INHIBITION OF LIPID PEROXIDATION IN MITOCHONDRIA ISOLATED FROM THE LIVER OF HYPOTHYROID RABBITS

A. I. Marzoev, A. V. Kozlov, and Yu. A. Vladimirov

UDC 616.441-008.64-092.9-07:616.36-008.939.15

KEY WORDS: chemiluminescence of mitochondria; phospholipids; hypothyroidism.

It was shown previously that in rabbits with experimental hypothyroidism lipid peroxidation (LPO) in liver mitochondria induced by Fe^{++} takes place at higher velocities than in mitochondrial lipids from normal animals [2]. The observed activation of LPO is evidently directly connected with changes in the composition of the mitochondrial lipids and, in particular, with an increase in their unsaturation index [4]. As can be judged from the kinetics of chemiluminescence, LPO activation was not due to a decrease in the antioxidant content in the organelles, for the rate of rise of the "slow flash" of chemiluminescence in mitochondria from hyperthyroid animals was lower than in those from normal animals [2].

The object of the present investigation was to study the effect of hypothyroidism on the development of LPO in mitochondria. In hypothyroidism the content of unsaturated fatty acids as a fraction of the total content of fatty acid chains is increased [5], although at the same time the index of unsaturation is reduced [4]. These changes may affect the rate of LPO in mitochondrial membranes.

EXPERIMENTAL METHOD

Rabbits weighing 1.8-2.5 kg were used. The hypothyroid animals took part in the experiments 6-8 h after thyroidectomy. Mitochondria were isolated by the method described previously [2]. Lipids were isolated from the mitochondria by the method of Bligh and Dyer [3]. The incubation medium for the mitochondria (1 mg protein/ml) contained 125 mM KCl, 2 mM KH_2PO_4, pH 7.4, and $FeSO_4$ in a final concentration of 10^{-3} M. Lipids were incubated in medium containing 120 mM KCl, 16 mM KH_2PO_4, pH 7.4, and $FeSO_4$ in a final concentration of 5×10^{-4} M. The temperature of the medium was 37°C. Chemiluminescence of the mitochondria and liposomes as a test for the velocity of LPO was recorded on an instrument of the type described in [1]. Chemiluminescence of samples of mitochondria and liposomes was measured by a scheme described in the previous communication, when material was isolated from control and experimental animals simultaneously and under identical conditions. The protein concentration was determined by the biuret reaction.

EXPERIMENTAL RESULTS

Hypothyroidism leads to a decrease in the velocity of LPO in mitochondria isolated from the animal liver. This was shown by a decrease in amplitude of the "slow flash" of chemiluminescence induced by addition of Fe^{++} and lengthening of the latent period of development of the "slow flash" (Fig. 1a; Table 1). Changes in the kinetics of chemiluminescence taking place in hypothyroidism were thus opposite to those observed previously in hyperthyroidism [2], which in general was to be expected. However, the mechanism of the increase in the parameter τ, observed also in liver mitochondria from hyperthyroid animals [2], remains unclear. Whatever the explanation, the data on the kinetics of luminescence of the mitochondria relating to the three parameters studied (decrease in amplitude of the slow flash, increase in the latent periods of development of the slow flash, a decrease in the rate of rise) are evidence of the slowing of LPO in liver mitochondria in hypothyroidism. As was observed previously [5] in hypothyroidism there is an increase in the fraction of unsaturated fatty acids in lipids of liver mitochondria, but at the same time there is a decrease in the viscosity of the

Biophysics Teaching and Research Complex, N. I. Pirogov Second Moscow Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 40-42, March, 1982. Original article submitted August 2, 1981.

