Regulation of Angiotensin II Receptors in Rat Brain During Dietary Sodium Changes

Kathryn Sandberg, Hong Ji, Kevin J. Catt

Abstract

Activation of the renin-angiotensin system by sodium deficiency is associated with reciprocal changes in the expression of angiotensin II receptors in adrenal glomerulosa and vascular smooth muscle cells. The effects of dietary sodium changes on the expression of brain angiotensin receptor subtype 1 (AT1) mRNAs were examined in rats maintained on normal, low, and high sodium intake for 3 weeks. Plasma aldosterone and renin activity were elevated in rats maintained on a low salt diet compared with normal rats and were reduced in rats maintained on a high salt diet. These results are consistent with previous findings on the effects of altered dietary sodium on the renin-angiotensin system. The expression of AT1A and AT1B receptor subtype mRNAs was determined by quantitative reverse transcriptase-polymerase chain reaction during changes in sodium intake. The results revealed that sodium deprivation enhanced the expression of AT1A receptors in decorticated brains by 164% compared with high sodium intake. Conversely, high sodium diet increased the expression of AT1A receptors by 155% in the brain compared with low sodium intake. These data suggest that AT1A and AT1B receptors play reciprocal roles in central mechanisms for the control of fluid homeostasis. Further analysis of the molecular biology of angiotensin II receptor regulation in the brain may provide new insights into the interplay between the renin-angiotensin system and blood pressure regulation and also into the role of angiotensin II in the pathogenesis of essential hypertension.

Key Words • angiotensin • receptors, angiotensin • diet, sodium-restricted • brain

Recently, two distinct Ang II receptor genes have been cloned in the rat. These two receptor subtypes share a high degree (95%) of amino acid homology. However, subtype 1B (AT1B) receptors consistently express threefold to fivefold greater affinity for Ang II agonists and antagonists than subtype 1A (AT1A), and dose-related differences between the two receptor subtypes are present during Ang II-induced calcium signaling. Furthermore, AT1A and AT1B receptors have been shown to be differentially regulated in the pituitary gland. The primary sites of pressor action for Ang II in the rat brain appear to be in the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT). Therefore, it is of interest that AT1B receptors recently have been shown to be more abundant than AT1A in the SFO and OVLT.

Dietary sodium restriction is known to activate the RAS and to increase circulating levels of Ang II. Experimental evidence suggests that an elevated level of Ang II in the brain will increase the expression of angiotensin receptors and is associated with an increase in blood pressure and pressor responses, the induction of thirst, and altered resistance of the renal and mesenteric vascular beds. In this study, we examine the effects of dietary sodium changes on the expression of both AT1 subtype mRNAs in the decorticated rat brain to address the molecular mechanisms involved in Ang II receptor regulation in the brain.

Methods

Materials

\[^3Pd\]Deoxyctydine 5'-triphosphate (dCTP) (>6000 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. Oligonucleotide primers were synthesized by Lofstrand, Gaithersburg, Md, or Midland, Midland, Tex. Superscript RNase H\(^{-}\) reverse transcriptase and transcription buffer were
Hormone Assays

Rats were killed by decapitation, and approximately 5 mL of blood was collected in the presence of EDTA (5 mmol/L) for determination of aldosterone, renin, and corticosterone levels. Plasma aldosterone concentrations were measured by radioimmunoassay after extraction with methylene chloride and fractionation by LH-20 chromatography.19 Renin activity was assayed as previously described.20 Plasma corticosterone concentrations were also measured by radioimmunoassay according to published methods.21

RNA Preparation

After decapitation, the brain was decorticated and immediately frozen in liquid nitrogen. Total RNA was prepared by the method of Chomczynski and Sacchi22 from six pools of five to six brains each for control, low, and high salt groups. Genomic DNA contamination was removed from total RNA samples (10 μg) by a 10-minute incubation with RNase-free DNase (10 U) at 37°C in transcription buffer, followed by phenol extraction. Total RNA (20 μg per lane) was electrophoresed through 2.2 mol/L formaldehyde/1% agarose gel and stained with ethidium bromide for assessment of RNA quality.

cDNA Synthesis

Total RNA (5 μg) was reverse transcribed with oligo(dT) primers and reverse transcriptase in a 20-μL reaction volume for 60 minutes at 42°C, as previously described.12

