Overview:
Approximately half of all dementias are of the Alzheimer-type; clinical diagnosis of Alzheimer-disease (AD) is based on the primary indicator of memory loss with progressive cognitive impairment. It is well known that both ageing and age associated neurodegenerative disorders such as Alzheimer’s disease is associated with varying degrees of cognitive impairment, which can cause significant morbidity. Although neuropathologically Alzheimer’s disease is characterized by the accumulation of extracellular plaques, consisting primarily of a low molecular weight amyloid-b (Ab) peptide, and intracellular neurofibrillary tangles of aggregated hyperphosphorylated tau protein, it is well documented that AD is not a single entity. At present, amyloid beta-protein (A-beta) 1-42 is thought to be the most important substance and main ingredient of senile plaques. The two major neuropathological hallmarks of AD are extracellular beta-amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). The production of Aβ, which represents a crucial step in AD pathogenesis, is the result of cleavage of Aβ precursor protein (β-APP) that is overexpressed in AD (Griffin WS, 2006). Along with this free radicals and oxidative stress have been implicated as the prime candidates mediating the behavioral impairments and memory deficits in age related neurodegenerative disorders.

The additional risk for cognitive impairment conferred developing cardiovascular disease and stroke, hypertension is also a risk factor for cognitive impairment is clearly indicated in the clinical data associated with long-term elevated blood pressure (Harrington F et al., 2000). Antihypertensive therapies may be beneficial in treatment and prevention of Alzheimer’s disease. Drugs acting via the renin–angiotensin system are considered and it is suggested that
these drugs may produce their effects via mechanisms other than by their antihypertensive actions. The renin-angiotensin system (RAS) exists extensively throughout many organs of animals. Active components of the RAS, such as Ang II, do not cross the blood-brain barrier peripheral RAS can only directly influence those cerebral regions, such as circumventricular areas, that lack the blood-brain barrier. Therefore, blood-borne Ang II has been postulated to interact with specific receptors in the neurons of circumventricular organs which may project to many other brain regions behind the blood-brain barrier beyond the actions of peripheral RAS components of RAS present in certain regions of the central nervous system (CNS), an independent RAS exits in the brain. All the components of the renin-angiotensin system have been located in the brain. In general, the brain Ang II receptors are closely associated with endogenous brain derived Ang II. The distribution of angiotensinogen within the brain generally correlates with the location of the Ang II receptors. Using evidence from animal studies, the role of angiotensin as a neurotransmitter and its involvement in the control of normal cognitive function is reported. Angiotensin antagonists and angiotensin converting enzyme (ACE) inhibitors are now commonly used for the treatment of hypertension. To the extent that they control hypertension and retard cerebrovascular damage they may also be expected to reduce the incidence of disease-related cognitive decline. Therefore, it suggests that manipulation of the renin–angiotensin system may improve cognitive performance, independent of their protective role against vascular damage.

3.1. Renin–angiotensin system (RAS)

The angiotensins are a group of related peptides, of which octapeptide
angiotensin II (Ang II) is the best known and the most studied. Synthesis of the angiotensins begins with the conversion of angiotensinogen, a circulating peptide from the liver, to angiotensin I (Ang I) by the enzyme renin, released from the juxtaglomerular apparatus of the kidney in response to decreased pressure within the renal arterioles. Ang II is then synthesized from Ang I by the action of angiotensin converting enzyme (ACE), which is present with the endothelium of most blood vessels but has highest concentrations within the pulmonary vasculature and in the brain including the nigrostriatal pathway and the basal ganglia. After cleavage of the precursor glycoprotein angiotensinogen by renin, ACE converts the produced Ang I into Ang II. Ang II is then metabolized to Ang III (angiotensin 2–8), Angiotensin IV (angiotensin3–8) angiotensin1–7 or angiotensin3–7. All of the angiotensins are pharmacologically active with markedly different potencies, although different rank order of potency in different tissues has indicated the existence of multiple angiotensin receptors (De Gasparo M et al., 2000).

3.1.1. Components of the Renin-Angiotensin System (Fig. 3.2)

Renin

The rate limiting step in the Ang II production is the amount of renin released by the kidney. Renin is synthesized, stored, and secreted into the arterial circulation by exocytosis of the granular juxtaglomerular cells of the kidney (Fig. 3.1). Renin is an aspartyl protease that attacks a restricted number of substrates. Its principal natural substrate is a circulating α2-globulin, angiotensinogen, secreted by hepatocytes. Renin cleaves the bond between residues 10 and 11 at the amino terminus of angiotensinogen to generate Ang I. The active form of renin is a glycoprotein that contains 340 amino acids. It is synthesized as a pre proenzyme
of 406 amino acid residues that is processed to prorenin, a mature but inactive form of the protein. Prorenin then is activated by removal of 43 amino acids from its amino terminus to yield active renin (Friis et al., 1999). Both renin and prorenin are stored in the juxtaglomerular cells and, when released, circulate in the blood. The concentration of prorenin in the circulation is approximately tenfold greater than that of the active enzyme. The half-life of circulating renin is approximately 15 minutes (de Gasparo et al., 2000). Angiotensinogen is the major substrate for the enzyme renin, which generates angiotensin I. This molecule, in turn, is converted to angiotensin II by an ACE. Angiotensin II then binds to its receptor to mediate many different cellular effects.

