Evidence to support a hypertensinogenic role of family 3A cytochrome P-450 (CYP3A) activity is that troleandomycin, a selective inhibitor of CYP3A, decreases both blood pressure and in vivo corticosterone 6β-hydroxylation in spontaneously hypertensive rats (SHR). Renal CYP3A activity is markedly increased in SHR compared with Wistar-Kyoto (WKY) rats. Cyclosporine acutely increases both systolic blood pressure and renal total cytochrome P-450 in SHR. We tested the hypothesis that the augmentation of blood pressure by cyclosporine is mediated by a further increase in renal CYP3A activity. Accordingly, we assessed the effect of troleandomycin administration on cyclosporine-induced systolic blood pressure increase and renal and hepatic microsomal CYP3A activity in SHR. Cyclosporine (5 mg/kg SC) given daily in 11-week-old SHR resulted in substantial augmentation of blood pressure after 6 days. This blood pressure increase was attenuated by troleandomycin (40 mg/kg) given either during or after development of hypertension. Cyclosporine increased renal (60%) but decreased hepatic (25%) microsomal CYP3A activity in SHR. In contrast, cyclosporine failed to produce any detectable increase in either blood pressure or renal CYP3A activity in WKY rats. Troleandomycin completely inhibited renal CYP3A activity measured after cyclosporine treatment of SHR, which correlated with its attenuation of the cyclosporine-induced blood pressure increase. These findings suggest that renal CYP3A could play an important role in acute cyclosporine-induced hypertension.

Key Words: rats, inbred SHR • cyclosporine • blood pressure • corticosterone • kidney
SBP in SHR. We further demonstrated that renal CYP3A catalytic activity and CYP3A protein were increased fivefold or more in SHR compared with WKY rats, whereas there was less than a onefold increase in the hepatic catalytic activity in SHR. These latter findings support a role for enhanced renal CYP3A in the basal blood pressure elevation in SHR compared with WKY rats. The purpose of this study was to test the hypothesis that CSA augments SBP in SHR by further increasing renal CYP3A activity.

**Methods**

**Experimental Protocol**

CSA was a gift from Sandoz Research Institute (East Hanover, NJ). It was administered to 11-week-old rats at a dose of 5 mg/kg body wt, as this dose was shown to increase blood pressure in SHR in the absence of significant changes in creatinine clearance; a higher CSA dose caused renal function impairment after 7 and 28 days of drug administration. CSA and TAO (40 mg/kg body wt SC) were administered in 0.5 mL/kg dimethyl sulfoxide as diluent. Ten-day and 6-day protocols were used. In the 10-day protocol, two groups of SHR were used: one (9 rats) received CSA for 10 days, and the other (14 rats) received TAO for 4 days (days 6 through 9) in addition to CSA. SBP was measured before the first dose of drug at time zero, on day 6, and 24 hours after the 10th consecutive dose of the drug (day 10). In the 6-day protocol, one group of WKY rats (6 rats received CSA) and four groups of SHR were used. The SHR groups were group 1 (9 rats, control), group 2 (9 rats, CSA-treated), group 3 (8 rats, CSA+TAO-treated), and group 4 (9 rats, TAO-treated). Basal SBP was measured just before the first dose of drug was injected at time zero. The rats received the drug for 6 consecutive days. The second SBP was determined 24 hours after the sixth dose of drug (day 6). TAO was given for 4 days (days 2 through 5).

**SBP Measurements**

Male SHR and WKY rats were obtained from Taconic Farms (Germantown, NY) at 10 weeks of age. SBP in awake rats was measured by the tail-cuff method using a photoelectric sensor and pulse amplifier (ITTC Life Science) connected to a two-channel recorder. Before the experiment, animals were maintained in a quiet room for 4 to 5 days, during which they were trained twice by measurement of SBP. For each measurement, rats were placed in a restraining cage for 5 minutes, after which successive readings were taken until four to five sequential readings were within a range of 15 mm Hg. The average was used as the SBP value.

**Measurement of CYP3A Catalytic Activity**

Liver and kidney from 11-week-old male SHR and WKY rats were homogenized in 100 mmol/L Tris-EDTA buffer (pH 7.4) in the presence of protease inhibitors (2 mmol/L hydrocinnamic acid, 0.5 mmol/L benzyl arginine, and 5 mmol/L benzamidine) and microsomes prepared as described by Grogan et al. Corticosterone 6β-hydroxylase activity was measured by a radiometric method. Typically, 0.5 mg of microsomes was incubated for 30 minutes at 37°C in 50 mmol/L KPO₄ buffer, 5 mmol/L MgCl₂, 10 mmol/L glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehydrogenase, 0.5 mmol/L NADP⁺, and 3 μCi [3H]corticosterone (10⁴ mol/L) in a total volume of 1.5 mL. Unlabeled corticosterone was obtained from Sigma Chemical Co, and [1,2-3H]corticosterone was obtained from New England Nuclear. The reaction was terminated by placing the mixture on ice followed by reversed solid-phase extraction, elution, and reversed-phase high-performance liquid chromatography (HPLC) as described below.

