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Effect of Brain Angiotensin II Receptor Antagonists and Antisense Oligonucleotide on Drinking and Renal Renin in Rats

Hyeon-kyeong Cho, Eun-kyoung Yang, Hee-suk Han, Won-jung Lee, and M. Ian Phillips[1]

Department of Physiology, School of Medicine, Kyungpook National University, Taegu 700−422, Korea; [1]Department of Physiology, College of Medicine, Box 100274, University of Florida, Gainesville, FL 32610, USA

The physiological roles of brain angiotensin II in mediating water deprivation-induced drinking and in regulating renal renin release were assessed in male Sprague-Dawley rats. Specific AT_1 receptor antagonists, losartan and SK 1080, and antisense oligonucleotide (AS-ODN) directed to AT_1 receptor mRNA were intracerebroventricularly (i.c.v.) administered in conscious unrestrained rats. When water was given 20 min after i.c.v. injection of AT_1 receptor antagonists in 48-h water-deprived rats, losartan and SK 1080 produced approximatly 20% and 50% decrease in 1-h water intake, respectively. In contrast, i.c.v. treatment of the AS-ODN to AT_1 receptor mRNA for 24-h did not alter 1-h water intake in 24-h water-deprived rats, but prevented the increase in overnight water intake after 24-h water-deprivation. Six-day i.c.v. treatment of AS-ODN did not alter either the basal plasma renin concentration or renal cortical levels of renin and renin mRNA. The present results suggest that endogenous brain Ang II plays an important role in thirst and water intake through AT_1 receptors, but further studies are required to elucidate its regulatory role in renal renin synthesis.

Key Words: Intracerebroventricular, Losartan, SK 1080, Water-deprivation, Renin mRNA

INTRODUCTION

The discrete existence of the brain renin-angiotensin system (RAS) and its role in central cardiovascular and fluid control have been demonstrated (Phillips et al, 1993). Two major receptor subtypes for angiotensin II (Ang II), AT_1 and AT_2, have been identified (Bumpus et al, 1991; Toney & Porter, 1993), and AT_1 receptors are mainly found in the brain areas known to be involved in body fluid and blood pressure homeostasis (Steckelings et al, 1992). The intracerebroventricular (i.c.v.) administration of Ang II in rats elicits a series of responses, such as induction of drinking and salt appetite, blood pressure increase, secretion of vasopressin and adrenocorticotropic hormone, and inhibition of renin secretion (Hoffman et al, 1977; Reid, 1984; Phillips, 1987; Ahn et al, 1992).

With the development of competitive specific Ang II receptor antagonists, it has been possible to evaluate the physiological role of endogenous Ang II in the brain. Recently we have shown that acute central administration of losartan, a specific AT_1 receptor antagonist, significantly impaired cardiovascular and vasopressin responses to hemorrhage in conscious rats (Lee et al, 1995) and drinking in 48-h water-deprived rats (Lee et al, 1996). These results suggest that the brain RAS plays a significant role in regulation of blood pressure during hemorrhage and drinking in water deprivation.

Synthetic antisense oligodeoxynucleotides (AS-ODN) have been used successfully to inhibit genetic expression by sequence-specific hybridization of mRNA and synthesis of a particular protein in a number of biological systems. (Crooke, 1993; Phillips & Gyurko 1995) Blocking specific gene expression by AS-ODN inhibits action of specific protein without multiple, nonspecific side effects exerted by most pharmacological drugs. Phosphodiester AS-ODN directed to

Corresponding to: Won-jung Lee, Department of Physiology, School of Medicine, Kyungpook National University, Taegu 700-422, Korea. (Tel) 82-53-420-6926, (Fax) 82-53-424-3349, (E-mail) wjleek@knu.ac.kr

AT_1 receptor or angiotensinogen mRNA significantly reduced blood pressure for several days in hypertensive animals with a single injection into the brain (Gyurko et al, 1993; Phillips et al, 1994; Wielbo et al, 1995). In contrast to the prolonged effect of AS-ODN, the hypotensive effect of i.c.v. losartan in spontaneously hypertensive rats lasted only for several hours. AS-ODN to AT_1 receptor mRNA inhibited drinking response to i.c.v. Ang II (Meng et al, 1994). However, effects of i.c.v. AS-ODN on physiological thirst, such as water deprivation-induced drinking have not been tested.

