Chapter 6

6. DISCUSSION

The present study was carried out using rats for investigation of ACEIs and ARBs on learning and memory tasks. Rats were pre-treated with two doses, each of ACEIs and ARBs and their passive avoidance learning, learning in elevated plus maze, and spatial learning and memory were assessed in scopolamine treated rats. The gross morphology of the hippocampal CA1, CA3 and dentate gyrus neurons was analysed and the degree of neuronal survival in these hippocampal subregions was quantified. ACh activity in the hippocampus of control, scopolamine and drug-treated groups was analysed by measuring the activity of choline acetyl transferase and acetylcholinesterase enzymes. Antioxidant activity of ACEIs and ARBs against scopolamine-induced oxidative stress was assessed by measuring the level of malondialdehyde, glutathione transferase, protein thiols and superoxide dismutase within the hippocampus.

6.1. Effect of ACEIs and ARBs on scopolamine-induced behavioural changes

Critical analysis of the results of the present study showed that exposure to scopolamine impaired the learning and memory processing activities in experimental rodents. Three behavioural paradigms were used in our study to assess learning and memory in rats. They were passive avoidance test, elevated plus maze test and Morris water maze test.

- Passive avoidance test:
Passive avoidance is a fear-aggravated task used to assess memory or retention in animal models of CNS disorders, particularly dementia. Rats, as a part of their normal behaviour, generally avoid bright illumination and prefer dim illumination. When placed in a brightly illuminated compartment connected with a dark enclosure, they rapidly enter the dark compartment and remain there. Once they receive an aversive consequence (foot shock) in the dark compartment, the animals modify their behaviour by staying in the bright compartment to avoid a noxious event, thus suppressing the learned habits of staying in the dark compartment. Since there is punishment to the natural exploratory drive of a rodent with a pulsating electric foot shock, this is clearly an aversive task.

In our present study, administration of scopolamine clearly produced memory deficits (amnesia) in rat performance in passive avoidance test as indicated by their shorter latency to enter the dark compartment in the memory retention test compared to the control group. Results of this study are consistent with earlier reports which showed that administration of scopolamine is associated with impaired learning and retrieval in the passive avoidance paradigm (Rush 1988; Hiramatsu and Inoue 1999; Jinghua et al., 2014). It has been suggested that muscarinic acetylcholine receptor antagonist modulates passive avoidance performance in rodents via cholinergic receptors that are located in anterior cingulate cortex (Riekkinen et al., 1995). This would corroborate the view that scopolamine is an amnesic drug that adversely affects learning and memory, probably by disrupting the central cholinergic neurotransmission.

Scopolamine-induced deleterious effect on memory may also involve the disruption of hippocampal theta rhythm that normally gets activated during exploratory behavior (Anagnostaras et al., 1999), and possibly by impairment of hippocampal LTP (Huerta and Lisman 1995). Hippocampal lesions have been shown to produce deficit in acquisition (Phillips and LeDoux 1992; Maren and Fanselow 1997) and a time-dependent retrograde
amnesia (Anagnostaras et al., 1999) that are selective for contextual fear. Since the hippocampus receives extensive cholinergic input from the medial septal nucleus (Amaral and Kurz 1985), it is expected that muscarinic-cholinergic antagonism will disrupt contextual fear acquisition to the extent that anticholinergic action can attenuate the function of the hippocampus.

Pre-treatment with ACEIs and ARBs showed that the mean latency of rats to enter the dark compartment after receiving the aversive stimulus were significantly higher compared to scopolamine group, indicating reversal of amnesia. This showed that scopolamine-treated rats after being exposed to aversive stimulation in the passive avoidance task, failed to remember the task on the following day, but this effect could be attenuated following pre-treatment with ACEIs and ARBs. Among the ACE inhibitors, a higher dose of ramipril and fosinopril was found to demonstrate better efficacy in reversing the memory deficits induced by scopolamine. Among the ARBs, telmisartan and olmesartan showed better efficacy in passive avoidance test compared to losartan and valsartan.

