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# Nongenomic Actions of Thyroid Hormones: From Basic Research to Clinical Applications. An Update

R. G. Ahmed[1], Paul J. Davis[2], Faith B. Davis[2], Paolo De Vito[3], Ricardo N. Farias[4], Paolo Luly[3], Jens Z. Pedersen[3] and Sandra Incerpi*

*Department of Biology, University of Rome 'Roma Tre', Viale Marconi, 446 00146 Roma, Italy;*

*[1]Department of Zoology, Beni-Suef University, Beni-Suef, Egypt; [2]Albany Medical College and Albany College of Pharmacy and Health Sciences, Albany, NY, USA; [3]Department of Biology, University of Rome 'Tor Vergata', Viale della Ricerca Scientifica, 00133 Roma, Italy; [4]INSIBIO (Conicet-UNT) Chacabuco 461 (4000) San Miguel De Tucuman, Tucuman, Argentina.*

**Abstract:** Extranuclear or nongenomic actions of thyroid hormones are unaffected by the inhibitors of protein synthesis, their site of action has been localized at the plasma membrane but also in the cytoplasm and organelles such as the mitochondria. This review takes into account recent major advances in nongenomic effects of thyroid hormones in nervous system, immune system and cardiovascular tissue, with a particular focus on the plasma membrane receptor integrin αvβ3. In nerve cells nongenomic effects of thyroid hormones point mainly to a direct modulation of several channels/receptors for the major neurotransmitters, even though more complex pathways have also been demonstrated. Certain neuroprotective actions have recently been described for thyronamines, and this may be relevant to Alzheimer's disease and multiple sclerosis. The immune system is also modulated nongenomically by thyroid hormones, through potentiation of the effects of cytokines such as IFN-γ or lipopolysaccharide, or through activators of STAT protein leading to activation of the mammalian target of rapamycin (mTOR) pathway, a highly-conserved kinase downstream target of nongenomic actions of thyroid hormone. The mTOR system is also involved in the cardioprotection mechanisms, where thyroid hormone signaling through the receptor integrin αvβ3 may play an important role that needs to be further studied. The identification of integrin αvβ3 as a plasma membrane receptor for thyroid hormones has provided a new perspective on the role of these hormones in cellular defense. Analogs of thyroid hormones, inhibitors and agonists at the integrin receptor for the hormone and mTOR inhibitors are evaluated as areas of emphasis for therapeutic research.

**Keywords:** Cardiovascular system, immune system, integrin, ion transport, MAPK, mTOR, $Na^+/K^+$-ATPase, nerve cells.

## INTRODUCTION

Thyroid hormones triiodothyronine ($T_3$) and thyroxine ($T_4$) give rise to a wide range of different effects on metabolism, growth and development [1, 2]. They are considered the main hormones controlling tissue growth, even though they are not the only ones since obviously steroid hormones, insulin and growth hormone also make their important contributions to the development and growth of the organism. This implies that thyroid hormones produce a wide range of effects and in practically every type of tissue. The major form of thyroid hormone secreted by the thyroid gland is $T_4$ (Fig. **1**), whereas $T_3$ is largely produced in target tissues by deiodination of $T_4$ [3]. It is $T_3$ that accounts for the majority of the actions of thyroid hormones; these effects are known to be mediated by the binding of $T_3$ to specific nuclear receptor proteins that modulate gene expression [1]. Being genomic effects they require hours for consequent protein synthesis and the biological response to become manifest. So far two different types of receptors, TRα and TRβ, have been identified, and the ligand-binding domains of both TRα and TRβ have been crystallized and structurally characterized in detail [4, 5]. But it is now well established that thyroid hormone also can act through nongenomic and extranuclear actions, with a time-course of seconds to minutes, and that these effects can be mediated by binding of $T_3$ or $T_4$ to receptors on the plasma membrane of different cells [6]. Although various reports of nongenomic actions of thyroid hormones began to appear in the literature more than 35 years ago, it is only in the last decade that more detailed information on the mechanisms behind these effects have become available, in particular after the identification in 2005 of integrin αvβ3 as a plasma membrane receptor of thyroid hormones [7].

The discovery of the integrin receptor for thyroid hormone was a major breakthrough which inspired and renewed research in the field, and it also led to the publication of a review in a special issue of *Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry* [Vol. 6, Number 3, June 2006]. The purpose of the present article is to provide

*Address correspondence to this author at the Department of Biology, University of Rome "Roma Tre, Viale Marconi 446, 00146 Roma, Italy; Tel: ++39-06-57336335; Fax: ++39-06-57336321; E-mail: incerpi@uniroma3.it

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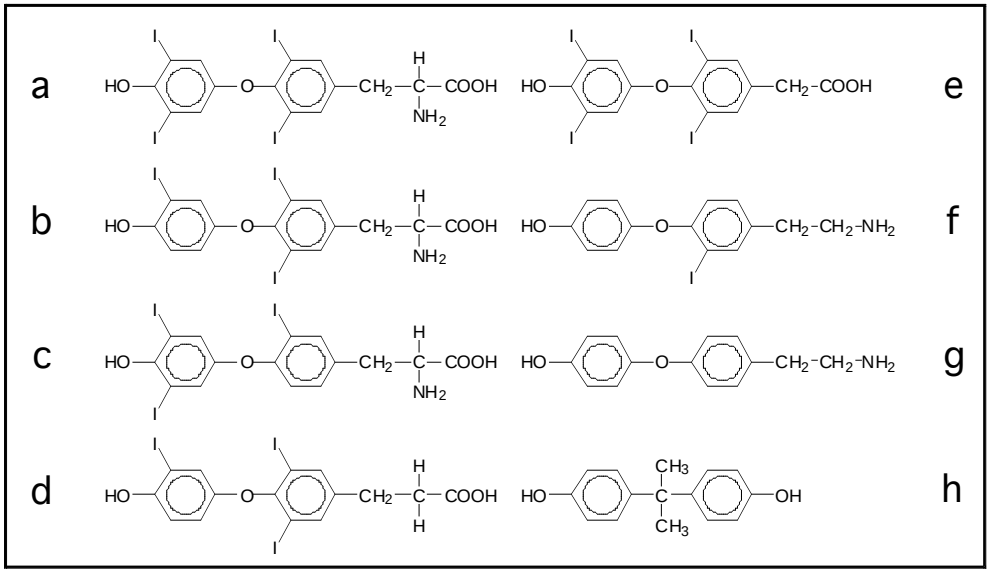


**Fig. (1). Thyroid hormones and related molecules.** The structures of thyroxine ($T_4$), triiodothyronine ($T_3$) and some of the metabolites and analogs mentioned in this review. (a) $T_4$; (b) $T_3$; (c) $rT_3$; (d) DITPA; (e) Tetrac; (f) $T_1AM$; (g) $T_0AM$; (h) Bisphenol A.

an update on our current understanding of nongenomic effects of thyroid hormones six years after the *IEMAMC* review in 2006. This update will include the major advances in our understanding of these important hormones made in the last few years, in particular the effects involving the integrin receptor. But there are other plasma membrane or cytosolic receptors that may mediate fast nongenomic effects, and there is also a possible direct effect of the hormones on channel/receptor structures. Another aspect of the mechanism that has been recognized recently is the contribution of mTOR to thyroid hormone physiology [8]. The nongenomic picture now appears to be just as variegated as the genomic one: different effects of thyroid hormones may be found in all kind of cells. The tissues where there has been more progress in research are the cardiovascular system, the nervous system and the immune system, the latter being a relatively new and little known topic [9]. We will focus mainly on these targets, but also give some information on the current situation for other tissues and physiopathological conditions.

## GENERAL NONGENOMIC MECHANISMS OF THYROID HORMONES

Nongenomic actions of thyroid hormones are mediated through transduction pathways involving cytoplasmic kinases such as mitogen-activated protein kinase (MAPK) [10-16] or phosphatidylinositol 3-kinase (PI3K) [17-22]. These kinases are early factors and may give rise downstream to specific nuclear long-term effects such as gene transcription and cell proliferation [23, 24]. Thus nongenomic and genomic actions of thyroid hormone can interface, leading to complex cellular events. The extranuclear effects may also depend on the initial binding of $T_3$ or $T_4$ to a receptor in the cytosol; this could be one of the TRs interacting with other proteins. Binding of thyroid hormone to the integrin αvβ3 receptor on the plasma membrane gives rise to a complex sequence of cellular events, leading to angiogenesis and tumor cell proliferation, and only a few of these events have been characterized to date, including the transcription of genes such as *ZAKI4* and *HIF-1α* [6, 25, 26]. Integrins are integral membrane proteins that are very important for extracellular matrix (ECM) protein interactions. They are heterodimers composed of an α-type and a β-type subunit, with a long extracellular domain for the binding to ECM proteins, a transmembrane domain, and a short cytoplasmic domain that interacts with cytoskeleton actin and other proteins [27, 28]. The binding of proteins or smaller molecules to integrins on either the outside or the inside of the plasma membrane may regulate important cell functions such as differentiation, proliferation, migration, tumor invasion, metastasis, gene expression, and cell survival [29]. Important physiological functions where integrins, particularly the integrin αvβ3, are involved are angiogenesis, wound-healing, tissue development and differentiation [30]. Dysregulation of integrins may play a role in the pathogenesis of several diseases affecting different tissues; overexpression of integrins has been reported to occur in several types of tumors. The integrin αvβ3 is involved in several mechanisms of tumor growth and invasion [31, 32]. This high expression of integrins in tumor cell make them a valuable target for cancer therapy, including antiangiogenic therapy. It is also known that viruses and bacteria may use integrins to enter the cells [33, 34].

## NERVOUS SYSTEM CELLS

Thyroid hormones are very important for the normal development and differentiation of the cells of the central nervous system (CNS). Deficiency of thyroid hormones during the perinatal period may result in severe mental and physical retardation. In addition to the genomic effects of thyroid hormones, the existence of nongenomic actions have been known for some time [35]. In astrocytes, $T_4$ is converted to $T_3$ by a 5'-deiodinase and $T_3$ is then transferred to neurons where it binds to nuclear receptors to regulate gene expression. It is likely that $T_3$ in these cells is directly regu-

lating gene expression of proteins such as myelin basic protein, neurotrophins and reelin [36]. Thyroid hormone — $T_4$, but not $T_3$ — can modulate processes such as actin polymerization and the extracellular arrangement of laminin, an ECM protein which has a primary role in nerve cell migration during CNS morphogenesis, through the interaction between integrins and components of the cytoskeleton [37-39]. Experimental hypothyroidism has been widely used to study the roles of thyroid hormones in brain and brain development. Hypothyroidism can be achieved by thyroidectomy of pregnant female animals or administration of antithyroid drugs such as 6-propyl-2-thiouracil (PTU), and the effects can be evaluated from the biochemical activities and morphological parameters of fetuses and newborns [40-42]. The systems mainly affected under these experimental conditions are: a) the neurotransmitter system; b) the expression and activities of plasma membrane ion transport systems ($Na^+/H^+$-exchanger, $Na^+/K^+$-ATPase and $Ca^{2+}$-ATPase); c) the neuronal cytoskeleton.

In nervous system cells, nongenomic actions of thyroid hormones may be initiated at a plasma membrane integrin or a G-protein, followed by activation of a second messenger cascade. Although fast compared to modulation of gene expression and protein synthesis, chemical messenger-based mechanisms are always less rapid than the direct interaction of a hormone molecule with a channel or transporter. For thyroid hormones this type of interaction was first shown by Martin *et al*. in 1996 [43] who found that direct binding of $T_3$ to the ionotropic $GABA_A$ receptor gave rise to a concentration-dependent, biphasic modulation of chloride currents in synaptoneurosomes. A similar direct interaction and modulation of nervous cells firing activity in CNS is also known for the neurosteroids, which is reasonable from an evolutionary point of view when dealing with cells where saving milliseconds of the reaction time may save small fishes from predation.

Involvement of the integrin receptor in the $T_4$-mediated increase of voltage-gated neuronal $Na^+$ currents [44] was found in sensory neurons, Rohn-Beard cells, of the zebrafish spinal cord [44]. The downstream signal transduction pathway passes through the p38 isoform of the MAPK pathway; $T_3$ did not affect the $Na^+$ currents in this experimental system. Studies on rat brain slices showed the modulation by $T_4$ of $Na^+$ current in prefrontal cortex, but this effect did not seem to involve integrin αvβ3 or another membrane receptor [45]. It could therefore be the result of a direct effect of the hormone on the channel, but this has not been shown so far. Genomic and nongenomic actions of thyroid hormones may interface to regulate the density of $Na^+$-channels and the activity of neurons. $T_3$ seems to be more active on the genomic pathway, whereas $T_4$ is more active on the nongenomic pathway in modulating basal activity of the $Na^+$ channels [45].