**Results**

Carrier corticosterone and 6β-OH-corticosterone (Steraloids) were added before extraction.

The microsomal incubate was extracted on a C₁₈ Sep-Pak cartridge (Waters Chromatography Division, Millipore Corp). The Sep-Pak cartridge was washed with 10 mL water and 2 mL of 30% MeOH, eluted with 2 mL of 100% MeOH, and dried under N₂. The sample was reconstituted in 30% MeOH/H₂O and chromatographed with standards on an automated HPLC system (Shimadzu) that included an autoinjector and Chromatopac (C-R4A) data processor. The 6β-OH-corticosterone was eluted from a C₁₈ column (5 μm, 4.6×250 mm; Rainin) by 41.5% MeOH at 0.8 mL/min for 20 minutes followed by 55% MeOH for 40 minutes. The standards (carrier corticosterone and 6β-OH-corticosterone) were monitored at 244 nm on a Shimadzu UV detector. Radioactivity was counted in a flow-through radioisotope detector (Beckman Instruments), and the results from both sensors were fed into the data processor. The [3H]6β-OH-corticosterone recovery, as a percentage of total radiolabel recovered, was used to calculate catalytic activity expressed as picomoles per minute per milligram protein.

**Measurement of CSA Blood Levels**

Whole-blood CSA level was measured using the Abbott TDX system (Abbott Laboratories). The TDX CSA assay uses fluorescence polarization immunoassay technology and competitive binding immunoassay methodology. Whole-blood CSA levels were determined in 11-week-old SHR after 6 days of drug treatment. One rat group (n=6) received CSA for 6 consecutive days, and the other group (n=6) received TAO.

**Statistical Analysis**

Results are reported as mean±SEM. ANOVA was used for comparison among different series. Student's t-test was used to compare two groups.

**Results**

Blood Pressure Responses to CSA and TAO

Daily CSA administration alone (Fig 1, Table 1) produced an increase in SBP from 155±1.27 to 178±2.06 mm Hg at day 6. SBP was essentially the same at day 10 (180±3.28 mm Hg). Thus, the maximal effect
TABLE 1. Effect of Cyclosporine and Troleandomycin on Systolic Blood Pressure In 11-Week-Old SHR and WKY Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 0</th>
<th>Day 6</th>
<th>Day 10</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>CSA (9)</td>
<td>155±1.27</td>
<td>178±2.06</td>
<td>180±3.28</td>
</tr>
<tr>
<td></td>
<td>CSA+TAO (14)</td>
<td>156±1.40</td>
<td>179±2.72</td>
<td>139±1.66*</td>
</tr>
<tr>
<td>SHR</td>
<td>Control (9)</td>
<td>150±1.28</td>
<td>149±1.66</td>
<td>. . .</td>
</tr>
<tr>
<td></td>
<td>CSA (9)</td>
<td>146±1.70</td>
<td>178±2.63</td>
<td>. . .</td>
</tr>
<tr>
<td></td>
<td>CSA+TAO (8)</td>
<td>155±2.68</td>
<td>140±3.78</td>
<td>. . .</td>
</tr>
<tr>
<td></td>
<td>TAO (9)</td>
<td>154±2.65</td>
<td>128±1.82</td>
<td>. . .</td>
</tr>
<tr>
<td>WKY</td>
<td>CSA (6)</td>
<td>105±1.79</td>
<td>106±1.86</td>
<td>. . .</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; CSA, cyclosporine; and TAO, troleandomycin. Values are mean±SEM. Group A followed an 11-day protocol; group B, a 6-day protocol. Numbers in parentheses indicate number of animals used in each treatment schedule.

Of CSA was achieved in 6 days, as previously described.\(^6\) TAO, an inhibitor of CYP3A, given for 4 days (days 5 through 9) virtually abolished the SBP increase by day 10. In fact, the value of 139±1.66 mm Hg was below the initial SBP and significantly different from the SBP in the series receiving CSA alone (\(P<.001\), Table 1). When TAO was administered (days 2 through 5) during the development of CSA-induced augmentation of SBP, it not only prevented the increase in SBP at day 6 (Fig 2, Table 1) but also reduced SBP from 155±2.68 mm Hg to a final value of 140±3.78 mm Hg (\(P<.01\)). This decrement produced by TAO in animals receiving CSA was essentially the same as found in the 10-day protocol (Fig 1, Table 1) but not the decrease in SBP produced in the animals treated by TAO alone (Fig 2). As noted in Fig 3, the percent change in SBP was significantly lower in TAO-treated rats compared with the decrease seen in CSA+TAO-treated animals (\(P<.05\)). In WKY rats, CSA did not change blood pressure.