There is a possibility that central Ang II may be involved in renal renin release. The i.c.v. Ang II decreased plasma renin activity (Eriksson & Fyhrquist, 1976; Malayan et al, 1979; Ahn et al, 1992; Weekley, 1992; McKinley et al, 1994), whereas i.c.v. infusion of losartan with a high dose increased plasma renin activity in sodium-depleted sheep (McKinley et al, 1994). We observed that chronic i.c.v. administration of losartan increased the basal plasma renin concentration 3-fold (Yang et al, 1996), although acute i.c.v. losartan had no effect on the plasma renin concentration (Lee et al, 1995) as reported by others (Baum et al, 1983; Berecek et al 1991; DePasquale et al, 1992). Different responses to acute and chronic i.c.v. losartan might be because longer period of time is required for more losartan to get to critical brain target regions inaccessible to acute losartan.

We therefore attempted in the present study to compare the effects of blockade of brain Ang II-AT_1 receptors with short acting receptor antagonists and long acting AS-ODN on water deprivation-induced drinking and renal renin release. We injected the antagonists and AS-ODN directly to the brain ventricles of conscious unrestrained rats.

METHODS

Animals and surgical procedures

Experiments were performed on conscious, unrestrained male Sprague- Dawley (SD) rats (250~300 g). Each animal was anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and a stainless steel cannula (22 gauge) was stereotaxically implanted into the left lateral cerebral ventricle following the procedure previously reported (Lee et al, 1995). The stereotaxic coordinates were 1.5 mm lateral, 0.8 mm posterior to the bregma, and 4.0 mm below the skull surface. The animals were allowed to recover for 3 days and were initially screened for correctness of cannula location by an Ang II test. The average water intake after i.c.v. injection of 10 ng Ang II was 6.8 ± 0.5 ml/15 min (n=25). Any rat that drank <4 ml/15 min was not used in the study.

Effect of i.c.v. AT_1 receptor antagonists on drinking in water-deprived rats

Before the experiment, water bottles were removed from the cages for 2 days. In the morning of the experiment, 48-h water-deprived rats remained unrestrained in individual home cages. The rat was injected i.c.v. with either an AT_1 receptor antagonist (losartan potassium, 50 μg=110 nmol or SK-1080, 10 μg=20 nmol) or artificial cerebrospinal fluid (aCSF) as the vehicle. SK-1080 is a new non-peptide AT_1 receptor antagonist synthesized in the Korean Research Institute of Chemical Technology (Lee et al, 1999). Water was given 20 min after the injection and water intake was measured for 60 min.

Effect of i.c.v. AS-ODN on drinking in water-deprived rats

Basal overnight water intake (17 : 00~9 : 00) of rats with i.c.v. cannula was measured in each metabolic cage. Next day at 17 : 00, a 50 μg dose of AS- or scrambled (SC)-ODN or 4 μl aCSF was administered i.c.v. and water bottles were removed for 24 h. Another overnight water intake was measured in 24-h water-deprived rats.

AS- and SC-ODN were synthesized 15-mers to base +63 to +77 of AT_1 receptor mRNA (Murphy et al, 1991). The ODN sequences were modified by backbone phosphorothioation and were synthesized in the DNA Synthesis Laboratory, University of Florida. The ODN sequences were as follows: AS: 5'-TAACTGTGCCTGCAA-3', SC: 5'-AATTGGTGTGTTTCGTTC-3'.