➢ **Elevated plus maze test:**

The elevated plus maze test has been considered as an indicator of short-term memory (Sharma and Kulkarni 1992). In this test, scopolamine-treated rats showed a significant decrease in transfer latencies on 1\textsuperscript{st} and 2\textsuperscript{nd} days, indicating impairment of acquisition and retention behaviour; this could be markedly attenuated by pre-treatment with higher doses of ramipril, fosinopril and telmisartan, and with both lower and higher doses of olmesartan. The precise mechanism of impaired memory induced by scopolamine in EPM is not clearly established; however it is believed that a possible involvement of cholinergic and NMDA-receptor blockade may play a role, the blockade being reversed following pre-treatment with ACEIs and ARBs.
Morris water maze test:

The MWM test is a well-established model for evaluating hippocampal-dependent memory deficits in experimental animals and has been used for the evaluation of drugs with neurocognitive enhancing ability. In the MWM task, the animal learns to swim in a water tank, guided by external cues, and climb up to a submerged platform. Based upon spatial information, this animal learns how to escape to a platform. Rats are natural swimmers, but in this task they just want to get out of the water and escape into the platform. In our study, administration of scopolamine produced severe deficits in both the acquisition and memory retention trials as indicated by their longer latencies to escape into the submerged platform. Scopolamine treated animals also spent lesser time in the target quadrant during the retention trial compared to control rats. Our results are consistent with earlier studies which described that administration of scopolamine does impair spatial learning and memory in experimental rodents (Fan et al., 2005; Jeong et al., 2008).

Scopolamine interferes with the navigation test in MWM by probably blocking the cholinergic signals to hippocampus and neocortex (Wenk et al., 1980; Alger et al., 2014) which are a prerequisite for mapping solution of the task. Further, scopolamine is believed to affect spatial memory by blocking the NMDA receptors located at the hippocampus and dorsomedial prefrontal cortex (Falsafi et al., 2012). In addition, scopolamine has also been shown to disrupt the hippocampal activity during spatial learning and memory by inducing a significant reduction in the activation of the hippocampus and by causing dissociation between hippocampus-based and striatal-based memory systems (Antonova et al., 2011).

Pre-treatment with ramipril (0.45 mg/kg) and fosinopril (1.80 mg/kg) could significantly attenuate the scopolamine-induced memory deficits in the water maze test as demonstrated by their shorter latencies to locate the hidden platform during the acquisition trials and longer time spent in the target quadrant during the retention trial, indicating their potential memory
enhancing effects. When the ARBs were compared with the scopolamine-group, rats that were pre-treated with telmisartan and olmesartan were found to show better performance in the water maze test compared to valsartan- and losartan-treated rats.

The improvement in cognitive function observed with ACEIs and ARBs in the behavioral paradigms of memory retention can be due to decrease in Ang-II or blockade of the AT₁ receptors which mediate the actions of Ang-II. Ang-II interferes with ACh release and reducing its synthesis may remove an inhibitory influence upon Ang-II, thereby improving memory in scopolamine-treated rats (Barnes et al., 1989; Bodiga and Bodiga 2013). The memory enhancement could also be due to conversion of Ang-II to Ang-IV, the latter being responsible for cognitive facilitation (Wright and Harding 2010). Furthermore, it has been shown that Ang-IV by activation of hippocampal AT₄ receptors can overcome the disruption of spatial memory accompanying treatment with the muscarinic receptor antagonist scopolamine (Pederson et al., 1998; Gard 2008), thus suggesting a role of angiotensin receptor subtypes in influencing cognition.

6.2. Effect of ACEIs and ARBs on scopolamine-induced changes in brain antioxidant status

Memory impairment in scopolamine-induced animal model is associated with altered status of brain OS. Previous studies have reported that scopolamine triggers the induction of ROS and cause free radical injuries associated with reduced activity of antioxidant enzymes like superoxide dismutase and glutathione peroxidase in the brain (Fan et al., 2005). Since OS is implicated in mediating cognitive impairment as commonly occurs in dementia, this study analysed the effects of RAS inhibitors on scopolamine-induced OS. The level of MDA, a marker of oxidative stress, was increased in scopolamine-treated group and was significantly
higher than control group. Levels of brain antioxidant enzymes including SOD, protein thiols and GST were also significantly decreased in scopolamine-treated rats compared to control group. Pre-treatment with ACEIs and ARBs was found to markedly ameliorate the scopolamine-induced increase in MDA level and increase the level of hippocampal antioxidant enzymes that were reduced by scopolamine.

