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Studies on Nootropic Activity of Ramipril and Losartan on Scopolamine-induced Amnesia in Rats

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The present work was carried out to evaluate the effects of angiotensin converting enzyme inhibitor as well as angiotensin receptor antagonist on cognitive functions impaired by scopolamine in rats. In Wistar rats of either sex amnesia was induced by administering scopolamine butyl bromide (2 mg/kg). Ramipril and losartan (1 and 2 mg/kg) were evaluated for their nootropic activity in terms of spatial memory and working memory using Morris water maze task. Ramipril (1 and 2 mg/kg) and losartan (1 and 2 mg/kg) have shown significant improvement in basal as well as scopolamine-impaired performance with respect to acquisition ($P<0.05$) and retention of memory ($P<0.05$) in both spatial and working memory. The observed results suggest that ramipril and losartan improve cognitive function with respect to spatial and working memory processes.

An enhanced life expectancy in developed countries has been accompanied by an increased number of people suffering from age-associated dementia. This syndrome not only causes a terrible reduction in the quality of life of the sufferer, it also places tremendous burden on both the career and the welfare systems[1]. The cognitive disorders are commonly associated with definable neuropathological, metabolic or toxic changes and are characterized by confusion, disorientation and memory disorders. Recently memory complaints and memory disorders are becoming more prevalent due to various factors such as natural (aging, physical and mental stress), environmental (excess levels of carbonmonoxide and carbondioxide, methyl mercury in atmosphere, aluminium in foods), iatrogenic (electro convulsive shock therapy and use of certain central nervous system depressants) and diseases (Alzheimer's disease, Brushfield-Wyatt disease, and Huntington's chorea)[2].

Several reports have indicated that angiotensin converting enzyme inhibitors captopril, enalapril and trandolapril have shown to improve learning and memory in active and passive avoidance tests comparable to the effects of the nootropic drug oxiracetam[3-5]. Previously Chalas and Conway[6] studied the involvement of angiotensin II in spatial learning in water maze by using Wistar rats at a dosage regimen of ramipril 2 and 10 mg/kg/day and losartan 10 and 30 mg/kg/day. They concluded that neither ramipril nor losartan reversed the scopolamine impaired spatial learning. Later Hirawa *et al.*[7] studied the long-term inhibition of renin angotensin system by cilazapril 0.0025 mg/ml in drinking water and E4177, an AT II receptor antagonist, in Dahl rats with passive avoidance test and concluded that, the long-term inhibition by cilazapril and E4177 improved the memory function. Since there are conflicting reports on the involvement of renin angiotensin system in learning and memory[6,7], in the present study ACE inhibitor-ramipril and angiotensin receptor antagonist, losartan were employed to assess the spatial and working memory tasks in scopolamine induced amnesia in rats.

MATERIALS AND METHODS

Wistar rats weighing between 90-130 g purchased from M/S Ghosh Enterprises, Kolkata were used. They were housed in colony cages in groups of 4-5 in the departmental animal house, at an ambient temperature with a 12 h light/

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dark cycle. The animals had free access of standard pellet chow and tap water. The animals were acclimatized to the laboratory environment for at least a week before experimentation. The present investigation confirms to the guidelines laid by the Institutional Animals Ethics Committee (Reg. no. 516/01/A/CPCSEA). Ramipril was a generous gift by Dr. Reddy's Laboratories Ltd. Hyderabad, Losartan was a gift sample from Sun Pharmaceuticals Ltd. Baroda and all other reagents used were of analytical grade.

Experimental design:

In the present study, Morris water maze[8] was used to study the effects of ACE inhibitor (ramipril) and angiotensin II receptor blocker (losartan) on spatial memory and working memory. The Morris water maze consists of a large circular tank (120 cm in diameter and 40 cm deep) filled with water upto 30 cm height (22-26°). The actual dimensions of the pool can be varied depending upon the space available and whether rats or mice are being tested. The water was made opaque by adding milk (1.5 l). A 10x15 cm rectangular escape platform is constructed of a water-resistant material and covered with material that allows the animal to remain on the top when it submerged. The platform is made heavy enough to remain upright when submerged or may be attached to the bottom of the pool. The platform is 28 cm in height so that it is submerged 2 cm below the level of water surface. The water temperature is maintained at 26°. The major advantage of the water maze task over the radial arm maze task is that the rats do not need to be water or food deprived, they are quite motivated to escape from the water. The task is also free from the errors of omission (or) abortive choices i.e., rat makes an attempt to find the platform on every trial.

