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# RENAL AND HEPATIC FAMILY 3A CYTOCHROMES P450 (CYP3A) IN SPONTANEOUSLY HYPERTENSIVE RATS

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**Abstract**—Troleandomycin (TAO), a selective family 3A cytochromes P450 (CYP3A) inhibitor, decreases enhanced *in vivo* corticosterone 6β-hydroxylation and blood pressure in spontaneously hypertensive rats (SHR). Corticosterone 6β-hydroxylation was measured in liver and kidney microsomes, to determine ontogeny and the effect of TAO on CYP3A activity at the organ level. SHR kidney CYP3A activity increased from 4 to 8 weeks, stabilized at 11 and 16 weeks, and was much higher than in control (Wistar–Kyoto, WKY) rats at all ages. Hepatic activity showed less consistency in strain difference. TAO produced a relatively large decrease in renal CYP3A activity compared with liver. Although renal *CYP3A* mRNA was not present in sufficient quantity for detection by northern blot analysis of total RNA, its presence was demonstrated in SHR by reverse transcriptase-polymerase chain reaction amplification. Correlations between renal CYP3A activity and systolic blood pressure in SHR and WKY rats with variations in age, strain and drug treatment are consistent with the role of the enzyme in the pathogenesis of blood pressure elevation in SHR.

*Key words:* SHR; CYP3A; troleandomycin; 6β-hydroxycorticosterone; hypertension

Corticosterone is the circulating substrate for extraadrenal production of 6β-OH-corticosterone. Although the predominant site of 6β-OHase§ activity in mammals is thought to be the liver [1], we have found that 6β-OH-corticosterone is also produced in kidney cells by a member(s) of the microsomal CYP3A [2, 3]. Immunohistochemical studies localized these enzymes to the collecting tubule in rat kidney [3]. Although P450 enzymes exhibit broad, but overlapping, isomeric and epimeric specificities in their metabolite patterns, CYP3As account for most of the steroid 6β-hydroxylase activity in mammals [3].

6β-OH-Corticosterone stimulates transepithelial active $Na^+$ transport in cultured cells derived from toad kidney (A6), possibly by a unique non-classical mechanism [4, 5]. Although there are numerous possibilities as to how CYP3A may contribute to hypertension, we speculate that excess 6β-OH-corticosterone made from corticosterone in kidney could be a stimulatory agonist for renal tubular $Na^+$ transport, resulting in a defect in renal $Na^+$ excretion. Indeed, excessive 6β-hydroxylation of cortisol in humans is associated with hypertension in several conditions, i.e. hypothyroidism, Cushing's syndrome, estrogen administration and preeclampsia [6–8]. Thus, increased glucocorticoid 6β-hydroxylation could be a risk factor or intermediate phenotype for hypertension. We previously demonstrated a 5- to 6-fold higher catalytic activity of CYP3A in 11-week SHR than in WKY rats [9]. TAO, a selective inhibitor of CYP3A [10–12], decreases blood pressure and *in vivo* 6β-hydroxylation of corticosterone in SHR [13]. Moreover, the TAO-sensitive component of blood pressure in SHR, which is 50% of the SHR/WKY strain difference at 11 weeks [14], could reflect the hypertensinogenic effect of an excess renal 6β-hydroxylation and serve as one of the phenotypes affecting blood pressure in this polygenic model.

In our earlier work [13], the developmental course of corticosterone 6β-hydroxylation in young SHR and WKY rats and the effect of TAO on 6β-hydroxylation were assessed *in vivo*, by measurement of the percentage of injected [$^3$H]corticosterone found as [$^3$H]6β-OH-corticosterone in the subsequent 24 hr urine. However, this technique does not identify sites of increased synthesis, mechanisms mediating the elevation of 6β-OH-corticosterone, or the specific CYP isozymes involved. In the current study, we have addressed the first problem by measuring CYP3A catalytic activity in liver and

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§ Abbreviations: 6β-OHase, steroid 6β-hydroxylase; CYP, cytochromes P-450; CYP3A, family 3A cytochromes P-450; *CYP3A* indicates mRNA; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto; TAO, troleandomycin; CA, cyclosporin A; RT, reverse transcriptase; PCR, polymerase chain reaction; and SBP, systolic blood pressure.

kidney of SHR and WKY rats of various ages. We have also used this approach to further characterize the effects of *in vivo* treatment with TAO.

