text{Glucose 6 phospholactone} + \text{NADPH} + H^+$$

SOD, CAT and GPx are considered the primary antioxidant enzymes, since they are involved in the direct elimination of active oxygen species. GST, GR and G6 PD are secondary antioxidant enzymes, which help in the detoxification of reactive oxygen species (ROS) by decreasing the peroxide levels (GST) or by maintaining a steady supply of metabolic intermediates like glutathione (GR) and NADPH (G6 PD) for the primary antioxidant enzymes. The nonenzymatic small molecular antioxidants include sulfhydryl compounds such as GSH and thiols; NADPH; ascorbate; $\alpha$ tocopherol; $\beta$- carotene; bilirubin; selenium; urate; butyl hydroxy toluene (BHT); butyl hydroxyanisole (BHA) etc.

Abnormality of antioxidant enzymes occurs during stress, the decreased activities of these antioxidant enzymes are due to decreased enzyme protein levels. Low antioxidant enzymes will cause the accumulation of free radicals, which can result in damage to DNA, RNA, lipids and proteins and perhaps finally leading to cancer (Sun Y, 1990).
2.4.3. Oxidative stress in CNS disorders

The nervous system, including the brain, spinal cord, and peripheral nerves, is rich in both unsaturated fats (which are prone to oxidation) and iron (Halliwell, 1992). The high lipid content of nervous tissue, coupled with its high metabolic (aerobic) activity, makes it particularly susceptible to oxidant damage (Dawson and Dawson, 1996). The high level of brain iron may be essential, particularly during development, but its presence also means that injury to brain cells may release iron ions that can lead to oxidative stress via the iron-catalyzed formation of reactive oxygen species (Gerlach et al., 1994).

There is substantial evidence that oxidative stress is a causative in the pathogenesis of major neurodegenerative diseases, including Parkinson's disease (Ebadi et al. 1996), Alzheimer's disease (Markesbery and Carney, 1999; Behl, 1999) as well as in cases of stroke, trauma, and seizures (Coyle and Puttfarcken, 1993; Facchinetti et al., 1998). Decreased levels of antioxidant enzyme activity are found in Parkinson's disease patients (Fahn and Cohen, 1992). Evidence of oxidative stress in the form of increased lipid peroxidation and oxidation of DNA bases is seen in the substantia nigra, the area of the brain affected in Parkinson's disease (Jenner, 1996). Similar increased lipid peroxidation and oxidation of DNA and proteins are seen in Alzheimer's disease (Retz et al., 1998).

A number of in vitro studies have shown that antioxidants, both endogenous and dietary, can protect nervous tissue from damage by oxidative stress. Uric acid, an endogenous antioxidant, was found to prevent neuron damage in rats, both in vitro and in vivo, from the metabolic stresses of ischemia (oxidative stress as well as exposure to the excitatory amino acid glutamate and the toxic compound cyanide) (Yu et al., 1998). Vitamin E was found to prevent cell death (apoptosis) in rat neurons subjected to hypoxia followed by oxygen reperfusion (Tagami et al., 1998). Both vitamin E and beta-carotene were found to protect rat neurons against oxidative stress from exposure to ethanol (Mitchell et al., 1999).
In a study, it was found that the risk for Parkinson's disease was lower for subjects who had higher dietary intakes of antioxidants, particularly vitamin E (de Rijk et al., 1997). The same group reported that a low dietary intake of beta-carotene was associated with impaired cognitive function in a group of persons aged 55-95; no such association was observed for either vitamins C or E (Jama et al., 1996).

Anxiety
There are several studies reporting the role of free radical in anxiety disorders. Tezcan et al (2003) and Kuloglu et al (2002) investigated FRs in obsessive-compulsive disorder (OCD) and found that OCD may be related to FRs.

Depression
Stress is believed to be a major predisposing factor in the development of depression. Pal and Dandiya (1994) reported cerebral GSH, a biological marker of depression. Experimental data from several studies indicate a lower antioxidant defense against lipid per oxidation, a lower blood GSH levels in patients with depression and a therapeutic benefit from antioxidant supplementation (Altschule et al., 1952; Ozcan et al., 2004). Yanik et al., (2004) demonstrated that the patients with major depression were exposed to oxidative stress and the potency of the oxidative stress was significantly related to the severity of the depression. Several studies reported that patient with major depression and minor depression had high levels of lipid peroxidation, and diminished antioxidant defenses (Bilici et al 2001; Tapia-Saavedra, 2005) and that oxidative stress was associated with a diminution of omega-3 fatty acids. Thus, it was proposed that oxidative stress could have a role in the etiology of the subtype of the depression related to diminution of omega-3 fatty acids (Tapia-Saavedra, 2005). Akhtar et al (2005) showed mice exposed to modified FST exhibited oxidative stress and increase of free radical generation as evidenced by a significant reduction in GSH levels and an increase in TBARS and catalase levels. Thus, the oxidative stress is involved in the biochemical mechanisms underlying depression.
Schizophrenia

Herken et al. (2001) investigated the importance of the FRs in schizophrenia subtypes, reported that oxidative stress might have a pathophysiological role in all subtypes of schizophrenia. Oxidative stress exists in schizophrenia based on altered antioxidant enzyme defense, increased lipid peroxidation and reduced levels of essential polyunsaturated fatty acids (EPUFA’s). The life style of schizophrenic patients is also pro-oxidative stress, i.e., heavy smoking, drinking, high caloric intake with no physical activity and treatment with pro-oxidant drugs. The patients in developed countries show higher levels of lipid peroxidation and lower levels of membrane phospholipids as compared to patients in the developing countries. Initial observations on the improved outcome of schizophrenia in patients supplemented with EPUFAs and antioxidants suggest the possible beneficial effects of dietary supplementation. Since the oxidative stress exists at or before the onset of psychosis the use of antioxidants from the very onset of psychosis may reduce the oxidative injury and dramatically improve the outcome of illness (Mahadik et al., 2001)

Cerebral ischemia

Although free radicals can be directly detected in brain tissue, their very low concentration and very short half-life make them difficult to measure. Therefore, in most studies, their production is often evaluated through indirect techniques, by measuring, for example, irreversible damage in biological macromolecules, or consumption of low molecular weight endogenous antioxidants. Evaluation or endogenous antioxidant systems or oxidized molecules in brain tissues shows an early oxidative stress as soon as 30-60 minutes after permanent ischemia. This oxidative stress is characterized by a drop in concentrations of reduced glutathione (GSH), vitamin C, vitamin E, and ubiquinol-10 (Kato et al., 2003) as well as by an increase in malondialdehyde (MDA) and lipid-conjugated diene levels. A marked increased in brain uric acid and decrease xanthine levels within the first hour’s after permanent ischemia in rats were observed. Other study demonstrated an immediate and important production of hydroxyl radicals and superoxide anions after ischemia. After reperfusion, free radical production is notably increased in damaged tissues (Kato et al., 2003). Concerning endogenous antioxidant enzyme activity, a
significant decline in catalase activity has been demonstrated at reperfusion onset, as well as a 48 hr delayed decline in GPx activity (Lerouet et al., 2002). Data on SOD are not consistent. One study showed that SOD activity was enhanced in ischemic penumbra, but not in the core (Toyoda et al., 1997), whereas another reported a decline of SOD activity at reperfusion onset (Lerouet et al., 2002). Taken together these studies demonstrate that cerebral ischemia induces an early and sustained oxidative stress that increases during reperfusion.
3.0. Materials and Methods
3.0. MATERIALS AND METHODS

3.1. ANIMALS
Swiss strain albino mice of either sex (25-30 g) and albino male wistar rats (350-400 g) supplied by the Central Animal House Facility of Jamia Hamdard, New Delhi were used. All animals were housed in cages kept at a temperature of 23 –30 °C with a natural light–dark cycle. They had free access to standard pellet diet (Amrut Laboratory rat and mice feed, NavMaharastra chakan oil mills Ltd, Pune) and tap water. The study was approved by the Animal Ethics Committee CPCSEA (Project no 134). Ethical norms were strictly followed during all experimental procedures.

3.2. DRUGS AND CHEMICALS
The studies utilized the following drugs and chemicals:

**DRUGS**
- Amphetamine sulphate
- Apomorphine HCl
- Diazepam
- Fluoxetine
- Haloperidol
- (R)-α- methylhistamine
- Thioperamide maleate

**CHEMICALS**
- Bovine albumin
- CDNB
- Copper Sulphate
- Double distilled water
- DTNB (Ellman Reagent)
- EDTA
- Ethanol

Sigma - Aldrich Chemical Pvt. Ltd. (USA).
Sigma - Aldrich Chemical Pvt. Ltd. (USA).
Ranbaxy (India).
Cadila (India).
Sigma - Aldrich Chemical Pvt. Ltd. (USA).
Sigma - Aldrich Chemical Pvt. Ltd. (USA).
Sigma - Aldrich Chemical Pvt. Ltd. (USA).

