

PREFACE

In the past five years, so much additional progress has been made in our understanding of both the basic and clinical aspects of a wide variety of thyroid disorders that a second edition of *Diseases of the Thyroid* was considered a necessary addition to the *Contemporary Endocrinology* series. Fresh perspectives also seemed warranted, so we recruited new authors for many of the chapters and believe that this edition will provide the reader with further insights into the pathophysiology and clinical presentation and treatment of thyroid disease. It includes topics ranging from neonatal thyroid screening, thyroid dysfunction during infancy and childhood, peripartum thyroid disorders, thyroid disease in the elderly, and the clinical relevance of the sodium/iodide symporter (NIS) to the pathogenesis and treatment of nodular goiter, thyroid cancer, thyrotoxicosis, and hypothyroidism. The worldwide problem of iodine deficiency and its eradication is also discussed, along with environmental goitrogens and iodine-induced thyroid disease.

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2

Nongenomic Actions of Thyroid Hormone

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CONTENTS

INTRODUCTION
EXPERIMENTAL DEFINITION OF NONGENOMIC ACTIONS OF THYROID HORMONE
SPECIFIC NONGENOMIC ACTIONS OF THYROID HORMONE
PRINCIPLES OF MECHANISMS OF NONGENOMIC ACTIONS OF THYROID HORMONE
SUMMARY
ACKNOWLEDGMENTS
REFERENCES

INTRODUCTION

Nonnuclear or nongenomic cellular actions of thyroid hormone (TH) are those that are independent of intranuclear liganding of the hormone and traditional nuclear thyroid-hormone receptors (TRs) (1,2). There are a number of such actions (Fig. 1), at least in part because they involve various organelles, specialized functions of the plasma membrane, and biochemical events in cytoplasm. Further, TH can nongenomically activate signal-transduction pathways in the cell that culminate in phosphorylation of certain nucleoproteins (3,4). Recognition of the existence of nongenomic actions of TH has provided a complex picture of the roles of TH in the cell beyond critical functions in regulating gene expression (5,6).

Nongenomic actions of TH have several functional qualities that distinguish them from nucleus-mediated actions of iodothyronines. Such qualities may include structure–activity relationships of the hormone—e.g., the dominant activities of L-thyroxine (T_4) (7) or 3,3',5'-triiodo-L-thyronine (rT_3) (8) in several nongenomic actions over that of 3,3',5-triiodo-L-thyronine (T_3), or, in some cases, onsets of action that are apparent in seconds (9,10) or minutes (11). The signal-transduction pathways on which TH acts nongenomically, such as the mitogen-activated protein kinase (MAPK) cascade (3,4,12) and cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) (3,13) or inositol phosphate pathways (14), have not been implicated in genomic actions of the hormone. Additionally, the calmodulin– Ca^{+} complex can play a role in certain nongenomic effects of T_4 and T_3 (15,17).

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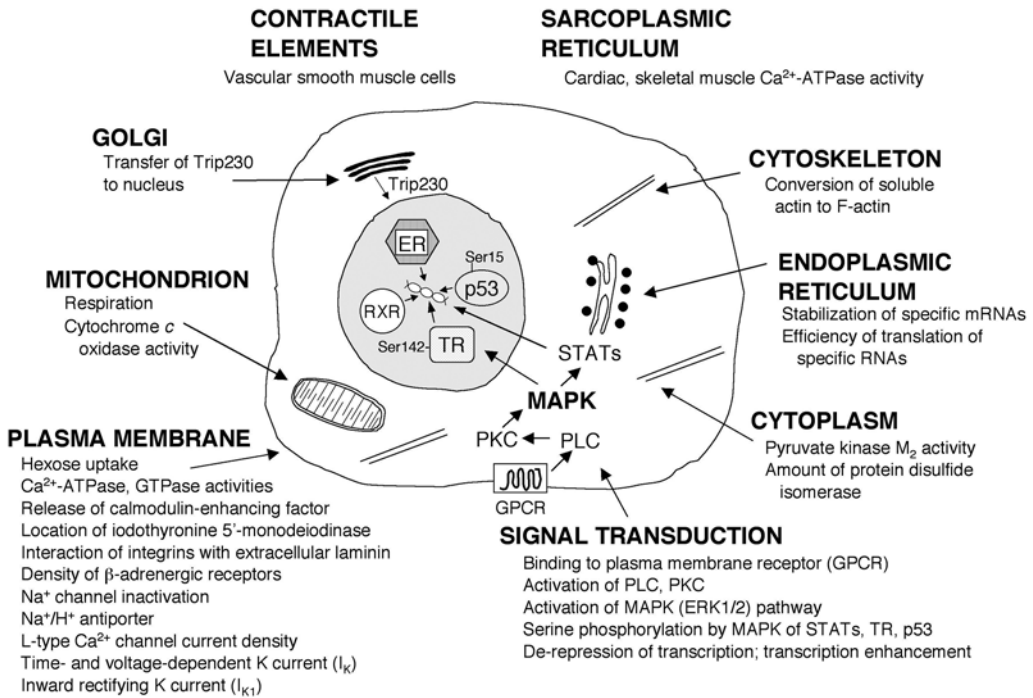


Fig. 1. Nongenomic actions of TH classified by site in an idealized cell. See text for specific cells in which actions have been described. The amount of plasma-membrane iodothyronine-5'-monodeiodinase activity and integrin-laminin interaction, as well as the distribution of protein disulfide isomerase between cytoplasm and endoplasmic reticulum, reflect the action of TH on the cytoskeleton. TH regulates the solubility of actin. The action of T_4 on cellular signal transduction is initiated at a cell-surface G protein-coupled receptor (GPCR), leading to activation of the MAPK (ERK1/2) pathway and serine phosphorylation of the nuclear thyroid-hormone receptor TR β -1 (TR) and p53. In the nucleus of T_4 -treated cells, the proteins TR, RXR, p53, and estrogen receptor (ER) are coimmunoprecipitated with activated MAPK (Lin H-Y, Davis PJ and Davis FB, unpublished observations), forming an "enhanceosome" that may then bind to DNA.

