Original Article

Effect of short time captopril administration on spatial memory in aging rats

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Abstract

Introduction: The brain renin-angiotensin system (RAS) has been reported having a pathological role in age-related impairment in learning and memory. Therefore, angiotensin converting enzyme inhibitors (ACEi) are expected to have positive effects on memory. Longtime treatment with captopril (an angiotensin converting enzyme inhibitor) significantly attenuates the age-related impairment in learning and memory.

Materials and Methods: In the present study, 24 month old male Wistar rats were divided into four experimental groups (n=8). Captopril treated groups received daily ip injections of captopril at doses of 5, 10, 15 mg/kg/day for one week, the forth group served as control and remained untreated. Learning process was assessed by the reference memory task in the Morris water maze. All rats received water maze training (4 trials/day for 5 days) to assess hippocampal dependent spatial learning and then received a 60-s probe test of spatial memory retention 24 h after the 20th trial.

Results: Over 5 days of training, captopril 5, 10, 15 mg/kg/day treatment significantly reduced the latency and path length to finding the escape platform. In probe trails (without platform), on the last day of training, the captopril -treated group spent significantly longer time in the platform quadrant than control animals. Among treated group, 10 /mg/Kg dosage of captopril induced the best rehearsals memory.

Conclusion: These results confirm the previous studies that ACEi have a positive influence on memory and it was noticeable that even short time treatment by captopril can improve spatial memory in the aged rats.

Introduction

The brain has a fully functional angiotensin system that mediates local generation of angiotensin II by the angiotensin-converting enzyme (Wright and Harding, 2010). The renin-angiotensin system (RAS) in the brain is well known to be involved in systemic blood pressure control (de Gasparo et al., 2000). Some recent reports have shown that this system has a

role in some additional functions in the central nervous system including regulation of cerebral blood flow and cerebral protection against stress, depression, seizure, alcohol consumption and memory consolation, with possible role in the etiology of Alzheimer's and Parkinson disease (De Bundel *et al.*, 2008; Mertens *et al.*, 2010; Pelegrini-da-Silva *et al.*, 2009; Prozherina, 2008; Vanderheyden, 2009; Wright *et al.*, 2008). Cognitive impairment and dementia are common serious health problems that impair quality of life in the elderly. Several lines of evidence suggest that learning and memory gradually decline with advancing age in humans (Albert, 1993; Beatty, 1988). This age-related impairment extends to spatial learning and memory tasks (Evans *et al.*, 1984; Light and Zelinski, 1983; Park *et al.*, 1990; Sharps and Gollin, 1987; Weber *et al.*, 1978). These age- related spatial learning and memory deficits have been attributed to alterations in the connections and function of hippocampal formation (De Leon *et al.*, 1997; Squire, 1992). In the brain, the angiotensin-converting enzyme is overexpressed in the hippocampus, frontal cortex, and caudate nucleus of both hemispheres in patients with dementia (Ohrui *et al.*, 2004). Previous reports indicate the possibility that treatment with antihypertensive agents can prevent the impairment of quality of life including cognitive performance(Fletcher, 1999; Fogari and Zoppi, 2004). Possible beneficial effects of RAS blockade on cognitive function are also being highlighted in the clinical field (Iwanami *et al.*, 2009; Wright and Harding, 2010). The angiotensin receptor blockers (ARBs), which selectively prevent the actions of AngII on receptors, are thought to have positive effects on cerebral blood flow and neuronal function (Maxwell and Hogan, 2010). An epidemiological study by Li et al. recently showed that male subjects treated with ARBs exhibited a significant reduction in the incidence and progression of Alzheimer disease (Li *et al.*, 2010). A recent report in rat demonstrates that lifetime treatment with the antihypertensive drug captopril significantly attenuates the age-related impairment in learning and memory by lowering arterial pressure (Wharton *et al.*, 2012; Wyss *et al.*, 2000, 2003). But the interpretation of this beneficial effect of ACE remains clouded. ACE

inhibitors were also demonstrated to reverse the amnesic effects of electroconvulsive shock in a passive avoidance task in mice, directly improve passive avoidance learning and improve its retention with a 24-h delay in mice (Raghavendra *et al.*, 2001) .But the nootropic effects of short time captopril treatment has been not reported in spatial memory. In the present study, we employed captopril with three different dosages in a short time treatment model using Morris water maze task to assess its ability to improve spatial memory in the aged rats.