The local administration *in vivo* of $T_4$ rapidly inhibited fast excitatory postsynaptic potentials in the dentate gyrus of the hippocampus with stimulation of the perforant pathway [46]. This effect was particularly significant in hypothyroid rats; a similar response was found using isolated hippocampal slices [46]. The perforant pathway together with the mossy fibers and Schaffer collateral represent the circuit of long-term potentiation, one of the sites of the long-term memory. Interestingly $T_3$ and $T_4$ produced opposite effects in the CA1 area of the hippocampus: $T_3$ increased the cell firing rate whereas $T_4$ decreased it. These effects were quite small on the basic firing activity, but they became very significant for the cell firing rate induced by norepinephrine. This work provides the first evidence that nongenomic actions of thyroid hormones lead to rapid changes in neuronal excitability in the hippocampus in adult euthyroid rats, depending on the relative levels of $T_3$ and $T_4$ [46].

The ionotropic receptors of glutamate, the main excitatory neurotransmitter of the CNS, are also affected by thyroid hormones through nongenomic mechanisms, both in primary cultures of hippocampal neurons and in slices [47]. Albeit in what are considered to be supraphysiologic concentrations, $T_3$ and $T_4$ were shown to decrease NMDA currents, and this effect was not competitive with glutamate or glycine binding [47]. Since NMDA receptors are targets for protein kinases [48], the hypothesis that thyroid hormone effects could act through PKC-mediated phosphorylation of the channel was tested, but this kinase was found not to be involved. The other ionotropic receptors involved in glutamate neurotransmission were AMPA and kainate, whose activity was inhibited by $T_3$ but not by $T_4$ [47], again opposite effects of $T_3$ and $T_4$ on nervous cells were observed [49].

Thyroid hormones have been known for many years to modulate the ionotropic GABA receptor, the main inhibitory neurotransmitter of the central nervous system, in different ways. The levels of glutamate and GABA, the synthesis and degradation of GABA, and its release and reuptake by neurons are all parameters depending on thyroid hormone actions; but, on the other hand, the GABAergic system also regulates the pituitary-thyroid axis at different points [50]. The majority of these effects are mediated by transcriptional mechanisms, but there is much evidence also for a direct action of thyroid hormones on ionotropic GABA receptors [40]. In cultured hippocampal neurons $T_3$ and $T_4$ in the micromolar concentration range decrease $GABA_A$-evoked currents [49, 51]. The antagonism was found to be non-competitive, as previously shown by different authors in synaptoneurosomes from rat brain [52, 53]. The hormone concentrations employed were quite high, but it is possible that thyroid hormone concentrations in the brain could be locally regulated, as in other tissues and cells, and high levels might be present in specific brain areas [49]. To identify elements of a possible nongenomic signaling pathway various experimental procedures were available, such as a pharmacological approach with respect to receptor phosphorylation, intracellular $Ca^{2+}$ measurements or the use of the $Ca^{2+}$ chelator BAPTA, the use of protein kinase blockers and of tetraiodothyroacetic acid (tetrac) or other probes of the integrin αvβ3 receptor [7, 14, 42, 54, 55]. None of these treatments were able to affect the inhibition by thyroid hormones of GABA-induced currents [51], implying that this effect may be the result of a direct interaction of the hormones with the $GABA_A$ receptor.

Thyroid hormones may have an important role also in neuroprotection. Repeated administration of $T_4$ is able to protect against brain ischemia [56, 57] and $T_4$ is known to support the integrity of the brain vascular system [58]. Fur-

thermore thyroid hormones may support the activity of the CNS by nongenomic/genomic modulation of primary and secondary transport systems such as the plasma membrane $Na^+/H^+$ exchanger, $Na^+/K^+$-ATPase, and $Ca^{2+}$-ATPase. The activation of the $Na^+/H^+$ exchanger by thyroid hormones [10, 16] may help a tissue to recover from ischemia/acidosis. Thyroid hormones are able to modify $Na^+/K^+$-ATPase activity [15, 19, 21, 22], thus contributing to maintenance of the $Na^+$ gradient across the plasma membrane and action potentials in excitable cells. Thyroid hormones also influence $Ca^{2+}$ levels by changing the activity of voltage-dependent calcium channels [42] and of the Ca-ATPase [59-61]. The activity of channels can be regulated not only at the GABA receptor or glutamate receptor levels, and through voltage-gated $Na^+$ channels, but also at the inward rectifier $K^+$ channel [62]. These effects support a general rule of thumb: thyroid hormones are important contributors to the maintenance of steady state. The $K^+$ channel in fact is activated by membrane hyperpolarization after an action potential and supports recovery and maintenance of resting membrane potential [63]. All of these effects can be considered neuroprotective, but there are other very interesting effects of thyroid hormones in the CNS. One of the more life-threatening forms of brain damage is glutamate excitotoxicity: an increase in the concentration of glutamate in the extracellular space in brain ischemia contributes to neuronal death, and chronic glutamate toxicity is implicated in the pathogenesis of neurodegenerative diseases. It has been shown that $T_3$ at 10 nM eliminates the gliotoxic effect of glutamate on cultured cerebellar astrocytes and enhances viability of astrocytes and neurons in co-culture [64]. The mechanism of neuroprotection involves the enhanced uptake of glutamate by glutamate transporters in astrocytes, due to both increased transporter activity and increased transporter expression. That is, glutamate excitotoxicity is expressed at the cell surface and relieved by cell uptake and metabolism of the glutamate. Protection by $T_3$ of rat hippocampal neurons against glutamate toxicity by a nongenomic mechanism has also been reported [47]. Once more, the nongenomic and genomic effects of thyroid hormones seem to interface, and in this case the interaction leads to neuroprotection.

In this context it could be useful to find a thyroid hormone analog that shows the positive neuroprotective effect of $T_3$ and may be used as a therapeutic device, without producing the other thyroid hormone actions. Interesting compounds in this respect have been recently proposed: the thyronamines. These are thyroid hormone derivatives with a decarboxylated side chain. Two thyronamines, one with a 3-iodine ($T_1AM$) and the other without iodine ($T_0AM$), have been tested by Scanlan and co-workers [65, 66] and seem to be particularly active as neuroprotectants. The thyronamines bind G-protein coupled receptors called TAAR1, rather than the intracellular thyroid hormone receptors; therefore, their mechanism must be largely nongenomic. A remarkable effect of thyronamines is lowering of the body temperature without giving rise to a homeostatic response. Therefore these agents might be used for neuronal rescue in the setting of ischemia. In fact, a thyronamine given *i.p*. to mice after cerebral artery occlusion promptly lowered the body temperature to 31 °C. The agent also reduced infarct volume by more than 30%. These effects are thought to be nongenomic and mediated by the TAAR1 receptor. Future studies will no doubt test these interesting molecules also in other possible pathological situations associated with brain damage, but also in a different context such as the cardiovascular system, where lowering body temperature — and thus metabolism — in the setting of ischemia can result in tissue salvage [67].

Related to neuroprotection is the *SELective AD indicator-1 (seladin-1*) gene which codes for a protein found to be deficient in Alzheimer's disease (AD) [68, 69]. The seladin-1 protein inhibits the proapoptotic protein caspase 3 and shows enzymatic activity as a hydroxysterol reductase that converts desmosterol to cholesterol. Thyroid hormones, mainly $T_3$, promotes the expression of the gene in neuronal precursor cells and therefore protects the precursor neuron cells from apoptosis through this protein [70]. The modulation of *seladin-1* gene expression may be a component of the effect of $T_3$ on embryonic fetal brain development [68]. $T_3$ also inhibits the expression of the β-*amyloid precursor protein (APP)* gene in neuroblastoma cells in culture [71]. The effect of thyroid hormone on APP gene splicing and in general on AD was summarized recently [72]. It is worthwhile to mention at this point that the serum protein transthyretin, one of the transporters of thyroid hormone, appears to have neuroprotective effects in ischemia, but has shown contradictory effects in AD models. It was found to increase the cleavage of the Aβ-peptide [73], but also to enhance the deposit of the peptide in vascular system of the mouse AD model [74]. Even though the role of thyroid hormones in the context of AD and other neurodegenerative diseases is not particularly clear at present, such aspects should be taken into account when considering the use of analogs of thyroid hormones, and also inhibitors of the αvβ3 integrin function, as possible therapeutic tools [57].

A new type of neuroprotective effect of thyroid hormone was recently described for experimental allergic encephalomyelitis rats, an animal model of multiple sclerosis [75]. In this system $T_3$ administration resulted in myelin sheath protection due to an increased remyelination/demyelination balance and improved nerve impulse propagation.

## IMMUNE SYSTEM CELLS

The last few years have brought increasing evidence confirming the importance of thyroid hormones in immune system function and physiology [8, 9, 76, 77]. In general, hyperthyroidism enhances the immune response in terms of antibody production, cell migration, lymphocyte proliferation and reactive oxygen species (ROS) production, whereas it is associated with decreased proinflammatory marker release, antioxidant enzyme production and immune functions [9]. Hypothyroidism often gives rise to the opposite effects for some of these parameters: it decreases the immune response, antibody production, cell migration and lymphocyte proliferation [77, 78]. However, certain of these effects have not yet been confirmed and therefore it is difficult to predict correlation between immune function and hyper- or hypothyroid conditions [79-82]. A connection between thyroid hormone and the immune system has been observed mainly in pathophysiologic conditions, but it can also been detected under normal physiological conditions. Hodkinson *et al*. [83] measured different characteristic parameters of the immune

system in a large group of healthy euthyroid subjects, together with the plasma thyroid hormone concentrations. They used these data to demonstrate a direct correlation of immune functions with low and high levels of $T_3$ and $T_4$, all within the normal physiological ranges of the hormones [83].

Thyroid hormones are able to potentiate the antiviral state induced by IFN-γ, a pro-inflammatory and pro-apoptotic cytokine that regulates host defense, on HLA-DR expression in CV-1 fibroblasts and HeLa cells, these cells are devoid of the classical nuclear receptors and therefore any effects observed must be due to hormone binding at the plasma membrane or in the cytosol [11]. The signal transduction mechanism was identified as a downstream activation of the MEK-MAPK signaling pathway that in turn activated STAT1α; this was before integrin αvβ3 was identified as a plasma membrane receptor for thyroid hormone. Interestingly, $T_4$ at the physiological concentration of $10^{-7}$ M was effective, while $T_3$ was effective only at the supraphysiological concentrations of $10^{-7}$ M. Tetrac, a product of thyroid hormone metabolism (Fig. **1**) and now used as a probe to test the involvement of the integrin receptor, was able to inhibit the effect of thyroid hormones, whereas membrane-impermeant $T_4$-agarose mimicked the effect, thus confirming that the hormone acted at the plasma membrane by a nongenomic mechanism [11]. The potentiation by thyroid hormone of IFN-γ antiviral activity depends on the MAPK phosphorylation and nuclear translocation and, as a result, on the STAT1α pathway activated by the cytokines. In fact, nuclear STAT1α was found in MAPK immunoprecipitates after treatment of cells for 30 min with 100 nM $T_4$. Thyroid hormone was able to increase the phosphorylation at Ser-727 of STAT1α, the same effect was achieved by the use of IFN-γ [11], whose activity is also influenced by other cytokines or lipopolysaccharide (LPS) [84-86].

LPS and IFN-γ are able to activate PI3K and mammalian target of rapamycin (mTOR), a highly conserved serine-threonine kinase involved in cell growth and protein synthesis in response to growth factors and nutrients by activation of p70S6 kinase and 4EBP1 [87]. mTOR regulates phosphorylation of STAT1α at Ser-727 in lung epithelial adenocarcinoma cells (A549) [88]. Activation of PI3K and mTOR pathways leads to the activation of STAT1-dependent transcription of pro-apoptotic and pro-inflammatory genes. The activation of STAT1 is mediated by PKC isoforms δ and ε, but also other kinases may be involved through an integrin-dependent interaction [89, 90].

