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RESEARCH ARTICLE

Captopril modifies angiotensin-converting enzyme but not choline acetyltransferase gene expression in the frontal cortex of renovascular hypertensive rats

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ABSTRACT

Background: The classic renin-angiotensin system (RAS) has a role in the cardiovascular system and water homeostasis in the body, but recently, the existences of this system with all of its components including angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensin receptors have been shown in the mammalian brain. Recent clinical studies suggest that treatment of hypertensive patients with the RAS affecting drugs, such as captopril improves their cognitive function. **Aims and Objectives:** This study was conducted to evaluate the effects of captopril on the frontal cortex levels of ACE and choline acetyltransferase (ChAT) in renovascular hypertensive rats receiving captopril. **Materials and Methods:** The rats were randomly divided into 3 groups of 8 animals; control, Goldblatt two kidney one clip (2K1C) hypertensive rats and Goldblatt 2K1C hypertensive rats received 5 mg/kg captopril. After 8 days of treatment; the rats were sacrificed and expression of ACE and ChAT in the frontal cortex were assessed by real-time polymerase chain reaction. The results were analyzed with two-way ANOVA test using SPSS software and the level of significance was set at $P < 0.05$. **Results:** Captopril treatment decreased the levels of ACE mRNA in the frontal cortex of renovascular hypertensive rats but has no significant effect in the ChAT mRNA levels. **Conclusion:** Captopril as a nootropic drug has no significant effect on the ChAT gene expression in the frontal cortex of renovascular hypertensive rats and it may exert its memory enhancing effect on the frontal cortex via a non-cholinergic mechanism.

KEY WORDS: Captopril; Renin-angiotensin System; Angiotensin-converting Enzyme; Choline Acetyl Transferase

INTRODUCTION

The renin-angiotensin system (RAS) is one of the best-studied enzyme cascade systems in the body. It controls fluid homeostasis, blood pressure and some behavioral and cognitive responses.[1] In response to a decrease in arterial blood pressure, the proteolytic enzyme renin releases from the juxtaglomerular cells in the kidney and acts on its inactive substrate angiotensinogen (Ang) to form the decapeptide Ang I. Ang I, in turn, is hydrolyzed at its carboxy terminal by angiotensin converting enzyme (ACE), which is present within the endothelium of most blood vessels, to form the active octapeptide angiotensin II (Ang II).[2] Ang II binds to one of its receptors named AT_1 and AT_2, and exerts its physiological effects via these G-protein coupled receptors.[3] It is now well established that apart from peripheral RAS, brain has an intrinsic RAS with all components present in the central nervous system. Both AT_1 and AT_2 receptors expressed in the brain. These receptors mediate most of the recognized actions of the RAS in the body including

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fluid homeostasis and blood pressure regulation.[3] It is reported that continuous activation of RAS is involved in the impaired cognitive functions,[4] and an increase in ACE activity has been reported in the brains of Alzheimer's disease (AD) patients. Some clinical evidence suggested that ACE is involved in cognitive deficits in AD patients because ACE inhibitors delayed the onset of dementia.[5] Although learning and memory have considered as complex cognitive processes and multiple mechanisms are involved in cognition, extensive research has established the relationship between cognitive impairment and the cholinergic system in the basal forebrain.[6] The aim of this study was to develop a model of renovascular hypertension by using Goldblatt's two kidneys one clip (2K1C) method and evaluating the levels of choline acetyltransferase (ChAT) mRNA as the main marker of cholinergic system activity as well as ACE mRNA level in the frontal cortex.

MATERIALS AND METHODS

Animals and Treatments

Three-month-old rats weighing 250-300 were housed 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 chew 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 3 groups of 8 animals; control, Goldblatt 2K1C hypertensive rats and Goldblatt 2K1C hypertensive rats received 5 mg/kg captopril.

Real-time Polymerase Chain Reaction (RT-PCR) Analysis

After 8 days of treatment, the rats in all treatment groups were sacrificed by decapitation, the brains were removed and frontal cortex tissues were extracted in three treatment groups. The tissue samples were stored in −70°C for later RNA extraction. RNA was extracted from frontal cortex tissue samples using the Gena Bioscience RNA extraction kit according to the manufacturer's instructions. Residual DNA was digested with 10 U RNase-free DNase (DNase I, TaKaRa) in the presence of 20 unit of RNase inhibitor at 37°C for 20 min. After heat inactivation for 10 min at 75°C in 2 mM EDTA, total RNA solution was removed for quantification. Concentration and purity of the DNase I-treated samples was measured using a NanoDrop ND-1000 spectrophotometer (A260/A280>1.8 and A260/A230>1.6). The integrity and stability of the RNAs confirmed by demonstrating the intact 28s and 18s bands on gel electrophoresis.