Materials and methods

Animals

Twenty-four-month-old rats weighing 400–450 g were obtained from the Aging Farm, which was established at our animal house and used as the aged rats. The aged rats were produced by keeping them for 21 months in the Aging Farm after purchase from the same breeder at 3 months old. They were housed two per cage in a temperature (23±1 °C) and light (12-h light/dark schedule; lights on at 8:00 am) controlled environment and were fed laboratory food and water *ad libitum*. All protocols for the experiments on animals were approved by the Research and Ethics Committee of Golestan University of Medical Sciences**.** Animals were randomly divided into 4 groups of 8 animals in each, including control group and captopril 5, 10, 15 mg/Kg groups respectively**.** Captopril was purchased from Sigma–Aldrich, dissolved in saline and administrated intraperitonally at dose of 5, 10, 15 mg/kg to rats 60 min before Morris water maze task for 6 days.

Morris water maze (MWM) task

The MWM used in our study was a black circular pool (160 cm diameter, 60 cm high) filled with water (30 cm depth) at 24 ± 2 °C. The pool was divided into four quadrants arbitrarily deigned Northeast (NE), Northwest (NW), Southeast (SE) and Southwest (SW). A submerged plexiglas platform (10cm x 10cm) was hidden 1cm below the water surface and placed in a constant location in the center of NW quadrant. Animals received 5 days of training with the hidden

platform, each day included 4 training sessions with a 60s intersession interval. Each trial was started by placing a rat with its face toward the wall of the pool at one of three start points. The start location was varied on each training trial and changed each day. The trial was terminated when the animal entered the platform. If the rat didn't find the platform within 60s it was placed on the platform by the experimenter for 15s. During acquisition of the spatial navigation task all groups were given one session of four trials each day (day 1-5; trial 1-20). Spatial memory was evaluated in the probe trial. On sixth day (trial 21), the platform was removed and animals were allowed to swim for 60s. The path of the animals in the maze was monitored using a computerized video tracking system (Maze router, urmia Instruments Inc). Parameters measured were the time taken to reach the platform (latency), swimming speed and swim path length (SPL) in the training trials. And during probe trials, the running time percentage within the quadrant of the water bath where the hidden platform had been placed in the training trials was calculated for each experimental animal.

Statistical analysis

Data were analyzed using SPSS 11.5 software and were plotted as mean ± SD. Comparison among the groups was made using analysis of variance (ANOVA) with a post hoc Tukey test or Repeated Measures ANOVA. Results were considered significantly different when $P<0.05$ and highly significantly different when $P<0.01$.

Results

Acquisition

The escape latency and distance traveled by rats to finding the hidden platform in the water maze task are presented in Fig. 1A&B. The ability of all experimental animals to find the platform progressively was improved over the 5 days of acquisition ($P<0.05$). However, repeated ANOVA measures of these data revealed that the performance on this task was differed between the control and each of three captopril treated groups in the first four trial days

($P<0.01$). Inspection of the data in these days showed that animals in captopril treated groups learned the task at a high rate and traveled a shorter distance and spent less time to find the escape platform than control group ($P < 0.01$), but in the final trial day the escape latency and path length was similar in all experimental groups. Furthermore, there was no significant difference in these data among the groups receiving captopril in each trial day .The analysis of swimming speed by using two-way ANOVA also showed no significant difference as training days progressed among groups and no interaction between days and group (Fig. 1C).
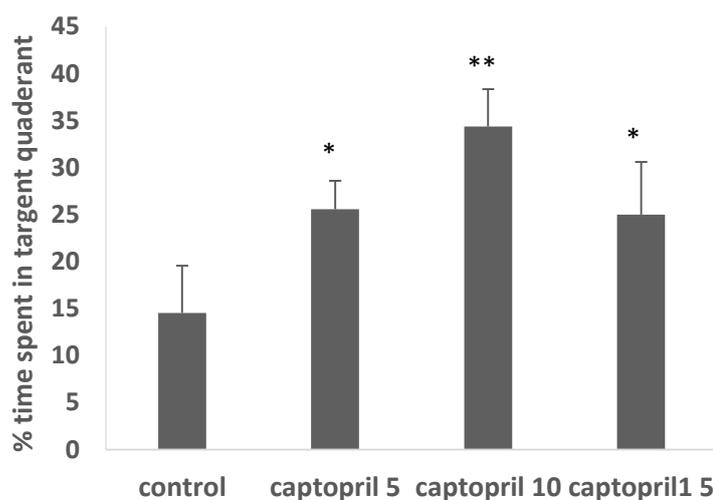
Probe trial