Synthesis of Polymerase Chain Reaction Products

Rat brain cDNA was primed with oligonucleotides (10 pmol each) based on the sequences of β-actin and AT1A and AT1B receptors (Table 2) and was amplified by polymerase chain reaction (PCR) for 20 (β-actin) or 27 (AT1A and AT1B) cycles unless otherwise specified. The amplification conditions were as follows: denaturation at 94°C for 45 seconds; primer annealing at 62°C, 53°C, or 57°C (β-actin, AT1A, and AT1B, respectively) for 45 seconds; and extension at 72°C for 1 minute using Taq polymerase in the presence of MgCl2 (concentration specified in Table 2) in a Perkin-Elmer Cetus DNA thermal cycler.

cDNA Probes

cDNA probes were prepared by PCR19 in the presence of 1 ng substrate (cloned β-actin, AT1A, or AT1B; [α-32P]dCTP (100 μCi per reaction), 2 μmol/L each of deoxyadenosine 5′-triphosphate, thymidine 5′-triphosphate, and deoxyguanosine 5′-diphosphate; 1.5 mmol/L (β-actin) or 4 mmol/L (AT1A and AT1B) MgCl2; 10 pmol each of antisense and sense specific primers (Table 2); and 2.5 U of Taq polymerase in a total volume of 20 μL. After PCR, the labeled DNA was separated from free nucleotides on nucleotide-push columns (Stratagene).

Quantitation of Angiotensin Receptor Expression

Ten microliters of the 50-μL reaction mixture was electrophoresed through 1% agarose gel and blotted onto Nytran membranes with a pressure blower (Stratagene). The blot was probed with labeled cDNA representing the open reading frame of AT1A or AT1B for Southern analysis.12 After hybridization and washing at high stringency, washed membranes were scanned using a radioanalytic imaging system (AMBIS Systems, Inc, San Diego, Calif).12

Results

The recent demonstration of the predominance of AT1B receptors in the circumventricular regions of the rat brain14 led us to examine whether angiotensin receptors in the brain were differentially regulated by alterations in sodium diet. Male Sprague-Dawley rats were maintained on low, normal, or high sodium diets for 3 weeks before death. To confirm the effectiveness of dietary sodium restriction, we measured plasma levels of renin activity, aldosterone, and corticosterone (Table 1). Sodium deprivation markedly increased aldosterone production (18-fold) and caused a significant increase in renin activity (1.4-fold). On the other hand, sodium loading substantially reduced aldosterone (>75%) and renin activity (>73%) compared with the group maintained on a normal diet. Rats maintained on the high sodium diet had increased levels (1.6-fold) of corticosterone compared with the control group. These results are consistent with previous reports demonstrating the effects of dietary sodium on the RAS.24,26

Immediately after death, rat brains were removed, decorticated (because the cortex is known to possess few Ang II receptors5), and frozen in liquid nitrogen. Total RNA was isolated from individual rat brains and subjected to electrophoresis to ascertain RNA integrity before cDNA was synthesized. No detectable degradation was observed in samples used for cDNA synthesis and amplification by the PCR. Oligonucleotide primers (Table 2) were chosen from the 3′ and 5′ untranslated regions (areas of these receptors that share <65% homology) to selectively amplify the coding region of AT1A or AT1B receptor cDNA. In initial experiments, annealing temperature and MgCl2 concentration were optimized for each set of primers to determine maximal primer efficiency (data not shown). Control experiments revealed that AT1A-specific primers did not amplify purified AT1B cDNA, nor did AT1B-specific primers amplify purified AT1A cDNA (<50 cpm/μg RNA). Genomic DNA contamination of our RNA preparations was minimized by treatment of RNA with RNase-free DNase before cDNA synthesis. Furthermore, AT1A- and AT1B-specific primers did not detectably

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**Table 1. Effect of Dietary Sodium on Concentrations of Plasma Aldosterone, Renin Activity, and Corticosterone Levels**

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Aldosterone, ng/dL</th>
<th>Renin Activity, (ng Ang/ml)/h</th>
<th>Corticosterone, μg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low sodium</td>
<td>530±37</td>
<td>4.9±0.65</td>
<td>16±3.7</td>
</tr>
<tr>
<td>Normal sodium</td>
<td>29±3.6</td>
<td>3.6±0.80*</td>
<td>15±2.8</td>
</tr>
<tr>
<td>High sodium</td>
<td>6.8±0.94</td>
<td>0.96±0.14</td>
<td>24±4.7</td>
</tr>
</tbody>
</table>

Ang I indicates angiotensin I. Hormone levels were determined in blood plasma obtained immediately after decapitation. Values are mean±SEM of 18 to 20 rats per group.

