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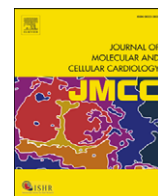


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## Review article

## Sensing pressure in the cardiovascular system: Gq-coupled mechanoreceptors and TRP channels

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## ABSTRACT

Despite the central physiological importance of cardiovascular mechanotransduction, the molecular identities of the sensors and the signaling pathways have long remained elusive. Indeed, how pressure is transduced into cellular excitation has only recently started to emerge. In both arterial and cardiac myocytes, the diacylglycerol-sensitive canonical transient receptor potential (TRPC) subunits are proposed to underlie the stretch-activated depolarizing cation channels. An indirect mechanism of activation through a ligand-independent conformational switch of Gq-coupled receptors by mechanical stress is invoked. Such a mechanism involving the angiotensin type 1 receptor and TRPC6 is proposed to trigger the arterial myogenic response to intraluminal pressure. TRPC6 is also involved in load-induced cardiac hypertrophy. In this review, we will focus on the molecular basis of pressure sensing in the cardiovascular system and associated disease states.

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How cells are able to convert a mechanical force into an electrical response (mechanosensory transduction) remains a major biological question. Cells respond to diverse mechanical stimuli including flow (i.e. shear stress), pressure (i.e. stretch) or osmotic stress (i.e. swelling or shrinking). These responses involve different types of ion channels including non-selective, potassium- and chloride-selective ion channels that can respond to either one or several mechanical stimuli.

The TRPC channels have recently been implicated in pressure sensing [1–7]. TRP channels are made of six transmembrane segments and a single pore (P) domain located between segments 5 and 6. Both the amino and carboxy terminal domains face the cytosol. There are 33 TRP channel genes in mammals subdivided into six subfamilies based on homology: TRPC (seven members), TRPM (eight members), TRPV (six members), and the more distantly related TRPML (three members), TRPP (eight members), and TRPA (one member) (for a review and more details, see [8–10]). These subunits associate as tetramers with possible heteromultimerization.

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Most TRP channels are non-selective cation channels permeable to  $\text{Ca}^{2+}$  and have been implicated in a large variety of sensory functions such as temperature sensing [11]. Several TRP channels including TRPA1, TRPC1, TRPC6, TRPV1, TRPV2, TRPV4, TRPM4, TRPM7 and TRPP2 have also recently been implicated in force sensing (for recent reviews see [7,9,12]). Indeed, TRPC6, TRPM4 and TRPV2 have been implicated in the osmotic and stretch sensitivity of arterial myocytes [3,13,14]. However, a direct role for any of these TRP channels in mechanosensory transduction remains highly controversial and the molecular basis of SACs (i.e. channels which are directly gated by force) still remains obscure (see [4,7,15]).

Nevertheless, the function of TRP subunits in mechanotransduction appears to be conserved during evolution. For instance, in yeast, Yvc1p, a vacuolar membrane protein that shows homology to TRPV channels, was shown to be responsible for hyperosmolarity-induced  $\text{Ca}^{2+}$  release [16–18]. Yvc1p is mechanosensitive and pressure at tens of millimeters of Hg activate this channel [17,18]. Moreover, a role for TRP channels in touch sensing or hearing has also been established through genetical approaches using model systems such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila* (for review see [9,19]).

In the present review, we will summarize the evidence that Gq-coupled receptors are directly activated by mechanical stress without the involvement of their ligands. Activation of these mechanoreceptors by pressure leads to the opening of receptor-operated diacylglycerol (DAG)-sensitive TRPC channels resulting in cell depolarization. We will discuss the possibility that this novel mechanosensitive pathway is implicated in a variety of cardiovascular pathologies including ventricular hypertrophy and atrial fibrillation, focusing on the angiotensin II type 1 receptor and the TRPC6 subunit.

## 1. The diacylglycerol-sensitive TRPC6 channel

All TRPCs, besides the pseudogene TRPC2, have been identified in cardiac myocytes, vascular smooth muscle and endothelial cells (for recent reviews see [20,21]). TRPC3/6/7 form a structural and functional subfamily with 70–80% homology. TRPC6 assembles either into homo or heterotetramers with TRPC3 and TRPC7. Members of the TRPC family contain four N-terminal ankyrin repeats and several protein binding-domains. Indeed, TRPC6 interacts with a number of adaptor proteins including cytoskeletal proteins (for review see [22]).