CDH, Mumbai
Sisco lab, Mumbai
Glaxo, Mumbai
Lab Distillation Assembly
Himedia, Mumbai
Himedia, Mumbai
Lab Supply
<table>
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<tr>
<td>FAD</td>
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<tr>
<td>Folin’s ciocalteau phenol</td>
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<tr>
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<tr>
<td>Potassium Chloride</td>
<td>CDH, Mumbai</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>CDH, Mumbai</td>
</tr>
<tr>
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<td>Reduced glutathione</td>
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<tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>Sodium Citrate</td>
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</tr>
<tr>
<td>Sodium dihydrogen phosphate</td>
<td>E-Merck, Mumbai</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>CDH, Mumbai</td>
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<tr>
<td>Sodium potassium tartrate</td>
<td>CDH, Mumbai</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>Himedia, Mumbai</td>
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<td>Thiobarbituric Acid</td>
<td>Loba, Mumbai</td>
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<td>Trichloroacetic Acid</td>
<td>Sisco lab, Mumbai</td>
</tr>
<tr>
<td>Triss-Buffer</td>
<td>CDH, Mumbai</td>
</tr>
<tr>
<td>Xanthine Oxidase</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>Xanthine</td>
<td>Himedia, Mumbai</td>
</tr>
<tr>
<td>Zinc Sulphate</td>
<td>CDH, Mumbai</td>
</tr>
</tbody>
</table>
3.3. INSTRUMENTS

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Manufacturer/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated plus maze</td>
<td>Fabricated in our laboratory</td>
</tr>
<tr>
<td>Grip strength meter</td>
<td>Panlab, Barcelona</td>
</tr>
<tr>
<td>Habitest™ (lickometer)</td>
<td>Coulbourn, USA</td>
</tr>
<tr>
<td>Photoactometer</td>
<td>Techno, India</td>
</tr>
<tr>
<td>Video path analyzer</td>
<td>Coulbourn, USA</td>
</tr>
</tbody>
</table>

3.3.1. ELEVATED PLUS MAZE (CROSS MAZE)

A plus shaped wooden maze was constructed based on the specifications of Itoh et al (1991) and Ragozzino et al (1998). For rats, the maze consisted of four symmetrical arms (55 cm long x 10 cm wide), wall height 12 cm with a central platform (25 cm diameter). The maze for mice consisted of four symmetrical arms (24 cm long, 8 cm wide), wall height 10 cm and a central platform (8x 8 cm).

3.3.2. GRIP STRENGTH METER

Grip strength meter (Panlab, Barcelona) consisted of a digital meter and is fixed on an iron platform for the support. The device is attached to a sensor, which in turn is attached to a steel mesh (rats mesh is different with that of mice). The latter provides grip to the animal. The instrument is used for the measurement of grip strength both in rats and mice. The digital display shows the readings.

3.3.3. HABITEST™ (LICKOMETER)

Habitest™ (Coulbourn, USA) was used for the measurement of anxiety state in mice. The instrument has habitest animal shocker, for providing the shock intensity which ranges from 0 to 5 milliamperes (mA) and also has a licker. The animal is placed in the habitest operant vertical cage which has long bars and water feeding bottle connected with licker and shoker, when mice licks, it gets the shock. The instrument records the total number of licks and shocks which can be displayed on the computer monitor.
3.3.4. PHOTOACTOMETER

Photoactometer (Techno, India) was used for the measurement of spontaneous closed field locomotor activity. There is a square closed arena equipped with infrared light (IR) sensitive photocells. When the animal crosses the IR light during activity testing, a count is observed on the digital display.

3.3.5. VIDEOPATH ANALYZER

Videopath analyzer (Coulbourn, USA) is used for recording locomotor activities of the animals. It consists of an open field chamber, a video camera fixed over the chamber by an adjustable rod, an activity monitor, programmer and printer. This instrument has a facility of simultaneously recording locomotor time, rest time, distance traveled and average speed of the animal along with other variables.

3.3.6. Other Equipments/Apparatus

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balances</td>
<td>(Dhona, India)</td>
</tr>
<tr>
<td></td>
<td>Electronic balance for weighing chemicals</td>
</tr>
<tr>
<td></td>
<td>Single pan spring balance for weighing tissue</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Remi Scientific Industry, Bombay</td>
</tr>
<tr>
<td>pH Meter</td>
<td>Control Dynamics, Bangalore</td>
</tr>
<tr>
<td>U.V Spectrophotometer</td>
<td>Shimadzu (UV-1601)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Distillation apparatus, feeding needles, glassware, oven, plastic ware, refrigerator, syringes etc.</td>
</tr>
</tbody>
</table>
3.4. PHARMACOLOGICAL EVALUATIONS

3.4.1. ANXIETY

3.4.1.1. Vogel's Conflict Test Using Habitest™ lickometer

Vogel's conflict test as described by Umezu (1999) in mice and Millan et al (2001) in rats was suitably modified. This method measures the ability of a drug to study the drinking behavior of water-deprived rodents exposed to a mild aversive stimulus. Briefly, mice were deprived of water for 24 hours. They were then placed in habitest operant cage for a training period in which they were allowed to drink water freely for 40 minutes (habituation procedure). The experiment was performed on the next day in which again water-deprived mice were put into the cage and allowed to drink water. Every 20th lick was punished by an electric shock (0.1 mA for 0.2 sec) delivered via the sipper tube. The whole procedure was repeated for 3 minutes. The total number of licks and shocks during the 3 minutes period was recorded.

3.4.1.2. Elevated Plus Maze

The effects of H3 receptor ligands on anxiety were assessed in mice using a plus maze as described by Pellow et al (1985) with some modifications. The mice were placed in the central square of the plus maze and the total time spent by the animal in the open or closed arm and the number of open or closed arm entries were recorded during a 5 min observation period. Confinement to closed arms is associated with more anxiety-related behavior. Anxiolytics show a significant increase in the percentage of time spent in the open arms and number of open arm entries.

3.4.2. DEPRESSION

3.4.2.1. Modified Forced Swimming Test

Modified forced swimming test as described by Cryan and co-workers (Cryan and Lucki, 2000; Cryan et al., 2002) was slightly modified. Briefly, the mice were forced to swim
Materials and Methods

individually in cylinder (40 cm height, 15 cm diameter) containing fresh water (temperature 22 ± 2° C) up to a height of 30 cm for 15 minutes. This constituted the ‘pre-test’ swim. Twenty-four hours later, each mouse was re-exposed to the swimming condition in a similar environment in a 6 minutes ‘test session’. The total duration of climbing, swimming and immobility in the last 5 minutes of the 6 minutes test session was recorded for each animal.

After the behavioral testing, the animals were decapitated and the brains were quickly removed for estimations of thiobarbituric acid reactive substance (TBARS), reduced glutathione and catalase levels as described in the subsequent sections (Biochemical Evaluations, Section 3.5).

3.4.3. SCHIZOPHRENIA

3.4.3.1. Bar Test

Haloperidol-induced catalepsy is a widely employed method for screening of neuroleptic drugs in rodents. Briefly, catalepsy was induced with haloperidol and determined every hour up to 4 hours by means of a standard bar test as described by Silva et al (1995). The phenomenon was measured as the time when the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high bar (0.4 cm diameter). The total time during which animal stayed on the bar (even if it climbed back up) was recorded for a maximum period of 300 seconds. Between determinations, the animals were replaced into their respective cages.

3.4.3.2. Amphetamine Induced Locomotor Activities

Inhibition of locomotor hyperactivity induced by dopaminergic drugs such as amphetamine has been a frequently used animal model to study neuroleptic drugs (Arnt, 1995; Salim et al., 2003). Briefly, mice were administered amphetamine (2 mg/kg s.c.) and locomotor activity was then recorded in a Video path analyzer. The animal was placed over a black paper in the open field chamber and the camera was activated. The animal could now be viewed on the monitor screen and its position could be adjusted using horizontal/vertical
controls. The desired number of sessions, duration and session intervals could be programmed on the activity programmer. Locomotor time, rest time, distance traveled and average speed of the animal were monitored and then recorded.

3.4.3.3. Apomorphine Induced Climbing Behavior
Apomorphine induced climbing behavior was followed as previously described (Costall et al., 1978, Moore et al., 1992). Briefly, mice were injected apomorphine (1.5 mg/kg s.c.) and were placed individually in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, and mesh size 3 mm). During the trial, the time when each mouse climbed on the inside of the wire cage was recorded over a 30 minutes period. Maximum time (i.e. maximum time spent in a single climb throughout the duration of apomorphine effect) and % climbing index (i.e. the % of time spent on climbing during 30 minutes period following the first climb) were calculated.

3.4.4. CEREBRAL ISCHEMIA

3.4.4.1 Middle Cerebral Artery Occlusion (MCAO) model of focal ischemia
Rats were anaesthetized with chloral hydrate 400mg/kg i.p, core temperature was maintained around 37 °C throughout the surgical procedure using heating lamp. A midline incision was made and the right common carotid artery, external carotid artery and internal carotid artery were exposed. A 4.0 nylon monofilament, the tip of which was rounded with the use of an open flame, was inserted into the external carotid artery and advanced into the internal carotid artery until a slight resistance was felt. Such resistance indicated that the filament had passed beyond the proximal segment of the anterior cerebral artery. At this point, the intraluminal filament blocked the origin of the MCA and occluded all sources of blood flow from the internal carotid artery, anterior cerebral artery and posterior cerebral artery. The nylon filament was allowed to remain in the place for 2 hours after which it was gently withdrawn until tip reached external carotid artery to allow the reperfusion of the ischemic region (Longa et al., 1989). In sham-operated rats, the external carotid artery was surgically prepared for the insertion of the filament, but the filament was not inserted.
The rats were then studied for motor performance test, grip strength test and spontaneous alternation behavior and then sacrificed for the estimation of oxidative stress markers including nitrate and nitrite levels as described in detail in Section 3.5 (Biochemical Evaluations).

### 3.4.4.2. Motor Performance Test

Photoactometer was used for the measurement of spontaneous closed field locomotor activity. Each rat was observed for a period of 10 minutes in a square closed arena equipped with infrared light sensitive photocells. During activity testing, only one animal was tested at a time (Lannert et al., 1998).

### 3.4.4.3. Grip Strength Test

A grip strength meter (Panlab, Barcelona) was used to assess the muscle relaxation of the animal. Briefly, the rats were allowed to hold the grid, which was then pulled back horizontally until the rat released its grip. This was recorded from a digital meter (Ali et al., 2004).

