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# Nongenomic Signaling Pathways Triggered by Thyroid Hormones and Their Metabolite 3-Iodothyronamine on the Cardiovascular System

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Thyroid hormones play a wide range of important physiological activities in almost all organism. As changes in these hormones levels—observed in hypothyroidism and hyperthyroidism—promote serious derangements of the cardiovascular system, it is important to know their mechanisms of action. Although the classic genomic actions which are dependent on interaction with nuclear receptors to modulate cardiac myocytes genes expression, there is growing evidence about $T_3$ and $T_4$-triggered nongenomic pathways, resulted from their binding to plasma membrane, cytoplasm, or mitocondrial receptors that leads to a rapidly regulation of cardiac functions. Interestingly both actions converge to amplify thyroid hormone effects on cardiovascular system. $T_3$ and $T_4$ nongenomic actions modify inotropic and chronotropic effects, cardiac action potential duration, cardiac growth, and myocyte shape by protein translation through protein kinases-dependent signaling cascades, which include PKA, PKC, PI3K, and MAPK, and changes on ion channels and pumps activity. In respect to the decreased systemic vascular resistance seen in hyperthyroidism, $T_3$ appears to activate NOS or ATP-sensitive $K^+$ channels. In addition, a novel biologically active $T_4$-derived metabolite has been described, 3-iodothyronamine, $T_1AM$, which also acts through membrane receptors to mediate nongenomic cardiac effects. This metabolite influences the physiological manifestations of thyroid hormone actions by inducing opposite effects from those stimulated by $T_3$ and $T_4$, such as negative inotropic and chronotropic effects. Therefore, beyond genomic and nongenomic effects of thyroid hormones, it is crucial for there to be an equilibrium between $T_3$ or $T_4$ and $T_1AM$ levels for maintaining cardiac homeostasis.

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The thyroid hormones, triiodothyronin ($T_3$) and thyroxine ($T_4$), play crucial physiological roles in nearly all organism tissues already studied (Oppenheimer et al., 1987). Although $T_4$ is the major secretion product of thyroid, $T_3$ is responsible for most of the biological effects ascribed to thyroid hormones, including the negative-feedback system over the thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) secretion by the hypothalamus and pituitary, respectively (Shupnik and Ridgway, 1987).

Two specific iodothyronine deiodinases (D1 and D2) found in different tissues, convert $T_4$ to $T_3$ by 5′-monodeiodination (Köhrle, 2000; Sabatino et al., 2005). This wide distribution leads to controversial data in the literature about the main $T_3$ source. While thyroid is described as the major source of circulating $T_3$ in rats (Chanoine et al., 1993), peripheral tissues are considered the main source of $T_3$ in humans, being 80% provided by D1 activity (Engler and Burger, 1984; Sabatino et al., 2005). Among the human tissues, kidney, liver, adrenal gland, intestine, skeletal muscle, thyroid, and heart display a significant amount of D1, indicating their critical role in thyroid hormone homeostasis and consequently in the thyroid hormone action (Sabatino et al., 2001, 2005; Gereben et al., 2008). In the heart, the occurrence of both deiodinases reflects the necessity of supplying this organ with $T_3$, evidencing its relevance to cardiac function (Sabatino et al., 2001, 2005). In human serum, $T_4$ levels are about 100 times higher than $T_3$. Although it is difficult to measure $T_3$ and $T_4$ content in human hearts, there are a few reports in the literature showing that $T_3$ cardiac concentrations in rats and mice range from 4.5 to 6 ng/tissue g, while $T_4$ cardiac concentrations range from 1.6 to 2 ng/tissue g (Trivieri et al., 2006; Liu et al., 2008). This slight difference between $T_3$ and $T_4$ levels in hearts when compared to serum, demonstrates the importance of $T_4$ local conversion to $T_3$. Indeed, some heart diseases induce local tissue hypothyroidism by altering cardiac thyroid hormone metabolism via the D1 and D2 deiodinases, whose activities appear to be exceptionally low in the heart (Gereben et al., 2008). Besides D1 and D2, there is a third monodeiodinase, D3, that catalyzes the $T_4$ conversion to reverse-$T_3$ ($rT_3$) and the $T_3$ conversion to diiodothyronine ($T_2$) by 5-monodeiodination and thereby terminating thyroid hormone action (Sabatino et al., 2005; Gereben et al., 2008).

Among the different tissues, $T_3$ has pivotal effects on the heart and cardiovascular system, acting as an important regulator of cardiac function and cardiovascular hemodynamics through its direct action on the cardiac myocytes, vascular smooth muscle cells (VSMCs), and endothelium (Klein and Ojamaa, 2001; Biondi and Klein, 2004). This affirmation is based on the fact that the increased or decreased thyroid hormone levels exert significant derangements on the cardiovascular system. While hyperthyroidism leads to an enhanced left ventricular systolic and diastolic function, increased prevalence of supraventricular tachyarrhythmias, ventricular hypertrophy,

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shortened action potential duration, hyperdynamic circulation with increased cardiac output, heart rate, pulse pressure and blood pressure and decreased vascular peripheral resistance, opposite changes are related to hypothyroidism (Klein and Ojamaa, 2001; Biondi and Klein, 2004). Most of these processes are described to be mediated by the classic genomic mechanism of steroid hormone action that takes days or months to be effective, once dependent on gene transcription. However, recent studies have shown that $T_3$ also acts in minutes to hours leading to changes in cardiac inotropism, chronotropism, and hypertrophy through receptors placed in the membrane and at other subcellular sites (Rudinger et al., 1984; Mylotte et al., 1985; Craelius et al., 1990; Sakaguchi et al., 1996; Vassy et al., 1997; Incerpi et al., 1999; Sun et al., 2000; Quesada et al., 2002; Schmidt et al., 2002; Wang et al., 2003; Kuzman et al., 2005; Kenessey and Ojamaa, 2006; Storey et al., 2006; Zinman et al., 2006).