The antioxidant potential of ACEIs and ARBs have been documented in several clinical trials involving type II diabetic and metabolic syndrome patients without hypertension (Yusuf et al., 2000; Kovesdy et al., 2008). Ang II is a potent generator of ROS through the activation of NADPH oxidases and consequently, mitochondrial ROS generation via the phenomenon of ROS-induced ROS release (Griendling et al., 2000; Harrison et al., 2003). ACEIs and ARBs by blocking Ang-II production may prevent activation of NADPH oxidase and endothelial NO synthase uncoupling (prevention of tetrahydrobiopterin oxidation), thereby increasing NO availability and enhancing the expression of extracellular SOD, a potent scavenger of superoxide anions (Fukai et al., 2000; Landmesser and Drexler 2002). Reduced vascular superoxide formation by the aforementioned mechanism may then contribute to reduction of OS induced by scopolamine.

A study indicated that ramipril increases NO availability, resulting in a direct scavenging of superoxide anion as well as causing downregulation of the oxidant generating system (Berkenboom et al., 1999). An ACEI, quinapril, has been shown to reduce plasma markers of oxidative stress in metabolic syndrome patients (Khan et al., 2004). Further, ACEIs including fosinopril and captopril have been shown to exhibit antioxidant properties and block LDL oxidation, lipid peroxidation and generation of MDA and 4-HNE in apoE -/- mice (Hayek et al., 1998, 1999; Salvayre et al., 2008). Olmesartan is one of the ARBs commonly used for patients at risk for coronary artery diseases due to its strong anti-inflammatory and antioxidant properties (Yoshida et al., 2004). Further, olmesartan has been shown to exert a
direct antioxidant effect independent of its AT₁ receptor blocking action (Kurita et al., 2008). ARBs were also proven to attenuate inflammatory and oxidative stress (Ando et al., 2004; Zhou et al., 2005) and to regulate the nitric-oxide synthase isoenzymes in the brain (Ito et al., 2002). Thus, treatment with ACEIs or ARBs have the potential to exert protective effects against OS that is one of the key factors contributing to the pathogenesis of AD.

6.3. Effect of ACEIs and ARBs on scopolamine-induced changes in brain cholinergic activity

Cholinergic deficits seen during memory impairment in AD and other types of dementia can be attributed to neuronal death, decreased ChAT synthesis, increased AChE and alterations in neuroimmunological responses. Therefore increasing ACh levels in the brain, either by decreasing the synaptic degradation of ACh (through inhibition of AChE) or by inducing the synthesis of ACh (through increased synthesis of ChAT), seem to be a useful strategy for development of anti-dementia drugs. In our study, scopolamine administration markedly reduced ChAT activity in the rat hippocampus compared to normal controls, which is in line with earlier reports suggesting that exposure to scopolamine causes severe decrease in cholinergic system reactivity indicated by reduced ACh levels and decreased ChAT activity (Heo et al., 2006; Xiang et al., 2012).

In the present study, pre-treatment with varying doses of ARBs and ACEIs showed a dose-dependent increase in ChAT activity. Compared to scopolamine, the results were significantly increased in rats that were pre-treated with higher dose of ramipril, higher dose of telmisartan and both lower and higher doses of olmesartan. ChAT is an enzyme involved in synthesis of ACh and our results suggests that molecules with an ability to restore the
synaptic ACh levels could have potential therapeutic value in the prevention or treatment of AD.

In the *in vitro* AChE estimation, the activity of hippocampal AChE, the enzyme involved in degradation of ACh was found to be increased in rats that were treated with scopolamine and our results are in line with earlier studies (Rubio et al., 2007; Lee et al., 2010). In this relation, it is noteworthy to mention that cholinergic hypofunction participates in the memory deficits induced by scopolamine. Since degeneration of cholinergic system is shown to correlate with Aβ plaques and neurofibrillary tangles in AD (Rees and Brimijoin 2003; Ballard et al., 2005), therapies designed to reverse the cholinergic deficit are in large measure based on the importance of cholinergic function in cognition. Pre-treatment with ACEIs and ARBs altered the scopolamine-induced changes in brain AChE, the effects being significant with ramipril, telmisartan and olmesartan. Findings from our study is supported by an earlier study which reported that administration of perindopril, a centrally acting ACEI can prevent elevation of brain AChE activity in scopolamine-induced amnesic rats (Tota et al., 2012).