TRPC6 has a ubiquitous expression with high levels in vascular smooth muscle cells and in cardiomyocytes [3,23]. In lung tissues and pulmonary arterial smooth muscle cells (PASMCS) obtained from patients suffering from primary pulmonary artery hypertension, the mRNA and protein expression of TRPC6 is increased compared to those from normotensive or secondary pulmonary hypertension patients [24]. Moreover, PDGF-mediated proliferation of PSMC is associated with c-Jun/STAT-3-induced upregulation of TRPC6 expression [25]. In the heart, immunolabeling experiments localized TRPC6 in T-tubular membranes of cardiomyocytes [5,6].

TRPC6 is a 28–37 pS inwardly and outwardly rectifying channel, with a relatively low selectivity for  $\text{Ca}^{2+}$  over  $\text{Na}^{+}$  ( $\text{PCa}^{2+}/\text{PNa}^{+}$ : 5). It shows a very low basal activity and is a receptor-operated channel (ROC). Indeed, TRPC6 has been shown to underlie the  $\alpha$ 1-adrenoceptor-activated non-selective cation channel in vascular smooth muscle cells and the vasopressin-activated cation channel in the aorta [23,26]. TRPC6 is directly activated by DAG, independently of protein kinase C [27] (also a feature of TRPC3, TRPC7, TRPC2 and TRPC5) and is stimulated by intracellular  $\text{Ca}^{2+}$  via a calmodulin-dependent mechanism. Its activity is reduced by protein kinase C-mediated serine phosphorylation, but stimulated by Fyn tyrosine kinase. Pharmacologically, TRPC6 is opened by flufenamate, but inhibited by the non-selective SAC blockers  $\text{La}^{3+}$ ,  $\text{Gd}^{3+}$ , GsMTx4 [28,29].

Surprisingly, TRPC6 knock-out mice show an elevated blood pressure, enhanced agonist-induced contractility of isolated aortic

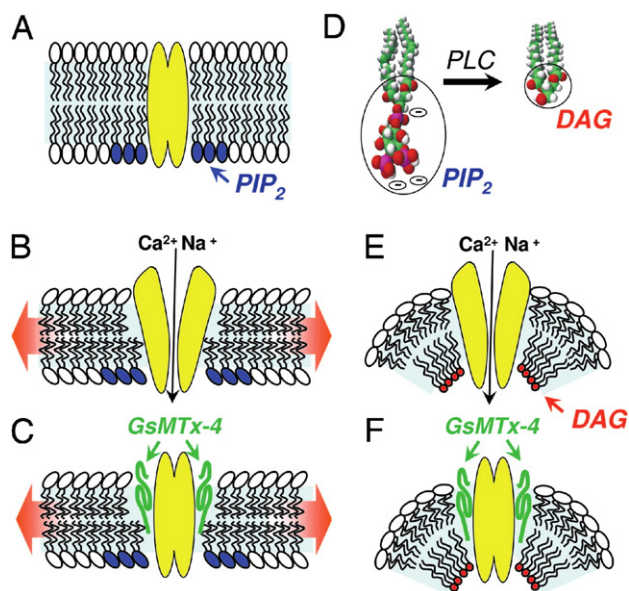
rings, and enhanced myogenic contractility in cerebral arteries [30]. Furthermore, TRPC3 expression is significantly increased in smooth muscle cells from TRPC6<sup>-/-</sup> mice. These cells have a higher basal cation entry and consequently a more depolarized membrane potential [30]. This depolarizing current is abolished by TRPC3 specific siRNA [30]. These results indicate that constitutively active TRPC3 channels are up-regulated in TRPC6<sup>-/-</sup> mice. Remarkably, the acute hypoxic vasoconstriction of pulmonary arteries, unlike the sustained contraction, is completely blunted in TRPC6<sup>-/-</sup> mice [31], indicating a specific involvement of TRPC6.

