ALTERATIONS IN THE CONTENTS OF CYTOCHROME P-450 AND ADRENAL FERREDOXIN IN ADRENALS OF SPONTANEOUSLY HYPERTENSIVE RATS[1]

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SUMMARY: The contents of cytochrome P-450 and adrenal ferredoxin were determined in the adrenals of spontaneously hypertensive rats of the Wistar-Kyoto strain and were compared with those in the adrenals of normotensive Wistar-Kyoto rats. Male rats between 10 and 12 weeks of age were employed in these studies. While the adrenal weight was only slightly decreased in the spontaneously hypertensive rats, the contents of both cytochrome P-450 and adrenal ferredoxin per 2 adrenals were found to be approximately 35% lower as compared to the normotensive controls. Subcellular fractionation of whole adrenal homogenates revealed that the observed decreases in the contents of cytochrome P-450 and adrenal ferredoxin were the result of alterations in the concentrations of these proteins in mitochondria. These observations are consistent with the suggestion that the pattern of steroidogenesis in the adrenal of the spontaneously hypertensive rat is altered.

In 1963, Okamoto and Aoki (1) developed a strain of spontaneously hypertensive rats (SHR) by selective inbreeding in a colony of Wistar rats. The SHR is currently considered to be the best animal model for human essential hypertension. At the time that the SHR strain was developed, normotensive rats (WKY) from the same stock colony of Wistar-Kyoto rats were propagated, and this strain has served as the best control for the SHR because of the genetically similar ancestry.

Although the etiology of the hypertension in the SHR remains unknown, the adrenal cortex may be involved in the pathogenesis of the disease since Aoki (2) has shown that bilateral adrenalectomy can prevent the development of the hypertensive condition and can also reduce the blood pressure to the normotensive range in rats which had become hypertensive. In addition,

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a number of investigators have observed both morphological and histochemical changes in the adrenal cortex of the SHR (3-6) together with an increased adenohypophyseal-adrenocortical activity (5). Furthermore, alterations in the secretory rates and/or plasma levels of adrenocortical steroids in the SHR have been reported under a variety of experimental conditions (7-9). These observations suggest that adrenal steroidogenesis is altered in the SHR and have prompted the present investigation on the adrenal contents of cytochrome P-450 and adrenal ferredoxin. Since cytochrome P-450 and adrenal ferredoxin are required for many of the reactions involved in adrenal steroidogenesis, including the conversion of cholesterol to pregnenolone, (10-14) changes in the contents of these two proteins in the adrenal of the SHR could lead to the observed alterations in the synthesis and secretion of the adrenocortical steroids.

METHODS

Male SHR and WKY animals were obtained from Laboratory Supply Company, Inc., Indianapolis, Indiana. At 10 to 12 weeks of age, rats were sacrificed by decapitation (between 9:00 A.M. and 10:00 A.M.) and the adrenals were excised and placed in 0.2 M Tris-HCl buffer, pH 7.4. The adrenals were then stripped of adhering fat, blotted, weighed and homogenized in 1.2 ml of 0.2 M Tris-HCl buffer, pH 7.4. Homogenates were then sonicated for a total of 2 min in 30-sec bursts at 4°C using a microtip probe with a Branson model W185 Sonifier at an output of 15 W (20 KHz) (14). Adrenal mitochondria and microsomes were prepared from unsonicated homogenates as previously described (14).

Adrenal ferredoxin contents were determined by electron paramagnetic resonance (EPR) spectrometry at 100°K using a Varian E-104A EPR spectrometer equipped with a variable temperature attachment. Quantitation was based on the magnitude of the $g = 1.94$ signal of the iron-sulfur protein which had been reduced by the addition of sodium dithionite as described previously (15,16). The instrument parameters were: modulation amplitude, 12.5 G; modulation frequency, 100 KHz; power, 50 mW; and temperature, 100°K.

The contents of cytochrome P-450 in the sample homogenates were determined according to the method of Estabrook et al. (17) using an Aminco DW-2 UV/VIS spectrophotometer. Quantitation was based on the difference in absorbance at 450 nm minus 510 nm in the CO + dithionite minus CO difference spectrum using a millimolar extinction coefficient of 100 mM^{-1} cm^{-1}. The contents of cytochrome P-450 in the mitochondrial and microsomal fractions were determined by the method of Omura and Sato (18). A millimolar extinction coefficient of 91 mM^{-1} cm^{-1} was used for the difference in absorbance between the reduced cytochrome P-450-CO complex and reduced cytochrome P-450 using the wavelength pair 450 and 490 nm.

Protein was determined by the microbiuret method (19) using bovine serum albumin as the standard. Systolic blood pressures of the animals were

determined by the tail plethysmographic method with an automated cuff inflator-pulse reading system manufactured by Technilab Instruments. Data were analyzed statistically by the group Student's *t* test.

RESULTS

Systolic blood pressures of 10 to 12 week-old male SHR animals were an average of 51 mm Hg higher than those of WKY animals of the same ages (Table I). Although the body weights of the SHR and WKY animals were not significantly different at this age, the adrenal weights were approximately 16% lower and total protein per two adrenals was about 7% less in the SHR.

