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Species-specific splicing and expression of angiotensin converting enzyme

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Abstract

Angiotensin converting enzyme (ACE) is a critical determinant in the pathogenesis of various cardiovascular diseases and in the control of male fertility. Multiple isoforms of ACE protein are present in body fluids and tissues, but their formation and functions *in vivo* remain to be investigated. To determine whether alternative splicing contributes to the formation of ACE isoforms, this study was designed to clone all possible spliced transcripts in rat. We found that the splicing of intron 13 in testicular *ACE* was species-dependent. Compared with human and mouse testicular *ACE*, rat testicular *ACE* (*rtACE*) retained intron 13 in its mature transcripts. The insertion of the intron 13 did not change or shift the reading frame. Cloning and characterization of the *rtACE* showed that, in addition to testicular tissue, it was wildly expressed in somatic tissues, such as lung, kidney, cardiac ventricle, and skeletal muscle from both genders. Furthermore, we demonstrated that the expression of *rtACE* was developmentally up-regulated in testicular tissue and increased during cardiac hypertrophy. Our data suggests that the inclusion of intron 13 produces a novel ACE isoform. This isoform likely participates in local angiotensin II formation in both somatic and germinal tissues, and associates with certain physiological or pathophysiological events.

Keywords: ACE isoforms; RNA splicing; Cloning; Male maturation; Cardiac hypertrophy

1. Introduction

ACE, a dipeptidyl carboxypeptidase, mainly converts angiotensin I into angiotensin II and degrades bradykinin into an inactive form [1]. It plays an important role in the pathogenesis of various cardiovascular diseases as well as in the control of male fertility [2,3]. In clinics, pharmacological inhibition of ACE has become an effective target in treatment of cardiovascular diseases, such as hypertension and cardiac dysfunction. Human *ACE* consists of 26 exons and lies in chromosome 17q23 [4]. Over the past decades, several isoforms of ACE protein have been identified, such as membrane-bound sACE, soluble sACE, and tACE [5]. In both man and mouse, sACE is predominately produced in lung and less expressed in the other tissues, whereas tACE is exclusively expressed in testicular tissue. The membrane-bound and soluble sACEs carry two similar extracelluar domains, referred to as the carboxyl- (C-) and amino- (N-) domains in proximity to the C- or N-terminus of ACE protein, respectively [6,7]. The tACE has only C-domain of the sACE protein [8]. A single N-domain of sACE and a soluble form of tACE are other isoforms that have been reported [9,10]. All such isoforms are originally from a same gene but distinct in exons. The membrane-bound sACE consists of all exons except for exon 13; the soluble sACE does not contain a membrane anchoring region (exon 26) [11], whereas tACE contains exons 13–26 [12].

The functions of ACE isoforms remain unclear. It was previously shown that null function of both somatic and testicular ACEs led to hypotension and male infertility in mice [2,13]. Introducing a soluble *sACE* into an *ACE*-deficient genetic background failed to rescue hypotension and renal structural damage [14]. A similar study found

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Abbreviations: ACE, angiotensin converting enzyme; tACE, testicular angiotensin converting enzyme; sACE, somatic angiotensin converting enzyme; SD, Sprague–Dawley; WKY, Wistar–Kyoto rat; SHR, spontaneous hypertensive rats; PCR, polymerase chain reaction.

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that a transgenic expression of *sACE* in the sperm of *ACE*-deficient mice did not restore the impaired male fertility [15]. Interestingly, a recent study reported that a *tACE* could substitute for the *sACE* to maintain normal renal structure and function [16]. Previously, we demonstrated that cardiac over-expression of *sACE* augmented a hypertrophied response in transgenic rats [17]. These evidences suggest that different ACE isoforms appear to play a role, either alone or compensating with each other. It is now accepted that sACE is responsible for the regulation of electrolyte balance, vascular tone, cell metabolism, and growth, while tACE is critical for male maturation [18,19].

It was speculated that ACE isoforms were generated from a single gene either by different transcription start sites or by proteolysis [8,11,20,21]. However, it was later reported that alternative splicing could contribute to the formation of the soluble somatic isoform [22]. To understand whether splicing produces novel ACE isoforms, and whether the these isoforms have any physiological and/or pathophysiological roles, we designed the present study to clone all possible spliced transcripts and determined the expression of these isoforms during male maturation and cardiac dysfunction.