### 3.4.4.4. Spontaneous Alternation Behavior (SAB)

SAB was assessed in a plus maze as described by Ragozzino et al (1998). The rats were placed in a four-arm cross arm maze. The maze (85 cm height) was constructed of wood painted gray and contained a central platform (25 cm diameter), from which radiated four symmetrical arms (55 cm long x 10 cm wide), with 12 cm wall. After being placed in the central platform, rats were allowed to traverse the maze freely for 12 minutes. The number and sequence of entries were recorded. An alternation was defined as entry into four different arms on an overlapping quintuple set. Five consecutive arm choices within the total set of arm choices constitute a quintuple set. A quintuple consisting of arm choices A, B, A, C, D was considered as an alternation while the set with A, B, A, C, B did not. Using this procedure percentage alternation and possible alternations were calculated.

\[
\text{Percentage alternation} = \frac{\text{Actual alternation}}{\text{Possible alternation}} \times 100
\]

\[
\text{Possible alternation} = \text{Number of arm entries} - 4
\]
3.5. BIOCHEMICAL EVALUATIONS

3.5.1. Estimation of Oxidative Stress Markers
Following the behavioral testing, the animals were decapitated and the brains were quickly removed, weighed and homogenized in ice-cold KCl phosphate buffer (0.1 M pH 7.4) 10 times (w/v) and centrifuged at 2000 rpm for 5 minutes at 4°C. The supernatant containing crude membrane was used for the estimation of TBARS and GSH. The remaining supernatant was again centrifuged at 10,000 rpm at 4°C for 20 minutes. The post mitochondrial supernatant was used for the study of antioxidant enzyme activities and protein estimation. Catalase and superoxide dismutase activities were determined immediately after sample preparation. Protein concentration was determined according to Lowry et al (1951) using purified bovine serum albumin as standard.

3.5.1.1. Lipid Peroxidation
Thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxidation was measured as described by Ohkawa et al (1979). Briefly, 0.1ml homogenate, 1ml of trichloroacetic acid (10%) and 1ml of thiobarbituric acid (0.67%) were added to all test tubes, covered with aluminum foil and placed in boiling water bath for 20 minutes. Test tubes were then shifted to crushed ice bath and then centrifuged at 6000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm using spectrophotometer against a reagent blank. The amount of MDA expressed as n moles of MDA formed per mg of protein in the tissue.

3.5.1.2. Reduced Glutathione
Glutathione was measured according to the method of Ellman (1957). The equal quantity of homogenate (w/v) and 10% trichloroacetic acid were mixed and centrifuged to separate the proteins. To 0.01ml of this supernatant, 2ml of phosphate buffer (pH 7.4), 0.5ml 5, 5'-dithiobisnitro benzoic acid (DTNB) and 0.4 ml of double distilled water was added. The mixture was vortexed and the absorbance was read at 412 nm within 15 minutes. The concentration of reduced glutathione was expressed as μg/mg protein in the tissue.
3.5.1.3. Catalase
Catalase activity was measured by the method of Claiborne (1985). A total of 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7). The reaction was started by the addition of 1 ml freshly prepared 30 mM $H_2O_2$. The rate of decomposition of $H_2O_2$ was measured spectrophotometrically at 240 nm. The activity of the catalase was expressed as n mol of $H_2O_2$ consumed /min/mg of protein.

3.5.1.4. Glutathione - S - transferase (GST)
GST activity was measured by the method of Habiq et al (1974). The reaction mixture consisted of 1.425 ml of phosphate buffer (0.1 M, pH 6.5), 0.2 ml reduced glutathion (1 mM), 0.025 ml CDNB (1mM) and 0.3 ml PMS (10% w/v) in a total volume of 2 ml. The change in absorbance was recorded as nM CDNB conjugate formed/minute/mg protein.

3.5.1.5. Glutathione Peroxidase (GPx)
Glutathione peroxidase activity was measured according to the procedure described by Mohandas et al (1984). The reaction mixture consisted of 1.44 ml phosphate buffer (0.05 M, pH 7), 0.1 ml of EDTA (1mM), 0.1 ml sodium azide (1mM), 0.05 ml glutathione reductase (1 enzyme unit/ml), 0.1 ml glutathione (1mM), 0.1 ml of NADPH (0.2 mM), 0.01 ml of $H_2O_2$ (0.25 mM) and 0.1 ml of PMS (10% w/v) in a final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nm NADPH oxidized/minute/mg protein.

3.5.1.6. Glutathione Reductase (GR)
Glutathione reductase activity was assayed by the method of Calberg and Mannervik (1975), as modified by Mohandas et al (1984). The assay system consisted of 1.65 ml of phosphate buffer (0.1 M, pH 7.6), 0.1ml NADPH (0.1mM), 0.1ml EDTA (0.5 mM), 0.05 ml oxidized glutathione (1 mM) and 0.1 ml PMS (10 % w/v) in a total volume of 2 ml. The enzyme activity was recorded by measuring the disappearance of NADPH at 340 nm and was calculated as nm NADPH oxidized/minute/mg protein.
3.5.1.7. Superoxide Dismutase (SOD)

Superoxide dismutase activity was measured by the method of Beauchamp et al (1971). The reaction mixture of total 1 ml consisted of 0.6 ml of phosphate buffer (0.5 M, pH 7.4), 0.1 ml PMS (10 % w/v), 0.1 ml xanthine (1mM), 0.1 ml NBT (57 μM) was incubated for 15 minutes at room temperature and reaction was initiated by the addition of xanthine oxidase (50 mU). The rate of reaction was measured by recording change in the absorbance at 550 nm due to the formation of formazan, a reduction product of NBT.

3.5.2 Measurement of Brain Nitrate and Nitrite Levels

The animals were decapitated and the brains were quickly removed. The levels of nitrate and nitrite were determined as described by Grisham et al (1995) with some modifications. Briefly the tissue was weighed and mixed with ten times (w/v) (20 mM) triss-buffer plus EDTA (2M, pH 7.4). The mixture was homogenized and centrifuged at 10,000 rpm for 10 minutes at 4 °C. 100 μl supernatant was taken and mixed with 0.4 ml distilled water and 0.3 ml NaOH (0.3N). The mixture was kept for 5 minutes and then added 0.3 ml of 5% ZnSO4 solution, again waited for 5 minutes and then centrifuged for 20 minutes at 10,000 rpm 4 °C. From the supernatant 100 μl sample was taken and incubated for 30 minutes in the presence of 10 U/ml nitrate reductase, 1 M HEPES buffer, 0.1 mM FAD, 1mM NADPH in a total volume of 500 μl as outlined in table 1 (sample 1 served as the blank). Following the incubation, 5 μl of lactate dehydrogenase (1500 U/ml) and 50 μl of 100 mM pyruvic acid were added to each test tube. Samples were then incubated for an additional 10 minutes at 37 °C. 1 ml of premixed Griess reagent (equal volumes of 0.2% (w/v) naphthylene ethylenediamine and 2% (w/v) sulfanilamide in 5% (v/v) phosphoric acid) was then added to each tube. After 10 minutes of incubation at room temperature, the absorbance of each sample was determined at 543 nm. The nitrate and nitrite levels were determined individually by using two sets of samples with nitrate reductase omitted from one set of samples. Griess positive reactions in these samples indicate the presence of nitrite levels only. Nitrate and nitrite concentrations were calculated using nitrate and nitrite standard curves. To determine the concentration of nitrate and nitrite in each sample, the following formula was used.
Total nitrate and nitrite (μ M) = \frac{(A_{543} \text{ sample} - A \text{ blank})(\text{dilution factor})}{\text{slope}}

The dilution factor used was 5 because the 100 μl sample was diluted to a total volume of 500 μl. The slope was determined experimentally using known concentrations of nitrate and nitrite.

Table 1: Experimental design using *Aspergillus Nitrate Reductase* for NO estimation (Grisham et al., 1995).

<table>
<thead>
<tr>
<th>Tube</th>
<th>Water</th>
<th>1M HEPES (pH 7.4)</th>
<th>Sample</th>
<th>0.1 mM FAD</th>
<th>1 mM NADPH</th>
<th>10 U/ml Nitrate reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>390 μl</td>
<td>25 μl</td>
<td>0</td>
<td>25 μl</td>
<td>50 μl</td>
<td>10 μl</td>
</tr>
<tr>
<td>2.</td>
<td>290 μl</td>
<td>25 μl</td>
<td>100 μl</td>
<td>25 μl</td>
<td>50 μl</td>
<td>10 μl</td>
</tr>
</tbody>
</table>
Materials and Methods

Standard calibration curve of Nitrate and Nitrite

Concentration (µM)

Optical Density (345 nm)

- concentrations of nitrate
- concentrations of nitrite
3.5.3. Protein Estimation Using Folin’s Reagent (Lowry et al., 1951)

Principle: Protein reacts with Folin’s ciocalteau phenol reagent to give coloured complex. The copper with the protein as in the biuret test and the reduction of phosphomolybdate by tyrosine and tryptophan present in the proteins.

Reagent required and their preparations:
Alkaline sodium carbonate solution (2% Na$_2$CO$_3$ in 0.1 NaOH): 100 ml of 0.1N NaOH solution was prepared by dissolving 400 mg of NaOH in distilled water and the volume was made upto 100 ml. then 2 gm of Na$_2$CO$_3$ was dissolved in 100 ml of 0.1N NaOH solution.
Copper sulphate sodium potassium tartarate solution (0.5% CuSO$_4$ in 1% Na-K tartarate): 0.5% CuSO$_4$ solution was mixed with 1% Na-K tartarate.
Alkaline solution: prepared on the same day of use by mixing 50 ml of reagent 1 and 1 ml of reagent 2.
Folin’s ciocalteau phenol reagent: The commercial reagent was diluted with 2 volumes of distilled water on the same day of use.
Standard protein: Bovine serum albumin (2 mg/ml): 10 ml of bovine serum albumin was dissolved in 5 ml of distilled water to get a solution of 2.0 mg/ml of protein.
Procedure: 5 ml of alkaline solution was added to 0.1 ml of supernatant and allowed to stand for 10 minutes. 0.5 ml of dilute Folin’s reagent was added and the tube was shaken to mix the solution. After 30 minutes the absorbance against appropriate blank was recorded at 750 nm.

