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Differential Gene Expression and Regulation of Type-1 Angiotensin II Receptor Subtypes in the Rat

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SUMMARY: A simplified and sensitive method for measuring expression levels of type-1 angiotensin II (AT_1) receptor subtypes, AT_{1A} and AT_{1B}, was established. The two receptor cDNAs were co-amplified and measured by polymerase chain reaction using primers based on the corresponding receptor subtype genes. Both AT_{1A} and AT_{1B} mRNAs were widely expressed in the rat tissues including adrenal gland, kidney, heart, aorta, lung, liver, testis, pituitary gland, cerebrum and cerebellum. AT_{1A} mRNA was predominantly expressed in the rat tissues examined except adrenal gland and pituitary gland where AT_{1B} mRNA was predominantly expressed. Sodium depletion did not change mRNA levels of AT_{1A} and AT_{1B} in the all tissues. However, both AT_{1A} and AT_{1B} mRNA levels in the heart and aorta were down-regulated by treatment with AT_1 specific antagonist, TCV 116. In contrast, AT_{1B} mRNA in the adrenal gland was mainly reduced by the treatment. These results suggest that the expression level of AT_{1B} mRNA in the adrenal gland depends on the activity of the renin-angiotensin-aldosteron system (RAAS) and both receptor subtypes mediate contraction and hypertrophy of the smooth and cardiac muscles via the RAAS.

Angiotensin II (Ang II), which is a biologically active peptide in the renin-angiotensin-aldosteron system (RAAS), plays an important role in the homeostasis of blood pressure, electrolyte balance and cardiovascular hypertrophy (1). Ang II interacts with pharmacologically distinct subtypes of cell-surface receptors, type I (AT_1) and type II (AT_2). AT_1 receptor is a G-protein coupled receptor and mediates the classical functions assigned to Ang II whereas the functions of AT_2 receptor remains uncertain (2).

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Recently, AT_1 receptor cDNA (AT_{1A}) has been cloned and characterized from bovine adrenal cells (3) and rat vascular smooth muscle cells (4). Furthermore, the second form of AT_1 receptor cDNA (AT_{1B}), which exhibited a high similarity relative to amino acid sequence of AT_{1A} receptor (95% identity), was cloned from the rat pituitary gland (5). AT_1 receptor subtypes, AT_{1A} and AT_{1B}, mRNAs were widely expressed in the various rat tissues, and the differential tissue expression of the two mRNAs were observed (5, 6).

In this report, we measured expression levels of AT_{1A} and AT_{1B} receptor mRNAs in the various rat tissues using a simplified and sensitive method which we developed. Furthermore, we studied the effects of sodium depletion and an AT1 receptor specific antagonist, TCV 116, on the expression and regulation of the two receptor mRNAs.

MATERIALS AND METHODS

Animals and treatments

Male Wistar rats (400-450 g) were purchased from Charles River Japan Inc. (Atsugi, Kanagawa, Japan). They were fed a standard laboratory diet (11 mmol Na/ 100g; Oriental Yeast Co., Tokyo, Japan) and tap water for one week before the experiment. Control group (n=3) was fed a standard laboratory diet for one week. Sodium-depleted group (n=3) was fed a low sodium diet (3 mmol Na/100 g; Oriental Yeast Co.) for one week and was administered furosemide (30 mg/kg/day, subcutaneously) on days 4, 5 and 6, as well as 4 hr before killing. AT_1 receptor specific antagonist-treated group (n=3) was fed a standard laboratory diet and was administered TCV 116 (1 mg/kg/day, orally) for one week. At the end of the experiment, all rats were anesthetized with ether and were killed by decapitation. The whole tissues of adrenal gland, aorta and pituitary gland, and small pieces of lung, liver, kidney, left ventricular muscle of heart, testis, cerebrum and cerebellum were removed from individual rats of the three groups. All tissues were immediately snap-frozen in liquid nitrogen and stored at -80 °C until used for the isolation of the tissue total RNA.