0007-4888/82/9303-0275$07.50

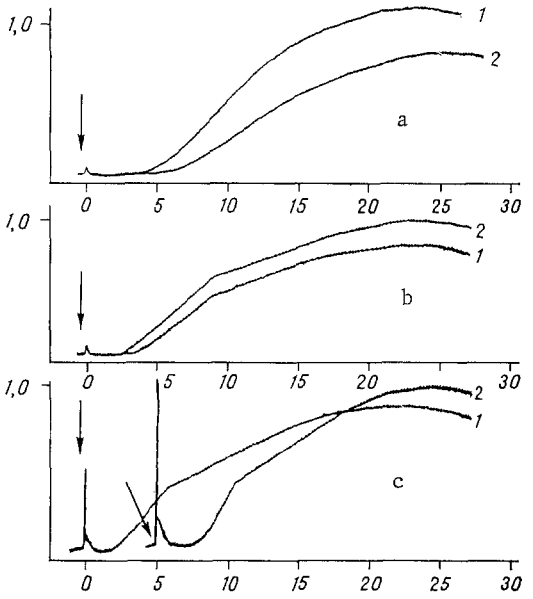

Fig. 1. Effect of concentration of thyroid hormones *in vivo* on chemiluminescence of mitochondria and liposomes: a) liver mitochondria from normal (1) and thyroidectomized (2) rabbits; b) liver mitochondria from normal (1) and hypothyroid (2) rabbits receiving single injection of thyroxine (300 μg/kg body weight) 41 h before isolation of the preparations; c) chemiluminescence of liposomes prepared from mitochondria of normal (1) and hypothyroid (2) rabbits receiving a single injection of thyroxine (300 μg/kg body weight) 41 h before isolation of preparations. Abscissa, time (in min); ordinate intensity of chemiluminescence (in relative units). Time of addition of iron indicated by arrow.

TABLE 1. Effect of Hypothyroidism on Chemiluminescence of Mitochondria

Parameter	Normal	Hypothyroidism
Amplitude of slow flash, %	100 (5)	74,7±4,9 (5)
Latent period, %	100 (5)	123,9±8,9 (5)
Value of τ, %	100 (4)	128,7±24,4 (4)

Legend. Here and in Table 2 values of parameters in normal animals taken as 100%; number of animals shown in parentheses.

membrane lipids, as shown by a shift of the transition temperature of the succinic oxidase system from 23 to 8°C. As a first approximation both these factors ought to accelerate LPO. However, it is evidently not so much the increase in the percentage of unsaturation of the lipids that is of decisive importance for the rate of oxidation of mitochondrial lipids in hypothyroidism as the decrease in the unsaturated index, as reported previously [4]. This is supported by the results of the present experiments in which mitochondria and lipids were isolated at different times after a single injection of L-thyroxine (330 μg/kg body weight). Between 12 and 72 h after injection of thyroid hormones changes opposite to those evoked by hypothyroidism take place in mitochondrial lipids and, in particular, in fatty acids [4, 5]. It will be clear from Fig. 1b, c and Table 2 that 41 h after injection of thyroxine into hypo-

TABLE 2. Effect of Single Injection of Thyroxine (300 μg/kg body weight) on Chemiluminescence of Mitochondria and Mitochondrial Lipids Isolated 41 and 65 h after Injection of Hormone

Object	Amplitude of slow flash, %	Latent period, %	Value of τ, %
Mitochondria after 41 h	124,5±0,5 (2)	88,1±8,9 (2)	94,3±4,8 (2)
Mitochondria after 65 h	142,8±10,82 (3)	63,1±3,9 (3)	78,9±9,1 (3)
Liposomes	126,9±6,5 (3)	91,1±5,6 (3)	90,9±11,1 (3)

thyroid rabbits definite changes pointing to intensification of LPO reactions in these objects took place in the kinetics of chemiluminescence of the mitochondria and liposomes. These changes were still increasing 65 h after injection of the hormone. In the course of isolation of lipids from the mitochondria, hydroperoxides accumulated in them as a result of autooxidation of unsaturated fatty acids. One test for the relative concentration of hydroperoxides is the "fast flash" of chemiluminescence in response to addition of Fe^{++} to lipid-containing systems [1]. The amplitude of the fast flash in lipids from mitochondria of animals treated with thyroxine was found to be 2-2.5 times higher than that in a suspension of mitochondrial lipids isolated from normal animals (Fig. 1c). This can be regarded as further evidence of a sharp increase in the velocity of LPO caused by injection of the hormone.

A fall in the thyroid hormone level in the body thus leads to slowing of the rate of LPO in the liver mitochondria. This is probably connected with a decrease in the unsaturation index of the fatty acids. This hypothesis is supported by the activation of LPO observed in response to a single injection of thyroxine, which restored this index within 40 h after injection.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. A. I. Marzoev, A. V. Kozlov, A. P. Andryushenko, et al., Byull. Eksp. Biol. Med., No. 3, 36 (1982).
3. E. G. Bligh and W. J. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
4. I. Y.-D. Chen and F. L. Hoch, Arch. Biochem., 181, 470 (1977).
5. A. J. Hulbert, M. L. Augee, and J. K. Raison, Biochim. Biophys. Acta, 455, 597 (1976).