Pergamon

0960-0760(95)00025-9

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1-6, pp. 89-94, 1995
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0960-0760/95 $9.50 + 0.00

Cytochrome P450(11β): Structure–Function Relationship of the Enzyme and its Involvement in Blood Pressure Regulation

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Cytochrome P450(11β) is deeply involved in the final steps of biosynthesis of mineralocorticoids. This paper deals with following issues about this enzyme. (1) The structure and function of the enzymes of various animal species are discussed. By making alignment of amino acid sequences of the enzymes, we identified peptide domains essential for the enzyme actions such as a putative steroid binding domain and a heme binding region. Estimates of molecular similarity among the P450(11β) family enzymes suggested that the enzymes having both 11β-hydroxylation activity and aldosterone (ALDO) synthetic activity of certain animals such as frog, cattle and pig are more similar to the ALDO synthases of the other animals, such as rat, mouse and human, than the 11β-hydroxylases of these animals. (2) The molecular nature of the P450(11β) family enzymes of genetically hypertensive rats as well as adrenal regeneration hypertension (ARH) rats is examined. (i) Mutation was found in the P450(11β) gene of Dahl's salt-resistant normotensive rat. Steroidogenic activity expressed by the mutated gene accounted well for abnormal plasma levels of steroid hormones in this rat. (ii) 11β-, 18- and 19-Hydroxylation activities of adrenal mitochondria prepared from spontaneously hypertensive rat (SHR), Wistar-Kyoto rat (WKY), and stroke-prone (SP)-SHR were not significantly different from each other. Levels of mRNA of ALDO synthase in adrenal glands of 50-week-old SHR was significantly lower than those of 10-week-old SHR, WKY and SHR-SP. (iii) No significant difference in 19-hydroxylation activity was found between adrenal mitochondria prepared from ARH rat and those from control rat. The level of message of ALDO synthase was lower in adrenal glands of ARH rat.

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1-6, pp. 89-94, 1995

STRUCTURE AND FUNCTION OF P450(11β) FAMILY ENZYMES

The molecular structures and functions of cytochromes P450(11β) of various animal species have been extensively studied. The conversion of 11-deoxycorticosterone (DOC) to aldosterone (ALDO) is catalyzed by a single molecule of P450(11β) in bovine [1, 2], porcine [3] and frog adrenal cortex. On the other hand in human [4, 5], rat [6-8], and mouse [9] adrenal cortex, the enzyme responsible for the 11β-hydroxylation of DOC which exists in the zonae fasciculata-reticularis differs from ALDO synthase present in the zona glomerulosa. The structural analysis of the two enzymes has revealed that the two belong to a P450(11β) family.

The primary structures of P450(11β) family enzymes reported to date are illustrated in Fig. 1. We developed a special system to name and classify these enzymes. The name "P450(11B0)" was used for the enzyme which catalyzes not only the 11β- and 18-hydroxylation of DOC but also the conversion of DOC to ALDO, and which seems to be by itself responsible for

