For the transition in erythrocyte membranes from patients with essential hypertension an almost 30% increase in the enthalpy of the temperature conversions were observed, evidently a sign of the high protein content of these membranes, corresponding to the C transition. It must be pointed out in this connection that according to data published by other workers [9, 10], the C transition is due to thermal conversion of the transmembrane protein in band 3, i.e., the protein responsible for anionic transport through the membrane. Further research will fill in the details of the molecular nature of this band, and also of the components responsible for B_1 and B_2 transitions, for which the changes in enthalpy of heat conversion were less marked.

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REGULATION OF PYRUVATE TRANSPORT IN MITOCHONDRIA BY THYROID HORMONES

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KEY WORDS: thyroidectomy; pyruvate; mitochondria.

Thyroid hormones are known to regulate the sensitivity of target cells to insulin and the insulin concentration in the blood serum [7]. The writers showed previously that the action of insulin on metabolism is mediated, at least partially, by changes in the activity of a cytoplasmic glycolipopeptide, which has been called insulin-dependent cytoplasmic regulator (IDR) [2]. Hormonal control of gluconeogenesis in the liver may be effected at the level of pyruvate transport from cytosol into mitochondria [9]. IDR is an endogenous inhibitor of pyruvate transport [10].

The object of this investigation was to study the role of thyroid hormones in the regulation of carbohydrate metabolism at the level of the pyruvate carrier.

EXPERIMENTAL METHOD

Rat liver mitochondria were isolated in 0.3 M sucrose containing 5 mM Tris-HCl, pH 7.4. Ca^{++} transport into mitochondria was measured by an ion-selective Ca^{++}-sensitive electrode, using a pH-metric method, by studying the kinetics of Ca^{++}/H^{+} exchange in the presence of phosphate as penetrating anion. Swelling of the mitochondrial suspension was measured from the change in optical density at 540 nm. Cytosol was obtained by centrifugation of homogenates of rat liver and diaphragm, prepared with mitochondrial isolation medium in the ratio of 1:1, at 30,000 g. To obtain the thermostable fraction the cytosol was heated for 7 min at 95°C,

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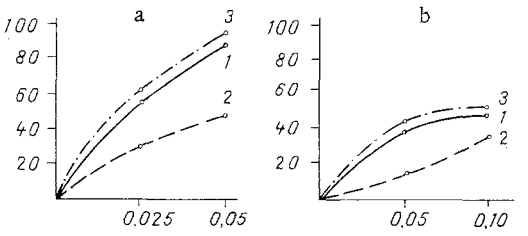

Fig. 1

Fig. 1. Effect of thermostable fraction of liver (a) and diaphragm (b) cytosol on calcium capacity of rat liver mitochondria. 1) Control; 2) thyroidectomy; 3) thyroidectomy + thyroxine. Incubation medium: 0.1 M KCl, 3 mM Tris-HCl, 5 mM succinate, 1 mM phosphate, 0.7 μg/ml rotenone, pH 7.1. Mitochondria: 1.5 mg protein/ml. Abscissa, volume of cytosol added (in ml); ordinate, change in Ca^{++} concentration (in %).

TABLE 1. Effect of Thyroidectomy and Thyroxine on Blood Insulin and Glucose Levels in Rats (M ± m)

Experimental conditions	Insulin, μU/ml	Glucose, mg%
Control	41,4±1,9 (17)	91,6±3,2 (10)
Thyroidectomy	15,9±0,8 (17)	63,3±2,5 (17)
Thyroidectomy + thyroxine (50 μg/100 g)	—	88,7±5,3 (9)

Legend. Number of experiments shown in parentheses.

followed by centrifugation of the denatured proteins. In the experiments with thryoidectomy [1] male rats weighing 80 g were used. The control rats 4 months after thyroidectomy weighed 220-260 g and the thyroidectomized rats 110-150 g. Thyroxine was injected intraperitoneally into the thyroidectomized rats in a dose of 50 μg/100 g daily for 4 days. The blood glucose concentration was determined by the orthotoluidine method [3]. The serum insulin concentration was determined by a radioimmunologic method using the "Insik-I-M" kit from CEA-IRE-Sorin.