Thyroid hormone $T_4$ — but not $T_3$ — can modulate the pericellular arrangement of laminin, an extracellular matrix protein which has a pivotal role in nerve cell migration during CNS morphogenesis, through the interaction between integrins and components of the cytoskeleton [37]. The same authors also showed that thyroid hormones are able to stimulate actin polymerization through nongenomic mechanisms, a process used by leukocytes and other cells able to migrate in response to chemoattractant molecules [91]. Cell migration to the inflammation site depends on a series of processes which are part of a typical immune response: rolling, adhesion and transmigration. The integrin αvβ3 is widely expressed in cells of the immune system, where it is probably involved in proliferation, differentiation and migration processes [8]. For THP-1 monocytes it has been shown that integrin αvβ3 and LPS are involved in the *Coxiella burnetii*-stimulated production of tumor necrosis factor [33].

The preceding evidence caused us to examine whether the integrin αvβ3 could be involved also in other effects induced by thyroid hormones in immune system cells. We found that thyroid hormones were able to increase ROS production, and that this effect could be inhibited by the RGD tripeptide recognized by several integrins, as well as by inhibitors of PI3K and ERK1/2 (wortmannin and PD98059, respectively) and by the flavoprotein inhibitor diphenylene iodonium, indicating a possible involvement of the plasma membrane NADPH oxidase [8]. ROS production may in turn support other typical activities of monocytes, such as cell migration [8].

One of the authors of this review (RNF) and co-workers have recently defined an interface between nongenomic and genomic actions of thyroid hormones on cell proliferation, and reported the up-regulation of nuclear thyroid hormone receptor and iNOS expression [76]. It was found that the cell proliferation effect initiated at the plasma membrane, since thyroid hormone covalently bound to agarose and thus excluded from the cell interior was capable of inducing cell proliferation, although to lower extent than the free hormone [76]. We may close this section by quoting a phrase from this work: 'Therefore, thyroid hormones activation of nongenomic signal through this integrin receptor would contribute to the physiologic interaction between immune cells during immune responses and would explain the immune deficit frequently observed in hypothyroid conditions' [76].

From this survey of thyroid hormone and the immune system, we conclude that the inhibitors/ligands of the integrin αvβ3, like tetrac, the RGD peptide or disintegrins sharing the same RGD sequence such as echistatin from *Echis carinatus* [92], could be tested as possible modulators of immune system functions.

## CARDIOVASCULAR SYSTEM

The cardiovascular system is a principal target of thyroid hormone [93]. A number of hormone actions are carried out through the classical nuclear receptors, mainly TRα1, but also via nongenomic effects that are becoming increasingly appreciated. Thyroid hormones exert their effects mainly by stimulating the synthesis of proteins that are important for the function of the heart: sarcoplasmic reticulum $Ca^{2+}$-ATPase, α-myosin heavy chain, β1-adrenergic receptor or voltage-gated channels, just to mention some of them [94]. Thyroid hormones are also able to affect heart function through stimulation of systolic contraction and diastolic relaxation by modulating the release and reuptake of calcium ions into and from the sarcoplasmic reticulum. Phospholamban, a membrane protein that is positively regulated by thyroid hormone, decreases the rate of muscle relaxation and contractility through inhibition of the calcium pump [95]. Proteins that are known to be regulated either positively or negatively by thyroid hormones are listed in Table **1**

Another important aspect of thyroid hormone function is the effect on lipid metabolism. The euthyroid subject who has no genetic dyslipidemia has normal levels of blood cho-

**Table 1. Negative and positive regulation of proteins by thyroid hormones in the heart.**

| Negative regulation | References | Positive regulation | References |
|---|---|---|---|
| β-Myosin heavy chain | [136, 137] | α-Myosin heavy chain | [136, 137] |
| Phospholamban | [138, 139] | Sarcoplasmic reticulum $Ca^{2+}$-ATPase | [138-140] |
| $Na^+/Ca^{2+}$ exchanger | [138, 139] | $Na^+,K^+$-ATPase | [140, 141] |
| α1-Adrenergic receptors | [145] | β1-Adrenergic receptors | [143-147] |
| Thyroid hormone receptor α1 | [142] | Voltage-gated potassium channels | [142] |
| Adenylate cyclase type V | [142] | Atrial and brain-natriuretic peptides | [142] |
| Adenylate cyclase type VI | [142] | Adenine nucleotide transporter 1 | [142] |
| Guanine nucleotide-binding protein $G_i$ | [142] | Guanine nucleotide-binding protein $G_s$ | [142] |
| $Ca^{2+}$-channels | [151] | Ryanodine receptors | [148] |
| $K^+$-channels (Kv 1.2, Kv 1.4) | [154] | $K^+$-channels (Kv1.5, Kv4.2, Kv4.3) | [149-151] |
| PKC-ε | [155-157] | Connexin-40, Connexin-43 | [152, 153] |

lesterol, since thyroid hormone promotes expression of the plasma membrane LDL receptor. In untreated hypothyroidism, levels of blood lipids, including cholesterol, are typically increased, but can be normalized by thyroid hormone therapy. This regulation of blood lipid is also important for the heart function, since high lipid levels can lead to atherosclerosis and increased risk of other cardiovascular pathologies. Hypolipidemic hormone analogs could help to control such life-threatening diseases, and should be devoid of undesirable effects of excess hormone action on the heart, including increased heart rate, atrial fibrillation, congestive heart failure and dilated cardiomyopathy [96, 97]..

The possibility of nongenomic contributions of thyroid hormone to regulation of cardiac mass and in cardioprotection have been investigated for several years [20, 98-100]. Thyroid hormones $T_3$ and $T_4$ can promote growth (hypertrophy) that involves enlargement of cardiac myocytes [99]. The hypertrophic process initiated by iodothyronines involves the activation of PI3K/PKB-Akt [98] and downstream targets such as mTOR [99, 100], and possibly glycogen synthase kinase-3β [GSK-3β;101]. The contributions of GSK-3β [102, 103] and mTOR [104] to the biochemistry of hypertrophy in the absence of thyroid hormone are clear, and inhibition of mTOR has been shown to prevent thyroid hormone-induced myocardial hypertrophy [100]. A very recent paper [105] showed that thyroid hormones are very important for the maturation of sheep fetal hearts with an involvement of mTOR, SERCA2 and also atrial natriuretic peptide (ANP). Is there a role for integrin αvβ3 in the maturation of the heart? Thyroid hormone signaling mediated by the integrin receptor in the heart has not been shown so far. A regulatory contribution by integrin αvβ3 in human cardiac fibroblasts has been recently described, where inhibition of the integrin is able to potentiate the BNP/cGMP response in cardiac fibroblasts [106]. Cardiac fibroblasts are important in cardiac remodelling and fibrosis through their proliferation, differentiation and secretion of extracellular matrix proteins; therefore modulation by integrin αvβ3 of ANP signaling in cardiac remodelling could be another target for thyroid hormone.

Aortic constriction triggers an inflammatory process that may contribute to cardiac dysfunction both in humans and in models of myocardial hypertrophy. It is important to define the mechanisms involved, and to identify tools able to suppress the inflammatory process. To study the role of mTOR in pathological hypertrophy Song *et al.* generated transgenic mice with specific overexpression of heart mTOR [107, 108]. These mice (mTOR-Tg) were protected against cardiac dysfunction induced by left ventricular pressure overload (transverse aortic constriction, TAC), and their hearts showed a low level of interstitial fibrosis compared to wild-type mice after 4 weeks of TAC. The mechanism of the inflammatory response is not known, but certainly proinflammatory cytokines such as IL-6 and IL-1β are involved in models of cardiac hypertrophy [109-111]. One week after TAC treatment IL-1β and IL-6 levels were increased significantly in wild type mice, but not in mTOR-Tg mice. *In vitro* experiments showed that transfection of HL-1 cardiomyocytes with *mTOR* inhibited the increase of IL-6 due to LPS, whereas treatment with the mTOR inhibitors rapamycin and PP242 prevented this effect [107]. In addition, *mTOR* overexpression reduced NF-κB-regulated transcription in the cardiomyocytes. Taken together, these data indicate that mTOR confers cardioprotection by suppression of the inflammatory response [107]. However, it should be mentioned that other authors previously have reported contrasting results; in wild type mice treated with inhibitors of mTOR after aortic costriction for 5 weeks, the hemodynamic parameters indicated protection [112], whereas rapamycin inhibited cardiac hypertrophy and improved cardiac performance in mice subjected to pressure overload [113].

The results of Song *et al.* [107] are important because they suggest continuation of the effort to modulate pharmacologically the expression of *mTOR* in several of the models of cardiac disease mentioned above. Many different factors are known to regulate mTOR activity or expression of the

*mTOR* gene. These include hormones, growth factors, and cytokines, and it is known that $T_3$ may act through mTOR as indicated above. The relationship between thyroid hormones and mTOR was first shown in fibroblasts [17, 18, 26] where nongenomic activation of mTOR was found to give rise to transcription of the genes for the calcineurin inhibitor *ZAKI-4* and hypoxia-inducible factor *HIF-1α*. Once more we see an interplay between nongenomic and genomic effects of thyroid hormones. These results have been confirmed in the last few years in several papers using different cell models, most cardiomyocytes [99, 100, 114] and also in rat hippocampus [115]. Is integrin αvβ3 actually involved in the mediation of mTOR? The evidence in the heart cells is, for the time being, still lacking. Very recently Gnoni and coauthors have shown that $T_3$ induces expression of the sterol-regulatory element binding protein-1 (SREBP-1) in HepG2 cells, by a nongenomic mechanism PI3K/Akt/mTOR-C1 pathway activation. The $T_3$ effect is partly inhibited by tetrac, a probe of the interaction of $T_3$ and the αvβ3 integrin receptor, and mimicked by $T_3$-agarose, an analog of $T_3$ that does not enter the cell [116].

From all these studies it appears that non-nuclear, non-transcriptional effects of thyroid hormone can be mediated by cytosolic/nuclear receptors or by a plasma membrane receptor to give complex physiological responses. These actions of thyroid hormones are rapid in onset but may lead to long-term effects.

At the end of this paragraph on the complex actions of thyroid hormones in heart, we note that from a pharmacological point of view there are several compounds worthy of being tested for their potential as therapeutic tools against cardiovascular disease. We have already mentioned thyronanime derivatives in other section of the present article and we refer the reader to the references quoted there. Another compound that was reported in IEMAMC 2006 as a possible tool in cardiovascular disease is 3,5-diiodothyropropionic acid (DITPA, Fig. **1**), reported to increase cardiac output and to lower left ventricular and diastolic pressure, improving ventricular relaxation after infarction, but without increasing the heart rate. DITPA decreases the inflammatory response and myocardial infarct size in animal models, when administered up to 3 days after myocardial ischemia. The positive effect of DITPA seems to be due to modulation of the inflammatory response (decrease of creatine kinase, IL-6, and monocyte chemoattractant protein [MCP-1; 117]. Other studies have shown an improvement of hemodynamic and metabolic parameters, but no real symptomatic benefits in patients [118, 119]. Clinical trials of DITPA for congestive heart failure have been terminated in the U.S. (see www.ClinicalTrials.gov). A recent study evaluating the efficiency of DITPA administration in four children with monocarboxylate transporter 8 (MCT8) deficiency has been published, and the results indicate that DITPA normalized elevated serum levels of $T_3$ and thyreotropic hormone, with a decrease in sex hormone-binding globulins, heart rate and ferritin; no adverse effects were reported. These data suggest that DITPA does not require the MCT8 transporter to enter the cells [120]. This is a very important result that stimulates future research on thyroid hormones analogs.

Bisphenol A (BPA), is a component of polycarbonate plastics and epoxy resins widely produced in the world and an environmental and human contaminant (Fig. **1**). The population exposed to BPA is at risk, particularly the high levels found in pregnant women (umbilical cord and fetal plasma) indicate that BPA can cross the maternal-fetal barrier [121, 122]. BPA shows estrogen agonist/antagonist properties, but is also able to bind to thyroid hormone receptor, acting as hormone antagonist, by preventing the binding of $T_3$ [123]. It has recently been reported [124] that low concentrations of BPA are able to suppress thyroid hormone transcription through a nongenomic effect involving integrin αvβ3 in CV-1 cells lacking nuclear thyroid hormone receptor and also TR-repleted 293T cells. BPA inhibits integrin αvβ3/c-Src/TR-β1 signaling pathways that are normally mediated by $T_3$ and $T_4$. This leads to inhibition of thyroid hormone gene transcription through recruiting the N-CoR (nuclear corepressor) or SMRT (silencing mediator of retinoic and thyroid hormone receptor) to nuclear thyroid hormone receptor. Once more a cross–talk or interface between the genomic and nongenomic effects of thyroid hormones has been shown.