2. Materials and methods

2.1. RNA isolation

SD, WKY, SHR, and mice (strain C57BL) were obtained from the animal facility of Max-Delbruck Center for Molecular Medicine (Berlin, Germany). RNA isolation was based on the manual of the TRIzol® Reagent (Invitrogen). Total RNA was suspended in freshly autoclaved water and kept at −80° prior to use.

2.2. Cloning of spliced ACE by 3- and 5-rapid amplification of cDNA end (RACE)

Five micrograms RNA from various tissues including kidney, ventricle, lung, and testis of adult SD rats was pretreated with DNase (2 U/μg) in 10 μL of PCR buffer (1.5 mM Mg^{2+}) plus 10 U RNasin at 37° for 30 min. The DNase was then inactivated by heating at 90° for 5 min.

For 3′-RACE, reverse transcription (RT) was performed in 20 μL consisting of 1× PCR buffer, 0.5 mM each dNTP, 0.5 μM primer PLA-P (5′-CGG AAT TCT CGA GAA $GC(T)_{12}$), 20 U of RNasin, 2 μg pretreated RNA and 100 U M-MLV. The reaction was carried out at 42° for 40 min and terminated by heat at 90° for 5 min. Subsequently, PCR was performed in 50 μL containing 1× PCR buffer, 0.2 mM each dNTP, 0.2 μM each primer (*ACE* specific primer, P11: 5′-GAG TTT CGT GCT ACA GTT CCA GTT-3′ or Pace3d-1: 5′-CAG TAA GCA GTG GCC AGA A-3′ and a common primer (CP): 5′-CGG AAT TCT CGA GAA GCT T-3′), 2 μL RT solution, and 1 U Taq polymerase (Gibco BRL) with thermal-cycles including the first denaturation at 95° for 2 min, 33 cycles of 95° for 20 s, 55° for 45 s and 72° for 2 min, and a final prolongation at 72° for 10 min.

For 5′-RACE: *ACE* specific primer (P1215-2: 5′-GAA CCT TCT TTA TGA TCC GCT T-3′) was used in place of PLA-P in the RT reaction. The RNA was removed by a mixture of RNases (RNase H plus RNase A1) after RT. The first strand DNA was A-tailed in 20 μL containing 1× terminal transferase (TdT) buffer, 1 mM dATP and 50 U TdT at 37° for 30 min. The second strand of cDNA was synthesized in 20 μL reaction system containing 1× Klenow buffer, 2.5 mM each dNTP, 0.5 μM PLA-P, 10 μL A-tailed first strand of DNA and 10 U DNA polymerase I at 14° overnight. PCR was preformed with primers P1215-2 and CP under the same condition as listed above.

The other segments of C-terminal ACE were amplified by RT–PCR with the primers: P16-1: 5′-GAC ATT TGA CGT GAG CAA CTT-3′/P16-2: 5′-GTC ATA GAT GTT GGA CCA A-3′ and P18-1: 5′-ACC TGC TAG GGA ACA TGT GG-3′/P18-2: 5′-GTT AGG TTG GCC TGT GAT TA-3′. The genomic DNA fragments from exon 13 to 14 from both mouse and rat were amplified with the primers: PmrtACE13: 5′-GGC TAC TCC AGG ACT GCC-3/ PmrtACE14-2: 5-TGC CAG TTG GCC TCT GCG T-3′.

PCR products were immediately ligated into pCR2.1 vector and transformed into DH5a (TA-cloning Kit, Invitrogen).

2.3. Colony hybridization

The colonies were blotted onto Hybond-N+ membrane (Amersham), and cross-linked by an UV-stratalinker (Stratagene, 1800). Prehybridization was done in 10 mL buffer containing 6× SSC (1× SSC: 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 1% sodium dodecyl sulfate (SDS), 500 μg/mL Herring sperm DNA, and 5× Denhart's solution at 68° for 1 hr. Hybridization was carried out for 3–4 hr following the addition of a radioactive labeled probe (fragment of exons 11–18 for 5-RACE and fragment of exons 23–26 for 3-RACE). The hybridized membrane was washed twice (30 min for each) at a high stringent condition (0.5% SDS in 0.1× SSC, 68°) and the radioactive signal was scanned and analyzed with Bio-Imaging Analyzer Bas 2000 (Fuji).

2.4. Sequence analysis

All positive clones were subjected to sequencing with either forward or reverse M13 primers (DNA sequencer-4000L, MWG-Biotech). Sequence analysis was performed with software Vector NTI. The protein motifs and glycosylation sites were predicted through web-based analysis tools at http://hits.isb-sib.ch/cgi-bin/PFSCAN, http://pir.georgetown.edu/, or http://www.cbs.dtu.dk/services/YinOYang/.

