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THYROID HORMONES AND REGULATION OF CELL RELIABILITY SYSTEMS

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INTRODUCTION

Reliability may be defined as a fundamental biological object property that causes stable and efficient functioning of biological objects under randomly varying environmental conditions and as a function of time. Key systems of cell reliability determine in particular: (1) inhibition of reactive oxygen species (ROS) and (2) repair of genetic structures from injuries caused by endogenous and induced mutagenesis. These systems seem to function in close co-operation, enhancing each other's action and thus increasing cell reliability. Such positive feedback may, in particular, considerably raise the reparogenic activity during antioxidant protection stimulation. It is known, for instance, that several DNA-repairing enzymes are energy- (1, 2) and Ca^{2+}-dependent (3, 4). At the same time, repair enzymes themselves (2) as well as key enzymes of energy metabolism (5), transport Ca^{2+}-ATPases (6) and hormone receptors involved in the maintenance of cellular Ca^{2+} homeostasis (7), all are highly sensitive to oxidation by ROS. In this connection, the most promising way of improving cell reliability is perhaps to look for natural substances that can stimulate directly both antioxidant protection and reparogenic activity. The present paper contains arguments supporting the idea that such substances may be thyroid hormones (TH), thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3), which regulate energy metabolism and Ca^{2+} status of target cells.

MATERIALS AND METHODS

Measurement of microsomal membranes' microviscosity. Sarcoplasmic reticulum (SR) vesicles from the smooth muscles of rabbit aorta were isolated according to (8). Changes in aorta SR membrane microviscosity were evaluated judging by the degree of pyrene eximerization. Pyrene eximerization was measured in 20 mM Tris-HCl, pH 6.8, 0.6 M KCl, and 0.25 M sucrose at a protein concentration of 0.5 mg/ml. Pyrene was dissolved in ethanol and slowly added to the incubation medium to a final

concentration 15 μM. Fluorescence was measured on a Hitachi MPF-4 spectrofluorimeter.

Assay of Ca^{2+}-ATPase activity and Ca^{2+} transport. Ca^{2+}-ATPase activity was determined as described previously (9). Active Ca^{2+} transport in the SR was examined at 37°C in a medium of the following composition: 120 mM KCl, 5 mM NaN_3, 5 mM $MgCl_2$, 3 mM ATP, 40 mM histidine, 40 mM HEPES pH 6.8, 10 mM sodium oxalate, 10 mM $CaCl_2$ containing 0.05 μCi ^{45}Ca (10 μM free Ca^{2+}). Incubation time was 15 min; microsomal protein concentration was 50 μg/ml. Reaction was stopped by 50 μl aliquot filtration through Millipore filters (HA, 0.45 μm). Ca^{2+} transport was also studied using a fluorescent chlortetracyline probe (CT). Measurements were performed at 25°C in a medium containing 20 μM CT, 120 mM KCl, 100 μM $Mg(CH_3COO)_2$, 20 mM HEPES pH 6.8, 5 mM $Na_2C_2O_4$, 2 mM ATP and 0.1 mg/ml protein.

Chemiluminescence assay. To obtain neutrophils, EDTA-blood from normal healthy volunteers was separated according to Ref. (10). Chemiluminescence measurements were performed on an LKB-1250 luminometer (LKB, Sweden) at room temperature. The reaction-mixture contained 10^6 cells/ml neutrophils in Krebs–Ringer phosphate buffer and 200 μM luminol. Chemiluminescence was induced by the addition of 1 μg 4β-phorbol 12-myristate 13-acetate or 0.5 ng pyrogenal (final vol 1 ml). Thyroxine and triiodothyronine were dissolved in 0.01 M NaOH; ionol was dissolved in ethanol. Agents were added 5 min before or 2 min after activation of neutrophils.

X-ray irradiation experiments. The main groups of rats were irradiated at a dose of 250 BER. X-ray irradiation at doses of 150 BER and 200 BER was also carried out to estimate the effect of thyroxine in terms of a dose reduction factor. Hepatectomy was performed after X-ray exposure. Animals were decapitated 26 hr after the operation. The number of hepatocytes with chromosome aberrations (fragments, bridges, or bridges and fragments) was estimated among 100 anaphases and early telophases, stained by the Feulgen method and with light green.

RESULTS AND DISCUSSION

Protection of Membrane Enzyme Systems Against Oxidative Stress: New Approaches

Current cell membrane antioxidant protection strategy aimed mainly at combatting lipid peroxidation requires significant correction. This

circumstance is caused by a growing amount of data about the leading contribution to the damaging effect of oxidative stress on the cell by the oxidation of membrane-bound enzymes and proteins regulating these enzymes (11, 12). Indeed, the direct enhancing effect on the damaging action can appear only when membrane enzyme systems are oxidated, especially those involved in the regulation of cellular ion homeostasis. At the same time, no efficient mechanisms that could improve the antioxidant resistance of membrane regulatory systems have been found so far (13). This may be associated with the fact that among classic antioxidants, which act mainly as scavengers for oxidants, there are still no compounds that would combine high activity with a wide spectrum of antioxidant action, exerting no toxic side effects. These circumstances stimulate the search for new ways of cell membrane antioxidant protection. We believe that these ways may include covalent modification of easily oxidizable target proteins, as well as the inhibition of toxic oxidant formation in the serum. In the first part of this paper it is demonstrated that it is feasible to provide protection from neutrophil-induced oxidative stress with the use of both protein phosphorylation in target cell membranes and inhibition of induction of this stress by thyroid hormones.

Structural and functional modification of sarcoplasmic reticulum membranes during oxidation. A good model of oxidative stress may be presented by oxidative attack induced by activated neutrophils, because these cells, under some pathologic conditions, make a significant contribution to membrane damage *in vivo* (14). Activated neutrophils produce a number of ROS: superoxide anion, hydroxyl radical, singlet oxygen, H_2O_2 and hypochlorite (HOCl) (14, 15). It should be noted that the term "oxygen free radicals" is not infrequently associated with all reactive oxygen intermediates including non-radical oxygen species; therefore, the term "ROS" seems preferable. Among ROS produced by activated neutrophils, of interest is HOCl which is not a free radical. HOCl is generated by myeloperoxidase and is the most toxic product of activation of these cells (16). It is noteworthy that HOCl is generated both within and outside the neutrophil, interacting non-enzymatically with nitrogen compounds to form long-living non-radical oxidants (16). These oxidants (chloramines) are lipophilic agents, and, therefore, their main target may be biologic membranes, in particular, SH-groups of membrane-bound proteins (17).