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24-29 September 1994.
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SPECIES        -21                    1                                            40
FROG    11B0   M LEKTAARQIG SCLMRCRTLD TTSPLWTGFS RLSTAPLIHE AREDGSLASQ ****TLPYEA
BOVINE  11B0                          MALWAKARVR MAGPWLSLHE ARLLGTRGAA APKAVLPFEA
PIG     11B0                          MAIWAKAEAW LAGPWLALNR ARTLGTRAVL APKGVLPFEA
RAT     11B2                          MALRVTADVW LARPWQCLHR TRALGTTATL APKTLKPFEA
MOUSE   11B2                          MALRVTADVW LARPWQCLHR TRALGTTATL APKTLQPFEA
HUMAN   11B2                          MALRAKAEVC VAAPWLCLQR ARALGTRAAR APRTVLPFEA
RAT     11B1                          MALRVTADVW LARPWQCLHR TRALGTTAKV APKTLKPFEA
MOUSE   11B1                          MALRVTTDVW LARPWQCLHR TRALGTTATL APKTLQPFEA
HUMAN   11B1                          MALRAKAEVC MAVPWLSLQR AQALGTRAAR VPRTVLPFEA
                                                                              100
FROG    11B0   IPTTGRSAWF NLFQFWRKNS FQHMHLAMEE NFQNLGPIYR EKLGTHNSVN IMLPQDVARL
BOVINE  11B0   MPRCPGNKWM RMLQIWKEQS SENMHLDMHQ TFQELGPIFR YDVGGRHMVF VMLPEDVERL
PIG     11B0   IPQFPGKKWM RVLQLWREQG FENNHLEMHQ TFQELGPIFR FDVGGRNMVL VMLPEDVERC
RAT     11B2   IPQYSRNKWL KMIQILREQG QENLHLEMHQ AFQELGPIFR HSAGGAQIVS VMLPEDAEKL
MOUSE   11B2   IPQYSRNKWL KMIQILREQG QENLHLEMHQ VFRELGPIFR HSVGKTQIVS VMLPEDAEKL
HUMAN   11B2   MPQHPGNRWL RLLQMWREQG YEHLHLEMHQ TFQELGPIFR YNLGGPRMVC VMLPEDVEKL
RAT     11B1   IPQYSRNKWL KMIQILREQG QENLHLEMHQ AFQELGPIFR HSAGGAQIVS VMLPEDAEKL
MOUSE   11B1   IPQYSRNKWL KMIQILREQG QENLHLEMHQ VFRELGPIFR HSVGKTQIVF VTLPEDVEKL
HUMAN   11B1   MPRRPGNRWL RLLQIWREQG YEDLHLEVHQ TFQELGPIFR YDLGGAGMVC VMLPEDVEKL
                                            *                                 160
FROG    11B0   FQSEGIFPRR MTMEAWSKHR ELRNHKQGVF LLNGEAWRSD RIILNKEVLS LAGVKKFLPF
BOVINE  11B0   QQADSHHPQR MILEPWLAYR QARGHKCGVF LLNGPQWRLD RLRLNPDVLS LPALQKYTPL
PIG     11B0   QKVEGLHPQR DVPGPWLAYR HLRGHKCGVF LLNGPTWRLD RLQLNPGVLS LQAMQKFTPL
RAT     11B2   HQVESILPRR MHLEPWVAHR ELRGLRRGVF LLNGAEWRFN RLKLNPNVLS PKAVQNFVPM
MOUSE   11B2   HQVESMLPRR MHLEPWVAHR ELRGLRRGVF LLNGPEWRLN RLRLNRNVLS PKAVQKFVPM
HUMAN   11B2   QQVDSLHPCR MILEPWVAIR QHRGHKCGVF LLNGPEWRFN RLRLNPDVLS PKAVQRFLPM
RAT     11B1   HQVESILPHR MPLEPWVAHR ELRGLRRGVF LLNGADWRFN RLQLNPNMLS PKAIQSFVPF