CYP3A Activity

Corticosterone 6β-hydroxylase activity (CYP3A) in the renal microsomes of SHR treated with CSA was 60% greater than the value found in control kidneys (\(P<.05\), Table 2). Concomitant TAO administration (CSA+TAO series) reduced renal catalytic activity in SHR to nondetectable levels (\(P<.05\)). In contrast to kidney, liver microsomes from CSA-treated SHR had 30% lower catalytic activity compared with those from control rats (\(P=NS\)). On simultaneous TAO administration, CYP3A activity was further reduced in liver compared with control (\(P<.05\), Table 2).

CYP3A enzyme activity was not detectable in kidney of control or CSA-treated WKY rats (Table 2), and thus no effect of CSA was discerned. Undetectable CYP3A activity is often the case in WKY kidney (the lower limit of detection of the catalytic assay is 0.05 pmol·min\(^{-1}\).

\[^{6}\]Hypertension\ Vol 24, No 4 October 1994

![Fig 2. Plot shows effect of cyclosporine (CSA), troleandomycin (TAO), and CSA+TAO on systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. SBP is shown as mean±SEM. Number of rats in each group is shown in parentheses. See Table 1 for results of statistical evaluation.](http://hyper.ahajournals.org/.)

![Fig 3. Bar graph shows percent change in systolic blood pressure (SBP) (±SEM) from day 0 to day 6 in rat groups shown in Fig 2. Spontaneously hypertensive rats were divided into control (C), cyclosporine (CSA), troleandomycin (TAO), and CSA+TAO groups. Wistar-Kyoto (WKY) rats were treated with CSA only. *\(P<.05\) by ANOVA.](http://hyper.ahajournals.org/.)
mg\(^{-1}\) protein). CSA decreased CYP3A activity by 33% in WKY liver, which was similar to the change in SHR liver and was not statistically significant.

**CSA Blood Levels**

After 6 days of drug treatment, average CSA level in the group receiving CSA alone was 558±37.4 ng/mL; the level in the CSA+TAO-treated group was 399±94.7 ng/mL. The values between the two groups were not significantly different.

**Discussion**

The major new findings of this study are (1) CSA produces an augmentation in blood pressure in SHR but not in WKY rats; (2) an augmented blood pressure response is associated with a CSA-induced increase in renal CYP3A activity in SHR (but not in WKY rats), and hepatic CYP3A is, if anything, decreased by CSA in SHR; and (3) TAO, which is considered a selective inhibitor of CYP3A, attenuated the CSA-induced augmentation of SBP, and this attenuated blood pressure response correlated with a marked reduction in renal CYP3A activity observed after CSA+TAO administration. The major new conclusion from this study is that a CSA-induced increase in renal CYP3A is of potential importance in CSA-induced hypertension. We believe that an understanding of such cellular mechanisms will be crucial to the design of future therapy to prevent CSA-induced hypertension in humans after heart and other solid organ transplantation.

Several potential etiologic factors contribute to CSA-induced hypertension. Indeed, evidence from our experiments indicates that more than one mechanism may be operative in SHR, as TAO did not abolish all of the CSA-induced blood pressure increase. Our reasoning is as follows: Previous work from our laboratory has demonstrated that TAO abolishes approximately 50% of the increment of SBP difference in SHR compared with WKY rats (50 mm Hg at 11 weeks) from a value of approximately 155 to 130 mm Hg.\(^{20}\) If all the SBP augmentation resulting from CSA administration to SHR were mediated by the concomitant increase in renal CYP3A, then TAO should decrease SBP in CSA-treated animals to the range of 130 mm Hg; ie, it should inhibit both the basal TAO-sensitive component and the CSA-stimulated component of blood pressure elevation. This was clearly not the case, because SBP fell only to 140±3.78 mm Hg in CSA+TAO-treated animals, a change that was significantly different from the effect of TAO alone (Fig 2, Table 1). In other words, if the effects on SBP of CSA (+25 mm Hg) and TAO (−25 mm Hg) are simply additive, then CSA+TAO treatment should produce no change in SBP, as observed in control animals given no drugs. However, the combined administration of the two drugs produced a significant decrease in SBP (Fig 2), indicating an inhibitory effect of TAO on the CSA-induced hypertension. We interpret these results to suggest that TAO inhibits a major component but not necessarily all of the CSA-induced augmentation in SBP. The absence of any hypertensive effect of CSA in WKY rats suggests an increased sensitivity of SHR to the hypertensinogenic effect of CSA. The increased CYP3A activity in SHR could contribute to the enhanced short-term hypertensinogenic response to CSA in this strain.

