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Changes of the Level of Cytochrome P-450 and NADPH-Cytochrome P-450 Reductase in Rats with Hereditary Degeneration of the Retina

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In animals with hereditary degeneration of the retina the level of cytochrome P-450 in the brain microsomal fraction is found to be higher than that in healthy animals. In rats with hereditary degeneration of the retina the activity of NADPH-cytochrome P-450 reductase is unchanged in all tissues examined except for the retina, where it is markedly higher than in healthy animals on postnatal day 90.

Key Words: *hereditary degeneration of the retina; cytochrome P-450; NADPH-cytochrome P-450 reductase; hemoglobin; microsomes*

In studies performed on rats with hereditary degeneration of the retina (HDR) we established that in the early postnatal stages (days 10-20) some metabolic changes found in the retina and pigment epithelium (PE) of the eye of diseased animals also manifest themselves in the cerebral cortex [3], evidently due to the fact that these tissues develop from the same presumptive region [5]. Specifically, it has been shown that the rate of induced lipid peroxidation is higher in the retina, PE, and cerebral cortex of affected animals. Research into the possible causes of this phenomenon (experiments have been performed on different subcellular fractions of the animal brain) has shown that in the early stages of postnatal ontogenesis (day 20) the total content of heme-free iron in the microsomal fraction of the cerebral cortex drops, and the ratio between the oxidized and reduced forms of iron markedly changes (in favor of the latter). In addition, in the retina and cerebral cortex the activity of glucose-6-phosphate dehydrogenase, a major enzyme of the hexose-monophosphate shunt producing NADPH, an effective iron-reducing agent in the cell, was found to be higher in diseased than in healthy animals.

In view of the data on changes in the content of heme-free iron in microsomes of the cerebral cortex of rats with HDR (probably the same changes also occur in the retina), it seemed of interest to elucidate whether the heme-containing components, along with the related NADPH-dependent systems, are altered in the microsomal membrane of affected animals. Accordingly, the aim of the present study was to determine the content and

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TABLE 1. Content of Cytochrome P-450 and Its Activity with Respect to Type I and II Substrates in Microsomes of the Brain and Liver of Wistar and Campbell Rats of Different Ages

Age, days	Strain	Content of cytochrome P-450, pmol/mg protein	Activity of cytochrome P-450, nmol product/mg protein×min	
			aminopyrine (type I substrate)	aniline (type II substrate)
		Brain		
45	Wistar	15.8±0.2 (20)	–	–
	Campbell	37.3±1.0 (20)*	–	–
90	Wistar	17.5±0.1 (10)	–	–
	Campbell	81.3±1.6 (20)*	–	–
		Liver		
20	Wistar	590.0±40.0 (6)	8.5±0.9 (8)	1.4±0.1 (6)
	Campbell	540.0±60.0 (6)	8.2±0.9 (8)	1.2±0.1 (6)
45	Wistar	1040.0±80.0 (6)	–	–
	Campbell	1160.0±70.0 (6)	–	–
90	Wistar	1200.0±100.0 (6)	11.9±2.2 (8)	1.6±0.1 (6)
	Campbell	1250.0±90.0 (6)	16.2±3.2 (8)	1.6±0.1 (6)

Note. Here and in Tables 2 and 3 the number of experiments is shown in parentheses; the reliability of differences at $p<0.05$ is shown by an asterisk.

activity of cytochrome P-450, a heme-containing protein of the microsomal membrane, and to compare these data with the activity of NADPH-cytochrome P-450 reductase in the target tissues (the retina, cerebral cortex, and, as a reference, the liver) in the early and later stages of postnatal ontogenesis. In addition, we thought it worthwhile to estimate the amount of hemoglobin, a major heme-containing soluble protein, in the blood of diseased and healthy animals at different stages of life.