Certain nonnuclear actions of TH appear to be homeostatic. That is, hormonal action on plasma membrane ion pumps or channels (18), on the compartmentalization of ions within the cell (19,20), or on the rate of transport of nonionic solutes, such as glucose (21), may contribute to the intracellular content of specific ions or glucose. The modulation of ion-channel or pump activities involves a large number of factors, and any role of the iodothyronines in such modulation is likely to be modest. Interestingly, nongenomic actions of TH can interface with genomic effects of the hormone. For example, enhancement of the antiviral activity of interferon- γ by TH is achieved genomically and nongenomically (22), and MAPK, nongenomically activated by T_4 , can serine-phosphorylate TR β -1, the nuclear receptor for T_3 (3). The hormone can also modify by an apparently nonnuclear mechanism the half-lives of specific messenger RNAs (mRNAs) (23), the transcription of whose genes may be affected by iodothyronines. The abundance of certain cell-surface adrenergic receptors may be regulated acutely and nongenomically by TH (24) or by nucleus-dependent processes (25). The plasma-membrane content of iodothyronine-5'-monodeiodinase is regulated nongenomically in astrocytes by T_4 and rT_3 (26) through a mechanism that involves a change in the solubility of actin (8).

The physiologic significance of nongenomic effects of TH observed at the cellular level in most examples remains to be determined in the intact organ or organism. Recent observations by Schmidt et al. (27), however, have substantiated acute cardiovascular effects of T_3 in human subjects, including effects on myocardial contractility and on vasomotor tone. These effects may be manifestations of hormone actions on cell-membrane ion channels (Na^+ -channel inactivation) (9,10), on sarcoplasmic-reticulum (SR) ion-pump activity (Ca^{2+} -adenosine triphosphatase [Ca^{2+} -ATPase]) (16,19), or on contractile elements in vascular smooth muscle (28). The principal cardiovascular actions of TH nonetheless are seen to be genomic in mechanism (29,30). There are several other nonnuclear cellular effects of the hormone whose systemic roles appear to be of interest. Among such effects are the action of 3,3'-diiodothyronine (T_2) on cytochrome *c* oxidase activity in mitochondria (31,32), an effect that may contribute to modulation by TH of cell and organ respiration, and which raises the possibility that deiodination of T_3 may result in a biologically important analogue. The effect of T_3 and T_4 on synaptosomal Ca^{2+} uptake (33) may provide insight into the effects of hypothyroidism on central nervous system (CNS) function.

The cellular or molecular mechanisms of most nongenomic actions of iodothyronines are incompletely understood, and specific extranuclear binding sites ("receptors") that are relevant to these actions have not yet been isolated or cloned for a number of such actions. However, evidence has been presented for the existence of a plasma-membrane receptor site for TH that is linked to the MAPK pathway (3,4). This putative site has characteristics of a G protein-coupled receptor that preferentially binds T_4 and tetraiodothyroacetic acid (3,12,34). A mechanism by which TH regulates the activity of cytoplasmic pyruvate kinase M_2 has been defined (35) and involves direct interaction of the hormone with an enzyme subunit. The relevant hormone-binding site (36), probably a signal-transducing protein kinase (37,38), and the role of the calmodulin- Ca^{2+} complex (15), have been described for the stimulation by TH of human erythrocyte Ca^{2+} -ATPase activity.

EXPERIMENTAL DEFINITION OF NONGENOMIC ACTIONS OF THYROID HORMONE

Secure definition of nongenomic effects of TH requires exclusion of the participation of TRs in hormonal action. Strategies for such exclusion include the experimental: (1) use of enucleated cell models, notably the human mature red cell (7,15); (2) use of cell lines in which the nuclear TR is not present (12,34); (3) use of cellular systems in which inhibition of protein synthesis (22) or of gene transcription (34) is achieved; (4) use of model systems whose rapid time courses of hormone action (seconds or a few minutes) preclude mediation by the cell nucleus (9,10); and (5) use of cell organelles. Examples of the last that have been used to study hormone action are mitochondria (39), synaptosomes (33,40), cytosol (35,37), and SR (20). Because certain nongenomic actions of TH require several hours to be manifested (41), a time course consistent with a nucleus-mediated effect does not eliminate the possibility of a nongenomic contribution of TH to a cellular event.

It is desirable in models of the nongenomic action of TH to link a particular effect—such as prolonged inactivation of the myocardial Na^+ channel (9,10) or stimulation of red-cell Ca^{2+} -ATPase activity (15)—to relevant physiologic events, such as, respectively, changes in myocardial Ca^{2+} content (via Na^+ - Ca^{2+} exchange) or myocardial contractility, or to red-cell Ca^{2+} content (18) or Ca^{2+} efflux (42). It is also desirable to identify relevant hormone-specific binding sites in the system in which a nongenomic event has been described. We have also excluded a genomic contribution in certain of our own studies by using TH analogs, such as

tetrac, that have virtually no intrinsic biologic activity at TRs, but are capable of inhibiting putative nongenomic actions of the hormone (3,12,22).

SPECIFIC NONGENOMIC ACTIONS OF THYROID HORMONE

Actions of Thyroid Hormone at the Cell Membrane

Functions of the plasma membrane include solute transport and regulation of selective permeability (ion channels), reception of primary messenger molecules at specific receptors, signal transduction, presentation of molecules with metabolic roles (such as enzymes) at the cell surface, and interaction of the cell surface with molecules in the extracellular matrix (ECM). These latter molecules are structural/adhesion substances of the interstitium that are important determinants of cell–cell relationships (43).

At the outset, it should be noted that the cell membrane contains several energy-dependent transport systems for TH (44,45). These systems are a mechanism for cellular uptake of iodothyronines, and are linked to the transport of specific amino acids (44,46) and to $\text{Na}^+\text{-H}^+$ exchange (47). The hormone-uptake systems themselves are not considered in this review as sites of action of TH. It would be of interest if these transport/uptake systems were initial participants in specific intracellular nongenomic actions of TH, but no such association has yet been established.