## MATERIALS AND METHODS

*Materials.* TAO was provided as a gift from Pfizer Inc. (New York, NY). [1,2-$^3$H]Corticosterone was obtained from New England Nuclear/DuPont (Boston, MA). Unlabeled corticosterone was obtained from the Sigma Chemical Co. (St. Louis, MO) and 6$\beta$-OH-corticosterone from Steraloids (Wilton, NH). Nytran Magna N$^+$, used for northern blot analysis, was obtained from Schleider & Schuell (Keene, NH). RNasin was purchased from Pharmacia (Piscataway, NJ), and Taq polymerase from Perkin Elmer-Cetus (Norwalk, CT). dNTPs, MLV-RT and oligo dT were obtained from Life Technologies (Gaithersburg, MD).

*Systolic blood pressure measurements.* SHR and WKY male rats were obtained from Taconic Farms (Germantown, NY) at 3, 7, 10 and 15 weeks of age. SBP was measured in conscious rats by the tail-cuff method using a photoelectric sensor and pulse amplifier (IITC LifeScience, Woodland Hills, CA) connected to a two-channel recorder [15]. Rats were maintained in a quiet room for 4–5 days prior to the experiment. Effects of training were controlled for in designated experiments (see Table 1) by measuring SBP twice during this period. To measure SBP, rats were placed in a restraining cage for 5 min at 28°. Successive readings were taken during the following 5- to 10-min period until 4 or 5 sequential readings were within a 15 mm Hg range. The average of these values was used as the SBP value. TAO was administered daily for 4 days. SBP was measured just prior to the first treatment and on day 4. The dose of TAO was 4 mg in 50 $\mu$L DMSO·100 g rat$^{-1}$·day$^{-1}$. This vehicle has been shown to have no effect on SBP over the 4-day period in either SHR or WKY rats [13]. Although higher doses of TAO induce CYP3A [11, 16], the dose used here was shown to inhibit 6$\beta$-hydroxylation of corticosterone, *in vivo*, without induction of hepatic family CYP3A (unpublished results).

*Measurement of CYP3A catalytic activity.* Liver and kidney from 11-week male SHR and WKY rats were homogenized in 100 mM Tris-EDTA buffer (pH 7.4) in the presence of protease inhibitors (2 mM hydrocinnamic acid, 0.5 mM benzoyl arginine and 5 mM benzamidine), and microsomes were prepared as described by Grogan *et al.* [2]. Corticosterone 6$\beta$-hydroxylase activity was measured by the radiometric method described previously [9].

*Urinary [$^3$H]6$\beta$-OH-corticosterone.* For *in vivo* measurement of CYP3A activity, rats were injected subcutaneously with [1,2-$^3$H]corticosterone (2 $\mu$Ci in 50 $\mu$L isotonic saline/100 g rat). They were placed in metabolic cages, and urine was collected for 24 hr. One-third volume of each urine was extracted on a $C_{18}$ cartridge (Sep-Pak, Waters/Millipore, Milford, MA). The cartridge was washed with 10 mL $H_2O$ and 2 mL 30% MeOH and eluted with 2 mL MeOH. The eluate was dried under $N_2$ and fractionated on a reverse phase $C_{18}$ HPLC column as described previously [16]. The 6$\beta$-OH-corticosterone was eluted from the $C_{18}$ column with 37% MeOH at 0.8 mL/min for 30 min, followed by 64% MeOH for 15 min. Radioactivity was measured by an in-line scintillation detector and used to calculate 24-hr urinary recovery of the [$^3$H]6$\beta$-OH-corticosterone, expressed as percent of [$^3$H]corticosterone injected into the rat.

*Northern blot analysis.* SHR and WKY kidney RNA was extracted by the method of Chirgwin *et al.* [17]. Thirty micrograms of total RNA was size-fractionated by electrophoresis on a denaturing formaldehyde agarose gel and electrophoretically transferred to Nytran Magna N$^+$. The filter was air dried, baked *in vacuo* for 1 hr, and prehybridized at 42° for 3–5 hr as described by Sambrook *et al.* [18]. The cDNA probes were labeled by nick translation using a kit from Life Technologies Inc. Hybridized filters were washed with 0.2× SSC (0.15 M NaCl and 0.015 M sodium citrate) and 0.2% SDS at 42°. Hybridized bands were then visualized by autoradiography.

