EFFECT OF THYROXINE ON TRANSMEMBRANE POTENTIAL OF RAT LIVER MITOCHONDRIA IN VITRO

T. Yu. Koval', A. I. Gagel'gans, and D. Kh. Khamidov*

UDC 612.352.015.3:577.121.7].014.46:615.357.441:577.175.444

KEY WORDS: transmembrane potential; thyroxine; rat liver mitochondria

Research into the mechanism of action of thyroid hormones on energy metabolism in the mitochondria and, in particular, their influence on transmembrane potential, has recently been published [3, 4]. As long ago as in 1971-72, the present writers [2] made successful attempts to determine the transmembrane potential by Mitchell's method [9, 10]. Since the mechanism of action of thyroid hormones on the energetic parameters of the mitochondria still remains an urgent problem, we decided to continue these experiments. It has been suggested [9] that the mitochondria membrane is impermeable for H^+ and OH^- ions. Accumulation of H^+ and OH^- in two aqueous phases separated by a membrane is used to form ATP from ADP + P_i, through the action of ATPase. The membrane potential ($\Delta\Psi$) is not determined entirely by the H^+ gradient (ΔpH), for an exchange-diffusion system, exchanging H^+ for cations and OH^- for anions operates in mitochondria. The membrane potential level required for ATP synthesis is approximately 200 mV at G = 9 kcal, and is maintained by activity of the respiratory chain. Since thyroid hormones are only slightly soluble in fat and reduce the resistance of artificial bilayer phospholipid membranes (BPM) only a very little, unlike classical uncouplers, which have been shown to reduce the resistance of BPM by several orders of magnitude [1], realization of the biological activity of thyroid hormones is evidently linked with a change in the properties of the membrane structure in the presence of active forms of iodine ($I^\cdot$, I^+); under these circumstances, however, the fact must be borne in mind that thyroid hormones may overcome the M phase, through their attachment to specific carrier proteins. An excess of thyroid hormones is considered to lead to uncoupling of oxidative phosphorylation, although Shears [11], who observed acceleration of oxygen consumption by isolated mitochondria in the presence of thyroxine (T_4) in vitro, did not observe any decrease in the efficiency of oxidative phosphorylation.

To represent the mechanism of the influence of T_4 and tri-iodothyramine (T_3) on coupling of respiration to phosphorylation, we measured the transmembrane potential (TMP) of rat liver mitochondria and studied the effect of I_2 and KI on TMP.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing about 200 g. For the experiments in vitro L-T_4 (from Reanal, Hungary) was used. Mitochondria were isolated from the liver by the usual method in isolation medium containing 0.25 M sucrose, 10 mM Tris-HCl, and 0.5 mM EDTA, and then washed and kept in isolation medium. TMP of the mitochondria was measured by means of a K^+-electrode. The antibiotic valinomycin can form a fat-soluble complex with K^+ ions and, in that form, can be transported across the membrane. Transport of K^+ ions correlated with membrane potential: the higher potential the more K^+ was carried by valinomycin inside the mitochondria. This antibiotic is used to determine the potential difference on the membrane by the Donnan or Nernst equation:

$$\Delta\psi = Z\Delta pK,$$

where $\Delta pK = \lg \frac{[K^+]_i}{[K^+]_o}$, Z = 59 mV at 25°C, and the indices i and o denote the medium inside and outside the mitochondria. With the aid of the detergent Triton X-100, which disrupts the

*Corresponding Member, Academy of Sciences of the Uzbek SSR.

Laboratory of Cytochemistry and Electron Microscopy, Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 567-570, November, 1987. Original article submitted July 14, 1986.