2.5. RT–PCR

Five micrograms testicular RNA from mouse, WKY, and SHR was pretreated with DNase and RT was performed as described in Section 2.2 except for PrtACE-2 (5′-ggg gta cca GCT TCT TTA TGA TCC GCT T-3′) that was used in place of PLA-P primer. Two microliter RT reaction was used for PCR, in which PrtACE-1 (5′-gct cta gag GGT CCT GAG TCA GGT GGC CAC T-3′) and PrtACE-2 were used. PCR products were visualized on 1.5% agarose gel after staining with ethidium bromide.

2.6. Western blot

The freshly collected tissues were homogenized in a buffer containing 50 mM Tris–Cl, 1 mM EDTA, 150 mM NaCl, and 5× Protease inhibitor's set (Roche) by PolyTron (PT-3100, Eberbach Corporation) at a speed of 15,000 rpm for 10 s. The samples were kept on ice for 1 min and then repeated the homogenization twice. The homogenized samples were centrifuged at 1000 *g* for 15 min to remove the debris and nucleus. The resultant supernatants were mixed with Triton® X-100 to a final concentration of 2%, and kept on ice for 1 hr. The samples were centrifuged at 13,000 *g* for 20 min. The supernatants that contained ACE were quantified by Bio-Rad protein assay. 20–250 μg of total proteins were subjected to 7.5% SDS–PAGE (Bio-Rad), and blotted onto polyvinylidene difluoride membrane (Bio-Rad). After blocking with 5% dried milk in PBS-T (0.25% Tween-20 in PBS), the membrane was incubated with a diluted (1:1000) goat-anti-ACE polyclonal antibody (sc-12187, Santa Cruz Biotechnology) at 4° overnight. The membrane was washed five times (10 min for each) in PBS-T, and incubated with rabbit anti-goat horseradish peroxidase (1:10,000; Sigma) for 1 hr at room temperature. After washed five times in PBS-T, the immunoreactive signal was detected by using ECLTM Western Blotting Detection Reagents (Amersham).

2.7. RNase protection assay

RT–PCR products from rat *ACE* by the primers PrtACE-1 and PrtACE-2, or from glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by the primers: Pga-1: 5′-act aag ctt GGA AAC TCA CCA TCT T-3′/Pga-2: 5′-aat gaa ttc GTG GTT CAC ACC CAT CAC AA-3′ were cloned into plasmid pBSK to form pBSKrtACE5d and pBSKrGA200, respectively. The radioactive labeled complementary RNA (cRNA) probes were made according to the *in vitro* transcription kit (Roche). In brief, the labeling was performed at 37° for 30 min in the reaction system that contained 1× transcription buffer, 0.5 mM of each 3 dNTP (ATP, CTP, and GTP), 2 μM UTP, 5 μL α-^{32}P-UTP (800 Ci/mmol, ICN Biomedicals, Inc.), 20 U RNasin, 0.5 μg linerized plasmid (EcoR1-pBSKrtACE5d, or Xho1-pBSKrGA200), and 10 U T7 RNA polymerase. The intact transcripts were fragmented and eluted from denaturing polyacrylamide gel (5%, 8 M Urea).

5–20 μg total RNA was pretreated with DNase, precipitated and suspended in 30 μL hybridization solution (22 μL deionized formamide, 6 μL 5× HS (0.2 M Pipes, 5 mM EDTA, and 2 M NaCl, pH 6.4), and 1–2 μL cRNA probe (1–5 × 10^4 cpm)). After denaturing at 90° for 5 min, the hybridization was performed at 45° overnight.

RNase treatment was carried out at 37° for 60 min by adding 270 μL digestion buffer (10 mM Tris–HCl, 5 mM EDTA, 0.3 M NaAc) containing 0.5 μg RNase A and 10 U RNase T1 (Roche). The RNases were then inactivated by addition of 50 μL stop-solution (35 μL of digestion buffer, 10 μL of 20% SDS, 3.5 μL of 10 mg/mL Proteinase K, and 1.5 μL of 10 mg/mL yeast tRNA) for 60 min (37°). The protected probe was precipitated and suspended in 12 μL loading buffer. After denaturing at 95° for 5 min, they were loaded on 5% denatured PAG gel (8 M Urea). The gel was finally dried and the radioactive signal was detected by Bio-Imaging Analyzer Bas 2000 after 4–18-hr exposure.