**Angiotensinogen**

Angiotensinogen, the substrate for renin, is an abundant globular glycoprotein (MW = 55,000 to 60,000) containing 13% to 14% carbohydrate. Angiotensinogen is produced constitutively and released into the circulation mainly by the liver. Angiotensinogen transcripts also are abundant in fat, certain regions of the CNS, and kidney. It is a member of the serpin family, although it is not known to inhibit other enzymes, unlike most serpins. The human angiotensinogen contains 452 amino acids and is synthesized as preangiotensinogen, which has a 24- or 33-amino acid signal peptide. The synthesis of angiotensinogen is stimulated by inflammation, insulin, estrogens, glucocorticoids, thyroid hormone and AngII (Staessen et al., 1999).
Fig 3.1. Schematic portrayal of the three major physiological pathways regulating renin release

Angiotensin converting enzyme (ACE)
ACE is secreted by pulmonary and renal endothelial cells and catalyzes the conversion of decapeptide Ang I to octapeptide Ang II (Niu et al., 2002). ACE is an ectoenzyme and glycoprotein with an apparent molecular weight of 170,000. Human ACE contains 1277 amino acid residues and has two homologous domains, each with a catalytic site and a Zn$^{2+}$-binding region. ACE has a large amino-terminal extracellular domain, a short carboxyl-terminal intracellular domain and a 17-amino acid hydrophobic region that anchors the ectoenzyme to the cell membrane. Circulating ACE represents membrane ACE that has undergone proteolysis at the cell surface by a secretase. ACE is nonspecific peptidase which cleaves dipeptide units from substrates with diverse amino acid sequences. Preferred substrates have only one free carboxyl group in the carboxyl-terminal amino acid and proline must not be the penultimate amino acid; thus the enzyme does not degrade Ang II. ACE is identical to kininase II, the enzyme that inactivates bradykinin and other potent vasodilator peptides. Although slow conversion of Ang I to Ang II occurs in plasma, the very rapid metabolism that occurs in vivo is due largely to the activity of membrane-bound ACE present on the luminal surface of endothelial cells throughout the vascular system (Beldent et al., 1995).

Angiotensin converting enzyme 2 (ACE 2) is a new component of the RAS that was discovered in 2000 (Donoghue et al., 2000). Although ACE 2 was first shown to cleave Ang I to Ang (1–9), which can be converted by ACE into the heptapeptide Ang (1–7), another study showed that ACE 2 hydrolysis of Ang II into Ang-(1–7) has a much higher efficiency (~ 400-fold) than that for Ang I to Ang (1–9). It has been established that Ang (1–7) mediates vasodilation, antiproliferation and apoptosis, therefore opposing the effects of Ang II. Using genetic and pharmacologic approaches, ACE 2 has been shown to have
protective effects in various tissues and to prevent overactive RAS associated
diseases, including hypertension (Xia et al., 2008). Thus, the new arm of the
RAS, the ACE 2-Ang-(1–7)-Mas axis, has been shown to be effective at
counter-regulating the effects of the classic ACE-Ang II-AT1R axis.

Fig. 3.2: Schematic of the components of the rennin angiotensin system.

**Angiotensin peptides**

Ang I is formed by the action of renin on angiotensinogen. Ang I appear to have
no biological activity and exist solely as a precursor to Ang II. Ang II and Ang
III acts as agonists at the specific receptors called Angiotensin receptors (AT
receptors) (de Gasparo et al., 2000). Ang IV binds with low affinity at the AT1
and AT2 receptor subtypes (Swanson et al., 1992), but with high affinity and
specificity at the AT4 receptor subtype. A specific binding site for Ang (1–7) has been reported (Ferrario, 2003), but not fully elucidated.

**Formation of Angiotensin peptides**

The tetradecapeptide angiotensinogen serves as a precursor to angiotensin peptides (Fig. 3.3). The decapeptide Ang I is formed by renin acting upon the amino terminal of angiotensinogen. Ang I is a substrate for ACE, a zinc metalloprotease that hydrolyzes the carboxy terminal dipeptide His-Leu to form Ang II (Unger et al., 2004). Ang II is converted to Ang III by glutamyl aminopeptidase A that cleaves the Asp residue at the N-terminal (Vauquelin et al., 2002). Membrane alanyl aminopeptidase N cleaves Arg at the N-terminal of Ang III to form Ang IV which is further converted to Ang (3–7) by carboxypeptidase P(carb-P) and propyl oligopeptidase cleavage of the Pro-Phe bond. Endopeptidases such as chymotrypsin are capable of cleaving the Val, Tyr and Ile residues along with dipeptidyl carboxypeptidase that cleaves the His–Pro bond, reducing Ang IV and Ang (3–7) to inactive peptide fragments and amino acid constituents. Ang II can also be converted to Ang (1–7) by Carb-P cleavage of Phe, by the newly discovered mono-peptidase ACE2 (Ferrario et al, 2004) or by ACE cleavage of the dipeptide Phe-His from Ang (1–9) (Vauquelin et al., 2002), and can be further converted to Ang (2–7) by AP-A acting at the Asp-Arg bond.

