Variability of Microsomal Oxidation and Porphyrin Metabolism in Rats

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An inverse relationship between erythropoiesis intensity and microsomal oxidation level has been detected during the early postnatal period in rats with high resistance to hypoxia.

Key Words: *hypoxia; erythropoiesis; microsomal oxidation; porphyrins*

The specific features of microsomal oxidation can form during the intrauterine development, when hypoxia can modulate functional and metabolic status of the progeny. It was demonstrated that rats resistant to hypoxia are characterized by low basal activities of enzyme systems of xenobiotic metabolism [1]. Therefore, high hypoxic resistance and low level of microsomal oxidation are observed during the early stages of postnatal development. Unfortunately, we failed to find the data characterizing variability of microsomal enzymes at this stage of ontogeny. The causes of individual differences in cytochrome P-450 activity during the early postnatal development are also unclear.

We compared the activities of microsomal and key enzymes of the heme biosynthesis in the liver of 14-day-old rats.

MATERIALS AND METHODS

The study was carried out on 14-day-old rats (180-200 g; n=176) from the litters of 20 outbred females (4-13 animals per litter). Microsomal oxidation was evaluated by the hexenal sleep duration and activities of microsomal enzymes (p-aniline hydroxylase and dimethylaniline demethylase). Hexenal was injected intraperitoneally (5% water solution) in a dose of 15 mg/kg and the duration of sleep (lateral posture) was determined. Recovery of the turning reflex was considered as the end of sleep [3].

Porphyrin metabolism was studied by activities of heme biosynthesis enzyme in liver homogenates: δ-aminolevulinate synthase (δ-ALA-synthase) [5], δ-aminolevulinate dehydrase (δ-ALA-dehydrase) [4], heme synthase [6], and the concentrations of δ-aminolevulinic acid (δ-ALA) and porphobilinogen [2]. Statistically significant differences were detected using Student's t test.

RESULTS

The animals were divided into two subgroups by the results of hexenal-induced sleep test. The subgroups were arbitrarily denoted as rapidly and slowly metabolizing. The duration of hexenal-induced sleep in the former was 2-fold shorter than in the latter (Table 1).

The data on microsomal enzyme activities are in good correlation with our results. In rapidly metabolizing rats, activities of dimethylaniline demethylase and of p-aniline hydroxylase were lower by 4 and almost 2 times compared to slowly metabolizing animals (Table 1). Hence, a clear-cut variability of microsomal oxidation was seen in animals, due to which the initially homogeneous group could be divided into two polar types. The hexenal test in this study reflects primarily the CYP2B1/2 dependent mono-oxygenation status; p-aniline hydroxylase activity and N-dimethylaniline demethylase activity are supported by the CYP2E form.

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TABLE 1. Microsomal Oxidation and Activities of ALA, Porphyrobilinogen, and Heme Synthesizing Enzymes

Parameter	Rapidly metabolizing (n=48)	Slowly metabolizing (n=32)
Microsomal oxidation status		
hexenal-induced sleep, min	47.00±1.33	101.0±14.5***
dimethylaniline demethylase, ng/mg protein/min	8.6±0.5	2.02±0.11***
p-aniline hydroxylase, ng/mg protein/min	0.230±0.012	0.14±0.01**
Porphyrin metabolism		
ALA synthase, μ/ml/min	0.179±0.012	0.054±0.005***
heme synthase, pm/ml/min	0.013±0.001	0.008±0.001***
ALA, μ/ml	0.200±0.014	0.0090±0.0001**
porphyrobilinogen, μ/ml	0.060±0.009	0.150±0.012**
ALA-dehydrase, μ/ml/min	0.102±0.028	0.186±0.091
Hematological values		
erythrocytes, 10^{12}/liter	3.46±0.06	3.50±0.08
hemoglobin, g/liter	89.0±1.5	91.0±1.2
mean hemoglobin content in erythrocyte, pg	26.00±0.51	26.0±0.5
reticulocytes, ‰	8.80±0.46	11.6±1.1***
erythroblasts, per 100 cells	1.20±0.23	7.70±0.32***
basophilic normoblasts, per 100 cells	3.20±0.13	9.00±0.58***
polychromatophilic normoblasts, per 100 cells	6.30±0.68	10.0±1.2*
oxiphilic normoblasts, per 100 cells	8.00±0.58	5.3±0.8*
red cell mitoses, per 100 cells	0.30±0.02	0±0
total count of erythroid cells per 100 cells	19.0±1.9	33.0±3.4***
erythroblast maturing index	0.75±0.04	0.47±0.04***
erythroblast maturing indicator	4.50±0.78	1.70±0.23**

Note. *p<0.05, **p<0.005, ***p<0.001 compared to the other group.

Hence, the results suggest the isoform-nonspecific variability of microsomal oxidation.

Further studies showed that the differences between slowly and rapidly metabolizing rats could be also traced at the level of heme biosynthesis in the liver. Activities of ALA-synthase and heme synthase were 2-fold reduced in slowly metabolizing rats. This led to a drop of ALA content (Table 1). Hence, the typological polarization of microsomal oxidation in rat pups was caused primarily by differences in heme biosynthesis. Low level of porphyrin metabolism in the liver is responsible for the formation of slow metabolism in animals.

On the other hand, reciprocal relationships between porphyrin metabolism in the liver and bone marrow are possible, because of different levels of iron support of erythropoiesis and liver cytochrome P-450 synthesis. This hypothesis is supported by the data on more rapid erythropoiesis in slowly metabolizing rats. Peripheral blood values indicate delayed maturation of red blood cells (Table 1). Relationship between high hypoxic resistance and erythropoiesis intensity explains the low level of microsomal oxidation in animals highly resistant to hypoxia.

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