Action of Thyroid Hormone on the Distribution of Specific Cell-Surface Proteins

The extrathyroidal (“peripheral”) conversion of T_4 to T_3 is accomplished by deiodinases. Iodothyronine-5'-monodeiodinase Type II (5'-DII) is located in the plasma membrane of astrocytes. The abundance of this enzyme in the cell membrane has been shown by Siegrist-Kaiser and coworkers to be under the control of T_4 and rT_3 (8). The nongenomic mechanism involved, as reported by Farwell et al. in the same laboratory, is the regulation by TH of actin polymerization and thus of the amount of F-actin stress fibers in the microfilament network that are available to interact with the plasma membrane (26). Unique features of this model are that T_3 is ineffective, and that rT_3 , usually seen to have little or no biologic activity, is equipotent to T_3 in causing actin polymerization (8) and the transfer of 5'-DII to endosomes (26). The molecular basis of this action of TH on actin is not yet known. The possibility that calmodulin is involved in the mechanism merits examination because of the importance of the calmodulin- Ca^{2+} complex to the interaction of F-actin with actin-associated proteins (48), and because calmodulin is implicated in three other nongenomic, membrane-associated actions of TH: (1) hexose uptake; (2) Ca^{2+} -ATPase activity; and (3) the release of a calmodulin-enhancing factor (*see next subheading*).

Of particular interest is that the effect of T_4 on actin also regulates the interaction of glial cells with laminin in ECM (49). Laminin is the principal component of ECM in the developing brain, and is bound to astrocytes by integrins. Integrins are plasma membrane-spanning molecules stabilized in the plasma membrane by actin microfilaments (43). It is possible that TH acts via this route to influence neuronal migration, synapse formation, and morphogenesis (40). It is not yet known whether control of actin polymerization by iodothyronines occurs in cells other than glial cells, nor is it understood whether T_4 and rT_3 control the cell-surface presentation of proteins other than 5'-DII.

It has recently been observed that the density of beta-adrenergic receptors (BAR) on the surface of chick embryonic cardiac myocytes can be increased nongenomically by T_3 (24). The change is small (10–15%) and is followed by a nucleus-dependent response that increases BAR density by 40%. It is not known whether rT_3 is effective in the nongenomic

component of this process; T_4 was shown to affect BAR density, albeit less well than T_3 . The nonnuclear TH response is inhibited by colchicine (24), indicating that the mechanism of the hormone-induced change in surface BAR density requires an intact microtubule system. The possibility exists that the acute BAR response to T_3 might contribute, via an enhanced response to endogenous beta-agonists, to the improvement in cardiac output that can be observed clinically in humans with administration of T_3 .

From the above observations it is clear that TH nongenomically influences the function of both microfilament (actin) and microtubule networks that are important to the function of cell-surface proteins. TH may also affect cell-membrane adenylate cyclase activity; this signal-transduction action is linked to solute (glucose) transport, and is described in the next section.

Action of Thyroid Hormone on Plasma-Membrane Transport Functions

The myocardial Na^+ channel is a sarcolemmal feature essential to development of the cell action potential. It also regulates Na^+-Ca^{2+} exchange by gating Na^+ entry into myocytes. Huang et al. (9) have shown in neonatal rat myocardial cells that inactivation of the Na^+ channel (i.e., the duration of opening of the channel after depolarization) is prolonged within seconds after application of T_3 . This prolongs the action potential of these cells and, by promoting cellular uptake of Na^+ , may activate the Na^+-Ca^{2+} exchanger, increase intracellular Ca^{2+} content, and cause an inotropic effect. Huang et al. have also described the structure-activity relationships of TH analogues on the Na^+ channel (9). Acute inotropic effects of TH have been reported in human subjects (27). The nongenomic effect of T_3 on rabbit cardiac Na^+ channels has been confirmed by Dudley and Baumgarten (10). These investigators have shown that the hormonal effect must occur at or near the extracellular face of the Na^+ channel, since application of T_3 to the cytoplasmic face of sarcolemmal patches was ineffective. It should be emphasized that a variety of factors in addition to TH, including second-messenger pathways (10), can influence channel gating.

It should also be noted that TH can acutely enhance the action of isoproterenol on peak L-type Ca^{2+} channel current density (50). Such an alteration may promote Ca^{2+} entry into the cell and enhance Ca^{2+} -induced Ca^{2+} release from SR stores. Interactions of effects of adrenergic agonists and nongenomic effects of TH action are also described below in regard to hexose uptake and to Ca^{2+} -ATPase activity.

Action-potential duration (APD) is prolonged in ventricular myocytes obtained from hypothyroid rats (51). Incubation of hypothyroid cardiomyocytes with 100 nM T_3 normalized APD. No hormonal effect on APD was observed when ventricular cells from euthyroid animals were exposed to TH. The action of TH can be viewed as a contribution to the basal APD. Sun has interpreted the mechanism of hormone action to be an effect on the time- and voltage-dependent potassium current (I_K), an important determinant of repolarization (51).

The myocardial inward rectifying potassium current (I_{K1}) maintains the resting membrane potential of ventricular myocytes. I_{K1} is acutely increased by T_3 (1 nM–1 μ M), as shown by Sakaguchi et al. (52). In studies they conducted on guinea pig ventriculocytes, these investigators reported onset of the effect at 5–15 min and a maximum effect at 25 min. This effect may contribute to the prolongation of APD caused by TH. It is not known whether this action of the hormone contributes to cardiac arrhythmias in the clinical setting of hyperthyroidism; these arrhythmias are largely atrial, rather than ventricular, in origin.

It should also be noted that T_3 has recently been shown to activate one class of voltage-regulated potassium channel by a wortmannin-inhibitable mechanism (53). This is the first description of a nongenomic action of TH that is apparently mediated by a

phosphatidylinositol-3'-kinase-dependent signal-transduction pathway. Rac, a Rho-guanosine-triphosphatase (GTPase), is also implicated in this potassium-channel effect of TH (53).

Another membrane-transport system nongenomically affected by TH is the Na^+/H^+ exchanger or antiporter. Experiments done on a rat skeletal myoblast cell line by Incerpi et al. (54) showed that T_3 or T_4 in nanomolar concentrations shortened the time of recovery of these cells from an acid load. Propylthiouracil blocked the action of T_4 in this model system, indicating that T_3 in fact mediates the effect on the antiporter. Protein kinase C (PKC) is involved in this action of TH, as is MAPK¹ (S. Incerpi, personal communication). In studies not involving iodothyronines, other laboratories have shown that MAPK modulates activity of the Na^+/H^+ exchanger (55).