The beneficial effect of RAS inhibitors on cholinergic activity could be attributed to their inhibitory influence on activity of brain Ang-II. Besides exerting a direct effect on inhibition of ACh release, Ang-II has been shown to cause a 25-35% reduction in activities of ChAT within the rat hippocampus (Micossi et al., 1992). This finding was supported by a study by which reported that administration of Hoe 065, a compound structurally related to ACEI, enhanced the activity of ChAT as well as the capacity of high-affinity choline uptake system (a rate-limiting step in the synthesis of ACh) in the rat brain (Wiemer et al., 1989). Further, an improvement of cholinergic function also leads to endothelium-dependent relaxation of cerebral arteries resulting in increased cerebral circulation that in turn leads to improved cognitive function (Faraci and Heistad 1998; Nicolakakis and Hamel 2011).
The fact that fosinopril, valsartan and losartan which were relatively weak and devoid of significant AChE inhibitory activity, but still showed comparable memory enhancing effect in the behavioural tests suggests that these drugs facilitate cognition possibly through mechanisms independent of their AChE enzyme inhibition. The memory enhancing effect shared by these drugs might be attributed to their effects on ChAT activity or to their antioxidant property, though additional mechanisms cannot be ruled out.

6.4. Effect of ACEIs and ARBs on scopolamine-induced changes in hippocampal morphology and degree of neuronal survival

Data generated from the present study demonstrated that administration of scopolamine induces profound memory deficits in rat performance in all the three paradigms of learning and memory tasks. This change in behavioural performance was accompanied by increased oxidative stress and was associated with signs of neurodegeneration in the hippocampus as evident by the deeply stained and shrunken neuronal cells in CA1, CA3 and dentate gyrus regions of the hippocampus. The exact mechanism responsible for this degeneration is not clear but it could be attributed partly to the generation of ROS which is known to impair antioxidant defences in hippocampus, cortex and cerebellum (Abraham et al., 2001; Patel et al., 2002).

Damage to the hippocampus could also be due to the dysregulated hippocampal ACh neurotransmission caused by administration of scopolamine. Endogenous ACh released from cholinergic terminals has been known to modify LTP and synaptic plasticity (Boyd et al., 2000; Shinoe et al., 2005), the major cellular mechanisms that underlie learning and memory. The hippocampus plays a crucial role in learning and memory and damage to the
hippocampus and its related systems thus can cause amnesia in humans and rats (Scoville and Milner 1957), as also observed in the current study using experimental rodents.

Analysis of the gross morphology of CA1, CA3 and dentate gyrus regions showed that administration of ACEIs and ARBs could prevent the scopolamine-induced structural abnormalities and preserve the neuronal cells in the hippocampal subregions. This strongly suggests that treatment with RAS inhibitors maintain cognitive function in rats probably through protection of the vascular vessels and neuronal cells responsible for memory function. Sakanaka and colleagues reported that a relationship exists between the latency of passive avoidance task and the cell density of the CA1 region (Sano et al., 1994) and the present study supports this finding as we observed that treatment with RAS inhibitors improved performance in the passive avoidance test, and it correlated with an increased number of cells in the hippocampal CA1 and CA3 regions. A possible mechanism by which these drugs may preserve the neuronal function could be due to the removal of the inhibitory influence of Ang-II on ACh release thus preserving the cholinergic function. Further, the hippocampus and the amygdala also receive intensive angiotensinergic synaptic inputs which can either promote or restrict the induction or expression of long-term plasticity (Lenkei et al., 1997). Ang-II suppresses LTP (Denny et al., 1991; Tashev and Stefanova 2015) and treatment with ACEIs or ARBs may facilitate synaptic plasticity by decreasing the Ang-II mediated effects. Another plausible mechanism that prevents damage to the hippocampal cells could be attributed to the ability of ACEIs and ARBs in ameliorating the oxidative damage in the brain.