Preparation of calibration standard curve for protein:
5 ml of bovine serum albumin solution (2 mg/ml) was prepared and different volumes of the same were taken in 10 test tubes. To all the tubes distilled water was added to make up the volume in each tube, 1 ml. The protein concentration in the above 10 tubes was estimated in the same way as for the sample. The graph was plotted between concentration of protein and optical density. The calibration standard plot was used to calculate the concentration of protein in each ml of sample.
Materials and Methods

Standard calibration curve for protein.

Concentration (mg/ml)

optical density (750 nm)
3.6. SELECTION OF DOSES

All drug solutions were prepared using double distilled water. RAMH was dissolved in sterile water for injection for intracerebroventricular (i.c.v.) administration. Thioperamide, fluoxetine and diazepam were administered intraperitoneally (i.p.) Doses of RAMH (5 μg per mouse), and THP (3.75, 7.5, 15 mg/kg i.p.) were based on our earlier work on mice (Vohora et al., 2001). The doses of THP in rats (5.5 mg/kg i.p, and 11 mg/kg i.p.) were derived from converting the mice doses (7.5 and 15 mg/kg i.p. respectively) to rats dose. Doses of haloperidol (2 mg/kg p.o.) (Pilot et al., 2002), amphetamine (2 mg/kg s.c.) (Arnt J., 1995), apomorphine (1.5 mg/kg s.c.) (Costall, 1978) and diazepam (1mg/kg and 2 mg/kg i.p.) (Umezu, 1999; Perez-Garcia et al., 1999) were based on earlier published reports. All the drugs were given in a volume of 10 ml/kg except RAMH, which was administered intracerebroventricularly (i.c.v.) in a volume not exceeding 5 μl using Hamilton’s micro liter syringe. The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears in mice. Control animals received the appropriate vehicle. Each mouse and rat received only one type of treatment and test and was not reused. All the observations were made 1 hour after THP, 30 minutes after fluoxetine and diazepam and 15 minutes after RAMH administration.
Table 2. Doses of drugs used in various studies.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Vogel's conflict test in mice</th>
<th>Elevated plus maze in mice</th>
<th>Modified forced swim test in mice</th>
<th>Catalepsy test in mice</th>
<th>Video path analyzer activity in mice</th>
<th>Apomorphine induced climbing in mice</th>
<th>Middle cerebral artery occlusion in rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>10 ml/kg</td>
<td>10 ml/kg</td>
<td>10 ml/kg</td>
<td>10 ml/kg</td>
<td>10 ml/kg</td>
<td>10 ml/kg</td>
<td>10 ml/kg</td>
</tr>
<tr>
<td>RAMH</td>
<td>5 µg</td>
<td>5 µg</td>
<td>5 µg</td>
<td>5 µg</td>
<td>5 µg</td>
<td>5 µg</td>
<td>-</td>
</tr>
<tr>
<td>THP</td>
<td>15 mg/kg</td>
<td>15 mg/kg</td>
<td>3.75, 7.5, 15 mg/kg</td>
<td>3.75, 7.5, 15 mg/kg</td>
<td>3.75, 7.5, 15 mg/kg</td>
<td>3.75, 7.5, 15 mg/kg</td>
<td>5.5, 11 mg/kg</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>-</td>
<td>-</td>
<td>10 mg/kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1 mg/kg</td>
<td>2 mg/kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

RAMH: R (α) methylhistamine; THP: Thioperamide.
Normal saline, diazepam, fluoxetine and thioperamide were given intraperitoneally; RAMH was given intracerebroventricularly; amphetamine and apomorphine were given by subcutaneous route and haloperidol was administered orally. All the observations were made using single dose studies and animals were not reused again.
3.7. STATISTICAL ANALYSIS

The results of all the experiments were expressed as Mean ± SEM (Standard error of Mean) and analyzed by ANOVA followed by Dunnett’s t test (Croxton et al., 1982, Armitage and Berry 1985). P values less than 0.05 were considered significant.
4.0. Results
4.0. RESULTS

4.1. ANXIETY

4.1.1. Effect of H₃ receptor ligands on the Vogel’s conflict test in mice.

Diazepam pretreatment (1 mg/kg i.p.) resulted in a significant increase in the number of licks \([F (3, 23) =4.031, P<0.01, \text{ANOVA}]\) and increase in number of shocks, the latter being statistically insignificant. No significant effects were observed with THP and RAMH \((P>0.05)\) (Table 1).

4.1.2. Effect of H₃ receptor ligands on the elevated plus maze in mice.

Administration of diazepam (2 mg/kg i.p.) resulted in a significant increase in the time spent in the open arms and a reduction in time spent in closed arms \([F (3, 19) =12.749, P<0.01, \text{ANOVA}]\). Neither THP nor RAMH significantly changed these variables. \((P>0.05)\) (Table 2).

Pretreatment with diazepam (2 mg/kg i.p.) resulted in a significant increase in the number of open arm entries \([F (3, 19) = 8.524, P<0.01, \text{ANOVA}]\). THP and RAMH did not produce any significant change in the number of open and closed arm entries. \((P<0.01)\) (Table 3)

4.2. DEPRESSION

4.2.1. Effect of histamine H₃ receptor ligands on modified Forced Swimming Test in mice.

Administration of thioperamide (7.5 and 15 mg/kg, i.p) dose-dependently decreased the immobility time \([F (6, 41) = 17.072, P<0.01, \text{ANOVA}]\) and increased the swimming time
[F (6, 41) = 19.029, P<0.01, ANOVA] However, the lower dose of THP (3.75 mg/kg, i.p.) and RAMH was without any effect. None of the two drugs affected climbing behavior. Fluoxetine administration significantly increased swimming and reduced immobility time (Table 4)

4.2.2. Effect of histamine H₃ receptor ligands on the levels of oxidative stress markers (TBARS, GSH, Catalase) in brain tissues of mice.

A significant reduction in GSH [F (7, 47) = 20.401, P<0.01, ANOVA] levels and an increase in catalase [F (7, 47) = 4.723, P<0.05, ANOVA] levels were observed in the brains of mice exposed to modified FST. Fluoxetine reversed the reduced GSH levels and elevated catalase levels. THP dose-dependently reversed the reduced GSH levels. It also reduced the elevated catalase levels, though statistically insignificant. RAMH pretreatment caused a reversal of elevated catalase but not reduced GSH levels. No significant change was however, observed in the TBARS (Table 5).

4.3. SCHIZOPHRENIA

4.3.1. Effect of histamine H₃ receptor ligands on catalepsy test in mice.

Haloperidol per se (but not THP and RAMH) produced catalepsy post 2, 3, 4 hours, and its peak effect was observed at 2 hours [F (7, 47) = 12.183, P < 0.01, ANOVA]. THP (3.75, 7.5 and 15 mg/kg i.p.) dose-dependently elevated the haloperidol induced increase in catalepsy times (P < 0.01). Pretreatment with RAMH significantly reversed such an effect of THP (15 mg/kg i.p.) (Table 6).

4.3.2. Effect of histamine H₃-receptor ligands on amphetamine induced locomotor activities in mice.

Administration of amphetamine per se produced significant increase in the locomotor time, distance traveled, and average speed [F (7, 47) = 3.831, P<0.01, ANOVA] but reduction in
the rest time. RAMH *per se* resulted in significant reduction \[ F(7, 47) = 4.224, P<0.01, \text{ANOVA} \] in locomotor time, distance traveled and average speed. No statistically significant changes in locomotor activity were observed with THP (15 mg/kg i.p.) *per se*. However, the latter drug at 3.75 and 7.5 mg/kg i.p., but not 15 mg/kg i.p., significantly decreased amphetamine-induced hyperactivity as shown by reduction in locomotor time, distance traveled and average speed \[ F(7, 47) = 4.050, P<0.01, \text{ANOVA} \] and increase in rest time. Pretreatment with RAMH (5 µg i.c.v) could partially reverse such effect of THP (3.75 mg/kg i.p.) (Table 7).

### 4.3.3. Effect of H₃ receptor ligands on apomorphine induced climbing behavior in mice.

The climbing behavior was observed with apomorphine (1.5 mg/kg s.c.) *per se* (though statistically insignificant). Pretreatment with THP (7.5 and 15 mg/kg i.p.) reduced the climbing behavior. Such an effect, was however, reversed in presence of RAMH (5 µg i.c.v) (Table 8).

### 4.4. CEREBRAL ISCHEMIA

#### 4.4.1. Effect of thioperamide on the oxidative stress markers in middle cerebral artery occluded (MCAO) rats.

TBARS levels were significantly elevated in the brains of MCA occluded rats (P<0.01). THP pretreatment (5.5 mg/kg and 11 mg/kg i.p.) followed by MCA occlusion significantly decreased the elevated TBARS levels \[ F(4, 29) = 14.262, P<0.01, \text{ANOVA} \]. MCA occlusion resulted in the significant reduction of glutathione levels as compared with sham group (P<0.01). THP pretreatment (at both the doses) could not show any significant reversal of such a reduction, though there was a tendency towards increasing the levels as compared to MCAO \[ F(4, 29) = 19.460, P<0.01, \text{ANOVA} \]. No change in catalase levels was observed in either MCAO or THP treated groups. MCA occlusion resulted in the
significant reduction of GST levels (P<0.01). THP pretreatment (at both the doses) was unable to show any reversal of reduction observed by MCAO. GPx activity was reduced in MCA occluded groups when compared with control, though statistically insignificant. Pretreatment with THP further reduced the GPx levels [F (4, 29) = 7.010, P<0.01, ANOVA]. Although the activity of GR was reduced following MCAO but was not statistically significant (P>0.05). A further reduction of GR was observed at both the dose levels of THP [F (4, 29) = 4.191, P<0.01, ANOVA]. A non-significant reduction of SOD was observed in the MCA occluded rats. Pretreatment with THP (at both the doses) further reduced SOD levels (Table 9).

