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Hepatic microsomal oxidative drug metabolism in the spontaneously hypertensive rat

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One of the most extensively studied animal models of human essential hypertension is the spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki [1] in 1962–1963 by means of selective inbreeding in a colony of Wistar rats. In 1974, Vainionpaa *et al.* [2] reported that the *in vivo* metabolism of hexobarbital, the *in vitro* oxidative metabolism of aminopyrine or of 3,4-benzpyrene, or the content of cytochrome P-450 in hepatic microsomes was not altered in SHR in comparison with normotensive Wistar rats. Willis and Queener [3], however, subsequently reported that pentobarbital sleeping times were significantly shorter in 10- to 14-week-old SHR than in Wistar–Kyoto rats (WKY), the normotensive control for SHR [1], and Hall *et al.* [4] have reported similar observations. More recently, Yates *et al.* [5] observed that the NADPH–cytochrome *c* reductase activity was slightly greater in hepatic microsomes of saline-pretreated SHR in comparison with the activity catalyzed by hepatic microsomes prepared from saline-pretreated normotensive Wistar rats. In an attempt to gain further insight into the effects of hypertension on the hepatic microsomal oxidative metabolism of xenobiotics, the present investigation was conducted using male SHR that were either developing hypertension or in a period of sustained hypertension. A preliminary account of this work has been published [6].

Male SHR were obtained from an inbred colony maintained at The University of Iowa and were of the forty-third through the forty-fifth generations when tracked back to the original pairing that resulted in the derivation of the strain. Male WKY were from a colony maintained in these facilities under the same conditions as were SHR. WKY are descended directly from a colony of Wistar–Kyoto rats from which SHR were originally isolated and are presently considered to be the most appropriate control for SHR [7]. Systolic arterial blood pressures of 10- to 13-week-old and 20- to 22-week-old unanesthetized animals were determined by a modification of the tail plethysmographic method described by Friedman and Freed [8]. Prior to pressure measurements, the rats were warmed to 35° for several minutes, and systolic arterial blood pressures were determined using an automated cuff-inflator-pulse detection system manufactured by Technilab Instruments (Pequannock, NJ). For each determination, three to five consecutive measurements were made on each animal that had been previously conditioned to the apparatus.

For the determination of hexobarbital sleeping times, hexobarbital was dissolved in saline made alkaline by the dropwise addition of 1 N NaOH and was administered intraperitoneally to 10- to 13-week-old animals at a dose of 200 mg/kg. The duration of sleep was determined from the time at which the righting reflex was lost to the time at which the rats regained the righting reflex. Upon regaining the righting reflex, rats were decapitated and trunk blood was collected. The plasma levels of hexobarbital were then determined as described by Cooper and Brodie [9].

Rats were fasted for 24 hr and then were decapitated. Livers were excised after perfusion *in situ* with ice-cold 0.154 M NaCl, and 10% (w/v) homogenates were prepared in ice-cold 0.25 M sucrose. The hepatic microsomal fraction was then isolated as described by Master *et al.* [10]. Protein was determined by the biuret method using bovine serum albumin as the standard [11].

Hepatic microsomal NADPH–cytochrome *c* reductase activity was determined at 25° as described by Masters *et al.* [10]. The *N*-demethylation of ethylmorphine catalyzed by hepatic microsomes was determined at 25° by measuring the rate of formation of formaldehyde employing the method of Nash [12] as modified by Cochin and Axelrod [13]. Each 7-ml reaction mixture contained 14 mg of microsomal protein, 8 mM ethylmorphine, 150 mM KCl, 10 mM $MgCl_2$, and 50 mM Tris–HCl buffer, pH 7.4. After the addition of 200 μM NADPH, 1-ml aliquots were removed every 30 sec for the determination of formaldehyde. Hepatic microsomal aniline hydroxylase activity was determined at 25° by measuring the rate of formation of *p*-aminophenol according to the method of Schenkman *et al.* [14]. The *O*-demethylation of *p*-nitroanisole catalyzed by

