

# **Nongenomic Actions of Thyroid Hormone**

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## **NONGENOMIC ACTIONS OF THYROID HORMONE**

Thyroid hormone actions have been mechanistically defined in terms of expression of specific genes that is modulated by the intranuclear complex of 3,5,3'-triiodo-L-thyronine ( $T_3$ ) with its nuclear receptor ( $TR\beta_1$ , TR). These are designated genomic mechanisms. Another set of mechanisms exist that are initiated at or near the plasma membrane, do not primarily involve gene transcription, may be consummated at the cell membrane (1-4), itself, or may have consequences in the cell nucleus. These nongenomic actions are mediated, at least in part, by signal transduction pathways (5-11). Nongenomic and genomic mechanisms of thyroid hormone action interface in the cell nucleus, where nuclear hormone receptors are acted upon by signal transduction pathway kinases whose activities are regulated from outside the nucleus by iodothyronines. These kinase actions alter transcriptional activity of receptors. This review will focus on nongenomic actions of thyroid hormone on plasma membrane functions, such as ion pumps or channels, on signal transduction and intracellular protein trafficking, and on the interface of nongenomic and genomic actions of the hormone in the nucleus. There are other nongenomic actions of iodothyronines that involve the mitochondrion (12), the actin cytoskeleton (13), certain cytoplasmic proteins (14) and ribosomes (15).

Nongenomic actions are usually rapid in onset (seconds to minutes), do not require protein synthesis and are independent of nuclear TR. A number of these actions are at least equally responsive to L-thyroxine ( $T_4$ ) and  $T_3$ , and in some cases are more responsive to  $T_4$ . The structure-activity relationships of thyroid hormone analogues in nongenomic actions of the hormone are sometimes novel. Tetraiodothyroacetic acid (tetrac) may block specific nongenomic actions of the hormone, e.g., activation of the mitogen-activated protein kinase (MAPK) signal transduction pathway, but is not, itself, an agonist; 3,3',5'-triiodo-L-thyronine (reverse  $T_3$ ,  $rT_3$ ) may be an agonist (cytoskeleton), as may be  $T_2$  (mitochondrion).

It is important to note that the model systems in which nongenomic actions are demonstrated are generally those in which the cells studied have been made thyroprival and then are acutely exposed to physiological concentrations of thyroid hormone analogues. In the intact organism, in contrast, ambient thyroid hormone levels are usually stable. Therefore we may conceive of many nongenomic actions of thyroid hormone as contributing to the setpoints or basal levels of activity of cellular functions. Some of these functions are homeostatic, as noted below.

Actions of thyroid hormone at the **plasma membrane** that are nongenomic in mechanism include increased activities of the  $Na^+/H^+$  exchanger (antiporter)(1),  $Na^+$  current (2), inward rectifying  $K^+$  current (3) and  $Ca^{2+}$ -ATPase (calcium pump)(4). Such actions may be relevant to regulation of intracellular pH and  $[Ca^{2+}]_i$  or to modulation of excitability in muscle cells. The nongenomic effect of  $T_3$  on the antiporter has been well-studied in rat myoblasts and has been shown to enhance the ability of the cell to recover from an acute

acid load by increasing the rate of export of protons. This rapid action of the hormone is blocked by exposure of cells to PD 98059, a pharmacologic inhibitor of the MAPK (ERK1/2) signal transduction pathway. A transient spike in  $[Ca^{2+}]_i$  also occurs in these cells with exposure to  $T_3$ . While  $T_4$  is active in this model, its effect is inhibited by propylthiouracil (PTU). In contrast,  $T_4$  is more active than  $T_3$  in stimulating membrane  $Ca^{2+}$ -ATPase activity in a variety of tissues. This has been shown to lower cytoplasmic  $Ca^{2+}$  concentration. It has been postulated that in muscle cells,  $T_4$ -enhanced activity of sarcoplasmic reticulum  $Ca^{2+}$ -ATPase contributes to the inotropic effect of the hormone. The actions of thyroid hormone on the inward rectifying  $K^+$  current and  $Na^+$  current are potential contributors to lowering of the arrhythmia threshold. Acute cardiovascular responses to thyroid hormone occur in organs (16), intact animals (review:17) and man (18).

One model of the plasma membrane-initiated action of thyroid hormone on **signal transduction** is the activation of the MAPK (ERK1/2) cascade(5,8,9). Upstream of MAPK in the cascade, the enzymes that are activated include protein kinase C (PKC). The  $Na^+/H^+$  antiporter is a substrate for activated MAPK and the  $Na^+$  current is subject to modulation by PKC. Downstream, MAPK, when activated by  $T_4$  at the cell surface, translocates immediately to the nucleus. Substrates of activated MAPK here include TR $\beta$ 1, signal transducer and activator of transcription (STAT)-1 $\alpha$  and p53. The interaction of MAPK with these nucleoproteins and serine phosphorylation of TR by MAPK alters the interactions of TR with its accessory (corepressor and coactivator) proteins and alters the transcriptional activity of STAT1 $\alpha$  and p53. The docking site for activated MAPK on TR has been identified (9) and a similar docking site motif exists on other members of the superfamily of nuclear hormone receptors. These nongenomic effects of thyroid hormone thus include 1) post-translational modification (phosphorylation) of TR, 2) altered states of interaction between TR and co-regulatory proteins, and 3) intracellular **protein trafficking**. Examples of the latter are nuclear translocation of MAPK, STAT proteins and certain accessory proteins, such as the TR coactivator, Trip230. Movement of TR, itself, into the nucleus has been shown by several laboratories to be subject to thyroid hormone control (19,20). The interaction of activated MAPK with specific nuclear transactivator proteins in a manner that alters transcriptional activity of the nucleoproteins exemplifies the **interface between nongenomic and genomic actions** of thyroid hormone. Recoverable from nuclear fraction of  $T_4$ -treated cells are complexes of activated MAPK and TR that also include p53, STAT1 $\alpha$  and other transactivators. Such complexes are enhanceosome-like.

The cell surface receptor for thyroid hormone has qualities of a family of structural membrane proteins (integrins) that connect the cell to extracellular matrix and are also linked intracellularly to the MAPK cascade.

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