Changes in Iron Content and Saturation of Plasma Transferrin in Rats with Hereditary Degeneration of the Retina

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The content and characteristics of plasma transferrin were studied in Campbell rats with hereditary degeneration of the retina and healthy Wistar rats in various periods of postnatal life. In Campbell rats aged 20 days the content of nonhemic iron was found to be 1.9 times lower, and the content of transferrin in the blood plasma 4.3 times lower than in healthy animals. The degree of transferrin saturation with iron in these animals was 2.3 times higher than in heathy rats. Differences in the content of transferrin and degree of its saturation with iron were leveled by days 45 and 90 of life. Apotransferrins of sick and normal rats did not differ in their capacity for saturation with iron.

Key Words: *hereditary degeneration of the retina; iron status; nonhemic iron; transferrin*

The iron ion within hemic and nonhemic iron-containing proteins is indispensable for the vital activity of all body tissues. A specific role of this ion has been demonstrated for brain tissues [8]. Iron has been found to enter the brain from transferrin (TF), a blood plasma protein which binds to specific receptors localized on the plasma membrane of endothelial cells of brain capillaries [10]. The mechanisms of iron release from the TF-TF receptor complex and of its transport are being actively studied at present [7]. The so-called iron status of the body influences the content of iron in the brain; this status is assessed by the amount of nonhemic iron in the blood plasma, the bulk of it being integrated in the complex with TF, and by the content of nonhemic iron in hepatocytes [11]. An impoverished iron status of the body, in contrast to a robust one, has a marked effect on the level of iron in the brain: the content of nonhemic iron in brain tissue drops, and various biochemical and functional disorders of the brain are observed [14].

Our previous experiments with pure-strain Campbell rats (with hereditary degeneration of the retina, HDR) showed a noticeable, about 40%, reduction of the content of nonhemic iron in the cerebrocortical microsomal fraction in the early periods of the disease (day 20 of life) in comparison with healthy animals [2]. This phenomenon might be due to the poor iron status of rats with HDR.

Our present research was aimed at examining the content and properties of plasma TF, one of the components characterizing the iron status of the body, in rats with HDR in comparison with healthy animals at various stages of the disease.

MATERIALS AND METHODS

Experiments were carried out with pure-strain Campbell rats of various age. Wistar rats were con-

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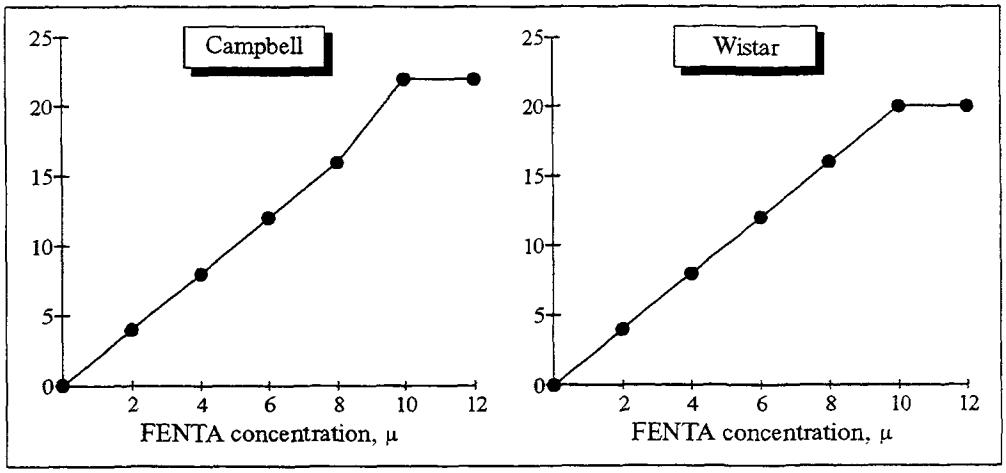

Fig. 1. Kinetics of serum apoTF saturation with iron in 20-day-old Wistar and Campbell rats. Ordinate: optical density at wavelength $\lambda = 470$ nm$\times 2.5 \times \times 10^{-3}$. ApoTF content in sample 0.14 μM.

trols. Electrophoresis of serum proteins of both rat lines was carried out in polyacrylamide gel using a nondenaturating buffer system [13] which was followed by staining with Coomassie Blue-R. Five μg of protein from a pool of sera of 8 rats of each age tested were put into wells. Rat plasma apoTF (Sigma) was used as a blank sample. In parallel with this, the content of nonhemic iron was measured in the remaining material by a previously described method [3]. After staining the gel plates were cut into strips which were scanned with an attachment to a Specord-M40 spectrophotometer at wavelength 600 nm. TF content was assessed by the size of the peaks on the densitogram by correlating it to the total size of the peaks of a known amount of protein applied. TF saturation with iron was estimated from the content of nonhemic iron in a serum aliquot by relating it to the TF content (in μmol) in the same preparation, bearing in mind that 1 mol of apoTF is capable of binding at most 2 mols of iron. Two series of experiments were carried out. An iron complex with nitriloacetic acid (FENTA) was used in studies of the kinetics of apoTF saturation with iron [6]. Isolation and partial purification of apoTF from rat serum were carried out as described previously [13] with some modifications. The protein content in the sample s was measured as previously [9]; the results were statistically processed after a method described earlier [1].

