Effect of losartan and enalapril on cognitive deficit caused by Goldblatt induced hypertension

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The present study was designed to evaluate the learning and memory, in an altered physiological state associated with increased blood pressure and activated renin angiotensin system in Wistar rats. The role of angiotensin in cognitive function was assessed by treatment with angiotensin converting enzyme (ACE) inhibitor enalapril (2 mg/kg), angiotensin I receptor (AT(1)) antagonist losartan (5 mg/kg) and their combination. The experimental renal hypertension was induced by the method of Goldblatt. Learning and memory was assessed using the radial arm maze test. Acetylcholine esterase (AChE) levels in the pons medulla, hippocampus, striatum and frontal cortex were measured as a cholinergic marker of learning and memory. Results indicate that in comparison to normotensive rats, renal hypertensive rats committed significantly higher number of errors and took more trials and days to learn the radial arm maze learning and exhibited memory deficit in the radial arm maze retrieval after two weeks of retention interval, indicating impaired acquisition and memory. Treatment with enalapril, losartan and their combination attenuated the observed memory deficits indicating a possible role of renin angiotensin system in cognitive function. AChE level was reduced in hippocampus and frontal cortex of renal hypertensive rats which could be attributed to the observed memory deficit in hypertensive rats. It can be concluded that, renal hypertensive rats had a poor acquisition, retrieval of the learned behavior, perhaps a possible disturbance in memory consolidation process and that this state was reversed with ACE inhibitor enalapril and AT I receptor antagonist losartan.

Keywords: Acetylcholine esterase, Angiotensin, Enalapril, Learning and memory, Losartan, Renal hypertension

Hypertension has been attributed to cognitive deficits, psychomotor impairments and neural modifications. Several studies have shown the role of brain angiotensins in cognitive function, for instance, bilateral micro-injection of angiotensin II in the hippocampal area improved learning in the shuttle box and food finding in the T-maze. Intra cerebroventricular (icv) administration of angiotensin II (2-8)3, angiotensin II (3-8)6 and angiotensin II (3-7)7 exhibited memory enhancing effects in aversively motivated learning. However, contradictory findings have also been reported9. The reports on cognitive effects of antihypertensive drugs like adrenoceptor blockers, calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor I (AT I) antagonists are contradictory in animals and in hypertensive subjects. An increased activation of brain cholinergic and adrenergic system in the hypertensive state has been demonstrated. These putative neurotransmitter systems were found to influence various behaviours including memory consolidation and memory retrieval and are reported to be modulated by the angiotensin system. The reports of ACE inhibitors and AT (1) antagonists in cognitive function in the hypertensive state were contradictory and most of the experiments were performed either in normal animals or with icv administration of angiotensin II in normotensive rats (NTR). Hence, the present study has been planned to evaluate the cognitive effects in an altered physiological state, associated with increased BP and activated renin angiotensin aldosterone system (RAAS). Since cholinergic dysfunction is correlated to cognitive impairments, AChE level was measured in different brain regions of NTR and renal hypertensive rats (RHR).

Materials and Methods

Animals — Inbred Wistar strain male Albino rats weighing 200-250 g and procured from the Central Animal House of the Institute were used. They were
housed in groups of 5-6 in colony cages at an ambient temperature of 25°C ± 2°C and 45-55% RH with 12:12 hr L:D cycle. They had free access to pellet chow (Brook Bond, Lipton India) and water ad libitum. Animals were exposed only once to the experiments and the experiments were performed between 0900 to 1700 hrs. The project was approved by the Institutional Animal Ethical Committee under the regulation of CPCSEA, New Delhi.

Surgical procedure — Experimental hypertension was induced in non-fasted rats by renal occlusion method of Goldblatt et al.18. Surgical anesthesia was induced by chloral hydrate (400 mg/kg, ip). Lumbar surgery took place in an aseptic condition, whereby the left kidney was exposed and the branch of renal artery, supplying its anterior portion was occluded with aneurysm clips. Muscle and skin were sutured separately and the rats were housed individually, until they recovered from the surgical wound. Rats were given amoxycillin (10 mg/kg) and ibuprofen (100 mg/kg) for 5 days, as a postoperative management once daily. After recovery, the animals were again housed in colony cages for acclimatization. A similar surgery was done to the control rats omitting the occlusion step with aneurysms clips. Systolic BP measurements were made by using tail-cuff blood pressure recorder (ITC INC Life Science Instruments) in conscious animals before the behavioral testing.

