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SEX-DEPENDENT DIFFERENCES IN THE EFFECTS OF PORTACAVAL ANASTOMOSIS ON HEPATIC MONOOXYGENASES IN RATS

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Abstract—Previous work revealed that portacaval anastomosis (PCA) in rats results in hepatic atrophy and marked decreases in components of the microsomal monooxygenase system such as cytochrome P-450. In the present study, the effects of PCA on hepatic monooxygenase activity were studied in more detail. We report that PCA, in general, produces effects resembling those of castration. Thus, in male rats, PCA depressed the activity of highly sex-dependent enzymes such as ethylmorphine and aminopyrine demethylases. Similar effects were produced by castration, and the combination of PCA and castration produced the same effect as either treatment alone. In male rats, non-sex-dependent enzymes such as aniline hydroxylase and NADPH–cytochrome *c* reductase were unaffected by either PCA or castration. By contrast, in female rats, neither PCA nor castration significantly affected microsomal monooxygenase activities. In male rats, PCA was accompanied by a 75% reduction in serum testosterone levels and a 6-fold increase in total estrogen levels. We conclude that these effects of PCA in male rats were due, in large measure, to a demasculinizing effect.

A sex difference in the response to certain drugs in rats has been known for several decades [1, 2]. Thus, following hexobarbital administration, longer sleeping times were observed in female than in male rats [3]. This phenomenon has largely been accounted for by the lower rates of metabolism in the female, which prolong the biological effects of the drug.

The magnitude of the sex difference in microsomal enzymes is dependent on the substrate employed [4]. Accordingly, hexobarbital and aminopyrine are metabolized more rapidly (two to four times) by microsomes from male rats than those from female rats, but no sex difference is observed in the metabolism of substrates such as aniline and zoxazolamine [4].

The sex difference in drug metabolism has been found to be androgen dependent, i.e. it can be abolished by castrating male rats and is restored by testosterone administration [3]. Although castration of female rats is without effect on drug metabolism, administration of estrogen to intact males impairs metabolism [5], presumably by antagonizing the effects of testosterone.

Diversion of the portal blood supply from the liver (portacaval anastomosis; PCA) in rats has been shown previously to result in hepatic atrophy, hepatocellular necrosis, and a reduction in certain aspects of the microsomal monooxygenase system [6–8]. Pector *et al.* [8] showed that the changes in hepatic function that follow PCA, such as impaired clearance of colloidal gold and bromsulfophthalein and reduced hepatic levels of cytochrome P-450, are not merely the result of ischemic hepatocellular necrosis. Anastomosis of a renal artery with the hepatic stump of the portal vein (hepatic "arterialization") prevented hepatic atrophy and cellular fibrosis and necrosis but had no effect on the decline of hepatic cytochrome P-450 produced by PCA. Portal blood, therefore, appears to be an essential factor for maintaining the hepatic monooxygenase system.

Preliminary work in our laboratory suggested that the effects of PCA on the hepatic monooxygenase system were more selective than previously realized. Examination of a number of microsomal drug metabolism variables in male rats suggested that the activities of highly sex-dependent enzymes such as ethylmorphine demethylase, aminopyrine demethylase, and aryl hydrocarbon hydroxylase (benzo[*a*]pyrene hydroxylase) (AHH) were markedly impaired after PCA, whereas non-sex-dependent enzymes such as biphenyl 4-hydroxylase and NADPH–cytochrome *c* reductase were unaffected by PCA. These findings suggested that the effects of PCA on the monooxygenase system in male rats resembled those of castration, adrenalectomy, starvation and a number of other manipulations, namely to reduce the magnitude of the sex difference by causing selective depression of the highly sex-dependent enzymes in the male to levels similar to those observed in normal females. This hypothesis was supported by the finding that male rats subjected to PCA exhibited impaired hepatic metabolism of estrogen and testicular atrophy [9]. These findings suggested that the effects of PCA on sex-dependent monooxygenases in male rats were due, in part, to a demasculinizing effect.

The purpose of the present investigation was to

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test this hypothesis by comparing the effects of castration and PCA, individually, and in combination, on microsomal monooxygenase activities in male and female rats.