Retention of the spatial training was assessed 24 h after the last training session with a 60 s free-swim probe trial using a new starting position. The parameters measured on the probe trial were time spent in the quadrant containing the platform during training (target quadrant). Post hoc analysis showed that the captopril treated groups showed significantly different experimental parameters from control ones ($P<0.05$). The captopril treated animals spent more time in the training quadrant compared to the control animals in the 60s probe trial (Fig. 2). In addition, among treated groups, the 10mg/ kg/day group spent more time in the training quadrant ($P<0.05$). Figure 3 shows representative plots (top view) of individual swim paths of one rat for each treatment group and probe trial. In these examples, the start location was in the north–west quadrant of the pool, and the platform had formerly been located in the north–east quadrant of the pool (indicated by small circle for illustration purposes only; note that no platform was present in probe trials).

Discussion

There are now several lines of evidence suggesting angiotensin II (AII) may disrupt memory formation. Recent studies using specific antagonists of the angiotensin receptor (N. M. Barnes et al., 1990; Barnes et al., 1991) and angiotensin converting enzyme inhibitors that block the formation of AgII have shown these treatments can improve memory
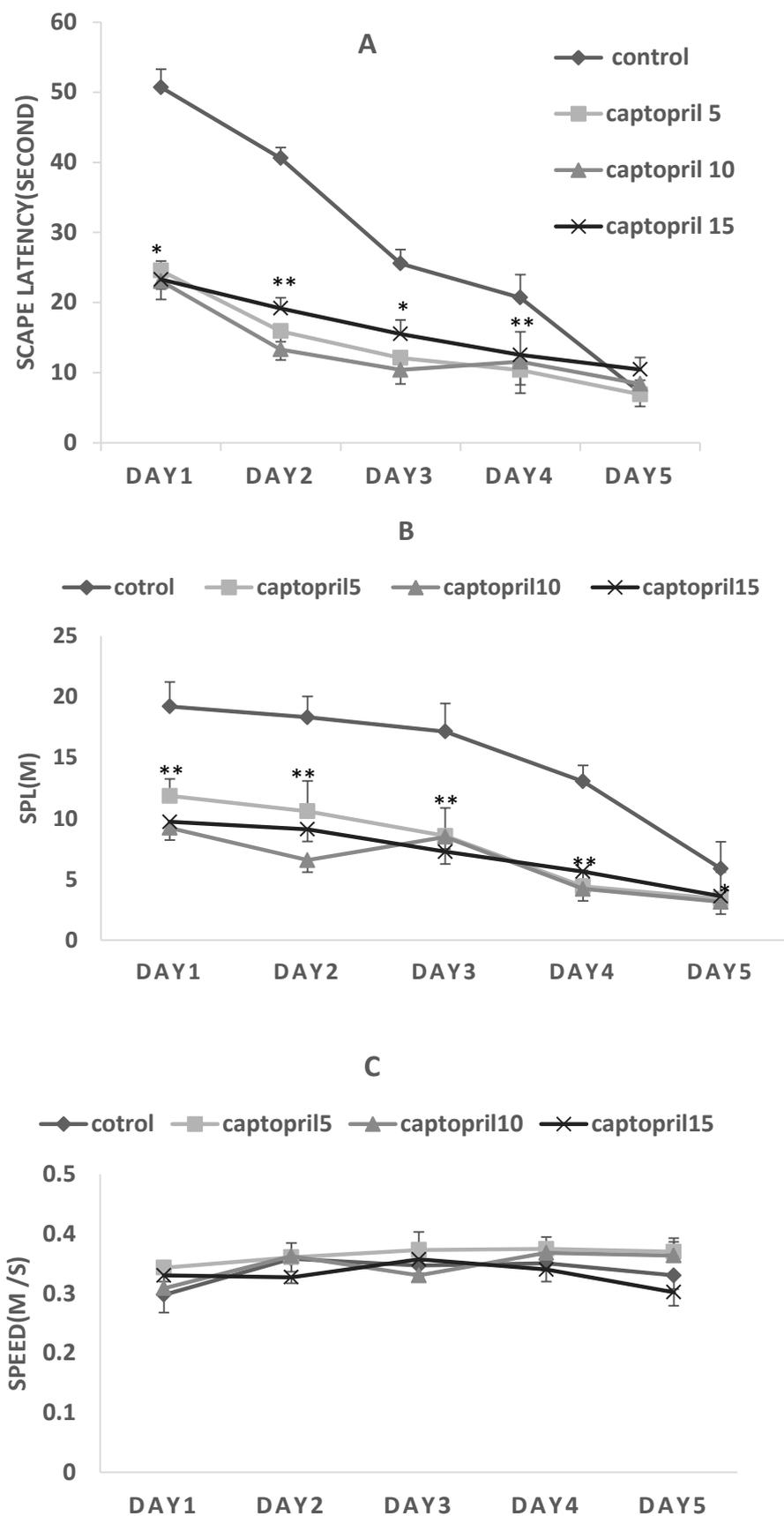
Fig 1:
Effect of short time treatment with captopril (7 days) on the performance of spatial memory acquisition phase in Morris water maze. Average scape latency (A), distance traveled (B), swimming speed (C) within each day (made up of four trials). Asterisks indicate a significant difference from control (*$P < 0.05$; **$P < 0.01$).