Behavioral studies

Locomotor activity — Rats were placed individually in photocell box, a 6 × 60 cm black metal chamber with a screen floor and a light-tight lid. Rats were placed in the chamber and allowed to acclimate for 2 min, then light beam breaks were counted for the next 5 min.

Radial arm maze19 — The effect of renal hypertension on learning and memory was evaluated in an eight-arm maze made of wood and elevated 50 cm from the floor. The arms, each 80 cm long and 10 cm wide were extended from an octagonal central platform, 35 cm across. Food cups (1 cm deep) were centered 2 cm from the end of each arm. The testing room contained many extra maze cues and was dimly lit while sessions were in progress. Initially, the animals were given drug free training for three days, for 10 min. On day 4, the rats were tested one session per day, all the eight arms were baited with 1 pellet of food each. Rats were allowed to move freely in the maze until it collected all the 8 pellets of food or 10 min time elapsed, whichever occurred first. The following parameters were recorded: (i) total number of errors; i.e., re-entry into baited arms that had been already visited during the session; (ii) total arm entries; and (iii) the number of days to learn the task. The rats were declared learnt after reaching the performance of committing one mistake on three consecutive days. After an interval of 14 drug free days of learning, the rats were again assessed once in the radial arm maze for memory retrieval.

Biochemical estimation

Acetylcholine esterase — Rats were sacrificed on the immediate day after the experiments. The brain was rapidly removed and the regions of frontal cortex, hippocampus, striatum and pons medulla were dissected out. The tissue samples were homogenized (approximately 20 mg of the tissue per ml of phosphate buffer, pH 8, 0.1 M) in a hand homogenizer. An aliquot (0.4 ml) of the homogenate was taken and mixed with 2.6 ml of phosphate buffer. To this, 100 μl of 5-5’-dithiobis-2-nitrobenzoic acid (Ellman’s reagent; 1 mM in phosphate buffer pH 8) reagent was added and the change in absorbance was observed spectrophotometrically19, at 412 nm every minute after the addition of 20 μl of the substrate acetylthiocholine iodide (0.01 mM). The enzyme activity was expressed as mmol/min/g tissue20.

The experimental design is given in Fig. 1.

Drugs — The drugs enalapril (EPL, 2 mg/kg; ENACE, Nicholas Piramal, Bombay, India), losartan (LSN, 5 mg/kg; LOSAR, Unisearch Laboratories, Bombay, India) and their combination (enalapril + losartan, 2+5 mg/kg) were suspended in 0.3% carboxy methyl cellulose and administered through per oral route once daily and experiments were performed 45 min after drug administration.

Fig. 1— Schematic diagram represents the experimental design.
**Statistical analysis** — Data are expressed as mean ± SE. The data on locomotor activity were subjected to Kruskal-Wallis one-way analysis of variance (ANOVA) followed by Mann-Whitney U test. Other behavioural and biochemical data were subjected to one-way ANOVA followed by Newman Keuls multiple comparison post hoc test, using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, California, USA). A *P* value of < 0.05 has been taken as significant.

**Results**

**Blood pressure** — Renal artery occlusion in rats significantly elevated the BP (194.0 ± 1.6 mm/Hg) in comparison to NTR (118.0 ± 0.4 mm/Hg). Treatment with EPL (2 mg/kg) (141.0 ± 0.8 mm/Hg), LSN (5 mg/kg) (147.0 ± 0.64 mm/Hg) and combination of EPL and LSN (2 + 5 mg/kg) (135.0 ± 0.85 mm/Hg), [F (4,25) = 9.51, *p* < 0.01], significantly lowered the BP. In NTR, no effect on BP was observed with these drugs.

**Locomotor activity** — Renal hypertensive rats showed increased spontaneous locomotor behavior in comparison to sham controls. Treatment with EPL, LSN and their combination attenuated (χ² = 12.35, *p* < 0.01) the observed increased locomotor activity in RHR. No effect on locomotor activity was observed in NTR with these drugs (Fig. 2).

