Role of TGF-β on cardiac structural and electrical remodeling

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Abstract: The type β transforming growth factors (TGF-βs) are involved in a number of human diseases, including heart failure and myocardial arrhythmias. In fact, during the last 20 years numerous studies have demonstrated that TGF-β affects the architecture of the heart under both normal and pathological conditions. Moreover, TGF-β signaling is currently under investigation, with the aim of discovering potential therapeutic roles in human disease. In contrast, only few studies have investigated whether TGF-β affects electrophysiological properties of the heart. This fact is surprising since electrical remodeling represents an important substrate for cardiac disease. This review discusses the potential role of TGF-β on cardiac excitation-contraction (EC) coupling, action potentials, and ion channels. We also discuss the effects of TGF-β on cardiac development and disease from structural and electrophysiological points of view.
Keywords: transforming growth factor, ion channel, cardiac electrophysiology

Introduction

The superfamily of type β transforming growth factors (TGF-β) includes TGF-β1, TGF-β2, and TGF-β3, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activins, and inhibins. At least 35 members of this superfamily have been discovered in vertebrates. These factors regulate a diverse array of cellular processes, including tissue development and repair. Their effects are mediated by binding to two types of functional receptors (TBRI and TBRII), which are single-pass transmembrane serine/threonine kinases that phosphorylate specific signal-transducing molecules termed Smads (Figure 1). Activated Smads can be translocated into the nucleus, bind to the DNA, and regulate the transcription of specific genes. The TGF-βs bind to and activate the type II receptor (TBRII), which in turn binds to and phosphorylates the type I receptor (TBRI). This yields to formation of a tetrameric complex of proteins, formed by two TBRIs and two TBRIIs. Once activated, the TBRI phosphorylates the C-terminus of receptor-associated Smads (or R-Smads, eg, Smad 3); see Chang and colleagues (2002) for an excellent and comprehensive review regarding signaling pathways and *in vivo* effects of TGF-β.

Even though C-terminal phosphorylation is the key event in Smads activation, other kinase pathways also regulate the Smad signaling. For example, both tyrosine kinase receptors to epidermal growth factor (EGF) and hepatocyte growth factor phosphorylate Smad2, and induce its nuclear translocation (de Caestecker et al 1998). In fact, the activation of Smads can be also induced by at least the following signaling pathways: the Erk mitogen-activated protein kinase (MAPK) and the Ca^{2+}/calmodulin-dependent protein kinase II (CamKII). Thus, similar to other signaling pathways, the TGF-β signaling exhibits cross-talk with a number of second messengers. Moreover, other molecules apart from Smad proteins interact with and regulate the activaty of TBRs, without apparent direct activation of Smads (eg, FK-506 binding protein). Finally, the activated receptor can also activate non-Smad signaling pathways, such as PP2A, Erk, JNK, PI3K, and p38MAPK (Derynck and Zhang YE 2003).

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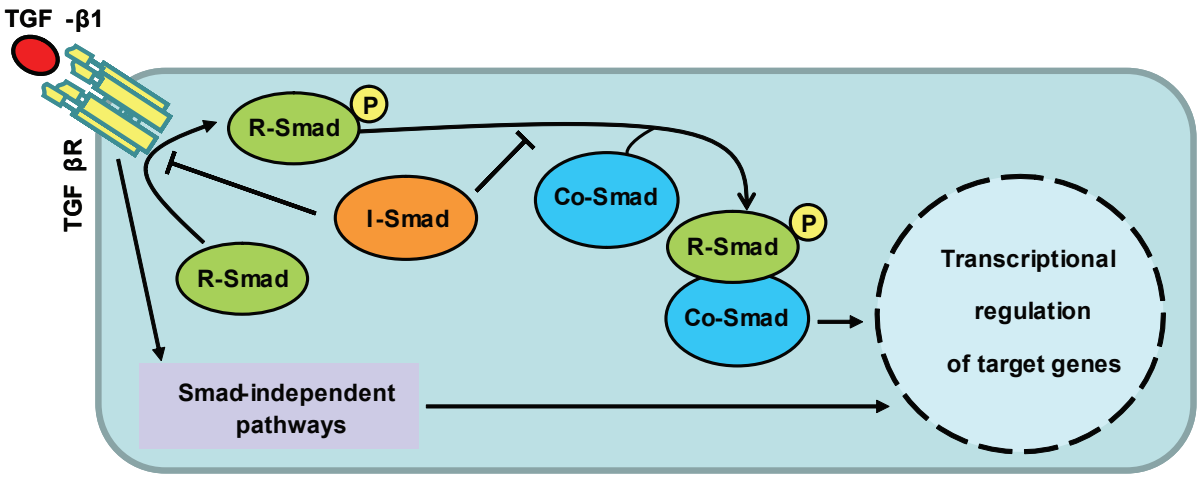

Figure 1 TGF-β1 and its general mechanism of action. Binding of TGF-β1 with its receptor (TGF-βR) activates intracellular signaling proteins termed Smads receptors (R-Smads, eg, Smad3), which are translocated into the nucleus following interaction with co-activator proteins termed co-Smads. Once in the nucleus, the Smads bind to the DNA and regulate transcription of specific genes. Inhibitory Smads, like Smad 6 and 7 prevent activation of R-Smads, by competitively inhibiting either its activation by the receptor, or its association with Co-Smads. Smads independent signaling pathways can also contribute to diversify responses to TGF-β1 (eg, MAPK kinases, and Rho GTPases).