## CONCLUSIONS

A comparison of the three tissues mainly discussed in this paper, the nervous system, the immune system, and the cardiovascular system, reveals that most of the nongenomic mechanisms involved varies widely among the different cell types. The main transduction pathways related to the nongenomic effects of thyroid hormones involving either membrane or cytosolic receptors are shown in Fig. **2**. Essentially it can be concluded that it is not possible to deduce mechanistic aspects for one cell type on the basis of results obtained for cells coming from another tissue or organ.

This review is an update of a paper previously published in a special issue of IEMAMC on the nongenomic effects of the steroid hormone receptors superfamily. The 2006 article pointed to membrane ion transport systems as one target of importance among nongenomic effects of thyroid hormones. The research of the last 6-8 years has progressed in terms of hormone actions in ion transport and other transport systems but not only. The $Na^+/H^+$ exchanger was shown to be activated in a nongenomic way by thyroid hormones in L-6 skeletal muscle cells in culture, exchanging the $Na^+$ ions with protons, according to the concentration gradient and with a 1:1 stoichiometry. This system may support recovery from the acid pH due to muscle activity, but also can protect nervous cells from ischemia. As to the $Na^+/K^+$-ATPase activity, we reported inhibition of this primary transport system by thyroid hormones in chick embryo hepatocytes at different levels of development. The increase in intracellular $Na^+$ ions together with a stimulation of the amino acid uptake System A represents an anabolic signal during development [21, 22, 125]. A relationship between the $Na^+/K^+$-ATPase and mTOR has been reported by several groups of investigators and also this effect appears to be very cell specific [126]. In 2003 Bassett *et al.* [127] published a table listing the nongenomic effects known at that time. We here present an upgraded version, Table **2**, which shows how the research involving the nongenomic effects of thyroid hormones has gone ahead.

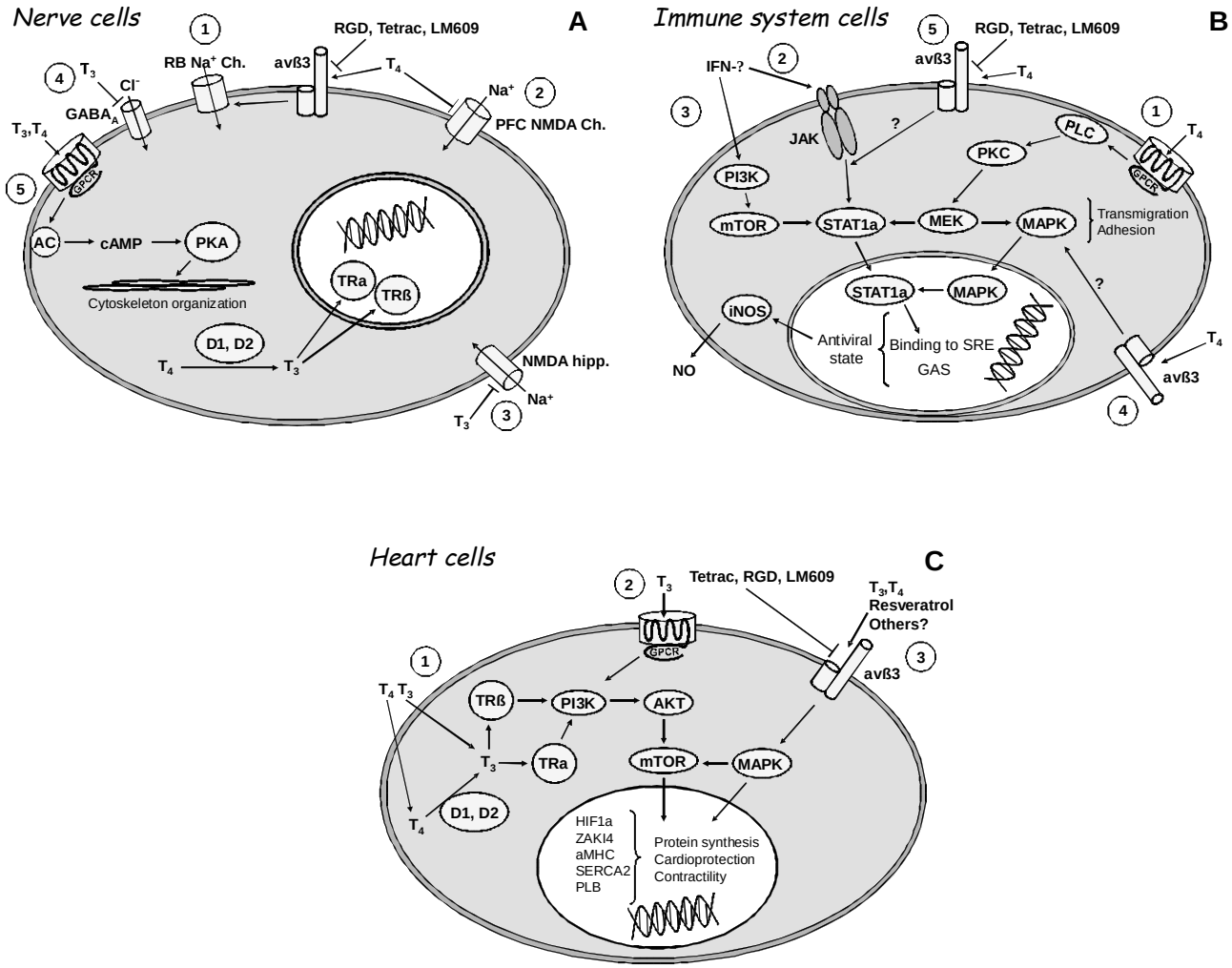


**Fig. (2). Nongenomic effects of thyroid hormones in the cell.** A schematic summary of the nongenomic mechanisms of thyroid hormones as described in the text for three different cell types. **Panel A.** Nerve cells. **1.** In the sensory neurons Rohn–Beard (RB), $T_4$ stimulates the voltage-dependent $Na^+$-Channel through integrin αvβ3. Major inhibitors of the integrin are shown: RGD peptide, tetrac, anti-αvβ3 LM609. **2**. In pre-frontal cortex (PFC) slices $T_3$ and $T_4$ inhibit the NMDA receptor /channel [45]. **3, 4.** $T_3$ inhibits the NMDA $Na^+$-channel and GABA receptor in hippocampal (hipp) neurons, probably through a direct interaction [49]. **5**. Modulation by $T_3$, $T_4$ of Protein Kinase A leading to reorganization of the cytoskeleton [42]. The modulation of gene expression is only suggested by the arrow to the nucleus, involving thyroid receptors (TR) and Deiodinases 1 and 2 (D1, D2). **Panel B.** Immune system cells and others. **1**. $T_4$ through G-protein coupled receptor (GPCR) potentiates the IFN-γ antiviral action, and **2** activation of signal transducer and activator of transcription (STAT1)-α, by Janus Kinase (JAK), that at the nuclear level can increase the antiviral state through binding to specific response elements (SRE) and IFN-γ activated sequences (GAS) [11] **3.** IFN- γ activates PI3K/Akt/mTOR pathway [88]. **4.** $T_4$ through integrin αvβ3 and activation of the MAPK pathway may modulate transmigration and adhesion proper ties, at the nucleus it stimulates iNOS and protein synthesis [76, 129]. **Panel C.** Heart cells. **1.** $T_3$, $T_4$ produce nongenomic effects also through the classical receptors located in the cytosol leading to activation of the PI3K/mTOR pathway, important modulator of protein synthesis, contractility and cardioprotection [99-101]. **2**, **3** $T_3$,$T_4$ and other small molecules such as resveratrol (resv) activate the PI3K/Akt/mTOR pathway through integrin αvβ3 [108], the hormones also through a GPCR receptor [99]. Modulation at the nucleus is indicated for hypoxic factor-1α (HIF-1α), the calcineurin-like protein ZAKI-4, α-Myosin Heavy Chain (α-MHC), sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA2), and phospholamban (PLB), [6].

There are many questions remaining with regard to the roles of nongenomic actions of thyroid hormones in nerve cells as well as cardiac and striated muscle cells. The neuroprotective and potentially cardioprotective effects of hormone analogs encourage additional pharmacological exploration, particularly where the cell surface receptor for the hormone on integrin αvβ3 is involved. This receptor separates hormone effects on ion transporters and on expression of certain genes from the traditional and important nuclear TR-dependent housekeeping functions. Hormone analogues can be formulated to limit hormone analog action to αvβ3-mediated resoponses. The potential importance of the mTOR pathway as a central junction of hormone action is substantial. We believe that inhibitors and activators of this pathway

**Table 2. Nongenomic effects of thyroid hormones. Selected nongenomic effects of thyroid hormones and tissues/cells where they have been found. Explanation of symbols: ↑ increase, ↓ decrease, ⇒ give rise to:**

| Actions of thyroid hormones | Cell type/tissue | Range | Signal transduction | Physiological function or final effect | References |
|---|---|---|---|---|---|
| $Ca^{2+}$-ATPase | ↑ Red blood cells<br>↑ Skeletal muscle<br>↑ Myocardium | $T_4$ 0.1 nM<br>$T_3$, $T_4$ 0.1 nM<br>$T_3$, $T_4$ 0.1 nM | Phospholipase C activation ? | ?<br>Muscle relaxation<br>Diastolic function | [130, 131]<br>[132]<br>[61] |
| 2-deoxyglucose transport | ↑ Rat thymocyte | $T_3$ 1 nM | $Ca^{2+}$/Adenylate cyclase/cAMP | Increase of glucose uptake | [133, 134] |
| Actin polymerization | ↑ Astrocytes culture | $T_4$, r$T_3$ 10 nM | Regulation of actin cytoskeleton through laminin-integrin interactions | Neuron arborization, axonal transport, brain development | [35-39] |
| Ionotropic GABA receptor | Rat brain synaptoneurosomes | $T_3$, $T_4$<br>μM range | Binding of the hormone to the GABA receptor | Inhibition of cell firing | [43, 52, 53] |
| Antiviral effect of IFN-γ | CV-1 fibroblasts, HeLa Cells | $T_3$, $T_4$ 100 nM | MEK/MAPK/STAT1α | Potentiation of antiviral effect | [11] |
| $Na^+/H^+$ exchanger | ↑ L-6 myoblasts<br>↑ Chick embryo hepatocytes | $T_3$ 1 nM<br>$T_4$ 100 nM<br>$T_3$,$T_4$ 1 nM | PKC/PI3K/MAPK pathway, $[Ca^{2+}]_i$ ↑<br>PKC/PI3K/MAPK pathway, $[Ca^{2+}]_i$ ↑ | Recovery from acidosis during muscle contraction<br>$[Na^+]_i$ ↑ + cell volume ↑ = mitogenic signal | [10, 16]<br>[21, 22] |
| Na+/K+-ATPase | ↑ Alveolar epithelial cells<br>↓ Chick embryo hepatocytes | $T_3$<br>10 nM-10 μM<br>$T_3$, $T_4$<br>0.1 nM-10 μM | PI3K/Akt pathway, Src kinase<br>PKC/PKA/$[Ca^{2+}]_i$ ↑ | Fluid clearance, recovery from respiratory syndrome<br>$[Na^+]_i$ ↑ + cell volume ↑ = metabolism ↑ ⇒ proliferation ↑ | [15,19]<br>[21, 22, 158, 159] |
| Amino acid transport System A | ↑ Chick embryo hepatocytes | $T_3$, $T_4$<br>0.1-100 nM | PKC/PI3K/MAPK pathway | Cell volume ↑ ⇒ cell metabolism ↑⇒ proliferation ↑ | [21, 22] |
| Angiogenesis | Chick chorioallantoic membrane assay | $T_4$ 0.1 nM<br>$T_3$ 100 nM | Integrin αvβ3/MAPK activation | Angiogenesis stimulation | [7] |
| Cancer cell proliferation | Glioma cells | $T_4$<br>1-100 nM | Integrin αvβ3/MAPK activation | Tumor cell growth increase | [25] |
| $V_m$–dependent $Na^+$ current | Sensory neurons | $T_4$ 10 nM | Integrin αvβ3/p38 MAP activation | Modulation of action potentials in embryonic nervous system | [44] |
| $V_m$-dependent $Na^+$ current | Rat prefrontal cortex | $T_4$ 10 μM | Direct interaction ? | Modulation of neuronal excitability | [45] |
| NMDA ionotropic receptor | Rat hippocampal neurons | $T_3$, $T_4$ 10 μM | Direct interaction ? | Inhibition of firing | [46, 47, 49] |
| GABA ionotropic receptor | Rat hippocampal neurons | $T_3$ 10 μM | Direct interaction ? | Inhibition of firing | [51] |
| Cell proliferation | T lymphocytes | $T_3$ 1 nM<br>$T_4$ 100 nM | Integrin αvβ3 ?<br>/PKCζ/ERK1/2/NF-κB/iNOS | Modulation of T cell proliferation | [8] |
| Cell migration | THP-1 monocytes | $T_4$ 10 μM | Integrin αvβ3/ROS/MAPK<br>Integrin αvβ3/ROS/PI3K/Akt | Stimulation of spontaneous migration | [62] |
| IR K channel | Guinea pig ventricular myocytes | $T_3$ 1 nM-1 μM | Direct channel opening ? | Shortening of action potential duration | [45] |