Glucose transporters (GLUTs) are a family of membrane-spanning proteins that facilitate glucose uptake by cells. The abundance of GLUT1 in a liver-derived cell line (ARL 15) has been shown by Weinstein and Haber (56) to be genomically regulated by TH. However, the stimulation by T_3 of glucose uptake (transporter activity) in this model cannot be fully explained by the increased abundance of GLUT1 protein induced by T_3 . Thus, it is postulated that iodothyronines nongenomically activate preexisting GLUT1 molecules in the cell membrane (57).

A series of studies by Segal and coworkers has substantiated a nongenomic effect of iodothyronines on 2-deoxy-D-glucose uptake in rat myocardium (58) and in thymocytes (21). T_3 is more effective than T_4 in the glucose-uptake models (21). Enhancement of glucose uptake by up to 20% above basal levels is achieved with nanomolar concentrations of T_3 (59). As described in the thymocyte, the action of TH involves an increase in calcium uptake ($^{45}\text{Ca}^{2+}$ accumulation) and in the cytoplasmic free-calcium concentration within 15–30 s after exposure of intact cells to T_3 (10^{-9} M) (60). A subsequent increase in thymocyte plasma-membrane adenylate cyclase activity occurs at 1–2 min (61). In the absence of extracellular Ca^{2+} , T_3 has no effect on glucose uptake, and cyclic adenosine monophosphate (cAMP) is required for hormone action. Further, the effect of TH is inhibited by β -adrenergic antagonists, specifically, β_1 antagonists (62), but not α -adrenergic antagonists. The Ca^{2+} -binding protein calmodulin is also implicated in this model (17).

This plasma-membrane effect of TH on glucose transport thus involves signal transduction (involvement of cAMP, calmodulin– Ca^{2+}), and can be modified by specific adrenergic receptors. The binding site (“receptor”) for TH in this model has not been described. Adenylate cyclase activity may also be increased nongenomically by iodothyronines in cat myocardial membranes (63) (see “Additional Membrane Actions of Thyroid Hormone” following).

The action of TH on membrane Ca^{2+} -ATPase (calcium pump) activity has been documented by several laboratories in erythrocytes (7,15,64), thymocytes (65), and cardiac (16,19) and skeletal muscle (20). This enzyme is a factor that maintains the erythrocyte intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) at less than 10^{-6} M (66). In the human red cell, the enhancement of Ca^{2+} -ATPase activity by physiologic concentrations of TH has been associated with increased $^{45}\text{Ca}^{2+}$ efflux (42) and decreased $[\text{Ca}^{2+}]_i$ (18). Several characteristics of the membrane-binding site (“receptor”) for the hormone that are relevant to Ca^{2+} -ATPase activity have been described (36,67). The structure–activity relationships for hormone analogues in the erythrocyte model have been extensively studied (7); T_4 is more active than T_3 , r T_3 and D-analogues are without effect, and 3,5-dimethyl-3'-isopropyl-L-thyronine (DIMIT) is active. Tetraiodothyroacetic acid (tetrac) is inactive, but blocks the action of T_4 (3,12,36).

The calmodulin–Ca²⁺ complex is the principal intracellular regulator of Ca²⁺–ATPase activity (68). The TH effect on Ca²⁺–ATPase requires the presence of calmodulin (15), and pharmacologic antagonists of calmodulin inhibit the stimulation of the enzyme by TH (15,16). One of the products of the phosphoinositide (PI) signal-transduction pathway, D-myoinositol-1,4,5-trisphosphate (InsP₃), has been shown to inhibit the stimulation by TH of red-cell Ca²⁺–ATPase activity (14). Two mechanisms of this inhibition of hormone activity are known: InsP₃ inhibits T₄- and T₃-binding to erythrocyte membranes (14), and InsP₃ interferes with the binding of calmodulin to Ca²⁺–ATPase *in situ* in the red-cell membrane (69). Thus, factors that activate the PI pathway can modify this nongenomic action of iodothyronines. Further, the presence of α₁-adrenergic receptors on red-cell membranes has been shown, and α₁-adrenergic agonists can inhibit the action of T₄ on red-cell Ca²⁺–ATPase activity (70).

The mechanism by which T₄ stimulates the activity of Ca²⁺–ATPase is believed to depend on PKC. PKC has been shown to activate Ca²⁺–ATPase (38), and we have described the stimulation of red-cell PKC activity nongenomically by T₄ (37). We have also shown non-nucleus-mediated enhancement of PKC activity by T₄ in HeLa cells (13). Red-cell membrane Ca²⁺–ATPase activity has been shown to be increased in hyperthyroidism and decreased in hypothyroidism (71). This is thought primarily to be a genomic effect. However, stable circulating levels of TH in eumetabolic subjects may contribute nongenomically to the set-point of enzyme activity. Selected plasma-membrane actions of TH are summarized in Table 1.

Action of Thyroid Hormone on Synaptosomes

Bellarbarba et al. have described T₃-binding sites in synaptosomes of developing rat brain (40). Recently, this group has shown that these hormone-binding sites are linked to G protein and GTPase activity in synaptosomes (72). These observations raise the possibility that via this nongenomic mechanism, TH may modify neurotransmission. Studies of the model that involve neurotransmitter and Ca²⁺ release/reuptake in the presence and absence of iodothyronines are required to define the possible physiologic role of TH in synaptosomes.

Effects of Thyroid Hormone on Signal-Transduction Pathways

Acting at the plasma membrane, iodothyronines can stimulate the MAPK (ERK1/2) signal-transduction cascade in several human and animal cell lines (3,4,12). That the action is initiated at the cell membrane is supported by observations that agarose-T₄ is as effective as T₄, that pertussis toxin blocks the hormonal effect, and that tetrac, a hormone analogue known to block binding of T₄ to the plasma membrane (36), inhibits activation by T₄ of MAPK (3,12). T₃ is less effective than T₄ in this model of hormone action (3). We have shown that upstream of MAPK itself, inhibition at the individual steps of MAPK kinase (MEK), Raf-1, Ras, PKC or phospholipase C (PLC) serves to block action of iodothyronines on the MAPK pathway (3,4,12) (Fig. 2).