*Reverse transcription and polymerase chain reaction (RT-PCR).* First strand DNA was synthesized from total RNA using MMLV-RT as described by Sambrook *et al.* [18]. Oligo dT was used as the primer with the following reagents in 100 $\mu$L of PCR buffer: 0.1 $\mu$mol dNTPs; 1 U RNasin; 200 ng oligo dT; 200 U MMLV-RT. An aliquot of first strand DNA was amplified by PCR using oligo dT as a 3′ primer and a 5′ consensus primer described below. The following components were used in 100 $\mu$L for amplification: primers, 300 pmol each; 20 nmol dNTPs; 2.5 U Taq polymerase. Amplification cycles (total 30) consisted of 1 min denaturation (94°), 1 min annealing (50°), and 2 min extension (72°); extension time was increased to 10 min in the last cycle. PCR products were electrophoresed on 0.5% agarose + 0.5% NuSieve gel and stained with EtBr. The DNA was denatured, neutralized and capillary transferred to a Nytran Magna N$^+$ filter. The filter was processed for hybridization as described above in northern blot analysis. A $^{32}$P-labeled 0.9 kb fragment of CYP3A1 (pDex12) [12] was used as the probe.

*Synthesis of consensus primer.* Using GC GENE, multiple sequence analysis was performed and alignment obtained for reported sequences of *CYP3A1* [19], *CYP3A2* [20] and *CYP3A6* [21], two rat liver and a rabbit liver CYP3A form, respectively. A consensus region was chosen that extended from bp 498 to 527 of CYP3A1 [19]: 5′-CAC CAG TGG AAG ACT CAA GGA GAT GTT CCC-3′. The oligonucleotide was synthesized on a Cyclone DNA synthesizer (Bioresearch Inc., Edison, NJ). This consensus sequence was used as a 5′ primer (see above).

*Statistical analysis.* The results in this study (figures and tables) are represented as means ± SEM. Student's *t*-test was used to compare two groups, e.g. strain differences in Fig. 1. ANOVA was used to evaluate the variation with age of each parameter in Fig. 1.

## RESULTS

Figure 1 shows the age dependence of SBP,

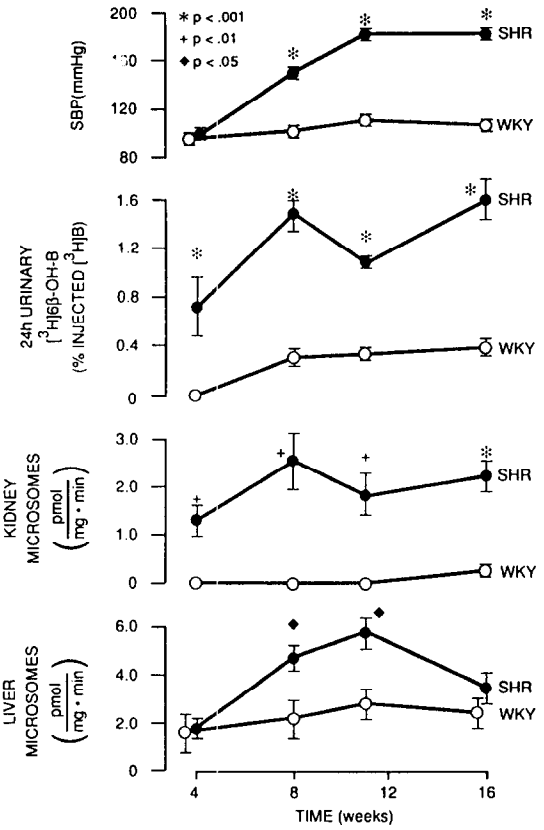


Fig. 1. Microsomal corticosterone 6β-hydroxylase activity in kidney and liver of SHR and WKY rats of various ages. Corticosterone 6β-hydroxylase activity was measured in 500 μg of microsomal protein by conversion of radiolabeled corticosterone. Values are expressed as means ± SEM; N = 4 for microsomal catalytic activity, 16–24 for systolic blood pressure (SBP) and 4–7 for urinary [$^3$H]6β-OH-corticosterone ([$^3$H]6β-OH-B) excretion. P values, comparing SHR with WKY measurements, are: (*) $< 0.001$; (+) $< 0.01$; and (◆) $< 0.05$. Results of SBP and urinary [$^3$H]6β-OH-corticosterone assays at 4, 8 and 16 weeks have been published previously [13]. Rats were not trained prior to SBP measurement in these experiments. See Results for temporal analysis of the various parameters by ANOVA.