Table 1. Systolic arterial blood pressures, hexobarbital sleeping times, and plasma concentrations of hexobarbital upon awakening in 10- to 13-week-old male SHR and WKY*

	Systolic arterial blood pressure (mm Hg; N = 14)	Hexobarbital sleeping time (min; N = 5)	Plasma concentration of hexobarbital upon awakening (μg/ml; N = 4)
SHR	169 ± 6†	41 ± 5‡	50.2 ± 2.9
WKY	128 ± 3	78 ± 11	57.4 ± 4.1

* Each value is the mean ± S.E. of the number of determinations indicated in parentheses.
† $P < 0.001$, when compared to WKY.
‡ $P < 0.01$, when compared to WKY.

hepatic microsomes was determined spectrophotometrically at 25° as described by Buening and Franklin [15], and the *O*-deethylation of 7-ethoxyresorufin was determined fluorometrically at 37° as described by Burke and Mayer [16, 17]. All enzymatic activities were linear with time and were directly proportional to the amount of microsomal protein used in the reaction mixtures.

Optical absorbance difference spectra were recorded at 25° with an Aminco DW-2 spectrophotometer in the split beam mode using microsomal suspensions which had been diluted to 1–2 mg protein/ml with 0.1 M potassium phosphate buffer, pH 7.4. The contents of cytochromes P-450 and b_5 in the hepatic microsomal suspensions were determined from carbon monoxide and NADH-reduced minus oxidized difference spectra, respectively, as described by Omura and Sato [18, 19]. Ethylmorphine-induced type I and aniline-induced type II binding spectra were determined as described by Schenkman *et al.* [14, 20]. Apparent spectral dissociation constants (K_s) and theoretical maximal absorbance changes (ΔA_{max}) were calculated from regression lines on double reciprocal plots of the change in absorbance as a function of substrate concentration using the wavelength pairs 388–422 nm for the type I spectral change and 430–394 nm for the type II spectral change.

At 10–13 weeks of age, the male SHR exhibited significantly greater systolic arterial blood pressures than did male WKY (Table 1). The data presented in Table 1 further demonstrate that 10- to 13-week-old male SHR exhibited significantly shorter hexobarbital sleeping times than did age-paired male WKY. Differences were not observed, however, in the plasma concentrations of hexobarbital in SHR and WKY upon awakening (Table 1), indicating that the shortened hexobarbital sleeping times in SHR may have resulted from an enhanced rate of hexobarbital metabolism. To investigate this possibility, variables associated with *in vitro* hepatic microsomal oxidative drug metabolism were examined. As seen from the data presented in Table 2, although the liver wet weights and the yields of hepatic microsomal protein were similar in SHR and WKY, the content of cytochrome P-450 was slightly, but significantly, greater in hepatic microsomes prepared from 10- to 13-week-old SHR. Significant differences were not observed in either the hepatic microsomal cytochrome b_5 content or NADPH–cytochrome *c* reductase activity (Table 2). While

Table 2. Body weights, liver weights, yields of microsomal protein, hepatic microsomal cytochromes P-450 and b_5 contents, and enzymatic activities of male SHR and WKY*