MATERIALS AND METHODS

The study was carried out on male Campbell rats of different ages with HDR. Wistar rats of the same ages and sex were used as the control. The microsomal fractions of the liver, retina, and brain were obtained after Karuzina *et al.* [4] and Vijayalakshmi *et al.* [14]. In hepatic microsomes the level of cytochrome P-450 was determined after Omura *et al.* [11], and in microsomes of the brain after Nabeshima [10]; the protein content in the sample was 1-2 and 3-5 mg for the liver and for the brain, respectively. The N-demethylase and p-hydroxylase activity of cytochrome P-450 was determined as described elsewhere [4], using aminopyrine as the type I substrate and aniline as the type II substrate (formaldehyde and p-aminophenol, respectively, being the reaction products). The protein content in the sample was 1.5 mg. The activity of NADPH-cytochrome P-450 reductase was measured after Strobel *et al.* [13], using the oxidized form of cytochrome C as the substrate; the protein content in the sample was 0.05-0.15 mg. The blood hemoglobin level was measured after Derviz [2]; erythrocytes were routinely quantitated in a Goryaev chamber [7]. The protein content was measured after Lowry [9]. The results were statistically processed using Student's *t* test [1].

RESULTS

The data on the content of cytochrome P-450 and on the activity of NADPH-cytochrome P-450 re-

TABLE 2. Activity of NADPH-Cytochrome P-450 Reductase in Microsomes of the Retina, Cerebral Cortex, and Liver of Wistar and Campbell Rats of Different Ages

Age, days	Strain	Activity of NADPH-cytochrome P-450 reductase, nmol reduced cytochrome C/mg protein×min		
		retina	cerebral cortex	liver
20	Wistar	8.0±0.4 (8)	6.7±0.2 (18)	93.8±1.5 (6)
	Campbell	8.1±0.3 (7)	6.2±0.1 (19)	78.8±3.3 (6)
45	Wistar	11.1±0.5 (8)	12.4±0.4 (24)	263.2±12.6 (6)
	Campbell	12.3±0.8 (8)	15.1±0.6 (15)	220.1±10.2 (6)
90	Wistar	1.9±0.2 (12)	7.7±0.2 (8)	75.0±4.5 (6)
	Campbell	5.2±0.1 (12)*	6.6±0.3 (12)	74.5±1.4 (6)

ductase in the retina, brain, and liver of diseased and healthy rats of different age are presented in Tables 1 and 2. Beginning from postnatal day 45, the microsomal content of cytochrome P-450 rose sharply (two-fold) in the brain of diseased rats as compared to that in healthy animals. At earlier stages it was impossible to measure the content of this component, this being consistent with published data on the formation of cytochrome P-450 in the membranes of brain microsomes during ontogenesis [6]. These differences became even more pronounced (4-fold) on postnatal day 90, whereas the activity of NADPH-cytochrome P-450 reductase at these stages did not change markedly in diseased as compared with healthy animals. These findings do not contradict the commonly accepted notions [6] that there are 10-20 mmol NADPH-cytochrome P-450 reductase for every mol cytochrome P-450 in health, this evidently being sufficient to reduce the excess cytochrome P-450 in the brain microsomes of diseased animals. Throughout the studied stages the content of cytochrome P-450 and the activity of NADPH-cytochrome P-450 reductase in microsomes of the liver of diseased rats did not differ from those in healthy animals. The ratio between the content of cytochrome P-450 and the activity of NADPH-cytochrome P-450 reductase in microsomes of the liver and brain of healthy animals approximates that reported in the literature for 90-day animals [14] (3.96 and 11.08, respectively, vs. 2.21 and 16.0 in our study). On the other hand, it is worthy of note that in the brain (the target tissue of the disease) of affected animals, in contrast to the hepatic tissue, this ratio sharply rose: for example, on day 45 it was twice as high as in health, being 2.45 in diseased and 1.29 in healthy rats, and on day 90 it was five times higher (12.30 and 2.21, respectively).