The HOCl effect on the structure (fluidity) of sarcoplasmic reticulum membranes in myocytes was studied, as well as Ca^{2+}-ATPase (EC 3.6.1.3) activity and Ca^{2+} transport in these membranes. It is known that SR plays the central role in the regulation of intracellular free Ca^{2+} concentration in muscle tissue; in its turn, myocyte cytosol Ca^{2+} level determines myocyte contractility.

As an object for studying HOCl action on the structure and function of SR membranes, we have chosen aorta smooth muscle because it has been found that vascular tone can be not only disturbed but also regulated both by free radicals (15) and HOCl (17).

SR microviscosity was determined by exciting hydrophobic pyrene probe fluorescence with the light at 280 nm and 334 nm wavelength for protein-bound and free lipids, respectively. The rate of pyrene diffusion in membranes and, consequently, the fluidity of lipid bilayer are characterized by the ratio of C/I_m where C is for pyrene concentration and I_m for pyrene monomer fluorescence intensity at 380 nm. It was found that (Fig. 1), under HOCl influence, SR membrane microviscosity, which is characterized by the slope value, is approximately 8% higher than in the control (free lipid zone) and approximately 15% higher than in the control in the protein-bound lipid zone. This finding supports the concept of the leading contribution made by protein oxidation to structural and functional changes of cell membranes during ROS attack (12).

Results of the study of HOCl influence on functional properties (ATPase activity and Ca^{2+} transport) of the main SR protein species — Ca^{2+}-ATPase, making at least 70% of total membrane protein (18) — are shown in Figure 2. As HOCl concentration increased from 10^{-7} M to 10^{-3} M (physiological HOCl level is approximately 10^{-6} M to 10^{-5} M), Ca^{2+}

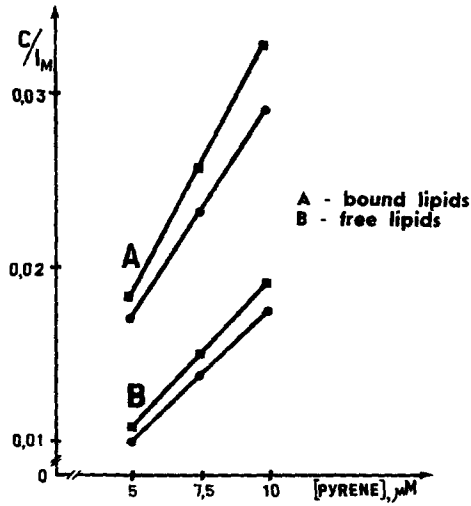

FIG. 1. Microviscosity of aorta sarcoplasmic reticulum membrane treated with HOCl. (•) baseline (no HOCl treatment); (■) treatment with 5×10^{-7} M HOCl (25°C, 5 min). See the text for further detail.

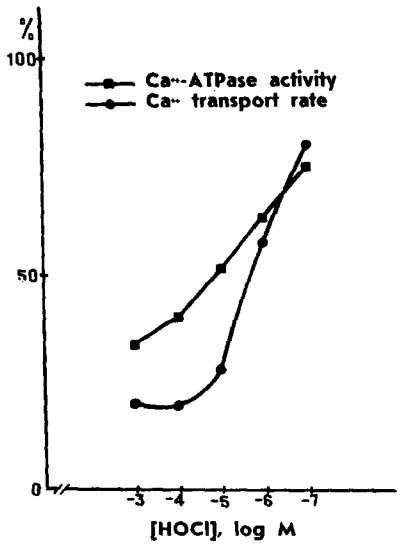

FIG. 2. ATP hydrolysis and ^{45}Ca transport rates in aorta sarcoplasmic reticulum treated with HOCl (25°C, 5 min). The corresponding values in the absence of HOCl taken as 100%.

transport rate decreased from 80 to 18%, respectively, as compared with the baseline level (before treatment with HOCl), whereas Ca^{2+}-ATPase activity inhibition was less pronounced: from 78 to 32% (Fig. 2). It should be noted that other ROS generated by activated neutrophils (superoxide anion, hydroxyl radical and H_2O_2), taken at physiologic concentration, did not influence the SR function (14).

Protective effect of methionine and dithiothreitol. It is known that easily oxidizable methionine (Met) and dithiothreitol (DTT) can act as scavengers for ROS. When aorta SR was pretreated with L-Met, a partial, and in the case of pretreatment with DTT, a complete protective effect of these compounds was found with respect to HOCl-inhibited active Ca^{2+} transport (Fig. 3). The difference thus observed in the protective effect between Met and DTT allows us to conclude that targets for HOCl action in SR are SH groups of membrane-bound proteins, in particular, Ca^{2+}, ATPase which contains over 20 thiol groups per mole (12). Indeed, DTT, apart from being able to act as a ROS scavenger, can also reduce oxidized SH groups in target proteins, and, therefore, under given experimental conditions it can be a more efficient antioxidant than Met.

Protective effect of protein phosphorylation. It is quite probable that one of the proteins attacked by HOCl may also be phospholamban,

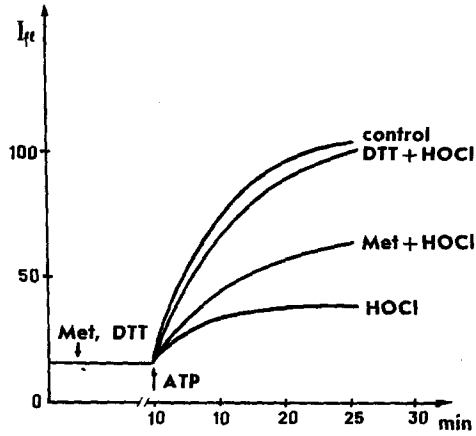

FIG. 3. Influence of HOCl on active Ca^{2+} transport rate in aorta sarcoplasmic reticulum pretreated with Met and DTT. Control: no antioxidants and HOCl. Met (5×10^{-3} M) and DTT (5×10^{-3} M) were added just before the addition of HOCl (treatment with 5×10^{-5} M HOCl: 25°C, 10 min). I_{fl}: CT fluorescence intensity (relative units).

an ATPase-associated low-molecular SR component found in slow skeletal, cardiac and smooth muscle. Like Ca^{2+}-ATPase, phospholamban contains a considerable amount of easily oxidizable Cys, Met and His residues (19). Phospholamban phosphorylation intensifies active Ca^{2+} transport (20), while dephosphorylated phospholamban can function as a Ca^{2+} channel (21).