The hippocampus, which is required for many forms of LTM in humans and animals appears to be an important site of BDNF action (Hall et al., 2000). The BDNF has been demonstrated to play a critical role in LTP (Korte et al., 1995) and thus has an important role in learning and memory (Cunha et al., 2010). BDNF expression in the hippocampus is increased during
contextual learning (Hall et al., 2000), and there is substantial evidence that suggests a critical role for BDNF in LTP at hippocampal synapses, Schaffer collaterals, CA1 synapse (Kang and Schuman 1995) and dentate gyrus (Messaoudi et al., 2002). BDNF transcription is regulated by cAMP-response element-binding protein (CREB) in the brain (Tyler et al., 2002). CREB is shown to regulate the expression of genes involved in neuroplasticity, cell survival, and LTM formation (Guzowski and McGaugh 1997; Kida et al., 2002). In this study, scopolamine-induced performance deficits in tests of cognitive function as well as corresponding signs of neurodegeneration may be due to decreased BDNF and CREB expression in the hippocampus. Scopolamine administration, besides inducing dysregulation of the cholinergic neuronal pathway and memory circuits in the CNS, has also been shown to reduce the expression of BDNF and CREB in the hippocampus and cause disruption in hippocampal function during working memory (Bekinschtein et al., 2008; Lee et al., 2014).

Treatment with an ARB, candesartan, at sub-hypotensive and therapeutic doses has been shown to afford neuroprotection after focal ischaemia, associated with increased activity of BDNF (Krikov et al., 2008). This effect is believed to be mediated through Ang-II induced stimulation of the AT₂ receptor (Alhusban et al., 2013). Another ARB, telmisartan, was also found to protect against cognitive decline via up-regulation of BDNF in the hippocampus of hypertensive rats, partly because of PPAR-gamma activation independent of blood pressure-lowering effect (Kishi et al., 2012). In the hippocampus, BDNF protects against ischemic cell damage (Ito et al., 2001). Thus, an increase in the level of BDNF could contribute to the neuroprotective effect exhibited by ARBs though it needs further investigation.
6.5. Possible factors attributed to differences among ACEIs

In our study, differences observed among different ACEIs with regard to cognitive improvement could be attributed to the unique chemical structure that is representative of each ACEI. Such variation further contributes to differences in pharmacokinetic properties such as lipophilicity, absorption, protein binding, plasma half-life, metabolism or elimination, ability to penetrate and bind tissue ACE, and differences in pharmacodynamic properties.

Our study demonstrated that ramipril was the most effective ACEI in reversing amnesia; the anti-amnesic activity was moderate for fosinopril while lisinopril at the given doses did not show significant effect in their ability to reverse scopolamine-induced memory deficits. Lack of significant anti-amnesic activity with lisinopril could be due to its poor lipophilicity, resulting in its lesser concentration in the brain (Raizada et al., 1993). Ramipril, fosinopril and lisinopril are all centrally acting ACEIs with an ability to cross the blood brain barrier (Cushman et al., 1989; Sink et al., 2009). However, differences in lipid solubility between these three drugs could be responsible for differences in their degree of penetration into the brain.

Studies have shown that inhibition of brain ACE in structures within the BBB is more evident with the more lipophilic ACEIs such as ramipril, perindopril or captopril while hydrophilic agents such as lisinopril or enalapril do not produce detectable inhibition of brain ACE. Lipophilicity is an important physicochemical property that governs the passage of drugs across cells and tissues; the higher the lipophilicity of the drug, the better is the tissue penetration and inhibition of tissue ACE. Additionally, at equipotent doses, it has been found that CSF penetration is greatest for the most lipophilic drug. ACEIs can inhibit brain tissue
ACE; however, each drug differs in the amount of ACE inhibition and brain tissue bioavailability is the major determinant of this effect that needs further evaluation.

Ramipril is the most effective agent for lowering BP and the most commonly prescribed ACEI, comprising 50% of prescription of ACEIs (Matchar et al., 2008). The ability of ramipril to cross the BBB is significant (Sink et al., 2009; Wharton et al., 2012) and meta-analyses have shown that BBB crossing antihypertensives elicit more pronounced cognitive benefits than non-centrally acting medications (Ohru et al., 2004b; Sink et al., 2009).