The significance of this nongenomic effect of TH has been explored by examining possible substrates of MAPK. It has been shown that TH-directed MAPK phosphorylates specific serines in a number of important cellular proteins. For example, T₄-activated MAPK phosphorylates serine-727 (Ser-727) of the signal-transducer and activator of transcription (STAT)-1α (12). This effect potentiates the signal generated at the cell surface by interferon (IFN)-γ—tyrosine phosphorylation of STAT-1α at Tyr-701 (73)—and results in significant enhancement by TH of the antiviral (12) and immunomodulatory (34) effects of IFN-γ in

Table 1
Selected Plasma Membrane Actions of Thyroid Hormone

Action	Cell	Result	Mechanism of action	Analogue	Ref. no.
Na ⁺ /H ⁺ antiporter stimulation	Rat myoblasts	Increased intracellular pH 5–10 min	PKC	T ₃ > T ₄	54
Sodium current (I _{Na})	Neonatal rat ventricular myocytes	Increased peak I _{Na} ; slight prolongation of I _{Na} ; inactivation 5 min	PKC; independent of β-blockade	T ₃ = T ₄ = 3,5-rT ₃ blocks T ₃ , T ₄ action	9
Inward rectifier potassium current (I _{K1})	Guinea pig ventricular myocytes	Increased open probability of channel, shortened action potential, 5–25 min	?G protein	T ₃ , triac	52
Action-potential duration	Hypothyroid rat ventricular myocytes	Shortened action potential	?G protein	T ₃ > T ₄	51
Ca ²⁺ -ATPase activity	Human erythrocytes, rabbit myocardial sarcolemma	Increased calcium pump activity	Calmodulin, PKC	T ₄ = T ₃	7,15,19,37

PKC, protein kinase C.

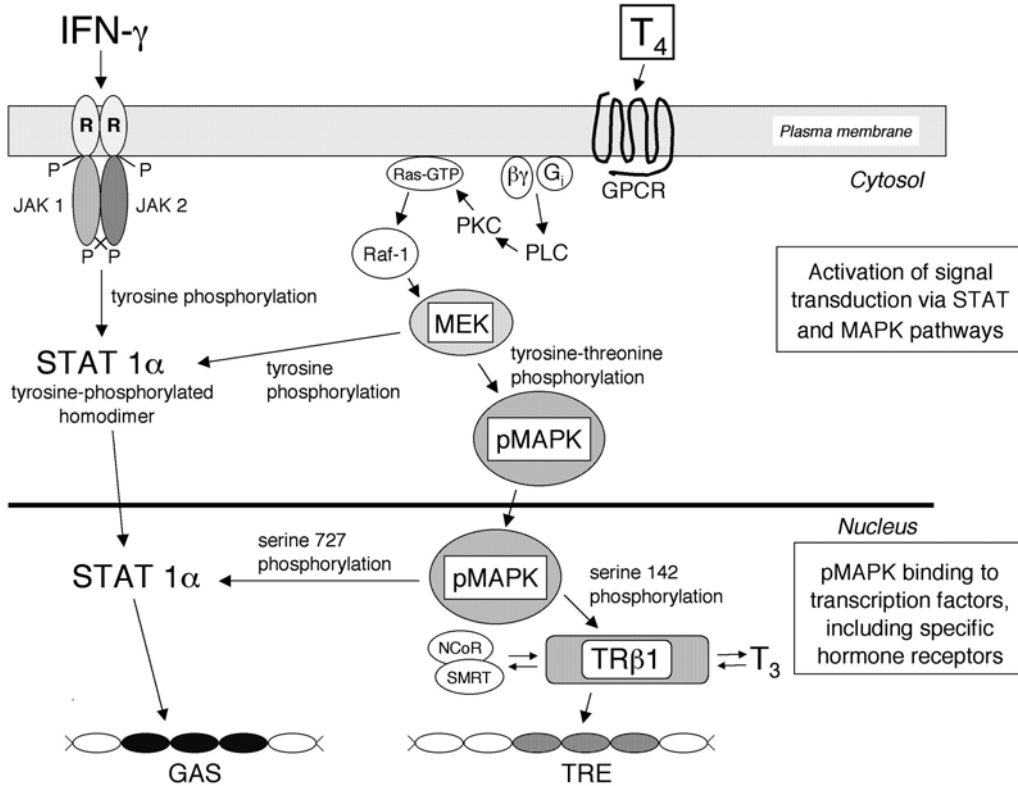


Fig. 2. Signal-transduction pathways by which T₄ affects specific nuclear proteins. T₄ interacts with a plasma-membrane G-protein-coupled receptor (GPCR) to initiate a protein kinase cascade including activation of PLC, PKC, Ras, Raf-1, and MAPK kinase (MEK). Phosphorylated by MEK, pMAPK translocates to the nucleus, forms an immunoprecipitable complex with TR β -1, and serine-phosphorylates this nuclear receptor at residue 142. Serine phosphorylation of TR β -1 is associated with dissociation of the corepressors NCoR and SMRT, previously bound to the unphosphorylated receptor. This T₄-directed MAPK action is thought to be sufficient to permit binding of receptor to thyroid-hormone response elements (TREs), but it is thought that substantial transcriptional activity results only in the presence of T₃. Cells treated with IFN- γ respond with phosphorylation and dimerization of the IFN- γ receptor (R), tyrosine phosphorylation of Janus kinases (JAK) 1 and 2, and tyrosine phosphorylation of STAT-1 α . This protein dimerizes, translocates to the nucleus, and binds to IFN- γ -activated sequences (GAS) on DNA. In the presence of T₄, STAT-1 α is serine-phosphorylated on residue 727 by pMAPK, enhancing by up to 100-fold the effect of submaximal concentrations of IFN- γ . Even in the absence of IFN- γ , T₄ also causes tyrosine phosphorylation of STAT-1 α by MEK, but this hormone action alone is not sufficient to cause increased expression of antiviral or other IFN- γ -responsive proteins.

human cell lines. T₄-activated MAPK phosphorylates Ser-15 of the transcription factor, p53, an oncogene-suppressor protein (4). This alters the transcriptional activity of p53, inferred from its modulation of expression of an immediate-early gene, *c-Jun* (4). MAPK activated by T₄ also phosphorylates Ser-142 of TR β -1, the nuclear receptor for T₃ (74). The significance of this phosphorylation step is that dissociation of TR from the corepressor proteins, NCoR (nuclear corepressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors) takes place with T₄ treatment (3).