We demonstrated earlier that phospholamban phosphorylation increased the resistance of this protein and Ca^{2+} transport into SR membranes against the influence of serine proteases (22). Taking into account the close interrelation between protein responsiveness to proteolysis and the degree of protein oxidation (11), mutual influences of HOCl and phosphorylation on ^{45}Ca transport rate were studied in aorta SR in the presence of exogenous cAMP, cGMP and calmodulin (Fig. 4). It has been established that earlier SR membrane HOCl treatment and subsequent protein phosphorylation led to a partial phosphorylation protective action against Ca^{2+} transport damaging under the 10^{-7} M HOCl treatment and the absence of a protective effect at the 10^{-3} M HOCl level. It should be noted in this connection that membrane-bound protein oxidation reduces their ability for phosphorylation (12). On the other hand, earlier SR protein phosphorylation completely protects Ca^{2+} transport against the 10^{-7} M HOCl damage action and partly preserves its protective effect even under the 10^{-3} M HOCl treatment of the membranes.

It may be believed that phosphorylation of SR regulatory proteins coupled with Ca^{2+}-ATPase (probably that of phospholamban) will change

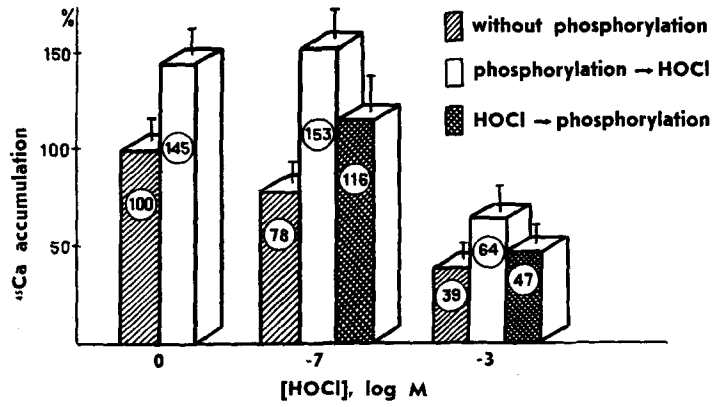

FIG. 4. Influence of phosphorylation and HOCl on ^{45}Ca accumulation in aorta sarcoplasmic reticulum. Phosphorylation was carried out for 10 min, 25°C in the presence of 10^{-6} M cAMP, 10^{-7} M cGMP and 10 μg/ml calmodulin. 100%: ^{45}Ca accumulation in microsomes in the absence of phosphorylation and HOCl treatment.

the conformation of these molecules, rendering SH and NH_2 groups less accessible to HOCl. Combined action of protein phosphorylation and SH-protectors may lead to a significant increase of antioxidant effect on a positive feedback basis, since it is known that oxidation attack causes inactivation of protein kinases, in particular of cysteine and methionine SH group-rich cAMP-dependent protein kinase (12).

It should be noted that antioxidant protection with the aid of DTT and protein phosphorylation, which has been demonstrated in this paper, seems to be efficient enough *in vitro*, but may be subject to some limitations when used *in vivo*. For instance, a possible toxic effect produced by millimolar DTT concentrations cannot be ruled out; the protective effect of phosphorylation is aimed only at proteins that contain phosphorylizable amino acid residues with hydroxyl groups; finally, antioxidant effects have been found in respect of only one, although the most toxic, ROS generated by neutrophils. It seems probable that one of the most efficient antioxidant protective effects *in vivo* might be the inhibition of ROS generation by activated neutrophils with the use of some substances of natural origin. In the next part of our paper it will be demonstrated that such substances may be represented by thyroid hormones acting at physiologic concentrations.

Antioxidant Action of Thyroid Hormones

Data about the influence of thyroid gland extract on the increase of SH group numbers in proteins and enzymes that have been lowered following X-ray irradiation appeared a rather long time ago (23) but remained

unexplained. Antioxidant TH action has been studied only insufficiently and has not yet attracted due attention. At the same time, it seems that this aspect of TH action may contribute considerably to generalized effects produced by thyroid gland hormones. The versatility of TH action suggests that these hormones are able to modify ROS injuries in protein, lipid and nucleic acid molecules by exerting their influence at different intracellular metabolic levels, that is, by interfering in the process of prooxidant and antioxidant enzyme and protein synthesis, by acting as antioxidants themselves and by inhibiting ROS induction. Each of these hypotheses is supported by experimental evidence which is presented below.

Regulation of TH and antioxidant enzymes. It was found that in response to low-dose TH injection in rats there was an increase in total activity of superoxide dismutase (an enzyme that removes superoxide radicals) in red blood cells and cardiomyocytes and a decrease in lipid peroxidation product contents (24). Under conditions of a considerable increase in T_4 content (hyperthyroidism), intensification of microsomal membrane lipid peroxidation in rat hepatocytes was also accompanied by a rise in activity of antioxidant enzymes, glutathione peroxidase and glutathione reductase, as compared with that observed in euthyroid animals (25). In cardiomyocytes of hyperthyroid rats, mitochondrial superoxide dismutase activity was found to be increased, the cytosol form of the enzyme being inhibited and lipid peroxidation being intensified (26). In contrast, in fetal hypothyroidism caused by the inhibition of T_3 generation in pregnant guinea pigs, no changes in catalase and cytosol-associated superoxide dismutase activities were observed, but a considerable decrease in the activity of a mitochondrial form of the enzyme was found in the lungs of hypothyroid fetuses as compared with that in the fetuses of animals that had not received T_3 blocker (27). It is believed that under physiological conditions T_3 is required for the synthesis of mitochondrial superoxide dismutase (27). This circumstance appears to be of special interest in connection with the fact that mitochondria are one of the main intracellular targets for TH action (28, 29).