2.8. Statistical analysis

Data are presented as means ± SEM. Comparisons between two groups were performed with two-sample *t*-test. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Cloning of 3-end of ACE cDNA

The cloning scheme and primers are shown in Fig. 1A. To amplify the 3-end of SD rat *ACE*, 3-RACE was performed with common primers (PLA-P and CP) and a specific primer (Pace3d-1 or P11). The PCR fragment was inserted into a vector pCR2.1. Thirty six positive clones were identified from PCR products amplified by Pace3d-1/CP by colony hybridization. Sequencing showed that 10 clones were identical with a size of 1341 bp (7 from lung, and 3 from heart), 19 clones were identical with a size of 626 bp (13 from testis, 4 from heart, and 2 from lung), and 7 clones were mis-ligated and disregarded. The clones with the size of 1341 bp were termed as ACE3d-L (Genbank accession no. AF539701), while those with a size of 626 bp were termed as ACE3d-S. The ACE3d-S was identical to the upstream of ACE3d-L, the later had an extended 3-end compared with the published rat *sACE* cDNA sequence (Genbank accession nos. U03734 and U03708). The stop codon (TGA) was seen at 398–400 bp, and two poly A signal sites, ATTAAA and AATAAA, were located at 601–606 and 1247–1252 bp in the sequence of ACE3d-L. The alignment of ACE3d-L with ACE3d-S and human as well as mouse *tACE* suggested that ATTAAA seems to be the poly A signal for rat *tACE*, (*rtACE*) whereas AATAAA was possibly for its

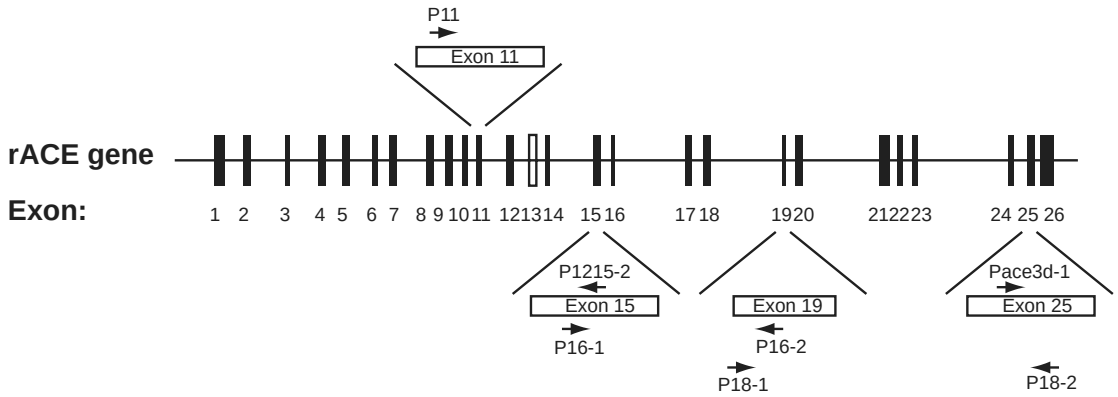

Fig. 1. Cloning scheme. A genomic arrangement of rat *ACE* gene was drafted. The black-filled vertical boxes represent the exons in *sACE*. The blank vertical box represents exon 13, which, together with exons 14–26, presents in *tACE*. The PCR primers, including P11, P1215-2, P16-1/P16-2, P18-1/P18-2, and Pace3d-1, were marked as arrows on exons 11, 15, 19, and 25 (blank horizontal boxes).

somatic *ACE*. ACE3d-S and ACE3d-L were likely the complete 3-end sequences of testicular and somatic *ACE*s, respectively. Furthermore, all positive clones from 3-RACE contained the sequence encoding the transmembrane region [11]. No positive clones were obtained when primers P11/CP were used.

3.2. Cloning of rtACE cDNA

The 5′- and 3′-end of *rtACE* were amplified by 5-RACE and 3-RACE, respectively. Three separate positive clones were obtained from 5-RACE using primer (P1215-2/CP). The rest of *tACE* cDNA was cloned from RT–PCR based on the reported sequence of *rACE* (Genbank accession no. NM_012544). The full-length cDNA sequence of SD *rtACE* (Genbank accession no. AF539425) was generated by joining these fragments together after contig. Interestingly, we found that all three independent clones from 5-RACE contained a 132-bp insert between exons 13 and 14 when aligned with mouse and human *tACE*. Compared with the cloned genomic DNA sequence of rat *ACE* (by PCR with primers PrtACE-1/PrtACE-2, data not shown), the insert was perfectly aligned with intron 13 sequence, indicating that the 132-bp insert in SD *rtACE* was intron 13. We demonstrated by sequencing that the first base of the intron 13 in rat was A, whereas of mouse or human was G. In addition, *rtACE* had a shorter nontranslated region compared with somatic *ACE* cDNA. The full length of *rtACE* cDNA was 2568 bp and, unlike the *sACE* cDNA, the *rtACE* had only a single poly A signal ATTAAA (2543–2548 bp) at 3-end. The translation likely starts from the first ATG (15–17 bp) and stops at TGA (2340–2342 bp) [23,24].