Isolation of the tissue total RNA

The tissue total RNA was extracted from each tissue by the protocol of ISOGEN (Nippon Gene Co. Ltd., Tokyo, Japan). The extracted RNA was finally suspended in ribonuclease-free water and was quantified by measuring the absorbance at 260 nm. At the same time, each RNA was subjected to Northern gel electrophoresis to check the quality of RNA.

Measurement for levels of AT_1 receptor subtype mRNAs

The tissue total RNA (20 μg) was subjected to first strand cDNA synthesis in 20 μl of reaction volume using oligo-dT primers (Boehringer

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                                        1
AT1A  GTGGAGTGAca.ggttcaaagcacactggcaatgtaatgccctga...ca  1396
AT1B  GTGGAGTGAgagggttcaaagcctgcaagtgaagtgatttcctgacagca  1210
                                        3

AT1A  gaagccagggcagcct.....ctgactaaatggcttacgaccaaaggacc  1441
AT1B  gaagccagaggaccatttgggctaagcagctcactcactaccgaaggagt  1260

AT1A  attcaccctgc.........ctcaggatctaagcagaaaa...atgcgct  1480
AT1B  gttcaacccccagcaatcctttcagggttgaagcagagaaagcactcagt  1310

AT1A  catcagactgtagataatgactaaaactctgagagaagactttgaaggag  1530
AT1B  gtacacattttcaaaagtgggaaacaaaagctttttcccctttaaaaca  1360

AT1A  taaccaagcaaagcc.................gtcttgcattaatagat  1562
AT1B  aattggagcaaagccactgttaaaagcctttttttgttgactgaataact  1410
                    4

AT1A  gatggctagccaaaggaag..agtcaggagctggatggattggtgggtct  1610
AT1B  gctttaaggacaatgtcagaaattgagtggctgtatggatttgggagtta  1460

AT1A  ggaaaacagttcgctggcagaaatgcaatctcatcagtctcc.ctttgct  1659
AT1B  gggaggaggtgtactagcagaaactgaatgtctccagtcccctctcaatt  1510

AT1A  atgttccttttgatttccacacgtaggtatgtggcccatgctaaatattt  1709
AT1B  ctgttacttttgatttccacttgaaggtatttagaagctattaaatctgt  1560

                                        2
AT1A  aggcaagaaacaagagatggaaatcaaggtcacttgttctgttcaactcc  1759
AT1B  agacaatcaaaaggagatgggaggtcaag.............agttt  1584
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Figure 1. Structures of PCR primers and the nucleotide sequences in the 3'-untranslated regions of AT_{1A} and AT_{1B} receptor cDNAs.

The underlines 1-4 are the sequences of synthetic oligonucleotide primers used for PCR described in Figures 2 and 3.

Mannheim, Mannheim, Germany) and reverse transcriptase (Seikagaku Kogyo, Co. Ltd., Tokyo, Japan). The reaction was carried out by the method described previously (7). After the reaction mixture was heated at 95 °C for 5 min, 1/10 volume of the mixture was directly used for amplification. PCR was carried out in 50 μl of reaction mixture in the presence of 0.5 μl of [α-^{32}P]dATP (185 TBq/mmol; Amersham International Plc., Bucks, UK) by denaturing 94 °C for 30 sec, annealing at 58 °C for 45 sec and elongating at 72 °C for 45 sec. The reaction was carried out by 25 cycles. The employed primers for AT_{1A} (primers 1 and 2) and AT_{1B} (primers 3 and 4) receptor cDNAs are shown in Figure 1. To amplify both subtype cDNAs in a single tube, each set of primers was constructed in the 3'-untranslated regions which were low homology in nucleotide sequences of the two receptor genes. Amplified cDNAs of AT_{1A} and AT_{1B} receptor subtypes gave a 385 base-pairs (bp) and a 204 bp fragments in length, respectively. Ten microlitter aliquot of PCR products were electrophoresed on 8% polyacrylamide gel and the gel was subsequently exposed to an X-ray film (Hyperfilm-MP; Amersham International Plc.) using intensifying screens for 2 hr. The scanning of autoradiographic images was performed with a digital densitometer (DMU-

33C, Toyo Co. Ltd., Osaka, Japan) for the quantification of the amplified cDNA products. Ratios between AT_{1A} and AT_{1B} mRNA expression levels were calculated by arbitrary OD units which were corrected by the length ratio of the two cDNA fragments (AT_{1A}/AT_{1B}= 204/385).

