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Effect of enzyme imprinting of liver microsomal monooxygenases upon lifespan of rats

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Abstract

Effect of the enzyme imprinting by phenobarbital upon alterations of hepatic microsomal monooxygenase activities and lifespan of Wistar rats has been studied. Phenobarbital-sodium (3.5 mg/100 g body weight per day, i.p.) was injected during 1–3 days after birth. This resulted in the enzyme imprinting of the liver microsomal monooxygenases, however, this effect being observed in female but not male rats. In the phenobarbital treated female rats of different age the duration of sleeping time was significantly lower than that in control animals, whereas it did not differ substantially in male rats. The cytochrome P-450 content increased by 34.5% in phenobarbital treated female rats in the age of 12 months in comparison with control animals. A mean lifespan of experimental female rats increased by 17.5% compared to the level of control animals and did not change in male rats. The analysis of survival of animals in Gompertz equation coordinates showed that enzyme imprinting by phenobarbital caused changes in the mortality patterns at different stages of ontogenesis in experimental female but not male rats. An inverse correlation was found between the duration of pentobarbital sleeping time and lifespan of female and male rats.

Keywords: Neonatal imprinting; Phenobarbital; Microsomal monooxygenase; Lifespan

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1. Introduction

The phenomenon of enzyme imprinting is well known. It was shown that the treatment of neonatally castrated male and female rats by testosterone propionate resulted in irreversible induction of the liver microsomal testosterone metabolizing enzymes (Gustafsson and Stenberg, 1974). Diethylstilbestrol treatment for days 2, 4 and 6 post partum resulted in decreased histidase activities in adult female rats (Lamartiniere, 1979). It was ascertained that treatment of newborn animals by enzyme inductors resulted in alterations of enzyme activities which were maintained over a period of several months. Daily injections of cortisol to rats during 2 weeks from the first day of birth gave rise to the induction of tyrosine aminotransferase activity in adulthood. The treatment of newborn mice by acetate-16a-isothiocyanopregnenolon (mixed-function oxydase inductor) resulted in the stable increase of arylhydrocarbon hydroxylase activity and this effect retained during 8 months (Salganik et al., 1982). Phenobarbital treatment of pregnant female mice initiated prolonged 30% rise of *p*-nitroanisole demethylase activity in the liver of offspring (Janai, 1979). In adult male rats after neonatal injection of phenobarbital the blood testosterone concentration and pathways of testicular androgenesis regulation were altered (Wani et al., 1996). It was shown that hyperoxia results in the induction of cytochrome P-450 sex-specific isoforms (Okamoto et al., 1993). Neonatal exposure to hyperoxia leads to feminization of male rats at the expense of a positive imprinting of testosterone metabolising cytochrome P-450 isoforms (Kikkawa et al., 1994).

In the publications referred to an increase of enzyme activities after neonatal injection of specific inductors was observed only during some weeks or months after inductors treatment. The investigations concerning an influence of enzyme imprinting upon mortality and lifespan of animals were not performed earlier. Recently it was shown that in Wistar rats with a high level of hepatic microsomal oxidation the lifespan is more prolonged versus animals with a low level of monooxygenases (Paramonova, 1989). All these data determined the goal of the present investigation—to study the influence of enzyme imprinting by phenobarbital upon the changes of liver microsomal monooxygenase activities during a whole lifetime, survival and lifespan.

2. Materials and methods

The experiments were performed with the use of 150 Wistar rats. Phenobarbital-sodium (Merck, Germany, 3.5 mg/100 g body weight per day, i.p.) was injected during 1–3 days after birth. At the age of 1 month the rats were separated according to sex and placed in cages, five animals in each, to monitor survival or death. The alterations of the liver microsomal oxydation system were assessed either by direct determination of cytochrome P-450 content or duration of pentobarbital sleeping time-pharmacological index which has inverse correlation with

microsomal monooxygenases activities of liver (Gubski, 1972). In a series of longitudinal experiments on the group of female rats aged 3, 5-, 8-, 11- and 20-months the duration of sleeping time was measured. All animals of both sexes which survived until the age of 22 months were subjected to the same investigation. The duration of sleeping time was estimated from the effect of lateral position after an injection of pentobarbital-sodium (2.5 mg/100 g body weight, i.p.) until the appearance of locomotor reactions. A part of the animals was sacrificed at 12 months of age and hepatic microsomes were prepared by differential centrifugation ($105\,000 \times g$). Cytochrome P-450 content was assayed spectrophotometrically according to the method of Omura and Sato (1964), assuming an extinction coefficient of 91 mM/cm. Protein concentrations were determined by the method of Lowry (Lowry et al., 1951). Analysis of survivorship curves was carried out in Gompertz equation coordinates: $R_t = R_0\, e^{\alpha t}$, where R_t is a mortality at t-instant of time, R_0 is hypothetical mortality at $t = 0$, and α is the coefficient of age-related growth of mortality. Results obtained are given as means and standard errors. Distinctions between control and treated groups were evaluated using Student's t-test and nonparametric U-test.