From our study, it may be concluded that the more lipophilic ACEIs such as fosinopril and ramipril may have been more readily taken up by the brain tissues to suppress the locally generated Ang-II within the brain and this may have resulted in their better efficacy compared to lisinopril. Lack of efficacy with lisinopril could be due to lesser inhibition of cortical and hippocampal ACE by lisinopril due to its poor lipophilicity, but also suggest that lisinopril probably can be taken up by the brain only when given in high doses. Hence, effects of lisinopril on cognitive function need to be further tested by administration of higher doses of the drug.

6.6. Possible factors attributed to differences among ARBs

Like ACEIs, differences in structural features and pharmacological characteristics also exist among ARBs which are responsible for variations in lipid solubility, volume of distribution, bioavailability, biotransformation, plasma half-life, elimination as well as receptor binding affinity. These different properties might drive the variations that are apparent in the pharmacodynamic effects of ARBs (Table 3-2).
In *in vitro* experiments analysing the Ang-II time-dependent dissociation of telmisartan, olmesartan, valsartan and losartan from membrane components containing human AT$_1$ receptors, the dissociation rate constant of each ARB was found to be 0.003248, 0.004171, 0.009946 and 0.01027 minutes (-1) respectively with corresponding dissociation time of 213, 166, 70 and 67 minutes, respectively (Kakuta et al., 2005). The half-life of telmisartan is considerably longer than that of other ARBs (24 hours) followed by olmesartan (12 hours), valsartan (10 hours) and losartan (6 hours) [Kakuta et al., 2005]. These structural and pharmacokinetic differences among ARBs may partly explain why telmisartan and olmesartan can block AT$_1$ receptors to a greater degree than other ARBs.

Although ARBs vary in their binding affinities in *in vitro* experiments, the significance of the binding affinities of the various ARBs in brain tissues need to be further analysed to understand if these variations are responsible for their protective function against cognitive decline. Further, except for losartan, all ARBs are highly selective for the AT$_1$ receptor, implying that the AT$_2$ receptor may be exposed to a higher concentration of Ang-II because of the renin–angiotensin feedback loop following ARB administration (Kivlighn et al., 1995). Besides regulating arterial BP, both AT$_1$ and AT$_2$ receptors are expressed in various brain areas including structures involved in cognition, behaviour and locomotion (Horiuchi et al., 2010; Mogi and Horiuchi 2012). AT$_2$ receptor is suggested to be an important regulator of brain functions with an ability to modulate neuronal excitability (Guimond and Gallo Payet 2012), neurite elongation, and neuronal migration (Gallo-Payet et al., 2011).

Another important feature of AT$_2$ receptor signalling is induction of NO and cGMP production, an effect which leads to an increase in CBF and enhanced spatial memory (Jing et al., 2012). These findings indicate that ARBs with higher selectivity for AT$_1$ receptor may indirectly lead to greater activation of AT$_2$ receptor, thereby exerting a beneficial effect on neuronal function and cognition.
As discussed earlier, lipophilicity is an important factor governing the penetration of a drug into the brain and the strongest evidence for brain penetration has been demonstrated with telmisartan which is consistent with its greater lipophilicity as compared to other ARBs. This may also partly explain the better effects of telmisartan on memory enhancement compared to other ARBs like losartan or valsartan. A study comparing the effects of telmisartan and losartan in AD model showed that at doses of 100 mg/kg administered for 28 days, telmisartan significantly increased avoidance scores in shuttle avoidance test compared to losartan, and it significantly decreased Aβ deposition that was not observed with losartan (Santos et al., 2005; Mogi et al., 2008). Penetration into the brain with losartan has shown variable results, sometimes suggesting that losartan or its metabolite cannot cross the BBB, while sometimes demonstrating central effects. Gard (2008) therefore concluded that not all strains of laboratory mice exhibit the same behavioural responses to losartan and thus, it is possible that different individuals may exhibit different cognitive responses to any of these ARBs.

Among ARBs, telmisartan also possess PPAR-gamma activation property which is known to reduce inflammation and oxidative stress, and promotes Aβ clearance in AD by increasing the CBF. PPAR-γ activation can also downregulate brain inflammation by inhibiting several functions associated with microglial activation and neuroinflammation (Zhang and Jiang 2015). Telmisartan’s ability to activate PPAR-gamma receptors might be an additional mechanism responsible for effective neuroprotection against scopolamine-induced amnesia.
6.7. Other plausible mechanisms of improved learning and memory with ACEIs and ARBs

A plausible mechanism by which RAS inhibitors may enhance memory could be attributed to their interaction with NMDA receptors. Ang-II interacts with NMDA receptors by producing an inhibitory action on NMDA-induced neuronal excitation in the hippocampus and amygdala (von Bohlen and Albrecht 2006). Since NMDA receptors are involved in mediating LTP, ACEIs and ARBs may help in LTP-facilitated memory enhancement by blocking the influence of Ang-II on NMDA receptor.