**Table 2. Contd….**

| Actions of thyroid hormones | Cell type/tissue | Range | Signal transduction | Physiological function or final effect | References |
|---|---|---|---|---|---|
| Increased expression of ZAKI-4α | Human skin fibroblasts | $T_3$ 10 nM | TR-β1 ⇒ PI3K/PKB/Akt/mTOR/ p70S6K | Nongenomic modulation of a genomic effect | [18] |
| Cardiac growth | Rat cardiomyocytes | $T_3$ 10 nM | TRα1 ⇒ PI3K/PKB/Akt/mTOR/ p70S6K | Physiological cardiac growth | [99] |
| Initiation of *HIF-α* gene transcription | Human glioblastoma U-87 MG cells | $T_3$ 1 nM | αvβ3 integrin/ Src kinase/ PI3K/ *HIF-α* gene expression | Nongenomic modulation of a genomic effect | [15, 19] |
| Cell proliferation | Human lung carcinoma cells (NCI-H510A) | $T_3$, $T_4$ 100 nM | αvβ3 integrin/ERk1/2 cross–talk with ER-α | Modulation of cell proliferation in lung cancer cells | [135] |
| Increased expression of SREBP-1 | HepG2 cells | $T_3$ 1 μM | αvβ3 integrin/PI3K/Akt/mTORC-1 | Nongenomic modulation of lipogenic genes | [116] |

may represent a new class of drugs in several pathological situations, such as cardiovascular hypertrophy, immune system functions, tumor cell growth, CNS disease and injury [128]. Several pharmacological therapies for brain injuries and traumatic disease involving the mTOR pathway are at present under investigation.

## ABBREVIATIONS

AMPA = γ-amino-3-hydroxy-5-methylisoxazol-4-propionic acid

ANP = Atrial natriuretic peptide

BPA = Bisphenol A

CNS = Central nervous system

DAG = Diacylglycerol

DITPA = 3,5-diiodothyropropionic acid

ECM = Extracellular matrix

GABA = γ-aminobutyric-acid

LPS = Lipopolysaccharide

MAPK = Mitogen activated protein kinase

MCP-1 = Macrophage chemoattractant protein-1

MCT8 = Monocarboxylate transporter 8

NMDA = N-methyl-D-aspartic acid

NOS = Nitric oxide synthase

PI3K = Phosphatidyl-inositol-3-kinase

PKA = Protein kinase A

PKC = Protein kinase C

RGD = Arginine-glycine-aspartate

ROS = reactive oxygen species

$rT_3$ = Reverse-$T_3$

SERCA = Sarcoplasmic reticulum $Ca^{2+}$-ATPase

SREBP-1 = Sterol-regulatory element binding protein-1

$T_3$ = 3,3',5-L-triiodothyronine

$T_4$ = L-thyroxine

TAC = Transverse aortic constriction

TETRAC = tetraiodothyroacetic acid

TH = thyroid hormone

TR = Thyroid hormone receptor

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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## REFERENCES

[1] Yen, P.M. Physiological and molecular basis of thyroid hormone action. *Physiol. Rev*., **2001**, *81*, 1097-1142.

[2] Pascual, A.; Aranda, A. Thyroid hormone receptors, cell growth and differentiation. *Biochim. Biophys. Acta*., **2012** doi: 10.1016/j.bbagen.2012.03.012.

[3] Bianco, A.C; Salvatore, D.; Gereben, B.; Berry, M.J; Larsen, P.R. Biochemistry, cellular and molecular biology and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev*., **2002**, *23*, 38-89.

[4] Wagner, R.l.; Apriletti, J.W.; McGrath, M.E.; West, B.L.: Baxter, J.D.; Fletterick, R.J. A structural role for hormone in the thyroid hormone receptor. *Nature*, **1995**, *378*, 690-697.

[5] Darimont, B.D.; Wagner, R.L.; Apriletti, J.W.; Stallcup, M.R; Kushner, P.J.; Baxter J.D.; Fletterick, R.J.; Yamamoto, K.R. Structure and specificity of nuclear receptor-coactivator interactions. *Genes Dev*., **1998**, *12*, 3343-3356.

[6] Cheng, S.-Y.; Leonard, K.L.; Davis, P.J. Molecular aspects of thyroid hormone actions. *Endocr. Rev*., **2010**, *31*, 139-170.

[7] Bergh, J.J.; Lin, H.-Y.; Lansing, L.; Mohamed, S.N.; Davis, F.B.; Mousa, S.; Davis, P.J. Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology*, **2005**, *146*, 2864-2871.

[8] De Vito, P.; Balducci, V.; Leone, S.; Percario, Z.; Mangino, G.; Davis, F.B.; Davis, P. J.; Affabris, E.; Luly, P.; Pedersen, J.Z.; Incerpi, S. Nongenomic effects of thyroid hormones on the immune system cells: new targets, old players. *Steroids*, **2012**, *77*, 988-995.

[9] De Vito, P.; Incerpi, S.; Pedersen, J.Z.; Luly, P.; Davis, F.B.; Davis, P.J. Thyroid hormones as modulators of immune activities at the cellular level. *Thyroid*, **2011**, *21*, 879-90.

[10] Incerpi, S.; Luly, P.; De Vito, P.; Farias, R.N. Short-term effects of thyroid hormones on the Na/H antiport in L-6 myoblasts: high molecular specificity for 3,3',5-triiodo-L-thyronine. *Endocrinology*, **1999**, *140*, 683-689.

[11] Lin, H.-Y.; Davis, F.B.; Gordinier, J.K.; Martino, L.J.; Davis, P.J. Thyroid hormone induces activation of mitogen-activated protein kinase in cultured cells. *Am. J. Physiol*., **1999**, *276*, C1014-C1024.

[12] Davis, P.J.; Shih, A.; Lin, H.-Y.; Martino, L.J.; Davis, F.B. Thyroxine promotes association of mitogen-activated protein kinase and nuclear thyroid hormone receptor (TR) and causes serine phosphorylation of TR. *J. Biol. Chem*., **2000**, *275*, 38032-38039.

[13] Shih, A.; Lin, H.-Y.; Davis, F.B.; Davis, P.J. Thyroid hormone promotes serine phosphorylation of p53 by mitogen-activated protein kinase. *Biochemistry*, **2001**, *40*, 2870-2878.

[14] Davis, P.J.; Davis, F.B.; Cody, V. Membrane receptors mediating thyroid hormone action. *Trends Endocrinol. Metab*., **2005**, *16*, 429-435.

[15] Lei, J.; Mariash, C.N.; Bhargava, M. Wattenberg, E.V.; Ingbar, D.H. $T_3$ increases Na-K-ATPase activity via a MAPK/ERK1/2-dependent pathway in rat adult alveolar epithelial cells. *Am. J. Physiol. Cell. Physiol*., **2008**, *294*, L749-L754.

[16] D'Arezzo, S.; Incerpi, S.; Davis, F.B.; Acconcia, F.; Marino, M.; Farias, R.N., Davis, P.J. Rapid nongenomic effects of 3,5,3'-triiodo-L-thyronine on intracellular pH of L-6 myoblasts are mediated by intracellular calcium mobilization and kinase pathways. *Endocrinology*, **2004**, *145*, 5694-5703.

[17] Moeller, L.C.; Cao, X.; Dumitrescu, A.M.; Seo, H.; Refetoff, S. Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor β through the phosphatidylinositol 3-kinase pathway. *Nucl. Recept. Signal*., **2006**, *4*, e020.

[18] Cao, X.; Kambe, F.; Moeller, L.C.; Refetoff, S.; Seo, H. Thyroid hormone induces rapid activation of Akt/protein kinase B-mammalian target of rapamycin-p70$^{S6K}$ cascade through phosphatidylinositol 3-kinase in human fibroblasts. *Mol. Endocrinol*., **2005**, *19*, 102-112.

[19] Lei, J.; Mariash, C.N.; Ingbar, D.H. 3,3',5-Triiodo-L-thyronine up-regulation of Na,K-ATPase activity and cell surface expression in alveolar epithelial cells is Src kinase- and phosphatidylinositide 3-kinase-dependent. *J. Biol. Chem*., **2004**, *279*, 47589-47600.

[20] Hiroi, Y.; Kim, H.H.; Furuya, F.; Huang, Z.; Simoncini, T.; Noma, K.; Ueki, K.; Nguyen, N.H.; Scanlan, T.S.; Moskowitz, M.A.; Cheng, S.Y.; Liao, J.K. Rapid nongenomic effects of thyroid hormone. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 14104-14109.

[21] Scapin, S.; Leoni, S.; Spagnuolo, S.; Fiore, A.M.; Incerpi, S. Short-term effects of thyroid hormones on the $Na^+$-$K^+$ATPase activity of chick embryo hepatocytes during development: Focus on signal transduction. *Am. J. Physiol. Cell. Physiol*., **2009**, *296*, C4-C12.

[22] Scapin, S.; Leoni, S.; Spagnuolo, S.; Gnocchi, D.; De Vito, P.; Luly, P.; Pedersen, J.Z.; Incerpi, S. Short-term effects of thyroid hormones during development: focus on signal transduction. *Steroids*, **2010**, *75*, 576-584.

[23] Tang, H.Y.; Lin, H.-Y.; Zhang, S.; Davis, F.B.; Davis, P.J. Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. *Endocrinology*, **2004**, *145*, 3265-3272.

[24] Moeller, L.C.; Dumitrescu, A.M.; Refetoff, S. Cytosolic action of thyroid hormone leads to induction of hypoxia-inducible factor-1α and glycolytic genes. *Mol. Endocrinol*., **2005**, *19*, 2955-2963.

[25] Lin, H.-Y.; Sun, M.; Tang, H.Y.; Luidens, M.K.; Mousa, S.A.; Incerpi, S.; Drusano, G.L.; Davis, F.B.; Davis, P.J. L-Thyroxine vs. 3,5,3'-triiodothyronine and cell proliferation: activation of mitogen activated protein kinase and phosphatidylinositol 3-kinase. *Am. J. Physiol. Cell. Physiol*., **2009**, *296*, C980-C991.

[26] Moeller, L.C.; Broecker-Preuss, M. Transcriptional regulation by nonclassical action of thyroid hormone. *Thyroid Res*., **2011**, *4*(Suppl. 1), 1-7.

[27] Switala-Jelen, K.; Dabrowska, K.; Opolski, A.; Lipinska, L.; Nowaczyk, M.; Gorski, A. The biological functions of β3 integrins. *Folia Biologica (Praha)*, **2004**, *50*, 143-152.

[28] Barja-Fidalgo, C.; Coelho, A.L.J.; Saldanha-Gama, R.; Helal-Neto, E.; Mariano-Oliveira, A.; De Freitas, M.S. Disintegrins: integrin selective ligands which activate integrin-coupled signaling and modulate leukocyte functions. *Braz. J. Med. Biol. Res*., **2005**, *38*, 1513-1520.

[29] Hynes, R.O. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*, **1992**, *69*, 11-25.

[30] Giancotti, F.G.; Ruoslahti, E. Integrin signaling. *Science*, *1999*, *285*, 1028-1032.

[31] Hosotani, R.; Kawaguchi, M.; Masui, T.; Koshiba, T.; Ida, J.; Fujimoto, K.; Wada, M.; Doi, R.; Imamura, M. Expression of integrin αvβ3 in pancreatic carcinoma: relation to MMP-2 activation and lymph node metastasis. *Pancreas*, **2002**, *25*, e30–35.