In humans, gain of function mutations of TRPC6 which increase  $\text{Ca}^{2+}$  influx lead to focal and segmental glomerulosclerosis (FSGS) in the kidney [32,33]. TRPC6 was identified as an essential component of the glomerular slit diaphragm, where it interacts with podocin [34]. Mutations in podocin similarly cause disruption of the kidney filter and focal segmental glomerulosclerosis. Finally, the OAG-induced currents are significantly augmented in oocytes coexpressing TRPC6 and podocin [34]. These observations indicate that increased activity of TRPC6 is associated with FSGS.

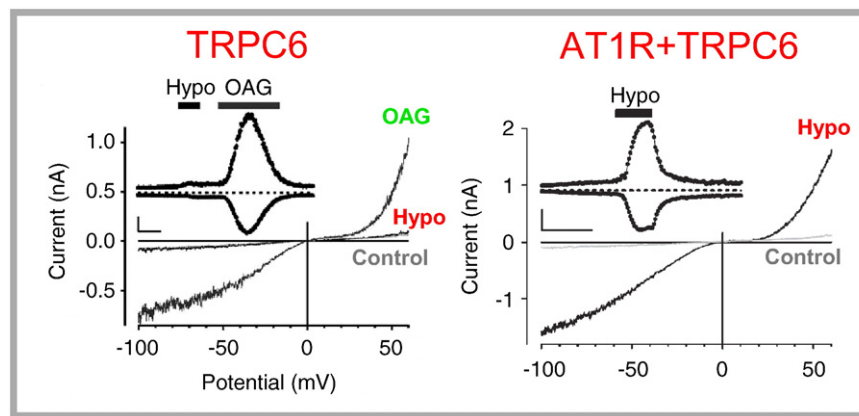
## 2. TRPC6 and arterial mechanotransduction

TRPC6 has recently been implicated in arterial pressure sensing [3,4]. Antisense oligonucleotides to TRPC6 decrease TRPC6 protein expression and attenuate arterial smooth muscle depolarization and constriction caused by elevated intraluminal pressure in cerebral arteries [3]. Suppressing the expression of TRPC6 also reduces the amplitude of the current elicited by cell swelling using a hypotonic solution [3].

TRPC6 has thus been proposed to be a direct sensor of both mechanically and osmotically induced membrane stretch [1,3] (Fig. 1). Pressure-induced activation of TRPC6 in transiently transfected HEK 293 or CHO cells was recorded in the presence of the phospholipase C inhibitor U73122 [1]. The stretch and OAG-mediated opening of TRPC6 is inhibited by the tarantula peptide, GsMTx-4, known to specifically inhibit mechanosensitive channels by modifying the external lipid-channel boundary [1] (Fig. 1).



**Fig. 1.** Model of TRPC6 activation by stretch or diacylglycerol and inhibition by the tarantula peptide, GsMTx-4. (A) The resting closed state of TRPC6. (B) Stretch induces a conformational change in TRPC6, resulting in its transition to the open state. (C) GsMTx-4 peptide inserts in the outer membrane leaflet and inhibits stretch-induced opening of TRPC6. (D) Receptor activation of phospholipase C hydrolyzes PIP<sub>2</sub> and releases DAG. (E) DAG in the inner leaflet induces a convex membrane curvature and opening of TRPC6. (F) GsMTx-4 inhibits channel opening by DAG. Figure from [1].



**Fig. 2.** Co-expression of TRPC6 with the AT1R yields a mechano- and osmo-sensitive current in the absence of angiotensin II. Whole cell currents from TRPC6 alone (left panel) or co-transfected TRPC6 and AT1R HEK293 cells (right panel). The inset shows the inward at outward currents induced by hypoosmotic (hypo) and OAG stimulation at  $\pm 60$  mV. In the absence of AT1R overexpression, there is no stimulation of current amplitude by cell swelling, although channels can be opened by OAG. The figure is modified from [4].