Since 1990, few works have been published concerning the nongenomic cardiac effects. With a better understanding of nonnuclear receptors, second messengers and effector proteins involved on thyroid hormones nongenomic actions in the cardiovascular system, there would be a greater chance to find new targets for drugs to improve the cardiac function, principally on hyper or hypothyroidism. Thus, this review will briefly comment on the classic genomic effects of thyroid hormones on the cardiovascular system and will focus on their nongenomic effects in order to put together the important findings in this field, to stimulate further studies in this area.

## General Aspects of Genomic Signaling Pathways

Classically, $T_3$ enters the cell membrane in a simple diffusion way, as described for the steroidal hormones (Farach-Carson and Davis, 2003), or via specific transport proteins such as an energy-dependent carrier that partially depends on the $Na^+$ gradient (Everts et al., 1996), a $Na^+$-independent organic anion-transporting polypeptide (OATP; Pizzagalli et al., 2002) or a $Na^+$-independent monocarboxylate transporter 8 (MCT8; Friesema et al., 2003). In the nucleus, $T_3$ interacts with the nuclear thyroid receptors (TR) $\alpha$1, $\alpha$2, $\beta$1, $\beta$2, and $\beta$3 (the last one is confined to the hypothalamic/pituitary axis where it mediates the negative regulation of TSH transcription) (Bassettt et al., 2003). The hormone–receptor complex recognizes specific thyroid responsive elements (TREs) in the promoters region of $T_3$-target genes, which, in combination with recruited cofactors, act as specific transcriptional activators (linked to TR$\alpha$1) or repressors (linked to TR$\alpha$2; Brent, 1994). The TRs can bind to TREs as homodimers or heterodimers, commonly with retinoid X receptor (RXR) (Brent, 1994; Bassettt et al., 2003; Farach-Carson and Davis, 2003).

The TREs and the related target genes could be positively or, to a lesser extent, negatively regulated by $T_3$ (Miller et al., 2001). In the first case, unligand TR shows a basal transcriptional repression of the target gene expression due to the recruitment of co-repressor proteins (like NCoR and SMRT), while $T_3$ binding leads to a conformational change in the receptor structure, dissociation of the co-repressors, and recruitment of co-activators (p160/SRC-1, p300/CBP and Trip230) (Rosenfeld and Glass, 2001; Bassettt et al., 2003; Farach-Carson and Davis, 2003). In the case of negative regulated TREs, unligand TR mediate constitutive gene expression and ligand binding ($T_3$) induces active repression of gene transcription via mechanisms that have not been precisely clarified yet (Bassettt et al., 2003).

$T_3$ responsiveness can further be altered by post-translational modifications of TRs, such as phosphorylation (Lin et al., 1992; Tzagarakis-Foster and Privalsky, 1998). It has been already shown that the TRs binding to TREs in the absence of $T_3$ could be triggered by kinases, suggesting that phosphorylation could participate in TR-mediated regulation of gene transcription (Lin et al., 1992; Tzagarakis-Foster and Privalsky, 1998).

### $T_3$-altered gene expression mediate changes in inotropic action, action potential duration and cardiac output

Myocyte contractile action depends on several factors, whose changes modify the cardiac systolic and diastolic function and consequently alter the cardiac output. Among these factors there are the velocity of fiber shortening, changes in the intracellular concentration of some ions like $Ca^{2+}$, $Na^+$, and $K^+$ and the sympathetic tonus (Bassettt et al., 2003; Farach-Carson and Davis, 2003). Therefore, alteration of cardiovascular system-specific gene expression caused by thyroid hormones and/or its metabolite activity (which will be discussed in the nongenomic action section) could contribute to changes on cardiac contractile function (Balkman et al., 1992; Bassettt et al., 2003; Farach-Carson and Davis, 2003).

In this view, $T_3$ treatment stimulates transcription of the $\alpha$-MHC gene and inhibits $\beta$-MHC mRNA production both in the heart, leading to increased $\alpha/\alpha$ myosin isoform and enhanced cardiac contractility ($\alpha/\alpha$ myosin isoform has higher ATPase activity and increased velocity of fiber shortening than $\beta/\beta$ myosin isoform) (Balkman et al., 1992; Danzi et al., 2003). $T_3$ upregulates the expression of the principal determinant of muscle contractility, SERCA2 (the main responsible for removing calcium from the cytosol) and downregulates expression of phospholamban (an inhibitory SERCA regulator), suggesting that induction of this ATPase may account for $T_3$ enhancement of cardiac output by relaxing the heart more rapidly (lusitropic effect) (Kiss et al., 1994; He et al., 1997; Shenoy et al., 2001; Pantos et al., 2007a).