urinary [$^3$H]6β-OH-corticosterone excretion, and renal and hepatic microsomal corticosterone 6β-OHase in SHR compared with WKY rats. Strain difference of each of the four parameters was evaluated by *t*-test at each age. SBP values were similar for SHR and WKY rats at 4 weeks, but were significantly higher in SHR at 8, 11 and 16 weeks. Urinary [$^3$H]6β-OH-corticosterone excretion and renal microsomal 6β-OHase were elevated significantly in SHR compared with WKY rats at 4 weeks, as well as at the subsequent ages. In contrast, hepatic 6β-OHase activity in SHR was not significantly different from WKY rats at 4 and 16 weeks. In WKY renal microsomes, corticosterone 6β-OHase was often not detectable [$< 0.05$ pmol·min$^{-1}$·(mg protein)$^{-1}$]. Although 6β-OHase was detected in 11-week WKY renal microsomes in an earlier study [9], it was detectable only at 16 weeks in the current study (Fig. 1).

In a separate set of statistical analyses, the variation with age of each parameter in Fig. 1 was evaluated by ANOVA, for each strain. The SBP at 8, 11 and 16 weeks in SHR was significantly greater ($P < 0.05$) compared with that at 4 weeks of age. The only other significant ($P < 0.05$) SBP difference at the various ages in SHR was the increase at 16 weeks compared with 8 weeks. There was no significant difference in SBP in WKY rats at any age or in urinary [$^3$H]6β-OH-corticosterone excretion at any age in either SHR or WKY rats. Renal microsomal 6β-OHase activity in SHR increased significantly ($P < 0.05$) from 4 to 8 weeks, but there was no significant difference between values at any other age. At three of the four ages studied in WKY rats, renal 6β-OHase activity was undetectable, so no ANOVA comparisons between ages were made in WKY rats. In SHR, liver microsomal 6β-OHase activity was significantly greater ($P < 0.05$) at 8, 11 and 16 weeks of age compared with 4-week rats and decreased significantly ($P < 0.05$) between 11 and 16 weeks. There was no significant age difference in WKY rat liver 6β-OHase activity values. To summarize strain difference and age comparisons, the following statements can be made. First, both urinary [$^3$H]6β-OH-corticosterone excretion and renal microsomal 6β-OHase activity were increased in SHR compared with WKY rats at age 4 weeks, prior to the development of overt hypertension in SHR. Second, hepatic microsomal 6β-OHase activity was not increased in SHR compared with WKY rats at 4 weeks and fell to near WKY levels between 11 and 16 weeks of age.

Consistent with our earlier reports [9, 13], TAO, the selective inhibitor of CYP3A, significantly decreased SBP in SHR, while having no significant effect in WKY rats (Table 1). Moreover, in preliminary dose–response studies, the TAO-induced decrement in SBP appears to be the maximum hypotensive effect of TAO [14]. This TAO effect in SHR was associated with the loss of all detectable renal 6β-hydroxylase activity (Table 1). In contrast, TAO treatment decreased hepatic catalytic activity by only 31% ($P < 0.05$), although the absolute magnitude of the decline in specific activity in liver was similar to that of kidney. Thus, in SHR the renal CYP3A seems to be relatively more susceptible than the hepatic isozymes to inhibition by TAO. In WKY rats, renal catalytic activity was undetectable both with and without TAO. However, hepatic catalytic activity was decreased 31%, identical to the decrease seen in TAO-treated SHR.

