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THYROID CONTROL OVER BIOMEMBRANES. II. RAT LIVER NUCLEI[1]

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Summary

Liver cell nuclei obtained from hypothyroid rats contain phospholipids in which the relative amounts of unsaturated fatty acids differ from those in normal nuclei: linoleic (18:2) and docosatrienoic (20:3) increase, γ-linolenic (18:3 ω6) appears, and arachidonic acid (20:4) decreases. The unsaturation index decreases by 7%, the ratio 20:4/18:2 by 63%. These changes resemble the pattern reported for the liver mitochondrial inner membrane, and are evidence for a defect in Δ5-desaturation in the livers of hypothyroid rats. The membrane-dependency of the activity of the nuclear glucose-6-phosphate phosphohydrolase is slightly but consistently different in hypothyroids and normals.

The fatty acid contents of the phospholipids extracted from the inner membranes of liver mitochondria (1-3) and from intact heart mitochondria (3; Shaw and Hoch, submitted for publication) are altered in rats made hypothyroid. These compositional abnormalities are accompanied by abnormal velocity-temperature relationships in processes of oxidative phosphorylation (1-3) and of transport (4). One injection of thyroid hormone corrects within 3 days some of the fatty acid abnormalities as well as the Arrhenius profiles, in liver more completely than in heart mitochondria. The pattern of depletion and excesses of hormone-responsive unsaturated fatty acids of the ω6 family in the liver mitochondria suggests that there is a defect in Δ5-desaturation in hypothyroidism (1-3). That in turn suggests that the phospholipids of other membranes in liver cells should also be abnormal. Altered lipid environment may account for the abnormal reactivity reported for the -SH groups in the proteins of microsomes from hepatocytes of hypothyroid rats (5). The generality of a defect in essential fatty acid metabolism in the liver is therefore examined here through a comparison of the nuclear membranes in normal and hypothyroid rats.

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Materials and Methods

Male Sprague-Dawley rats were obtained from Spartan Research (Haslett, MI) and maintained on Tekland Mills Mouse & Rat Diet. Thyroidectomized male rats from the same source, obtained when their weights were between 50 g and 100 g, were injected with ^{131}I as iodide and maintained for at least 3 weeks on a low-iodine vitamin-enriched synthetic diet (Nutritional Biochemicals Co., Cleveland) plus $CaCl_2$ in their drinking water. The synthetic diet is necessary because ordinary laboratory diets supply enough hormone to relieve some of the signs of hypothyroidism (6). The comparisons between these groups of rats on different diets seem justifiable because of our findings on liver mitochondria (1-3), as discussed below. Oxidative phosphorylation and ADP translocation are similar in mitochondria from normal rats fed either a standard diet or the low-iodine diet plus 0.0005% KI in their drinking water.

Rats were fasted overnight before decapitation and excision of their livers. Liver nuclei were prepared by a combination of methods described by (7). The liver was pulped in a tissue press (Harvard Apparatus, Millis, MA). Using a Teflon-glass homogenizer, a concentrated homogenate of 2 rat livers was prepared in ice-cold 0.32 M sucrose containing 3 mM $MgCl_2$. The homogenate was diluted to 80 ml with the 0.32 M sucrose-3 mM $MgCl_2$ solution, and centrifuged at 700 g for 10 minutes at 4°C. The pellet was resuspended in 25 volumes of 2.2 M sucrose containing 1 mM $MgCl_2$, using a very loose-fitting ground glass homogenizer. Following centrifugation at 50,000 xg in a swinging bucket rotor (Beckman SW 25.1) for 90 min, the supernatant was discarded. The pellet which contained the nuclei was resuspended in 25 ml of 0.32 M sucrose-3 mM $MgCl_2$ and collected by centrifugation for 10 minutes at 700 g. The final pellet was suspended in 0.32 M sucrose-3 mM $MgCl_2$ for phospholipid extraction, or in 0.25 M sucrose-0.05 M Tris HCl, pH 7.0-2.25 mM KCl-5 mM $MgCl_2$ for activity measurements. Protein content was measured by a rapid biuret method (8).

Phospholipids were extracted and fatty acids were analyzed as described for rat heart mitochondria elsewhere (Shaw and Hoch, submitted for publication). Up to 80% of the phospholipids of rat liver nuclei are in the nuclear membrane (7). To determine whether the observed changes in the nuclear membrane fatty acids of hypothyroid rats affected the behavior of a process dependent on that membrane, the activity of the nuclear glucose-6-phosphate phosphohydrolase was measured according to (9) in a reaction mixture containing 10 mM NaCl; 20 mM cacodylate buffer and 20 mM Hepes buffer, pH 7.0; 5 mM glucose-6-phosphate; and a total volume of 1.4 ml. To start the reaction, 0.1 ml of a nuclear suspension containing 10 mg of protein per ml was added. The reaction was stopped in 10 min by adding 0.9 ml of 10% TCA, and Pi in the supernatant was measured using acid molybdate and 0.16% Elon in 0.48% $NaHSO_3$ as the reducing agents. This enzyme activity is reported to be closely associated with the integrity of the membrane (9, 10). The temperature dependence of enzyme activity was measured at 8 or 9 points between 0° and 40°, using intact nuclei. Arrhenius plots were calculated from the velocity-temperature data, and energies of activation (Ea) and correlation coefficients (r) were determined from least mean square lines. As a potentially more sensitive method for estimating membrane dependency, a cooperative inhibition of the phosphohydrolase activity and calculated values for the Hill coefficients were examined (11, 12).