The TGF-β signaling is involved in a number of human pathologies, including lung fibrosis (Willis and Borok 2007), renal and liver injury (Breitkopf et al 2005; Böttinger 2007), Alzheimer (Masliah et al 2001), cancer (Roberts and Wakefield 2003), and cardiac remodeling (Bujak and Frangogiannis 2007; Burstein and Nattel 2008). The role of TGF-β on cardiac pathophysiology began to be elucidated ~20 years ago by Thompson and colleagues (1988). Basically, what they found was that ventricular myocytes from the infarcted myocardium overexpresses TGF-β1 protein and mRNA. Soon thereafter, Potts and Runyan (1989) suggested a role for TGF-β signaling in promoting development of the heart. To this date, a great deal of information regarding to the effects of TGF-β on cardiac architecture has been accumulated. In fact, significant efforts are currently been made to discover potential therapeutic roles for TGF-β signaling in cardiac pathology (Narine et al 2004; Ng et al 2004; Li et al 2005; Liao 2005; Okada et al 2005).

Role of TGF-β in cardiac development

The TGF-β signaling is essential to epithelial–mesenchymal transformation (EMT). This is an embryonic phenomenon that determines formation of cardiac valves and the septa. Specifically, the EMT involves endothelial cells that migrate into an expanded extracellular matrix (or the cardiac jelly) where they proliferate and differentiate into mesenchymal cells. Subsequently, locally expanded swellings of cardiac jelly and mesenchymal cells form what is known as endocardial cushion tissue, which undergoes an extensive remodeling from bulbous swellings to eventual thinly tapered heart valves (Nakajima et al 2000).

A number of studies show that the TGF-β superfamily signaling is essential for heart development. For example, TGF-β1, -β2, and -β3, as well as BMP-2, -4, -6, and -7, are all expressed at specific regions and stages of development of the immature heart. In addition, several receptors (ALK2, ALK3, and ALK5) and downstream molecules (Smad5 and Smad6) are important in cardiac morphogenesis. Moreover, BMP-2-null mice either do not have a heart, or develop a very retarded and malformed heart, mice carrying mutations in BMP-5 or -7 die before birth with multiple defects in heart development, and mice deficient in BMP-6 or -7 have delayed cardiac cushion formation, which results in subsequent valve and septation defects, as well as a premature death due to heart failure (Chang et al 2002). Isoforms of the TGF-β subfamily are also important for heart development. For example, specific antibodies against the corresponding receptors inhibit EMT (Potts and Runyan 1989; Boyer et al 1999; Brown et al 1999), the heart of TGF-β2-null mouse embryos have specific defects in the development of the valves and septa (Sandford et al 1997), and both TGF-β2 and TGF-β3 are critical to the initiation and regulation of EMT (Chang et al 2002). Thus, several TGF-βs are required to achieve proper valve morphogenesis, and according to this, there are numerous of non-compensated functions between the three different isoforms of the TGF-β subfamily. For example, TGF-β2 null-mice exhibit multiple defects that are not overlapping with TGF-β1- and TGF-β3-null mice

(Sandford et al 1997), and TGF-β2 promotes cardiac cushion formation by activating a unique set of downstream mediators (ie binding to a third type receptor or TBRIII, which in turn modifies the functional TBRI-TBRII signaling complex (Brown et al 1999). Moreover, TGF-β2 and TGF-β3 exert distinct effects in EMT during valve formation (Boyer et al 1999), and TGF-β1 plays a critical role in adult cardiac structural remodeling (Bujak and Frangogiannis 2007; Burstein and Nattel 2008). Below we describe the major alterations associated to cardiac remodeling, as well as the corresponding TGF-β effects.

Structural remodeling of the ventricle

Cardiac structural remodeling stands for several morphological changes that can be induced by the following physiological and pathophysiological factors, cytokine and neurohumoral stimuli, hemodynamic load, an altered function of mutant proteins, and a loss of contractile mass from prior infarction (Figure 2). The involved changes include alterations on the expression levels of structural proteins, as well as an increase on the following parameters: proliferation rate of fibroblast, deposition of extracellular matrix (ECM) constituents or fibrosis, and the size of cardiac myocytes or hypertrophy. Ventricular hypertrophy accompanies many forms of heart disease, such as ischemic disease, hypertension, heart failure, and valvular disease. Initially, hypertrophy helps the heart to meet the needs of the body (compensated hypertrophy). However, in response to a more severe, or prolonged stimuli, the hypertrophy becomes inadequate (decompensated hypertrophy), and contributes *per se* to the genesis of ischemia, arrhythmia, and heart failure (see the vicious circles that are indicated with gray arrows in Figure 2,). Some alterations in gene expression that are