MOUSE   11B1   YQVESTHPCR MPLESWIVHR ELRGLGRGVF LLNGPEWYFD RLQLNPNVLS PKAVQKFVPL
HUMAN   11B1   QQVDSLHPHR MSLEPWVAYR QHRGHKCGVF LLNGPEWRFN RLRLNPEVLS PNAVQRFLPM
                                                                              220
FROG    11B0   LDEAAADFVT FMKKRMSKNT RGSLTVDLYA DLFRFTLEAS SYVLYGQRLG LLEEHPNADT
BOVINE  11B0   VDGVARDFSQ TLKARVLQNA RGSLTLDIAP SVFRYTIEAS TLVLYGERLG LLTQQPNPDS
PIG     11B0   VDGVARDFSQ ALRARVMQNA RGSLTLDIKP SIFRYTIEAS NLVLFGERLG LLAHQPNPES
RAT     11B2   VDEVARDFLE ALKKKVRQNA RGSLTMDVQQ SLFNYTIEAS NFALFGERLG LLGHDLNPGS
MOUSE   11B2   VDMVARDFLE TLKEKVLQNA RGSLTMDVQQ SLFNYTIEAS NFALFGERLG LLGHDLSPGS
HUMAN   11B2   VDAVARDFSQ ALRKKVLQNA RGSLTLDVQP SIFHYTIEAS NLALFGERLG LVGHSPSSAS
RAT     11B1   VDVVARDFVE NLKKRMLENV HGSMSINIQS NMFNYTMEAS HFVISGERLG LTGHDLKPES
MOUSE   11B1   VDGIARDFVD NLKKKMLESV HGSFSMDFQS SVFNYTIEAS HFVLFGERLG LIGRDLSPDS
HUMAN   11B1   VDAVARDFSQ ALKKKVLQNA RGSLTLDVQP SIFHYTIEAS NLALFGERLG LVGHSPSSAS
                                                                              280
FROG    11B0   LRFISAVETV LKTTLPLLYY PHQILQLFQT RLWNEHMHAW DVIFEQADRC IQNIYQEYCL
BOVINE  11B0   LNFIHALEAM LKSTVQLMFV PRRLSRWMST NMWREHFEAW DYIFQYANRA IQRIYQELAL
PIG     11B0   LDFIHALEVM FKSTVQLMFM PRSLSRWTST GTWKEHFEAW DCIFQYANKA IQRLYQELTL
RAT     11B2   LKFIHALHSM FKSTTQLLFL PRSLTRWTST QVWKEHFDAW DVISEYANRC IWKVHQELRL
MOUSE   11B2   LKFIHALHSM FKSTSQLLFL PKSLTRWTST RVWKEHFDAW DVISEYANRC IWKVHQELRL
HUMAN   11B2   LNFLHALEVM FKSTVQLMFM PRSLSRWISP KVWKEHFEAW DCIFQYGDNC IQKIYQELAF
RAT     11B1   VTFTHALHSM FKSTTQLMFL PKSLTRWTST RVWKEHFDSW DIISEYVTKC IKNVYRELAE
MOUSE   11B1   LKFLHTLHSM FKTTTQLLYL PRSLTRWTST RVWKENLESW DFISEYVTKC IKNVYRELAE
HUMAN   11B1   LNFLHALEVM FKSTVQLMFM PRSLSRWTSP KVWKEHFEAW DCIFQYGDNC IQKIYQELAF
                                                                              340
FROG    11B0   GQERGYSGIM AELLLQAELP LDSIKANITE LMAGGVDTTA MPLLFTLFEL ARNPSVQREL
BOVINE  11B0   GHPWHYSGIV AELLMRADMT LDTIKANTID LTAGSVDTTA FPLLMTLFEL ARNPEVQQAV
PIG     11B0   GHPWHYSGVV AELLTHANMT VDAIKANSID LTAGSVDTTA YPLLMTLFEL ARNPEVQQAL
RAT     11B2   GSSQTYSGIV AALITQGALP LDAIKANSME LTAGSVDTTA IPLVMTLFEL ARNPDVQQAL
MOUSE   11B2   GSSQTYSGIV AELISQGSLP LDAIKANSME LTAGSVDTTA IPLVMTLFEL ARNPDVQKAL
HUMAN   11B2   NRPQHYTGIV AELLLKAELS LEAIKANSME LTAGSVDTTA FPLLMTLFEL ARNPDVQQIL
RAT     11B1   GRQQSWSVI* SEMVAQSTLS MDAIHANSME LIAGSVDTTA ISLVMTLFEL ARNPDVQQAL
MOUSE   11B1   GRPQSWSVT* AELVAERTLS MDAIQANSME LIAGSTDTTS TPLVMTFFEL ARNPDVQQAL