MATERIALS AND METHODS

Animals. Male and female Sprague–Dawley rats (225–250 g), purchased from Taconic Farms, New York, were fed Purina laboratory chow and tap water *ad lib.* End-to-side portacaval anastomosis (PCA) was performed by the procedure of Lee and Fisher [10] using 7–0 silk and a continuous suture technique. Castration or ovariectomy was performed concurrently as necessary.

Two weeks after surgery, blood was collected by heart puncture between 8:00 and 9:00 a.m., and the serum was stored at −40° until assayed. The animals were killed by cervical dislocation and livers were rapidly excised and placed on ice. Liver homogenates (20%) were prepared in cold 150 mM KCl–50 mM Tris buffer (pH 7.4) containing 1 mM EDTA, in a Potter-type homogenizer with a motor-driven Teflon pestle and were centrifuged at 9000 *g* for 20 min. The supernatant fraction was further spun at 105,000 *g* for 60 min. The resultant microsomal fraction was resuspended in the homogenizing medium.

Biochemical measurements. NADPH–cytochrome *c* reductase activity was assayed by the method of Williams and Kamin [11] as described by Gigon *et al.* [12]. Microsomal cytochromes P-450 and b_5 were determined by the methods of Omura and Sato [13]. The *N*-demethylation of ethylmorphine and aminopyrine was estimated by assay of formaldehyde [14], and aniline hydroxylase activity by measurement of *p*-aminophenol [15].

Benzo[*a*]pyrene hydroxylase (AHH) activity was measured by the method of Nebert and Gelboin [16] using 3-hydroxybenzo[*a*]pyrene (provided by Dr. Harry Gelboin, NCI, NIH) as standard.

Plasma hormonal levels were measured by radioimmunoassay using [^{3}H]estrogen and [^{3}H]testosterone, Radioimmunoassay Pak, New England Nuclear Corp. (Boston, MA). Microsomal protein was measured according to the method of Lowry *et al.* [17] with bovine serum albumin as standard. Statistical comparisons were made using Student's *t*-test. $P < 0.05$ was considered to be significant.

RESULTS

It is clear from Table 1 that castration of male or female rats had no significant effect on either liver or body weight, whereas PCA significantly reduced both variables in both sexes. The loss in body weight following PCA has been suggested to result from inanition while the etiology of the hepatic atrophy, although abundantly documented [6–8], remains controversial. The combined effects of PCA and castration were similar to those produced by PCA alone (Table 1).

NADPH–cytochrome *c* reductase [18] and cytochrome b_5, two non-sex-dependent components of the microsomal electron transfer chains, were not significantly affected by PCA or castration, in male or female rats (Table 2). Cytochrome P-450 levels, shown by others to exhibit a small but statistically significant sex difference [18–20], responded similarly to the two treatments in both sexes (Table 2). Thus, PCA decreased cytochrome P-450 by 40% in intact male rats and 20% in female rats, but castration was without effect on this parameter.

The effects of PCA and castration on drug oxidation in male rats were correctly predicted by our hypothesis. Ethylmorphine and aminopyrine demethylases as well as AHH activity, which are sex-dependent enzymes, were all reduced dramatically (35–70%) in male rats by PCA, castration, and PCA plus castration (Table 3). The combined effects of PCA and castration were similar to the effects of PCA or castration alone. By contrast, aniline hydroxylase, an enzyme which displays no sex difference [4], was unaffected by these procedures, alone or in combination.

In female rats, as expected, none of the mono-

Table 1. Effects of PCA on liver and body weights, and on microsomal protein in rats*

	Male rats			
	Control	PCA	Castration	Castration and PCA
Body weight (g)	320 ± 7	216 ± 9†	296 ± 6	208 ± 13†
Liver weight (g)	11.47 ± 0.50	4.73 ± 1.12†	11.00 ± 0.61	4.98 ± 1.11†
Liver/body weight × 10^2	3.80 ± 0.05	2.25 ± 0.16†	3.72 ± 0.05	2.42 ± 0.24†
Microsomal protein (mg/g liver)	28.0 ± 1.0	23.8 ± 0.9†	26.8 ± 0.7	25.5 ± 0.8
	Female rats			
	Control	PCA	Castration	Castration and PCA
Body weight (g)	303 ± 4	234 ± 10†	296 ± 12	253 ± 17†
Liver weight (g)	7.85 ± 1.24	4.01 ± 0.82†	8.11 ± 1.53	4.11 ± 0.91†
Liver/body weight × 10^2	3.14 ± 0.16	1.73 ± 0.10†	3.07 ± 0.22	1.63 ± 0.13†
Microsomal protein (mg/g liver)	23.8 ± 1.5	23.3 ± 2.1	23.7 ± 1.3	24.1 ± 1.0

* Values are means ± S.E. (N = 12).
† $P < 0.001$ vs control animals.