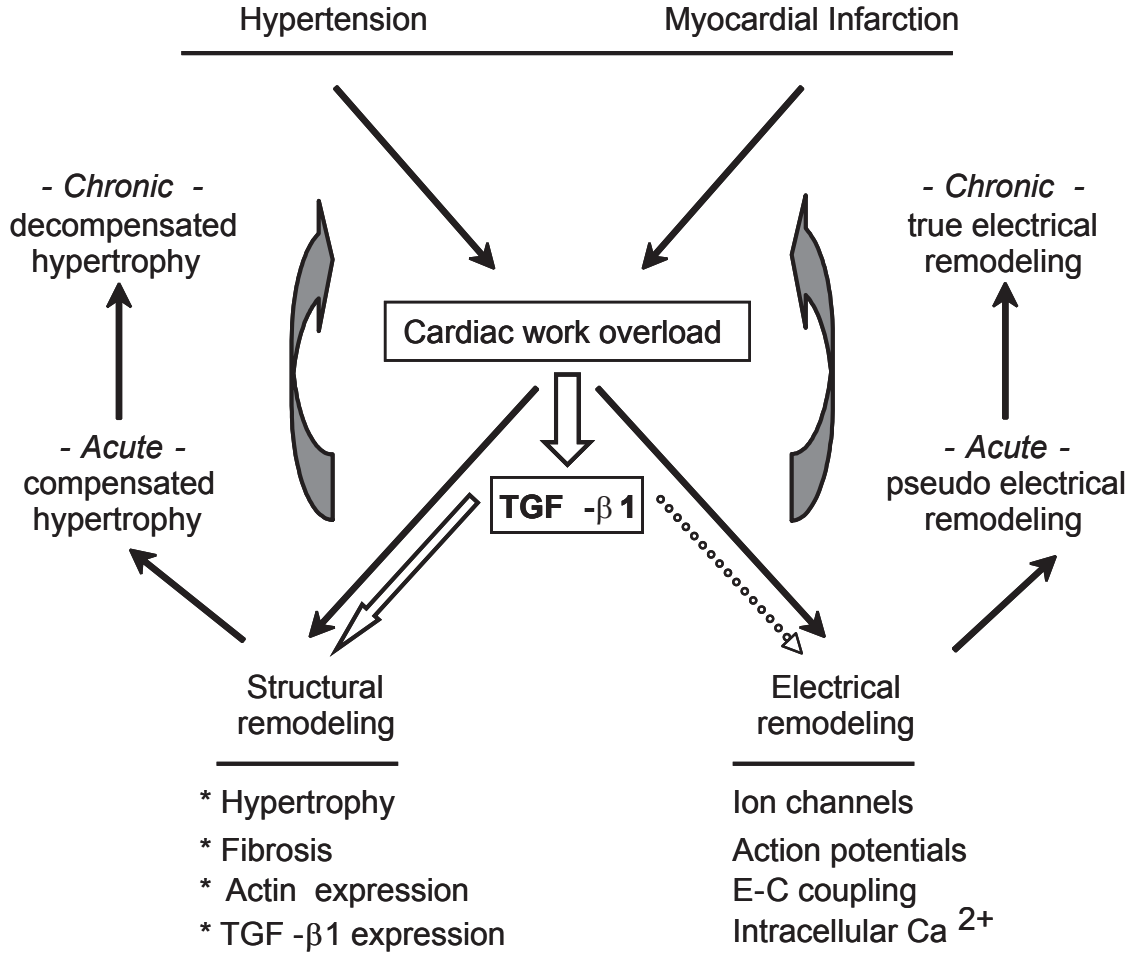

Figure 2 Role of TGF-β on ventricular remodeling. Certain human diseases provoke cardiac work-overload, which in turn promotes several changes known as structural and electrical remodeling. Initially, these changes help the heart to meet the needs of the body, by means of optimizing cardiac output (*acute remodeling*). However, in response to prolonged stimuli the remodeling becomes inadequate and contributes *per se* to cardiac dysfunction (vicious circles indicated by *gray arrows*). Interestingly, work-overload also increases the expression levels of TGF-β1. In fact, TGF-β1 *per se* reproduces most of the hallmarks associated to structural remodeling (*asterisks*), including its own overexpression. TGF-β1 may also provoke electrical remodeling *(dotted arrow*), but the corresponding effects have not been thoroughly investigated.

involved in hypertrophy are switching from adult α-myosin heavy chain (α-MHC) to fetal β-myosin heavy chain (β-MHC), and reexpression of skeletal α-actin. Altogether, this pattern of gene expression mimics that seen during embryonic development and is thus called re-induction of the "fetal-gene program". Thus, hypertrophy seems to be a programmed reversion toward a more fetal phenotype (Bers 2001; Katz 2002; Hill 2003).

Activation of the renin–angiotensin system (RAS) has been implicated in the generation of cardiac remodeling (hypertrophy and fibrosis). In fact, inhi bition of angiotensin II (or Ang II, the effector molecule of the RAS), by angiotensin-converting enzyme (ACE) inhibitors or Ang II type 1 (AT_1) receptor antagonists prevents structural remodeling of the ventricle. Interestingly, while Ang II directly promotes growth in neonatal cells, it does not in adult cardiomyocytes, suggesting Ang II indirectly promotes hypertrophy. In fact, Ang II exerts its *in vivo* profibrotic and hypertrophic effects by increasing the expression levels of TGF-β1 (Rosenkranz 2004).

Role of TGF-β in structural remodeling of the ventricle

The proposed role for TGF-β1 in cardiac disease is illustrated in Figure 2. Both neonatal and adult cardiomyocytes synthesize and release the three mammalian isoforms of the TGF-β subfamily (ie, TGF-β1, TGF-β2, and TGF-β3), although it is the TGF-β1 and TGF-β3 isoforms that predominate (Long 1996). The cardiac myocytes increase expression levels of TGF-β1 in response to myocardial stress in an animal model of myocardial infarction (Thompson et al 1988). Accordingly, expression levels of TGF-β1 mRNA are also increased in left ventricular myocardium of patients with either idiopathic hypertrophic cardiomyopathy (Li et al 1997) or dilated cardiomyopathy (Pauschinger et al 1999), as well as in animal models of norepinephrine-induced hypertrophy (Bhambi and Eghbali 1991), progressive coronary artery occlusion (Wünsch et al 1991), and pressure overload (Villarreal and Dillmann 1992). In both experimental models and humans TGF-β1 is particularly overexpressed in myocardium during transition from stable hypertrophy to heart failure. Thus, TGF-β1 is considered an essential mediator of cardiac adaptation to work overload. In fact, TGF-β1 represents one of the few markers that discriminate between compensated and decompensated hypertrophy (in addition to increased collagen content) (Rosenkranz 2004).