RESULTS

Table 1 presents the results of analysis of densitograms reflecting the TF content in the sera of healthy rats and those with HDR. As is seen from the table, the content of TF in healthy animals virtually did not change from day 20 to day 45 of postnatal life, this being in line with a previous report [11]. By day 90 of postnatal life the level of TF had markedly dropped in normal animals. As for rats with HDR, on day 20 of life the content of TF was 4.3 times lower than in normal controls; these differences leveled out by day 45 and completely disappeared by day 90: at this time the TF level of rats with HDR was the same as in normal controls. We have been unable to find reports about a reduction of the content of this protein during various changes of the iron status of the body or in disease, except for the so-called transferrinemias [5]. It is, however, known that in rats fed diets deficient in iron the content of nonhemic iron in the plasma is reduced, with the content of TF increased, while in animals fed iron-rich diets the iron TF content remains unchanged [11].

Besides the significant difference in the content of TF in rats with HDR vs. healthy ones in the early periods of the disease, the nature of the changes in the content of this protein in ontogenesis is worthy of note. Its plasma level rises 4.8-fold from day 20 to day 45 of life, whereas in control rats the TF content remains unchanged.

The next step in our study was to assess TF saturation with iron in rats with HDR and in healthy animals *in vivo*. Remembering that virtually all the nonhemic iron of the blood plasma is bound to TF [4], we measured the content of nonhemic iron in the sera of rats with HDR and normal animals of various age (Table 1). In normal controls the serum content of nonhemic iron was maximal on day 20 and then markedly dropped. In rats with HDR the serum level of nonhemic iron was virtually unchanged in the same period. The most marked difference in the content of nonhemic iron in the animals with HDR in comparison with healthy ones was observed on day 20 of life (a 1.9-fold drop), by day 45 the difference was 18%, and by day 90 the content of nonhemic iron was the same in the sick and healthy rats. Table 1 presents estimated data on TF saturation with iron in health and disease in vari-

TABLE 1. Serum Content of TF and Nonhemic Iron and TF Saturation with Iron (% of Maximal Saturation) in Wistar and Campbell Rats of Different Age ($n=16$)

Age of animals, days	Rat line	TF, mg/ml (densitogram)	Nonhemic iron, μg/ml	TF saturation with iron
20	Wistar	4.3	1.74±0.04	29.0
	Campbell	1.0	0.93±0.08*	66.4
45	Wistar	4.2	1.48±0.01	25.0
	Campbell	4.8	1.22±0.06*	30.0
90	Wistar	2.6	1.05±0.03	28.8
	Campbell	2.6	0.96±0.88	26.4

Note. n=number of animals in experimental series; asterisk shows reliability of differences at $p<0.05$.

ous periods of life. It is evident that in 20-day-old rats with HDR TF saturation with iron in 2.3 times higher than in healthy controls (66.4 and 29.0%, respectively), whereas by days 45 and 90 of life TF saturation with iron becomes similar in rats of both lines.

The next series of experiments was devoted to a study of apoTF saturation with iron in animals with HDR and controls (the kinetic curves are presented in Fig. 1). These experiments were carried out with apoTF of 20-day-old rats. It is evident that apoTF of rats of the two lines did not differ in capacity for saturation with iron.

Assessing the results, we may conclude that the iron status of the body in rats with HDR deteriorates in the early period of disease development (day 20 of life), there being a reduced content of nonhemic iron in the serum and a change in the level of plasma TF and its saturation with iron. It is known from published data that in health the peak level of iron absorption by rat brain tissues is observed on days 15-21 of life [12]. Our previous study revealed that the same period (day 20) is associated with a reduction of the content of nonhemic iron in the microsomal fraction of the cerebral cortex of rats with HDR. The present research revealed a reduced level of nonhemic iron in the blood serum and a lower level of TF in the same period, but the degree of TF saturation with iron was 2.3 times higher in animals with HDR than in healthy ones. The affinity of TF receptors for TF is known [11] to increase in parallel with the saturation of this protein with iron, this possibly permitting the brain of animals with HDR to partially compensate for the deficit of this ion. Considering the intricate mechanisms responsible for iron release from the complex with TF in tissues (the contribution of Cl^- ions, the cellular level of $NADPH_2$, etc.), we cannot rule out other hypotheses explaining the phenomenon of increased TF saturation with iron in rats with HDR.

In conclusion we should like to emphasize that the detected changes in the content of nonhemic iron and TF (and TF saturation) in the blood plasma of rats with HDR on day 20 of life are somewhat unusual, because, along with the reduced plasma content of nonhemic iron (characteristic of common iron deficiency), the pattern of changes related to TF is different. For example, for a deficit of nonhemic iron in the plasma, the content of TF is known to increase to compensate for it and its saturation with iron to decrease [11]. In Campbell rats the content of TF dropped, while the degree of its saturation with iron drastically increased. Assessment of other components characterizing the iron status of rats with HDR (the content of nonhemic iron in hepatocytes, the content of iron, and the degree of saturation with it, of liver ferritin, and its ferroxidase properties) appears to be of interest in this connection.

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