**3.2. Central Renin Angiotensin System**

Active components of the RAS, such as Ang II, do not cross the blood-brain barrier (Harding JW et al, 1988), and peripheral RAS can only directly influence
those cerebral regions, such as circumventricular areas, that lack the blood-brain barrier. Therefore, blood-borne Ang II has been postulated to interact with specific receptors in the neurons of circumventricular organs (CVOs) which may project many other brain regions behind the blood-brain barrier. Beyond the actions of peripheric RAS components in certain regions of the central nervous system (CNS), an independent RAS exits in the brain. Early observations in the 1970s in dog and rodent brains suggested the possibility of a centrally localized RAS. Renin was isolated in the dog brain (Ganten D et al., 1971), and Ang II binding sites were determined in rat brain (Sirrett NE, 1977). Immunohistochemical experiments revealed the distribution of angiotensinogen, Ang I, Ang II and renin in several brain regions of rats. Ang II immunoreactivity was localized both to neurons and vessels in the brainstem, cerebellum and hypothalamus, whereas angiotensinogen and Ang I was found in cellular localization in hypothalamic nuclei. In addition, in human brain Ang II immunoreactivity has been shown in the basal ganglia, cortex, hypothalamus,
thalamus, brainstem and cerebellum. Thus, Ang II in the brain has been suggested to act as a neurotransmitter regulating thirst, drinking, antidiuretic hormone secretion, facilitating vasopressor effects and hormone secretion such as adrenocorticotrophic and luteinizing hormones. Ang II is also involved in the regulation of neurotransmitters such as noradrenaline and 5-hydroxytryptamine (5-HT), and inhibits acetylcholine release. Today, it is well established that the brain has its own intrinsic RAS with all its components present in the CNS.

**Fig 3.3:** Schematic representation of renin angiotensin system.
3.2.1. Angiotensin Receptors

Angiotensins act through two specific G protein coupled receptors (GPCRs), designated AT1 and AT2. The AT1 receptor has a high affinity for losartan, a low affinity for PD 123177, and a low affinity for CGP 42112A (a peptide analog). In contrast, the AT2 receptor has a high affinity for PD 123177 and CGP 42112A but a low affinity for losartan. The AT1 and AT2 receptors have little sequence homology. Most of the known biological effects of Ang II are mediated by the AT1 receptor. Consistent with its functional preeminence, the AT1-receptor gene contains a polymorphism (A1166C) that is associated with hypertension, hypertrophic cardiomyopathy, coronary artery vasoconstriction, and aortic stiffness, while preeclampsia is associated with the development of activating autoantibodies against the AT1 receptor. Potential roles for the AT2 receptor include antiproliferative, proapoptotic, vasodilatory, and antihypertensive effects.

3.2.2. Angiotensin Receptor–Effecter Coupling

AT1 receptors activate a large array of signal-transduction systems to produce effects that vary with cell type and that are a combination of primary and secondary responses. The relative importance of these myriad signal-transduction pathways in mediating biological responses to Ang II is tissue-specific. Other receptors may alter the response to AT1-receptor activation (e.g., AT1 receptors heterodimerize with bradykinin B2 receptors, which enhances Ang II sensitivity in preeclampsia). Signaling from AT2 receptors is mediated largely by Gi. Consequences of AT2-receptor activation include activation of phosphatases, K⁺ channels, and bradykinin and nitric oxide (NO) production and inhibition of Ca²⁺ channel function.
3.2.3. Cerebral distribution of angiotensin receptors subtype in the brain

At the end of the 1980s, AT1 and AT2 angiotensin receptors that bound Ang II with equal potency were identified, and their specific antagonists were developed. AT1 receptors are, expressed in brain areas regulating autonomic and hormonal responses. AT1 receptors are heterogeneously regulated in a number of experimental conditions. In contrast to the AT1 receptor, AT2 receptors are expressed at low density in adult brain but are up-regulated under pathological conditions. AT4 receptors have more recently been identified as being identical with insulin-regulated aminopeptidase. Therefore, the angiotensin AT4 ligand (Ang IV) is also a potent competitive inhibitor of the insulin regulated aminopeptidase (Lew et al., 2003). CVOs have a reduced blood-brain-barrier because of the presence of fenestrated capillaries and are thought to represent the interface between the RASs of the periphery of the body and the CNS. Indeed, the CVOs, including the anterior pituitary gland, area postrema, median eminence, organum vasculosum of the lamina terminalis, and the subfornical organ possess Ang-II-binding sites (Landas et al. 1980). Apparently, the distribution of AT1 receptors is highly conserved across mammalian species, and in many brain areas, there is a considerable overlap of the distribution of AT1 and AT2 receptor. In certain areas, such as the cortex, AT2 receptors are almost absent. However, high densities of Ang-IV-binding sites can be found both in the cortex and in other brain areas associated with memory function (e.g., hippocampus and amygdala).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AT1 receptor</th>
<th>AT2 receptor</th>
<th>AT4 receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>Ang II&gt;Ang III&gt;Ang I</td>
<td>Ang III&gt;Ang</td>
<td>Nle&gt;Ang IV</td>
</tr>
</tbody>
</table>
### Chapter 3: Review of Literature