[32] Landen, C.N.; Kim, T.J.; Lin, Y.G.; Merritt, W.M.; Kamat, A.A.; Han, L.Y.; Spannuth, W.A.; Nick, A.M.; Jennnings, N.B.; Kinch, M.S.; Tice, D.; Sood, A.K. Tumor-selective response to antibody-mediated targeting of αvβ3 integrin in ovarian cancer. *Neoplasia*, **2008**, *10*, 1259-1267.

[33] Dellacasagrande, J.; Ghigo, E.; Hammami, T.; Toman, S.R.; Raoult, D.; Capo, C.; Mege, J.L. αvβ3 integrin and bacterial lipopolysaccharide are involved in *Coxiella burnetii*-stimulated production of tumor necrosis factor by human monocytes. *Infect. Immun*., **2000**, *68*, 5673-5678.

[34] Chiodelli, P.; Urbinati, C.; Mitola, S.; Tanghetti, E.; Rusnati, M. Sialic acid associated with αvβ3 integrin mediates HIV-1 Tat protein interaction and endothelial cell proangiogenic activation. *J. Biol. Chem*., **2012**, *287*, 20456-20466.

[35] Koibuchi, N.; Chin, W.W. Thyroid hormone action and brain development. *Trends Endocrinol. Metab*., **2000**, *11*, 123-128.

[36] Siegrist-Kaiser, C.A.; Juge-Aubry, C.; Tranter, M.P.; Ekenbarger, D.M.; Leonard, J.L. Thyroxine–dependent modulation of actin polymerization in cultured astrocytes: A novel, extranuclear action of thyroid hormone. *J. Biol. Chem*., **1990**, *265*, 5296-5302.

[37] Farwell, A.; Dubord-Tomasetti, S.A. Thyroid hormone regulates the extracellular organization of laminin on astrocytes. *Endocrinology*, **1999,** *140*, 5014-5021.

[38] Farwell, A.P.; Dubord-Tomasetti, S.A.; Pietrzykowsky, A.Z.; Stachelek, S.J.; Leonard, J.L. Regulation of cerebellar neuronal migration and neurite outgrowth by thyroxine and 3,3',5'-triiodothyronine. *Develop. Brain Res*., **2005**, *154*, 121-135.

[39] Farwell, A.P.; Dubord-Tomasetti, S.A.; Pietrzykowsky, A.Z.; Leonard, J.L. Dynamic nongenomic actions of thyroid hormone in the developing rat brain. *Endocrinology*, **2006**, *147*, 2567-2574.

[40] El-Bakry, A.M.; El-Ghareeb, A.-W.A.; Ahmed, R.G. Comparative study of the effects of experimentally-induced hypothyroidism and hyperthyroidism in some brain regions in albino rats. *Int. J. Dev. Neurosci*., **2010**, *28*, 371-389.

[41] Ahmed, O.M.; Ahmed, R.G.; El-Gareib, A.W.; El-Bakry, A.M.; Abd El-Tawab, S.M. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: II-The developmental pattern of neurons in relation to oxidative stress and antioxidant defense system. *Int. J. Dev. Neurosci*. **2012**, *30*, 517-537.

[42] Zamoner, A.; Pessoa-Pureur, R. Nongenomic actions of thyroid hormones: Every why has a wherefore. *Immun. Endoc. Metab. Agents Med. Chem*., **2011**, *11*, 165-178.

[43] Martin, J.V.; Williams, D.B.; Fitzgerald, R.M.; Im, H.K.; Vonvoigtlander, P.F. Thyroid hormonal modulation of the binding and activity of the GABAA receptor complex of brain. *Neuroscience*, **1996,** *73*, 705-713.

[44] Yonkers, M.A.; Ribera, A.B. Nongenomic thyroxine regulation of voltage-gated sodium current during embryonic development. *Immun. Endocr. Metab. Agents Med. Chem*., **2011**, *11*, 157-164.

[45] Cao, J.H.; Pan, J.: Lin, H.-Y.; Davis, F.B.; Zhou, M.; Davis, P.J. L-Thyroxine attenuates pyramidal neuron excitability in rat acute prefrontal slices. *Immun. Endocr. Metab. Agents Med. Chem*. **2011**, *11*, 152-156.

[46] Caria, M.A.; Dratman, M.B.; Kow, M.-L.; Mameli, O.; Pavlides, C. Thyroid hormone action: Nongenomic modulation of neuronal ex-

citability in the hippocampus. *J. Neuroendocrinol*., **2009**, *21*, 98-107.

[47] Losi, G.; Garzon, G.; Puia, G. Nongenomic regulation of glutamatergic neurotransmission in hippocampus by thyroid hormones. *Neuroscience*, **2008**, *151*, 155-163.

[48] Traynelis, S.F.; Wollmuth, L.P.; McBain, C.J.; Menniti, F.S.; Vance, K.M.; Ogden, K.K.; Hansen, K.B.; Yuan, H. Myers, S.J., Dingeldine, R. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev*., **2010**, *62*, 405-496.

[49] Puia, G. Nongenomic modulation of ligand-gated ionotropic receptors by thyroid hormones. *Immun. Endocr. Metab. Agents Med. Chem*., **2011**, *11*, 179-187.

[50] Wiens, S.C.; Trudeau, V.L. Thyroid hormone and gamma-aminobutyric acid (GABA) interactions in neuroendocrine systems. *Comp. Biochem. Physiol. A Mol. Integr. Physiol*., **2006**, *144*, 332-344.

[51] Puia, G.; Losi, G. Thyroid hormones modulate $GABA_A$ receptor-mediated currents in hippocampal neurons. *Neuropharmacology*, **2011,** *60*, 1254-1261.

[52] Chapell, R.; Martin, J.; Machu, T.K.; Leidenheimer, N.J. Direct channel-gating and modulatory effects of triiodothyronine on recombinant $GABA_A$ receptor. *Eur. J. Pharmacol*., **1998**, *349*, 115-121.

[53] Martin, J.V.; Padron, J.M.; Newman, M.A.; Chapell, R.; Leidenheimer, N.J.; Burke, L.A. Inhibition of the activity of the native γ-aminobutyric acid A receptor by metabolites of thyroid hormones: correlations with molecular modeling studies. *Brain Res*., **2004,** *1004*, 98-107.

[54] Sarkar, P.K.; Durga, N.D.; Morris, J.J.; Martin, J.V. *In vitro* thyroid hormone rapidly modulates protein phosphorylation in cerebrocortical synaptosomes from adult rat brain. *Neuroscience*, **2005**, *137*, 125-132.

[55] Sarkar, P.K. L-Triodothyronine differentially and nongenomically regulates synaptosomal protein phosphorylation in adult rat brain cerebral cortex: role of calcium and calmodulin. *Life Sci*., **2008,** *82*, 920-927.

[56] Rami, A.; Krieglstein, J. Thyroxine attenuates hippocampal neuronal damage caused by ischemia in the rat. *Life Sci*., **1992**, *50*, 645-650.

*[57]* Lin, H.-Y.; Davis, F.B.; Luidens, M.K.; Mousa, S.A.; Cao, J.H.; Zhou, M.; Davis, P.J. Molecular basis for certain neuroprotective effects of thyroid hormone. *Front. Mol. Neurosci*., **2011**, *4*, 1-6.

*[58]* Schlenker, E.H.; Hora, M.; Liu,Y.; Redetzke, R.A.; Morkin, E.; Gerdes, A.M. Effects of thyroidectomy, $T_4$, and DITPA replacement on brain blood vessel density in adult rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **2008**, *294*, R1504-R1509.

*[59]* Davis, F.B.; Cody, V.; Davis, P.J.; Borzynski, L.J; Blas, S.D. Stimulation by thyroid hormone analogues of red blood cells $Ca^{2+}$ ATPase activity *in vitro*. Correlations between structure and biological activity in a human cell system. *J. Biol. Chem*., **1983**, *258*, 12373-12377.

*[60]* Davis, F.B.; Davis, P.J.; Blas, S.D. Role of calmodulin in thyroid hormone stimulation *in vitro* of human erythrocyte $Ca^{2+}$-ATPase activity. *J. Clin. Invest*., **1983**, *71*, 579-586.

*[61]* Mylotte, K.M.; Cody, V.; Davis, P.J.; Davis, F.B.; Blas, S.D.; Schoenl, M. Milrinone and thyroid hormone stimulate myocardial membrane $Ca^{2+}$-ATPase activity and share structural homologies. *Proc. Natl. Acad. Sci. USA*, **1985**, *82*, 7974-7978.

*[62]* Sakaguchi, Y.; Cui, G.; Sen, L. Acute effects of thyroid hormone on inward rectifier potassium channel currents in guinea pig ventricular myocytes. *Endocrinology*, **1996**, *137*, 4744-4751.

*[63]* Bichet, D.; Haass, F.A.; Jan, L.Y. Merging functional studies with structures of inward-rectifier $K^+$ channels. *Nature Rev. Neurosci*., **2003**, *4*, 957-967.

*[64]* Mendes-de-Aguiar, C.B.; Alchini, R.; Decker, H.; Alvarez-Silva, M.; Tasca, C.L.; Trentin, A.G. Thyroid hormone increases astrocyte glutamate uptake and protects astrocytes and neurons against glutamate toxicity. *J. Neurosci. Res*., **2008**, *86*, 3117-3125.

*[65]* Gompf, H.S.; Greenberg, J.H.; Aston-Jones, G.; Ianculescu, A.G.; Scanlan, T.S.; Dratman, M.B. 3-Monoiodothyronamine: the rationale for its action as an endogenous adrenergic-blocking neuromodulator. *Brain Res*., **2010**, *10*, 130-140.

*[66]* Piehl, S.; Hoefig, C.S.; Scanlan, T.S.; Kohrle, J. Thyronamines – past, present and future. *Endocr. Rev*., **2011**, *32*, 64-80.

*[67]* Doyle, K.P.; Suchland, K.L.; Ciesielski, T.M.; Lessov, N.S.; Grandy, D.K.; Scanlan, T.S.; Stenzel-Poore, M.P. Novel thyroxine derivatives, thyronamine and 3-iodo-thyronamine, induce transient hypothermia and marked neuroprotection against stroke injury. *Stroke*, **2007**, *38*, 2569-2576.

*[68]* Greeve, I.; Hermans-Borgmayer, I.; Brellinger, C.; Kasper, D.; Gomez-Isla, T.; Behl, C.; Levkau, B.; Nilsch, R.M. The human DIMINUTO/DWARF1 homolog seladin-1 confers resistance to Alzheimer's disease-associated neurodegeneration and oxidative stress. *J. Neurosci*., **2000**, *20*, 7345-7352.

*[69]* Peri, A.; Serio, M. Neuroprotective effects of the Alzheimer's disease-related gene seladin-1. *J. Mol. Endocrinol*., **2008**, *41*, 251-261.

*[70]* Benvenuti, S.; Luciani, P.; Cellai, L.; Deledda,C.; Baglioni, S.; Saccardi, R.; Urbani, S.; Francini, F.; Squecco, R.; Giuliani, C.; Vennelli, G.B; Serio, M.; Pinchera, A.; Peri, A. Thyroid hormones promote cell differentiation and up-regulate the expression of the seladin-1 gene in *in vitro* model of human neuronal precursors. *Exp. Neurol*., **2008**, *197*, 437-446.

*[71]* Belandia, B.; Latasa, M.; Villa, A.; Pascual, A. Thyroid hormone negatively regulates the transcriptional activity of the β-amyloid precursor protein gene. *J. Biol. Chem*., **1998**, *273*, 30366-30371.

*[72]* Tan, Z.S.; Vasan, R.S. Thyroid function and Alzheimer's disease. *J. Alzheimers Dis*., **2009**, *16*, 503-507.

*[73]* Costa, R.; Gonçalves, A.; Saraiva, M.L.; Cardoso, I. Transthyretin binding to A-β peptide – impact on A-β fibrillogenesis and toxicity. *FEBS Lett*., **2006**, *582*, 936-942.

*[74]* Wati, H.; Kawarabayashi, T.; Matsubara, E.; Kasai, A.; Hirasawa, T.; Kubota, T.; Harigaya, Y.; Shoji, M.; Marda, S. Transthyretin accelerates vascular Aβ deposition in a mouse model of Alzheimer's disease. *Brain Pathol*., **2009**, *19*, 48-57.

*[75]* Dell'Acqua, M.L.; Lorenzini, L.; D'Intino, G.; Sivilia, S.; Pasqualetti, P.; Panetta, V.; Paradisi, M.; Filippi, M.M; Baiguera, C.; Pizzi, M.; Giardino, L.; Rossini, P.M.; Calzà, L. Functional and molecular evidence of myelin- and neuroprotection by thyroid hormone administration in experimental allergic encephalomyelitis. *Neuropathol. Appl. Neurobiol*., **2012**, *38*, 454-470.