Table 2. Effects of PCA on the hepatic microsomal mixed function oxidase system in rats*

		Intact rats		Castrated rats	
		Control	PCA	Control	PCA
NADPH–cytochrome *c* reductase†	M	192.9 ± 6.0	198.6 ± 10.0	172.8 ± 7.6	167.8 ± 7.8
	F	169.9 ± 12.3	157.6 ± 8.7	164.3 ± 8.2	154.1 ± 6.1
Cytochrome P-450‡	M	0.821 ± 0.24	0.501 ± 0.017§	0.688 ± 0.028	0.471 ± 0.022§
	F	0.713 ± 0.029	0.561 ± 0.019§	0.730 ± 0.031	0.591 ± 0.040§
Cytochrome b_5‡	M	0.475 ± 0.008	0.447 ± 0.011	0.444 ± 0.010	0.429 ± 0.013
	F	0.466 ± 0.013	0.419 ± 0.023	0.435 ± 0.012	0.441 ± 0.002

* Values are means ± S.E. (N = 12). M: male; F: female.
† Expressed in nmoles·min^{-1}·(mg protein)$^{-1}$.
‡ Expressed in nmoles/mg protein.
§ $P < 0.001$ vs control animals.

Table 3. Effects of PCA on hepatic microsomal drug metabolism in rats*

		Intact rats		Castrated rats	
		Control	PCA	Control	PCA
Ethylmorphine demethylation†	Male	12.01 ± 0.30	5.35 ± 0.37‡	6.09 ± 0.45	4.18 ± 0.36
	Female	2.41 ± 0.10	2.20 ± 0.17	1.59 ± 0.05	1.93 ± 0.10
Aminopyrine demethylation†	Male	10.70 ± 0.30	6.99 ± 0.28‡	7.19 ± 0.32	6.33 ± 0.36
	Female	4.86 ± 0.21	4.31 ± 0.28	3.83 ± 0.30	4.17 ± 0.26
Benzo[*a*]pyrene hydroxylation§	Male	516 ± 24	144 ± 24‡	272 ± 28	140 ± 12‡
	Female	50 ± 8	50 ± 10	22 ± 2	21 ± 3
Aniline hydroxylation†	Male	0.61 ± 0.02	0.61 ± 0.05	0.51 ± 0.03	0.54 ± 0.05
	Female	0.67 ± 0.02	0.71 ± 0.04	0.51 ± 0.03	0.58 ± 0.03

* Values are means ± S.E. (N = 12).
† Expressed in nmoles·min^{-1}·(mg protein)$^{-1}$.
‡ $P < 0.002$ vs control.
§ Expressed in pmoles·min^{-1}·(mg protein)$^{-1}$.

oxygenase activities were affected significantly by PCA (Table 3). On the other hand, ovariectomy reduced enzyme activity by 20–50%, and similar small changes were seen in animals subjected to ovariectomy plus PCA.

Circulating levels of sex hormones were altered dramatically by the two surgical procedures. In male rats, serum testosterone levels were reduced 75% by PCA, 95% by castration, and to an intermediate level by the combined manipulations (Table 4). Total estrogen levels increased about 6-fold in males following PCA or PCA plus castration, but were unaffected by castration alone.

DISCUSSION

Data presented in this manuscript support the view that the effects of PCA on microsomal drug metabolism in rats resemble those produced by castration. Thus, like the effects of castration, the effects of PCA were observed only in males and were expressed only on enzymes which are highly sex-dependent. A reflection of this similarity can be found in the effects of the two procedures on plasma testosterone levels in males. PCA produced a reduction of 75%, while castration evoked a 95% decrease. Following PCA in males, the 75% reduction in

Table 4. Effect of PCA on circulating serum hormonal levels in rats*

	Testosterone (ng/ml)	Total estrogen (pg/ml)	
	Male	Male	Female
Intact	4.03 ± 0.38	7 ± 4	142 ± 45
Castration	0.14 ± 0.06†	8 ± 3	80 ± 20
PCA	1.07 ± 0.63†	40 ± 12†	353 ± 121†
Castration and PCA	0.58 ± 0.39†	46 ± 13†	228 ± 84

* Values are means ± S.E. (N = 12).
† $P < 0.001$ vs intact animals.

plasma testosterone was accompanied by a 6-fold increase in total plasma estrogen. Whether the relationship between these alterations is causal or not, the net result would certainly be a demasculinization of the animal and reduction in the activities of hepatic enzymes that are androgen-dependent.

