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Response of NADPH cytochrome c reductase and cytochrome P-450 in hepatic microsomes to treatment with phenobarbital—Differences in rat strains

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During a study [1] on the effect of age on microsomal enzymes, it was observed that the induction of NADPH cytochrome *c* reductase from male Fischer rats treated with phenobarbital was much less pronounced than the induction reported [2–4] for most other strains of rat. The rate of hepatic drug metabolism has been shown to depend on the strain of an animal species [5–8] used for investigation; in addition, pre-exposure to substrates (cf. Refs. 9 and 10) also affects these enzymes. In order to determine the reason for the initial observation, the effects of phenobarbital administration to both Fischer and Sprague–Dawley rats have been investigated. The results reported here suggest that the differences in the basal levels of NADPH cytochrome *c* reductase and the response to phenobarbital are not caused by preinduction, but, instead, are a reflection of differences in the two strains of rat.

Male Fischer (240–250 g) and Sprague–Dawley (260–270 g) rats were maintained in steel cages suspended above pine-chip bedding for 1 week prior to each experiment. Phenobarbital (8 mg/100 g body weight) was injected subcutaneously once each day for the number of days specified. Rats treated with phenobarbital were sacrificed 24 hr after the final injection, and all rats were sacrificed after a 15-hr fast.

Rough and smooth microsomes were separated by centrifugation for 21 hr at 25,000 rev/min on a discontinuous sucrose gradient, essentially as described by Rothschild [11]. Cytochrome P-450 was assayed according to Omura and Sato [12], aminopyrine demethylase was measured at 37° [13] and formaldehyde quantitated by the method of Nash [14]. The isolation of microsomes and procedures for the remaining enzymatic and chemical assays have been described elsewhere [1]. All preparative procedures were carried out at 0–4°, and freshly prepared microsomes were employed for all determinations.

The specific activity of NADPH cytochrome *c* reductase in total microsomes from untreated Fischer rats was double that in microsomes from untreated Sprague–Dawley rats (Table 1); this difference was significant ($P < 0.001$). The concentration of cytochrome P-450 was lower in untreated Fischer rats, but not significantly different from the concentration in microsomes from untreated Sprague–Dawley rats. After 3 days of treatment with phenobarbital, the levels of these enzymes reached their maximum in both strains of rat. After this treatment, the specific activity of NADPH cytochrome *c* reductase in Sprague–Dawley rats increased by 107 per cent over controls. These values are in general agreement with the report of others [3]. In contrast, the same treatment with phenobarbital caused an increase of only 28 per cent in the specific activity of NADPH cytochrome *c* reductase in Fischer rats, which was significantly different ($P < 0.01$) from the results for the Sprague–Dawley rats. The relative induction of cytochrome P-450 in total microsomes was also statistically different ($P < 0.001$) in these two strains; a 146 per cent increase over control values was observed in the Fischer rat and a 48 per cent increase in Sprague–Dawley rats (Table 1).

Rough and smooth microsomes were prepared to determine whether microsomal heterogeneity contributed to these differences. As can be seen from Table 1, both enzymes were approximately 2-fold more concentrated in the smooth microsomal fraction from Fischer rats and, as has been reported [15, 16] from Sprague–Dawley rats. The specific activity of NADPH cytochrome c reductase was higher in both microsomal fractions from untreated Fischer rats than from untreated Sprague–Dawley rats. During induction by phenobarbital, a larger relative increase in NADPH cytochrome *c* reductase activity was observed in both microsomal subfractions from Sprague–Dawley rats, and, again, the relative increase in cytochrome P-450 concentrations was greater in both microsomal subfractions from Fischer rats than from Sprague–Dawley rats.

Red cedar bedding has been reported [17, 18] to contain volatile compounds which enhance the rate of drug metabolism. The possibility that a compound in pine-chip bedding was inducing NADPH cytochrome *c* reductase in Fischer rats was tested by maintaining these animals for 1 week in isolation cages above either pine-chip or autoclaved cotton bedding. All volatile, high molecular weight compounds would have been removed from the autoclaved cotton by steam distillation. No difference (less than 6 per cent) was observed between these groups of Fischer rats

Table 1. Comparison of specific enzymatic activity of microsomal NADPH cytochrome *c* reductase and cytochrome P-450 from untreated rats and rats treated with phenobarbital*

Strain of rat	Microsomal fraction	No. of injections with phenobarbital	NADPH cytochrome *c* reductase (μmoles cytochrome *c* reduced/min/mg protein at 25°)	Cytochrome P-450 (nmoles/mg protein)
Fischer	Total	0	0·135 ± 0·019 (6)	0·585 ± 0·083 (4)
	Total	3	0·173 ± 0·019 (4)	1·48 ± 0·15 (4)
	Smooth	0	0·17 (2)	0·87 (2)
	Smooth	3	0·20 (2)	1·8 (2)
	Rough	0	0·082 (2)	0·28 (2)
	Rough	3	0·10 (2)	0·88 (2)
Sprague–Dawley	Total	0	0·067 ± 0·026 (6)	0·754 ± 0·16 (3)
	Total	3	0·139 ± 0·038 (6)	1·13 ± 0·14 (5)
	Smooth	0	0·12 (6)	0·93 (2)
	Smooth	3	0·17 (2)	1·6 (2)
	Rough	0	0·065 (2)	0·42 (2)
	Rough	3	0·090 (2)	0·55 (2)

* Numbers represent the mean ± standard deviation. Two animals were used for each experiment and the number of experiments is in parentheses. The injection schedule is described in Methods.

in either the specific activity of NADPH cytochrome *c* reductase or the concentration of cytochrome P-450.

Several other effects of phenobarbital treatment were assayed. No strain-related difference ($P > 0.1$) was observed either for the relative increase in liver weight or the induction of aminopyrine demethylase. Microsomal protein, corrected for preparative losses using glucose 6-phosphatase as a marker, increased by 25 per cent in both strains; this increase in microsomal protein is known to be mainly a proliferation of smooth endoplasmic reticulum [15, 16]. The specific activity of glucose 6-phosphatase and NADH cytochrome *c* reductase decreased by 36–50 per cent in each strain during treatment with phenobarbital, as reported by others [1, 16, 19, 20].

The higher specific activity of NADPH cytochrome *c* reductase in untreated Fischer rats and the relatively poor inducibility of this enzyme after phenobarbital administration thus appeared to be a strain-related variable. The increase in cytochrome P-450, aminopyrine demethylase activity, liver weight and microsomal protein after phenobarbital administration, and the decrease of NADH cytochrome *c* reductase and glucose 6-phosphatase activity suggest that the animals were neither preinduced nor resistant to phenobarbital. Inadvertent preinduction by the bedding was ruled out. In addition, the strain-related differences observed for NADPH cytochrome *c* reductase and cytochrome P-450 in total microsomes from Fischer and Sprague–Dawley rats were also observed in both microsomal subfractions. It appears, therefore, that these functionally linked enzymes of the NADPH electron transport chain of microsomes [9, 21, 22], which proliferate differently during development [23], are also regulated in an independent or non-concerted manner in normal rats and rats treated with phenobarbital.

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