4.4.2. Effect of thioperamide on behavioral parameters in middle cerebral artery occluded (MCAO) rats.

A reduction was observed in the locomotor count of MCA occluded rats and it was reversed on pretreatment with THP (5.5 mg/kg and 11 mg/kg i.p.) but the results were not found to be statistically significant. No significant change was observed in the grip strength of control, sham and THP treated groups. There was no change in the percentage alternation of animals both in the MCAO group and those treated with thioperamide. However, a significant reduction in the possible alternation was observed in both the group of animals (Table 10).

4.4.3. Effect of thioperamide on the levels of nitric oxide (NO) in middle cerebral artery occluded (MCAO) rats.

MCA occluded rats showed significant reduction of nitrate and nitrite levels (P<0.01) as compared to control and sham groups. However, pretreatment with THP (at both the doses) elevated the levels of nitrite [F (4, 29) = 19.310, P<0.01, ANOVA] [F (4, 29) = 26.057, P<0.01, ANOVA] (Table 11).
Table 1. Effect of H₃ receptor ligands on the Vogel’s Conflict Test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n=6)</th>
<th>Number of licks (Mean ± SEM)</th>
<th>Number of shocks (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>50.83 ± 13.32</td>
<td>4.66 ± 2.76</td>
</tr>
<tr>
<td>2.</td>
<td>Diazepam (1 mg/kg i.p.)</td>
<td>143.83 ± 22.09*</td>
<td>12.0 ± 5.29</td>
</tr>
<tr>
<td>3.</td>
<td>Thioperamide (15 mg/kg i.p.)</td>
<td>66.33 ± 16.80</td>
<td>2.66 ± 0.88</td>
</tr>
<tr>
<td>4.</td>
<td>RAMH (5 µg i.c.v.)</td>
<td>86.33 ± 26.35</td>
<td>5.16 ± 1.81</td>
</tr>
</tbody>
</table>

Data represents as Mean ± SEM

n= no of animals=6

*P<0.05 vs Gp 1

Significance by one – way ANOVA followed by Dunnett’s t test.
Table 2. Effect of H₃ receptor ligands on the time spent in open and closed arm of the elevated plus maze in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n=6)</th>
<th>Time spent in open arms (sec, mean ± SEM)</th>
<th>Time spent in closed arms (sec, mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>48.64 ± 14.17</td>
<td>238.32 ± 16.41</td>
</tr>
<tr>
<td>2.</td>
<td>Diazepam (2 mg/kg i.p.)</td>
<td>237.15 ± 5.20**</td>
<td>41.48 ± 4.73**</td>
</tr>
<tr>
<td>3.</td>
<td>Thioperamide (15 mg/kg i.p.)</td>
<td>39.62 ± 21.13##</td>
<td>284.82 ± 21.09###</td>
</tr>
<tr>
<td>4.</td>
<td>RAMH (5 μg i.c.v.)</td>
<td>41.27 ± 18.52##</td>
<td>190.62 ± 46.07##</td>
</tr>
</tbody>
</table>

Data represents as Mean ± SEM
n= no of animals=6
**P<0.01 vs Gp 1;  ## P<0.01 vs Gp 2
Significance by one – way ANOVA followed by Dunnett’s t test
Table 3. Effect of H₃ receptor ligands on the number of open and closed arm entries of the elevated plus maze in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n=6)</th>
<th>Open arm entries (mean ± SEM)</th>
<th>Closed arm entries (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>4.0 ± 1.22</td>
<td>5.80 ± 1.60</td>
<td></td>
</tr>
<tr>
<td>2. Diazepam (2 mg/kg i.p.)</td>
<td>9.80 ± 2.22</td>
<td>3.0 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>3. Thioperamide (15 mg/kg i.p.)</td>
<td>1.20 ± 0.37‡‡</td>
<td>2.20 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>4. RAMH (5 μg i.c.v.)</td>
<td>1.80 ± 0.80‡‡</td>
<td>2.80 ± 0.73</td>
<td></td>
</tr>
</tbody>
</table>

Data represents as Mean ± SEM
n= no of animals=6
*P<0.05 vs Gp 1;  ‡‡ P<0.01 vs Gp 2
Significance by one – way ANOVA followed by Dunnett’s t test
Table 4. Effect of H₃ receptor ligands on modified forced swimming test (FST) in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n=6)</th>
<th>Climbing time (sec)</th>
<th>Swimming time (sec)</th>
<th>Immobility time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control i.p.</td>
<td>6.35 ± 1.08</td>
<td>103.70 ± 11.43</td>
<td>180.93 ± 11.41</td>
</tr>
<tr>
<td>2.</td>
<td>Control icv</td>
<td>4.30 ± 0.87</td>
<td>130.09 ± 11.94</td>
<td>159.13 ± 11.46</td>
</tr>
<tr>
<td>3.</td>
<td>RAMH (5 μg i.c.v.)</td>
<td>6.62 ± 1.97</td>
<td>121.67 ± 18.85</td>
<td>153.10 ± 17.82</td>
</tr>
<tr>
<td>4.</td>
<td>Fluoxetine (10 mg/kg i.p.)</td>
<td>6.05 ± 1.17</td>
<td>253.94 ± 9.72**</td>
<td>36.60 ± 10.28**</td>
</tr>
<tr>
<td>5.</td>
<td>THP (3.75 mg/kg i.p.)</td>
<td>9.33 ± 0.58</td>
<td>75.65 ± 7.12</td>
<td>200.1 ± 6.11</td>
</tr>
<tr>
<td>6.</td>
<td>THP (7.5 mg/kg i.p.)</td>
<td>8.14 ± 0.91</td>
<td>166.86 ± 14.41**</td>
<td>125.39 ± 15.01*</td>
</tr>
<tr>
<td>7.</td>
<td>THP (15 mg/kg i.p.)</td>
<td>5.92 ± 0.95</td>
<td>191.15 ± 18.72**</td>
<td>87.71 ± 19.19**</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM
n = no of animals
Significance by one - way ANOVA followed by Dunnett’s t test.
* P< 0.05; **P <0.01 vs. control i.p.
Table 5. Effect of histamine H₃ receptor ligands on the levels of oxidative stress markers (TBARS, GSH, Catalase) in brain tissues of mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n= 6)</th>
<th>TBARS (n moles of MDA formed/mg protein)</th>
<th>GSH (µ moles / mg protein)</th>
<th>Catalase (n mol H₂O₂ consumed /minutes/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control- No FST</td>
<td>0.88 ± 0.16</td>
<td>97.72 ± 12.8</td>
<td>4.30 ± 0.71</td>
</tr>
<tr>
<td>2.</td>
<td>Control i.p. + FST</td>
<td>1.62 ± 0.38</td>
<td>26.29 ± 5.38*</td>
<td>22.25 ± 6.54**</td>
</tr>
<tr>
<td>3.</td>
<td>Control icv + FST</td>
<td>1.38 ± 0.06</td>
<td>25.18 ± 2.80**</td>
<td>15.66 ± 1.28*</td>
</tr>
<tr>
<td>4.</td>
<td>RAMH (5 µg i.c.v.) + FST</td>
<td>1.13 ± 0.34</td>
<td>13.22 ± 0.93**</td>
<td>12.95 ± 0.76</td>
</tr>
<tr>
<td>5.</td>
<td>Fluoxetine (10 mg/kg i.p.) + FST</td>
<td>1.30 ± 0.03</td>
<td>37.66 ± 1.57**</td>
<td>11.89 ± 1.24</td>
</tr>
<tr>
<td>6.</td>
<td>THP (3.75 mg/kg i.p.) + FST</td>
<td>1.84 ± 0.35</td>
<td>48.29 ± 4.0 **</td>
<td>8.0 ± 0.45</td>
</tr>
<tr>
<td>7.</td>
<td>THP (7.5 mg/kg i.p.) + FST</td>
<td>1.12 ± 0.19</td>
<td>54.69 ± 5.11**</td>
<td>9.24 ± 1.33</td>
</tr>
<tr>
<td>8.</td>
<td>THP (15 mg/kg i.p.) + FST</td>
<td>0.64 ± 0.05</td>
<td>70.11 ± 6.98*</td>
<td>9.78 ± 0.97</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM

n = no of animals

Significance by one – way ANOVA followed by Dunnett’s t test.

* P< 0.05; **P <0.01 vs. control No FST
Table 6. Effect of H3 receptor ligands on catalepsy test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (n = 6)</th>
<th>1 Hour</th>
<th>2 Hour</th>
<th>3 Hour</th>
<th>4 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>1.36 ± 0.15</td>
<td>1.20 ± 0.06</td>
<td>1.29 ± 0.08</td>
<td>1.23 ± 0.07</td>
</tr>
<tr>
<td>2.</td>
<td>Haloperidol (2 mg/kg p.o.)</td>
<td>27.99 ± 7.72</td>
<td>159.45 ± 22.37**</td>
<td>92.54 ± 14.93*</td>
<td>95.72 ± 31.38*</td>
</tr>
<tr>
<td>3.</td>
<td>Thioperamide (15 mg/kg i.p.)</td>
<td>18.78 ± 5.13</td>
<td>39.91 ± 11.65</td>
<td>33.80 ± 8.81</td>
<td>58.71 ± 19.33</td>
</tr>
<tr>
<td>4.</td>
<td>RAMH (5 μg i.c.v.)</td>
<td>58.32 ± 38.62</td>
<td>38.83 ± 11.54</td>
<td>29.92 ± 11.45</td>
<td>51.30 ± 20.78</td>
</tr>
<tr>
<td>5</td>
<td>Thioperamide (3.75 mg/kg i.p.) + Haloperidol (2 mg/kg p.o.)</td>
<td>87.63 ± 17.01*</td>
<td>156.34 ± 10.83**</td>
<td>80.46 ± 4.76**</td>
<td>65.70 ± 14.15</td>
</tr>
<tr>
<td>6</td>
<td>Thioperamide (7.5 mg/kg i.p.) + Haloperidol (2 mg/kg p.o.)</td>
<td>146.72 ± 21.31***</td>
<td>168.18 ± 12.80**</td>
<td>254.91±14.84**</td>
<td>237.27±30.68***</td>
</tr>
<tr>
<td>7</td>
<td>Thioperamide (15 mg/kg i.p.) + Haloperidol (2 mg/kg p.o.)</td>
<td>189.18 ± 26.4***</td>
<td>273.64 ± 7.10***</td>
<td>280.18±2.41**</td>
<td>295.35±2.55***</td>
</tr>
<tr>
<td>8</td>
<td>Thioperamide (15 mg/kg i.p.) + RAMH (5μg i.c.v.) + Haloperidol (2 mg/kg p.o.)</td>
<td>64.80 ± 8.80</td>
<td>118.25 ± 7.09***</td>
<td>109.02 ± 8.04**</td>
<td>100.07 ± 8.87**</td>
</tr>
</tbody>
</table>