Determination of the contents of cytochrome P-450 and adrenal ferredoxin in homogenates prepared from the adrenals of these animals revealed that the total amounts of cytochrome P-450 and adrenal ferredoxin per two adrenals were 35% and 34% lower, respectively, in the SHR as compared to the WKY (Table II). When cytochrome P-450 and adrenal ferredoxin contents are expressed on the basis of either adrenal protein or adrenal wet weight, significant decreases in the specific contents of these two proteins are also seen in the SHR (Table II).

In order to localize where the decreases in cytochrome P-450 and adrenal ferredoxin occur in the adrenal of the SHR, the contents of these two proteins were determined in mitochondrial and microsomal fractions which were prepared from the adrenals of 11-11½ week-old animals. The results of these experiments (Table III) indicated that the contents of cytochrome P-450 and adrenal ferredoxin per mg protein in the mitochondrial fraction of SHR adrenals were 32% and 27% lower, respectively, as compared to their contents in mitochondria prepared from the adrenals of WKY animals. In contrast, no differences in the content of cytochrome P-450 per mg protein were observed in the microsomal preparations derived from the adrenals of SHR and WKY animals. The presence of adrenal ferredoxin was not detected in the microsomal preparations.

DISCUSSION

The results of a number of recent studies indicate that adrenal

Table I: Comparison of body weight, blood pressure, adrenal weight, and adrenal protein between SHR and WKY animals.

Parameter	WKY	SHR
Body weight (g)	252 ± 7	232 ± 8†
Systolic B.P. (mm Hg)	151 ± 3	202 ± 5*
Adrenal weight (mg/2 adrenals)	47.4 ± 1.4	39.8 ± 5.3*
Adrenal protein (mg/2 adrenals)	14.3 ± 0.4	13.3 ± 0.2**

Male rats 10 to 12 weeks of age were used. The values given represent the mean ± S.E.M. of results obtained with 17 WKY animals and 25 SHR animals.

†$P > 0.05$ when compared with WKY.

*$P < 0.001$; **$P < 0.02$ when compared with WKY.

Table II: Comparison of cytochrome P-450 and adrenal ferredoxin contents in homogenates prepared from adrenals of SHR and WKY animals.

Component	WKY	SHR
Cytochrome P-450		
nmoles/2 adrenals	3.36 ± 0.18	2.18 ± 0.11*
nmoles/mg adrenal wet wt	0.071 ± 0.003	0.055 ± 0.003*
nmoles/mg adrenal protein	0.230 ± 0.009	0.165 ± 0.009*
Adrenal ferredoxin		
nmoles/2 adrenals	6.32 ± 0.16	4.18 ± 0.09*
nmoles/mg adrenal wet wt	0.126 ± 0.002	0.101 ± 0.002*
nmoles/mg adrenal protein	0.449 ± 0.017	0.317 ± 0.008*

Male rats 10 to 12 weeks of age were used. Values given represent the mean ± S.E.M. of results obtained with 17 WKY animals and 25 SHR animals.

*$P < 0.001$ when compared with WKY.

steroidogenesis is altered in the Wistar-Kyoto spontaneously hypertensive rat: changes have been observed in the secretory rates of corticosterone (8), deoxycorticosterone (8), 18-hydroxydeoxycorticosterone (8), and aldosterone

Table III: Ratios of the specific contents of cytochrome P-450 and of adrenal ferredoxin in mitochondria and microsomes prepared from adrenals of SHR and WKY animals.

Subcellular fraction	Component	SHR/WKY[a]
Mitochondria	cytochrome P-450	0.68 ± 0.08
	adrenal ferredoxin	0.73 ± 0.02
Microsomes	cytochrome P-450	0.94 ± 0.04

[a]Ratios were calculated from the determined nmoles of each component per mg mitochondrial or microsomal protein. Values represent the mean ± S.E.M. of the results of 3 experiments each involving 6-8 male SHR and WKY animals 11 to 11½ weeks of age. Adrenal ferredoxin was not detected in adrenal microsomes.

(7,9) and in the plasma levels of corticosterone following stress (20). In addition, quantitative ultrastructural studies by Nickerson (6) have shown that both the volumes of the cells, mitochondria, and lipid droplets and the surface area of the mitochondrial membranes are significantly less in the zona fasciculata of the SHR adrenal than in the adrenal of WKY animals.

The data reported in the present communication suggest that altered contents of cytochrome P-450 and adrenal ferredoxin in the adrenal of the SHR may be responsible for the altered patterns of adrenal steroidogenesis reported by others. The results of these studies demonstrate that the total contents of both cytochrome P-450 and adrenal ferredoxin in the adrenal of the SHR are significantly reduced at 10 to 12 weeks of age. Cytochrome P-450 has been shown to catalyze the side-chain cleavage of cholesterol (10) and the steroid 11β- and 18-hydroxylase activities in adrenocortical mitochondria (11,12), and adrenal ferredoxin is required for these cytochrome P-450-catalyzed steroid hydroxylations (14,15). Cytochrome P-450 also catalyzes the 21-hydroxylation of progesterone in rat adrenocortical microsomes (13). Adrenal ferredoxin, however, is not required for this

microsomal hydroxylation (15). The fact that the specific contents of both cytochrome P-450 and adrenal ferredoxin are significantly reduced in the mitochondrial compartment of the SHR adrenal suggests that the activities of one or more mitochondrial steroid hydroxylases are altered. Further studies are necessary to determine which activities are affected.

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