[76] Barreiro Arcos, M.L.; Sterle, H.; Paulazo, M.A.; Valli, E.; Klecha, A.J.; Isse, B.; Pellizas, C.G.; Farías, R.N.; Cremaschi, G.A. Cooperative nongenomic and genomic actions on thyroid hormone mediated-modulation of T cell proliferation involve up-regulation of thyroid hormone receptor and inducible nitric oxide synthase expression. *J. Cell. Physiol*., **2011**, *226*, 3208-3218.

[77] Klecha, A.J.; Genaro, A.M.; Lysionek, A.E.; Caro, R.A.; Coluccia, A.G.; Cremaschi, G.A. Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis. *Int. J. Immunopharmacol*., **2000**, *22*, 491-500.

[78] Klecha, A.J.; Genaro, A.M.; Gorelik, G.; Barreiro Arcos, M.L.; Silberman, D.M.; Schuman, M.; Garcia, S.I.; Pirola, C.; Cremaschi, G.A. Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *J. Endocrinol*., **2006**, *189*, 45-55.

[79] Marino, F.; Guasti, L.; Cosentino, M.; De Piazza, D.; Simoni, C.; Piantanida, E.; Cimpanelli, M.; Klersy, C.; Bartalena, L.; Venco, A.; Lecchini, S. Thyroid hormone regulation of cell migration and oxidative metabolism in polymorphonuclear leukocytes: clinical evidence in thyroidectomized subjects on thyroxine replacement therapy. *Life Sci*., **2006**, *78*, 1071-1077.

[80] Fabris, N.; Mocchegiani, E.; Provinciali, M. Pituitary-thyroid axis and immune system: a reciprocal neuroendocrine-immune interaction. *Horm. Res*., **1995**, *43*, 29-38.

[81] Foster, M.P.; Jensen, E.R.; Montecino-Rodriguez, E.; Leathers, H.; Horseman, N.; Dorshkind, K. Humoral and cell–mediated immunity in mice with genetic deficiencies of prolactin, growth hormone, insulin–like growth factor-1, and thyroid hormone. *Clin. Immunol*., **2000**, *96*, 140-149.

[82] Jafarzadeh, A.; Poorgholami, M.; Izadi, N.; Nemati, M.; Rezayati, M. Immunological and haematological changes in patients with hyperthyroidism or hypothyroidism. *Clin. Invest. Med*., **2010**, *33*, E271-E279.

[83] Hodkinson, C.F.; Simpson, E.E.A.; Beattie, J.H.; O'Connor, J.M.; Campbell. D.J.; Strain, J.J.; Wallace, J.M.W. Preliminary evidence of immune function modulation by thyroid hormones in healthy men and women aged 55-70 years. *J. Endocrinol*., **2009**, *202*, 55-63.

[84] Decker, T.; Stockinger, S.; Karaghiosoff, M.; Muller, M.; Kovarick, P. IFNs and STATs in innate immunity to microorganisms. *J. Clin. Invest.*, **2002**, *109*, 1271-1277.

[85] Refaeli, Y.; Van Parijs, L.; Alexander, S.I.; Abbas, A.K. Interferon-γ is required for activation-induced death of T lymphocytes. *J. Exp. Med*. **2002**, *196*, 999-1005.

[86] O'Shea, J.J.; Gadina, M.; Schreiber, R.D. Cytokine signalling in 2002: New surprises in the Jak/Stat pathway. *Cell*, **2002**, *109*(Suppl.), S121-S131.

[87] Schmelzle, T.; Hall, M.N. TOR, a central controller of cell growth. *Cell*, **2000**, *103*, 253-262.

[88] Kristof, A.S.; Marks-Konczalik, J.; Billings, E.; Moss, J. Stimulation of signal transducer and activator of transcription-1 (STAT1)-dependent gene transcription by lipopolysaccharide and interferon-γ is regulated by mammalian target of rapamycin. *J. Biol. Chem*., **2003**, *278*, 33637-33644.

[89] Rhee, S.H.; Jones, B.W.; Toshchakov, V.; Vogel, S.N.; Fenton, M.J. Toll-like receptors 2 and 4 activate STAT1 serine phosphorylation by distinct mechanisms in macrophages. *J. Biol. Chem*., **2003**, *278*, 22506-22512.

[90] Ivaska, J.; Bosca,L.; Parker, P.J. PKCε is a permissive link in integrin-dependent IFN-γ signalling that facilitates JAK phosphorylation of STAT1. *Nat. Cell. Biol*., **2003**, *5*, 363-369.

[91] Weiner, O.D.; Servant, G.; Welch, M.D.; Mitchison, T.J.; Sedat, J.W.; Bourne, H.R. Spatial control of actin polymerization during neutrophil chemotaxis. *Nat. Cell. Biol*., **1999**, *1*, 75-81.

*[92]* Marcinkievicz, C.; Vijay-Kumar, S.; McLane, M.A,; Niewiarowski, S. Significance of RGD loop and C-terminal domain of echistatin for recognition of αIIbβ3 and αVβ3 integrins and expression of ligand-induced binding site. *Blood*, **1997**, *90*, 1565-1575.

*[93]* Warner, A. ; Mittag, J. Thyroid hormone and the central control of homeostasis. *J. Mol. Endocrinol*., **2012**, 49, R29-R35.

[94] Arsanjani, R.; McGarren, M.; Bahl, J.J.; Goldman, S. Translational potential of thyroid hormone and its analogs. *J. Mol. Cell. Cardiol*., **2011**, *51*, 506-511.

[95] Kiss, E.; Jakab, G.; Kranias, E.G.; Edes, I. Thyroid hormone-induced alterations in phospholamban protein expression. Regulatory effects on sarcoplasmic reticulum $Ca^{2+}$ transport and myocardia relaxation. *Circ. Res*., **1994,** *75*, 245-251.

[96] Dillman, W. Cardiac hypertrophy and thyroid hormone signalling. *Heart Fail. Rev*., **2010**, *15*, 125-132.

[97] Siu, C.W.; Zhang, X.H.; Yung, C.; Kung, A.W.; Lau, C.P.; Tse, H.F. Hemodynamic changes in hyperthyroidism-related pulmonary hypertension: a prospective echocardiographic study. *J. Clin. Endocrinol. Metab*., **2007**, *92*, 1736-1742.

[98] Diniz, G.P.; Cramasco Takano, A.P.; Bruneto, E.; Goulart da Silva, F.; Nunes, M.T.; Barreto-Chaves, M.L. New insight into the mechanisms associated with the rapid effects of $T_3$ on AT1R expression. *J. Mol. Endocrinol*., **2012**, *49*, 11-20.

[99] Ojamaa, K. Signaling mechanisms in thyroid hormone-induced cardiac hypertrophy. *Vasc. Pharmacol*., **2010**, *52*, 113-119.

[100] Kuzman, J.A. ; O'Connell, T.D. ; Gerdes, A.M. Rapamycin prevents thyroid hormone-induced cardiac hypertrophy. *Endocrinology*, **2007**, *148*, 3477-3484.

[101] Kuzman, J.A.; Gerdes, A.M.; Kobayashi, S.; Liang, Q. Thyroid hormone activates Akt and prevents serum-starvation-induced cell death in neonatal rat cardiomyocytes. *J. Mol. Cell. Cardiol*., **2055**, *39*, 841-844.

[102] Michael, A.; Haq, S.; Chen, X.; Hsich, E.; Cui, L.; Walters, B.; Shao, Z.; Bhattacharya, K.; Kilter, H.; Huggins, G.; Andreucci, M.; Periasamy, M.; Solomon, R.N.; Liao, R.; Patten, R.; Molkentin, J.D.; Force, T. Glycogen synthase kinase 3β regulates growth, calcium homeostasis, and diastolic function in the heart. *J. Biol. Chem*., **2004**, *279*, 21383-21393.

[103] Murphy, E.; Steenbergen, C. Inhibition of GSK-3β as a target for cardioprotection, the importance of timing, location, duration and degree of inhibition. *Expert Opin. Ther. Targets*, **2005**, *9*, 447-456.

[104] Wang, X.; Proud, C.G. The mTOR pathway in the control of protein synthesis. *Physiology*., **2006**, *21*, 362-369.

[105] Chattergoon, N.N.; Giraud, G.D.; Louey, S.; Stork, P.; Fowden, A.L.; Thornburg, K.L. Thyroid hormone drives fetal cardiomyocytes maturation. *FASEB J*., **2012**, *26*, 397-408.

[106] Huntley, B.K.; Ichiki, T.; Sangaralingham, S.J.; Chen, H.H.; Burnett Jr., J.C. B-type natriuretic peptide and extracellular matrix protein interactions in human cardiac fibroblasts. *J. Cell Physiol*., **2010**, *225*, 251-255.

[107] Song, X.; Kusakari, Y.; Xiao, C.Y.; Kinsella, S.D.; Rosenberg, M.A.; Scherrer-Crosbie, M.; Hara, K.; Rosenzweig, A.; Matsui, T. mTOR attenuates the inflammatory response in cardiomyocytes and prevents cardiac dysfunction in pathological hypertrophy. *Am. J. Physiol. Cell Physiol*., **2010**, *299*, C1256-C1266.

[108] Davis, P.J.; Davis, F.B.; Lin, H.-Y. Roles and controls of mTOR in the heart. Focus on "mTOR attenuates the inflammatory response in cardiomyocytes and prevents cardiac dysfunction in pathological hypertrophy". *Am. J. Physiol. Cell. Physiol*., **2010**, *299*, C1250-C1252.

[109] Honshu, S.; Nishikawa, S.; Amano, K.; Zen, K.; Adachi, Y.; Kishita, E.; Matsui, A.; Katsume, A.; Yamaguchi, S.; Nishikawa, K.; Isoda, K.; Riches, D.W.; Matoba, S.; Okigaki, M.; Matsubara, H. Pressure-mediated hypertrophy and mechanical stretch induces IL-1 release and subsequent IGF-1 generation to maintain compensative hypertrophy by affecting Akt and JNK pathways. *Circ. Res*., **2009**, *105*, 1149-1158.

[110] Vistnes, M.; Wæhre, A.; Nygård, S.; Sjaastad, I.; Andersson, K.B.; Husberg, C.; Christensen, G. Circulating cytokine levels in mice with heart failure are etiology dependent. *J. Appl. Physiol*., **2010**, *108*, 1357-1364.

[111] Yndestad, A.; Damås, J.K.; Øie, E.; Ueland, T.; Gullestad, L.; Aukrust, P. Role of inflammation in the progression of heart failure. *Curr. Cardiol. Rep*., **2007**, *9*, 236-241.

[112] Gao, X.M.; Wong, G.; Wang, B.; Kiriazis, H.; Moore, X.L.; Su, Y.D.; Dart, A.; Du, X.J. Inhibition of mTOR reduces chronic pressure-overload cardiac hypertrophy and fibrosis. *J. Hypertens*., **2006**, *24*, 1663-1670.

[113] McMullen, J.R.; Sherwood, M.C.; Tarnavski, O.; Zhang, L.; Dorfman, A.L.; Shioi, T.; Izumo, S. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation*, **2004**, *109*, 3050-3055.

[114] Pantos, C.; Mourouzis, I.; Galanopoulos, G.; Gavra, M.; Perimenis, P.; Spanou, D.; Cokkinos, D.V. Thyroid hormone receptor alpha1 downregulation in postischemic heart failure progression: the potential role of tissue hypothyroidism. *Horm. Metab. Res*., **2010**, *42*, 718-724.

[115] Sui, L.; Wang, J.; Li, B.-M. Administration of triiodo-L-thyronine into dorsal hippocampus alters phosphorylation of Akt, mammalian target of rapamycin, p70S6 kinase and 4E-BP1 in rats. *Neurochem. Res*., **2008**, *33*, 1065-1076.

*[116]* Gnoni, G.V.; Rochira, A.; Leone, A.; Damiano, F.; Marsigliante, S.; Siculella, L. 3,5,3'-Triodo-L-thyronine induces SREBP-1 expression by non-genomic actions in human HepG2 cells. *J. Cell Physiol*., **2011**, *227*, 2388-23897.