Data presented as Mean ± SEM; n = no of animals = 6; * P < 0.05, **P < 0.01 vs Gp. 1; ***P <0.01 vs Gp. 2, ****P < 0.01 vs Gp. 3. Significance by one - way ANOVA followed by Dunnett's t test.
Table 7. Effect of H3 receptor ligands on locomotor activity of mice in a video path analyzer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n=6)</th>
<th>Locomotor time (sec)</th>
<th>Rest time (sec)</th>
<th>Distance traveled (cm)</th>
<th>Average speed (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>172.16 ± 24.82</td>
<td>722.83 ± 25.63</td>
<td>3389.33 ± 538.9</td>
<td>220.67 ± 34.63</td>
</tr>
<tr>
<td>2.</td>
<td>Amphetamine (2 mg/kg s.c.)</td>
<td>256.33 ± 18.89**</td>
<td>643.66 ± 18.89*</td>
<td>4823.16± 364.76**</td>
<td>310.67 ± 21.93*</td>
</tr>
<tr>
<td>3.</td>
<td>RAMH (5 µg i.c.v.)</td>
<td>165.83 ± 14.22##</td>
<td>707.16 ± 17.53</td>
<td>2974.5 ± 236.5##</td>
<td>201.66 ± 14.41##</td>
</tr>
<tr>
<td>4.</td>
<td>Thioperamide (15 mg/kg i.p.)</td>
<td>204.33 ± 6.79</td>
<td>668.33 ± 19.83</td>
<td>3836.66 ± 256.17</td>
<td>241.5 ± 19.87</td>
</tr>
<tr>
<td>5.</td>
<td>Thioperamide (3.75 mg/kg i.p.) + Amphetamine (2 mg/kg s.c.)</td>
<td>160.33 ± 12.11####</td>
<td>770.66 ± 19.99##</td>
<td>2876.5 ± 68.00##</td>
<td>190.50 ± 7.92##</td>
</tr>
<tr>
<td>6.</td>
<td>Thioperamide (7.5 mg/kg i.p.) + Amphetamine (2 mg/kg s.c.)</td>
<td>189.66 ± 7.82##</td>
<td>740.00 ± 14.90##</td>
<td>3045.66 ± 64.28##</td>
<td>206.16 ± 10.67##</td>
</tr>
<tr>
<td>7.</td>
<td>Thioperamide (15 mg/kg i.p.) + Amphetamine (2 mg/kg s.c.)</td>
<td>202.83 ± 8.36</td>
<td>717.66 ± 19.59</td>
<td>3450.5 ± 385.73##</td>
<td>229.5 ± 25.59##</td>
</tr>
<tr>
<td>8.</td>
<td>THP (3.75 mg/kg i.p.) + RAMH (5 µg i.c.v.) + Amphetamine (2 mg/kg s.c.)</td>
<td>192.16 ± 20.87##</td>
<td>722.66 ± 17.48##</td>
<td>3257.5 ± 292.76##</td>
<td>204.66 ± 16.86##</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM; n = no of animals = 6; * P< 0.05; ** P< 0.01 vs Gp. 1, * P< 0.05; ## P<0.01 vs Gp. 2.; Significance by one - way ANOVA followed by Dunnett's t test.
Table 8. Effect of H3 receptor ligands on apomorphine induced climbing behavior in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (n=6)</th>
<th>Max time (sec)</th>
<th>% Climbing index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>14.58 ± 1.69</td>
<td>7.85 ± 1.93</td>
</tr>
<tr>
<td>2.</td>
<td>Apomorphine (1.5 mg/kg s.c.)</td>
<td>382.54 ± 142.56</td>
<td>35.33 ± 13.60</td>
</tr>
<tr>
<td>3.</td>
<td>Thioperamide (3.75 mg/kg i.p.) + Apomorphine (1.5 mg/kg s.c.)</td>
<td>418.29 ± 187.68*</td>
<td>29.35 ± 11.63</td>
</tr>
<tr>
<td>4.</td>
<td>Thioperamide (7.5 mg/kg i.p.) + Apomorphine (1.5 mg/kg s.c.)</td>
<td>268.98 ± 87.29</td>
<td>24.02 ± 7.96</td>
</tr>
<tr>
<td>5.</td>
<td>Thioperamide (15 mg/kg i.p.) + Apomorphine (1.5 mg/kg s.c.)</td>
<td>3.59 ± 1.48</td>
<td>0.61 ± 0.13&quot;</td>
</tr>
<tr>
<td>6.</td>
<td>Thioperamide (15 mg/kg i.p.) + RAMH (5 µg i.c.v.) + Apomorphine (1.5 mg/kg s.c.)</td>
<td>94.62 ± 24.36</td>
<td>8.39 ± 1.54</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM

n = no of animals = 6

*P < 0.05; vs Gp. 1, #P < 0.05; vs Gp. 2.

Significance by one-way ANOVA followed by Dunnett’s t test.
Table 9. Effect of thioperamide on the oxidative stress markers in middle cerebral artery occluded (MCAO) rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (n=6)</th>
<th>TBARS (n moles MDA/mg proteins)</th>
<th>GSH (μ moles GSH/mg protein)</th>
<th>CAT (n moles of H₂O₂ consumed /min/mg protein)</th>
<th>GST (n moles of CDNB conjugate formed /min/mg protein)</th>
<th>GPx (n moles of NADPH oxidized /min/mg protein)</th>
<th>GR (n moles of NADPH oxidized /min/mg protein)</th>
<th>SOD (n moles formazan formed /min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle Control</td>
<td>0.57 ± 0.23</td>
<td>97.35 ± 10.48</td>
<td>10.29 ± 6.21</td>
<td>110.84 ± 14.70</td>
<td>38.55 ± 6.26</td>
<td>40.86 ± 4.27</td>
<td>47.24 ± 12.24</td>
</tr>
<tr>
<td>2.</td>
<td>Sham Operated</td>
<td>0.76 ± 0.05</td>
<td>90.08 ± 8.31</td>
<td>19.84 ± 5.29</td>
<td>97.55 ± 8.98</td>
<td>31.67 ± 9.20</td>
<td>47.71 ± 9.61</td>
<td>48.71 ± 13.23</td>
</tr>
<tr>
<td>3.</td>
<td>MCAO</td>
<td>14.83 ± 3.80**##</td>
<td>25.65 ± 1.27**##</td>
<td>12.79 ± 0.83</td>
<td>51.94 ± 3.14**##</td>
<td>17.25 ± 4.65</td>
<td>25.12 ± 11.81</td>
<td>30.39 ± 0.67</td>
</tr>
<tr>
<td>4.</td>
<td>Thioperamide</td>
<td>0.24 ± 0.11††</td>
<td>31.97 ± 9.22**##</td>
<td>7.20 ± 1.24</td>
<td>23.85 ± 3.52**##</td>
<td>3.63 ± 0.81**##</td>
<td>14.26 ± 1.21**##</td>
<td>15.91 ± 1.93**##</td>
</tr>
<tr>
<td>5.</td>
<td>(5.5 mg/kg i.p.)</td>
<td>0.16 ± 0.05††</td>
<td>47.75 ± 4.11**##</td>
<td>10.37 ± 1.20</td>
<td>46.72 ± 7.35**##</td>
<td>10.14 ± 2.39**##</td>
<td>17.93 ± 0.64**##</td>
<td>18.91 ± 5.26**##</td>
</tr>
<tr>
<td></td>
<td>+ MCAO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represents as Mean ± SEM n= no of animals=6 *P<0.05, **P<0.01 vs Gp 1; # P<0.05, ## P<0.01 vs Gp 2; †† P<0.01 vs Gp 3; Significance by one – way ANOVA followed by Dunnett’s t test.
Table 10. Effect of thioperaomite on behavioral parameters in middle cerebral artery occluded (MCAO) rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (n=6)</th>
<th>Locomotor Count</th>
<th>Grip Strength (Kg)</th>
<th>Percentage Alternations</th>
<th>Possible Alternations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle Control</td>
<td>305.66 ± 64.78</td>
<td>0.38 ± 0.04</td>
<td>80.36 ± 5.80</td>
<td>12.83 ± 1.93</td>
</tr>
<tr>
<td>2</td>
<td>Sham Operated</td>
<td>295.50 ± 31.94</td>
<td>0.35 ± 0.03</td>
<td>85.19 ± 5.44</td>
<td>8.83 ± 1.35</td>
</tr>
<tr>
<td>3</td>
<td>MCAO</td>
<td>174.84 ± 21.82</td>
<td>0.47 ± 0.05</td>
<td>97.61 ± 2.38</td>
<td>4.00 ± 0.93**</td>
</tr>
<tr>
<td>4</td>
<td>Thioperaomite (5.5 mg/kg i.p.) + MCAO</td>
<td>305.16 ± 47.91</td>
<td>0.38 ± 0.08</td>
<td>86.94 ± 6.09</td>
<td>3.83 ± 0.30**</td>
</tr>
<tr>
<td>5</td>
<td>Thioperaomite (11 mg/kg i.p.) + MCAO</td>
<td>327.33 ± 60.87</td>
<td>0.39 ± 0.04</td>
<td>90.27 ± 6.24</td>
<td>4.83 ± 0.30**</td>
</tr>
</tbody>
</table>