The $\beta$1R number is also altered in the heart in the presence of $T_3$. It may be responsible, at least in part, for the enhanced catecholamine sensitivity of $\beta$1R-coupled cardiac responses in the hyperthyroidism state (Williams et al., 1977; Tse et al., 1980). Decreased levels of Gi in ventricular cells exposed to $T_3$ may also contribute to the increased $\beta$-adrenergic sensitivity (Carvalho-Bianco et al., 2004).

Alterations in ion transporters expression by $T_3$ are also described. These includes upregulation (Kim et al., 1987) or downregulation of L-type $Ca^{2+}$ channels, despite an increased $Ca^{2+}$ current (Watanabe et al., 2005), upregulation of ($Na^+/K^+$) ATPase (Philipson and Edelman, 1977; Orlowski and Lingrel, 1990; Kamitani et al., 1992; Forini et al., 2004), and controversial regulation of $K^+$ channels with downregulation of a specific subunit of the delayed rectifier $K^+$ channels (Shimoni, 1999) in contrast with upregulation of voltage-gated $K^+$ channels (Fiset et al., 1997). In addition, opposite results shows that $T_3$ increase the $Na^+/Ca^{2+}$ exchanger expression (Hojo et al., 1997; Shenoy et al., 2001) while, in hypothyroidism, an increase was observed in mRNA and protein levels of the $Na^+/Ca^{2+}$ exchanger (Boerth and Artman, 1996).

### $T_3$-altered genes expression mediate cardiac hypertrophy

Thirty years have passed since Sanford et al. (1978) reported that $T_3$-induced cardiac hypertrophy is accompanied by a predominant increase in protein synthesis with a minor contribution from reduced protein degradation.

There are also indirect $T_3$-induced hypertrophy mechanisms that require activation of other systems, like the renin-angiotensin system (RAS) (Diniz et al., 2007). In this view, it was reported that the cardiac hypertrophy observed in hyperthyroid groups is related to enhanced cardiac levels of renin and angiotensin II (Ang II) without involving the sympathetic nervous system (Kobori et al., 1997) and with the local RAS playing the primary role in the development of

hyperthyroidism-induced cardiac hypertrophy (Kobori et al., 1999). It seems that increases in TGFβ1 levels are involved, at least in part, in the Ang II-mediated $T_3$-induced cardiac hypertrophy (Diniz et al., 2007). In agreement with these results, treatment with angiotensin type 1 receptor ($AT_1$) antagonists or angiotensin converting enzyme (ACE) inhibitors has been shown to attenuate thyroid hormones-promoted cardiac hypertrophy (Kobori et al., 1997; Pantos et al., 2005). Recent evidence indicates that intracellular $Ca^{2+}$ overload may be the link between RAS and thyroid hormones (Su et al., 2008).

In addition, it has been reported that new components of the RAS, which act as a counter-regulatory mechanism for the main axis ACE/Ang II/ $AT_1$, such as Angiotensin-(1–7) [Ang-(1–7)], its receptor Mas and angiotensin-converting enzyme-2 (ACE2), also have their levels altered accordingly to the thyroid status. It was observed that cardiac levels of Ang-(1–7) and Mas are increased in thyroid hormone-induced cardiac hypertrophy, suggesting that ACE2/Ang-(1–7)/Mas may be acting as a counter-regulatory mechanism during the myocardial hypertrophy stimulated by thyroid hormones (Barreto-Chaves et al., 2010).

## General Vision of Nongenomic Signaling Pathway

The mechanism of action of thyroid hormones has been described to begin in the cell nucleus and to require participation of specific receptor proteins in the nuclear compartment. Otherwise, some actions of thyroid hormones have now been assumed to involve novel extranuclear mechanisms in a variety of cells (Bassettt et al., 2003; Farach-Carson and Davis, 2003). $T_3$ actions that: (i) do not require the formation of a nuclear complex containing $T_3$ and TR, (ii) are completely independent of new mRNA transcription and protein synthesis, and (iii) occur within seconds to minutes, are refered as nongenomic effects (Farach-Carson and Davis, 2003).