 0007-4888/87/0011-1550$12.50

TABLE 1. Effect of K^+ Concentration in Medium on TMP of Rat Liver Mitochondria

K^+ concentration in medium		TPM, mV
mg-eq	mM	
0,01952	0,21	57,7
0,01426	0,144	75,7
0,009164	0,093	108,8
0,003726	0,0388	138,2
0.0012	0.013	150

Legend. Incubation medium: 150 mM sucrose, 5 mM succinate, 50 mM choline chloride, 15 mM Tris-HCl (pH 7.5).

membrane, the K^+ concentration inside the mitochondria after equilibration with valinomycin was calculated. It was considered that 1 mg of mitochondrial protein contains 5 µl of H_2O. To measure the shifts of pK a glass electrode from the set provided with the LUP-01 laboratory pH-meter was used. The comparison electrode was an Ag-AgCl electrode immersed in 1N KCl, and connected through a 30% agar bridge to a measuring cell with a capacity of 2-2.5 ml. To determine the K^+ concentration in the incubation medium it was evaporated down to one-tenth of its original volume, 0.1-0.2 ml of the product was introduced into a cuvette, and, by means of a calibration curve, the quantity of K^+ (in mg-eq) in the incubation medium was calculated. The scale of the automatic writer was calibrated in each experiment against KCl solution of known concentrations. The protein concentration was determined by Lowry's method [8].

EXPERIMENTAL RESULTS

It was shown previously that the higher the K^+ concentration in the medium, the lower the membrane potential of rat liver mitochondria [10]. We repeated these experiments and the resulting curve reflecting this dependence agreed with data in the literature [10] (Table 1).

The membrane potential difference on the M phase (the permeability barrier of the mitochondria) is essentially an electrochemical potential, for it consists of electrical and chemical components: $\Delta p = \Delta\Psi - Z\Delta pH$, where $Z = 59$ mV at 25°C, Δp is the "proton-motive force", equal to 230 mV according to Mitchell, $\Delta\Psi$ is the electrical potential (in mV) formed by electron transport across the redox chain, and ΔpH is the chemical potential (in pH units) existing as a result of the pH gradient between the inner and outer space of the mitochondria. These two components of the electrochemical potential are coupled together and undergo reciprocal changes when the K^+ ion concentration in the medium varies. Elevation of the K^+ level leads to a decrease in the electrical component and an increase in the chemical component of the electrochemical potential difference (Δp) [9]. What probably takes place in this case is diffusion transport of K^+ ions until equality is reached between the number of H^+ and K^+ ions in the medium and mitochondria. The force used to set in motion the diffusion of K^+ ions through the M phase changes the electrical potential, and this leads to a change in the chemical component also. This is evidently what was observed in the experiments described above.

Thyroid hormones are uncouplers of oxidative phosphorylation. It was shown previously that after administration of T_4 the quantity of esterified phosphate per unit of O_2 consumed is reduced, the rate of oxidation of substrates in state 4 is increased, and this leads to a decrease in the value of the respiratory control (RC) [1, 2]. According to some workers, uncoupling agents must also lead to a fall of membrane potential [5, 9]. It can therefore be postulated that the effects of thyroid hormones on mitochondria are mediated through a reduction of TMP.

To test this hypothesis a series of experiments was carried out in which TMP was measured in the presence of different concentrations of T_4 in the incubation medium. A curve of the resulting dependence is reproduced in Fig. 1. TMP was calculated after incubation of mitochondria with the hormone for 2 min. With an increase in the duration of incubation with