Data represents as Mean ± SEM
n= no of animals=6
**P<0.01 vs Gp 1; # P<0.05, vs Gp 2;
Significance by one – way ANOVA followed by Dunnett’s t test
Table 11. Effect of thioperamide on the levels of nitric oxide (NO) in middle cerebral artery occluded (MCAO) rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (n=6)</th>
<th>Nitrate (µ Moles / liter)</th>
<th>Nitrite (µ Moles / liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle Control</td>
<td>31.98 ± 0.63</td>
<td>2.16 ± 0.24</td>
</tr>
<tr>
<td>2.</td>
<td>Sham Operated</td>
<td>27.24 ± 2.37</td>
<td>2.48 ± 0.13</td>
</tr>
<tr>
<td>3.</td>
<td>MCAO</td>
<td>19.85 ± 2.10</td>
<td>1.53 ± 0.02</td>
</tr>
<tr>
<td>4.</td>
<td>Thioperamide</td>
<td>15.51 ± 0.43</td>
<td>3.08 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>(5.5 mg/kg i.p.) + MCAO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Thioperamide</td>
<td>20.66 ± 0.60</td>
<td>3.29 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>(11 mg/kg i.p.) + MCAO</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represents as Mean ± SEM
n= no of animals=6
*P<0.05, **P<0.01 vs Gp 1; # P<0.05, ## P<0.01 vs Gp 2; †† P<0.01 vs Gp 3;
Significance by one – way ANOVA followed by Dunnett’s t test
5.0. Discussion
5.0. DISCUSSION

Presynaptic control via histamine H\textsubscript{3} receptors (autoreceptors) is an important mechanism of histamine-mediated neurotransmission. The CNS effects of selective ligands of these receptors are currently an area of great interest. While experimental evidence from several studies indicate a variety of central effects following H\textsubscript{3} receptor stimulation or blockade and a therapeutic potential of H\textsubscript{3} receptor blockers in epilepsy and cognitive function disorders (Alguacil, Perez-Garcia, 2003), little is known about the role of these ligands in neuropsychiatric disorders (anxiety, depression and schizophrenia) and cerebral ischemia. There are, however, indicators (both direct and indirect) for the involvement of HA in these conditions requiring an effort in this direction and hence our study. Nonetheless, the understanding of underlying mechanisms in the pathogenesis of above-mentioned disorders is incompletely understood and hence attempts have been made to explore novel targets for these conditions. We chose to explore H\textsubscript{3} receptor ligands for these conditions as histaminergic neuronal system has now gained the status of a regulatory system for whole brain activity (Wada et al., 1991). In addition, a highly localized CNS distribution of H\textsubscript{3} receptors with extensive modulatory role is indicative of the vast potential for the ligands of these receptors in various CNS disorders.

ANXIETY

The present study evaluated the effects of selective H\textsubscript{3} receptor ligands on Vogel's conflict test (VCT) and elevated plus maze (EPM) in mice. The standard drug diazepam (1 mg/kg i.p.) produced significant antianxiety effects in both the paradigms. The test drugs (THP and RAMH), however, at the doses used, were without any significant effect. Our results are in agreement with those of Perez-Garcia et al (1999) where no anxiolytic effects were observed in rats treated with, either RAMH, THP or clobenpropit (another H\textsubscript{3} receptor antagonist) in the EPM. However, the predictive validity of latter test has some limitations i.e. it fails to detect the anxiolytic effects of buspirone-like drugs. Thus, it is advisable to test drugs in other models of anxiety such as VCT. We did not find any significant change in the latter test as well, though there was a tendency to increase anxiety-like behavior following THP. Infact, H\textsubscript{3} receptor antagonists have shown to induce experimental anxiety.
in mice in EPM and object recognition task (Yuzurihara et al., 2000; Bongers et al., 2004). Anxiolytic drugs such as benzodiazepines and 5 HT1A receptor agonists buspirone decrease histamine turnover in rodents (Chickai et al., 1993), suggesting that increase in central histamine with H3 receptor antagonists, could push the system too far and exacerbate existing anxiety. The differential effects observed in our study could be related to the doses employed and the animal species used. Our results, however, suggest a lack of significant effect on anxiety following either histamine receptor stimulation or blockade.

DEPRESSION

The results of the present study show a dose dependent antidepressant effect following thioperamide, a selective H3 receptor antagonist, in the modified FST. RAMH, a selective histamine H3-receptor agonist was, however, without any effect. This indicates that the antidepressant effect of THP may not be related to H3-receptor mediated increase of histamine release. It is well known that H3 receptors regulate the release of not only histamine but also a variety of other neurotransmitters including NA, 5-HT, DA, ACh and GABA in the CNS (Schlicker et al., 1988; Vellinga et al., 1992; Schlicker et al., 1993, 1994; Vohora et al., 2004). Thus, it is possible that THP exerted its antidepressant effect via modulation of these transmitters. One of the major advantages of modified FST over the traditional Porsolt test (Porsolt, 1977; 2000) is that it can differentiate swimming behavior sensitive to selective serotonin reuptake inhibitors (SSRIs) and 5-HT receptor agonists with that of the climbing behavior sensitive to tricyclic antidepressants (TCA) and drugs with selective action on catecholaminergic transmission (Cryan and Lucki, 2000; Cryan et al., 2002). Thus, in the present study, fluoxetine, a SSRI significantly increased the swimming time and reduced the immobility time. Further, thioperamide (both at 7.5 and 15 mg/kg) reduced the immobility time with a simultaneous increase in the swimming time without significantly affecting the climbing behavior. Thus, it is likely that the antidepressant effect produced by THP is exerted through an H3-receptor mediated increase of 5-HT release in the brain. Our results are consistent with those of other workers demonstrating the antidepressant effect of THP and other selective H3 receptor antagonists such as clobenpropit (Perez-Garcia et al., 1999). We, however, provide the first evidence
Discussion

for its effect on modified FST and a possible role for serotonergic mechanism in its antidepressant effect.

Recent evidence indicates that oxidative stress plays an important role in the pathogenesis of depressive illness. Experimental data from several studies indicate a lower antioxidant defense against lipid per oxidation, a lower blood GSH levels in patients with depression and a therapeutic benefit from antioxidant supplementation (Altshule et al., 1952; Ozcan et al., 2004). In our study, mice exposed to modified FST exhibited oxidative stress and increase of free radical generation as evidenced by a significant reduction in GSH levels and an increase in TBARS and catalase levels. Our results, thus, provide further evidence for the involvement of oxidative stress in the biochemical mechanisms underlying depression. Pretreatment with THP (3.75 - 15 mg/kg) effectively reversed the FST-induced reduction in GSH levels thus corroborating the earlier reports of GSH being a cerebral substrate in depression (Pal and Dandiya, 1994). Though a similar reversal was also observed in the TBARS levels, it was not found to be statistically significant. A dramatic increase in catalase levels was observed following FST. The enzyme is known to play a major role in cellular antioxidant defense by decomposing hydrogen peroxide thereby preventing the generation of hydroxyl radical (Ho et al., 2004). Pretreatment with THP could reverse such an elevation of catalase levels. Our results thus indicate an antioxidant potential for THP. This is probably the first experimental evidence for an antioxidant activity reported with any H1-receptor antagonist. This opens up exciting possibilities for the use of THP in various conditions associated with oxidative stress. Surprisingly, RAMH normalized the elevated catalase levels but did not show such an effect on GSH levels. Probably, these differential effects of RAMH on parameters of oxidative stress are responsible for a lack of its effect on modified FST.

To conclude, THP (but not RAMH) shows antidepressant effect in the modified FST by significantly reducing swimming and immobility times. Further, it causes a reversal of FST induced oxidative stress. Our study, thus, indicates that the antidepressant effect of THP could be related to either its ability to modify serotonergic transmission as evidenced by its effect on swimming behavior or via its antioxidant property.
SCHIZOPHRENIA

The present study also evaluated the effects of histamine H₃ receptor ligands on neuroleptic-induced catalepsy, apomorphine-induced climbing behavior and amphetamine-induced locomotor activity in mice. THP and RAMH per se did not affect the catalepsy times. However, THP dose-dependently increased the catalepsy times when co-administered with haloperidol. Further, pretreatment with RAMH suppressed this effect indicating the involvement of H₃ receptors. Our result is in agreement with Pillot et al reporting the potentiation of haloperidol-induced catalepsy by ciproxifan, another H₃ receptor antagonist (Pillot et al., 2002). Our results on catalepsy are, however, in contrast with the earlier report of Morisset and co-workers (Morisset et al., 1999) who failed to detect the potentiation of haloperidol-induced catalepsy by THP in mice but demonstrated the same in rats. However, they have used a 0.25 mg/kg i.p. dose of haloperidol in mice. This suggests that the potentiation of catalepsy is also dependent on the dose of haloperidol.

It has been reported that catalepsy induced by neuroleptic is associated with a concomitant increase in brain histamine content in mice (Yagi et al., 1990). Further, i.c.v. histamine and l-histidine has been reported to potentiate haloperidol-induced catalepsy in mice while H₁ - receptor blockers or histamine synthesis inhibitors antagonize it (Mulay et al., 1981; Yagi et al., 1990). Thus, the potentiation of histamine-induced catalepsy by THP could be related to an H₃ receptor mediated increase of histamine release in brain (Arrang et al., 1987). Recently, THP also potentiated the effect of risperidone on catalepsy (Zhang et al., 2005). The potentiation of neuroleptic-induced catalepsy by H₃ receptor antagonists has been suggested to result from a direct synergistic interaction between H₃ and D₂ receptors leading to enhanced activation of striatopallidal neurons (Morgan et al., 1991; Pillot et al., 2002). Another possible mechanism by which this potentiation can be explained is that H₃ receptors besides regulating the release of histamine also regulate the release of other neurotransmitters especially 5-HT. Increased availability of brain 5-HT due to the blockade of H₃ receptors could have resulted in worsening of catalepsy as 5-HT reuptake blockade (by e.g. fluoxetine) is known to exacerbate extrapyramidal side effects of antipsychotics.
Several clinical reports support this hypothesis, for example, fluoxetine can produce Parkinson-like extrapyramidal side effects (Meltzer et al., 1979; Chouinard et al., 1992; Ames et al., 1993; Jansen et al., 1993; Katai et al., 1993).