Effect of i.c.v. AS-ODN on renal levels of renin mRNA and protein

Rats were given two i.c.v. injections of 4 μl aCSF, or 50 μg/4 μl AS- or SC-ODN at 48 h intervals. Two days after the second injection, rats were decapitated, and the trunk blood samples were collected

for measurement of plasma renin concentration. The kidney was removed, renal cortex was isolated and frozen in liquid nitrogen, and then kept in a deep freezer (−70°C) until total RNA isolation.

Plasma and renal cortical renin levels were measured with radioimmunoassay (Lee et al, 1995) and renal renin mRNA levels were measured with Northern blot with methods previously reported (Jo et al, 1996). Briefly, total RNA was extracted from renal cortex according to the method of Chomczynski & Sacchi (1987). For Northern blot analysis, 20 μg total RNA was subjected to electrophoresis on a 1% agarose-formaldehyde denaturing gel and transferred to a nitrocellulose membrane (Amersham). The filter was baked, prehybridized, and hybridized to full-length cDNA for rat renin (provided by Dr. Kevin R. Lynch, University of Virginia) and 18 S probe, both labeled with ^{32}P. The filter then was washed and exposed to x-ray film.

Statistical analysis

Results are expressed as Mean±SEM. Statistical analysis was performed by unpaired or paired *t*-test. Differences were considered statistically significant at a value of $P<0.05$.

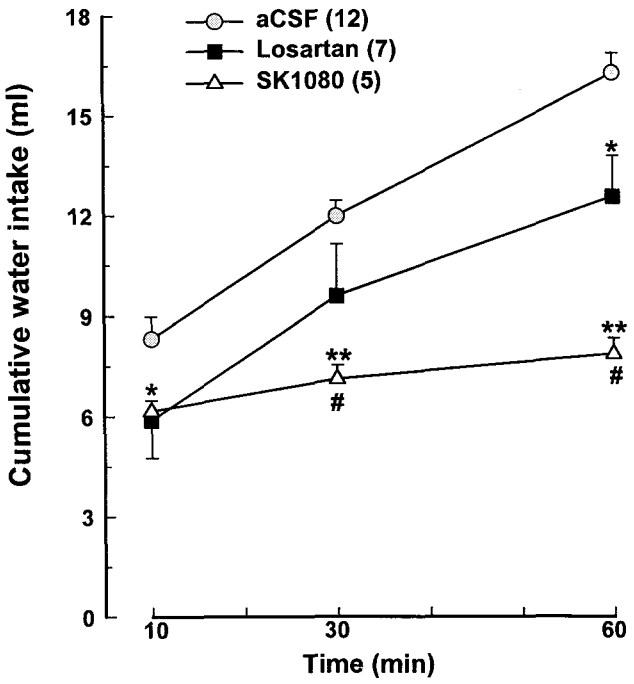

Fig. 1. Effect of i.c.v. administration of AT1 receptor antagonists (50 μg losartan and 10 μg SK-1080), or artificial cerebrospinal fluid (aCSF) on water intake of rats deprived of water for 48 h. Numbers in parentheses represent numbers of rats. $^{*}P<0.05$, $^{**}P<0.01$, vs. controls given aCSF, $^{\#}P<0.05$, SK-1080 vs. losartan.

RESULTS

Experiment 1

The effects of i.c.v. administration of AT_1 receptor antagonists on water intake in 48-h water-deprived SD rats are shown in Fig 1. Water intake of the control rats injected with aCSF was 16.0±0.67 ml/h. With an AT1 receptor antagonist, losartan, there was approx. 20% inhibition of drinking (12.6±1.06 ml/h). However, another AT_1 receptor antagonist, SK-1080 with one-fifth the molar dose of losartan, produced a significantly greater inhibition in drinking (7.9±0.48 ml/h) than losartan.