HUMAN   11B1   SRPQQYTSIV AELLLNAELS PDAIKANSME LTAGSVDTTV FPLLMTLFEL ARNPNVQQAL
                          *                                *  *              400
FROG    11B0   REEIRKAEAQ NPNDLNQLLN SLPLLKGAIK ETLRLYPVGI TVQRHLIKDI VLHNYHIPAG
BOVINE  11B0   RQESLVAEAR ISENPQRAIT ELPLLRAALK ETLRLYPVGI TLEREVSSDL VLQNYHIPAG
PIG     11B0   RQESLAAAAR ISENPQKAIT ELPLLRAALK ETLRLYPVGI FLDRCVTSDL VLQNYHIPAG
RAT     11B2   RQETLAAEAS IAANPQKAMS DLPLLRAALK ETLRLYPVGG FLERILNSDL VLQNYHVPAG
MOUSE   11B2   RQESLAAEAS IAANPQKAMS DLPLLRAALK ETLRLYPVGG FLERILSSDL VLQNYHVPAG
HUMAN   11B2   RQESLAAAAS ISEHPQKATT ELPLLRAALK ETLRLYPVGL FLERVVSSDL VLQNYHIPAG
RAT     11B1   RQESLAAEAS IVANPQKAMS DLPLLRAALK ETLRLYPVGS FVERIVHSDL VLQNYHVPAG
MOUSE   11B1   RQESLAAEAS IAANPQKAMS DLPLLRAALK ETLRLYPVRT FLERILSSDL VLQNYHVPAG
HUMAN   11B1   RQESLAAAAS ISEHPQKATT ELPLLRAALK ETLRLYPVGL FLERVASSDL VLQNYHIPAG
                                       a                          *  #        460
FROG    11B0   TLVQVGLYPM GRSPLLFQDA LRYDPARWLK RED**TNFKA LAFGFGSRQC IGRRIAETEI
BOVINE  11B0   TLVKVLLYSL GRNPAVFARP ESYHPQRWLD RQGSGSRFPH LAFGFGVRQC LGRRVAEVEM
PIG     11B0   TLVKVLLYSL GRNPAVFARP ERYHPQRWLD NQGSGTRFPH LAFGFGMRQC LGRRLAQVEM
RAT     11B2   TLVLLYLYSM GRNPAVFPRP ERYMPQRWLE RK***RSFQH LAFGFGVRQC LGRRLAEVEM
MOUSE   11B2   TLVLLYLYSM GRNPAVFPRP ERYMPQRWLE RK***RSFQH LAFGFGVRQC LGRRLAEVEM
HUMAN   11B2   TLVQVFLYSL GRNAALFPRP ERYNPQRWLD IRGSGRNFHH VPFGFGMRQC LGRRLAEAEM
RAT     11B1   TFVIIYLYSM GRNPAVFPRP ERYMPQRWLE RK***RSFQH LAFGFGVRQC LGRRLAEVEM
MOUSE   11B1   TVLNVNLYSM GRNPAVFPRP ERYMPQRWLE RK***RSFKH LAFGFGVRQC LGRRLAENEM
HUMAN   11B1   TLVRVFLYSL GRNPALFPRP ERYNPQRWLD IKGSGRNFYH VPFGFGMRQC LGRRLAEVEM
                                                  503 b
FROG    11B0   TLFLMHMLKN FQIDTVSKDD IKTVFGFILM PEKPPLLTFR PI
BOVINE  11B0   LLLLHHVLKN FLVETLEQED IKMVYRFILM PSTLPLFTFR AIQ
PIG     11B0   LLLLHHVLKN FLVETLVQED IKMIYRFIMT PSTLPLLTFR AIS
RAT     11B2   LLLLHHMLKT FQVETLRQED VQMAYRFVLM PSSSPVLTFR PIS
MOUSE   11B2   MLLLHHILKT FQVETLRQED VQMAYRFVLM PSSEPVLTFR PVS
HUMAN   11B2   LLLLHHVLKH FLVETLTQED IKMVYSFILR PGTSPLLTFR AIN
RAT     11B1   LLLLHHMLKT FQVETLRQED MQMVFRFLLM PSSSPFLTFR PVS
MOUSE   11B1   MLLLHHVLKS FHVETQEKED VRMAYRFVLM PSSSPLLTFR PVN
HUMAN   11B1   LLLLHHVLKH LQVETLTQED IKMVYSFILR PSMCPLLTFR AIN
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Fig. 1—*legend opposite.*