Results

Table I shows the fatty acid contents, as mole fractions percent of the total, of the phospholipids extracted from nuclei obtained from the livers of normal euthyroid and hypothyroid rats, hereafter referred to as N and H nuclei, respectively.

TABLE I

Fatty Acid Contents of Phospholipids Extracted from Nuclei obtained from the Livers of Normal and Hypothyroid Rats, in Mole Fractions Percent*

Fatty Acid	Normal (5)	Hypothyroids (5)
16:0	22.3 ± 1.5	21.7 ± 0.3
18:0	24.4 ± 0.4	19.0[a] ± 0.3
18:1	7.2 ± 0.3	11.0[a] ± 0.5
18:2	10.2 ± 0.4	20.4[a] ± 0.8
18:3	0	0.3[a] ± 0.04
20:3	0.5 ± 0.07	1.0[a] ± 0.05
20:4	29.7 ± 0.3	22.1[a] ± 0.4
22:3	0.7 ± 0.04	0.6 ± 0.03
22:4	0.7 ± 0.04	0.7 ± 0.04
22:5	0.6 ± 0.02	0.3[a] ± 0.02
22:6	2.2 ± 0.08	1.2[a] ± 0.04
Unsaturation Index	169 ± 0.9	157[a] ± 1.1
20:4/18:2	2.9 ± 0.1	1.1[a] ± 0.04
Mean C length	18.1	17.8

[a] $p<0.001$

*Liver nuclei were prepared and extracted, and phospholipid fatty acids were determined, as described under Methods. The unsaturation index is Σ(mole fraction x number of unsaturated bonds). The mean C length is Σ(mole fraction x number of carbon atoms).

One major component, 16:0, and two minor components, 22:3 and 22:4, are present in equal proportions in both groups. The differences in the contents of the others are highly significant. In H nuclei the contents of 18:1, 18:2, 18:3 and 20:3 are increased, and the contents of 18:0, 20:4, 22:5 and 22:6 are decreased, as compared with N nuclei. The unsaturation index is lower in H nuclei by 7.1%, mainly due to the contribution of the 25% decrease in 20:4, which is not balanced out by the 100% increase in 18:2. The ratio of 20:4/18:2, an indication of desaturating activity, in H nuclei is about one third of that in N nuclei. The average carbon length of the fatty acids is similar in H and N nuclei.

In inner membrane vesicles obtained from the mitochondria of the livers of normal rats on standard lab chow, as compared with those from normal rats on the low-iodine diet plus KI, the content of 18:1 is halved and 22:6 is doubled; 22:4 is not detected (it is 2.2% in the vesicles from rats on low-iodine); and 22:5 is increased (2). Thus the low 18:1 and the high 22:5 and 22:6 contents in N nuclei versus H nuclei seem to be accounted for by the diet (see Materials

and Methods). The chief remaining differences, the decrease in 20:4 and the accumulation of 18:2, 18:3 and 20:3, together with the low unsaturation and 20:4/18:2 ratio, seem attributable to the hypothyroid state, because the identical differences in mitochondrial vesicles are corrected by thyroid hormone injection of hypothyroid rats kept on the low-iodine diet.

The 18:3 that appears in H nuclei is of the same ω family as the 20:3 and the 22:3, because the plot of log retention time on GLC versus the length of the fatty acid carbon chains (13) is linear. The three acids have the same relationship when extracted from liver mitochondrial inner membranes from hypothyroid rats, and it was concluded that they are of the ω6 family (1).

Arrhenius plots of glucose-6-phosphate phosphohydrolase activity in nuclei from normal rats have a slight transition at about 22°C, and a reproducible ($p<0.02$) difference of 2.8 kcal/mole in the energy of activation (Ea) above and below the transition temperature (Fig. 1). Nuclei from hypothyroid rats