<table>
<thead>
<tr>
<th>Selective antagonist</th>
<th>CGP46027, Candesartan, Losartan, Telmisartan</th>
<th>PD123 319, PD123177, PD121981</th>
<th>Leual3-AngIV, Divalinal-AngIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coupled to G-protein</td>
<td>G-protein</td>
<td>G-protein</td>
<td>Tyrosine kinase</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>↑Ca2+, ↑IP3, DAG, ↓Adenylyl cyclase, Src, JAK/STAT, PL-A, -C and -D, ↑Prostaglandins</td>
<td>↓cGMP/↑cGMP, ↑Prostaglandins PL-A2, NO</td>
<td>Gab1, Grb 2, Grb10, PI3K, PLC-8, SHP2, Shc</td>
</tr>
<tr>
<td>Structure</td>
<td>359 amino acids; 7 transmembrane domains</td>
<td>363 amino acids; 7 transmembrane domains</td>
<td>dimer linked by disulfide bonds</td>
</tr>
<tr>
<td>Molecular size</td>
<td>41-42 kDa</td>
<td>40-41 kDa</td>
<td>α 50 kDa, β 140 kDa</td>
</tr>
</tbody>
</table>

Characteristic features of various angiotensin receptors

#### 3.2.3.1. AT1 receptors

The human AT1 gene is located on chromosome 3q and codes for a protein of 40–42 kDa (359 amino acids). Most species express a single autosomal AT1 gene, but two related AT1\(_A\) and AT1\(_B\) receptor genes are expressed in rodents. These two receptors are 95% identical in their amino acid sequences. They also seem to be similar in terms of ligand binding and activation but differ in their
tissue distribution, chromosomal localization, and transcriptional regulation (de Gasparo et al. 2000).

The AT1 receptor subtype is a G-protein-coupled receptor with signaling via phospholipase-C and calcium. Thus, the angiotensin ligand binds to the AT1 receptor and induces a conformational change in the receptor protein that activates G proteins that, in turn, mediate signal transduction. This transduction includes several plasma membrane mechanisms including phospholipase-C, phospholipase-A2, and phospholipase-d-adenylate cyclase, plus L-type and T-type voltage sensitive calcium channels (de Gasparo et al., 2000). This AT1 receptor (now designated AT1\textsubscript{A}) is also coupled to intracellular signaling cascades that regulate gene transcription and the expression of proteins that mediate cellular proliferation and growth in many target tissues. Expression cloning was used to isolate the cDNAs encoding this receptor protein (Murphy et al., 1991) and it was found to be a 7-transmembrane domain protein consisting of 359 amino acids with a mass of approximately 41. Subsequently, a second AT1 subtype was discovered and designated AT1\textsubscript{B} that was also cloned in the rat, and in human (Konoshi et al., 1994). This subtype is approximately 92–95% homologous with the amino acid sequence of the AT1\textsubscript{A} subtype. Of these two isoforms the AT1\textsubscript{A} subtype appears to be responsible for the classic functions associated with the brain angiotensin system. The AT1 receptor subtype is maximally sensitive to Ang II, but is also responsive to Ang III.

**Cerebral Distribution and Regulation of Expression of AT1 receptors**

Median eminence, amygdale, anterior pituitary gland, area postrema, dorsal motor nucleus of the vagus, inferior olivary nucleus, habenula, lateral olfactory tract, locus coeruleus, interpeduncular nucleus, vasculosum of the lateral
terminalis, olfactory bulbs, paraventricular nucleus. The regulation of AT1 receptor expression is of intense current interest. Limited available evidence supports the notion that AT1 receptor expression is modulated by angiotensin II itself and cAMP and may be regulated by protein kinase C and tyrosine phosphorylation. Previous binding studies have shown that vascular angiotensin receptors undergo homologous downregulation, suggesting that angiotensin II may regulate the expression of its own receptor. Angiotensin II has been shown to reduce AT1 receptor mRNA expression by 50% after 4-6 hours in cultured vascular smooth muscle cells and mesangial cells.

3.2.3.2. AT2 receptors
This receptor protein also evidences a 7-transmembrane domain characteristic of G-protein coupled receptors; however it shows only about 32–34% amino acid sequence identity with the rat AT1 receptor. The AT2 receptor protein includes a 363 amino acid sequence (40 kDa) with 99% sequence agreement between rat and mouse, and 72% homology with human (de Gasparo et al., 2000). Even though this AT2 receptor possesses structural features in common with members of the 7-transmembrane family of receptors, it displays few if any functional similarities with this group, although it does appear to be G-protein coupled (de Gasparo et al., 2000). The AT2 receptor subtype appears to be maximally sensitive to Ang III, but Ang II also serves as a ligand at this receptor subtype.