RESULTS AND DISCUSSION

Figure 2 shows autoradiographic images of the amplified cDNA products generated from AT_{1A} and AT_{1B} receptor subtype mRNAs in the various tissues of the control rats. Two forms of AT_1 receptor mRNAs were specifically amplified and detected in all tissues examined. In the aorta, heart, kidney, testis, cerebrum and cerebellum, ratios between AT_{1A} and AT_{1B} mRNA expression levels (AT_{1A}/AT_{1B} ratios) revealed approximately 1~2 (Table 1). Although, in the lung and liver, AT_{1A} receptor mRNA was predominantly expressed (AT_{1A}/AT_{1B} ratios were 4.08 and 8.50, respectively), AT_{1B} mRNA was predominantly expressed in the adrenal gland and pituitary gland (AT_{1A}/AT_{1B} ratios were 0.54 and 0.32, respectively). Kakar et al. (5, 6) reported that AT_{1B} was predominantly expressed in the adrenal gland and pituitary gland in the rat, and they speculated that AT_{1B} receptor mediates secretions of aldosterone from the adrenal gland and ACTH and prolactin from the pituitary gland.

The effect of sodium depletion on the expression of AT_{1A} and AT_{1B} receptor mRNAs was investigated. We compared expression levels of AT_{1A} and AT_{1B} receptor mRNAs between the control and sodium-depleted

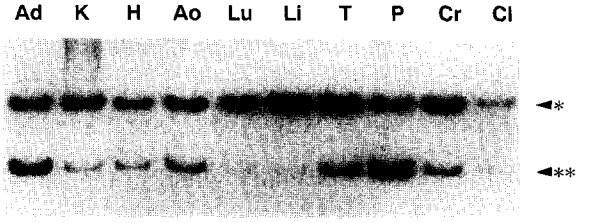

Figure 2. Autoradiographic images of the amplified cDNA products generated from tissue AT_{1A} and AT_{1B} receptor mRNAs of the control rats.

Ad, adrenal gland; K, kidney; H, heart; Ao, aorta; Lu, lung; Li, liver; T, testis; P, pituitary gland; Cr, cerebrum and Cl, cerebellum. *AT_{1A} cDNA product (385 base-pairs) and **AT_{1B} cDNA product (204 base-pairs) generated from 2 μg of tissue total RNAs were observed in each lane.

Table 1. Comparison with relative amounts of AT_{1A} and AT_{1B} receptor mRNAs in the various tissues of Wistar rats

tissues	relative amounts of mRNAs†		AT_{1A}/AT_{1B}§
	AT_{1A} mRNA	AT_{1B} mRNA	
adrenal gland	1.0	1.0	0.54
aorta	1.1	0.6	1.02
heart	0.9	0.3	1.75
kidney	1.1	0.3	1.83
lung	0.9	0.1	4.08
liver	1.6	0.1	8.50
testis	1.7	0.6	1.45
pituitary gland	0.9	1.5	0.32
cerebrum	1.2	0.5	1.40
cerebellum	0.3	0.1	1.72

†Relative amounts of tissue AT_{1A} and AT_{1B} mRNA are expressed as mean ratios in comparison with the corresponding mRNA levels in the adrenal gland (n=3). §AT_{1A}/AT_{1B}= mean ratios between AT_{1A} and AT_{1B} mRNA levels in the control rat tissues (n=3).