Fig 2: Water maze Mean (±SEM) percentage of time spent in target quadrant. Morris water maze during probe trials. Asterisks indicate a significant difference from control (*P < 0.05; **P < 0.01).
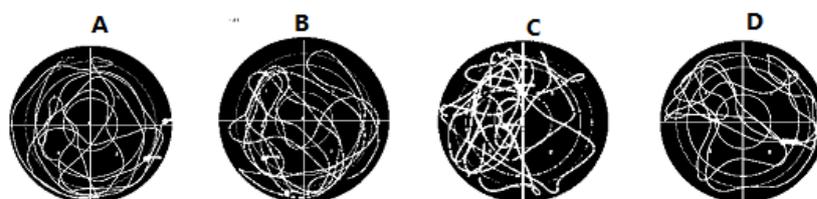
Fig 3: Tracings of the typical swim patterns in the probe trials (trial 21) at the control (A), captopril5 (B), 10 (C), 15 (D) mg/Kg group tested. The escape platform was on North West quadrant.

function in aged experimental animals (Costall *et al.*, 1989; Flood and Morley, 1993; Mondadori and Etienne, 1990) . In the present study we examined the effects of ACE inhibition on spatial memory in aged rats. Animals were treated for one week with three different doses of captopril, and their performance in a reference memory task-Morris water maze- was compared to controls. Rats treated with 5, 10 and 15 mg/kg/day doses of captopril performed better than the control group in the water maze task. Latency and path length to finding the escape platform was significantly reduced in captopril treated animals. Surprisingly, in the first day of testing, captopril treated rats found the platform significantly faster than the control animals, and were consistently better at the task than control group up to fifth day

and in the final trial day all rats found the platform in the similar time. In a probe trial, when the platform was removed from the pool, the captopril -treated group spend significantly longer time in the platform quadrant than control animals, demonstrating a better ability of remembering the position of the platform. Among treated group, 10 /mg/Kg dosage of captopril induced the best rehearsals memory. The positive effects of the ACEi treatment was reported previously in rat models of memory impairment in Morris water maze task in the diabetics rats (Jenkins and Chai, 2007; Manschot *et al.*, 2003; Wyss *et al.*, 2003), four months treatment by enalapril from the onset of diabetes, significantly attenuated memory impairment in the Morris water maze tasks, compared to untreated-diabetics(Manschot *et al.*,