3. Results

The experiments performed showed that the injection of phenobarbital to newborn rats resulted in the enzyme imprinting of liver microsomal monooxygenases, however, this effect was observed in female but not male rats. In the longitudinal investigations it was shown that the duration of the sleeping time in phenobarbital treated female rats of different age was significantly lower than that in control animals (Table 1).

The duration of sleeping time in male rats did not differ substantially neither at the age of 3.5 months (control, 12.5 ± 0.8 min; phenobarbital treated, 13.1 ± 1.1 min; $n = 7$, $P > 0.2$) nor at the age of 11 months (33.4 ± 1.4 and 32.2 ± 1.0 min, respectively; $n = 7$, $P > 0.2$).

Table 1
Duration of sleeping time (min) in female control and neonatally phenobarbital treated rats ($n = 6$)

Group of animals	Age (months)			
	3.5	8	11	20
Control	16.8 ± 2.6	18.6 ± 3.4	48.2 ± 8.6	67.8 ± 4.2
Treated	3.4 ± 0.8**	6.6 ± 1.2*	38.0 ± 9.5	41.1 ± 3.2**

* $P < 0.05$.
** $P < 0.01$.

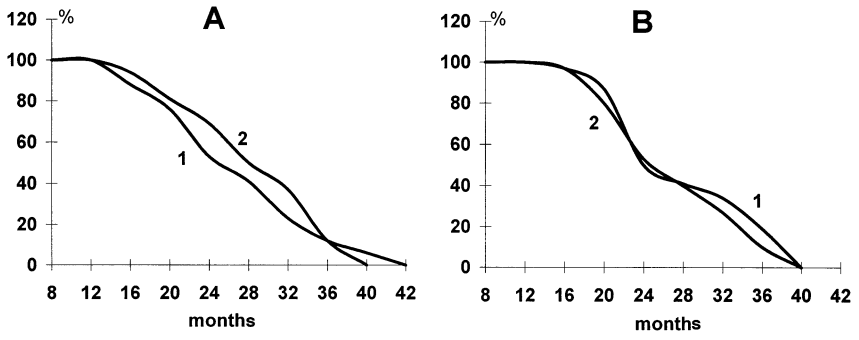

Fig. 1. The influence of neonatal injection of phenobarbital upon lifespan of female (A) and male (B) rats. 1, Control; 2, phenobarbital treated.

The measurement of cytochrome P-450 content in the liver of rats at the age of 12 months showed a 34.5% ($P < 0.02$) increase from 0.406 ± 0.010 nmol/mg (control, $n = 7$) to 0.546 ± 0.008 nmol/mg (phenobarbital treated, $n = 7$) in females and the lack of changes in males (0.862 ± 0.012 nmol/mg and 0.838 ± 0.017 nmol/mg, respectively; $n = 7$, $P > 0.2$). These data correlate with the changes of sleep duration and indicate for a stable rise of the hepatic monooxygenase activity after neonatal injection of phenobarbital in female rats.

In the studies of the survival level it was shown that mean lifespan of the phenobarbital treated female rats increased by 17.5% ($P < 0.05$) in comparison with the level of control animals (27.4 and 23.4 months, respectively). These results are presented in Fig. 1A. At the same time, neonatal injection of phenobarbital did not exert any substantial effect upon the mean lifespan in male rats (Fig. 1B). The mean lifespan in control males was 24.3 months and was 23.9 months in phenobarbital treated ($P > 0.2$). As seen from Fig. 1B, the survival curves practically did not differ until the age of 24 months, after which the mortality of control males rises at a higher rate than in phenobarbital treated animals. In phenobarbital treated females (Fig. 1A) the survival is considerably higher in all stages of ontogenesis and rather diminishes in latest terms of life in comparison with control rats.

Analysis of the survival of animals in Gompertz equation coordinates offers to represent the curves as linear function and to estimate the constant R_0 (the parameter of mortality in early stages of life) and α (the rate of mortality rise in later stages).

The equation of regression $\ln R_t = \ln R_0 + \alpha t$ assumes the form: $\ln R_t = 2.19 + 0.09t$ for control female rats and $\ln R_t = 0.96 + 0.15t$ for phenobarbital treated animals. As is seen in Fig. 2A and equations of regression, phenobarbital treated female rats reveal reduction of mortality rate in early stages of ontogenesis ($\ln R_0 = 0.96 \pm 0.40$) versus control ($\ln = 2.19 \pm 0.18$, $P < 0.02$) and increase of mortality rate in later stages of ontogenesis ($\alpha = 0.15 \pm 0.02$ and $\alpha = 0.09 \pm 0.01$, respectively, $P < 0.02$).