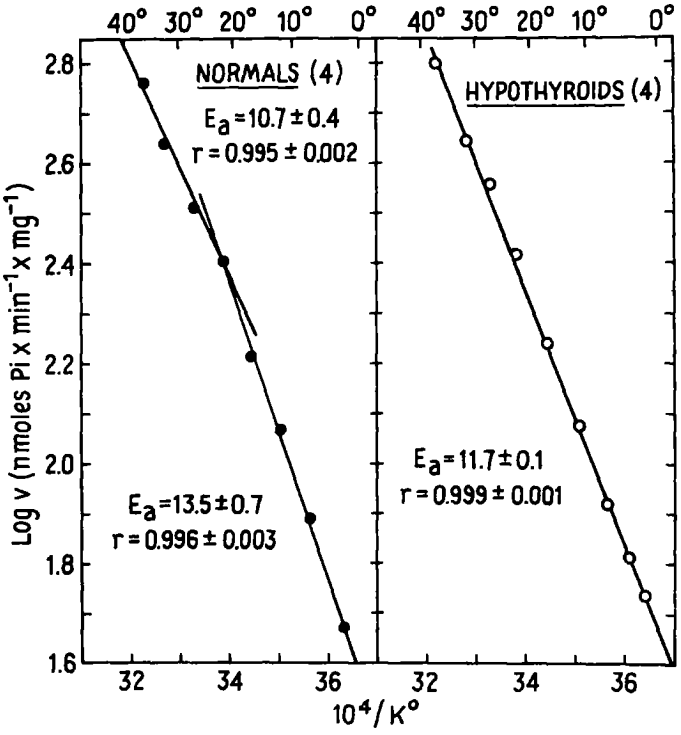

FIG. 1

Arrhenius plots of glucose-6-phosphate phosphohydrolase activity in nuclei from the livers of a representative normal and hypothyroid rat. The lines are calculated by a least squares method, and the means of regression coefficients in 4 experiments in each group are used to calculate the energy of activation (Ea); the means ± SEM of Ea and of the correlation coefficients (r) are shown.

consistently show no transition up to about 37°, and the Ea is intermediate between the two values observed in normal nuclei. Absolute phosphohydrolase activities at any single temperature in the range studied are not significantly different in H and N nuclei, however.

Arrhenius plots of membrane-dependent processes are said to be unreliable in detecting transitions that involve a difference of less than 3 kcal/mole, but enzyme inhibitions that show cooperativity change the degree of cooperativity when the Ea of the inhibition changes by about 0.8 kcal/mole (11, 12). Because of the relatively small differences between the Arrhenius profiles, we examined the highly cooperative inhibition of the phosphohydrolase by ethanol. Calculations of the Hill coefficient, $\underline{n}$, for ethanol inhibition at 37°, at concentrations between 0.05 M and 0.5 M, showed for 4 experiments with normal nuclei, $\underline{n} = 3.49 \pm 0.21$, $\underline{r} = 0.971 \pm 0.019$; and for 4 experiments with nuclei from hypothyroid rats, $\underline{n} = 4.38 \pm 0.36$, $\underline{r} = 0.995 \pm 0.001$. The 25% difference does not quite reach acceptably significant levels ($p<0.1$).

Discussion

The nuclei obtained from hypothyroid rats contain membrane phospholipids that have a fatty acid composition different from that found in normal euthyroid rats. One group of these fatty acids, that includes the essential acid 18:2 and some of its metabolites, 18:3, 20:3 and 20:4, has the same pattern of redistribution and almost the same relative contents that are seen in liver mitochondrial inner membranes in hypothyroidism: the first three are increased and 20:4 is decreased in content (1-3). In the membrane of H nuclei γ-linolenic acid (18:3 ω6) appears; it also appears in the mitochondria in the identical minor amount. The 20:3 that is increased in H liver nuclei, and more so in H liver mitochondria, is also ω6; its increase does not, therefore, denote an essential fatty acid deficiency, because such deficiencies are characterized by accumulation of 20:3 ω9 (14). The similar accumulation in at least two liver organelle membranes of 20:3 and its precursors 18:2 and 18:3, and the depletion of 20:4, are consistent with a defect in a desaturation step 20:3 → 20:4. This Δ5-desaturation is catalyzed by a cytochrome b_5 system in liver microsomes that includes a protein specific for 20:3 (15). The Δ6-desaturation 18:2 → 18:3 involves a different substrate-specific protein (16), and seems intact in hypothyroidism.

The significance of the changes in liver nuclear membrane composition in hypothyroidism for the function of the nucleus is not yet certain. Small but consistent changes are detected in the Arrhenius profiles of the membrane-dependent glucose-6-phosphate phosphohydrolase activity, with a definite linearity in hypothyroidism (Fig. 1). The inhibitory action of ethanol, which presumably depends on its effects on the lipids, shows only a 25% increase in the Hill coefficient. In liver mitochondria, the Arrhenius profiles for oxidative phosphorylation and ADP translocation become linear (1-4) and the Hill coefficient for dinitrophenol stimulation of respiration changes from positive to negative in hypothyroidism (17). The small changes in the nucleus may reflect a relatively greater independence of nuclear processes from the nuclear membrane (18). Alternatively, the small response of the phosphohydrolase activity to altered membrane composition may only reflect the insensitivity of this particular enzyme.

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