EXPERIMENTAL RESULTS

After thyroidectomy the stimulating action of catalytic quantities of the thermostable fraction of rat liver cell cytosol on Ca^{++} transport in liver mitochondria was weakened (Fig. 1). It was shown previously that the action of the thermostable fraction of cytosol on Ca^{++} transport in the mitochondrion is due to the presence of IDR in it. Consequently, after thyroidectomy, IDR activity in the liver was reduced. A similar effect was found also in experiments in which IDR activity was measured in the diaphragm of control and thyroidectomized rats (Fig. 1). The cause of the decrease in IDR activity in the target organs for insulin after thyroidectomy was evidently a fall in the serum insulin concentration (Table 1). Injection of physiological doses of thyroxine (50 μg/100 g) caused both an increase in the blood glucose concentration and an increase in IDR activity in the liver and diaphragm of the thyroidectomized rats. Hypoglycemia and hypoinsulinema have been described in the literature after thyroidectomy [7], but the intensity of the effects was much less than in the present experiments. This was evidently due to the longer times elapsing after thyroidectomy in the present experiments (4-5 months). The hypoglycemic effect of thyroidectomy also depends on the rats' age at operation, for in the present experiments hypoglycemia was more marked after thyroidectomy on rats weighing initially 80 g than in the series of experiments in which the initial weight of the rats was 120 g.

It is well known that both insulin and thyroid hormones stimulate glucose utilization *in vivo* [5, 7]. Meanwhile thyroid hormones activate gluconeogenesis whereas insulin inhibits it [5, 8]. The hypoglycemia and hypoinsulinemia after thyroidectomy are thus the result of inhibition of gluconeogenesis [7].

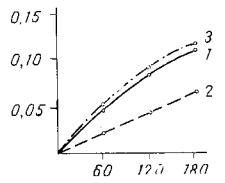

Fig. 2. Swelling of de-energized rat liver mitochondria in iso-osmotic ammonium pyruvate solution. 1) Control; 2) thyroidectomy; 3) thyroidectomy + thyroxine. Incubation medium: ammonium pyruvate 120 mM, Tris-HCl 10 mM, phosphate 3 mM, rotenone 1.0 μg/ml, 2,4-dinitrophenol 10^{-4} M, EDTA 1 mM, pH 7.4. Mitochondria 0.4 μg protein/ml. Abscissa, time (in sec); ordinate, changes in optical density.

Thyroid hormones are known to depress the sensitivity of target organs to the action of insulin [7]. Our data show that injection of physiological concentrations of thyroid hormones caused an increase in the serum insulin concentration and IDR activity in the target organs. IDR is an endogenous inhibitor of gluconeogenesis [10] and, for that reason, the increase in IDR activity in the liver is compensatory in character relative to the stimulating action of thyroxine on gluconeogenesis [8].

Recent investigations have shown that hormonal regulation of gluconeogenesis in the hepatocytes can take place at the level of pyruvate transport from cytosol into mitochondria, for low activity of the monocarboxylate barrier limits gluconeogenesis from lactate and pyruvate [9]. Catecholamines and glucagon stimulate pyruvate transport indirectly, by increasing the H^+ gradient on the mitochondrial membrane as a result of activation of the respiratory chain [6]. IDR inhibits pyruvate transport through a decrease in activity of the pyruvate carrier in the liver mitochondrial membrane [9]. The stimulating action of thyroid hormones on gluconeogenesis may perhaps incorporate activation of pyruvate transport from cytosol into mitochondria. It is shown in Fig. 2 that after thyroidectomy swelling of de-energized rat liver mitochondria is inhibited in an iso-osmotic solution of ammonium pyruvate. Under these conditions the rate of swelling is known to be limited by the permeability of the inner mitochondrial membrane for the anion [4]. Consequently, after thyroidectomy pyruvate transport into mitochondria is inhibited. This effect cannot be the result of a change in the H^+ ion gradient, for the experiments were carried out in the presence of 2,4-dinitrophenol. Administration of physiological doses of thyroxine to thyroidectomized rats stimulated pyruvate transport into rat liver mitochondria.