Cerebral distributions and regulation of expression of AT2 receptor
The highest levels of AT2 sites are found in the amygdala, medial geniculate, hypoglossal nucleus, inferior olivary nucleus, lateral habenula, caudate putamen, globus pallidus, locus coeruleus, thalamus, inferior colliculus, and ventral
tegmental area. Structures with the greatest densities of the AT4 subtype include anterior pituitary gland, caudate-putamen, globus. The most striking evidence for the regulation and potential importance of AT2-binding site expression comes from developmental biology studies. Several researchers have shown pharmacologically that AT2-binding sites are more abundant in embryonic and neonatal than in adult tissue (Grady EF et al., 1991).

3.2.3.3. The AT4 receptor

The AT4 subtype was originally identified in bovine adrenal membranes (Swanson et al., 1992). These characterization studies indicated that the AT4 receptor subtype is distinct from the AT1 and AT2 sites given that ligands known to bind to these sites do not bind at the AT4 site (Swanson et al., 1992). It was determined that Ang IV binds at the AT4 site reversibly, saturably, and with high affinity. This AT4 site has been found in a variety of mammalian tissues including adrenal gland, bladder, colon, heart, kidney, prostate, brain, and spinal cord. Recent identification explore that AT4 receptor system is the insulin-regulated membrane aminopeptidase (IRAP). In addition the AT4 receptor subunit exhibits a molecular weight of between 160 and 190 kDa as determined by reduced SDS-polyacrylamide gel electrophoresis. This receptor appears to be a trimer as suggested by results from a non-reducing gel that indicates two additional subunits.

Cerebral distributions and regulation of expression of AT4 receptors

Amygdale, anterior pituitary gland, caudate putamen, nucleus cerebellum, cerebral neocortex, globus pallidus, habenula, hippocampus, inferior colliculus, lateral geniculate, locus coeruleus, nucleus accumbens, nucleus
basalis of meynert. AT4 receptor and that the vast majority of small peptide receptors are G protein-linked, it would be predicted that the AT4 receptor might be a serpentine, G protein-linked receptor as well. The initial events that characterize AT4 receptor intracellular signaling mechanisms are presently unknown. Nevertheless, downstream targets appear to include immediate early genes. Intracerebroventricular infusion of Ang IV in rats induces c-Fos expression in brain regions associated with cognition.

### 3.3. Renin angiotensin system and memory impairment

Several studies have investigated the AD-related RAS alterations in the brain (Ge J et al., 1996). ACE activity was found to be elevated in the cortex, medial hippocampus, parahippocampal gyrus and caudate nucleus of AD patients (Arregui A et al., 1982). In addition, AT1 and AT2 (Ge J et al., 1996) have also been found to be increased in AD cortex suggesting an augmented brain RAS activity during the disease process. Since the suppressing effect of Ang II on acetylcholine release is well established (Barnes JM et al., 1991), and acetylcholine is one of the major neurotransmitter systems heavily implicated in AD pathology, the enhanced RAS activity may represent an additional factor contributing to cognitive impairment in AD. Interestingly, neurons containing Ang II found in the striatum and hippocampus of AD patients were found to participate in AD-related plaque formations, and Ang II immunoreactive neurons in those patients were mostly distorted showing neurodegenerative changes. Ang II acting as a neuropeptide in those brain regions may be directly involved in AD pathology.

#### 3.3.1. Effect of RAS affecting drugs on learning and memory
3.3.1.1. Animal Studies

It was reported that ACE inhibition improved memory function in aged rats (Basso et al., 2005). Streptozotocin diabetic rats, which had impaired memory in the water maze task, demonstrated improved performance after inhibition of ACE by enalapril (Manschot et al., 2003). Further, memory impairment in spontaneously hypertensive rats, Wistar-Kyoto rats and in scopolamine induced memory deficit rodents was reversed by ACE inhibition by captopril. In another study, rats made hypertensive by Goldblatt method had a poor acquisition and retrieval of the learned behavior but this state was reversed by ACE inhibition (Srinivasan et al., 2005). Recently, experimental studies also revealed the beneficial effects of ACE inhibition in cerebral hypoperfusion and Aβ induced models of memory impairment in rodents (Kumaran et al., 2008).

Further, treatment with AT1 receptor blockers prevented memory impairment induced by scopolamine in experimental models (Shepherd et al., 1996; Raghavendra et al., 1998, 2001). Recently there is growing evidence suggesting that AT2 receptor plays an important role in cognitive function. Barnes et al., (1991) reported that, PD123, 177, an AT2 receptor antagonist, enhanced memory and reversed scopolamine induced memory impairment in mice using habituation test. On the contrary, mice lacking AT2 receptor gene are significantly impaired in their performance in a spatial memory and active avoidance tasks. Further, it has also been showed that AT2 receptors do not play any role in memory function (Shepherd et al., 1996).