The nongenomic actions of thyroid hormones are dependent on the activation of plasma membrane receptor(s) or subcellular located receptor(s), mainly found at the cytoplasm, nucleus, and mitochondria (Bassettt et al., 2003; Farach-Carson and Davis, 2003; Saelim et al., 2004). Although the cell surface receptor(s) for thyroid hormone has not been sequenced or cloned yet, it has been suggested that this receptor could be distinct from the nuclear one (Bassettt et al., 2003; Farach-Carson and Davis, 2003). It appears to be located on integrin αVβ3, a heterodimer protein that interacts both with extracellular matrix proteins and thyroid hormones (Bergh et al., 2005). The Arg-Gly-Asp (RGD) recognition site in this integrin is critical for the thyroid hormone binding (Bergh et al., 2005; Cody et al., 2007). Other molecules like stilbene resveratrol (Lin et al., 2006, 2008) and dihydrotestosterone (Lin et al., 2009a) also bind to this integrin in the vicinity of the RGD domain (Davis et al., 2009). At first, it was believed that there was a single site capable for binding $T_3$ or $T_4$ in the integrin receptor at or near the RGD domain. However, Lin et al. (2009b) suggested that the hormone-binding domain is constituted of two binding sites. One site solely binds $T_3$ and activates the phosphatidylinositol 3-kinase (PI3K) pathway leading to cytoplasm-to-nucleus shuttling of TRα1 and into transcription of the hypoxia-inducible factor-1α gene. The second site binds both $T_3$ and $T_4$ and appears to trigger PKC, Ras, Raf1, and MEK, resulting in tyrosine phosphorylation, activation, and nuclear translocation of MAPK (ERK1/2) that in turns: (i) interacts with TR, resulting in a MAPK/TR complex that binds, and phosphorylates p53 leading to a decreased transcriptional activity of this suppressor protein oncogene (Shih et al., 2001; Bassettt et al., 2003; Lin et al., 2007, 2008); and (ii) phosphorylates STAT1, STAT3 (Lin et al., 1999; Bassettt et al., 2003), and the cytoplasmic TRβ1 (Cao et al., 2009) that translocate to nucleus for activation of gene transcription (Lin et al., 1999; Bassettt et al., 2003; Cao et al., 2009; Davis et al., 2009). These MAPK (ERK1/2)-mediated nongenomic effects of thyroid hormones induce angiogenesis (Davis et al., 2004; Bergh et al., 2005) and tumor cell proliferation (Hiroi et al., 2006; Lin et al., 2009b), while the PI3K pathway is not linked to cell proliferation, but to trafficking of different intracellular proteins (Lin et al., 2009b). The translocation of cytoplasmic TRα1 and TRβ1 to nucleus suggests that genomic and nongenomic effects of thyroid hormones may act together to potentiate each other—this point will be better discussed in the next section. In hepatocytes, $T_4$ is also thought to induce DAG formation in a biphasic manner: first dependent on the PLC activity, and subsequentialy through a PKC-dependent activation of PLD (Kavok et al., 2001).

In rat alveolar epithelial cells, there is a cytoplasmic TRβ1, which interacts with $T_3$, but apparently not with $T_4$, to activate the PI3K pathway. As a result, a stimulation of the ($Na^+$/$K^+$)ATPase activity is detected, accompanied by an augment of its insertion at the plasma membrane (Lei et al., 2004). Recently, it was shown that MAPK (ERK1/2) activation precedes the stimulation of the PI3K pathway (Lei et al., 2008) suggesting the involvement of the cell integrin αVβ3 receptor once it is capable of activating both MAPK (ERK1/2) and PI3K (Lin et al., 2009b).

In neurons and astroglial cells, there was also demonstrated a cytoplasmatic truncated thyroid hormone receptor isoform (TRα-derived polypeptide—TRΔα1) that has $T_4$ or reverse $T_3$ ($r T_3$) as ligands and is insensitive to $T_3$. The result of this binding is a remodeling of the intracellular actin pattern that supports cell motility, required for the normal development of the central nervous system (Leonard et al., 1994; Farwell et al., 2006).

### $T_3$-mediated nongenomic signaling pathways involved in inotropic action, action potential duration and cardiac output

Nongenomic actions of $T_3$ in the heart (summarized in Fig. 1) have been described on membrane ion transporters localized at the plasma membrane, in the cytoplasm and in cellular organelles, through mechanisms that involve distinct signaling cascades with further activation of protein kinases (Bassettt et al., 2003; Farach-Carson and Davis, 2003).

Schmidt et al. (2002) showed that $T_3$ enhanced myocardial contractility and reduced systemic vascular resistance in normal adult males within 3 min, evidencing a nongenomic mechanism of thyroid hormone in vivo. In addition, it was demonstrated that thyroid hormones restore to basal, elevated intracellular calcium concentration, and also delayed its intracellular elevation. These actions lead to an improvement of myocardium function and to the prevention of intracellular calcium overload through the activation of β-adrenergic receptors coupled to the AMPc/PKA signaling pathway that results in the enhancement of SERCA2 activity (Zinman et al., 2006). Together with the fact that $T_3$ upregulates the expression of SERCA2 and downregulates the expression of phospholamban, it seems that this is an example of interface between genomic and nongenomic actions of $T_3$ that converge to amplify its effects on cardiac contractility and output.

In accordance, a study by Mylotte et al. (1985) demonstrated that the myocardial plasma membrane $Ca^{2+}$-ATPase (responsible for $Ca^{2+}$ extrusion on the plasma membrane) is also involved in the thyroid hormone-enhanced cardiac output since this pump is activated in response to $T_4$ through a PKC-dependent transducing pathway (Mylotte et al., 1985; Enyedi et al., 1996). Besides the greater plasma membrane $Ca^{2+}$-ATPase-mediated $Ca^{2+}$ efflux, there is also an increased $Ca^{2+}$ influx due to an amplified L-type $Ca^{2+}$