Unactivated MAPK resides in cytoplasm (75). When activated by T₄ at the cell surface, MAPK translocates to the nucleus and phosphorylates the substrates mentioned above. Interestingly, TH-activated MAPK is recovered from nuclear fractions of treated cells in

a coimmunoprecipitated complex that includes the substrate proteins as well as retinoid X receptor (RXR) (Fig. 1), a member of the nuclear superfamily of hormone receptors that heterodimerizes with TR (76). In cells not exposed to physiologic concentrations of T_4 in vitro, no such association of nucleoproteins occurs, and activated MAPK is not found in the nucleus. Such nucleoprotein complexes have been described in signal-transduction models as “enhanceosomes” (77,78) when transcriptional activity of a specific transactivator is increased by protein–protein interactions. In the cellular models we describe, the increased activity of STAT-1 α complexed with MAPK, as promoted by T_4 in interferon-treated cells (12,34), would qualify as an enhanceosome. Undetermined in such a complex is whether inclusion of factors such as TR influence the behavior of STAT-1 α , or whether the latter, in the presence of T_4 , may modulate the activity of TR. It is thought that such associations of nucleoproteins enhance the ability of transactivators to bind to DNA (77).

The ability of TH to activate PKC (37) and MAPK may provide a mechanism by which the hormone can change the activity of certain membrane ion channels and pumps, as suggested above. It is known, for example, that PKC can modulate the activity of the plasma membrane Na^+/H^+ exchanger (79), Na^+ channel (80), and Ca^{2+} -ATPase (38), and that MAPK may modulate the activity of the Na^+/H^+ antiporter (81).

Additional Plasma-Membrane Actions of Thyroid Hormone

Stimulation of myocardial adenylate cyclase by TH in a membrane (particulate) fraction was described more than 25 yr ago by Levey and Epstein (63). They showed that T_4 (5×10^{-6} M) increased cAMP accumulation in this system by 20–45% within 3 min of exposure of membranes to the hormone. RT_3 was active, but less so than T_4 , and the action of T_3 was equivalent to that of T_4 . The dependence of the model on greater than physiologic levels of TH, and the apparent responsiveness of the cyclase to D- T_4 , make this report difficult to interpret.

T_4 promotes the release from human erythrocyte membranes of a soluble, heat-stable calmodulin-enhancing factor that binds to calmodulin and increases by sixfold the ability of native calmodulin to stimulate 3',5'-cyclic nucleotide phosphodiesterase activity (82). This enhancing factor resembles a “calmodulin activator” reported to occur in an animal model of hypertension (83). The possibility that such a factor might relate to the actions of TH on the cytoskeleton, and that this might involve participation of the calmodulin- Ca^{2+} complex, is attractive, but has not yet been studied.

Action of Thyroid Hormone on the Cytoskeleton

REGULATION OF ACTIN POLYMERIZATION BY IODOTHYRONINES

The regulation by TH of actin polymerization in astrocytes is intimately related to the effects of the hormone on specific plasma-membrane proteins (e.g., iodothyronine-5'-monodeiodinase (26) and the interaction of membrane integrin molecules with laminin in the ECM [49]), and has been described above. Another feature of the nongenomic actions of iodothyronine on the cytoskeleton is regulation of the cellular distribution of protein disulfide isomerase (84), an enzyme that is important to the processing (folding) of nascent proteins in the endoplasmic reticulum (ER) (see “Actions of Thyroid Hormone at the Endoplasmic Reticulum” following). It is important to determine whether these actions are general or are limited to the specialized cells (astrocytes) in which they have been initially described.

Because of its actions on these structural elements of cells that are linked to the plasma membrane, TH may be speculated to regulate cell shape. This possibility has not yet been explored.

Action of Thyroid Hormone on Sarcoplasmic Reticulum

STIMULATION OF Ca^{2+} -ATPASE ACTIVITY OF SARCOPLASMIC RETICULUM

The Ca^{2+} -ATPase of the plasma membrane exports Ca^{2+} ; the SR of striated and cardiac muscle also contains a Ca^{2+} -ATPase, which is directed vectorially to import Ca^{2+} into SR (85). These enzyme activities are different structurally and biochemically, but, like the Ca^{2+} -ATPase of the plasma membrane, that of the SR of skeletal muscle (20) and of myocardium (16,19) is stimulated in vitro by physiologic concentrations of T_4 and T_3 . Increased SR uptake of Ca^{2+} enhances muscle relaxation by decreasing the Ca^{2+} concentration in the sarcoplasm around actin-myosin bundles. Enhanced uptake of Ca^{2+} by SR can also promote inotropy by increasing the pool of Ca^{2+} potentially available before muscle excitation. Because hyperthyroidism is associated with a shortened muscle-relaxation time and increased force of contraction, the nongenomic effects of TH on SR Ca^{2+} metabolism may be implicated in these clinically observed actions of the hormone. However, such a speculation should be limited to very acute effects of the hormone on muscle. TH is known to induce the expression of genes for Ca^{2+} -ATPase (86), and such effects probably explain what is observed clinically in terms of skeletal- and cardiac-muscle function. Further, chronic TH treatment in animal models is associated with an increase in Ca^{2+} concentration in sarcoplasm, rather than a decrease (87).

Actions of Thyroid Hormone at the Endoplasmic Reticulum

ACTIONS OF THE HORMONE ON STABILITY OF SPECIFIC mRNAs

Vandenbrouck et al. have shown that T_3 increases the half-life ($t_{1/2}$) of apolipoprotein AI (apoAI) mRNA by two-to-threefold over that of control mRNA in HepG2 cells not exposed to the hormone (23). Stabilization by TH of specific mRNAs has been reported by other investigators (88), whereas shortening of mRNA $t_{1/2}$ by TH has also been reported (89). Although such changes may be genomic in origin, Puymirat et al. have shown that T_3 stabilizes specific mRNA by a mechanism that is independent of gene transcription and the processing of transcripts, and which requires activation of a serine-threonine kinase-dependent pathway (90). Because we know that TH can nongenomically stimulate such kinase activity (e.g., PKC) (37), the stabilization of mRNAs by T_3 may in some cases work through a nongenomic mechanism.