the biosynthesis of both glucocorticoid and mineralocorticoid under the physiological conditions of the relevant animals. The name "$P450$(11B1)" was used for 11β-hydroxylase which also catalyzes 18- and 19-hydroxylations of DOC, but not ALDO production. This enzyme is responsible for the production of glucocorticoid. The name "$P450$(11B2)" was used for ALDO synthase which catalyzes the conversion of DOC to ALDO with 11β- and 18-hydroxylated intermediates.

Sequences particularly conserved among the $P450$(11B) enzymes are marked by underlines in the figure. These sequences may construct several domains in the three-dimensional structure of $P450$(11B) enzyme, which play important roles in exerting the enzyme's catalytic action or in maintaining the enzyme's stability. Among these domains the one containing a Cys serving as the fifth coordinating ligand to heme iron (underline b) and the domain serving as a putative steroid binding site (underline a) have been particularly pointed out before [6].

The number of amino acid residues conserved among all the $P450$(11B)s cited in Fig. 1 is 150 residues. 28, 4, and 120 residues are conserved only between $P450$(11B0)s and $P450$(11B2)s, between $P450$(11B0)s and $P450$(11B1)s, and between $P450$(11B1)s and $P450$(11B2)s, respectively (Table 1). Because $P450$(11B1) and $P450$(11B2) in a certain animal species exist in different zones of the same adrenal cortex and are thought to be probably derived from a common ancestor gene, it is reasonable to find that they are mostly similar to each other.

It is worth noticing that significantly more amino acid residues are conserved between $P450$(11B0) and $P450$(11B2) than between $P450$(11B0) and $P450$(11B1), even if the sequences from evolutionarily distant animal species such as amphibians and mammalians are considered on equal terms in this comparison. This may be reasonable, if the fact is taken into account that both $P450$(11B0) and $P450$(11B2), but not $P450$(11B1), have ALDO synthetic activity (Table 1).

An interesting question which still remains to be answered is: why is the step of conversion of DOC to ALDO catalyzed by one enzyme in some animal species, but by two enzymes in the other species? Unfortunately we don't have any reasonable answer to this question at present.

Table 1. Molecular nature of P450 (11β) enzymes

AA conserved/ 502 residues		Activity		
		Aldo	11-Beta	Animal
$P450$ (11B0)		+	+	Frog Cattle Pig
	28			
$P450$ (11B2)	4	+	+	Rat Mouse Human
	120			
$P450$ (11B1)		−	+	Rat Mouse Human

150 AA are conserved among the three.

MUTATIONS IN $P450$(11B1) GENE OF DAHL RATS

Since the development by Dahl *et al.* [10] of selectively bred rats that were sensitive to the hypertensive effect of high salt diet (DS) and their counterparts that were resistant to the salt diet (DR), extensive investigations have been carried out to explore the mechanism underlying the blood pressure (BP) regulation of these rats. The most notable among them from the viewpoint of steroid metabolism was the study reported by Rapp and Dahl [11] who determined the steroidogenic activities of adrenal mitochondria of these rats. They found that the ratio of corticosterone (B) production (11β-hydroxylation) to 18(OH)DOC production (18-hydroxylation) catalyzed by the adrenal mitochondria of two strains of rats cosegregated with their BP levels, the ratios for DS and DR rats being about 2 and 4, respectively. Based on an assumption that B- and 18(OH)DOC-productions were mainly due to the action of a single $P450(11\beta)$ enzyme, they proposed that $P450$(11B1) may be mutated between the two strains of rat.

Having succeeded in cloning cDNAs of $P450$(11B1) and $P450$(11B2) from rat adrenal cortex [6, 7], we attempted to clone the $P450$(11B1) cDNAs from DS and DR rats and to see whether or not the proposition by Rapp and Dahl was verified. We isolated the clones by using a combined technique of first reverse-transcribing their mRNAs and then performing polymerase chain reaction on the resultant cDNAs [12]. The nucleotide sequence of the DS-cDNA was found to be

Fig. 1 (*opposite*). Primary sequences of $P450(11\beta)$ family enzymes. For naming of the enzymes, see the text. The amino acid sequences of frog $P450$(11B0) and porcine $P450$(11B0) are unpublished results from the authors' laboratory (manuscripts submitted). The sequences of bovine $P450$(11B0), rat $P450$(11B2), mouse $P450$(11B2), human $P450$(11B2), rat $P450$(11B1), mouse $P450$(11B1), and human$P450$(11B1) were cited from the references 18, 7, 9, 5, 6, 9 and 4, respectively. Cleavage site for extension peptide is marked by arrowhead. Positions where amino acid alterations were found for DR-$P450$(11B1) are marked by *. Putative steroid binding site and heme binding region are underlined and marked by a and b, respectively. The Cys served as the fifth ligand to heme iron is marked by #.