While the pharmacodynamic interaction at the histaminergic, dopaminergic and serotonergic system could play an important role, the existence of a pharmacokinetic interaction may not be ruled out. Both ciproxifan and THP are inhibitors of the cytochrome P450 enzymes, responsible for metabolizing risperidone and haloperidol (Zhang et al., 2005). It has been reported recently that imidazole H3 receptor antagonists potentially inhibited the metabolism of haloperidol and risperidone. Thus, a pharmacokinetic interaction could partly contribute to such a potentiation by THP. However, the reversal of THP on catalepsy by RAMH strongly suggests the involvement of pharmacodynamic interaction involving H3 receptors.

The inhibitory effect of thioperamide on amphetamine-induced hyperactivity has already been documented by other workers (Itoh et al., 1984; Clapham et al., 1994). Reversal of amphetamine-induced hyperactivity has been routinely used as a screening model to assess the antipsychotic activity of a compound (Arnt et al., 1995). In our study, THP *per se* did not affect the locomotor activity of mice. THP, however significantly reduced amphetamine-induced locomotor activity (the lower dose being more effective). However, the inhibition by RAMH of the promoting effect of THP on haloperidol-induced catalepsy strongly suggests the involvement of H3 receptors. The mechanism for this reduction was attributed to the increase in levels of histamine by THP, which could further reduce the dopamine stores. As dopaminergic neurons are involved in locomotor activity, the relative deficiency of dopamine could eventually lead to reduction of amphetamine-induced locomotor activity.

Apomorphine, a dopamine receptor agonist, exhibited climbing behavior. The climbing behavior is known to be elicited by stimulation of dopamine receptors in the striatum (Schlicker et al., 1993). THP at 15 mg/kg i.p. but not 3.75 and 7.5 mg/kg i.p. reduced apomorphine-induced climbing. This suggests that the histaminergic mechanisms may be
closely related to the dopaminergic systems, and play an important modulatory role in behaviors induced by dopaminergic agents. Further, as such a reduction by THP was at least partially reversed in presence of an H3 receptor agonist, a role of H3 receptor mechanism is likely in the modulation of the climbing behavior induced by apomorphine.

Taken together, our results indicate an antipsychotic-like profile of THP, a selective histamine H3 receptor antagonist, in mice. While the potentiation of haloperidol-induced catalepsy may result from both pharmacodynamic and pharmacokinetic interaction, its effects on other models viz apomorphine-induced climbing and amphetamine-induced locomotor activity is suggestive of an interaction mainly at a pharmacodynamic level. It is interesting to note that atypical antipsychotic drugs also enhance histamine turnover in the brain (Morisset et al., 1999). Our results suggest a potential for H3 receptor antagonists in improving the refractory cases of schizophrenia. As H3 receptor antagonists are known to improve cognitive and vigilance deficits (Vohora et al., 2004) and have antidepressant effects (Perez-Garcia et al., 1999; Akhtar et al., 2005), their use in schizophrenia may alleviate some of the negative symptoms observed in such patients. However, our observations are preliminary and further experimental investigations including an exact biochemical correlation with histamine and dopamine levels, are warranted to provide a more conclusive view.

CEREBRAL ISCHEMIA

In the present study, cerebral ischemia was induced by MCA occlusion for 2 h followed by 22 h reperfusion after which the oxidative stress markers were estimated in the whole brain of rats. We found an elevation of TBARS levels at 24 h of MCA occlusion as compared to sham treatment indicating lipid peroxidation and the involvement of oxidative stress in the neuronal injury. In addition to an increase in TBARS levels, a significant reduction in the GSH levels was observed following MCAO. Our results on TBARS and GSH are, thus, in consistent with the extensive number of experimental studies indicating an increase in free radical production in the post-ischemic period particularly after reperfusion (Yavuz et al., 1997; Sinha et al., 2001; Sinha et al., 2002; Gupta et al., 2002 a, b; Chaudhry et al., 2003).
This is further strengthened by a decline observed in the levels of antioxidant enzymes including GST, GPx, GR and SOD, though the last two being statistically insignificant.

In order to determine whether free radicals constitute a valuable therapeutic target in stroke, various antioxidant strategies have been evaluated both experimentally and clinically (Takasago et al., 1997; Haley 1998; Yamaguchi et al., 1998; Mary et al., 1999; Lees et al., 2001; Vender Worp et al., 2002; Lees et al., 2003; Salom et al., 2004; Toyoda et al., 2004). Recently we have reported an antioxidant potential of THP, a selective H₃ receptor antagonist, in forced swim test (FST) induced oxidative stress in mice (Akhtar et al., 2005). Further, in view of the recent evidences for the role of histamine and histaminergic agents in cerebral ischemia (Adachi et al., 2001; Adachi 2002, Yamamoto et al., 2004; Adachi 2005), we evaluated the role of THP in the present model. Pretreatment with THP (at both 5.5 and 11 mg/kg i.p) reversed the MCAO induced increase in TBARS. Though a tendency towards an increase GSH was also observed following THP, it was found to be statistically insignificant. Our results on TBARS are in agreement with the recent report of Adachi (Adachi, 2005) in which THP alleviated brain infarction in MCA occluded and this was shown to correlate well with an increase in striatal histamine levels. It is well known that H₃ receptors are densely localized in cerebral cortex, striatum and hippocampus (Arrang et al., 1983, 1987), which could be a factor in alleviation of brain infarction in the above study. However, they observed the effects of THP in combination with L-histidine and not per se. Thus, additional mechanisms (other than augmentation of central histaminergic activity) could be responsible. Our study provides one such possible mechanism for the protective effect of THP against cerebral ischemia.

Contrary to the effects on TBARS and GSH, THP (at both the doses), in our study, could not reverse the reduced levels of antioxidant enzymes. Paradoxically, it further reduced some of these enzymes including GPx, GR and SOD. While the reason for such an effect observed by THP is not clear, one possible explanation for insignificant reduction could be due to a delayed decline of such antioxidant enzymes following MCA occlusion in the groups treated with THP. Lerovet et al (2002) demonstrated a 48 h decline in the activity of some of the antioxidant enzymes following MCAO. This could be responsible for
insignificant reduction in the levels of catalase, GR and SOD following MCAO. Further, the data on SOD has also not been consistent in most of the studies (Lerovet et al., 2002). Further follow-up studies after 48 h and 72 h of MCAO and simultaneous measurement of histamine levels are required to investigate the exact role and the possible mechanism for the effect of THP in cerebral ischemia. It seems to be difficult to explain the dual effects (both pro-and antioxidant) of THP observed in the present study and the antioxidant effects observed in our earlier study on FST. Needless to emphasize that the neuronal injury in MCAO is far more intense as compared to that in FST.

We did not find any significant change in the grip strength, and percentage alternation of animals following MCAO and THP pretreatment. Locomotor activity was reduced slightly after MCAO but it was reversed on pretreatment with THP.

Accumulating evidence supports the idea that an over activity of excitatory amino acid transmitter systems and subsequent excessive stimulation of N-methyl-D-aspartic acid (NMDA) receptors play a significant role in the pathogenesis of ischemic neuronal injury. It is hypothesized that the rise in intracellular Ca\(^{2+}\) levels associated with NMDA receptor over-stimulation is the trigger of brain injury following cerebral ischemia. However, NO is a small diffusible molecule that appears to play a crucial role in many physiological, pathological and pharmacological processes. In the CNS, NO has been reported to function as neurotransmitter. Evidence that NO mediates the physiological effects of glutamate and participates in the development of ischemic brain damage strongly suggests that NO would also be involved in the mechanisms underlying the ischemic neuronal injury. It has been shown that there is an increase in nitric oxide production during brain injury and ischemic damage (Nakashima et al., 1999). This NO production may lead to toxicity by reacting with superoxide and producing peroxynitrite (Margaill et al., 2005). Contrary to an increase in NO production, we found a decrease in the level of nitrite and nitrate following MCAO. It is possible that some homeostatic mechanisms are operative that try to trigger the biochemical events i.e. compensatory reduction in the levels of NO. Another possibility is that chloral hydrate, the anesthetic used in the present study, could possibly interfere with the results obtained since it is known to antagonize glutamate receptors and this could
have resulted in decrease NO levels. While we do not have a clear explanation to a reduced NO following MCAO, our findings are in agreement with the investigations on NO synthase inhibitors where a decrease (Nowicki et al., 1991; Buisson et al., 1992; Nagafugi et al., 1992), an increase (Weissman et al., 1992; Yamamoto et al., 1992; Kuluz et al., 1993; Zhang et al., 1993) or no change (Dawson et al., 1992; Buchan et al., 1994) in the levels of cerebral infraction volume were reported. THP did not produce any significant change in the nitrite levels following MCAO. There was, however, a slight reversal of the reduced nitrite levels following MCAO.

To conclude, augmentation of histaminergic activity by blockade of histamine H₃ receptors could be a novel approach in cerebral ischemia. However, our results are preliminary and raise doubts on the use of THP in cerebral ischemia in view of its dual effects observed on oxidative stress markers. A detailed investigation on the same and simultaneous estimation of histamine and NO levels at different time intervals of MCA occlusion would probably give a clear picture of its possible use in cerebral ischemia and the possible mechanisms.