Similarities between the effects of PCA and those of castration were most obvious in male rats. Thus, both procedures, independently or in combination, impaired to about the same extent the highly sex-dependent enzymes—ethylmorphine demethylase, aminopyrine demethylase, benzo[*a*]pyrene hydroxylase (AHH) and cytochrome P-450—but had no effect on the sex-independent enzymes—NADPH–cytochrome *c* reductase, cytochrome b_5 and aniline hydroxylase (Tables 2 and 3). The changes in sex-dependent enzymes were accompanied by marked increases in serum estrogen coupled with reductions in serum testosterone.

PCA produced no significant effects on monooxygenase activity in female rats (Table 3). However, ovariectomy alone produced a small but significant (20–50%) impairment of monooxygenase activity in female rats. The effect of castration alone or in combination with PCA was most pronounced on AHH, the activity of which was reduced by about 50%. With that exception, the effects of castration on monooxygenase activity ranged from 10 to 30%.

Therefore, in male rats, the effects of PCA on microsomal monooxygenases resembled those of castration very closely. In female rats, PCA, as expected, was without effect on microsomal enzymes while castration caused a slight reduction.

In summary, our data indicate that in general terms PCA and castration produced very similar effects on hepatic monooxygenase activities in male and female rats. First, neither surgical procedure significantly affected, in either sex, enzymes known not to be dependent on sex, namely NADPH–cytochrome *c* reductase, cytochrome b_5 and aniline hydroxylase. Second, PCA and castration selectively depressed the highly sex-dependent enzymes in male rats, namely, ethylmorphine demethylase, aminopyrine demethylase, AHH and cytochrome P-450 but did not alter these activities in female rats. The net effects of PCA, like castration, are to selectively depress sex-dependent enzymes, only in male rats, resulting in a narrowing or disappearance of the sex difference.

REFERENCES

1. J. S. Nichols and D. H. Barron, *J. Pharmac. exp. Ther.* **46**, 125 (1932).
2. G. O. Holck, M. A. Kanan, L. M. Mills and E. L. Smith, *J. Pharmac. exp. Ther.* **60**, 323 (1937).
3. G. P. Quinn, J. Axelrod and B. B. Brodie, *Biochem. Pharmac.* **1**, 152 (1958).
4. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
5. R. Kato, *Drug Metab. Rev.* **3**, 1 (1974).
6. E. Rubin, F. Hutterer, J. Ohshiro and J. H. Jacobson, *Proc. Soc. exp. Biol. Med.* **127**, 444 (1968).
7. P. Vassanelli and E. Chiesara, *Archs int. Pharmacodyn. Thér.* **196**, 158 (1972).
8. J. C. Pector, S. Verbeustel and J. P. Lambilliotte, *Digestion* **12**, 144 (1975).
9. E. Dordal, S. Glagov and T. S. Bebanos, *Archs Path.* **83**, 49 (1967).
10. S. H. Lee and B. Fisher, *Surgery* **50**, 668 (1961).
11. C. H. Williams and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
12. P. L. Gigon, T. E. Gram and J. R. Gillette, *Molec. Pharmac.* **5**, 109 (1969).
13. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
14. T. Nash, *Biochem. J.* **55**, 416 (1953).
15. Y. Imai, A. Ito and R. Sato, *J. Biochem., Tokyo* **60**, 417 (1966).
16. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **248**, 6242 (1968).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
18. S. E. D. Masry, G. M. Cohen and G. J. Mannering, *Drug Metab. Dispos.* **2**, 267 (1974).
19. S. E. D. Masry and G. J. Mannering, *Drug Metab. Dispos.* **2**, 279 (1974).
20. G. M. Cohen and G. J. Mannering, *Drug Metab. Dispos.* **2**, 285 (1974).