3.3. Tissue expression of rtACE

The tissue expression profile of *rtACE* was determined by RNase protection assay (Fig. 2A and B) and Western blot analysis (Fig. 2C). In RNase protection assay, the probe that encompassed a portion of the intron 13 and the cDNA sequence of rtACE from exon 14 to 15 was used to distinguish somatic and testicular *ACE*. The protected band from testis (*rtACE*) was 330 nucleotide (nt) long, whereas that from lung (*sACE*) was 260 nt. The *rtACE* transcripts were clearly detected in brain, kidney, lung, skeletal muscle, and left ventricle from both genders (Fig. 2A and B). To further prove that the rtACE was expressed in the somatic tissues, the Western blot analysis was performed. The antibody used in the Western blot recognizes the carboxy terminus of ACE protein. It can react with both somatic and testicular ACE isoforms from mouse, rat and human. Our results showed that both rtACE and sACE proteins were detected in the somatic tissues, such as heart and kidney (Fig. 2C). Notably, the rtACE was significantly larger than mouse tACE protein while the molecular weights of their sACEs are similar (Fig. 2C).

3.4. The splicing of intron 13 in mouse, WKY and SHR

In order to determine whether the insert exists in the other strains and species, the fragment spanning between exons 13 and 15 was amplified by RT–PCR. Our results showed that the amplified PCR products from both WKY and SHR shared the same size and was 132 bp longer than that amplified from mouse (Fig. 3A). This suggests that, unlike mouse *tACE*, all *tACE* transcripts from WKY, and SHR carry the intron 13. Protein alignment suggested that the insert does not shift the reading frame in rat tACE (Fig. 3B).

3.5. Developmental regulation of rtACE in testicular tissues

The expression of *rtACE* was determined in the testis from 2- and 6-week-old rats, and corrected by the loading control (GAPDH). We found that *rtACE* is lowly expressed in 2-week-old rats (0.1132 ± 0.018), but highly expressed in 6-month-old rats (0.5235 ± 0.032) (Fig. 4A and B).