TH structure and antioxidant effect. Thyroxine and triiodothyronine belong to compounds possessing phenolic ring; such a structure of TH molecules suggests the antioxidant properties of these hormones (30). Indeed, antioxidant action of T_4 was demonstrated in microsomes (31) as well as in a model reaction of methyl oleate oxidation (32). It was also found that T_4 at a concentration of 10^{-7} M inhibited the development of chemiluminescence in rat hepatic mitochondria (33). In this case, antioxidant activity of T_4 was virtually not inferior to that of such a classic

antioxidant as alpha tocopherol and was by one or two orders higher than that of estrogenic steroid hormones (33).

TH and neutrophil respiratory burst inhibition. As already mentioned neutrophil activation is accompanied by the generation of the products possessing strong oxidant properties. A variety of effectors such as chemotactic peptide formylmethionylleucylphenylalanine (fMet–Leu–Phe), immune complexes, complement component 5a, lectin concanavalin A, etc., can interact through receptors and trigger numerous responses in neutrophils, including respiratory burst (34). It would be intriguing to suggest that TH antioxidant effect may also be caused by the inhibition of a specific neutrophil receptor–ligand interaction. The existence of the latter mechanism would allow TH to exert an effective antioxidant influence at physiological concentrations. Evidence in favor of this suggestion is provided by the T_4 and T_3 inhibition of a superoxide production of neutrophils activated with fMet–Leu–Phe by way of receptor mechanism (35). However, these authors demonstrated the antioxidant TH action only at high hormone concentrations (10^{-5}–10^{-6} M).

Our experiments revealed the inhibitory effect of physiologic T_4 and T_3 concentrations in the reaction of ROS generation by activated human neutrophils (Fig. 5A). For neutrophil activation 4β-phorbol 12-myristate 13-acetate (PMA) was used, which induces nonspecific neutrophil activation by stimulating protein kinase C (36) as well as pyrogenal – lipopolysaccharide from *Salmonella typhi* cell wall, whose effect on neutrophils is realized by way of specific interaction with receptors (37). Luminol-dependent

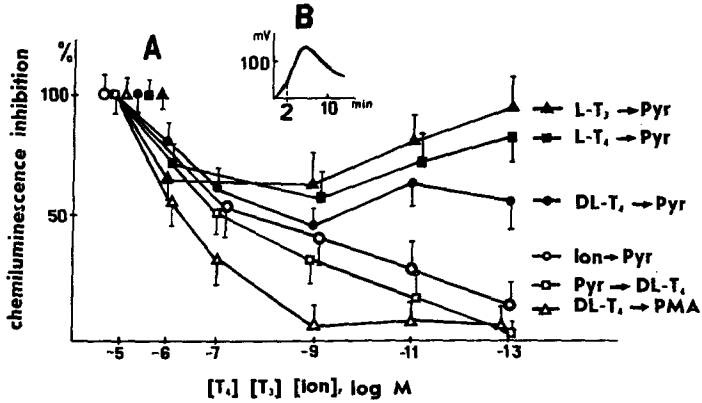

FIG. 5. T_4-, T_3-, and ionol-induced inhibition of human neutrophils chemiluminescence. Ion: ionol; Pyr: pyrogenal. See the text for further detail.

chemiluminescence inhibition in PMA-activated cells preincubated with DL-T_4 for 5 min was observed only at high hormone concentrations (10^{-5}–10^{-7} M). On the contrary, chemiluminescence inhibition in neutrophils activated by pyrogenal was observed at TH concentrations as low as 10^{-11}–10^{-13} M. The greatest inhibitory activity was observed in L-T_3 and L-T_4 as compared with that in DL-thyroxine. At the same time, if the hormone was added 2 min after neutrophil activation (at the beginning of respiratory burst product accumulation (Fig. 5B)), the antioxidant effect persisted only in cases of high DL-T_4 concentrations. Under our experimental conditions the antioxidant effect produced by TH was significantly stronger as compared with that of such a potent antioxidant as 4-methyl-2,6-di-isobutyl phenol (ionol) whose antioxidant activity in its turn is higher than that of alpha tocopherol (32).

The inhibitory effect of high T_4 concentrations in the neutrophil activation reaction seems to be nonspecific and caused by antioxidant properties of TH molecules, which act, in this case, as scavengers for ROS. Lipophilic TH molecule incorporation into neutrophil membrane at hormone concentrations ranging from 10^{-5} to 10^{-9} M may also change the viscosity of the lipid bilayer and thus inhibit specific antioxidant action of TH. This action manifests itself at low TH concentrations (10^{-11} to 10^{-13} M) and probably is caused by specific inhibition of pyrogenal interaction with receptors or other neutrophil membrane components.

Antioxidant effect produced by low TH concentrations has been established in the reaction of luminol-dependent luminescence inhibition in activated neutrophils. This circumstance may attract additional attention to the manifestation of antioxidant TH action *in vivo*. For instance, it is known that this luminescence is to a significant extent caused by HOCl (38) whose inhibitory effect on the activity of membrane-bound enzymes is the most pronounced as compared to those exerted by other ROS.

Further investigation into the antioxidant TH effect would be of undoubted interest. This interest is to a considerable extent caused by a possible TH influence on the processes of mutagenesis and genetic structure repair from injuries induced by ROS.

Thyroid Hormones and Repair Stimulation

The genetic structure repair system is one of the most efficient cell reliability systems (39, 40). Thus, repair mechanisms determine DNA native structure maintenance in the course of normal template processes, spontaneous mutagenesis level and cell resistance against the influence of DNA-tropic injuring agents (39, 41). As is known, the intensity of genetic structure repair processes depends on repair enzyme activity which in turn is a function of numerous cellular activity parameters

as follows: energy balance (1, 42, 43), ion homeostasis (3, 4) protein synthesis intensity (41, 42), DNA methylation process (44), etc. That is why, when searching for agents that would favor genetic structure restoration and increase genome reliability, a cue should be taken from substances potentially capable of influencing these parameters and primarily from those of natural origin. To such substances hormones may belong. Changing the synthesis of nucleic acids and proteins, hormones can influence genetic structure repair systems and, accordingly, mutagenesis (45, 46).