*Mean renin activity of control group is skewed because of two rats with unusually high renin levels; median value for normal sodium control group is 2.9 (ng Ang/ml)/h.
amplify RNA that had not been reverse transcribed (<80 cpm/μg RNA).

The amplified products were analyzed by Southern blotting using specific angiotensin receptor probes that were synthesized by PCR in the presence of [α³²P]dCTP (Table 2). An equivalent amount of probe was used to directly compare the hybridization signal obtained with AT₁A- and AT₁B-specific probes. After washing under identical conditions, the hybridization signal was quantitated by using a radioanalytic imaging system. We used a quantitative PCR assay to examine the effects of salt diet on angiotensin receptor mRNA because expression levels of angiotensin mRNA are relatively low in the brain, and PCR has the distinct advantage of being several-fold more sensitive than Northern analysis.

In Fig 1, the amount of radioactivity of the 1100-bp fragments amplified by PCR is shown to be linearly related to amplification cycle number for both AT₁A and AT₁B receptors. Under these conditions, incorporation was measurable at cycle 10 and was linear through cycle 30. Fig 2 demonstrates that there is also a linear relation between the amount of cDNA and radioactivity in the amplified fragments for both receptors after 27 cycles. Thus, product accumulation is dependent on the amount of substrate under these assay conditions between 15 and 500 ng RNA.

To directly compare the AT₁A with AT₁B receptor expression, we examined product accumulation from 500 ng of reverse-transcribed RNA after 27 cycles. Fig 3 demonstrates that rat AT₁B receptor expression is increased after 3 weeks of sodium deprivation compared with control (142%, P<0.05) and high sodium (164%, P<0.05) diets. On the other hand, AT₁A receptor expression was conversely affected by the altered sodium diets; high salt intake increased AT₁A receptor expression compared with control (135%, P<0.05) and low salt (155%, P<0.05) diets.

Discussion

Previous work has shown that the Ang II–induced drinking response is decreased in estrogen-treated rats and that this change is related to a reduction in the number of Ang II receptors in the brain. The latter finding, together with the observations that estrogen selectively attenuates AT₁B receptor expression in the pituitary and is predominantly expressed in brain regions involved in thirst, suggests that AT₁B receptors

![Graph](image-url)
AT,B were 4250, 409, and 311 cpm/mg RNA, respectively. Analytic Imaging system. Data are expressed as amount of AT W were hybridized with radiolabeled O-actin, AT 1A-, and AT 1B- specific primers (Table 2). Amplified products were electrophoresed and transferred to Nytran membranes. Southern blots were hybridized with radiolabeled O-actin, AT 1A-, and AT 1B- specific probes. Hybridization signals were analyzed by a radioanalytic imaging system. Data are expressed as amount of AT 1A (open bars) and AT 1B (shaded bars) per B-actin product accumulation (cpm/µg RNA); each point is the mean±SEM of six observations. Control levels of production for O-actin, AT A, and AT B per O-actin product accumulated were 4250, 409, and 311 cpm/mg RNA, respectively.

In conclusion, the present studies have demonstrated that changes in angiotensin receptor expression in the brain occur during altered sodium balance and are characterized by reciprocal changes in mRNA levels for the AT 1B and AT 1A receptors. Whereas sodium restriction led to increases in AT 1B receptor expression, alter-
ations in dietary sodium caused converse changes in AT1A receptor expression. It is likely that such reciprocal regulation of brain angiotensin receptors contributes to the maintenance of fluid homeostasis during changes in sodium intake. An understanding of the individual roles of the Ang II receptor subtypes in the physiology and pathophysiology of the RAS may provide new information regarding the regulation of blood pressure and pathogenesis of arterial hypertension.

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References
Regulation of angiotensin II receptors in rat brain during dietary sodium changes.
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