Experiment 2

The effects of i.c.v. administration of AS-ODN on AT_1 receptor mRNA and resultant effects on water intake in 24-h water-deprived rats are shown in Fig 2. The water deprivation increased the overnight water intake of the control rats injected with aCSF

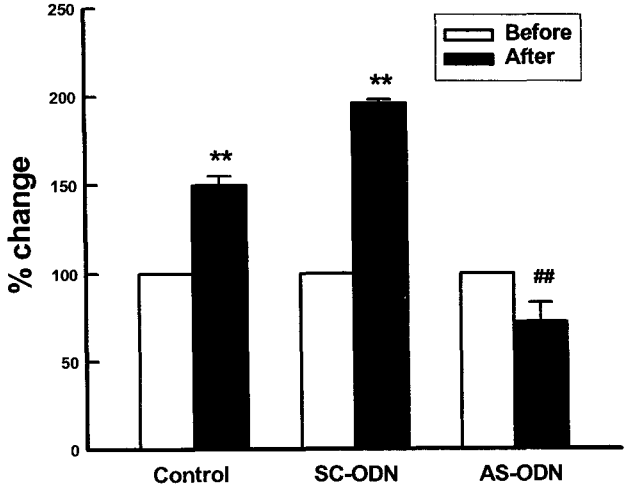

Fig. 2. Effect of i.c.v. administration of AS-ODN for AT1 receptor mRNA on overnight water intake for 16 h before and after 24 h water deprivation (n=5 in each group). $^{**}P<0.01$, before vs. after, $^{\#\#}P<0.01$, vs. controls given aCSF.

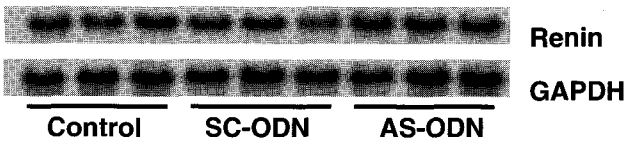

Fig. 3. Representative autoradiogram of Northern blot for renin and GAPDH mRNAs in the kideny from the rats i.c.v. injected twice at 48 h interval with AS-ODN for AT1 receptor mRNA (n=6 in each group).

Table 1. Basal plasma renin concentration (PRC) and renal renin content (RRC) in rats i.c.v. injected twice at 48 h interval with AS-ODN for AT_1 receptor mRNA

	aCSF	SC-ODN	AS-ODN
PRC, ng/ml/h	5.9±0.6	8.8±1.5	6.3±0.99
RRC, μg/mg protein/h	9.1±0.9	11.6±1.3	8.9±0.56

Values are mean±SE of 6 rats in each group.

or SC-ODN from 37.8±0.57 or 17.0±1.62 ml/16 h to 56.7±1.60 or 32.6±1.70 ml/16 h after the water deprivation (% increase: 49.7±4.03 or 95.8±8.50), respectively. However, with AS-ODN, overnight water intake was even decreased from 28.2±3.93 to 22.5±2.9 ml/16 h after 24-h water deprivation (% decrease: 22.9±9.46).

Experiment 3

The effects of i.c.v. AS-ODN on the plasma and renal renin levels, and renal renin mRNA are shown in Table 1 and Fig 3, respectively. Four-day i.c.v. treatment of AS-ODN altered neither the plasma renin concentration nor the levels of cortical renin and renin mRNA.

DISCUSSION

The present study demonstrates that blockade of the brain AT_1 receptor with antagonists and AS-ODN significantly inhibited the drinking response in water-deprived rats. However, the efficacy of AT_1 receptor antagonist and AS-ODN was different. Centrally administered AT_1 receptor antagonist produced more than 50% inhibition of 1-h water intake in 24-h water-deprived rats when water was given 20 min after the i.c.v. injection. In contrast, i.c.v. treatment of AS-ODN targeted to the AT_1 receptor mRNA for 24 h did not alter 1-h water intake after 24-h water deprivation (data not shown), but markedly decreased overnight water intake after 24-h water deprivation.