In northern blot analyses, specific cDNA probes for three different mammalian liver *CYP3A* mRNAs, rat *CYP3A1* [12], rat *CYP3A2* [20] and rabbit *CYP3A6* [21], gave strong signals with appropriate hepatic total RNA controls from female rats treated with phenobarbital, male rats treated with phenobarbital, and rabbits treated with rifampicin, respectively, in order to induce the specific corresponding *CYP3A* mRNA (not shown). Uninduced liver did not demonstrate *CYP3A* mRNA by northern blot. Similarly, none of the three probes

Table 1. Effect of troleandomycin (TAO) treatment on liver and renal corticosterone 6β-hydroxylase (6β-OHase) activity of 11-week male SHR and WKY rats

| | SHR | | | WKY | | |
|---|---|---|---|---|---|---|
| | | Microsomal 6β-OHase (pmol/min/mg protein) | | | Microsomal 6β-OHase (pmol/min/mg protein) | |
| | SBP | Kidney | Liver | SBP | Kidney | Liver |
| Control | 157 ± 2.5 | 1.9 ± 0.29 | 5.9 ± 0.55 | 108 ± 4.9 | 0 | 2.9 ± 0.33 |
| TAO | 128 ± 4.0 | 0 | 4.1 ± 0.30 | 102 ± 6.3 | 0 | 2.0 ± 0.32 |
| P | < 0.001 | < 0.001 | < 0.05 | NS | | NS |

Rats were given 40 mg/kg TAO s.c. daily for 4 days. Controls received only DMSO. Systolic blood pressure (SBP, mm Hg) was measured both before and after TAO administration in 18 SHR and 6 WKY rats. All rats were trained twice by SBP measurements, prior to experiments during their maintenance period (4–5 days) in a quiet room. When catalytic activity was not detectable (< 0.05 pmol/min/mg protein), it was assigned a value of zero (see Materials and Methods). Values for catalytic activity are means ± SEM, N = 4. A two-tailed *t*-test was used to determine statistical significance.

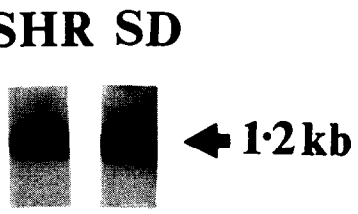


Fig. 2. Southern blot analysis of the product of reverse transcriptase-polymerase chain reaction (RT-PCR) amplification, utilizing a consensus CYP3A 5′ primer and oligo dT as a 3′ primer. The DNA products were separated on agarose gel, capillary transferred and hybridized at high stringency to a 0.9 kb Pst fragment of the *CYP3A1* cDNA (pDex12), as described in Materials and Methods. Lane 1: RT-PCR product obtained using SHR kidney RNA. Lane 2: positive control, PCR product obtained using Sprague–Dawley liver RNA.

gave a signal with total RNA from SHR or WKY kidney, even with low stringency hybridization, suggesting that *CYP3A* mRNA is present in kidney at levels too low for detection by northern blot analysis (not shown). However, the presence of renal *CYP3A* mRNA was demonstrated by PCR. PCR with SHR kidney RNA using oligo dT and the consensus primer for CYP3A1, CYP3A2 and CYP3A6 (see Materials and Methods) yielded a 1.2 kb product, consistent with the 1.7 to 2.0 kb reported for full-length *CYP3A* mRNA [12, 20, 21]. As seen in Fig. 2, this product hybridized at high stringency with pDex12, a 0.9 kb fragment of *CYP3A1* cDNA [12].

## DISCUSSION

Three new observations, which address the possibility that increased renal CYP3A may be pathogenetic in SHR, resulted from this study. First, renal corticosterone 6β-OHase activity increased from 4 to 8 weeks, remained at similar levels at 11 and 16 weeks and was much higher than WKY renal 6β-OHase activity at all four ages (Fig. 1). Second, strain differences and temporal changes in renal catalytic activity roughly correlated with differences in SBP between SHR and WKY rats. Indeed, renal microsomal catalytic activity and the *in vivo* index of 6β-OHase activity both were increased prior to development of overt hypertension in SHR compared with WKY rats, i.e. at 4 weeks, consistent with causality of the increased renal CYP3A activity in the hypertension. Third, the relatively large decrease in renal microsomal corticosterone 6β-OHase activity, following TAO treatment, was consistent with the substantial decrease in SBP produced in 11-week SHR by this selective CYP3A inhibitor. We now know that only 50% of the SBP elevation in SHR (strain difference compared with WKY rats) is inhibited by the maximal effective dose of TAO [14]. Thus, only 50% of the blood pressure elevation is likely to be CYP3A related in this polygenic model of hypertension. Nonetheless, each of these findings is consonant with our hypothesis that elevated renal CYP3A activity could be more important than hepatic CYP3A in the genesis of elevated blood pressure in SHR. However, hepatic CYP3A activity is also higher in SHR than in WKY rats at 8 and 11 weeks (Fig. 1, Table 1), as originally described by Schenkman *et al.* [22], which merits further discussion.