2003). Previous studies have demonstrated that learning and memory decline happens during aging in the rat, especially between 12 and 24 months of age(Frick *et al.*, 1995; Shukitt-Hale *et al.*, 2004; van der Staay and de Jonge, 1993; Wyss *et al.*, 2000). Furthermore, long-term administration of the ACE inhibitor captopril improved rat performance in the normotensive as well as hypertensive rats (Barnes *et al.,1991*). In this study we used 24month old rats but unlike the above mentioned researches, we administered captopril only for one week and like them, we have seen improvement of memory although for a much shorter captopril treatment time. In consistence of our results, in a habituation task in mice over 7 days, very low doses of the ACE inhibitors, captopril and ceranapril improved basal performance, reversed the impaired performance caused by the muscarinic receptor antagonist, scopolamine, and also improved the impaired performance of aged rats (Costall *et al.*, 1989).Surprisingly captopril in our data in the first day of acquisition reduced the scape latency. This may suggest that an increase in cerebral perfusion is responsible for the immediate improvement of memory. Consistent with these findings, subcutaneous administration of a single dose of ramipril improved retention of a footshock active avoidance task in STZ-diabetic mice (Zimmerman *et al.*, 2002). Matin and colleague in their study purposed captopril would alleviate cognitive function by improving endothelium dependent dilation of cerebral artery in the hypertensive rats (Matin, 2014) . In contrast, Wyss and colleagues have not seen any improvement in the performance of young or aged SHRs treated with the anti-hypertensive drug, hydralazine in the Morris water maze task but they have reported in the last day all rats found scape platform in the same time (Wyss *et al.*, 2003) . So it seems that five day of training is enough for the old rats to find the scape platform even without need to captopril in probe trial and all groups of captopril treated rats spent more time in the target quadrant. It may represent the beneficial role of captopril in the memory rehearsal. Among our captopril treated group, 10 /mg/Kg dosage of captopril showed the best rehearsals memory. Other studies have shown

that more conventional doses of ACE inhibitors can significantly improve memory in different conditioned avoidance paradigms. Ramipril administration in a dose of 0.5-1.5 mg/kg improved retention when administered immediately after training in an active avoidance task in rats (Flood and Morley, 1993). ACE inhibitors were also demonstrated to reverse the amnesic effects of electroconvulsive shock in a passive avoidance task in mice (Mondadori and Etienne, 1990). It has also been shown that intracerebroventricular (i.c.v.) administration of renin disrupts the learning of a passive avoidance response and this effect was antagonised by an ACE inhibitor as well as AT1 receptor antagonists, but not by an AT2 antagonist (DeNoble *et al.*, 1991). Captopril also might exert its effect without vascular action, for example by a direct effect on the brain neurotransmitters (Handa *et al.*, 1991). Acetylcholine is one of the most important neurotransmitters involved in cognition and memory. Many studies have demonstrated a relation between memory deficits and a decline in the cholinergic system function in the brain(Laursen *et al.*, 2014; Lippa *et al.*, 1980; Vanderheyden, 2009) . It has been shown that scopolamine can prevent the memory -enhancing effect of Captopril, suggesting that ACEIs, by preventing the formation of Ang II, may remove the inhibition of brain cholinergic system (J. M. Barnes *et al.*, 1990). Neurohistochemical studies have shown that angiotensin II, in a concentration-dependent manner in rat tissue can inhibit potassium-stimulated release of acetylcholine from slices of rat entorhinal cortex. So, it has been hypothesized that the potential cognitive enhancing properties of ACE inhibitors may reflect their role in preventing the formation of AT II and thus, remove an inhibitory modulatory effect of cholinergic function (J. M. Barnes *et al.*, 1990). Angiotensin converting enzyme in the brain by hydrolyzing some neuropeptides, such as bradykinin, enkephalin and substance P, might contribute in memory and learning processes (Hooper, N. M., Kenny A. and Turner A., J., 1985; Skidgel *et al.*, 1984). Opposing effects have also been reported; when AT2 infused into hippocampus after training, Ang II produced a dose-dependent amnesic effect in the inhibitory avoidance task in rats,

which was blocked by the AT_2 antagonist ,but not by the AT_1 antagonist, losartan (Kerr *et al.*, 2005). Our data indicate that short time captopril treatment can improve spatial memory in the aged rat in the water maze task and its immediate effect on memory probably is due to its effect on cerebral perfusion or its possible neuromodulatory effects.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

References

Albert M. Neuropsychological and neurophysiological changes in healthy adult humans across the age range. Neurobiol. Aging 1993; 14: 623–625.

Barnes JM, Barnes NM, Costall B, Horovitz ZP, Ironside JW, Naylor RJ, et al. Angiotensin II inhibits cortical cholinergic function: implications for cognition. J. Cardiovasc. Pharmacol. 1990; 16: 234–238.

Barnes NM, Champaneria S, Costall B, Kelly ME, Murphy DA, Naylor RJ. Cognitive enhancing actions of DuP 753 detected in a mouse habituation paradigm. Neuroreport 1990; 1: 239–242.