Among hormones participating in genetic homeostasis modification, a particular place seems to belong to TH. Thyroid hormones possess the widest spectrum of action of all mammalian hormones. Triiodothyronine receptors are present in nuclei of cells of all tissues, being especially numerous in tissues of target organs (liver, heart, etc.) (47–50). There are also reports on TH receptors present in plasma and mitochondrial membrane (28, 51). Thyroid hormones control a number of the most important biochemical reactions of protein, carbohydrate, lipid and water-salt metabolism as well as cell growth and differentiation (52–54). Thyroid hormones stimulate protein synthesis at both transcription and translation levels, in particular by activating DNA-dependent RNA polymerase (53, 55). Noteworthy is the influence exerted by TH on the synthesis of energy metabolism enzymes (5, 50, 56) and on DNA methylation–demethylation (57), since these processes to a considerable extent underlie repair system activity.

Thyroid hormone influence on cell energy production. The thyroid gland is one of the first among endocrine glands involved in primary nonspecific reactions of the organism to irritants of low and moderate intensity (58). As is known, even minor deviations from normal blood T_4 and T_3 concentrations will result in multiple changes that can be observed in virtually all organs and tissues (52–54). In this respect, especially noteworthy is the influence exerted by TH on oxidative phosphorylation.

It should be noted that a considerable number of authors regard extranuclear effects produced by TH as unimportant or non-physiological ones. At the same time, a rapid oxidative phosphorylation stimulation, which was not caused by the influence on protein synthesis, could be observed when mitochondria from the liver of hypothryoid rats were treated with low T_3 concentrations (beginning from 30 pM) (29, 59). A protein acting as T_3 receptor has been isolated from inner mitochondrial membranes of rat hepatocytes and characterized (59). Binding of T_3 to this protein causes immediate activation of oxidative phosphorylation (28). Most probably, the stimulating effect produced by TH on energy-exchange processes in the cell may be realized both by protein synthesis changes (5) and by direct interaction with mitochondrial receptors. In both cases, the

result may consist in the stimulation of genetic structure repair enzyme activities.

Thus, it has been found that it is ATP that ensures normal DNA-ligase activity level and acts as a cofactor of these enzymes in mammalian cells (1, 2). The importance of the oxidative phosphorylation system and its regulation for DNA repair processes is evidenced by the fact that enzymatic reactions with the participation of polynucleotide ligases, DNA ligases and DNA polymerases become inhibited under conditions of even a slight ATP deficiency (60). Repair of DNA radiation-induced injuries depends on the level of ATP obtained by way of oxidative phosphorylation or anaerobic glycolysis (61).

Influence of thyroid hormones on cell calcium homeostasis. In addition to oxidative phosphorylation, intracellular calcium level also may control the modification of genetic structure integrity. It has been established that a number of DNA repair enzymes, in particular endonucleases, are Ca^{-1P}-dependent (3, 4). Moreover, Ca^{2+} is necessary for maintaining and stabilizing the chromosomal DNA–protein complex (62) as well as for RNA synthesis (63). On the other hand, membrane permeability for ions, including Ca^{2+} status of the cell (49, 64–66), is under TH control. Thryoid hormones can control ion homeostasis of a target cell, activating *de novo* synthesis of cell-membrane ion channel and pump proteins (65, 66). Thus, for example, T_4 effect on the expression of cardiomyocyte sarcoplasmic reticulum Ca^{2+}-ATPase gene has been found (67), which may contribute considerably to the long-term Ca^{2+} level regulation in the cytosol of these cells.

It is known that distribution of intracellular Ca^{2+} in some tissues, such as liver, is regulated in part by energy-linked mitochondrial Ca^{2+} transport. T_3 profoundly accelerates rat liver mitochondrial Ca^{2+} efflux evoked by the oxidized state of mitochondrial NAD(P)H (68). This effect was observed at low T_3 administration level (5 μg per 100 g body weight daily) and required at least 3 days of exposure. According to the data of de Giovanni *et al.* (69), T_4 and T_3 at physiological intracellular concentration induce prompt and extensive Ca^{2+} transport change in rat liver mitochondria, which is accompanied by stimulation of respiration. The rapid enhancement of oxidative phosphorylation coupling induced by TH, as shown by Sterling *et al.* (59), could explain the rapid increase of Ca^{2+} transport by the same mitochondrial preparations (69), suggesting a central role of TH as regulators of mitochondrial and cellular activities.

It is believed that in different tissues the effect produced by TH, which are hydrophobic compounds, may be realized also through plasma membrane phosphoinositide cycle components (70). In this case, membrane-bound enzyme activities undergo changes (71, 72). For instance, Ca^{2+}-ATPase in

the plasmalemma of cardiomyocytes and red blood cells increased under the influence of physiological (10^{-9} to 10^{-10} M) T_4 and T_3 concentrations *in vitro* and *in vivo* (70, 73, 74). This stimulating effect of TH was not associated with the genetic apparatus activation in the cell nucleus by hormones but was most probably caused by the increase in Ca^{2+}-ATPase responsiveness to Ca^{2+} and calmodulin (70). High T_4 and T_3 concentrations did not change the activity of this enzyme or exerted an inhibitory effect (73). Under the influence of T_4 at a concentration of 10^{-6} M, an increase in Ca^{2+} accumulation in the sarcoplasmic reticulum of cardiomyocytes was observed (75).

It was found also that T_3 interaction with the cell membrane of rat thymocytes *in vitro* led to an immediate (within few seconds) Ca^{2+} uptake from the extracellular medium and probably to Ca^{2+} release from the intracellular depots. These events resulted in Ca^{2+}–calmodulin complex formation, adenylate cyclase activity and cAMP level increase (76, 77) as well as, probably, in phosphorylation of a receptor-dependent Ca^{2+} channel or of a functionally associated protein (78). *In vitro* results were confirmed by *in vivo* experimental findings. For instance, intensification of ^{45}Ca uptake by the tissues of rat ventricle, aorta and diaphragm could be observed as early as 2 min after T_3 injection at low concentrations (0.01 μg to 0.1 μg/100 g body weight). Inorganic Ca^{2+} channel blockers inhibited T_3-induced calcium uptake. As it had been found in the study of plasmalemmal Ca^{2+}-ATPase, high T_3 concentrations also caused the inhibition of ^{45}Ca uptake via Ca^{2+} channels (78). Thus, TH probably can exert both long-term (at the cell nuclei level) and a rapid, nuclear effect-independent influence on active and passive Ca^{2+} transport systems in the plasmalemma, mitochondrial and endoplasmic reticulum membrane of target cells. Cytosol Ca^{2+} concentration growth induced by physiological TH levels may in its turn lead to the intranuclear activation of DNA repair enzymes.