**Radial arm maze learning** — RHR learned the radial arm maze, with increased number of trials, more days, and committed more errors in comparison to normotensive vehicle treated rats. EPL, LSN and their combination treated RHR took less trials (F (4,25) = 14.92, *p* < 0.01), committed less errors [F (4,25) = 9.04, *p* < 0.05] to learn the radial arm maze in comparison to hypertensive vehicle treated groups. However, the number of days to learn the task remained unchanged in comparison to vehicle treated RHR (Table 1). Further, the drug treated RHR differed in their learning pattern in comparison to the

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**Table 1** — Effect of enalapril (EPL) and losartan (LSN) and its combination on radial arm maze learning and relearning in normotensive and renal hypertensive rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Learning (after 14 days)</th>
<th>Retrieval (after 14 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trials/day</td>
<td>Errors/day</td>
</tr>
<tr>
<td>NTR (vehicle)</td>
<td>8.66 ± 056</td>
<td>0.66 ± 0.3</td>
</tr>
<tr>
<td>EPL (2 mg/kg)</td>
<td>8.43 ± 075</td>
<td>0.43 ± 0.2</td>
</tr>
<tr>
<td>LSN (5 mg/kg)</td>
<td>8.73 ± 061</td>
<td>0.73 ± 0.3</td>
</tr>
<tr>
<td>EPL + LSN (2 + 5 mg/kg)</td>
<td>8.70 ± 065</td>
<td>0.70 ± 0.2</td>
</tr>
<tr>
<td>RHR (vehicle)</td>
<td>10.81 ± 030</td>
<td>2.81 ± 0.6</td>
</tr>
<tr>
<td>EPL (2 mg/kg)</td>
<td>8.40 ± 059</td>
<td>0.40 ± 0.5</td>
</tr>
<tr>
<td>LSN (5 mg/kg)</td>
<td>8.62 ± 058</td>
<td>0.62 ± 0.5</td>
</tr>
<tr>
<td>EPL + LSN (2 + 5 mg/kg)</td>
<td>8.50 ± 073</td>
<td>0.50 ± 0.2</td>
</tr>
</tbody>
</table>

*P* values: *< *0.05 and **< *0.01 versus respective vehicle-treated groups; *< *0.05 and *< *0.01 versus respective normotensive groups (One way ANOVA followed by Newman Keuls test).

NTR: normotensive rats, RHR: renal hypertensive rats
respective control group indicating altered learning of hypertensive rats with drugs’ treatment (Fig. 3).

**Radial arm maze retrieval** — In comparison to NTR, RHR committed more errors and took more trials in radial arm maze retrieval, 14 days after learning. However, EPL (2 mg/kg), LSN (5 mg/kg) and LSN + EPL treated hypertensive rats committed less errors \( F(4,25) = 5.06, p<0.05 \) and took less number of trials \( F(4,25) = 6.12, p<0.05 \) in comparison to vehicle treated group. Retrieval of normotensive rats with the treatment of EPL, LSN and their combination remains unaltered (Table 1).

**Biochemical estimation**

**Acetylcholine esterase** — Experimentally induced renal hypertension significantly decreased the AChE concentration in the hippocampus and frontal cortex regions in comparison to NTR. LSN alone and its combination with EPL increased the AChE level in the hippocampus \( F(4,25) = 24.86, p<0.01 \) and cortical regions \( F(4,25) = 137.7, p<0.001 \) of both NTR and RHR in comparison to their respective vehicle treated groups. Further, LSN also elevated the AChE level in pons medulla \( F(4,25) = 5.87, p<0.05 \) and striatal regions \( F(4,25) = 6.15, p<0.05 \) of RHR. EPL (2 mg/kg) did not alter the AChE level in hypertensive rats. In NTR, EPL increased the hippocampal \( F(4,25) = 28.79, p<0.001 \) and cortical \( F(4,25) = 79.32, p<0.001 \) AChE level in comparison to vehicle treated normotensive rats. Similar findings were observed with combination treatment (Table 2).

**Discussion**

The results of the present study indicate that experimental hypertension in rats produced acquisition and memory deficit as indicated by increased trials, working memory errors in the radial arm maze learning and retrieval. Treatment with ACE inhibitor EPL, AT(1) antagonist LSN and their combination reversed the observed memory and acquisition deficit in RHR. These results indicate that the blockade of angiotensin may have beneficial

![Graph showing effect of enalapril and losartan on number of errors in every 20% trials](image.png)