However, a more recent study performed in COS, CHO and HEK cells transiently transfected with TRPC6 failed to confirm a “direct” mechanosensitivity of this channel [4,15]. Instead, these findings show that TRPC6 *per se* is not mechanosensitive and that, in contrast to the initial claim [1] (Fig. 1), membrane stretch does not primarily gate TRPC6 [4]. Nevertheless, when co-expressed with the AT1R receptor, TRPC6 can be dramatically stimulated by cell swelling or positive pressure even in the absence of the agonist angiotensin II [4] (Fig. 2). There is no evidence for a direct protein interaction between TRPC6 and AT1R. Importantly, other Gq/11-coupled receptors including M5R, H1R, ETAR and V1AR can substitute for AT1R, whereas Gs-coupled receptors such as the  $\beta_2$ -adrenergic receptor as well as tyrosine kinase receptors do not link mechanical stimuli to TRPC6 activation. Importantly, other DAG-sensitive TRPC channels TRPC3 or TRPC7 can replace TRPC6 and be activated by osmotic stimuli when co-expressed with AT1R [4].

An agonist-independent activation of Gq/11-coupled receptors, which subsequently signals to TRPC channels in a G protein- and phospholipase C-dependent manner is involved in SAC activity [4]. Indeed, channel activation by stretch or swelling is blocked by PLC inhibition (contrasting with the initial study see [1]) or by GDP $\beta$ s, which impairs G protein activation [4,35], but opened by diacylglycerol [35]. Mechanoactivation of AT1R is inhibited by inverse agonists such as losartan, but not by neutral antagonists [4,36,37]. Remarkably, increasing the AT1 receptor density in mechanically unresponsive A7R5 cells renders these cells mechanosensitive [4]. It has not yet been determined whether increasing the expression of Gq alone may also result in an enhanced mechanosensitivity of TRPC6. Because the expression patterns of TRPC3 and TRPC6 overlap in vascular cells, a heteromeric TRPC3/TRPC6 channel complex has been proposed to be the native molecular correlate of the mechano-gated non-selective cation channel in arterial myocytes [30].

The myogenic tone of cerebral and renal arteries is profoundly diminished by the inverse angiotensin II AT1 receptor agonist losartan, independently of angiotensin II secretion, suggesting that such a mechanism may be involved in arterial autoregulation [4]. These findings further demonstrate that Gq/11-coupled receptors function as sensors of membrane stretch in VSM cells and induce TRPC channel opening, cell depolarization in cerebral and renal arteries and consequent myogenic contraction [4]. Nevertheless, it should be noted that native Gq-coupled receptors are endogenously present in every cell type, including HEK and A7R5 cells. However, in the absence of AT1R overexpression, there is no activation of TRPC6 by membrane stretch [4,15]. Therefore, it remains to be determined whether mechanical activation of AT1R receptor is physiologically relevant and not only observed in experimental conditions where its expression is abnormally high.

### 3. Conformational switch of AT1R by mechanical stress

The AT1R is a typical member of the G protein-coupled receptor (GPCR) family, the structure of which is characterized by seven-transmembrane spanning helices with an extracellular N-terminus and a cytoplasmic C-terminus. The AT1R can be activated by mechanical stress through an angiotensin II-independent mechanism [4,36,37]. Mechanical stress of AT1R not only activates extracellular-signal-regulated kinases and increases phosphoinositide production *in vitro*, but also induces cardiac hypertrophy [37]. Importantly, AT1R-mut1 receptor, a mutated form of the AT1R which cannot bind AngII, can also be activated by mechanical stress [37]. Moreover, stretch activates ERKs in cardiomyocytes prepared from angiotensinogen (ATG)<sup>-/-</sup> mice, which do not express AngII, while candesartan inhibits this activation [37].

Mechanically activated receptors adopt an active conformation, allowing for productive G protein coupling and recruitment of  $\beta$ -arrestin-2 as demonstrated by bioluminescence resonance energy transfer technique [4]. Again mechanoactivation of AT1R is inhibited by the inverse agonist of the AT1R candesartan and losartan.

Cell stretch induces an anticlockwise rotation and a shift of transmembrane domain (TMD) 7 into the ligand-binding pocket [36] (Fig. 3). As an inverse agonist, candesartan suppresses the stretch-induced helical movement of TMD7 through the binding of its carboxyl group to specific residues of the receptor [36]. The tight binding of candesartan to the AT<sub>1</sub> receptor stabilizes the receptor in the inactive conformation, preventing its shift to the active conformation (Fig. 3).