Barnes NM, Costall B, Kelly ME, Murphy DA, Naylor RJ. Cognitive enhancing actions of PD123177 detected in a mouse habituation paradigm. Neuroreport 1991; 2: 351–353.

Beatty WW. Preservation and loss of spatial memory in aged rats and humans: Implications for the analysis of memory dysfunction in dementia. Neurobiol. Aging 1988; 9: 557–561.

Costall B, Coughlan J, Horovitz ZP, Kelly ME, Naylor RJ, Tomkins DM. The effects of ACE inhibitors captopril and SQ29,852 in rodent tests of cognition. Pharmacol. Biochem. Behav. 1989; 33: 573–579.

De Bundel D, Smolders I, Vanderheyden P, Michotte Y. Ang II and Ang IV: unraveling the mechanism of action on synaptic plasticity, memory, and epilepsy. CNS Neurosci. Ther. 2008; 14: 315–339.

De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. Pharmacol. Rev. 2000; 52: 415–472.

De Leon MJ, George AE, Golomb J, Tarshish C, Convit A, Kluger A, et al. Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease. Neurobiol. Aging 1997; 18: 1–11.

DeNoble VJ, DeNoble KF, Spencer KR, Chiu AT, Wong PC, Timmermans PBMWM. Non-peptide angiotensin II receptor antagonist and angiotensin-convering enzyme inhibitor: effect on a renin-induced deficit of a passive avoidance response in rats. Brain Res. 1991; 561: 230–235.

Evans GW, Brennan PL, Skorpanich MA, Held D. Cognitive mapping and elderly adults: Verbal and location memory for urban landmarks. J. Gerontol. 1984; 39: 452–457.

Fletcher A. Quality of life in the management of hypertension. Clin. Exp. Hypertens. N. Y. N 1993 1999; 21: 961–972.

Flood JF, Morley JE. Dose-response differences in the ability of ramipril to improve retention in diabetic mice. Eur. J. Pharmacol. 1993; 240: 311–314.

Fogari R, Zoppi A. Effect of antihypertensive agents on quality of life in the elderly. Drugs Aging 2004; 21: 377–393.

Frick KM, Baxter MG, Markowska AL, Olton DS, Price DL. Age-related spatial reference and working memory deficits assessed in the water maze. Neurobiol. Aging 1995; 16: 149–160.

Handa K, Sasaki J, Tanaka H, Kiyonaga A, Matsunaga A, Shindo M, et al. Effects of captopril on opioid peptides during exercise and quality of life in normal subjects. Am. Heart J. 1991; 122: 1389–1394.

Hooper, N. M., Kenny A. and Turner A., J. The metabolism of neuropeptides. Neurokinin A (substance K) is a substrate for endopeptidase-24.11 but not for peptidyl dipeptidase A (angiotensin-converting enzyme). Biochem. J. 1985; 231: 357–361.

Iwanami J, Mogi M, Iwai M, Horiuchi M. Inhibition of the renin-angiotensin system and target organ protection. Hypertens. Res. Off. J. Jpn. Soc. Hypertens. 2009; 32: 229–237.

Jenkins TA, Chai SY. Effect of chronic angiotensin converting enzyme inhibition on spatial memory and anxiety-like behaviours in rats. Neurobiol. Learn. Mem. 2007; 87: 218–224.

Kerr DS, Bevilaqua LRM, Bonini JS, Rossato JI, Köhler CA, Medina JH, et al. Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. Psychopharmacology (Berl.) 2005; 179: 529–535.

Laursen B, Mørk A, Plath N, Kristiansen U, Frank Bastlund J. Impaired hippocampal acetylcholine release parallels

spatial memory deficits in Tg2576 mice subjected to basal forebrain cholinergic degeneration. Brain Res. 2014; 1543: 253–262.

Light LL, Zelinski EM. Memory for spatial information in young and old adults. Dev. Psychol. 1983; 19: 901–906.

Li N-C, Lee A, Whitmer RA, Kivipelto M, Lawler E, Kazis LE, et al. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. BMJ 2010; 340: b5465.

Lippa AS, Pelham RW, Beer B, Critchett DJ, Dean RL, Bartus RT. Brain cholinergic dysfunction and memory in aged rats. Neurobiol. Aging 1980; 1: 13–19.