Perhaps more importantly, TGF-β1 reproduces most of the hallmarks seen in structural remodeling (Figure 2, asterisks). Specifically, TGF-β1 induces expression levels of ECM constituents by cardiac fibroblasts (ie, fibrillar collagen, fibronectin, and proteoglycans), self-amplifies its own expression in both cardiac myocytes and fibroblast (Desmouliére et al 1993; Long 1996), stimulates the proliferation of fibroblasts and their phenotypic conversion to myofibroblasts (Sappino et al 1990; Walker et al 2004) and provokes "fetal" contractile protein gene expression in cardiomyocytes (Parker et al 1990). Additionally, TGF-β1 mediates the cardiac hypertrophy induced by Ang II (Schultz Jel et al 2002), overexpression of TGF-β1 in transgenic mice results in hypertrophic growth of ventricular myocytes (Nakajima et al 2000), heterozygous TGF-β1 (±)-deficient mice exhibit decreased fibrosis of the aging heart (Brooks and Conrad 2000) and functional blockage of TGF-β1 signaling *in vivo* by neutralizing antibodies prevents myocardial fibrosis and dysfunction in pressure overloaded hearts (Kuwahara et al 2002).

Structural remodeling of the atrium

Atrial remodeling, which stands for "any persistent change in atrial structure or function", promotes the occurrence or maintenance of atrial fibrillation (AF), by acting on the fundamental mechanisms of the arrhythmia (Thompson et al 1988). Accordingly, AF can be induced by several physiological and artificial stimuli (Figure 3), such as heart failure (Li et al 1999; Lee et al 2006), senescence (Anyukhovsky et al 2002; Hayashi et al 2002), atrial dilatation (Eckstein et al 2008), and rapid atrial rate of stimulation (Morillo et al 1995). Structural remodeling and in particular interstitial fibrosis represents the major promoter of AF. This is because fibrosis provokes disruption of electrical conduction among adjacent myocytes, due to an increased deposition of extracellular matrix, which disrupts the normal myocardial ultrastructure. In fact, transgenic mice overexpressing cardiac TGF-β1 develop atrial fibrosis, heterogeneous conduction, and AF (Verheule et al 2004). Additionally to atrial fibrosis, myocyte loss by either apoptosis or necrosis accompanies AF (Burstein and Nattel recently reviewed structural alterations associated to AF (Burstein and Nattel 2008)).

The cellular mechanisms that underlie cardiac fibrosis are similar to fibrosis in other epithelial organs. Accordingly, fibroblasts represent the principal cellular mediators of cardiac fibrosis. The increased number of these cells was initially thought to originate from proliferation of resident fibroblasts, bone marrow cells, and epithelial cells (derived through EMT). More recently, cardiac fibroblasts were also shown to be derived from endothelial cells, via endothelial–mesenchymal transformation (EndMT) (Kisseleva and Brenner 2008).

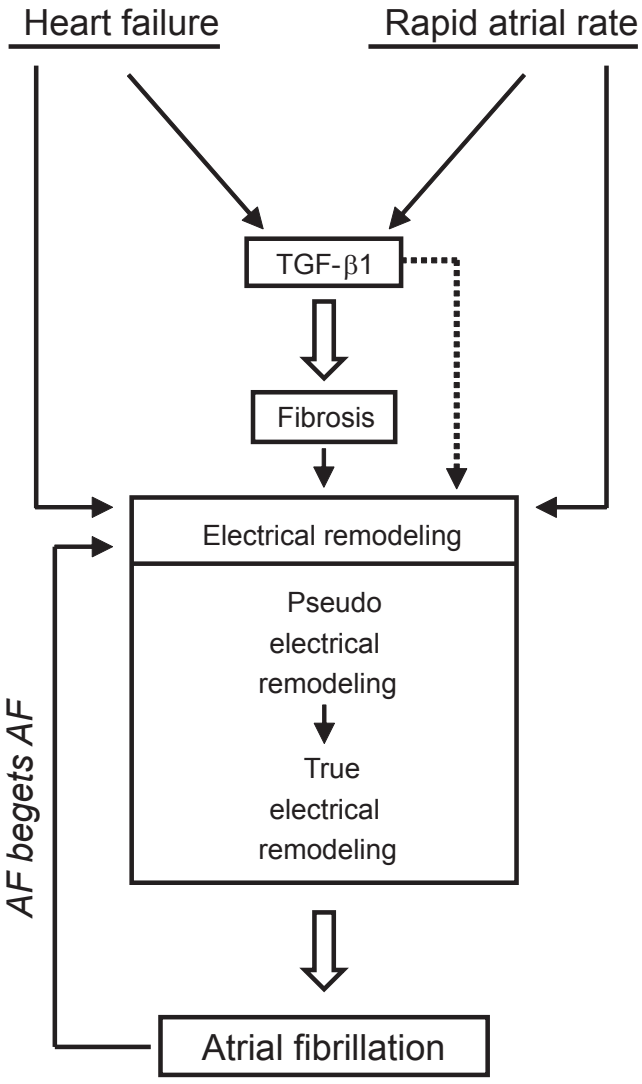

Figure 3 Role of TGF-β on atrial remodeling. Certain physiological and pathophysiological conditions (ie, heart failure, rapid atrial rate of electrical stimulation, aging, and atrial dilatation) promotes the development of atrial fibrosis, which in turn provokes AF. Atrial fibrosis is developed in parallel to increased expression of TGF-β1, a well known pro-fibrogenic agent. Interestingly, a compound named pirfenidone that exerts its biological actions by inhibiting the synthesis of TGF-β1, also prevents development of both atrial fibrosis and AF.[41] This suggests a cause-effect relationship, by which TGF-β1 promotes development of atrial fibrosis, and thereby AF. Recent evidences obtained from cultured cells suggest that TGF-β1 also provokes electrical remodeling (Table 1). These potentially *in vivo* electrophysiological effects by TGF-β1 could be attributed to a direct effect on ion channels expression and function (*dotted arrow*). Alternatively, they could be mediated by structural remolding (eg, *fibrosis*). Once established, electrical remodeling provokes AF and a vicious circle begins – termed atrial fibrillation begets atrial fibrillation, or *AF begets AF*.