POSSIBLE ACTION OF THYROID HORMONE ON EFFICIENCY OF TRANSLATION OF mRNA

T_3 induces apolipoprotein B (apoB) gene expression in rat hepatocytes (HepG2 cells); the hormone enhances this genomic effect by increasing the efficiency of translation of apoB mRNA (91). These several effects of TH are complicated by the ability of T_3 to increase the degradation rate of the apoB protein. Thus, the influence of iodothyronines on abundance of apoB protein is the algebraic sum of three rate effects. Whether the change in efficiency of translation is nongenomic is unclear. However, the ability of the TH to regulate compartmentalization of factors important to the ER, such as protein disulfide isomerase (PDI) (84), by nongenomic mechanisms increases the likelihood that the change in efficiency of translation of mRNA induced by TH does have a nongenomic component. About 25% of astrocyte PDI monomeric subunit (glial-p55) is cytoplasmic, but via a hormone-induced mechanism related to actin polymerization, cytoplasmic glial-p55 redistributes to the particulate fraction (ER) and becomes associated with actin (84). PDI is catalytic in the synthesis of disulfide-bond-containing proteins, and is also a component of a triacylglycerol transfer protein (MTP) that facilitates insertion of lipids into nascent lipoproteins within the ER (92). Such effects could increase the efficiency of protein synthesis.

Actions of Thyroid Hormone in Cytoplasm

REGULATION OF PYRUVATE KINASE ACTIVITY BY T₃

TH is bound by several cytosolic proteins (93–95). The functions of these proteins and of the hormone–protein interaction are largely undefined. However, Ashizawa et al. have shown that T₃ binds reversibly to cytoplasmic pyruvate kinase M2 (PKM₂) monomer (p58), and that this hormone–protein interaction prevents the association of monomers into the enzymatically active PKM₂ tetramer (35). The hormone does not bind to tetrameric PK. Fructose-1,6-diphosphate promotes tetramer formation, thereby activating the kinase and stimulating glycolysis. The affinity of PKM₂ monomer for T₄ is about 50% that of T₃, and rT₃-binding is negligible. These findings were originally made in a human epidermoid carcinoma-cell line, but the interaction of T₃ with human erythrocyte PK has also been reported (96). Thus, this cytoplasmic glycolytic enzyme system is a definitive example of nongenomic regulation by TH of enzyme activity. To the extent that T₃ diminishes PK activity, the hormone limits ATP generation by the glycolytic pathway and the availability of pyruvate to other cellular pathways, such as the citric-acid cycle.

REGULATION OF ACCESS OF THYROID HORMONE TO THE CELL NUCLEUS BY CYTOSOLIC PROTEINS

We have shown that cytosolic binding proteins for iodothyronines can restrict nuclear uptake of T₃ and T₄ (93). We do not know whether these hormone–protein complexes maintain a steady-state intracellular pool of unbound TH whose availability is determined by stable off/on kinetics, or whether the binding of TH might be varied by interaction of intracellular factors with cytosolic binding proteins that would adjust the intracellular free hormone concentration. However, a cytosolic thyroid hormone-binding protein (CTBP) described in rat kidney by Hashizume et al. can facilitate nuclear uptake of T₃ in the presence of nicotinamide adenine dinucleotide phosphate (NADP) (95) and in association with the formation of a T₃-CTBP-(NADP) complex. The T₃-CTBP-(NADPH) complex is not associated with nuclear uptake of the hormone, and may serve to stabilize the cytoplasmic pool of T₃. The NADP:NADPH ratio thus appears to be a regulator of nuclear uptake of T₃. The activity of the pentose-phosphate shunt, source of the NADPH, may by this mechanism modulate the availability of TH to the nucleus.

ACTION OF THYROID HORMONE ON CYTOSOLIC PROTEIN KINASE ACTIVITIES

PKC activity in rabbit mature erythrocyte cytosol is stimulated *in vitro* by physiologic concentrations of TH (37). T₄ is more active than T₃ in this model. When these observations were made, only diacylglycerol (DAG) and DAG-like compounds (phorbol esters) were recognized as stimulators of PKC. Other laboratories have confirmed that TH stimulates PKC (97). It has been reported that PLC activity is stimulated by iodothyronines (12), an effect by which PKC activity could be enhanced via elaboration of DAG. 1,25-Dihydroxyvitamin D₃ (98) and a variety of phospholipids have been shown to modulate PKC activity. PKC is a serine–threonine kinase (99). As noted, Puymirat, Etongue-Mayer, and Dussault have recently shown that T₃ stimulates cytosolic serine–threonine kinase activity in neuroblastoma cells, and that such activity is involved in stabilizing acetylcholinesterase mRNA (90). It is not yet clear whether this kinase activity is indeed PKC.

We have shown that TH potentiates the antiviral activity of homologous recombinant IFN- γ in HeLa cells by more than 100-fold (41). This action of the hormone has both genomic and nongenomic components (22). The nongenomic component represents a recently recognized postnuclear pathway that is independent of protein synthesis and that involves the action of

two signal-transduction enzymes, PKC and PKA (13). That is, inhibition of either PKC or PKA activity prevents postnuclear-pathway enhancement by TH of IFN- γ action. In addition, the potentiating effect of TH in the IFN model can be wholly reproduced by the addition of both phorbol ester (to stimulate PKC activity) and 8-Br-cAMP (to stimulate PKA), but not by the addition of only one of these agents (13). To our knowledge, this is the first observation of the reproduction of an action of TH by components of a signal-transduction pathway and supports the concept that nongenomic actions of TH can involve such pathways.

Because the IFN-HeLa cell model described above has a genomic component that is activated by rT₃ (22), it has been possible to show, using sequential submaximal concentrations of rT₃ and T₄ in a time paradigm that allows separation of nucleus-dependent and post-nuclear effects of thyroid hormone, that the nongenomic action of the hormone can potentiate its genomic effect (22). The molecular mechanism by which TH stimulates PKC and PKA activities in the IFN- γ system is not yet known. We believe that the physiologic relevance of the system is potentiation, by circulating levels of T₄, of the early cytokine response in host defense.

It should also be noted that TH can enhance the immunomodulatory activity of IFN- γ , i.e., HLA-DR antigen expression (100). This action of TH, however, appears to involve both nongenomic (signal transduction) and genomic mechanisms.