Hepatic as well as renal CYP3A activity increased from 4 to 8 weeks of age in SHR, coinciding with a large SBP increase (Fig. 1). However, unlike renal corticosterone 6β-OHase activity, hepatic activity was not significantly higher in SHR than in WKY rats at 4 weeks and decreased to a value similar to WKY rats by 16 weeks (Fig. 1). Although inhibition of SBP by TAO was associated with inhibition of both renal and hepatic CYP3A catalytic activity, the renal activity decreased to undetectable levels (Table 1). Observations of others support the primacy of increased renal CYP over hepatic CYP in the genesis of hypertension in SHR [23]. More specifically, we have observed recently that corticosterone 6β-OHase

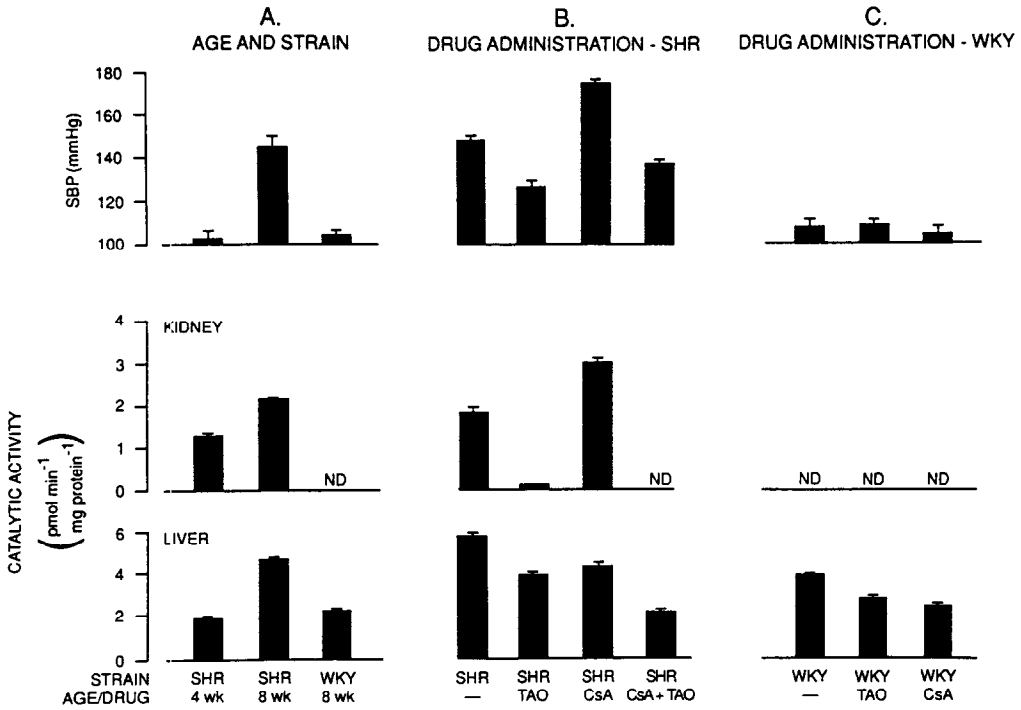


Fig. 3. Summary of correlations of SHR and WKY systolic blood pressure (SBP) with renal and hepatic microsomal corticosterone 6β-OHase catalytic activity as to strain, age and drug treatments. Data are from this study and Ref. 24 (see Discussion). Data shown in panels B and C were from 11-week-old rats. ND = not detectable. Values are means ± SEM. N = 4 for studies using CA (from Ref. 24).