Role of TGF-β in structural remodeling of the atrium

A number of stimuli that promotes AF converge in increasing expression levels of TGF-β1, which in turn provokes interstitial fibrosis (Figure 3). However, while upregulation of TGF-β1 represents a key event in inducing fibrosis, other growth factors are known to synergize with TGF-β1 for this effect. For example, connective tissue growth factor (CTGF), and platelet-derived growth factor (PDGF). Interestingly, while TGF-β1 promotes fibrosis and increases collagen gene expression by acting through Smad proteins signaling pathway (Piek et al 1999; Hao et al 2000; Evans et al 2003), inhibiting the activity of PI3K reduces TGF-β induced EMT, suggesting a role for Smad-independent pathways in fibrosis (Bakin et al 2000). In fact, the p38MAPK pathway is also believed to be required for TGF-mediated EMT (Bakin et al 2002).

Ang II has been also implicated in promoting the activation of fibroblasts, as well as the synthesis of extracellular matrix proteins, such as collagen and proteoglycan. Additionally, Ang II regulates production of matrix metalloproteinases (eg, MMP-2), as well as the breakdown of collagen IV (Mehta and Griendling 2007). Interestingly, cardiac fibroblasts exposed to Ang II overexpress both fibronectin and TGF-β1 (Moriguchi et al 1999). Actually, the profibrotic effects of Ang II are largely explained by its ability to increase expression levels of TGF-β1 (Kisseleva and Brenner 2008).

There are several differences between atrial and ventricular structural remodeling. For example, while at the ventricle TGF-β1 provokes hypertrophy, this is prevented by TGF-β1 at the atrium (Nakajima et al 2000). Additionally, compared to the ventricle, the atrial tissue is more susceptible to develop fibrosis (Nakajima et al 2000; Hanna et al 2004). Moreover, leukocyte infiltration, cell death, apoptosis and MAP kinase activation, are larger in atrium, occur earlier, and are more transient in atrium compared to ventricle (Hanna et al 2004). In a very interesting study, Burstein and colleagues (2008) investigated the different behavior of atrial and ventricular fibroblasts. Basically, they found the atrial fibroblasts show enhanced reactivity, which contributes to greater atrial fibrotic responses. Apparently, a different behavior between atrial and ventricular fibroblasts is due to an increased expression of the PDGF receptor in the atrium (Burstein et al 2008).

Electrical remodeling

Cardiac excitation-contraction (EC) coupling consists of intracellular Ca^{2+} release that occurs in response to activation of type 2 ryanodine receptors (RyR2s, located at the sarcoplasmic reticulum or SR). Intracellular Ca^{2+} release is triggered by Ca^{2+} entering through voltage dependent Ca^{2+} channels (L-type Ca^{2+} channels, or L-channels) located at the sarcolemma (termed Ca^{2+} induced Ca^{2+} release, or CICR) (Fabiato and Fabiato 1979). This combination of Ca^{2+} influx and release transiently

increases intracellular free Ca^{2+} concentration (termed Ca^{2+} transient), which in turn activates the contractile machinery. The relaxation process occurs following termination of the Ca^{2+} transient, which depends mostly on the removal of cytosolic Ca^{2+} by the activities of: sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), sarcolemmal Na^+/Ca^{2+} exchanger (NCX), sarcolemmal Ca^{2+}-ATPase, and mitochondrial Ca^{2+} uniport (Bers 2002). Ca^{2+}-dependent inactivation of L-channels, on the other hand, guarantees fast termination (in milliseconds) of the triggering Ca^{2+} influx (Bers and Perez-Reyes 1999; Catterall 2000).

In addition to L-current (I_{CaL}), cardiac myocytes express often a voltage-dependent T-type Ca^{2+} current (T-current, or I_{CaT}) whose physiological relevance remains elusive (Bers and Perez-Reyes 1999). Voltage-dependent Na^+ channels generate also quickly activating inward currents (I_{Na}), which are at least partially responsible for the fast depolarizing upstroke of the action potential (AP) (Balser JR 1999; Clancy and Kass 2005). Repolarization, on the other hand, is favored by inactivation of inward currents, but depends primarily on the activation of the following K^+ currents – which are often composed of two or more subcomponets: inward rectifier (I_{K1}), transient outward (I_{to}), delayed rectifier (I_{Ksus}), and a time independent background current (I_{KP}) (Snyders 1999; Clancy and Kass 2005). Thus, as in other excitable cells APs of the cardiac myocytes depend on a delicate balance on the activity of several ion channels and transporters. Consequently, heart disease is commonly associated to several channelopathies, which are not only restricted to inherited disorders, but involve also acquired alterations in either the expression or post translational modification of ion channels (Clancy and Kass 2005; Tomaselli and Marbán 1999; Marbán 2002). The observation that tachyarrhythmias modify electrophysiological properties in the same manner as cardiac disease has lead to the concept "electrical or electrophysiological remodeling" (Figures 2 and 3). However, the precise alterations involved in this process, regarding to ion channel function and expression remain controversial (Pandozi and Santini 2001).