	4-week-old		10- to 13-week-old		20- to 22-week-old	
Variable	SHR	WKY	SHR	WKY	SHR	WKY
Body weight (g)	49 ± 5	59 ± 2	226 ± 6	245 ± 6	314 ± 10	347 ± 12
Liver wet weight (g)	2.4 ± 0.2	2.6 ± 0.2	8.2 ± 0.2	8.3 ± 0.4	9.4 ± 0.3	8.9 ± 0.3
Yield of microsomal protein (mg/g liver)	8.1 ± 1.2	8.0 ± 0.9	10.7 ± 0.6	11.2 ± 0.5	10.0 ± 0.7	11.2 ± 0.9
Cytochrome P-450 (nmoles/mg protein)	0.52 ± 0.02	0.60 ± 0.03	0.83 ± 0.02†	0.74 ± 0.02	0.87 ± 0.06	0.84 ± 0.06
Cytochrome b_5 (nmoles/mg protein)	0.33 ± 0.02	0.32 ± 0.03	0.42 ± 0.01	0.40 ± 0.01	0.46 ± 0.04	0.50 ± 0.03
NADPH–cytochrome *c* reductase activity [nmoles cytochrome *c* reduced · min^{-1} · mg $protein^{-1}$]	122 ± 13	118 ± 8	118 ± 10	111 ± 8	123 ± 11	122 ± 13
Ethylmorphine *N*-demethylase activity [nmoles HCHO formed · min^{-1} · mg $protein^{-1}$]	3.4 ± 0.4	3.9 ± 0.4	5.7 ± 0.4‡	4.1 ± 0.3	6.8 ± 0.6	5.8 ± 0.6
p-Nitroanisole *O*-demethylase activity [nmoles *p*-nitrophenol formed · min^{-1} · mg $protein^{-1}$]			1.34 ± 0.07§	1.11 ± 0.06		
Aniline hydroxylase activity [nmoles *p*-aminophenol formed · min^{-1} · mg $protein^{-1}$]			0.21 ± 0.01	0.23 ± 0.01		
7-Ethoxyresorufin *O*-deethylase activity [nmoles resorufin formed · min^{-1} · mg $protein^{-1}$]			0.076 ± 0.015	0.083 ± 0.014		

* Each value is the mean ± S.E. of at least five experiments.
† $P < 0.01$, when compared to age-paired WKY.
‡ $P < 0.005$, when compared to age-paired WKY.
§ $P < 0.05$, when compared to age-paired WKY.

Table 3. Type I spectral change produced by the addition of ethylmorphine to hepatic microsomes prepared from male SHR and WKY*

Age	SHR	WKY
4-Week-old male rats (N = 4)		
ΔA_{max}/2 mg protein	0.012 ± 0.001	0.011 ± 0.001
ΔA_{max}/nmole cytochrome P-450	0.011 ± 0.001	0.010 ± 0.001
K_s (μM)	59 ± 5	50 ± 3
10- to 13-Week-old male rats (N = 5)		
ΔA_{max}/2 mg protein	0.029 ± 0.002†	0.019 ± 0.002
ΔA_{max}/nmole cytochrome P-450	0.017 ± 0.001‡	0.013 ± 0.001
K_s (μM)	58 ± 11	50 ± 7
20- to 22-Week-old male rats (N = 4)		
ΔA_{max}/2 mg protein	0.024 ± 0.002	0.024 ± 0.004
ΔA_{max}/nmole cytochrome P-450	0.015 ± 0.001	0.015 ± 0.001
K_s (μM)	56 ± 5	52 ± 2

* Each value is the mean ± S.E. of the number of determinations indicated in parentheses.

† <0.01, when compared to age-paired WKY.

‡ <0.05, when compared to age-paired WKY.

the hepatic microsomal content of cytochrome P-450 was found to be only about 12 per cent greater, the hepatic microsomal ethylmorphine *N*-demethylase activity was approximately 40 per cent greater and the hepatic microsomal *p*-nitroanisole *O*-demethylase activity was approximately 20 per cent greater in 10- to 13-week-old SHR (Table 2). In contrast, significant differences in either the hepatic microsomal aniline hydroxylase or 7-ethoxyresorufin *O*-deethylase activities were not observed between 10- to 13-week-old male SHR and WKY (Table 2). Since at least two cytochromes P-450 mediate the hepatic microsomal oxidative *O*-demethylation of *p*-nitroanisole [21] and since the hepatic microsomal oxidative metabolism of ethylmorphine and 7-ethoxyresorufin is mediated by different forms of cytochrome P-450 [16, 17], these observations suggest that there is an increased content of only a limited number of cytochromes P-450 in hepatic microsomes of 10- to 13-week-old male SHR.