**Fig. 3** — Effect of enalapril (EPL) and losartan (LSN) and its combination on learning graph (errors committed in every 20% trials) of renal hypertensive and normotensive rats in radial arm maze performance. [Values are mean ± SE from 6 animals in each group: NTRV: normotensive vehicle, NEPL: normotensive enalapril, NLSN: normotensive losartan, NEPLSN: normotensive enalapril + losartan, RHRV: hypertensive vehicle, REPL: hypertensive enalapril, RLSN: hypertensive losartan, REPLSN: hypertensive enalapril + losartan]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pons medulla</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Frontal cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR (vehicle)</td>
<td>4.35 ± 0.12</td>
<td>4.86 ± 0.05</td>
<td>6.01 ± 0.14</td>
<td>7.17 ± 0.27</td>
</tr>
<tr>
<td>EPL (2 mg/kg)</td>
<td>3.96 ± 0.09</td>
<td>6.06 ± 0.04</td>
<td>5.95 ± 0.30</td>
<td>8.44 ± 0.26</td>
</tr>
<tr>
<td>LSN (5 mg/kg)</td>
<td>4.77 ± 0.21</td>
<td>5.57 ± 0.29</td>
<td>6.54 ± 0.04</td>
<td>8.03 ± 0.15</td>
</tr>
<tr>
<td>EPL + LSN (2 + 5 mg/kg)</td>
<td>4.87 ± 0.35</td>
<td>7.63 ± 0.56</td>
<td>6.33 ± 0.06</td>
<td>8.68 ± 0.43</td>
</tr>
<tr>
<td>RHR (vehicle)</td>
<td>3.97 ± 0.04</td>
<td>3.26 ± 0.18</td>
<td>5.40 ± 0.20</td>
<td>3.43 ± 0.21</td>
</tr>
<tr>
<td>EPL (2 mg/kg)</td>
<td>4.53 ± 0.26</td>
<td>3.94 ± 0.09</td>
<td>5.11 ± 0.15</td>
<td>3.60 ± 0.22</td>
</tr>
<tr>
<td>LSN (5 mg/kg)</td>
<td>4.70 ± 0.29</td>
<td>4.06 ± 0.10</td>
<td>6.33 ± 0.24</td>
<td>4.01 ± 0.06</td>
</tr>
<tr>
<td>EPL + LSN (2 + 5 mg/kg)</td>
<td>4.05 ± 0.50</td>
<td>6.68 ± 0.23</td>
<td>5.68 ± 0.69</td>
<td>5.06 ± 0.08</td>
</tr>
</tbody>
</table>

*Values: *<0.05 versus respective vehicle treated groups; *<0.05 and *<0.01 versus respective normotensive groups (One way ANOVA followed by Newman Keuls test) NTR: normotensive rats; RHR: renal hypertensive rats
cognitive effects in the hypertensive state. In
contradiction, direct administration of angiotensin
in the brain improved the cognitive function in
aversively motivated learning. Further, bilateral
microinjection of angiotensin II into the hippocampal
area improved learning in the shuttle box. In the T-
maze, angiotensin II administration increased the
efficiency of food finding. All these reports clearly
indicate the facilitatory role of angiotensin II in the
learning and memory process on direct
administration. However, contradictory findings have
also been reported. The discrepancies of the
present findings in hypertensive state may be
attributed to the altered physiological conditions;
where the BP is elevated and RAAS is activated. Hence,
the memory deficit observed with RHR and its
reversal by EPL and LSN indicate the importance of
RAAS in memory formation.

Hypertensive animals committed an increased
number of working memory errors in the radial arm
maze acquisition and failed to show the retrieval of
the learned task after two weeks retention interval
indicating poor acquisition and memory deficit. It
may be attributed to the alteration in the long-term
potentiation (LTP) pathway, as LTP is influenced by
angiotensin resulting in poor consolidation process.
Though in hypertensive state increased adrenergic
and serotonergic activity have been reported, learning
deficits in hypertensive animals were observed in the
present study, indicating the role of other biochemical/neurohumoral alterations that could be
responsible for the observed deficit. EPL, LSN and
its combination treated hypertensive rats produced better
retrieval in the radial arm maze, further confirming
the role of angiotensin in cognitive function and
memory consolidation. In the present study, decreased
AChE concentration was observed in hypertensive
rats especially in the hippocampal and cortical
structures. Intact cholinergic activity has been
implicated to have a better working memory. Earlier
reports indicate that, ACE inhibitors and AT
antagonist produced better learning and memory
performance in hypertensive animals by enhancing
the cholinergic activity which is in accord to the
present study. All these results clearly indicate that
in hypertensive state there is a possible alteration in the
cholinergic function and memory consolidation
process. The possible restoration of the cholinergic
activity with the antihypertensive drugs could be
attributed to its beneficial cognitive effects.

Therefore, it can be concluded that the
hypertensive animals had both acquisition and
memory deficits of the learned behaviour in radial
arm maze, indicating a possible altered memory
consolidation process and it was reversed with
ACE inhibitor EPL (2 mg/kg), AT(1) antagonist LSN
(5 mg/kg) and the combination of EPL + LSN (2 + 5
mg/kg).

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