For RT-PCR, the cDNA was synthesized from 1 µg of DNaseI-treated total RNA using prime script RT reagent kit (TaKaRa) with random hexamer and oligo dT primers following the manufacturer's protocol. The forward and reverse PCR primers for the 2 genes were designed accordance to the RT-PCR conditions, using perlprimer software (Bio-Rad, USA), and the sequences are listed in Table 1. For each gene, the cDNA amplified by specific primers using Taq Polymerase Kit Data Analysis (TaKaRa) and correct product was confirmed by running on gel electrophoresis.

Statistical Analysis

Data were presented as mean±standard deviation (SD). Relative target gene expression and mRNA level were analyzed with two-way ANOVA using SPSS 16.0 statistical analysis software. $P < 0.05$ was considered statistically significant.

RESULTS

The levels of ACE and ChAT mRNA in the frontal cortex are shown in Figure 1. The ACE levels in hypertensive group showed a significant increase compared to controls ($P < 0.05$). Captopril decreased these change to an extent above controls, but the increased ACE levels in hypertensive+captopril group were not significant. ChAT mRNA levels differences in the three treatment group were not statistically significant. Quantitative RT-PCR analysis of ACE and ChAT genes in the frontal cortex of treatment groups. mRNA levels were measured using gene-specific primers, and the values were normalized to β-actin (*$P < 0.05$, mean ± SD, $n = 3$).

DISCUSSION

The results of this study indicated that experimental hypertension in rats increases the expression of ACE gene in the frontal cortex. Captopril treatment decreased the ACE

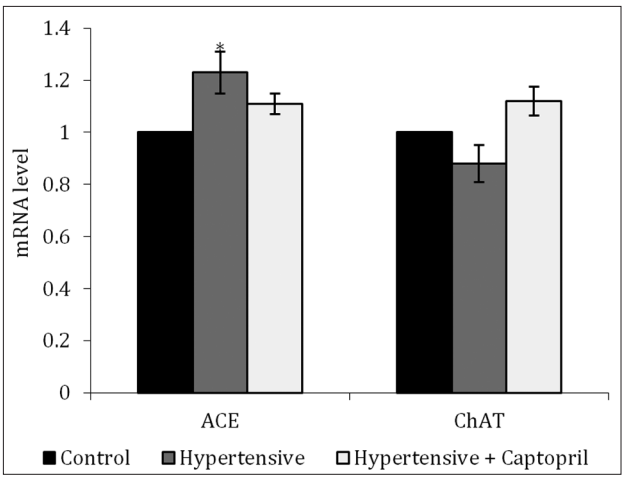

Figure 1: Levels of angiotensin converting enzyme and choline acetyl transferase mRNA in different groups

Table 1: Primer names and sequences used for qRT-PCR

Genes	Forward primer	Reverse primer
β-actin	AAGATCAAGATCATTGCTCCTC	CTCAGTAACAGTCCGCCT
ACE	TTGACGTGAGCAACTTCC	CAGATCAGGCTCCAGTG
ChAT	TCATTAATTTCCGCCGTCTC	AGTCCCGGTTGGTGGAGTC

qRT-PCR: Quantitative real-time polymerase chain reaction, ACE: Angiotensin converting enzyme, ChAT: Choline acetyl transferase

gene expression in the frontal cortex to near control level. In chronic renovascular hypertensive rats, elevated Ang II levels in different brain areas such as hypothalamus have reported.[7] The cognitive deficits observed in hypertensive animal models has been attributed to different levels of brain RAS activity.[8] On the other hand, ACE inhibitors such as captopril and AT_1 blockers such as losartan have shown cognitive enhancer effects,[9-11] and Sepehri et al. reported that short time captopril administration improves spatial memory in aged rats.[12] Tota et al. have suggested the nootropic effect of these drugs is related to their interference with cholinergic system.[13] The frontal cortex receives extensive innervation from basal forebrain cholinergic neurons.[14] Thus, this region might be susceptible to Ang II-induced changes in acetylcholine synthesis and release. ChAT is the most sensitive marker of cholinergic neurons in the central nervous system. However, in our study, the observed decrease in ChAT mRNA in the frontal cortex of renovascular hypertensive rats receiving captopril was not statistically significant. Although multiple neurotransmitter systems are involved in the learning and memory process, the cholinergic pathway projecting from basal forebrain to the cerebral cortex and hippocampus play a key role in mechanisms of learning and memory.[15] To our knowledge, to date, there is no report on the effect of ACE inhibitors on the frontal cortex ChAT mRNA levels in renovascular hypertensive rats in the literature. In recent years, several research works have reported the nootropic effects of ACE inhibitors and AV_1 blockers on learning and memory deficits in renovascular hypertensive and SHR rats.[16-18] The main proposed mechanism for this effect is the interaction of these drugs with the brain cholinergic system. Indeed, we observed the significant increase in ChAT mRNA level in captopril-treated hypertensive rats (unpublished data), but this was nonsignificant in the frontal cortex.

CONCLUSION

It seems that the memory enhancing effect of captopril in renovascular hypertensive rats acts independent from basal forebrain cholinergic projection to the frontal cortex.

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