Repair of thyroid hormones in X-ray irradiation. It is believed that no other factor is subject to such a strict regulation as is ionizing radiation; a clear dose–effect correlation is characteristic, for instance, also of DNA repair enzyme activities (79). As the enzymatic repair systems are being improved, cell reliability in relation to the damaging effect produced by ionizing radiation grows by 2 orders of magnitude (79). At the same time, among a number of agents reducing the damage caused by ionizing radiation in the human or animal organism there are still no preparations that would be able to stimulate genetic structure repair upon administration after X-ray irradiation.

Ye. N. Antipenko and his colleagues were the first to find the antiradiation effect produced by T_4 (80–83). Data concerning the influence of varying T_4

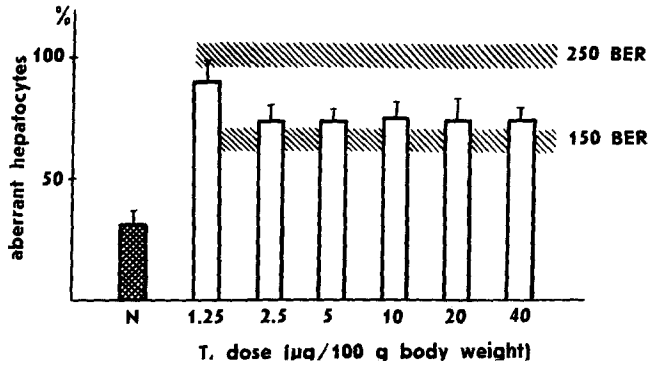

FIG. 6. T_4 reparogenic effect after X-ray irradiation. 100%: aberrant cells proportions under 250 BER irradiation. N: unirradiated. T_4 administration to rats on days 3 through 7 after irradiation. (Data, Ye. Antipenko and O. Timchenko).

doses on chromosome restoration from X-ray irradiation-induced lesions are presented in Figure 6. In this experiment the hormone was administered after irradiation in order to exclude a possible radiosensitizing TH effect demonstrated earlier (84). After general X-ray irradiation at a dose of 250 BER, T_4 administration to rats (25–40 μg per 100 g of body weight daily on days 3 through 7 after irradiation) caused a reduction in number of hepatocytes with aberrant chromosomes (Fig. 6). It is important to note that the reparogenic effect of T_4 was revealed at low (physiological) doses of the hormone. For instance, a minimum effective T_4 dose appeared to be equal to 2.5 μg per 100 g body weight corresponding to 1/2 of daily hormone production by the normally functioning thyroid gland in adult

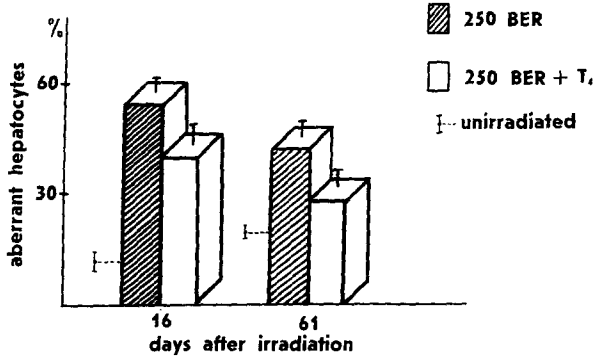

FIG. 7. Conservation of T_4 reparogenic effect. Dotted lines: aberrant cells proportions in unirradiated rats. T_4 administration to rats on days 3 through 15 after irradiation.

rats (54, 85). Reparogenic effect of T_4 administered on day 3 through day 15 after irradiation persisted for at least 2 months (Fig. 7) and was considerable: dose reduction factor reached 1.3–1.4.

Antiradiation effect of T_4 is probably brought about by way of T_4 influence on the DNA repair system that depends on oxidation and phosphorylation coupling. Thus, reparogenic action of T_4 was reduced in the presence of dinitrophenol dissociating oxidation and phosphorylation (86). Influence of TH on Ca^{2+} homeostasis in target cells also cannot be ruled out as one of the possible mechanisms underlying the reparogenic effect. This suggestion is supported by the data on the increase in the number of chromosome aberrations in X-irradiated cultured human leucocytes in the presence of calcium-binding EGTA (87).

Additional evidence to the fact that antiradiation effect of T_4 is associated with hormone action on the repair system is provided by the following observations. Thus, it is known that treatment with neutrons causes a high number of virtually irreparable double breaks and a significantly higher (as compared with that produced by X-ray irradiation) damage to repair systems: antiradiation effect of T_4 could not be observed after irradiation with neutrons (88).

It is important to note that T_4 seems to influence prereplicative repair systems which eliminate a major part of injuries as compared to replicative and postreplicative processes (39, 40). For instance, experiments with human peripheral blood lymphocyte culture determined that cytogenetic T_4 effects revealed *in vivo* were about at G_0 phase of the cell cycle and might have been caused by a direct hormone interaction with a target cell (89, 90).

The principal importance should be emphasized of a repair effect produced by T_4 administered 3 days after irradiation (81, 83) since self-dependent genetic structure repair processes are completed within a few hours after irradiation (84). It thus follows that the cytogenetic effect of T_4 may be regarded as a stimulation of reparative potentialities still available.

Reparogenic effect of T_4 manifested itself in rats irradiated at a dose of 250 BER on days 3 to 7 from the onset of radiation sickness, that is, exactly at that time when the demand for TH was increased due to the impairment of thyroid gland function (80, 83). After irradiation at a dose of 250 BER, it is during this period that not only the maximum dissociation between oxidation and phosphorylation occurs (91) but also the antioxidant activity is inhibited to the greatest extent (32). Thus, the best time for administration of T_4, which probably modifies these systems, in order to make use of T_4 reparogenic activity should be the period of maximum inhibition of oxidative phosphorylation and antioxidant protection.