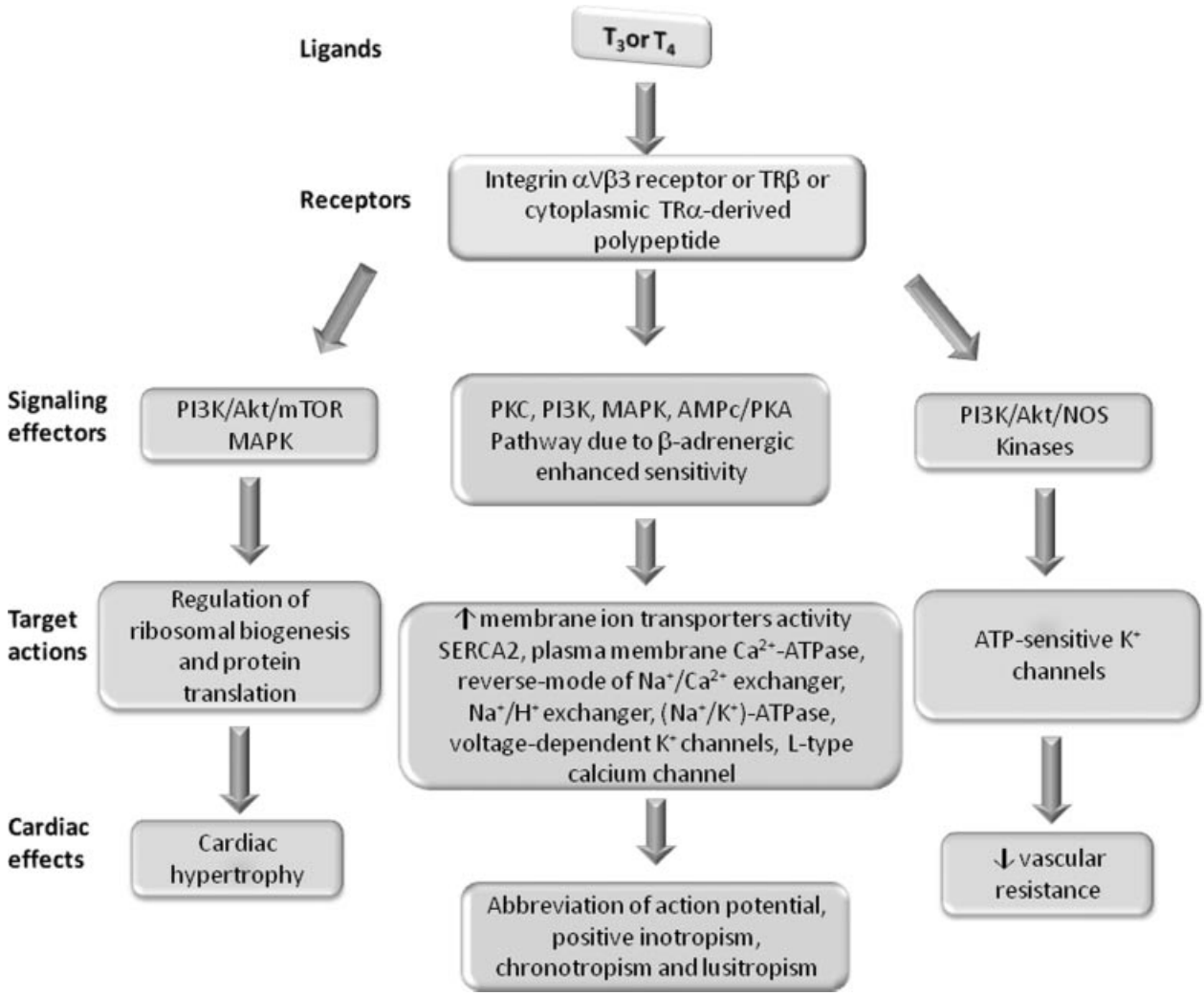


**Fig. 1. A scheme of the thyroid hormones, $T_3$ and $T_4$, nongenomic cardiac actions, including possible receptors for binding, the signaling effectors involved, the target actions and the final cardiac effects.**

current through the activation of the adenylate cyclase cascade by thyroid hormones (Watanabe et al., 2005). By the way, $T_3$ could enhance the cardiac contractility through an indirect nongenomic manner, which is dependent on action potential duration. Acute $T_3$ exposure increases $Na^+$ influx via tetrodotoxin-sensitive inward $Na^+$ current and thereby stimulates the reverse-mode of $Na^+/Ca^{2+}$ exchanger (Wang et al., 2003). This action results in increasing the intracellular $Ca^{2+}$ content. Although this leads to a positive inotropic effect, it can also contribute to the development of $Ca^{2+}$-mediated atrial tachy-dysrythmias that were usually attributed to chronic genomic effects of elevated $T_3$ on the atrial muscle (Wang et al., 2003). Recently, it was observed that thyroid hormone administration immediately after the induction of myocardial infarction in rats improves cardiac contractility by reducing the expression of PKCε and PKCα and increasing the expression of HSP70 in the myocardium (Pantos et al., 2007a). As PKCε has been involved in the inhibition of the L-type $Ca^{2+}$ channel in hearts (Hu et al., 2000), its suppression by thyroid hormones (Pantos et al., 2007a; Mitasikova et al., 2009) would potentiate the activation of $Ca^{2+}$ channels.