Manschot SM, Biessels GJ, Cameron NE, Cotter MA, Kamal A, Kappelle LJ, et al. Angiotensin converting enzyme inhibition partially prevents deficits in water maze performance, hippocampal synaptic plasticity and cerebral blood flow in streptozotocin-diabetic rats. Brain Res. 2003; 966: 274–282.

Matin N. Captopril treatment improves endothelium dependent dilation and middle cerebral artery remodeling in hypertensive rats (841.8). FASEB J. 2014; 28: 841.8.

Maxwell CJ, Hogan DB. Antihypertensive agents and prevention of dementia. BMJ 2010; 340

Mertens B, Vanderheyden P, Michotte Y, Sarre S. The role of the central renin-angiotensin system in Parkinson's disease. J. Renin-Angiotensin-Aldosterone Syst. JRAAS 2010; 11: 49–56.

Mondadori C, Etienne P. Nootropic effects of ACE inhibitors in mice. Psychopharmacology (Berl.) 1990; 100: 301–307.

Ohrui T, Tomita N, Sato-Nakagawa T, Matsui T, Maruyama M, Niwa K, et al. Effects of brain-penetrating ACE inhibitors on Alzheimer disease progression. Neurology 2004; 63: 1324–1325.

Park DC, Cherry KE, Smith AD, Lafronza VN. Effects of distinctive context on memory for objects and their locations in young and elderly adults. Psychol. Aging 1990; 5: 250–255.

Pelegrini-da-Silva A, Rosa E, Guethe LM, Juliano MA, Prado WA, Martins AR. Angiotensin III modulates the nociceptive control mediated by the periaqueductal gray matter. Neuroscience 2009; 164: 1263–1273.

Prozherina YA. Involvement of angiotensin II and angiotensin IV in producing the individual characteristics of defensive and feeding behavior in rats. Neurosci. Behav. Physiol. 2008; 38: 563–572.

Raghavendra V, Chopra K, Kulkarni SK. Comparative studies on the memory-enhancing actions of captopril and losartan in mice using inhibitory shock avoidance paradigm. Neuropeptides 2001; 35: 65–69.

Sharps MJ, Gollin ES. Memory for object locations in young and elderly adults. J. Gerontol. 1987; 42: 336–341.

Shukitt-Hale B, McEwen JJ, Szprengiel A, Joseph JA. Effect of age on the radial arm water maze-a test of spatial learning and memory. Neurobiol. Aging 2004; 25: 223–229.

Skidgel RA, Engelbrecht S, Johnson AR, Erdös EG. Hydrolysis of substance P and neurotensin by converting enzyme and neutral endopeptidase. Peptides 1984; 5: 769–776.

Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. 1992; 99: 195–231.

Van der Staay FJ, de Jonge M. Effects of age on water escape behavior and on repeated acquisition in rats. Behav. Neural Biol. 1993; 60: 33–41.

Vanderheyden PML. From angiotensin IV binding site to AT4 receptor. Mol. Cell. Endocrinol. 2009; 302: 159–166.

Weber RJ, Brown LT, Weldon JK. Cognitive maps of environmental knowledge and preference in nursing home patients. Exp. Aging Res. 1978; 4: 157–174.

Wharton W, Stein JH, Korcarz C, Sachs J, Olson SR, Zetterberg H, et al. The effects of ramipril in individuals at risk for Alzheimer's disease: results of a pilot clinical trial. J. Alzheimers Dis. JAD 2012; 32: 147–156.

Wright JW, Harding JW. The brain RAS and Alzheimer's disease. Exp. Neurol. 2010; 223: 326–333.

Wright JW, Yamamoto BJ, Harding JW. Angiotensin receptor subtype mediated physiologies and behaviors: new discoveries and clinical targets. Prog. Neurobiol. 2008; 84: 157–181.

Wyss JM, Chambless BD, Kadish I, van Groen T. Age-related decline in water maze learning and memory in rats: strain differences. Neurobiol. Aging 2000; 21: 671–681.

Wyss JM, Kadish I, van Groen T. Age-related decline in spatial learning and memory: attenuation by captopril. Clin. Exp. Hypertens. N. Y. N 1993 2003; 25: 455–474.

Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR, et al. Superoxide Mediates the Actions of Angiotensin II in the Central Nervous System. Circ. Res. 2002; 91: 1038–1045.