This is partially due to the different experimental models that are commonly used in heart disease. Another source of variation is that the alterations change during disease progression or transition to failure. In fact, different times of exposure to the pathophysiologic substrate may result in either "pseudo" (acute) or "true" (chronic) electrical remodeling (Figures 2 and 3). Pseudo-remodeling have short onset and offset kinetics (minutes), and involve alterations in the activity (or functional properties) of a fixed number of ion channels, transporters, and pumps (Pandozi and Santini 2001). In contrast, true electrical remodeling is associated with alterations in the expression levels of functional ion channels, transporters, and pumps. Accordingly, alterations on mRNA levels encoding to these proteins are commonly observed. True electrical remodeling has therefore, long onset and offset kinetics, last for a long time (hours or days) and is not functional or metabolic (Pandozi and Santini 2001; Allessie 1998).

Electrical remodeling of the ventricle

Shortenings of action potential duration (APD) and effective refractory period (ERP) are typical of atrial fibrillation (see below, Electrical remodeling of the atrium). In contrast, rapid rates of stimulation prolong both the APD and the ERP at the ventricle. Prolongation of the APD is characteristic of ventricular cells and tissues on which heart failure is induced by a number of mechanisms, including pressure and volume overload, genetic, metabolic, ischemia/infarction, and chronic pacing tachycardia. In certain models of ventricular failure prolongation of the APD is associated, as for the atrial myocytes, to reductions in I_{CaL}, I_{to}, and expression levels of the corresponding genes. It is interesting thus that opposite alterations in APD can be related to changes in the same ionic currents (I_{CaL} and I_{to}). However, the relative contribution of these channels to the AP differs substantially between atrium and ventricle, and this contributes to explaining the differences in APD between the two cavities.

Electrical remodeling of the ventricle also involves a parallel reduction in the amplitude and the rate of decay of Ca^{2+} transients, which provokes a reduced capability to develop force. Interestingly, while I_{CaL} is reduced in certain models of HF, it does not necessarily contribute to explain the reduced Ca^{2+} transients. This is because many reports show no change in I_{CaL} in the face of a strong depression of both contractions and Ca^{2+} transients (Kääb et al 1996; Gómez et al 1997). Instead, reduction of Ca^{2+} transients and the consequent contractile dysfunction, are both due to depletion of SR Ca^{2+}, which may result from RyR2-dependent Ca^{2+} leak, an increased Ca^{2+} extrusion through the NCX, or a reduced function of SERCA (Bers 2001; Wehrens et al 2005).

Electrophysiological effects of TGF-β on the ventricle

The reported effects of TGF-β1 on cardiac electrophysiology are summarized on Table 1. Pioneer studies that investigated these effects were performed on confluent cultures of ventricular myocytes, forming a syncytium (Neylon et al 1994; Kimura 1997; Carrillo et al 1998). Roberts and colleagues

Table 1 Modulation of cardiac electrophysiology by TGF-β1

Preparation	Results	References
Ventricle		
Cultured neonatal rat myocytes	A chronic exposure (48 h) to TGF-β1 increases the frequency of spontaneous Ca^{2+} oscillations, and increases the caffeine-sensitive intracellular Ca^{2+} store	Neylon et al 1994[71]
Cultured neonatal rat myocytes	In ~4 d TGF-β1 moderately (16%) decreases the spontaneous beating rate and increases the IP_3-sensitive intracellular Ca^{2+} store	Kimura et al 1997[73]
Cultured neonatal rat myocytes	TGF-β1 increases in 24 h both beating rate and expression levels of mRNA encoding the Na^+/Ca^{2+} exchanger	Carrillo et al 1997[72]
Cultured adult rat myocytes	TGF-β1 decreases in ~4 h the rate of myocyte shortening and relaxation, delays the peak of Ca^{2+} transients, and slows its rate of decay (termed "contractile dysfunction")	Li et al 2008[75]
Atria		
Atrial tissue from TGF-β1 overexpressing mice	TGF-β1 overexpression provokes vulnerability to atrial fibrillation and decreases epicardial conduction velocity	Verheule et al 2004[46]
Neonatal rat myocytes	TGF-β1 reduces in 1–2 d I_{CaL}, nifedipine-sensitive charge movement, and expression levels of $Ca_V1.2$ mRNA	Avila et al 2007[87]
Neonatal rat myocytes	TGF-β1 chronically (1–2 d) reduces current densities associated to I_{Na}, I_{K1}, and I_{Ksus}	Ramos-Mondragón et al 2008[88]

(1992) found that a chronic exposure of neonatal rat ventricular myocytes to TGF-β1 increases spontaneous beating rate of the syncytium. Conceivable, this effect could be at least partially explained by TGF-β1 provoking an increased SR Ca^{2+} content, and a higher frequency of spontaneous intracellular Ca^{2+} oscillations. Accordingly, both of these effects were subsequently reported by Neylon and colleagues (1994). More recently, Carrillo and colleagues (1998) reported that the increase in beating rate is also associated to a higher expression of the Na^+/Ca^{2+} exchanger, which could prepare cells to deal better with the Ca^{2+} overload associated to high frequency of Ca^{2+} oscillations. While these studies support the notion that TGF-β1 increases beating rate of the syncytium, Kimura (1997) reported an opposite effect, which was associated to an increased IP_3-sensitive intracellular Ca^{2+} store. Currently, molecular bases for this apparent contradiction remain unknown.