3.3.1.2. Human Studies

An increase in ACE activity has been reported in the Alzheimer’s disease (AD) brain (Miners et al., 2008). Clinical evidences suggested that ACE is involved in
cognitive dysfunction/dementia in AD patients because ACE inhibitors delayed onset of dementia (Davies et al., 2011) and significantly decreased the ACE activity in cerebrospinal fluid (CSF) (He et al., 2006). Further, studies showed that ACE inhibitors positively influenced cognitive function independent of their blood pressure lowering effects, with patients displaying better results than those on diuretics and beta blockers. Clinical studies also showed that chronic AT1 receptor blockade improves memory in elderly hypertensive patients (Fogari et al., 2006). The extensive research in last two decades has shown some novel physiological and pathological role of central RAS including memory function. Several studies tried to demonstrate involvement of RAS in the cognitive deterioration in human and animal species. However, the exact role played by central RAS in memory impairment and associated factors like cholinergic hypofunction, impaired cerebral circulation, oxidative stress and altered brain energy metabolism has not investigated properly. Therefore, we have undertaken this exercise to explore role of central RAS components in these pathological conditions associated with memory dysfunction. This study utilized colchicine and AF64A induced memory deficit models to achieve the objectives of this study.

3.4. Drugs acting on Renin Angiotensin System

Drugs acting on renin angiotensin system include angiotensin converting enzyme inhibitors and AT1 receptor blockers. ACE inhibitors like perindopril, lisinopril, captopril etc. inhibits ACE activity thereby preventing formation of Ang II. AT1 receptor blockers such as candesartan, losartan, telmisartan etc. antagonize action of Ang II at AT1 receptor. These drugs are clinically used for the treatment of hypertension and other cardiovascular disorders.
3.4.1. **Angiotensin converting enzyme (ACE) inhibitors**

Accompanying the therapeutic benefits of ACE inhibitors (captopril, enalapril, ramipril, ceranapril) in treating hypertension, congestive heart failure, and following mild cardiac infarction, there appears to be facilitated cognitive functioning and feelings of well-being. There was no change with propranolol treatment, and a decline in those patients placed on methyldopa. Blood pressure was equivalently controlled in all three treatment groups. (Barnes et al., 1991) posited that elevated brain Ang II levels may interfere with acetylcholine (Ach) release that in turn interferes with cognitive processing (Barnes et al., 1991). According to this hypothesis ACE inhibitors may facilitate cognitive functioning by reducing the synthesis of Ang II, thus removing an inhibitory influence upon Ach release (Barnes et al., 1990). Barnes et al. have also reported Ang II-induced interference with potassium-mediated release of (3H)-Ach from rat entorhinal cortex slices. The enzymatic action of ACE is not limited to cleavage of angiotensin I. ACE can metabolise bradykinin and therefore can modulate inflammation, a key factor in Parkinson’s disease. Substance P can also be hydrolysed by ACE, and this feature might be important since a primary loss of substance P has been linked with the pathogenesis of several neurological diseases. Several studies have been undertaken to evaluate the neuroprotective and neuro restorative effects of ACE inhibitors in animal models of Parkinson’s disease (Jenkins et al., 1999). Independent of their ability to inhibit ACE activity, ACE inhibitors are by themselves capable of scavenging reactive oxygen species (ROS) (Lopez-Real A et al., 2005). Contradictory results have been reported on the role of the sulphydryl group in these ROS scavenging properties. According to some authors, only ACE inhibitors with a sulphhydryl
group are capable of scavenging ROS whereas others suggest that the free radical scavenging properties are independent of the sulphhydryl group. ACE inhibitors such as captopril are potent free radical scavengers (Obata T et al., 2008).

3.4.2. AT1 receptor antagonists:
Ang II was suggested to directly act on central RAS receptors to enhance associative memory and learning possibly with differential effects on acquisition, storage and recall. Since the selective AT1 antagonist losartan was found to abolish the Ang II induced improvement in object recognition, the cognition improving effects of Ang II were suggested to be transmitted by AT1 (Kulakowska A et al., 1996). However, subsequent contradictory findings showed that losartan was also able to facilitate spatial and short-term working memory, and to reverse scopolamine induced cognitive deficits (Raghavendra V el al., 1998). Investigations using ACE inhibitors, on the other hand, supported the hypothesis that Ang II suppression may have cognitive enhancing effects. These drugs were found to enhance learning in rats. Daily administration of captopril, an ACE inhibitor, improved retention and learning deficits in aged mice. Ang II inhibits acetylcholine release (Barnes JM et al.1990). Therefore, enhanced acetylcholine release may be responsible for the cognitive improvement after the administration of ACE inhibitors decreasing Ang II levels (Barnes JM et al., 1990). Indeed, findings that Ang II injections suppress long term potentiation in the hippocampus (and amygdale and that this effect is AT1-mediated suggest that direct Ang II effects on AT1 may cause cognitive impairment. Thus, the long-term treatment of hypertension with captopril, but not with enalapril, another ACE inhibitor, improves quality of life in elderly
humans, providing additional clinical evidence for the positive effects of Ang II suppression. However, there is also evidence for a bimodal action of Ang II on learning, showing an inhibitory action at low doses, and a facilitatory effect at higher doses (Baranowska D et al., 1983).

Ang IV and AT4 agonists have been postulated to be positively implicated in memory acquisition and retrieval. Central administration of Ang IV stimulates exploratory locomotor behavior, improves recall in passive avoidance situations and facilitates memory retention in rodents (Braszko JJ et al., 1988), whereas AT4 agonists are able to reverse scopolamine or Bilateral perforant pathway lesion-induced memory deficits. Recently, AT4 has been isolated and has shown to be the insulin-regulated aminopeptidase (IRAP). IRAP is a metallopeptidase and has been shown to cleave a number of peptides in the brain. The distinct facilitating effects of Ang IV on memory have been related to its binding to IRAP and inhibition of IRAP’s enzymatic activity.