Table 2. Steroid production from DOC by adrenal mitochondria of hypertensive rats

Rat	Corticosterone	18(OH)DOC	19(OH)DOC
	[nmol/min/mg (%)]		
WKY	34.2 (100)	21.6 (63.2)	1.05 (3.1)
SHR	29.9 (100)	19.0 (63.5)	0.89 (3.0)
SHR-SP	33.0 (100)	20.0 (60.6)	0.86 (2.6)
ARH	16.6 (100)	10.3 (62.0)	0.51 (3.1)
Control	27.1 (100)	16.4 (60.5)	0.92 (3.4)

The adrenal mitochondria were prepared from WKY, SHR and SHR-SP rats (10-week-old male each), and ARH and control rats (8-week-old female each) on the 7th day after the surgery. The steroidogenic activity of the mitochondria was assayed under the reconstitution system of *P*450 electron transport.

identical to that of wild type (Sprague–Dawley rat) cDNA, while that of the DR-cDNA contained 6 nucleotide substitutions causing 5 amino acid alterations (R127 → C, V351 → A, V381 → L, I384 → L and V443 → M; shown by bold letters in Fig. 1). As expected *P*450(11B1)-DS expressed in COS cells converted DOC to B and 18(OH)DOC in a ratio of 1.72, a value which agreed well with the one (1.64) previously reported for the wild type enzyme. In contrast the ratio obtained for *P*450(11B1)-DR was 4.35. Taking into account the fact that the ratio of plasma concentrations of B and 18(OH)DOC in DS rat was 1.27 and that in DR rat was 4.17, it could be concluded that the plasma levels of 11- and 18-hydroxylated steroids reflected the enzyme activity of *P*450(11B1) in the adrenal cortex well.

It is interesting to note that the positions of three amino acid residues mutated in *P*450(11B1), namely the positions 381, 384 and 443, are very near to the above-mentioned two domains which were supposed to be important for the enzyme functions.

The finding that DR rat rather than DS rat has the mutation in *P*450(11B1) gene strongly suggests that *P*450(11B1) in the rat adrenal cortex somehow functions in BP regulation of the animal by producing the 11- and 18-hydroxylated steroids, which may have some mineralocorticoid activity. The mutation of *P*450(11B1) gene may have occurred in DR rat to compensate for the primary hypertensinogenic action of NaCl.

*P*450(11B1) AND *P*450(11B2) IN SPONTANEOUSLY HYPERTENSIVE RATS

The mitochondria prepared from the adrenal glands of SHR, SHR-SP and WKY rats (10-week-old males; average systolic BPs of SHR, SHR-SP and WKY were 185, 205 and 125 mmHg, respectively) were assayed for hydroxylation rates of DOC under the reconstitution system of *P*450 electron transport (Table 2). No significant difference in the relative activities of 11β-, 18- and 19-hydroxylations was found between these animals, indicating that mutation which might affect the enzymatic activity did not occur in the *P*450(11B1) genes of these animals.

Levels of mRNAs of *P*450(11B1) and *P*450(11B2) expressed in the adrenal glands were estimated by RT-PCR method described before [12] (Fig. 2). No significant difference in those levels of *P*450(11B1) was found between the strains. In contrast the level of message of *P*450(11B2) gene of 50-week-old SHR (male; systolic BP, 186 mmHg) was significantly lower than those of 10-week-old SHR (male; systolic BP,