Cognitive facilitation by ACEIs may also be directly related to their inhibitory effect on Ang-II. Inhibiting synthesis of Ang-II releases inhibition of potassium-induced exocytosis of ACh leading to facilitation of memory consolidation. ACEIs also increase the availability of Ang I facilitating formation of Ang (1-9) and Ang (1-7), resulting in the increased formation of Ang (2-7) and Ang (3-7). These in turn result in an increase in AT₄ receptor activation and memory facilitation. ACEIs also have a role in enhancing the activity of Kallikrein Kinin System which promotes endothelial production of NO and prostacyclin (Linz et al., 1995). This contributes to improved endothelial dysfunction and reduced OS, factors which are both implicated in AD (Costa-Neto et al., 2008).

ACEIs may also enhance memory by increasing brain substance P, a neuropeptide that plays an important role in inflammation, learning and memory mainly through its high affinity neurokinin 1 receptor (Hasenohr et al., 2000; Carvalho et al., 2008). Further, substance P is known to augment the activity of neprilysin, an Aβ-amyloid degrading enzyme. Since ACE degrades neprilysin, the administration of an ACEI would be expected to potentiate β-amyloid degradation (Wright and Harding 2010), a factor that may facilitate cognitive dysfunction in DAT.
ARBs facilitate cognitive function by blocking AT\(_1\) receptors and thereby preventing activation by Ang- II (Wright and Harding 1997). AT\(_1\) receptor blockade makes additional unbound Ang-II available for conversion to Ang-III and then to Ang-IV that activates the AT\(_4\) receptor subtype and facilitates cognition (Wright and Harding 2008; Davis et al., 2006). In scopolamine-induced amnesia model, Ang-IV and its analogues have been shown to facilitate acquisition by binding to the AT\(_4\) receptor subtype and compensating for dysfunctions of the cholinergic system (Wright and Harding 2008). Thus, many of the memory facilitating effects initially attributed to Ang-II may be due to the conversion of Ang-II to Ang-III and then to Ang-IV, which then acts at the AT\(_4\) receptor subtype and exert cognitive enhancing effects.

ACEIs and ARBs by reducing Ang-II (a potent vasoconstrictor) cause dilatation of cerebral blood vessels and may attenuate the hypoperfusion of cerebral cortex. Improved CBF may also be a contributing factor in improving cognitive function in dementia. Another mechanism by which RAS inhibitors may benefit patients with cognitive impairment may be due to improved insulin resistance (IR) since IR is known to decrease ACh synthesis and cause subsequent memory impairment (Rasgon and Jarvik 2004).

In the current study, effects of ACEIs and ARBs on BP was not recorded since numerous studies have shown that these drugs may prevent cognitive decline in patients with hypertension or heart failure, independent of their blood pressure lowering effect. In normotensive young adults, an ARB was shown to improve memory performance and reversed the detrimental effects of scopolamine highlighting the cognitive enhancing potential of ARBs in normotensive subjects (Mechael et al., 2011). Takeda et al., (2009) suggested that olmesartan at a dose of 1 mg/kg can prevent A\(\beta\)-induced cognitive impairment in experimental model of AD without significant change in BP. Another study conducted on Tg2576 mice showed that valsartan delivered at 10 or 40 mg/kg/d body weight of mice for
five months failed to produce a statistically significant change in either systolic, diastolic or mean arterial pressure in normotensive Tg2576 mice (Wang et al., 2007). A study in hypertensive rats found that lifelong treatment with captopril, but not hydralazine (a thiazide diuretic), significantly attenuated age-related cognitive impairment despite equal control in BP, supporting the contention that the mechanism of preservation of learning and memory may be independent of their BP lowering effect (Wyss et al., 2003).

The above findings thus indicate that ACEIs or ARBs may exert pleiotropic effects, including those on cognition, independent of their BP lowering effect.