3.5. Experimental models of memory impairment

3.5.1. Colchicine induced models of memory impairment

Central administration of microtubule disrupting agents can result in cell death associated with cognitive impairment, which resemble the microtubule dysfunction in AD (Flaherty et al., 1989) Colchicine, as a microtubule-disrupting agent, (James and Dennis, 1981), produces marked destruction of hippocampal granule cells, mossy fibers and septohippocampal pathways (SHC; a cholinergic link between medial septum and vertical limb of diagonal band). It induces neurofibrillary degeneration by binding to tubulin, the principal structural protein of microtubule which is associated with loss of cholinergic neurons and decrease in acetylcholine transferase, thereby resulting in
impairment of learning and memory (Emerich DF et al., 1990). Colchicine has been reported to mediate cascade of actions. The central administration of colchicine elevates GLU/GABA ratio in cortex of mice brain (Yu et al., 1997). The relative increase in the GLU activity exerts neurotoxic effect by generating the hydroxyl radicals. Secondly, Laurence and coworkers reported an increase in the NADPHd-positive neurons in the different hypothalamic area after central administration of colchicine, which indirectly suggest increase in production of NO synthase and NO in the brain (Dufourney L et al., 2000). The released NO can cause neurotoxicity by multiple mechanisms: (a) by interacting with superoxide anions and resulting in peroxynitrite formation, which has cytotoxic activity; (b) by nitrosylation of enzymes, e.g., phosphokinase-C and glyceraldehydes-3-phosphate dehydrogenase, which results in inhibition of glycolysis; (c) by causing DNA mutation and strand breaking, which in turn stimulates polyADP-ribosylation of proteins, and ultimately causing massive depletion of cellular energy. The glucose/energy metabolism impairment in the brain has been reported to impair the ability to scavenge the free radicals (Beal, 1995) and cause neuronal damage (Sims et al, 1987). Also, the deficit in cerebral regulation of glucose is known to cause impairment of learning and memory. Thus, it was our contention that the central administration of colchicine causes increase in free radical generation and the consequent oxidative stress leads to cognitive impairment. Thus, the colchicine model is relevant to deterioration of cognitive functions, microtubule destruction and decrease in ChAT activity (Bensimon et al, 1991).

3.5.2. Ethylcholine aziridinium ion (AF64A) induced models of memory impairment
In recent years, a number of choline analogues have been suggested as potential tools in developing selective animal models with central cholinergic hypofunction (Fisher et al., 1986; Mantione et al., 1981). The Na+-dependent, high affinity choline transport system (HACHT) at cholinergic nerve terminals is considered to be the rate limiting step for the synthesis of acetylcholine (ACh), and interference to such a process should provide a useful means for experimental reduction of cholinergic activity (Fisher et al., 1986). Reversible inhibitors of HACHT, e.g., hemicholinium-3, are expected to transiently impair cholinergic function in vivo. Several studies have shown memory retention defects in animals treated with these inhibitors (Freeman et al., 1979). Certain mustard analogues of choline, which are irreversible inhibitors of HACHT, have been shown to cause a specific reduction of presynaptic cholinergic parameters in vivo, suggesting that cholinergic terminals degenerate as the results of treatment with these compounds (Mantione et al., 1981). One of these compounds, ethylcholine aziridinium ion (AF64A), has been shown to impair the retention of a passive avoidance task and disrupt acquisition of a radial-arm maze task in rats after ICV injection. Ethylcholine mustard aziridinium ion (AF64A) has been proposed as a specific cholinergic neurotoxin, because intracerebroventricular (ICV) administration of AF64A to rats selectively destroys the central cholinergic system and reduces the number of presynaptic cholinergic markers, such as high affinity choline uptake, choline acetyltransferase activity, acetylcholine (ACh) release and ACh level (Leventer et al., 1987). It also causes impairment of memory performances including a deficit of the avoidance response in mice and rats (Gower et al., 1989). The reductions in cholinergic markers are long-lasting (Leventer et al., 1987).
3.5.3. Scopolamine induced memory impairment

Studies have shown that the cholinergic system plays an important role in learning and memory (Birks, 2006). The impairment in central cholinergic function is the most important cause of memory decline in AD (Kang et al., 2005). Therefore, cholinesterase inhibitors are used for the symptomatic treatment of cognitive impairment in humans and indeed these are the only drugs available for symptomatic treatment of AD (Ago et al., 2011). Scopolamine, a cholinergic muscarinic receptor antagonist, interferes with memory in animals and humans, particularly the processes of learning acquisition and short-term memory (Jeong et al., 2008). Scopolamine significantly increases acetylcholinesterase (AChE) and malondialdehyde (MDA) levels in the cortex and hippocampus (Jeong et al., 2008). Further, scopolamine has also been reported to impair cerebral blood flow and brain glucose utilization in animals and humans (Nakano et al., 2001). Therefore, scopolamine induced memory impairment model is extensively used for screening of potential antiamnesic agents.