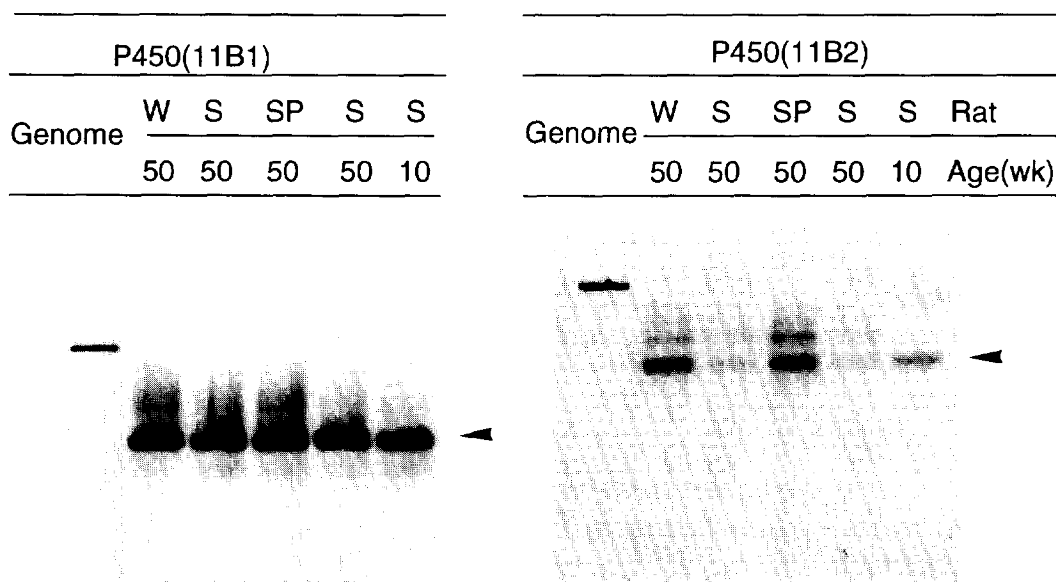

Fig. 2. Level of *P*450(11B) mRNA in adrenal glands of SHR, SHR-SP and WKY rats. The total RNAs prepared from the adrenal glands of old or young rats were used as the initial templates for RT-PCR. The RT-PCR products were hybridized with *P*450(11B1)- or *P*450(11B2)-specific oligonucleotide probe as described in Ref. [13]. The lanes on the left of each panel indicate cloned genomic DNAs of *CYP*11*B*1 and *CYP*11*B*2, respectively, as positive controls. Arrowheads indicate the RT-PCR products.

180 mmHg), SHR-SP (50-week-old male; systolic BP, 260 mmHg) and WKY (50-week-old male; systolic BP, 120 mmHg). Further study is needed to elucidate the meaning of these observations.

19-OXIDATION PATHWAY CATALYZED BY $P450(11B1)$ OF ARH RATS

It is well known that 19-hydroxylation of DOC is catalyzed by $P450(11\beta)$. A series of investigations by Ohta *et al.* [14, 15] has shown that the purified preparation of bovine $P450(11\beta)$ in the reconstitution system of electron transport catalyzes not only the 19-hydroxylation of DOC, but also further oxidative conversions of 19(OH)DOC to 19-oxo-DOC, and of 19-oxo-DOC to 19-oic-DOC. The 19-oic-derivative then seemed to be converted to 19-nor-DOC nonenzymatically during storage. Nonaka and Okamoto [16] also demonstrated that rat $P450(11B1)$ expressed in non-steroidogenic COS cells converted DOC to 19(OH)DOC at a considerable rate.

Gomez-Sanchez *et al.* [17] in 1979 reported the presence of 19-nor-DOC, a potent mineralocorticoid, in the urine of sodium-fed rats whose adrenal glands were in the course of regeneration after enucleation. They proposed that this steroid may be responsible for developing hypertension in these rats (adrenal regeneration hypertension rat; ARH rat). Taking both this fact and our previous findings into consideration, we postulated that the 19-oxidation pathway of DOC might be strongly expressed in the regenerating adrenal glands, the first step of which would be catalyzed by $P450(11B1)$. Therefore the expression of $P450(11B)$ enzymes in ARH rat was compared with that in sham-operated control rat.

Assayed under the reconstitution system of $P450$ electron transport, the rate of conversion of DOC to hydroxylated products by the adrenal mitochondria prepared from ARH rats 1 week after the enucleation seemed to be lower than that from the control rats (Table 2). But the relative rates of 11β-, 18- and 19-hydroxylations of ARH rats were not significantly different from those of control rats. This suggests that the level of $P450(11B1)$ activity during the adrenal regeneration was suppressed rather than enhanced, and no specific activation of 19-hydroxylation occurred in the adrenal mitochondria of ARH rats under our assay conditions.

The level of expression of $P450(11B2)$ gene of ARH rats, however, seemed to be significantly lower than that of control rats (data not shown). This fact must be further explored in the future.

CONCLUSION

$P450(11B1)$ (11β-hydroxylase) and $P450(11B2)$ (ALDO synthase) are the two enzymes intimately involved in the mineralocorticoid production in human and rat adrenal cortex. Therefore a possibility that the abnormal expression of these enzymes may cause the anomaly of BP regulation in rats was examined. The results revealed that the Dahl's salt resistant (DR) rat expressed a mutated $P450(11B1)$ gene in the adrenal cortex and had altered plasma steroid hormone levels. In the cases of SHR rat and ARH rat, the levels of expression of $P450(11B2)$ in their adrenal glands were significantly lower than the control rats, physiological meanings of which must await further exploration.

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