*[117]* Abohashem-Aly, A.A.; Meng, X.; Li, J.; Sadaria, M.R.; Ao, L.; Wennergren, J.; Fullerton, D.A.; Raeburn, C.D. DITPA, a thyroid hormone analog, reduces infarct size and attenuates the inflammatory response following myocardial ischemia. *J. Surg. Res*., **2011**, *171*, 379-385.

*[118]* Hassan Talukder, M.A.; Yang, F.; Nishijima, Y.; Chen, C.-A.; Xie, L.; Mahamud, S.D.; Kalyanasundaram, A.; Bonagura, J.D.; Periasamy, M.; Zweier J.L. Detrimental effects of thyroid hormone analog DITPA in the mouse heart: increased mortality with *in vivo* acute myocardial ischemia-reperfusion. *Am. J. Physiol. Heart Circ. Physiol*., **2011**, *300*, H702-H711.

*[119]* Pingitore, A.; Iervasi, G.; Gerdes, M.A. Letter by Pingitore *et al.* regarding article, DITPA (3,5-diiodothyropropionic acid), a thyroid hormone analog to treat heart failure: Phase II trial Veterans affairs cooperative study. *Circulation*, **2010**, *121*, e240.

[120] Verge, C.F.; Konrad, D.; Cohen, M.; Di Cosmo, C.; Dumitrescu, A.M.; Marcinkowski, T.; Hameed, S.; Hamilton, J.; Weiss, R.E.; Refetoff, S. Diiodothyropropionic Acid (DITPA) in the treatment

**[121]** of MCT8 deficiency. *J. Clin. Endocrin. Metab*., **2012**, **[**Epub ahead of print].

*[122]* Vanderberg, L.N.; Hauser, R.; Marcus, M.; Olea, N.; Welshons, W.V. Human exposure to bisphenol A (BPA). *Reprod. Toxicol*., **2007**, *24*, 139-177.

*[123]* Vandenberg, L.N.; Chahoud, I.; Heindel, J.J.; Padmanabhan, V.; Paumgartten, F.J.; Schoenfelder, G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ. Health Perspect.*, **2010**, *118*, 1055-1070.

*[124]* Moriyama, K.; Tagami, T.; Akamizu, T.; Usui, T.; Saijo, M.; Kanamoto, N.; Hataya, Y.; Shimatsu, A.; Kuzuya, H.; Nakao, K. Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J. Clin. Endocrinol. Metab*., **2002**, *87*, 5185-5190.

[125] Sheng, Z.-G.; Tang, Y.; Liu, Y.-X.; Yuan,Y.; Zhao,B.-Q.; Chao, X.-J.; Zhu, B.-Z. Low concentrations of bisphenol A suppress thyroid hormone receptor transcription through a nongenomic mechanism. *Toxicol. Appl. Pharmacol*., **2012**, *259*, 133-142.

[126] Ahmed, R.G. Maternal-Fetal Thyroid Interactions. In: Intech Open Access Publisher (ISBN: 980-953-307-179-9), Chapter 5, University Campus, STeP Ri Slavka Krautzeka 83/A 51000 Rijeka, Croatia. **2012**, Pp. 125-156.

[127] Tian, J.; Li, X.; Liang, M.; Liu, L.; Xie, J.X.; Ye, Q.; Kometiani, P.; Tillekeratne, M.; Jin, R.; Xie, Z. Changes in sodium pump expression dictate the effects of ouabain on cell growth. *J. Biol. Chem*., **2009**, *284*, 14921-14929.

[128] Bassett, J.H.D.; Harvey, C.B.; Williams, G.R. Mechanisms of thyroid hormone receptor-specific nuclear and extra-nuclear actions. *Mol. Cell. Endocrinol*., **2003**, *213*, 1-11.

[129] Ma, X.M.; Blenis, J. Molecular mechanisms of mTOR-mediated translational control. *Nat. Rev. Mol. Cell Biol*., **2009**, *10*, 307-318.

[130] Barreiro Arcos, M.L; Klecha, A.J.; Genaro, A.M.; Cremaschi, G.A. Immune system modulation by thyroid axis includes direct genomic and nongenomic actions of thyroid hormones on immune cells. *Immun. Endoc. Metab. Agents Med. Chem*., **2010**, *10*, 1-10.

[131] De Mendoza, D.; Moreno, H.; Farias, R.N. Membrane cooperative enzymes. High molecular specificity for blocking action of thyroxine on triiodothyronine effect in rat erythrocyte and *Escherichia coli* systems. *J. Biol. Chem.*, **1978**, *253*, 6255-6259.

[132] Galo, M.G; Uñates, L.E.; Farías, R.N. Effect of membrane fatty acid composition on the action of thyroid hormones on ($Ca^{2+}$ + $Mg^{2+}$)-adenosine triphosphatase from rat erythrocyte. *J. Biol. Chem*., **1981**, *256*, 7113-7114.

[133] Warnick, P.R.; Davis, P.J.; Davis, F.B.; Cody, V.; Galindo, J.Jr.; Blas, S.D. Rabbit skeletal muscle sarcoplasmic reticulum $Ca^{2+}$-ATPase activity: stimulation *in vitro* by thyroid hormone analogues and bipyridines. *Biochim. Biophys. Acta* **1993**, *1153*, 184-190.

[134] Segal, J.; Ingbar, S.H. *In vivo* stimulation of sugar uptake in rat thymocytes. An extranuclear action of 3,5,3'-triiodothyronine. *J. Clin. Invest*. **1985**, *76*, 1575-1580.

[135] Segal, J.; Ingbar, S.H. Evidence that an increase in cytoplasmic calcium is the initiating event in certain plasma membrane-mediated responses to 3,5,3'-triiodothyronine in rat thymocytes. *Endocrinology*, **1989**, *124*, 1949-1955.

[136] Meng, R..; Tang, H.Y.; Westfall, J.; London, D.; Cao, J.H.; Mousa, S.A.; Luidens, M.; Hercbergs, A.; Davis, F.B.; Davis, P.J.; Lin, H.Y. Crosstalk between integrin αvβ3 and estrogen receptor-α is involved in thyroid hormone-induced proliferation in human lung carcinoma cells. *PLoS One*. **2011**, *6*(11):e27547.

[137] Morkin, E. Regulation of myosin heavy chain genes in the heart. *Circulation*, **1993**, *87*, 1451-1459.

[138] Tribulova, N.; Knezl, V.; Shainberg, A.; Seki, S.; Soukup. T. Thyroid hormones and cardiac arrhythmias. *Vasc. Pharmacol*., 2010, 52, 102-112.

[139] Shenoy, R.; Klein, I.; Ojamaa, K. Differential regulation of SR calcium transporters by thyroid hormone in rat atria and ventricles. *Am. J. Physiol*., **2001**, *281*, H1690-H1696.

[140] Kiss, E.; Jakab, G.; Kranias, E.G.; Edes, I. Thyroid hormone-induced alterations in phospholamban protein expression. Regulatory effects on sarcoplasmic reticulum $Ca^{2}$+ transport and myocardial relaxation. Circ. Res., **1994**, *75*, 245-251.

[141] Brik, H.; Shainberg, A. Thyroxine induces transition of red towards white muscle in cultured heart cells. *Basic Res. Cardiol*., **1990**, *85*, 237-246.

[142] Awais, D.; Shao, Y.; Ismail-Beigi, F. Thyroid hormone regulation of myocardial Na/K-ATPase gene expression. *J. Mol. Cell Cardiol*., **2000**, *32*, 1969-1980.

[143] Cini, G.; Carpi, A.; Mechanick, J.; Cini, L.; Camici, M.; Galetta, F.; Giardino, R.; Russo, M.A.; Iervasi, G. Thyroid hormones and the cardiovascular system: Pathophysiology and interventions. *Biomed. Pharmacother*., **2009**, *63*,742-753.

[144] Disatnik, M.H.; Shainberg, A. Regulation of beta-adrenoceptors by thyroid hormone and amiodarone in rat myocardiac cells in culture. *Biochem. Pharmacol*., **1991** ,*41*, 1039-1044.

[145] Golf, S.; Lovstad, R.; Hansson, V. Beta-adrenoceptor density and relative number of beta-adrenoceptor subtypes in biopsies from human right atrial, left ventricular, and right ventricular myocard. *Cardiovasc. Res*., **1985**, *19*, 636-641.

[146] Gross, G.; Lues, I. Thyroid-dependent alterations of myocardial adrenoceptors and adrenoceptor-mediated responens in the rat. *Naunyn Schmiedebergs Arch. Pharmacol*., **1985**, *329*, 427-439.

[147] Hammond, H.K.; White, F.C.; Buxton, I.L.; Saltzstein, P.; Brunton, L.L.; Longhurst, J.C. Increased myocardial beta-receptors and adrenergic responses in hyperthyroid pigs. *Am. J. Physiol*., **1987**, *252*, H283-H291.

[148] Hoit, B.D.; Khoury, S.F.; Shao, Y.; Gabel, M.; Liggett, S.B.; Walsh, R.A. Effects of thyroid hormone on cardiac beta-adrenergic responsiveness in conscious baboons. *Circulation*, **1997**, *95*, 592-598.

[149] Xia, H.J.; Dai, D.Z.; Dai, Y. Up-regulated inflammatory factors endothelin, NF-κB, TNF-α and iNOS involved in exaggerated cardiac arrhythmias in L-thyroxine-induced cardiomyopathy are suppressed by darusentan in rats. *Life Sci*. **2006**, *79*, 1812-1819.

[150] Shimoni, Y.; Banno, H. Alfa-adrenergic modulation of transient outward current in hyperthyroid rabbit myocytes. *Am. J. Physiol*., **1993**, *264*, H74-H77.

[151] Watanabe, H.; Ma, M.; Washizuka, T.; Komura, S.; Yoshida, T.; Hosaka, Y.; Hatada, K.; Chinushi, M.; Yamamoto, T.; Watanabe, K.; Aizawa, Y. Thyroid hormone regulates mRNA expression and currents of ion channels in rat atrium. *Biochem. Biophys. Res. Commun*., **2003**, *308*, 439-444.

[152] Watanabe, H.; Washizuka, T.; Komura, S.; Yoshida, T.; Hosaka, Y.; Hatada, K.; Aizawa, Y.; Chinushi, M.; Yamamoto, T.; Ma, M.; Watanabe, K. Genomic and nongenomic regulation of L-type calcium channels in rat ventricle by thyroid hormone. *Endocr. Res*., **2005**, *31*, 59-70.

[153] Stock, A.; Sies, H. Thyroid hormone receptors bind to an element in the connexin43 promoter. *Biol. Chem*., **2000**, *381*, 973-979.

[154] Almeida, N.A.; Cordeiro, A.; Machado, D.S.; Souza, L.L.; Ortiga-Carvalho, T.M.; Camposde-Carvalho, A.C.; Wondisford, F.E.; Pazos-Moura, C.C. Connexin40 messenger ribonucleic acid is positively regulated by thyroid hormone (TH) acting in cardiac atria via the TH receptor. *Endocrinology*, **2009**,*150*, 546-554.

[155] Sunagawa, M.; Yamakawa, M.; Shimabukuro, M.; Higa, N.; Takasu, N.; Kosugi, T.; Electrophysiologic characteristics of atrial myocytes in levo-thyroxine-treated rats. *Thyroid*, **2005**, *15*, 3-11.

[156] Hamasaki, Y.; Shinohara, O.; Ishida, H.; Hayashi, Y.; Nakazawa, H. Decreased protein kinase C-epsilon expression in hypetrophied cardiac ventricles induced by triiodothyronine treatment in the rat. *Life Sci*., **2000**, *67*, 1859-1868.

[157] Mitasikova, M.; Lin, H.; Soukup, T.; Imanaga, I.; Tribulova, N. Diabetes and thyroid hormones affect connexin-43 and PKC-ε expression in rat heart atria. *Physiol. Res*., **2009**, *58*, 211-217.

[158] Rybin, V.; Steinberg, S.F. Thyroid hormone represses protein kinase C isoform and activity in rat cardiac myocytes. *Circ. Res*., **1996**, *79*, 388-398.

[159] Incerpi, S.; De Vito, P.; Luly, P.; Spagnuolo, S.; Leoni, S. Short-term effects of thyroid hormones and 3,5-diiodothyronine on membrane transport systems in chick embryo hepatocytes. *Endocrinology*, **2002**, *143*, 1660-1668.

[160] Incerpi, S.; Scapin, S.; D'Arezzo S.; Spagnuolo. S.; Leoni S. Short-term effect of thyroid hormone in prenatal development and cell differentiation. *Steroids* **2005**, *70*, 434-443,

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