Two possibilities may explain the component of CSA-induced blood pressure increase that was not sensitive to TAO. First, there could be an element of renal failure produced by CSA administration, which accounts for a portion of SBP elevation, although the daily dose used here (5 mg/kg body wt) did not produce a detectable change in creatinine clearance.\(^{6}\) Controversy exists as to the role of Na\(^+\) balance in the nephrotoxicity produced by a CSA dose higher than used here.\(^{16,17}\) We have made no attempt to control sodium intake or measure renal function in our studies. Second, the TAO-insensitive component could be due to an increase in CYP-dependent arachidonate metabolism to bioactive products, which are thought to contribute to basal blood pressure elevation in SHR compared with WKY rats.\(^{2,24}\) Not only are these metabolites increased in SHR compared with WKY kidney,\(^{24}\) but their production by SHR renal microsomes is further increased by CSA administration.\(^{21}\) Based on its apparent selective inhibition of CYP3A,\(^{13,14}\) TAO would not be expected to inhibit this other class of CYP (CYP4A) that metabolizes arachidonate.

The mechanism by which enhanced 6β-OH-corticosterone production might exert a basal hypertensive effect in SHR, and its augmentation by CSA, is uncertain. However, we have shown that 6β-OH-corticosterone is an agonist for increased Na\(^+\) transport in cultured epithelia derived from amphibian kidney (A6 cells).\(^{10}\) Most of the active transepithelial Na\(^+\) transport stimulation produced by corticosterone in the cells appears to be mediated by 6β-OH-corticosterone,\(^{11}\)
which is produced in the effector cell by CYP3A. We speculated that increased 6β-OH-corticosterone production in some segment of the nephron might cause a defect in renal Na⁺ excretion. Such a renal defect in Na⁺ excretion is postulated for essential hypertension in humans and in SHR. However, the renal Na⁺-retentive mechanism hypothesized may be necessary but not sufficient to produce a component of basal blood pressure elevation in SHR. It is also possible that excess renal CYP3A exerts a hypertensinogenic effect by some mechanism other than excess 6β-OH-corticosterone production.

Since pharmacokinetic interaction between CSA and TAO could occur, measured levels of CSA at the end of drug treatment. CSA levels were not significantly different between the two groups of SHR (CSA-treated and CSA+TAO-treated), indicating that the effect of TAO on SBP was not simply the result of an alteration in CSA levels.

Experimental and clinical studies have shown that the most common mechanism of CSA-induced hypertension relates to renal vasoconstriction, a suppressed renin-angiotensin system, and a salt-dependent hypertension with a tendency toward extracellular fluid volume expansion. Nephrotic effects per se have been suggested as a cause of CSA-induced hypertension. Studies have also shown that CSA results in progressive increased peripheral vascular resistance leading to pregnerular vasoconstriction, and it also causes a progressive narrowing of afferent arterioles secondary to smooth muscle proliferation, both resulting in diminished renal blood flow and increased mean arterial pressure. CSA-induced increased sympathetic tone also contributes to intrarenal vasoconstriction and proximal tubule sodium avidity in the innervated kidneys of extrarenal transplant recipients and those receiving CSA for autoimmune disorders. The renal vasoconstriction could also result from imbalance in the production of vasoconstrictor eicosanoids and/or failure of vasodilator prostaglandin synthesis. The endothelin vasoconstrictor axis has recently been suggested to play a role in CSA-induced hypertension. CSA stimulates endothelial cell synthesis of endothelin, which may mediate renal vasoconstriction in CSA-treated rats.

The extent to which renal CYP3A activity and 6β-OH-corticosterone production may relate to the endothelin vasoconstrictor axis remains to be defined. In rats, CSA is metabolized by CYP3A in intestine and liver. CSA metabolism by these organs as well as by kidney has also been demonstrated in humans. Whether any of the CYP3A metabolites are hypertensinogenic is unknown.

Further investigation is needed to determine the exact mechanism by which the CSA increase of renal CYP3A in SHR might contribute to and interact with a host of vasoactive mechanisms influencing hypertension. However, our results add another dimension to the pathogenetic mechanisms of CSA-induced hypertension, suggesting that CYP3A may play a significant role in this disorder.

Acknowledgments
This work was supported by Grant-in-Aid No. 91007390 from the American Heart Association. A.K.B. is a recipient of a postdoctoral fellowship grant from the Virginia Affiliate of the American Heart Association. The authors gratefully acknowledge the expert technical assistance of Lora Kramer.

References


Augmented arterial pressure responses to cyclosporine in spontaneously hypertensive rats. 
Role of cytochrome P-450 3A.
A K Basu, S Ghosh, P K Mohanty and C O Watlington

Hypertension. 1994;24:480-485
doi: 10.1161/01.HYP.24.4.480

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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