We were unable to determine the content of cytochrome P-450 in the retina of diseased and healthy rats. There are indications in the literature that only cytochrome B_5 is present in this tissue [12]. The activity of NADPH-cytochrome P-450 reductase in the retina of diseased rats was unchanged from that in healthy animals till postnatal day 45 (as it was in the cerebral cortex), but, in contrast to that in the brain, on postnatal day 90 it was markedly (3 times) higher than in healthy rats. Evidently, at this stage a marked change in the activity of NADPH-cytochrome P-450 reductase in the retina of diseased animals should be regarded as the result of destructive processes due to degeneration of photoreceptor cells in HDR. As to the fact that cytochrome P-450 was not discovered in the retina, it cannot be ruled out that in this case electrons are transferred to another acceptor, specifically, to cytochrome B_5. The increased content of cytochrome P-450 in brain microsomes of diseased animals on days 45 and 90 can probably be explained in terms of reported data demonstrating that damage to the peripheral visual analyzer (the retina) results in destructive changes in its central parts (the visual cortex) [8], which suggests that a change occurs in the normal metabolism.

Table 1 also shows the activity of cytochrome P-450 with respect to the type I and II substrates (aminopyrine and aniline) in the hepatic microsomes of rats of both strains in the early (day 20) and late (day 90) postnatal stages. The activity of this component in liver microsomes did not reliably differ between the animals of the two strains. We were unable to determine the activity of cytochrome P-450 with respect to the said substrates in the microsomes of the retina and brain in diseased and healthy animals. This is in accord with published findings that the content of the isoforms specific to these substrates is approximately just 5% of the total content of cytochrome P-450 in the brain tissue [6].

The results of measurements of the hemoglobin content, as well as of the erythrocyte count, in the blood of diseased and healthy rats of different ages are summarized in Table 3. As is seen from the table, these indexes did not differ between diseased and healthy rats throughout these stages.

Thus, our findings allow for the conclusion that in the microsomal fraction of the brain (target tis-

TABLE 3. Hemoglobin Content and Erythrocyte Count in the Blood of Wistar and Campbell Rats of Different Ages

Age, days	Strain	Hemoglobin content, g/liter	Erythrocyte count, T/liter
10	Wistar	91.1±3.4 (8)	3.1±0.2 (10)
	Campbell	78.6±5.7 (8)	2.8±0.2 (10)
20	Wistar	100.7±4.8 (6)	4.1±0.3 (6)
	Campbell	96.6±10.4 (6)	4.4±0.3 (6)
45	Wistar	122.3±6.2 (6)	5.3±0.4 (6)
	Campbell	105.8±8.1 (6)	5.0±0.3 (6)
90	Wistar	154.5±5.8 (6)	6.9±0.1 (6)
	Campbell	145.6±9.3 (6)	6.7±0.3 (6)

sue of the disease) the content of cytochrome P-450, a heme-containing protein, changes markedly in rats with HDR, whereas the activity of NADPH-cytochrome P-450 reductase does not differ from that in healthy animals. However, it should be mentioned that these alterations manifest themselves far later than changes in the heme-free components of the microsomal membrane and are evidently associated with the activation of compensatory processes in the damaged tissues. In the rats with HDR the content of hemoglobin, a soluble heme-containing protein, is unchanged throughout the studied postnatal stages. On the whole, our findings corroborate our previous data that in this disease it is precisely the microsomal fraction of target tissues where changes in iron-containing proteins occur.

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Hypolipidemic Effect of 6-Oxa-D-Homo-Isoestrone and Its Methyl Ester

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Administration of methyl ester to ovariectomized rats is shown to lower cholesterol and normalize the serum lipoprotein spectrum. 6-Oxa-D-homo-8-isoestrone also reduces the serum cholesterol level, but does not normalize the lipoprotein spectrum. Neither the cholesterol content nor the serum lipoprotein spectrum is affected by estradiol.

Key Words: *cholesterol; lipoproteins; ovariectomy; estrogen analogs*

There are published data indicating a marked decrease in total serum cholesterol (Ch) and, correspondingly, low-density lipoproteins (LDL) with a simultaneous increase in α-Ch and, accordingly, high-density lipoproteins (HDL) in postmenopausal women and women who have undergone an ovariectomy in accordance with medical indications [8]. At the same time, the concentration of serum triglycerides (TG) rises in such patients, posing an independent risk factor of the development of atherosclerosis and coronary artery damage [3]. In view with this, several studies are underway to seek

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