To our knowledge, only one study has investigated the electrophysiological effects of TGF-β1 in adult ventricular myocytes (Roberts et al 1992). What these authors found is that ~4 h of exposure to TGF-β1 provokes "contractile dysfunction", characterized by a significant reduction on the maximum rate of myocyte shortening and re-lengthening. Additionally, TGF-β1 reduces the time to peak and the rate of decay of intracellular Ca^{2+} transients, in the absence of significant alterations on I_{CaL}. Molecular mechanisms underlying these effects point to an enhanced reactive oxide species (ROS) production. However, downstream molecules other than ROS have yet to be elucidated.

For instance, the absence of alterations on I_{CaL} could be interpreted to suggest that contractile dysfunction and effects on Ca^{2+} transients could both arise from a possible regulation on the Ca^{2+} handling proteins of the SR. However, neither the SR Ca^{2+} content nor spark properties (frequency and amplitude) were altered by TGF-β1, even thought this factor reduced the phosphorylation state of phospholamban, which in turn modulates SERCA. We should keep in mind that I_{CaL} was recorded under whole-cell patch clamp conditions, whereas estimations of mechanical properties and intracellular Ca^{2+} were performed in intact (ie, nonpatched) myocytes (Li et al 2008). This represents an important limitation, since misleading conclusions can be drawn from comparing results of different conditions. For example, intracellular Ca^{2+} can be strongly influenced

by dialysis of the cytosol under whole-cell patch-clamp conditions. Finally, we should keep in mind that these data were obtained in cultured myocytes and the situation may be different *in vivo*.

Electrical remodeling of the atrium

An important step toward understanding atrial fibrillation (AF) was the discovery that once initiated, AF alters atrial electrical properties, in a manner that favors the maintaining of the arrhythmia (ie, electrical remodeling, as a vicious circle (Wijffels et al 1995)). Initially, high frequencies of atrial activation (ie, rapid atrial rate; Figure 3) leads to what is known as a short-term APD (action potential duration) adaptation to rate, which is due to rapid (minutes) functional changes in ion channels and transporters (basically, what happens is that at high frequencies of stimulation, the atrium responds with a shortening of the APD). Thus, rapid atrial rate stimulation provokes "pseudo" electrical remodeling (Figure 3).

This short-term adaptation can be at least partially explained by an increase in Ca^{2+}-dependent inactivation of I_{CaL}(Yagi et al 2002). Accordingly, cytosolic Ca^{2+} overload is also involved, which may activate intracellular signaling pathways that play a prominent role in the subsequent "true" electrical remodeling (Nattel 1999). Following this short-term adaptation, the atrial tachycardia begins to produce discrete changes in the expression levels of specific genes (in hours or days). Specifically, the proteins or mRNAs encoding to the principal subunits of I_{CaL}(Yue et al 1999; Brundel et al 2001), (but see Brundel and colleagues [Christ et al 2004]), I_{Na} (Yue et al 1999), I_{Kur} (Lai et al 1999), and I_{to} (Yue et al 1999) are downregulated, whereas the corresponding subunits of I_{K1} and $I_{K,Ach}$ are upregulated (Dobrev et al 2001; Gaborit et al 2005) in a similar time-course and magnitude as the corresponding ionic currents (Pandozi and Santini 2001; Allessie 1998; Nattel et al 2007; Nattel et al 2008).

True ionic remodeling (ie, changes in ion channel expression) permanently reduce the APD, the capability of APD to adapt to rate, and the effective refractory period (ERP). Altogether, these changes give rise to a vicious circle (Figure 3). This is because decrease ERP promotes AF, by decreasing the wavelength (distance traveled by the electrical wave during the ERP, which determines the minimum path length that can support electrical reentry). This in turn allows the atrium to accommodate a larger number of reentry circuits, decreasing the chance of spontaneous termination. Thus, ionic remodeling can be seen as a protective mechanism against the initial Ca^{2+} overload. However, the protective effect occurs at the expense of promoting the maintenance of AF, which nonetheless eventually alters Ca^{2+} homeostasis (Nattel et al 2008).

Effects of TGF-β on electrical remodeling of the atrium

An interesting effect of TGF-β1 is that it can provoke an increased susceptibility to develop atrial fibrillation. This was discovered in 2004 by Verheule and colleagues (2004), using transgenic mice overexpressing cardiac TGF-β1. These animals also present a decreased epicardial conduction velocity on the right atria, as well as a more heterogeneous conduction on the left atria. Since no differences in action potential properties were found, Verheule and colleagues (2004) concluded that atrial fibrosis induced by TGF-β1 is sufficient to enhance the inducibility of AF (as opposed to electrical remodeling) (2004). Unfortunately, they did not investigate I_{CaL} or a potential loss of the capability of APs to adapt to high rate of stimulation, which would have suggested alterations on I_{CaL}. In keeping with this possibility, we have previously reported that TGF-β1 reduces expression levels of I_{CaL}, in neonatal rat atrial myocytes (Avila et al 2007). Moreover, in these cells TGF-β1 also reduces current densities associated to I_{Na}, I_{Ksus}, and I_{K1} (Ramos-Mondragón 2008). Thus, at least in cardiomyocytes from neonatal rat TGF-β1 reproduces electrophysiological alterations that are commonly seen in AF.