Study protocol for spatial memory:

Wistar rats of either sex, weighing 90-130 g, were divided into 5 groups each containing 5 animals. The treatment schedule was as follows, group I comprised of saline-treated control animals, group II animals were treated with 1 mg/kg, p.o. ramipril, group III animals received 2 mg/kg, p.o. ramipril, group IV animals were treated with 1 mg/kg, p.o. losartan and Group V animals received 2 mg/kg, p.o. losartan.

In the test, rat was released with its head pointed towards the side of the water pool. Time was recorded to find the submerged platform. The time taken by the rat to find a hidden platform in a water pool after previous exposure to the set up, using only available external cues was determined as a measure of spatial memory. Then rat was allowed to remain on platform for 10 s. Then rat was removed from the pool and it was placed in holding cage for 15 s. Again the rat was released from same place and time for reaching the submerged platform was recorded. Likewise ten trials were conducted and average time to reach the submerged platform was recorded. Similar experimental conditions (time for starting of trials, lighting) were maintained throughout the study.

Drugs were administered orally according to the above depicted dosage schedule. After 90 min all the five groups were exposed to the above training schedule. This procedure was repeated at 24 h interval until each subject acquired minimum time interval that required to reach the submerged platform in the pool. After complete training all groups were treated with scopolamine butyl bromide (2 mg/kg i.p.), 30 min later they were treated with daily doses of ramipril, losartan and vehicle, after 90 min they were tested for spatial memory. The schedule was continued with daily doses of ramipril, losartan and vehicle until they return to normal state from scopolamine-induced amnesia.

Study protocol for working memory:

Wistar rats of either sex, weighing 90-130 g were divided into 5 groups each containing 5 animals. The treatment schedule was similar to that used in the spatial memory experiment described above. This protocol was performed after the acquisition phase of testing has been completed. It is important that the rats have demonstrated that they knew the location of hidden platform before beginning. This protocol has also been referred to as a reversal test.

In this test rat was released with its head pointed towards the side of the water pool. Time to find the submerged platform was recorded. Then the rat was allowed to remain on platform for 10 s. The rat was then removed from the pool and was placed in a holding cage for 15 s. The submerged platform was moved to a new location. Then rat was released from same place and time for reaching the submerged platform was recorded. In this manner the hidden platform was changed to all four quadrants and five trials were conducted in each quadrant and average time to reach the submerged platform in each quadrant was recorded.

Drugs were administered orally according to the above depicted dosage schedule. After 90 min all the five groups were exposed to the above training schedule. This procedure was repeated at 24 h interval until each subject acquired

minimum time interval that required to reach the submerged platform in the pool. After complete training all groups were treated with scopolamine butyl bromide (2 mg/kg, i.p), 30 min later they were treated with daily doses of ramipril, losartan and vehicle, after 90 min they are tested for working memory. The schedule is continued with daily doses of ramipril, losartan and vehicle, until they return to normal state from scopolamine-induced amnesia.

Statistical analysis:

All the values were expressed as mean±S.D. The data were analyzed as follows, using multiple group 2 way repeated measures ANOVA. A level of $P<0.05$ was considered as statistically significant. Tukey's test was performed to find the significant difference at $P<0.05$.

RESULTS

Effects of ramipril and losartan on scopolamine induced spatial memory impairment:

In saline-treated rats, the mean latency in finding the platform decreased gradually during the training period of 4 consecutive days ($P<0.05$). The scopolamine treated rats consistently took longer time to reach the platform than the initial values and the effect of scopolamine treatment was statistically significant ($P<0.05$).