These results provide compelling evidence that the AT1R shows a conformational switch upon mechanical stimulation, leading to receptor activation in the absence of the ligand AngII [4,36,37].

Importantly, the SAC blocker GsTMx4 does not prevent AT1R stimulation by stretch [36]. It is not yet clear how the mechanical force is transmitted to the AT1R. It might be possible that the increased tension in the membrane causes its thinning and triggers the tilting of TM7 in the AT1R to avoid hydrophobic mismatch [36].

### 4. TRPC6 in cardiac mechanotransduction

The heart, which is linked to force generation, is in turn sensitive to both external and internal mechanical forces. In a classic example, the heart can be stopped by an external impact applied to the chest that does not cause tissue damage, a phenomenon first described more than 100 years ago as *commotio cordis* (for review see [38]). Internal feedback such as a heart-rate response to lung filling and the observation that catheterization can induce ectopic beats when the

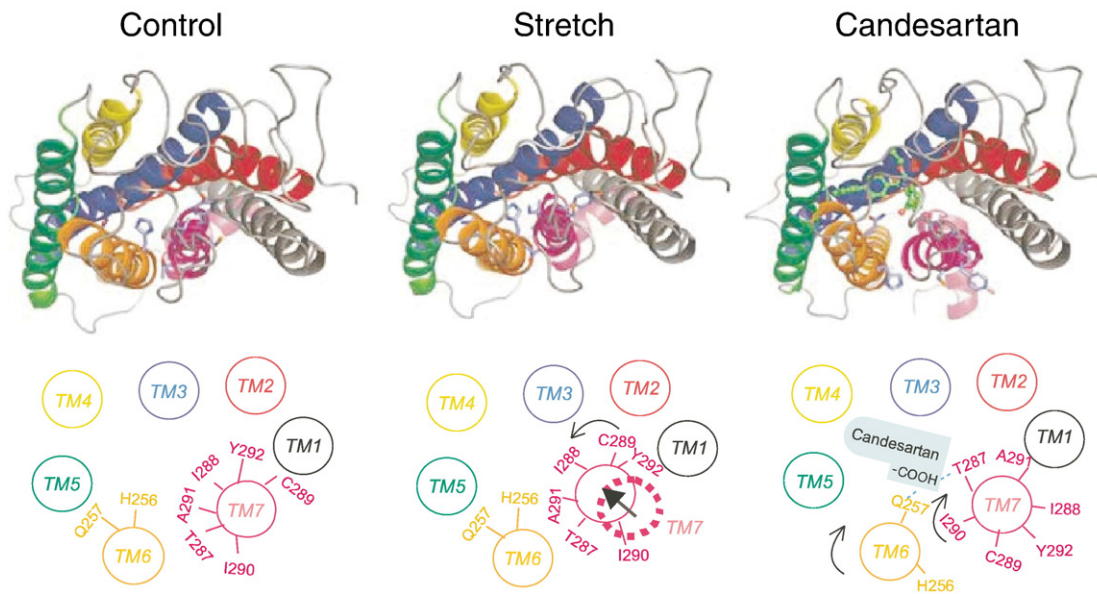


Fig. 3. Molecular model of stretch-induced changes in the conformation of the AT1R and inhibition by the inverse agonist candesartan. Figure is modified from [36].

catheter-tip approaches the endocardium further underscores the importance of mechanoelectric feedback (MEF) in cardiac function (for review see [39–41]). However, we are only beginning to appreciate the subtle effects of force on cardiac function and the relevance for pathology. Both hyperpolarizing  $K^+$ -selective channels and depolarizing non-selective SACs are implicated in cardiac MEF [42,43]. Stretch depolarizes the resting membrane potential of

ventricular myocytes, accelerates the initial action potential repolarization phase and retards the later repolarization phase [44]. Often stretched myocytes generate pacemaker-like depolarizations that lead to extra systoles [44]. Recently, evidence has pointed towards TRPC6 channels as possible players in cardiac cell mechanotransduction [5,6].