The Gompertz equation for control male rats assumes the form: $\ln R_t = 1.62 + 0.12$ and for phenobarbital treated male rats: $\ln R_t = 2.02 + 0.11t$. Graphical representations of these equations demonstrate that males of control and treated groups do not differ either in duration of lifespan or in age-related dynamics of mortality (Fig. 2B).

Calculation of constants of Gompertz equation allows to conclude that enzyme imprinting by phenobarbital caused changes in the mortality patterns of treated female but not male rats.

For estimation of a relationship between activity of liver microsomal oxidation enzymes and lifespan all survivor rats were tested for duration of pentobarbital sleeping time. The duration of sleeping time in control male rats at the age of 22 months was 54.1 ± 3.2 min and did not differ from the level observed in phenobarbital treated animals, 51.9 ± 4.1 min ($P > 0.2$). At the same time there were significant differences in sleeping time duration in the female rats. Thus, the duration of sleeping time in control females was higher by 52.5% ($P < 0.05$) as compared to phenobarbital treated animals (92.4 ± 4.1 and 43.9 ± 2.7 min, respectively).

An inverse correlation was found between the duration of pentobarbital sleeping time and lifespan of rats ($r = -0.47$ for males, $P < 0.05$; $r = -0.65$ for females, $P < 0.05$).

4. Discussion

The results of the investigation have indicated that the neonatal injection of phenobarbital, an inductor of the microsomal oxydation enzymes, brings about the stable changes of the liver detoxication function and influences essentially the lifespan of female rats. In phenobarbital treated animals, the cytochrome P-450 content increases by 34.5% and the mean lifespan is prolonged by 17.5%. The pattern of mortality also undergoes changes, being especially marked at the ages from 20 to 32 months, when the rate of mortality grows significantly in the control versus phenobarbital treated animals. At the age of 36 months the suvivals of

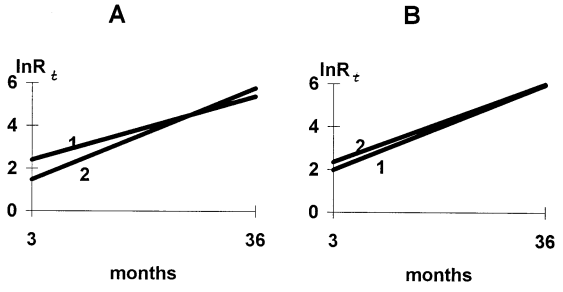

Fig. 2. The survival of female (A) and male (B) rats in Gompertz equation coordinates. 1, Control; 2, phenobarbital treated.

control and phenobarbital treated female rats do not differ. As far as the mean lifespan is a statistical value, individual animals may exhibit rather prolonged lifespan (in the experiments approximately 3% of control population).

In aged animals the duration of pentobarbital sleeping time increased in both the control and treated rats. However, the rats injected with phenobarbital in the neonatal period displayed significantly lower values of this parameter at all age stages. As was shown in previous publications, the values of pentobarbital sleeping time duration had great variabilities and differed in individual rats, particularly in females, from 20 to 180 min. The lifespan in 'short-term sleeping' rats was more prolonged than in the 'long-term sleeping' animals (Paramonova, 1989).

It should be noted that according to the presented data the duration of sleeping time in phenobarbital treated female rats at 22-month age varied from 23 to 57 min and that in control animals from 59 to 233 min. An enzyme imprinting by phenobarbital leads to the decrease of this index and converts treated female rats to the subpopulation of 'short-term sleeping' animals, having an impact upon lifespan of phenobarbital treated rats.

It is of great interest that the phenomenon of enzyme imprinting is registered only in female rats. Similar results were received by other authors (Bagley and Hayes, 1983), who observed a prolonged, during 20 weeks, rise of cytochrome P-450 content and monooxygenase activity of microsomes in female rats, in contrast to males, treated by phenobarbital in the first 5 days after birth. It was shown that following the neonatal phenobarbital injection to rats at therapeutic doses (40 mg/kg) the levels of testosterone secretion altered at different age periods. The level of blood serum testosterone decreased during the period around birth until puberty and increased after puberty and in adulthood. Moreover, the regulation of testicular steroidogenesis was disturbed (Wani et al., 1996). Probably, these changes of the hormonal status in males after phenobarbital administration may account for the sex differences in its influence on the rat lifespan.

Thus, an injection of phenobarbital to the female rats at first 3 days of life has resulted in an increase, during a whole lifetime, of the liver microsomal oxidation level and substantially influenced the mortality and lifespan of animals. The present research shows that in the studies on the hepatic microsomal oxidation system it is important to take into account the sex differences, because the levels of monooxygenase activities and their age-related changes differ greatly in male and female rats (Fujita et al., 1982, 1991; Kitani, 1984).

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