Molecular bases for downregulation of I_{CaL} by TGF-β1 point to a decreased expression of mRNA encoding to $Ca_V1.2$, the principal subunit of L-type Ca^{2+} channels. Accordingly, TGF-β1 also provokes reduction in the amount of immobilization-resistant charge movement, which reflects voltage sensor's activity of $Ca_V1.2$. Moreover, inhibition of I_{CaL} by TGF-β1 cannot be reverted by okadaic acid, an inhibitor of protein phosphatases, supporting the notion that this effect is not due to a possible reduction in the permanent phosphorylation state of the channels (Avila et al 2007).

As previously discussed (see Electrical remodeling of the atrium), molecular mechanisms underlying reduction of I_{CaL} in AF include an altered function of $Ca_V1.2$. For example, I_{CaL} is functionally reduced in AF by at the least the following mechanisms: 1) Ca^{2+}-dependent inactivation (Yagi et al 2002). 2) Reduced phosphorylation state due to either increased activity of protein phosphatases (Christ et al 2004), or impaired regulation by src kinase

(Greiser et al 2007). 3) Increased S-nitrosylation, due to decreased expression of the antioxidant glutation (Carnes et al 2007). Remarkably, I_{CaL} reduction has been also attributed to reduced expression levels of $Ca_V1.2$.(Yue et al 1999; Brundel et al 2001).

The question of whether TGF-β1 actually regulates I_{CaL} in adult atrial myocytes still remains to be solved. A recent study suggests increased *in vivo* levels of TGF-βs (in AF induced by rapid atrial pacing) do not significantly alter expression levels of $Ca_V1.2$ (Chen et al 2007). In contrast, the following evidences suggest possible functional effects. TGF-β1 increases intracellular levels of reactive oxide species (ROS) in adult ventricular myocytes (Li et al 2008). On the other hand, increased ROS levels can provoke a reduced density of I_{CaL} in adult atrial myocytes (Carnes et al 2007). Thus, conceivable, in adult atrial myocytes TGF-β1 could decrease I_{CaL}, by means of increasing levels of ROS. Undoubtedly, this possibility deserves to be elucidated.

Perspectives

Recently, arrythmogenic right ventricular dysplasia type 1 (ARVD1) was associated to mutations in the gene encoding TGF-β3 (Beffagna et al 2005). ARVD1 is a progressive myocardial disease characterized by ventricular arrhythmias that lead to sudden unexpected death. The reported mutations increase expression levels of TGF-β3, which might in turn provoke the arrhythmia by promoting fibrosis and electrophysiological effects. In 2000, Brooks and Conrad (Brooks and Conrad 2000) reported that senescent animals from TGF-β1 heterozygous (±) mice exhibit a decreased amount of myocardial fibrosis compared to controls. Additionally, these mice develop increased left ventricular compliance, live longer, and do not present a normal increase in diastolic pressure. This suggests decreased levels of the growth factor by loss of one TGF-β1 allele prevents development of myocardial stiffness induced by fibrosis. Alternatively, decreased levels of TGF-β1 in heterozygous (±) mice could also prevent development of a possible normal "contractile dysfunction" in senescent animals, similar to the electrophysiological effects that TGF-β1 exerts on cultured adult ventricular myocytes (Roberts et al 1992), see also Table 1.

On the other hand, Kubin and colleagues (2005) reported that in the absence of serum TGF-β1 reduces in 6 days the beating rate of adult rat ventricular myocytes, but does not alter the corresponding morphological properties. Thus, it will be interesting to investigate a possible relationship between the acute contractile dysfunction (Roberts et al 1992), and the chronic decrease in beating rate (Kubin et al 2005).

There are a number of unanswered questions regarding electrophysiological effects by TGF-β. To this date only the type 1 TGFβ has been used to perform electrophysiological studies (see Table 1). However, there are numerous of noncompensated functions among the three isoforms of the TGF-β subfamily (see the section *role of TGF-β in cardiac development*). An interesting prediction would be that different TGF-βs preferentially regulate specific regions of the heart. Support to this view comes from the fact that TGF-β1 exerts opposite effects on atrial and ventricular hypertrophy (inhibition and stimulation, respectively (Nakajima et al 2000)). Moreover, while transgenic mice overexpressing TGF-β1 develop increased vulnerability to atrial fibrillation (Verheule et al 2004), mutations in the gene encoding TGF-β3 are linked to a genetically determined myocardial dystrophy (ie, arrythmogenic right ventricular dysplasia type 1 or ARVD1 (Beffagna et al 2005)). TGF-β2, on the other hand, is preferentially involved in mitral valve disease (Ng et al 2004) and certain cardiovascular anomalies (Sandford et al 1997; Bartram 2001). Thus, we anticipate elucidating effects of the two other isoforms will be of paramount relevance.

The beneficial effects of certain neurohumoral blockers on cardiac disease can be at least partially explained by their ability to inhibit electrical remodeling (Katz 2002; Nattel 2002; Shinagawa et al 2003). Nevertheless, while certain drugs can be successfully used for the treatment of atrial fibrillation, they often result in potentially lethal alterations on ventricular electrophysiology. These opposite effects could be potentially explained by the aforementioned differences in electrical remodeling of the atrium and the ventricle. Thus, the identification of factors that selectively control ion channel expression and function in atrial vs ventricular myocytes, could be advantageous to find a more effective treatment of cardiac disease.

In conclusion, it has been recognized during the last 20 years that TGF-β plays a critical role in cardiac disease. Accordingly, significant efforts are been currently made to discover potential therapeutic roles for TGF-β signaling in cardiac pathology.(Li et al 2005; Narine et al 2004; Ng et al 2004; Liao 2005; Okada et al 2005). Thus, we believe that unraveling the corresponding electrophysiological effects may contribute to improve the clinical use of TGF-β-related agents, by providing the grounds for more rational and specific therapies.

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