(CYP3A) activity is increased in kidney and decreased in liver microsomes by CA administration [24], as previously shown for total CYP [25], which is associated with an augmented SBP [26] that is inhibited by TAO [24]. A summary of the numerous measurements of SBP and renal and hepatic microsomal corticosterone 6β-OHase in SHR and WKY rats is shown in Fig. 3. Shown are comparisons with strain, ontogeny and drug treatments including the effects of CA mentioned above, which dissociate the changes in SBP and renal CYP3A from changes in hepatic CYP3A.

The failure of three CYP3A cDNA probes to show detectable hybridization with total renal RNA of either SHR or WKY rats suggests a low mRNA copy number in kidney. Moreover, immunoreactive CYP3A is detected by immuno-histochemistry only in collecting ducts of Sprague–Dawley [3], SHR and WKY rats.* Synthesis of an RT-PCR product of the expected size (1.2 kb) using a consensus (CYP3A1, 3A2 and 3A6) 30 mer as a 5′ primer demonstrates the presence of renal *CYP3A* mRNA in SHR kidney (Fig. 2). This finding will not only facilitate cloning and sequencing of cDNA from SHR kidney but indicates the feasibility of measuring renal *CYP3A* mRNA in SHR and WKY by quantitative PCR if needed. However, measurement of *CYP3A* mRNA by northern blot or ribonuclease protection assay of poly $A^+$ mRNA should be tried first.

* Aniko Naray-Fejes-Toth and Geza Fejes-Toth, personal communication. Cited with permission.

A role for CYP3A in the hypertension of SHR does not rule out the potential role of CYP4A and its products, the ω and ω-1 hydroxylated metabolites of arachidonic acid [23, 27] or other bioactive CYP products. It should also be noted that 6β-OH-corticosterone may not be the only potentially hypertensinogenic product of CYP3A. Conclusive evidence for the hypertensinogenic role of renal CYP3A requires definitive genetic studies in SHR, showing the inheritance of increased renal CYP3A by a single gene as well as cosegregation of blood pressure with the enhanced renal CYP3A phenotype.

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## REFERENCES

1. Peterson R, Metabolism of adrenal cortical steroids. In: *The Human Adrenal Cortex* (Ed. Christy NP), pp. 87–189. Harper & Row, New York, 1971.
2. Grogan WMcL, Phillips VM, Schuetz EG, Guzelian PS and Watlington CO, Corticosterone 6β-hydroxylase in A6 epithelia: A steroid-inducible cytochrome P-450. *Am J Physiol* **258**: C480–C488, 1990.
3. Schuetz EG, Schuetz JD, Grogan WMcL, Naray-Fejes-Toth A, Fejes-Toth G, Raucy J, Guzelian P, Gionela K and Watlington CO, Expression of cytochrome P450