Acute effects of $T_3$ were also observed in $Na^+$ currents on ventricular myocytes. The major effect seems to be a slowing of current inactivation, where PKC is suggested to be involved (Harris et al., 1991; Huang et al., 1999) as well as pertussis toxin-sensitive G protein (Sen et al., 2002). This action may account for the prolongation of the action potential that is observed after acute addition of $T_3$ (Craelius et al., 1990) and may underlie once more, the propensity for arrhythmias in hyperthyroidism. However, Sakaguchi et al. (1996) found that $T_3$ acutely infused to guinea pig ventricular cells enhances the inward rectifier $K^+$ current. If this effect persists, it would in part explain the abbreviation of the action potential seen in chronic hyperthyroid conditions (Felzen et al., 1989). This shortened action potential duration could also be attributed to an acute $T_3$ effect on voltage-dependent $K^+$ channels (Sun et al., 2000). It seems that the cytoplasmic receptor TRβ2 regulates the activity of these $K^+$ channels in the plasma membrane through a PI3K/Rac GTPase-dependent mechanism (Storey et al., 2002, 2006). In other words, it means that this receptor is effective to transduce a rapid signaling in a way that does not require the nucleus or binding to DNA (Storey et al., 2006).

$T_3$ acutely stimulates the $Na^+/H^+$ exchanger in rabbit heart (Doohan et al., 1997), an effect that could be dependent on the MAPK (ERK1/2) (Gekle et al., 2001; D'Arezzo et al., 2004), on the PKC activity as seen in rat skeletal muscle (Incerpi et al., 1999) or even on PI3K activity as observed in $Na^+/H^+$ exchanger in chick embryo hepatocytes (Incerpi et al., 2002). This $T_3$-activated $Na^+$ uptake through $Na^+/H^+$ exchanger seems to negatively contribute to myocyte survival since inhibition of this antiporter in the heart has been shown to improve myocyte survival in the setting of ischemia (Avkiran, 1999). However, conversely to the $Na^+$ uptake by $Na^+/H^+$ exchanger, Doohan et al. (1997) have observed that $T_3$ enhances $(Na^+/K^+)$ATPase activity and $Na^+$ efflux, which would avoid the increase in intracellular $Na^+$ concentrations, as well as its deleterious effects. The $T_3$-induced stimulation of $(Na^+/K^+)$ATPase activity occurs both by PI3K/PKB pathway (Lei et al., 2004) and by MAPK (Lei et al., 2008) in adult rat epithelial alveolar cells, and therefore this cascade may also be activated on cardiomyocytes. The positive genomic (upregulation of $(Na^+/K^+)$ATPase expression) and nongenomic effect of $T_3$ on the $Na^+$ pump in myocytes can be considered synergistic effects that, once more, show the interface between genomic and nongenomic actions.

### $T_3$-mediated nongenomic signaling pathways to change cell surface proteins

On $T_3$-altered gene expression section it was reported that the increase in $\beta$1R number in the plasma membrane was a result of protein synthesis. However, other studies using chick embryonic ventricular myocytes showed that the $\beta$1R density was slightly enhanced only 2 h after $T_3$ addition, this action being blocked by colchicines, which suggests the involvement of microtubules and that $T_3$ is also able to enhance the myocyte sensitivity to catecholamines by nongenomic mechanisms (Vassy et al., 1997).

### $T_3$-mediated nongenomic signaling pathways involved in hypertrophy, cardiac development and remodeling

The activation of the PI3K/Akt/mTOR pathway by IGF-1 has been implicated in determining heart size and physiologic cardiac growth (Fujio et al., 2000). In this context, Kuzman et al. (2005) demonstrated that $T_4$ treatment promotes activation of the Akt signaling pathway in a rat isolated myocyte preparation that includes phosphorylation of mTOR and eNOS. Moreover, Kenessey and Ojamaa (2006) also showed that a cardiomyocyte culture treated with $T_3$ rapidly activates PI3K (which appeared to be linked with the cytoplasmic TR$\alpha$1 through its p85$\alpha$ subunit, independently of $T_3$ binding) leading to Akt phosphorylation that, in turns, translocates to the nucleus and promoted mTOR phosphorylation. As mTOR is important to regulate ribosomal biogenesis and protein translation, the signaling pathway described in these studies may underlie at least one of the nongenomic mechanisms by which $T_3$ regulates physiologic cardiac growth (Fig. 1). In accordance, it was observed that mutations of TRs reported in many human cancers allow a more effective binding of the mutated receptor to p85$\alpha$ subunit of PI3K, enhancing the activation of PI3K signaling that consequently results in increased cell proliferation, motility, migration, and metastasis (Furuya et al., 2009). A PI3K/Akt dependent cascade is also involved in the $T_3$ role of switching titins (giant sarcomere proteins involved in myocardial distensibility and mechanosignaling) during cardiac development (Krüger et al., 2008). It was recently demonstrated that the $T_3$-induced activation of PI3K/Akt/mTOR and the $T_3$-induced cardiomyocyte hypertrophy were completely abolished by using $AT_1$ receptor siRNA (Diniz et al., 2009). These results reinforce the contribution of the RAS in mediating the hypertrophy promoted by $T_3$.

In rat neonatal cardiomyocytes, $T_3$ was shown to induce changes on myocyte shape and geometry improving remodeling in failing hearts through the ERK pathway (Fig. 1). This cascade did not require alterations of cell size or protein synthesis and was independent of changes in the levels of phospho-Akt and phospho-p38 MAPK (Pantos et al., 2007b).