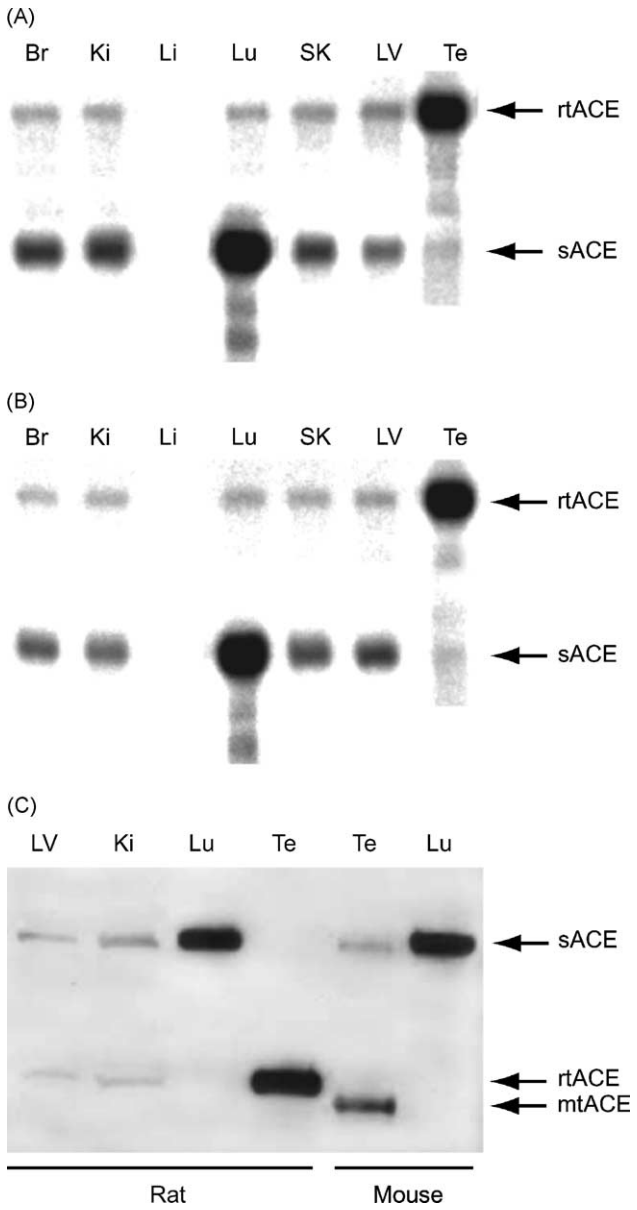

Fig. 2. Tissue expression of rtACE. (A and B) RNase protection assay. In panel A, the RNAs were from brain (Br), kidney (Ki), liver (Li), lung (Lu), skeletal muscle (SK), left ventricle (LV), and testis (Te) of male SD rat, and in panel B, the tissues were from female SD rat except for Te, which was used as a control. (C) Western blot analysis. The proteins were from SD rats and C57BL mice. Left ventricle (LV) (200 μg); kidney (Ki) (200 μg); lung (Lu) (20 μg), and testis (Te) (20 μg from rat, and 10 μg from mouse). mtACE: mouse testicular ACE.

3.6. Regulation of tACE gene expression in cardiac hypertrophy

Since *rtACE* is expressed in cardiac tissues, we investigated whether the cardiac expression of *rtACE* could be altered under pathophysiological conditions. It was found that the expression of *sACE* (0.4307 ± 0.043) in SHR was enhanced compared with age-matched WKY (0.1846 ± 0.044, $N = 6$, $P = 0.01$). Similar to *sACE*, the expression of *rtACE* was increased (0.2077 ± 0.003 in SHR vs. 0.1013 ± 0.009 in WKY, $N = 6$, $P = 0.01$) after corrected by the internal control (GAPDH). (Fig. 4C and D).

4. Discussion

The initial purpose of our study was to determine whether alternative splicing existed in *ACE* gene *in vivo*, and whether the splicing contributed to the formation of ACE isforms. The *sACE* contains two homologous domains of eight exons (4–11 and 17–24, respectively), each of which carries a catalytic active site. Thus, if alternative splicing exists and produces active ACE isoforms, it is likely located between exons 12 and 16, or between exons 25 and 26, which do not affect ACE activity [12]. It is also possible that exon 12 jumps to exon 26, forming a single N-domain ACE. To examine these possibilities, we attempted to clone all possible spliced transcripts between exons 12 and 16 by 5-RACE (P1215-2/CP), and between exons 25 and 26 by 3-RACE (Pace3d-1/CP), and from the jump from exon 12 to 26 by 3-RACE (P11/CP).

First, no alternatively spliced transcripts were cloned from P11/CP (data not shown), indicating that there were no transcripts which was responsible for the generation of single N-domain ACE. This may exclude the possibility that a single N-domain ACE is produced by splicing. Second, two types of rat ACE 3-ends, ACE3d-L and ACE3d-S, were cloned from Pace3d-1/CP, both contained the sequence encoding the transmembrane region. This suggests that the soluble ACE isoforms, under physiological condition, is unlikely to be generated via 3-end splicing of rat *ACE* gene, which is different from the cultured human endothelial cells [22].

Interestingly, we found that (1) all cloned 5-end sequences of SD rat *tACE* by 5-RACE carried intron 13, (2) RT–PCR and RNase protection assay demonstrated that all ACE transcripts contained the intron 13, (3) immunoblot analysis proved that the rat tACE protein was bigger than mouse's, as was in correspondence with the calculated tACE molecular weights of rat (88.16 kDa) and mouse (84.15 kDa), and (4) intron 13-containing transcripts presented in all rat strains detected. These suggested that the rat tACE retained the translated intron 13, likely producing a novel ACE isoform. A previous study has shown that a certain portion of mouse tACE cloned from the cDNA library also carried the intron 13 insert [8]. Unfortunately, we did not detect such tACE transcripts by RT–PCR from mouse testicular RNA. This would indicate that the intron 13-containing tACE may be minor or just the premature transcripts in mouse. It has been known that human tACE does not carry the intron 13 [25]. Thus, the splicing of intron 13 seems species-dependent although the significance remains unknown. Protein alignment indicated that the insertion of intron 13 did not change or shift the reading frame of rat *tACE* compared with mouse and human *tACE* as well as rat *sACE*. The amino acid sequence (S-N-K) and