3.5.4. Streptozotocin induced memory impairment model

Streptozotocin (STZ) is a betacytotoxic drug which, following peripheral administration at high doses, selectively destroys insulin secreting β-cells in the pancreas and causes type I diabetes mellitus in adult animals (Szkudelski, 2001). Central injection of STZ, in a sub diabetogenic dose, in rodents had been found to cause impairment in learning and memory without causing systemic metabolic changes and diabetes mellitus. The mechanism of central STZ action and its target cells molecules have not yet been clarified but a similar mechanism of action to that in the periphery has been recently suggested. In the
periphery, STZ selective β-cells toxicity results from the drug’s chemical structure which allows it to enter the cell via the GLUT2 glucose transporter. GLUT2 may also be responsible for the STZ induced effects in the brain as GLUT2 also is reported to have regional specific distribution in the mammalian brain. Decreased levels of ATP have also been reported following STZ-ICV treatment. The chemical structure of STZ also suggests this compound may produce intracellular free radicals, nitric oxide (NO) and hydrogen peroxide; indeed evidence of increased oxidative stress has been found in the brain of STZ (ICV) treated rats (Szkudelski, 2001; Saxena et al., 2008, 2010; Agrawal et al., 2009).

Investigations of cholinergic transmission in STZ treated rats revealed a decrease in choline acetyltransferase (ChAT) activity in the hippocampus. This is followed by a significant increase in acetylcholinesterase (AChE) activity (Agrawal et al., 2009). Recently, it has also been reported that administration of STZ caused neurodegeneration and a reduction in cerebral circulation in animals (Tota et al., 2010; Saxena et al., 2010).

3.5.5. Brain cholinergic system and dementia

For a long time it is known that agents such as tropane alkaloids that are today known to block muscarinic cholinergic receptors, result in memory deficits and cognitive disturbances. The involvement of central cholinergic function in learning and memory was first postulated by Deutsch (1971), and later further corroborated by pharmacological studies demonstrating that centrally acting anticholinergic drugs impaired cognitive performance in young healthy patients to a level detectable in dementia disorders, while enhancement of central
cholinergic function improved the performance of aged patients (Drachman, 1977).

A vast number of experimental studies have confirmed that administrations of anticholinergic drugs produce dose-dependent deleterious effects on cognitive performance in animals as well as in humans, providing evidence for the important role of the central cholinergic system in realizing cognitive processes. Cholinergic lesions by electrolytic or excitotoxic methods as well as application of the cholinergic immunolesion strategy (Wiley et al., 1991) that produces a more specific and selective destruction of basal forebrain cholinergic cells and stimulation of basal forebrain cholinergic cells provided experimental tools to further emphasize this view. Based on the assumption that the progressive decline of cognitive function mainly results from decreased cholinergic neurotransmission, pharmacological approaches to enhance basal forebrain cholinergic pathways have been developed for symptomatic and palliative therapy. A number of acetylcholinesterase inhibitors including tacrine, donepezil, rivastigmine and galantamine have been proved to enhance cholinergic synaptic efficacy by preventing the breakdown of acetylcholine and thus elevating and/or maintaining its level in the synaptic cleft and to prolong its action on postsynaptic muscarinic and nicotinic receptors (Birks, 2006).

3.5.6. Oxidative stress and memory impairment (Fig. 3.4.)

A free radical is an atom or molecule with an unpaired electron in its outer orbit, a state that makes it highly unstable and reactive. Free radicals are formed during normal metabolism, and free radical injury occurs within living cells when the generation of reactive oxygen species exceeds intrinsic antioxidant ability. This situation is also referred to as oxidative or oxidant stress. The brain
may be particularly vulnerable to oxidative damage, because it has high energy requirements and a high oxygen consumption rate; is rich in peroxidizable fatty acids; contains high levels of transition metals, which may catalyze the formation of the reactive hydroxyl radical; and has a relative deficit of antioxidant defenses compared with other organs (Floyd, 1999). There is a growing body of evidence suggesting that oxidative injury is involved in the pathogenesis of dementia. This concept originally derived from the free radical hypothesis of aging, which states that the age-related accumulation of reactive oxygen species results in damage to major cell components (Beal, 1995). Oxidant stress to the central nervous system predominantly manifests as lipid peroxidation because of its high lipid content and unusually high concentration of polyunsaturated fatty acids that are particularly susceptible to oxidation. This may in turn promote the formation of additional reactive oxygen species, and by so doing enhance protein and DNA oxidative damage. Recently, increased activity of the antioxidant enzyme, superoxide dismutase, has been reported in the cerebrospinal fluid (CSF) with aging, suggesting a possible reactive compensatory process secondary to this increased oxidant stress with time. If free radical injury is important in the initiation or progression of AD, then therapy to reduce oxidative injury and augment endogenous antioxidant defenses might prevent, delay, or ameliorate the disease process and diminish its human and social consequences. Evidence in both in vitro and animal studies suggests that treatment with antioxidant agents may be useful in neurological disorders, including AD (Pratico and Delanty, 2000).
Fig. 3.4: Oxidative stress and memory impairment (Alzheimer’s disease)

3.6. References


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Chapter 3: Review of Literature