Having demonstrated that the hepatic microsomal monooxygenations of certain xenobiotics are indeed altered in male SHR at 10 to 13 weeks of age, heaptic microsomal oxidative drug metabolism was next investigated in 4-week-old and 20- to 22-week-old animals to determine if these alterations were dependent upon the severity of the hypertensive condition. At 4 weeks of age, the systolic arterial blood pressures of SHR maintained in this colony are only slightly (i.e. 12 mm Hg) greater than those of age-paired WKY [22]. Thus, 4-week-old SHR are in the initial stages in the development of the hypertensive condition. At this age, differences were not observed between male SHR and WKY in either body weight, liver wet weight, or yield of hepatic microsomal protein (Table 2). The data presented in Table 2 further show that significant differences were not observed between SHR and WKY in either the hepatic microsomal contents of cytochromes P-450 and b_5 or in the hepatic microsomal ethylmorphine *N*-demethylase and NADPH–cytochrome *c* reductase activities. Similar results were obtained when 20- to 22-week-old SHR and WKY were employed (Table 2), although SHR at this age are in a period of sustained hypertension (systolic arterial blood pressures of 197 ± 4 mm Hg) which continues until death [23].

To determine if the greater rate of ethylmorphine *N*-demethylation catalyzed by hepatic microsomes prepared from 10- to 13-week-old male SHR resulted from an enhanced interaction of ethylmorphine with oxidized cytochrome P-450, the type I spectral change produced by the addition of ethylmorphine to hepatic microsomes was examined. The data presented in Table 3 show that the theoretical maximal absorbance change (ΔA_{max}) calculated for the ethylmorphine-induced type I binding spectrum was significantly greater in hepatic microsomes prepared from 10- to 13-week-old SHR. The difference in ΔA_{max} between SHR and WKY was significant when expressed both on the basis of microsomal protein (53 per cent greater in SHR) and on the basis of the hepatic microsomal cytochrome P-450 content (31 per cent greater in SHR). Although the ΔA_{max} was greater in SHR, a significant difference was not found in the apparent K_s value determined for the interaction of ethylmorphine with ferric cytochrome P-450 in SHR and WKY. In contrast to the enhanced interaction observed between ethylmorphine and cytochrome P-450 in hepatic microsomes prepared from SHR, significant differences were not found in either the ΔA_{max} or the apparent K_s value determined for the interaction of aniline with oxidized hepatic microsomal cytochrome P-450 (data not presented). Consistent with the observation that alterations in hepatic microsomal oxidative drug metabolism in male SHR are age-dependent, differences were not observed between male SHR and WKY at either 4 or 20–22 weeks of age in the theoretical maximal absorbance change (ΔA_{max}) or in the apparent K_s value determined for the interaction of ethylmorphine with hepatic microsomal cytochrome P-450 (Table 3).

The results of this study are in disagreement with those of Yates *et al.* [5] who did not observe a significant difference in the content of hepatic microsomal cytochrome P-450 between 10- to 14-week-old saline-pretreated male SHR and normotensive Wistar rats. However, Yates *et al.* did observe a greater hepatic microsomal NADPH–cytochrome *c* reductase activity in the saline-pretreated SHR. This discrepancy may be due to the use of different controls in the two studies: Yates *et al.* [5] employed an inbred Wistar rat as the control for SHR whereas WKY were employed as the SHR control in the present study.

In summary, the findings of the present study indicate that essential hypertension is not always associated with alterations in hepatic microsomal xenobiotic monooxygenase activity, but rather, the hepatic microsomal oxidative metabolism of certain xenobiotics may only be altered during certain stages in the development of the hypertensive condition. This relationship would explain the discrepancies between the findings of Vainionpaa *et al.* [2] who used older SHR and of Willis and Queener [3] and Yates *et al.* [5] who used 10-to 14-week-old SHR.

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