Thyroid hormones can thus stimulate gluconeogenesis at the level of pyruvate transport from cytosol into mitochondria. The results of stimulation of gluconeogenesis are a fall in sensitivity to insulin and an increase in the insulin concentration in the blood serum and IDR activity in the insulin target organs. The presence of strong correlation between the serum insulin concentration and IDR activity in the liver and diaphragm is evidence that sensitivity to insulin is regulated by physiological concentrations of thyroid hormones, not at the level of insulin binding by receptors on the plasma membrane, but at the level of interaction between intracellular insulin mediators and contrainsular hormones.

The decrease in sensitivity of the hepatocytes to the inhibitory action of insulin and IDR on gluconeogenesis, at the level of pyruvate transport from cytosol into mitochondria, may be due both to an increase in activity of the pyruvate carrier (Fig. 2) and to an increase in the H^+ ion gradient on the mitochondrial membrane on account of an increase in sensitivity of the hepatocytes to catecholamines.

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PHOTOGENERATION OF SINGLET OXYGEN BY PSORALENS

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KEY WORDS: psoralens; singlet oxygen; luminescence.

Furocoumarins (psoralens), in conjunction with near UV irradiation (UV-A, 315-400 nm), are widely used for the treatment of psoriasis and other skin diseases (PUVA-therapy) [1]. The therapeutic effect is connected with the photochemical activity of furocoumarin. Besides their therapeutic effects, they also give side-effects — erythema, changes in the mechano-electrical properties of the skin, etc. [7, 9]. The role of singlet oxygen ($^1O_2$) in the photobiological action of furocoumarins has recently been discussed in the literature. Experiments have shown that these compounds can generate $^1O_2$ during irradiation in solutions [9]. However, the basic information has been obtained by the use of indirect chemical methods of detection of $^1O_2$, which permit a different interpretation. Much more reliable results can be obtained by means of photosensitized luminescence of oxygen, which has recently been found in solutions of many sensitizers [2-6, 8]. Preliminary data obtained by the writers previously showed that such luminescence is also observed in solutions of 8-methoxypsoralen [10].

The aim of the present investigation was to determine the excitation spectra of luminescence of $^1O_2$ and quantum yields of $^1O_2$ generation in solutions of three furocoumarins: psoralen, 8-methoxypsoralen (8-MOP), and angelicin. The structural formulas and absorption spectra of these compounds are given in Fig. 1.

EXPERIMENTAL METHOD

Luminescence of oxygen was measured on instruments with photomultipliers described previously [3, 8]. The furocoumarins were generously provided by Professor G. Rodiguerro (University of Padua, Italy). CCl_4 (analytical grade), obtained from VEB Laborchemie, Apold, East Germany) was used as the solvent. Since this solvent, on excitation in UV rays, gave relatively intensive luminescence in the region of oxygen emission, it was purified by distillation immediately before the measurements. Distillation weakened luminescence by about one order of magnitude.

EXPERIMENTAL RESULTS

Excitation Spectra of Luminescence of $^1O_2$. Illumination of the furocoumarins in CCl_4 was shown to lead to the appearance of luminescence with a maximum at 1272 nm, corresponding to the $^1\Delta_g$-state of oxygen. The emission spectrum is given, for example, in [3, 4]. Excitation spectra of this luminescence, measured in the region of long-wave absorption bands of furocoumarins, are illustrated in Fig. 2. The accuracy of measurements of the spectra in the region of short-wave absorption bands was found to be insufficient because of superposition with luminescence of the solvent, and the short-wave maxima of luminescence excitation are therefore not shown in Fig. 2. It will be clear from Fig. 2 that in solution of psoralen the

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