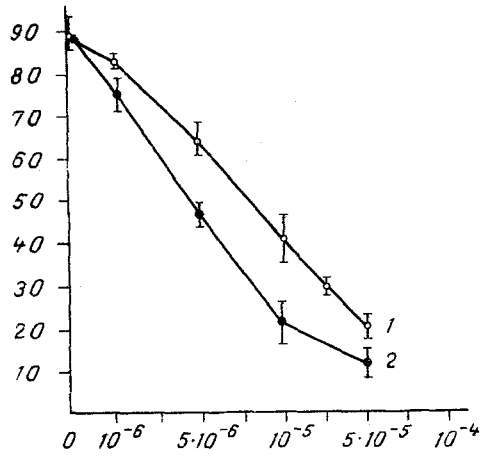

Fig. 1

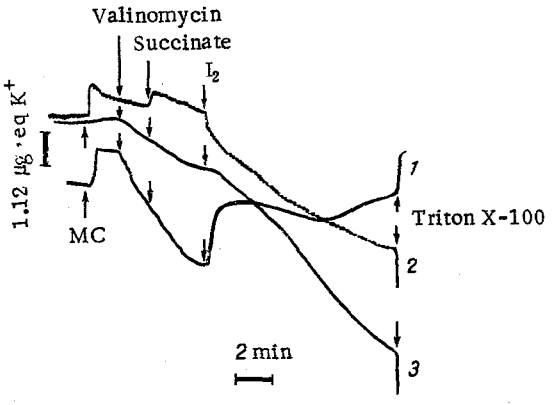

Fig. 2

Fig. 1. Effect of thyroid hormones in vitro on time of 100% abolition of TMP. Abscissa, concentration (in M); ordinate, time of 100% abolition of TMP (in min). 1) T_4; 2) T_3.

Fig. 2. Action of I_2 in vitro on various parameters of rat liver mitochondria (MC). 1) TMP; 2) transhydrogenase reaction (fluorometric measurement of NADPH concentration); 3) swelling of mitochondria (as shown by scattering of light by mitochondrial suspension). Measurements made simultaneously. Valinomycin used in a concentration of 10^{-4} M, succinate $5\cdot10^{-3}$ M, I_2 $5\cdot10^{-5}$ M.

T_4 or T_3, TMP fell more rapidly, but at the beginning a definite latent period was needed after addition of the hormones, evidently so that they could penetrate to the reaction centers. Depending on the hormone concentration, the latent period could vary significantly. Under the influence of T_4 in a concentration of $3.8\cdot10^{-6}$ M the latent period was 3-4 min, falling to 2 min when the T_4 concentration was 10^{-5} M and 0.5-1 min when it was $5\cdot10^{-5}$ M; in the presence of T_4 in a concentration of $2\cdot10^{-4}$ M, TMP began to fall immediately after addition of the hormone.

The rate of fall of TMP was inversely proportional to the concentration of thyroid hormones added to the measuring cell, and in the present experiments T_3 was about 30-40% more effective than T_4. Let us dwell on the mechanism of the observed action of thyroid hormones on TMP, for we know that T_4 increases proton conductance on bilayers, but not significantly. There is evidence in the literature that I_2 and thyroid hormones have a similar effect on cell metabolism [1]. It has been suggested [6] that the biological action of thyroid hormones is connected with the removal of iodine from the T_4 and T_3 molecules. A universal criterion which, in the opinion of the authors cited, enables both the "physiological" and the "toxic" effects of thyroid hormones to be evaluated, is the degree of reduction of the electrical resistance of the membrane or the increase in ionic permeability of the membrane as the result of the free-radical membranes of T_4 degradation, with liberation of I_2 from its molecules in the form of I_0 or I^+. Unlike the classical uncouplers, T_4 probably acts as a donor of "active" iodine in membrane structures. We studied the effect of I_2 on TMP and found that its action resembles that of T_4 and T_3: TMP was reduced, the mitochondria swelled, and NADPH was oxidized (Fig. 2). In a concentration of 10^{-6} M I_2 abolished TMP instantly, i.e., according to the parameters studied I_2 was more effective than T_4. The use of solutions of KI for these purposes did not lead to a fall in TMP, i.e., I^- cannot change the permeability of biological membranes. The existance of the lag period in the action of T_4 in experiments in vitro is perhaps connected with activation of endogenous mitochondrial phospholipase, an enzyme producing hydrolysis of lipids, by thyroid hormones, which leads to the formation of a certain quantity of fatty acids which, in turn, are classical uncouplers of oxidative phosphorylation, and as a result of this, to a fall of TMP. It has in fact been shown that the fall of TMP is blocked by phospholipase inhibitors [7].