The interface between nongenomic and genomic actions of thyroid hormones is also observed for cardiomyocyte growth once it was recently reported that the long-term effects of thyroid hormones on the expression of SERCA2, $\alpha$- and $\beta$-MHC and cardiomyocyte growth were reverted by using specific inhibitors of ERK1/2, p38-MAPK, PKC$\delta$, and Akt (Iordanidou et al., 2010).

### $T_3$-altered effects in the heart vasculature

The increase in cardiac output observed in hyperthyroidism is a result of the combination of the increased cardiac function (inotropic and chronotropic effects) and changes in the cardiovascular hemodynamics (lowered systemic vascular resistance and increased blood volume) (Klein and Ojamaa, 2001). Vargas et al. (1995) and Napoli et al. (2007) suggested that reduced vascular resistance could be secondary to an increased vascularity and/or to alterations in the vascular control mechanisms that favor vasodilatation.

The precise mechanism of the decline in systemic vascular resistance in hyperthyroidism is not known and there are some contradicting results on this subject. Some authors suggest the involvement of nitric oxide (NO), as nitric oxide synthase (NOS) activity seems to be upregulated in tissues primarily related to blood pressure control in hyperthyroid rats (Quesada et al., 2002) and in VSMCs of rat aortas when exposed to $T_3$ (Carrillo-Sepúlveda et al., 2010). Indeed, it was recently reported that the PI3K/Akt-signaling pathway plays a role in $T_3$-induced NO production by VSMCs (Carrillo-Sepúlveda et al., 2010) and by endothelial cels (Hiroi et al., 2006) (Fig. 1). Conversely, hypothyroidism, with reduced cardiac output, is associated with impaired endothelium-dependent vasodilatation (Delp et al., 1995; Moreno et al., 2003; Taddei et al., 2003) and reduced aortic NOS activity (Quesada et al., 2002).

It is also known that endothelial shear stress regulates the expression of eNOS (Xiao et al., 1997) and maybe its increase as a result of the augmented cardiac output, leads to a raise in NO production. There are other factors that could contribute to increased NOS activity, including a direct nongenomic effect of $T_3$ (Chakrabarti and Ray, 2000) or the increased release of vasoactive molecules such as Ang II (Hennington et al., 1998) or endothelin (Hirata et al., 1995), which are increased in hyperthyroid rats, and are known to stimulate NO production (Marchant et al., 1993; Singh et al., 1994).

There are evidences that the RAS plays an important role in the development of hypertension derived from $T_4$ chronic treatment and NOS inhibition, once losartan ($AT_1$ antagonist) significantly attenuated this type of hypertension (Rodriguez-Gomez et al., 2003). In a compensatory way, it seems that $T_3$ may act as a negative regulator of the RAS, since it indirectly (Ichiki et al., 1998) and directly (Fukuyama et al., 2003) inhibits vascular $AT_1$ expression.

Nevertheless, these studies, Yoneda et al. (1998) and Ojamaa et al. (1996) showed that $T_3$-induced vasodilatation is independent of NO production. Furthermore, the participation of cAMP and cGMP on vascular smooth muscle cell relaxation was also excluded (Ojamaa et al., 1996). In isolated skeletal muscle, glibenclamide decreased the effect of $T_3$, implicating ATP-sensitive $K^+$ channels in the mechanism of hormonal action (Park et al., 1997) (Fig. 1). Thus, it seems suitable to postulate that specific ion channels could be involved in the $T_3$-decreased coronary and systemic vascular resistance, probably

via protein kinases that have been shown to be involved in $T_3$ or $T_4$ nongenomic responses.

### 3-Iodothyronamine ($T_1AM$)

Besides, $T_4$ deiodination to produce $T_3$, it was recently discovered that deiodination and decarboxylation of $T_4$ could generate a biologically active metabolite, $T_1AM$, whose actions are opposite to those induced by $T_4$ or $T_3$ (Scanlan et al., 2004). The D1, D2, and D3 deiodinases involvement in thyronamine biosynthesis has already been described (Piehl et al., 2008), revealing once again their participation in thyroid hormone balance that would implicate in thyroid hormones actions (Gereben et al., 2008). This endogenously formed metabolite was found in heart, brain, liver, and blood and activates a novel aminergic system coupled to the trace amine-associated receptor (TAAR), a member of the orphan GPCR-related family (Scanlan et al., 2004; Zucchi et al., 2006; Frascarelli et al., 2008). As there are nine TAAR subtypes (Lindemann et al., 2005) and at least five were detected in rat heart tissue (Chiellini et al., 2007), some experiments suggest that iodothyronamine effects are not mediated solely by TAAR1 (Chiellini et al., 2007; Frascarelli et al., 2008) and possibly are also mediated by TAAR8a, since it has already been shown that this receptor is one of the TAAR subtypes preferentially expressed in the cardiac tissue (Zucchi et al., 2006).