Actions of Thyroid Hormone on Mitochondria

STIMULATION OF RESPIRATION BY THYROID HORMONE

The stimulatory action of TH on cell and tissue respiration has been widely acknowledged. Respiration in isolated mitochondria has been shown to be enhanced by TH (39,101). There are a number of mechanisms by which iodothyronines could stimulate mitochondrial respiration. These include: (1) enhanced activity of ADP-ATP (adenine nucleotide) translocase (AdNT), promoting the uptake of ADP; (2) stimulation of the tricarboxylic acid (TCA) cycle, producing intermediates that are sources of electrons; (3) promotion of phosphate uptake; (4) stimulation of F₀F₁-ATPase (ATP synthase), perhaps by dissipation of the H⁺ gradient across the inner mitochondrial membrane; and (5) stimulation of the electron-transport chain. The precise site of action of TH on mitochondria has not been firmly established, but a substantial body of evidence is consistent with an action of the hormone on AdNT (39,102).

Of interest is the recent finding that analogs of TH can stimulate the activity of cytochrome *c* oxidase isolated from rat liver (31) and bovine heart (103) mitochondria. T₃ has no effect on the activity of this enzyme, whereas diiodothyronines (3,3'-T₂, 3,5-T₂) are active. Diiodothyronines are apparently capable of rapidly stimulating mitochondrial respiration (104,105), but do not have an important effect on the whole-organism metabolic rate. A *bona fide* action of iodothyronines on cytochrome *c* oxidase activity is capable of enhancing electron transport. Arnold, Goglia, and Kadenbach (32) have recently reported that T₂ binds to subunit Va of the cytochrome *c* oxidase of bovine heart mitochondria, and as a consequence reduces ATP binding by subunit IV of the oxidase. ATP binding promotes an allosteric change in the oxidase that inhibits respiration, so that the cellular affect of T₂ binding is the stimulation of mitochondrial respiration.

Thyroid Hormone Action on Contractile Elements of Cells

Ojaama, Klemperer, and Klein have shown that application of T₃ to isolated rat aortic vascular smooth-muscle cells (VSMCs) causes relaxation of the cells (28). This occurs sufficiently rapidly to exclude a genomic mechanism. Such an action could contribute importantly to the reduction in peripheral vascular resistance that is associated with

hyperthyroidism or with increased cardiac output when T_3 is administered in the setting of weaning patients from cardiopulmonary bypass (29). The molecular mechanism by which TH can alter VSMC tone is not yet known.

PRINCIPLES OF MECHANISMS OF NONGENOMIC ACTIONS OF THYROID HORMONE

From the foregoing discussion, it is clear that our understanding of the mechanisms of nongenomic actions of iodothyronines is incomplete. However, there are several interesting features of what is known about the highly varied mechanisms so far implicated in the nongenomic actions of these hormones. First, there are examples of the “receptor” as “effector.” In the case of PKM₂ monomer, for example, the binding site for T_3 must be linked to an allosteric change in the cytosolic protein that prevents the self-association of monomers into the enzymatically active PKM₂ tetramer. That mitochondrial AdNT itself is a receptor for T_3 is another example of the receptor as effector, albeit open to contention. The stimulation by T_2 of mitochondrial cytochrome *c* oxidase also appears to be of a receptor–effector nature.

Second, the apparent receptors for nongenomic actions of iodothyronines can have features remarkably different from those of nuclear receptors for T_3 . The human red-cell receptor for TH that is linked to Ca²⁺–ATPase activity binds T_4 and T_3 equally well (67); it also binds tetrac in a manner that precludes binding of T_4 and T_3 , but that does not lead to the activation of ATPase (36). The mitochondrial cytochrome *c* oxidase binding site recognizes diiodothyronines, but not T_3 . The unknown mechanism by which iodothyronines regulate the polymerization of actin is activated by r T_3 and T_4 , rather than by T_3 .

Third, there are examples in which complex interactions of signal-transduction-pathway components are interposed between putative TH receptors and nongenomic actions of the hormone. The stimulation of the MAPK pathway and its consequences on nucleoprotein activity is a primary example of the action of TH on signal transduction. The effects of the hormone on plasma-membrane hexose uptake and on Ca²⁺–ATPase activity appear to reflect the action of TH on signal-transducing kinases. We have also suggested, as described earlier, that several of the nonnuclear effects of iodothyronines on plasma-membrane ion channels and on the Na⁺/H⁺ exchanger may be mediated by hormonal actions on MAPK or PKC.

Fourth, genomic and nongenomic mechanisms may interface in certain models of TH activity. Examples of this are the effects of T_4 -directed MAPK on serine phosphorylation of TR, and the observations that TH can affect both expression of the SR Ca²⁺–ATPase gene and, by a nongenomic effect, the activity of the gene product. Another possible example of interfacing of genomic and nongenomic mechanisms in the activity of TH is the stimulation of transcription of the apoB gene in association with the posttranscriptional stabilization of apoB mRNA.

SUMMARY

A number of nongenomic actions of TH have been substantiated in the past decade. Experimental conditions in which the existence of nongenomic actions of TH is suspected must securely exclude contributions from nuclear TRs. The physiologic importance of nongenomic actions of iodothyronines is largely speculative, but contributions by such actions to the basal activity of certain homeostatic mechanisms, such as ion-channel transport or the activity of the Na⁺/H⁺ exchanger, appear likely. For most of these actions, relevant

specific hormone receptors and mechanisms remain to be identified. Actions of TH on signal-transduction pathways do offer possible mechanisms for action of the hormone at the plasma membrane and on the activity of nucleoproteins. Serine phosphorylation of TR β -1 and p53 by T₄-directed MAPK, for example, alters the behavior of these nucleoproteins, and specific serine phosphorylation of STAT-1 α by MAPK explains the potentiation by TH of the antiviral effects of IFN- γ on human cells in vitro. It appears likely that nongenomic effects of the hormone contribute to the regulation of intracellular protein trafficking and to the state of the cytoskeleton in certain cells. Recent evidence for binding of T₂ by a subunit of mitochondrial cytochrome *c* oxidase is one explanation for the effect of TH on cellular respiration.

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