Both the drugs ramipril (1 mg/kg, 2 mg/kg) and losartan (1 mg/kg, 2 mg/kg) have shown significant improvement in basal ($P<0.05$) as well as scopolamine-impaired performance ($P<0.05$) with respect to acquisition and retention of memory (Table 1). Both the drugs ramipril and losartan have offered the same degree of dose independent nootropic activity with respect to spatial memory.

Effects of ramipril and losartan on scopolamine-induced working memory impairment:

In saline-treated rats, the mean latency in finding the platform in all the four quadrants decreased gradually during the training period of 3 consecutive days ($P<0.05$). The scopolamine-treated rats consistently took longer time to reach the platform than the initial values and the effect of scopolamine treatment was found to be statistically significant ($P<0.05$).

Both the drugs ramipril (1 mg/kg, 2 mg/kg) and losartan (1 mg/kg, 2 mg/kg) have shown significant improvement in basal as well as scopolamine impaired performance in all the four quadrants ($P<0.05$) with respect to acquisition and retention of memory (Table 2). Both ramipril and losartan have offered the same degree of nootropic activity with respect to working memory.

DISCUSSION

The Morris water maze task has been extensively used to study the neurological mechanisms that underlie spatial navigation to influence specific cognitive processes[9,10]. The same task can also be used to test working memory by changing the hidden platform from one quadrant to another quadrant.

In the past two decades, a great deal has been learnt about the renin angiotensin system (RAS) in the brain. The RAS is one of the best-studied enzyme neuro peptide systems in the brain. The diversity of localisation of this peptide throughout the brain has implied a variety of potential

TABLE 1: EFFECT OF RAMIPRIL, AND LOSARTAN ON SCOPOLAMINE INDUCED SPATIAL MEMORY IMPAIRMENT

Treatment	Latency to reach the platform in sec (mean ± S.D)				
	Day 0	Day 1**	Day 4	Day 5***	Day 6
Control	12.4±3.04	12.1±4.09	7.80±1.82†	15.5±2.49	11.6±3.88†
Ramipril (1mg/kg)	13.1±4.55	6.57±1.63*	5.12±0.79†	13.9±3.32	6.25±1.13*,†
Ramipril (2 mg/kg)	13.5±4.05	4.97±0.23*	4.82±1.20†	12.3±1.75	6.16±0.88*,†
Losartan (1mg/kg)	13.0±3.23	7.3S±1.16*	5.78±1.71†	11.3±1.36*	6.84±1.63*,†
Losartan (2 mg/kg)	14.4±3.94	6.64±1.01*	5.05±0.85†	12.1±1.17*	6.82± 0.99*,†

Values are mean ±SD; Repeated measure ANOVA, n=5 in each group, **Day from which animals were treated, ***Day on which animals were treated with scopolamine butyl bromide (2mg/kg i.p), *significantly different compared to day-1 values, $P<0.05$ (Tukey's test), †significantly different compared to day-5 values, $P<0.05$ (Tukey's test).

functions. Besides its classical role in the regulation of blood pressure and body fluid homeostasis, it has more subtle functions involving complex mechanisms such as learning and memory[11].

Preclinical and clinical studies suggest that angiotensin converting enzyme (ACE) may have a role in the modulation of cognitive and memory process in the rat and humans. The activity of the enzyme ACE has been found to be increased in the hippocampal regions of patients dying with Alzheimer's disease. In addition, angiotensin II has been shown to impair performance in various learning and memory paradigms in animals. Enalapril and captopril block the inhibitory effect of endogenous angiotensin II on the cholinergic memory centres and thereby leads to reinforcement of memory, especially current memory[3].