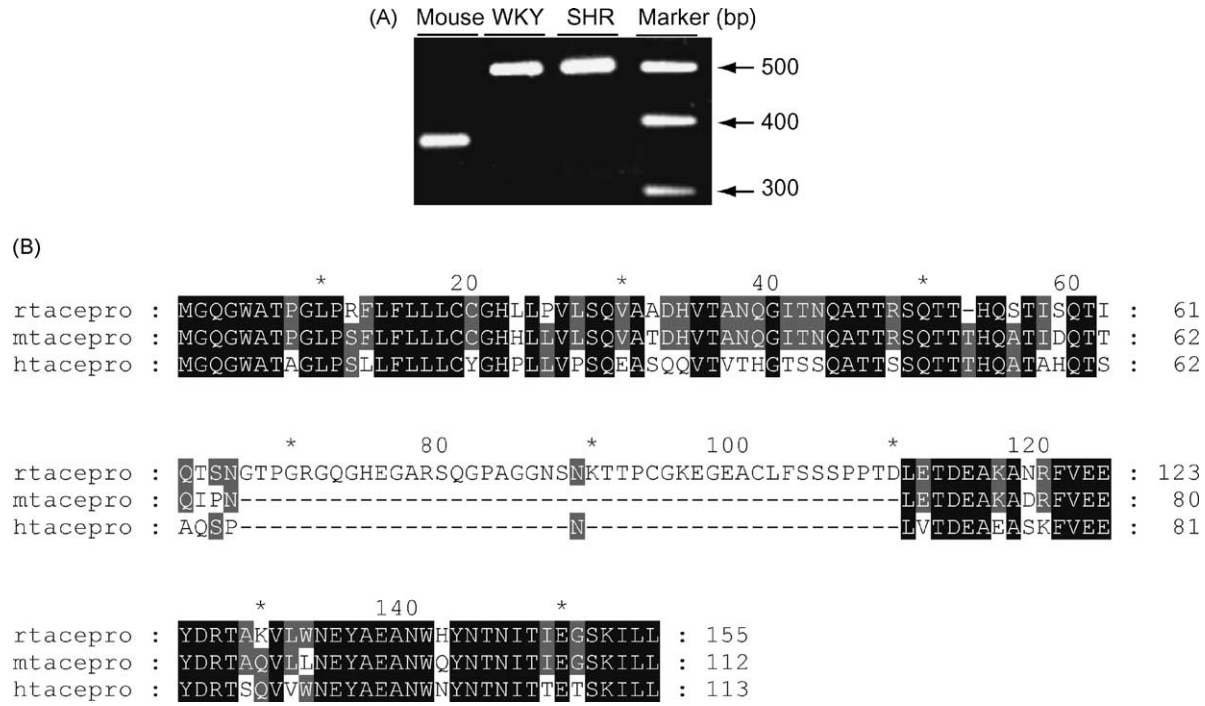

Fig. 3. Detection of intron 13 splicing in other rat strains and species. (A) RT–PCR. RNAs were from the testicular tissues of mouse (C57BL), WKY, and SHR. (B) Alignment of ACE proteins flanking the translated intron 13 (GTPGR–SPPTD). Rtacepro, mtacepro, and htacepro are the protein sequences of rat, mouse, and human testicular ACE, respectively.

(N-K-T-T) in the translated intron 13 was aligned to a potential protein kinase C phophorylation site [ST]-x-[RK] and an N-glycosylation site N-{P}-[ST]-{P}, respectively. These suggest that the insertion of intron 13 might have effects on ACE's function. Our immunoblot analysis revealed that the molecular weight of rtACE protein was higher than that of mouse tACE. The increased molecular weight of rtACE protein seems unlikely caused by different