3A in amphibian, rat, and human kidney. *Arch Biochem Biophys* **294**: 206–214, 1992.
4. Duncan R, Grogan W, Kramer L and Watlington CO, Corticosterone's metabolite is an agonist for $Na^+$ transport stimulation in A6 cells. *Am J Physiol* **255**: F736–F748, 1988.
5. Grogan WM, Fidelman ML, Newton DE, Duncan RL and Watlington CO, A corticosterone metabolite produced by A6 (toad kidney) cells in culture: Identification and effects on $Na^+$ transport. *Endocrinology* **116**: 1189–1194, 1985.
6. Frantz AG, Katz FH and Jailer JW, 6-Beta-Hydroxycortisol: High levels in human urine in pregnancy and toxemia. *Proc Soc Exp Biol Med* **105**: 41–43, 1960.
7. Katz FH, Lipman MM, Franz AG and Jailer JW, The physiologic significance of 6$\beta$-hydroxycortisol in human corticoid metabolism. *J Clin Endocrinol Metab* **22**: 71–77, 1962.
8. Yamaji T, Motohashi K, Murakawa S and Ibayashi H, Urinary excretion of 6$\beta$-hydroxycortisol in states of altered thyroid function. *J Clin Endocrinol Metab* **29**: 801–806, 1969.
9. Ghosh S, Grogan WM, Basu A and Watlington CO, Renal corticosterone 6$\beta$-hydroxylase in the spontaneously hypertensive rat. *Biochim Biophys Acta* **1182**: 152–154, 1993.
10. Chang TKH, Gonzalez FJ and Waxman DJ, Evaluation of triacetyloleandomycin, $\alpha$-naphthoflavone and diethyldithiocarbamate as selective chemical probes for inhibition of human cytochromes P450. *Arch Biochem Biophys* **311**: 437–442, 1994.
11. Watkins PB, Wrighton SA, Schuetz EG, Maurel P and Guzelian PS, Macrolide antibiotics inhibit the degradation of the glucocorticoid responsive cytochrome P-450p in rat hepatocytes *in vivo* and in primary monolayer culture. *J Biol Chem* **261**: 6264–6271, 1986.
12. Wrighton SA, Schuetz EG, Watkins PB, Maurel P, Barwick J, Bailey BS, Hartle HT, Young B and Guzelian P, Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to glucocorticoid-inducible cytochrome P-450 of the rat. *Mol Pharmacol* **28**: 312–321, 1985.
13. Watlington CO, Kramer LB, Schuetz EG, Guzelian P, Zilai J, Grogan WMcL, Gizek F and Schoolwerth AC, Corticosterone 6$\beta$-hydroxylation correlates with blood pressure in spontaneously hypertensive rats. *Am J Physiol* **262**: F927–F931, 1992.
14. Basu A, Hagley RD, Ghosh SS, Kramer LB, Nance WE and Watlington CO, Maternal environment defines blood pressure and its response to troleandomycin in spontaneously hypertensive rats. *Am J Hypertens*, in press.
15. Bunag R and Butterfield J, Tail-cuff blood pressure measurement without external preheating in awake rats. *Hypertension* **4**: 898–903, 1982.
16. Wrighton SA, Maurel P, Schuetz EG, Watkins PB, Young B and Guzelian PS, Identification of the cytochrome P-450 induced by macrolide antibiotics in rat liver as the glucocorticoid responsive cytochrome $P\text{-}450_{p}$. *Biochemistry* **24**: 2171–2178, 1985.
17. Chirgwin J, Przybyla AE, McDonald RJ and Rutter WJ, Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**: 5294–5299, 1980.
18. Sambrook J, Frisch EF and Maniatis T, *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
19. Gonzalez FJ, Nebert DW, Hardwick JP and Kasper CB, Complete cDNA and protein sequence of a pregnenolone 16$\alpha$-carbonitrile-induced cytochrome P-450: A representative of a new gene family. *J Biol Chem* **260**: 7435–7441, 1985.
20. Gonzalez FJ, Song B-J and Hardwick JP, Pregnenolone 16$\alpha$-carbonitrile-inducible P-450 gene family: gene conversion and differential regulation. *Mol Cell Biol* **6**: 2969–2976, 1986.
21. Dalet C, Clair P, Daujat M, Fort P, Blanchard J and Maurel P, Complete sequence of cytochrome P-450 3c cDNA and presence of two mRNA species with 3′ untranslated regions of different lengths. *DNA* **7**: 39–46, 1988.
22. Schenkman JB, Thummel KE and Farreau LV, Physiological and pathophysiological alterations in rat hepatic cytochrome P-450. *Drug Metab Rev* **20**: 557–584, 1989.
23. Sacerdoti D, Escalente B, Abraham NG, McGiff JC, Levere RD and Schwartzman ML, Treatment with tin prevents the development of hypertension in the spontaneously hypertensive rat. *Science* **243**: 388–390, 1989.
24. Basu A, Ghosh SS, Mohanty PK and Watlington CO, Augmented arterial pressure responses to cyclosporine in spontaneously hypertensive rats: Influence of renal family 3A cytochrome P-450. *Hypertension* **24**: 480–485, 1994.
25. Mayer RD, Berman S, Cockett ATK and Mahin D, Differential effects of cyclosporin on hepatic and renal heme, cytochrome P-450 and drug metabolism. *Biochem Pharmacol* **38**: 1001–1007, 1989.
26. Lustig S, Stern N, Eggena P, Tuck ML and Lee DBN, Effect of cyclosporine on blood pressure and renin-aldosterone axis in rats. *Am J Physiol* **253**: H1596–H1600, 1987.
27. Levere RD, Martasek P, Escalente B, Schwartzman ML and Abraham NG, Effect of heme arginate administration on blood pressure in spontaneously hypertensive rats. *J Clin Invest* **86**: 213–219, 1990.