It is remarkable that T_4 action as a reparogen under ionizing irradiation

conditions was reduced in 18- to 19-month-old rats as compared with that in 3 to 5-month-old animals (83), probably due to the changes at the receptor–ligand interaction level. Thus, a reduced sensitivity to a variety of growth factors and hormones is a common finding in senescent tissue; in some cases there is an age-dependent reduction in the number of receptors.

It is quite probable that TH may not only stimulate genetic structure repair but also, under certain circumstances, cause the decrease in ROS-induced oxidation of repair system components. Thus, DNA repair enzymes are SH-containing, lipid-dependent and membrane-bound (2). Some energy metabolism enzymes with a high cysteine and methionine content per mole such as, for example, creatine kinase (5) as well as cellular membrane Ca^{2+}-ATPases (6) and hormone receptors, involved in maintaining Ca^{2+} homeostasis (7) may also be good targets for ROS-induced attack. Thus, the reparogenic potential of TH can be efficiently protected against the action of oxidative stress. Finally, TH-dependent repair stimulation can also exert a protective effect in the synthesis of antioxidant enzymes (as already mentioned, synthesis of some of these enzymes is in its turn controlled by TH). Possible ways of regulating the key cell reliability systems with thyroid hormones are shown in Figure 8.

In general, stimulation of cell reliability systems with TH may prove to be important for correcting conditions caused by errors in energy- and Ca^{2+}-

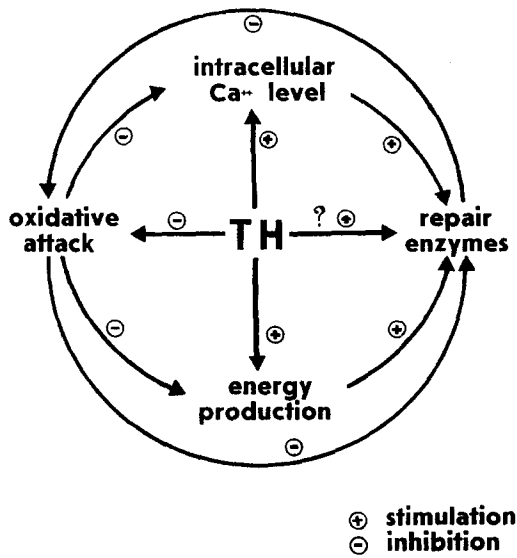

FIG. 8. Possible ways of thyroid hormones (TH) influence on cell reliability key systems.

dependent DNA repair under extensive ROS attack. Thus, in particular, study of TH influence on carcinogenesis may be of considerable interest.

Thyroid Hormones and Carcinogenesis

The influence of TH on carcinogenic mechanisms cannot be doubted. Thus, results of a follow-up examination of 2,000 patients with thyroid gland dysfunction look really impressive: neoplastic diseases occurred subsequently only in 0.5% of cases in hyperthyroid patients but as frequently as in 12% of cases in thyroidectomized and myxedematic patients (92). An extremely rare occurrence of cancer in patients with Basedow's disease has been confirmed by numerous observations (93, 94). In the experimental study it was also found that hyperthyroidism causes the increase in anti-tumor resistance (94). On the other hand, it was shown that T_4 stimulated the increase in tumor weight and metastatic index in mouse sarcoma (95). It should be noted that in this case T_4 concentrations several times as high as physiological ones were used. It was shown also (96) that hepatic tumor induced by chemical carcinogens did not develop in thyroidectomized rats. (As already mentioned, in thyroidectomized rats the number of aberrant hepatocytes increased under the influence or ionizing radiation (81).) Exposure of embryonic hamster cells to T_3 was accompanied by cell transformation into tumor ones under ionizing irradiation (97). It is noteworthy that T_3 in that experimental study was administered prior to irradiation, which did not permit ruling out a radiosensitizing effect produced by this hormone.

In general, however, the data presented on the diversified effect of TH on carcinogenesis may not conflict. It is likely that such a TH effect corresponds with established concepts of a two-stage mechanism of carcinogenesis. Thus, at the stage of initiation consisting in some kind of damage to DNA, the reparogenic and antioxidant TH potential may be important, whereas at the stage of promotion, which favors the manifestation of the transformed phenotype, the proliferative TH effect may be the determinant one. It should be noted that prereplicative DNA repair, which seems to be under TH control (88, 89), inhibits carcinogenesis only at the very beginning of this process (98).

Possible TH action at the stage of carcinogenesis initiation may be indicated also by an observation that ROS play an important role in this case (99, 100), while different antioxidants (alpha-tocopherol, ascorbic acid, selenium, etc.) are also anticarcinogens (101). For instance, the low efficiency of DNA repair in tumor cells may be associated with oxidative damage to prereplicative repair enzymes (98). It should be noted that, as there are stages in the process of tumor induction that are connected with the increase and decrease in general antioxidant activity of cells,

antioxidant administration at different doses and at different stages of carcinogenesis will lead to different effects (32). For instance, low (but not high!) doses of inhibitors from the class of shielded phenols during the initial period of carcinogenesis result in a reduced tumor occurrence, while administration of inhibitors late in the tumor induction period leads to the opposite effect (32).

Association between carcinogenesis and DNA repair impairment is obvious: unrepaired or poorly repaired injured DNA segments form a base for mutations, and mutations occurring at certain genome sites (protooncogenes) transform a normal cell into a malignant one, inducing oncogene generation. A considerable number of human hereditary diseases are known that are based on the deficiency of certain individual prereplicative DNA repair mechanisms: Xeroderma pigmentosum, Fanconi's anemia, ataxia-telangiectasia, progeria, etc. (102–104). One common feature is characteristic of these diseases, that is, an increased predisposition towards the development of malignant tumors and leucoses (105). For example, the probability of skin cancer development is 1,000 times higher in patients with Xeroderma pigmentosum as in the general population (105). In these patients, a marked correlation was found between the severity of DNA repair deficiency, mutation incidence and early development of tumors (106). For ataxia-telangiectasia a specific mutation was found, a translocation of the long arm of chromosome 14. Lymphocyte clones bearing this translocation seem to be leucotic cell precursors (107).