Reasons for different responses to AT_1 receptor antagonist and AS-ODN in the present study are not clear, but may be related to differences in distribution properties of the drugs. Inhibitory effects of AT_1 receptor antagonists are usually observed within 10 and 20 min after i.c.v. injection (Lee et al, 1995 & 1996). However, the route of i.c.v. injection of AS-ODN does not seem to be optimal to reach all target cells involved in the responses. AS-ODN is known to be avidly taken up by cells and are concentrated in cells close to the injection site (Ogawa et al, 1995; Phillips & Gyurko, 1995). The AT_1 AS-ODN reduced AT_1 receptor levels in the hypothalamus, which is most proximal to the site of injection, whereas it did not significantly reduce that of the brainstem, which is rich in AT_1 receptors but distal to the injection site. These results suggest that AT_1 receptors in the hypothalamus area would be exposed to the highest concentration of the oligomers, resulting in reduced delivery of the oligomers to more distal site such as the brainstem. Nonetheless, significant reduction of overnight water intake in water deprived rats treated with the AS-ODN in the present study supports the previous suggestion of the necessary involvement of AT_1 receptors in the ventricular regions for the water deprivation-induced dipsogenic action.

We observed suppression of plasma renin concentration after i.c.v. administration of Ang II (Ahn et al, 1992), as reported by other investigators in various species (Eriksson & Fyhrquist 1976; Malayan et al, 1979; Iwata et al, 1985; McKinley et al, 1994). Suppression of renal renin secretion rate in response to centrally administered Ang II was also observed in renal perfusion models of both the constant flow and constant pressure (Weekley, 1992). This result may suggest that changes in intrarenal hemodynamics do not mediate the response. However, in the medial basal forebrain lesioned rats (Weekley, 1992) and hypophysectomized dogs (Malayan et al, 1979), renal renin secretion was not decreased following i.c.v. injection of Ang II. Central administration of Ang II slightly decreased renal nerve activity (Unger et al, 1985), and renal denervation attenuated the central Ang II induced suppression of plasma renin activity

(Iwata et al, 1985). Taken together, these results may indicate an involvement of Ang II in the brain areas in the efferent path of the renal renin response.

Although large doses of Ang II administered directly into the brain can decrease the renin release, the physiological role of endogenous brain Ang II in regulation of renal renin release has not been clearly demonstrated. In sodium-depleted sheep, i.c.v. infusion of losartan with a high dose increased the plasma renin activity (McKinley et al, 1994). We observed that the basal plasma renin concentration increased 3-fold after chronic blockade of central AT_1 receptors with losartan (Yang et al, 1996), but was not altered after acute i.c.v. losartan (Lee et al, 1995). Different responses of the basal plasma renin concentration to acute and chronic i.c.v. losartan might be ascribed to the requirement of longer period of time for more losartan to get to critical brain target regions which is not easily accessible to acute losartan. To test this possibility in the present study, we injected the long acting phosphorothioated AS-ODN targeted to the AT_1 receptor mRNA into the brain twice at 48 h intervals for more effective blockade of the synthesis of the AT_1 receptors. After 4-day i.c.v. treatment of AS-ODN to AT_1 receptor, however, we observed no alteration in the basal plasma renin concentration, and renal levels of renin mRNA and protein. Chronic central blockade of Ang II with the AS-ODN failed to increase the basal plasma renin concentration unlike the chronic effect of losartan. On the other hand, a single i.c.v. injection of the same AS-ODN to the AT_1 receptor mRNA used in the present study produced a marked decrease in blood pressure for longer than a week in spontaneously hypertensive rats (Gyurko et al, 1993). Reasons of the lack of the plasma renin concentration response in the present study are not clear, but may be in part explained by that even repeated i.c.v. injections was not enough for the AS-ODN to effectively block the synthesis of the AT_1 receptors in critical brain areas. Further studies are required to clarify whether endogenous brain Ang II plays a significant physiological role in regulation of renal renin release.

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