The data given above suggest that the observed fall of TMP of rat liver mitochondria under the influence of T_4 and T_3 in experiments in vitro after a certain lag period is associated, in part, with removal of active forms of iodine from the hormone molecules and also with activation of mitochondrial phospholipases, which taken together, lead to a fall of TMP and to uncoupling of respiration and phosphorylation.

LITERATURE CITED

1. A. I. Gagel'gans, "Ion transport in mitochondria and the action of thyroid hormones," Dissertation for the Degree of Candidate, Tashkent (1970).
2. Yu. A. Vladimirov, A. I. Marzoev, S. L. Turchina, and V. A. Pechatnikov, Byull. Éksp. Biol. Med., No. 2, 167 (1984).
3. A. I. Marzoev, S. L. Turchina, V. A. Pechatnikov, and Yu. A. Vladimirov, Byull. Éksp. Biol. Med., No. 12, 30 (1982).
4. V. P. Skulachev, Accumulation of Energy in the Cell [in Russian], Moscow (1969).
5. A. I. Gagel'gans, G. A. Gaidina, L. A. Gol'ber, et al., Thyroid Hormones [in Russian], Tashkent (1972).
6. M. C. Beatrice, J. M. Palmer, and D. R. Pfeiffer, J. Biol. Chem., 255, 8663 (1980).
7. J. Grunstein and A. Wynn, J. Theor. Biol., 26, 343 (1970).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
9. P. Mitchell, FEBS Symp., 17, 219 (1969).
10. P. Mitchell and J. Moyle, Eur. J. Biochem., 7, 471 (1969).
11. S. B. Shears and J. R. Bronk, Biochem. J., 178, 505 (1979).

ACTION OF CYSTEAMINE, AN ULCEROGENIC AGENT, ON GLUTATHIONE AND GLUTATHIONE-DEPENDENT ENZYME LEVELS IN THE GASTRODUODENAL MUCOSA OF RATS

S. A. Morenkova, T. U. Tabutsadze, L. M. Fedorova, and V. P. Masenko

UDC 615.31.03:616.33-002.44-021.7].015.4:616.33-018.73-008.939.626].076.9

KEY WORDS: cysteamine; rat gastrointestinal trace; glutathione

Clinical and experimental studies have shown that the mucous membrane of the gastrointestinal tract, like the liver, contains high concentrations of glutathione and a broad spectrum of glutathione-dependent enzymes, which help to inactivate toxic compounds, lipid peroxidation (LPO) products, intracellular intermediates, drugs, and so on [5, 11, 14]. It has also been suggested that an unusually high level of reduced glutathione in the gastrointestinal mucosa may be a very important factor protecting the cells against the action of ulcerogenic agents [7, 9].

The aim of this investigation was to study the possible connection between the degree of activity of glutathione-dependent enzymes and the concentration of reduced and oxidized forms of glutathione in the mucous membrane of different parts of the gastroduodenal zone and the action of cysteamine, a specific ulcerogenic agent.

EXPERIMENTAL METHOD

Experiments were carried out on 120 female Wistar rats weighing 160-180 g. An experimental model of duodenal ulcer (DU) was produced by a single subcutaneous injection of cysteamine (from Fluka, Switzerland) in a dose of 30 mg/100 g, by the method in [13]. Under thiopental anesthesia the mucous membrane was removed from different parts of the stomach (fundus and antrum) and from DU, 2 weeks after injection of the compound, fixed in liquid nitrogen, and kept until use at −70°C.

Glutathione peroxidase activity was revealed by the method in [6] and glutathione reductase was determined in the modification of Gerasimov et al. [2]. Glutathione-S-transferase activity was studied with the aid of 1-chloro-2,4-dinitrobenzene as substrate [12].

Concentrations of reduced and oxidized glutathione were measured spectrofluorometrically [4]. Protein was determined by Lowry's method [10].

Laboratory of Biochemistry, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 570-572, November, 1987. Original article submitted September 30, 1986.

0007-4888/87/0011-1553$12.50