groups. We failed to detect significant differences in the levels of both AT_{1A} and AT_{1B} receptor mRNAs in 10 different rat tissues after treatment with sodium depletion in comparison with the control group. This result suggests that both AT_{1A} and AT_{1B} receptors may not mediate fluid homeostasis during the altered sodium intake in the adrenal gland or pituitary gland. Iwai et al. (8) reported that sodium depletion positively modulated the expression of AT_1 receptor (AT_{1A}+AT_{1B}) gene in the adrenal gland (2.3-fold increase compared with control rats). However, we failed to detect an increase in the levels of adrenal AT_1 receptor mRNA. This discrepancy will be due to the differences in a degree of sodium depletion and/or the method of measurement for AT_1 receptor mRNA. Recently, Sandberg et al. (9) cloned a novel Ang II receptor (AT_3) which was most abundant in the adrenal cortex and pituitary gland in the rat. They suggested that AT_3 receptor could be mainly regulated during altered sodium intake and could mediate the fluid homeostasis. However, AT_3 receptor reported by Sandberg et al. is very similar to AT_{1B} in respect to the nucleotide sequence in the cording region and the tissue distribution.

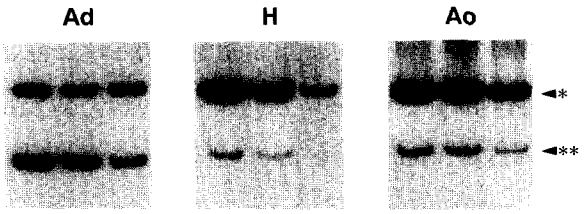

Figure 3. Changes in expression levels of AT_{1A} and AT_{1B} receptor mRNAs after treatment with sodium depletion or with an AT_1 receptor specific antagonist, TCV 116.

Ad, adrenal gland; H, heart and Ao, aorta. Left lane, control group; middle lane, sodium-depleted group and right lane , TCV 116-treated group. *AT_{1A} cDNA product (385 base-pairs) and **AT_{1B} cDNA product (204 base-pairs) generated from 2 μg of tissue total RNAs were observed in each lane.

Next, the effect of an AT_1 receptor specific antagonist, TCV 116, on the expression of AT_1 receptor mRNAs in the adrenal gland, heart and aorta was studied to examine whether both AT_{1A} and AT_{1B} receptors depend on the activity of the RAAS. Levels of both AT_{1A} and AT_{1B} receptor mRNAs were significantly decreased in the heart (AT_{1A}; 33% and AT_{1B}; 33% of the control level, $p<0.01$) and aorta (AT_{1A}; 48% and AT_{1B}; 67% of the control level, $p<0.05$) by treatment with TCV 116 for one week (Fig. 3). Interestingly, although AT_{1A} receptor mRNA did not change in the adrenal gland after treatment with TCV 116, the level of AT_{1B} receptor mRNA was significantly decreased by treatment with TCV 116 (35% of the control level, $p<0.01$). Iwai et al. (10) reported that administration of Dup 753, an AT_1 receptor antagonist, reduced the expression level of AT_1 receptor mRNA (50% of the control level) and the prolonged infusion of Ang II increased the expression level of this receptor mRNA (240% of the control level) in the adrenal gland. Down-regulation of TCV 116 or Dup 753 on the expression of AT_1 receptor mRNA was mainly caused by reducing the expression level of AT_{1B} receptor mRNA which depended on the activity of the RAAS in the adrenal gland. However, in other tissues, there were no significant differences in the levels of AT_1 receptor mRNAs between TCV 116-treated group and the control group.

In conclusion, a simplified and sensitive method for measuring AT_{1A} and AT_{1B} receptor mRNAs was established and the expression levels of the two receptor mRNAs in the various rat tissues were clearly demonstrated. The expression levels of both AT_{1A} and AT_{1B} receptor mRNAs were down -regulated by treatment with an AT_1 receptor specific antagonist in the aorta and heart, and the expression level of AT_{1B} receptor mRNA was mainly down-regulated by the treatment in the adrenal gland. However, the expression levels of AT_1 receptor mRNA did not change after treatment with sodium depletion in the all tissues. These results suggest that the expression level of AT_{1B} receptor mRNA mainly depends on the RAAS in the adrenal gland, and both AT_{1A} and AT_{1B} receptors mediate the contraction and hypertrophy in cardiovasculatures via the RAAS.

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