In a study carried out by Chalas[6], animals were trained for 5 d before treatment and concluded, that ramipril and losartan failed to improve the scopolamine-induced

TABLES 2: EFFECT OF RAMIPRIL AND LOSARTAN ON SCOPOLAMINE INDUCED WORKING MEMORY IMPAIRMENT

Quadrant	Treatment	Latency to reach the platform in s (mean±SD)				
		Day 0	Day 1**	Day 3	Day 4***	Day 5
I	G I	11.5±2.96	15.6±5.47	6.55±1.42†	12.3±2.20	7.95±1.15†
	G II	12.8±3.05	5.91±1.12*	4.24±0.71†	7.64±1.07*	4.31±0.9*,†
	G III	11.6±2.38	6.06±1.10*	4.12±0.88†	7.10±1.13*	4.07±0.84*,†
	G IV	11.5±1.84	6.88±1.28*	4.84±0.83†	7.83±1.30*	4.07±0.84*,†
	G V	12.3±2.72	6.50±1.05*	4.49±0.42	7.03±0.73*	4.54±0.35*
II	G I	12.7±3.20	15.4±9.84	5.73±1.90†	11.3±1.83	6.20±1.64†
	G II	12.3±3.07	5.72±0.96*	4.6 ± 0.59†	8.12±1.09*	7.09±0.76
	G III	11.9±3.2	5.24±0.85*	3.21±0.38	5.74±0.24*	3.23±0.42*
	G IV	12.3±3.47	5.34±1.27*	3.64±0.71	6.23±1.04*	3.71±0.81*
	G V	12.5±2.65	4.03 ± 0.41*	3.14±0.39	5.91±0.71*	3.36±0.35
III	G I	15.7±5.67	12.8±7.43	5.18±1.37†	11.6±0.76	8.08±1.31†
	G II	16.1±4.62	4.70±0.51*	3.28±0.29†	6.15±0.52*	3.36±0.59*,†
	G III	11.6±2.42	4.26±0.75*	3.08±0.42†	6.21±0.55*	3.06±0.40*,†
	G IV	11.5± 2.56	4.30±0.54*	3.32±0.36†	5.92±0.75*	3.36±0.29*,†
	G V	16.7±3.15	3.67±0.49*	3.28±0.54†	5.62±0.50*	3.32±0.51*,†
VI	G I	12.2±3.40	10.1±4.12	6.15±0.81†	10.8±1.21	8.86±1.03†
	G II	13.3±4.38	7.55±1.72*	4.92±0.77†	8.70±0.99	4.94±0.95*,†
	G III	11.4±3.36	5.74±0.77*	4.03±0.64	6.43±0.54*	4.07±0.65*
	G IV	13.8±4.22	6.90±1.35*	4.94±0.56†	7.36±0.90*	4.98±0.57*,†
	G V	12.0±2.82	6.47±0.70*	4.75±0.44†	7.07±0.39*	4.75±0.41*,†

G I– Control group, G II– ramipril (1 mg/kg), G III– ramipril (2 mg/kg), G IV– losartan (1 mg/kg), G V – losartan (2 mg/kg), Values are mean±SD: Repeated measures ANOVA, n=5 in each group. **Day from which animals were treated, ***Day on which animals were treated with scopolamine butyl bromide (2 mg/kg, i.p), *significantly different compared to day-1 values, $P<0.05$ (Tukey's test), †significantly different compared to day-4 values, $P<0.05$ (Tukey's test).

impairment in cognitive function. Later, in a long-term study carried out by Hirawa *et al*[7], the cognitive dysfunction was reported to be improved with ACE inhibitor and AT II receptor antagonist. In the present study the animals were treated on day -1 itself, and each animal was passed through 10 trials on each day till it receives scopolamine. Surprisingly ramipril and losartan treated animals have shown an improvement in cognitive function in day-1 itself, compared to day-1 of saline treated group.

In the present study, ramipril and losartan significantly improved the performance in normal and scopolamine treated rats including acquisition and retention of memory with respect to spatial and working memory. Both the drugs have shown the same degree of nootropic activity. The observed nootropic activity of ramipril and losartan may be attributed to ACE and angiotensin II Type I receptor inhibition. As both the drugs have shown same degree of nootropic activity, ACE inhibition or angiotern II type I receptor inhibition may be contributing equally in the cognitive and memory function. The observed results suggest the involvement of renin angiotensin system in the modulation of cognitive and memory function with respect to spatial and working memory processes.

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