As already mentioned, an anticarcinogenic TH effect observed in clinical practice cannot always be reproduced in experimental studies. This discrepancy may be caused by the fact that high carcinogen doses used in the latter case may be too high for the repair capacity of the cell, whether it is physiologic or TH-stimulated. On the whole, it is likely that the wide spectrum of TH action including the repair potential of these hormones may to a considerable extent determine the versatile influence exerted by TH on carcinogenesis. This influence may depend, in particular, on the stage of the process, on the dose of hormones and, probably, on the nature of mutagen.

Noteworthy is the potential possibility of TH application in oncological practice. This possibility is based on the use of the reparogenic potential of these hormones and is associated with the improvement of antitumor radiation therapy. It is known that the only approach to the problem of antitumor radiation therapy improvement has for a long time consisted in the optimization of spatial distribution of the absorbed dose with the use of new radiation sources and methods (108). Progress in radiobiology led to the discovery and subsequent employment of radiomodifiers which mostly increased tumor cell sensitivity to irradiation (109). Additional progress in radiation therapy may be achieved with the use of substances selectively

protecting normal tissues, which would allow increasing the exposure dose to affect the tumor. In our opinion, the role of such substances may be played by TH which stimulate the repair of genetic structures injured by irradiation of healthy tissue cells but do not act in such a way on the transformed cells. Thus, the fact that normal and tumor cells differ in their sensitivity to TH has been established (110, 111). This phenomenon may be caused, among other things, by structural changes in hormone- and DNA-binding sites of T_3 in the chromatin of the transformed tissue. In this case, receptors are transformed into oncogene protein with impaired hormone-binding ability (112).

In the experimental study of T_4 reparogenic properties, the dose reduction factor judged by the level of cells bearing chromosome aberrations reached 1.3–1.4 (see above). Retention of such a dose reduction factor under antitumor radiation therapy conditions would mean the possibility of intensifying the radiation load on a tumor by approximately 40%. It is known, meanwhile, that a 10–20% increase in the dose absorbed by a tumor may raise the efficiency of treatment approximately by a factor of 4 (113) and reduce tumor recurrence incidence by more than an order of magnitude (108). Therefore, on condition that TH reparogenic efficiency is the same in human individuals, the use of these hormones for the improvement of antineoplastic radiation therapy looks very promising. It should be noted that, when developing such an approach, it is necessary to take into account the possible radiosensitizing effect of TH under conditions of repeated exposure to ionizing radiation, as well as the altered thyroid status of the organism at the time of irradiation. Thus, a study of the nature of endocrine disorders in oncological patients revealed a decrease in TH levels irrespective of tumor localization but in a direct relation to the stage of carcinogenesis (114).

Conclusion

In search of agents that would increase cell reliability, a cue should be taken from natural compounds that are potentially able to influence the repair system as well as the antioxidant one. Such compounds may be TH. It was shown that T_4 acted as a reparogen even after the self-dependent chromosome repair from injuries has been completed. The genetic effect produced by T_4 was found at hormone concentrations exceeding the daily thyroxine production by the normally functioning thyroid gland by factors of 1.5 to 2 not only in irradiated animals, but also in unexposed ones (58, 81). At the same time, 1.5- to 2-fold blood TH level fluctuations were found also in human individuals under physiological stress (54, 115). This fact allows us to suggest that changes in the thyroid gland activity make a contribution to the formation of the genetic load.

According to our data, TH antioxidant activity also manifests itself within

the range of physiological T_3 and T_4 concentrations. This circumstance attracts additional attention to TH as potential modulators of cell reliability systems. At the same time, no systematic studies are being performed at present in this direction. For instance, in reviews dedicated to the problems of repair and mutagenesis (116–118) classification and native antioxidant application (15, 101, 119, 120), TH are not mentioned at all.

Results of the examination of cell reliability systems with the use of TH may appear of practical importance for the solution of a broad circle of problems such as those concerning protection against ionizing radiation, antineoplastic radiation therapy improvement, and carcinogenesis. It should be emphasized that the wide spectrum of TH effects to a significant extent causes the ambiguity of TH influence on these processes. Therefore, a search for conditions under which one of the specific TH effects (for example, the reparogenic potential) is most pronounced, is required. In this connection, it is necessary to take into account, in particular the dose and duration of TH administration, the time of the administration (i.e., before or after exposure to mutagen), the intensity and nature of mutagen, thyroid status and age of the organism, as well as specific features of target cell population reproduction.

SUMMARY

Data and arguments are presented that provide evidence of a role played by thyroid hormones (TH) in cell reliability improvement. This role may be determined by synergistic TH action on the following key cell reliability systems: (1) reactive oxygen species (ROS) attack inhibition, and (2) genetic structure repair from injuries inflicted in the course of endogenous and induced mutagenesis.

(1) New approaches to ROS oxidation defence were examined. It has been shown that Ca^{2+}-ATPase and, probably, regulatory proteins of cell membranes may be the main target for oxidative attack. Protein phosphorylation as well as use of dithiothreitol will lead to a protective action against Ca^{2+} transport damaging in aorta smooth muscle sarcoplasmic reticulum under oxidation by HOCl, the most toxic ROS of activated neutrophils, whereas thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) validly inhibit chemiluminescence in human neutrophils activated by pyrogenal, a lipopolysaccharide from *Salmonella typhi* cell wall. As this takes place, TH most likely block neutrophil stimulation at the receptor–ligand interaction level. In this case L-T_4 and L-T_3 antioxidative effect is greater than that of DL-thyroxine and much greater than that produced by such a potent antioxidant as 4-methyl-2,6-diisobutyl phenol.

(2) T_4 acts as reparogen in rat liver cells under X-ray irradiation when a dose measuring one-half of daily hormone production by the

normally functioning thyroid gland is administered to animals. Ionizing radiation dose reduction factor reached 1.3–1.4 following T_4 administration. Reparogenic effect of T_4 persists for at least 2 months from the moment the hormone has been administered and can be reduced in the presence of dinitrophenol.

It is important to note that antioxidant and reparogenic TH potential can manifest itself within the range of physiologic concentrations of these hormones. Therefore, stimulation of cell reliability systems with TH may prove to be important for correcting conditions caused by errors in energy- and Ca^{2+}-dependent DNA repair under extensive ROS attack. In particular, taking into account different responsiveness of normal and neoplastic tissues to TH, the use of TH reparogenic as well as antioxidant potential may contribute significantly to the improvement of antitumor radiotherapy efficacy.

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