As this metabolite does not interact with nuclear thyroid hormone receptors (Chiellini et al., 1998) but interact with TAAR1, it has been hypothesized that cardiac $T_1AM$ influences are also mediated via nongenomic effects (Fig. 2). It has been demonstrated that $T_1AM$ reduces cardiac output, heart rate, systolic pressure, and coronary flow in isolated heart preparation within minutes (Chiellini et al., 2007). These effects seem to be a result of the reduced amplitude and duration of the calcium transient due to: (i) the abolishment of the L-type $Ca^{2+}$ current facilitation by membrane depolarization, (ii) the reduced ryanodine binding in the sarcoplasmatic reticulum (SR) calcium release channel, an effect that does not appear to be the result of a direct interaction between $T_1AM$ and the SR channel, (iii) the significant increase in $Ca^{2+}$ leak, determined with the SR channel closed and, (iv) the action potential prolongation by reducing the transient outward current ($I_{to}$) and a background current ($I_{k1}$; Ghelardoni et al., 2009).

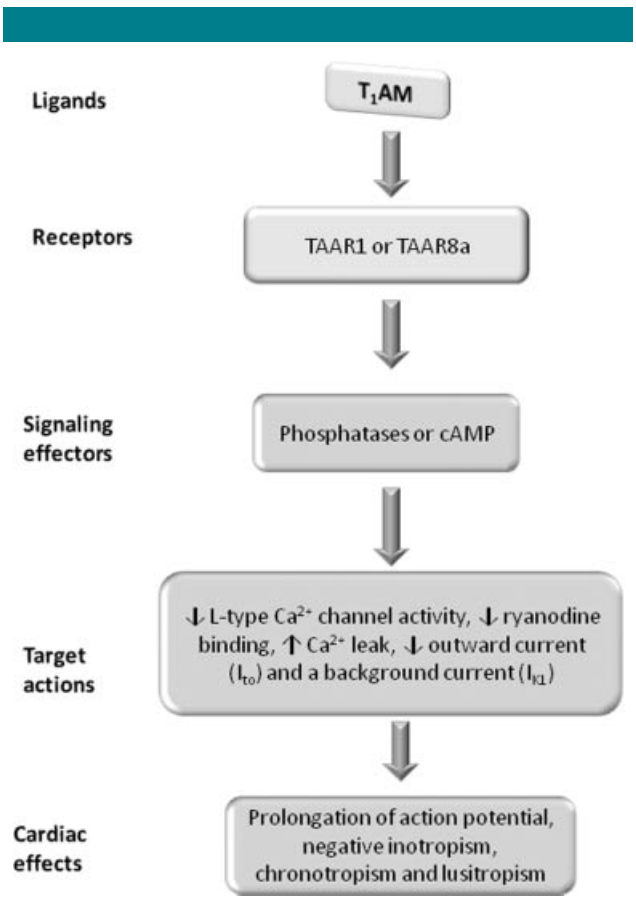


**Fig. 2. A scheme of the $T_1AM$ cardiac actions, including the receptors, the signaling effectors, the target actions and the final cardiac effects.**

Some studies proposed different signaling pathways to explain these negative inotropic and chronotropic $T_1AM$ effects. It was shown that a tyrosine kinase inhibitor remarkably increases them, whereas a tyrosine phosphatase inhibitor blocks them, suggesting that $T_1AM$ might induce the dephosphorylation of critical tyrosine residues (Chiellini et al., 2007). AMPc, PKA, PKC, calcium-calmodulin kinase II, PI3K, or MAPK appears not to be involved in the $T_1AM$ cardiac effects (Chiellini et al., 2007). However, Scanlan et al. (2004) previously reported that recombinant rat, mouse, chimp, and human TAAR1s heterologously expressed in HEK293 cells or *Xenopus oocytes*, rapidly leads to stimulation of cAMP production when exposed to $T_1AM$. These apparently contradictory findings might be explained by a localized increase in cAMP content—that could activate signaling pathways able to reduce cardiac inotropic and chronotropic state—or by activation of different signaling pathways coupled to TAAR1 in a native cellular environment. Furthermore, as previously mentioned, different TAAR subtypes may account for the effects of $T_1AM$ in the heart, such as TAAR8a (Zucchi et al., 2006; Chiellini et al., 2007). In fact, the signaling pathways triggered by $T_1AM$ in cardiomyocytes to result in modulation of cardiac function are still not completely elucidated.

### Future Perspectives

As we have shown in this review, thyroid hormones act via different pathways to play physiological roles in the cardiovascular system. Although general genomic effects have been well described for years, some nongenomic effects have been brought to scene and appear to converge and amplify genomic thyroid hormone effects in the cardiovascular system. Beyond genomic and nongenomic actions, thyroid hormones are also metabolized to a newly recognized biological active product, $T_1AM$, which acts through an aminergic system to trigger cardiovascular effects opposite to those described for $T_3$ and $T_4$. Therefore, a balance between $T_3$ or $T_1AM$ formation from $T_4$ could trigger a fast or slow signal transduction cascade resulting in an efficient mechanism of maintaining cardiac homeostasis. Changes in this equilibrium might contribute to the cardiovascular alterations that appear concomitantly to thyroid disease. It seems that a more complete elucidation of the effectors involved in $T_3$, $T_4$, and $T_1AM$ pathways, as well as a better understanding of the generation, distribution, and metabolic processing of this novel endogenous metabolite will be of great importance as targets for the treatment of cardiovascular diseases.

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