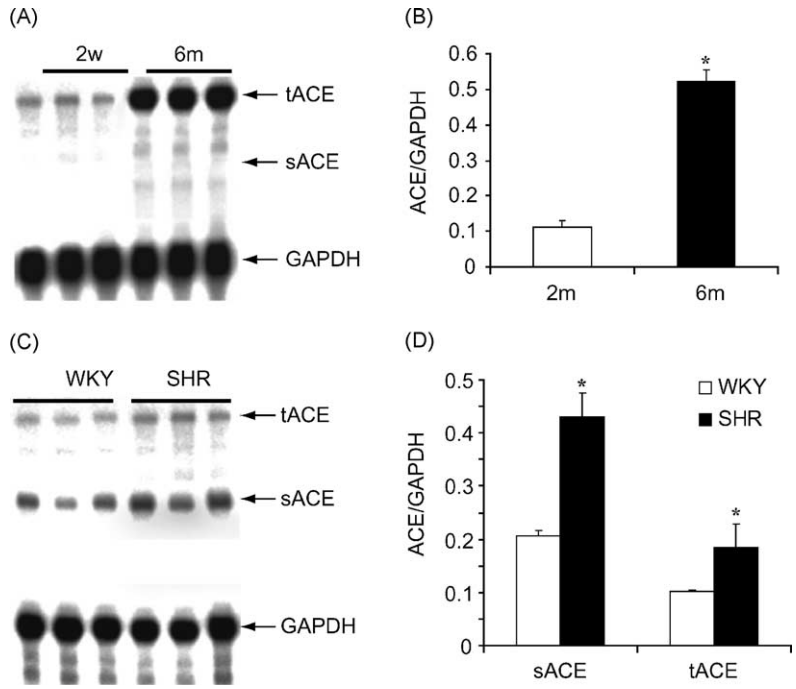

Fig. 4. Regulation of *ACE* expression. (A) RNase protection assay. RNAs were from the testicular tissues of SD rats at 2-week-old (2w) and 6-month-old (6m). tACE: 330 nt; sACE: 260 nt, and GAPDH: 200 nt. The band densities of tACE relative to GAPDH were plotted in panel B. (C) RNase protection assay (same probes were used as in panel A). RNA was from the left ventricles of WKY and SHR at the age of 2 weeks. The band densities of tACE and sACE relative to GAPDH were plotted in panel D. $^{*}P < 0.05$.

degrees of glycosylation between two species, since rtACE (without intron 13) and mouse tACE shared the same number of the predicted potential glycosylation sites. The increase in rtACE molecular weight is likely due to the insertion of intron 13. Additionally, we demonstrated at both transcriptional and protein levels that *rtACE* was also expressed in many somatic tissues other than testicular tissues. This suggests that rtACE might be involved in the regulation of the renin–angiotensin system (RAS) in tissues. The similar expression pattern of *sACE* and *tACE* may also support the notion that both *ACE*s had shared the same origin in the evolution [12].

The mechanisms of how intron 13 was maintained in rtACE remained unknown. By comparing the intron–exon border sequences among the species, we found that the first base of intron 13 in mouse and human is G [8,23], whereas in rat ACE is A that is different from the consensus sequence "GT/AG." However, whether the A to G substitution leads to the inclusion of intron 13 in rtACE needs to be further investigated. In order to determine whether any other splicing was present among the exons, we cloned the full-length cDNA sequence. No other alternative splicing was identified.

To further characterize the role of rtACE under physiological and pathophysilogical conditions, the expression of *rtACE* was examined in rat testis during different developmental stage and in cardiac tissues when hypertrophy was formed in SHR. We found that *rtACE* was weakly expressed in the testis from premature rats (2-week-old) but was highly expressed in mature rats (6-month-old). This indicates that the expression of *rtACE* is developmentally regulated, and points out the importance of an increase in *rtACE* expression during the maturation of male fertility. SHR exhibits organ damages, including cerebra lesions, myocardial lesions, and nephrosclerosis, which are similar to those in human [26]. SHR spontaneously develops cardiac hypertrophy in newborn or even at an earlier stage when it remains in uterus [27]. It has been documented that cardiac ACE is up-regulated upon cardiac hypertrophy, myocardial infarction, and heart failure, suggesting that the RAS is involved in the process of cardiac disorders [28–31]. We determined the expression of rtACE in left cardiac ventricles from both SHR and its control WKY. It was found that, similar to the *sACE*, *rtACE* was up-regulated in SHR at the age of 2 weeks when cardiac hypertrophy developed. The increased expression of *rtACE* in left ventricles indicates that it might also participate in the process of cardiac disorders.

Taken together, our study suggests that the splicing of intron 13 is species-dependent. The alternative splicing of intron 13 in rat strains results in a novel ACE isoform, rtACE. The rtACE presents three features distinct from human and mouse tACE: (1) rtACE contains intron 13, (2) it is widely expressed in somatic tissues other than the testicular tissue, and (3) it is up-regulated in ventricles during cardiac hypertrophy. These suggest that the rat RAS may be more complicated than that previously expected. The contribution of rtACE to local RAS should not be ignored when determining the effects of RAS in the pathogenesis of hypertension and cardiac defects in rat models.

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