At negative membrane potentials, stretch of mouse ventricular cardiomyocytes activates a nearly voltage-independent  $Gd^{3+}$  and

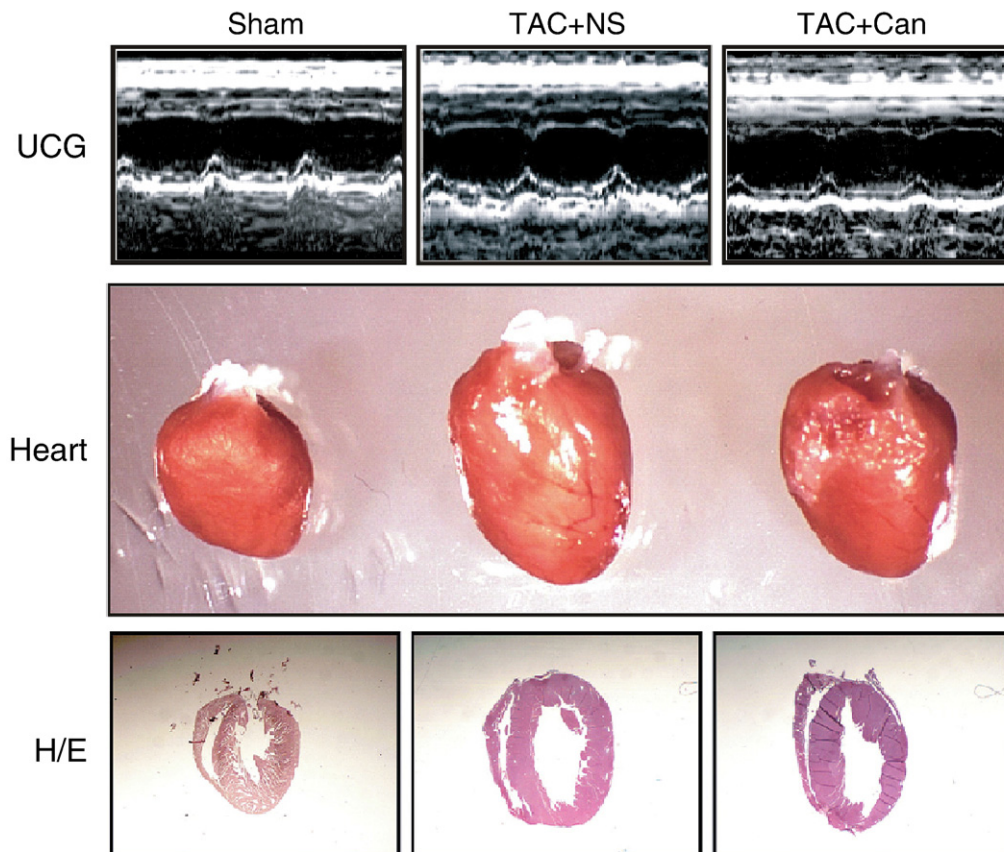


Fig. 4. Pressure overload (TAC)-induced cardiac hypertrophy in  $ATG^{-/-}$  mice. Mice were treated with saline (NS) or candesartan (Can). Top, M-mode echocardiograms, middle, gross appearance of the heart, bottom, sections stained by H and E. Figure modified from [37].

GsMTx-4-sensitive non-selective cation conductance [6]. Antibodies against TRPC6 prevent activation of this mechanosensitive conductance. Stretch-induced changes in membrane currents are absent in cells whose T-tubules had been removed. In absence of stretch, this current can also be activated by OAG and other amphipaths or with the TRPC6 opener flufenamate [6]. These findings further indicate that in ventricular cardiac cells, TRPC6 is also involved in the stretch-sensitive depolarizing current. It remains to be determined whether this stretch-activated current is sensitive to the AT1R inverse agonists as previously described in vascular smooth muscle cells [4].

The stretch-induced increase in force production of ventricular muscle is biphasic [5]. An abrupt increase in force coincides with the stretch, which is then followed by a slower force response (SFR) that develops over minutes [5]. The SFR is accompanied by a slow increase in the magnitude of the intracellular  $Ca^{2+}$  transient. Application of  $GdCl_3$  or GsMTx-4 reduces the magnitude of the SFR, suggesting that SACs possibly including TRPC6 are major contributors of the ventricular physiological SFR [5].

Recent findings also implicate TRPC6 in cardiac disease states including hypertrophy and possibly atrial fibrillation as discussed in the following sections.

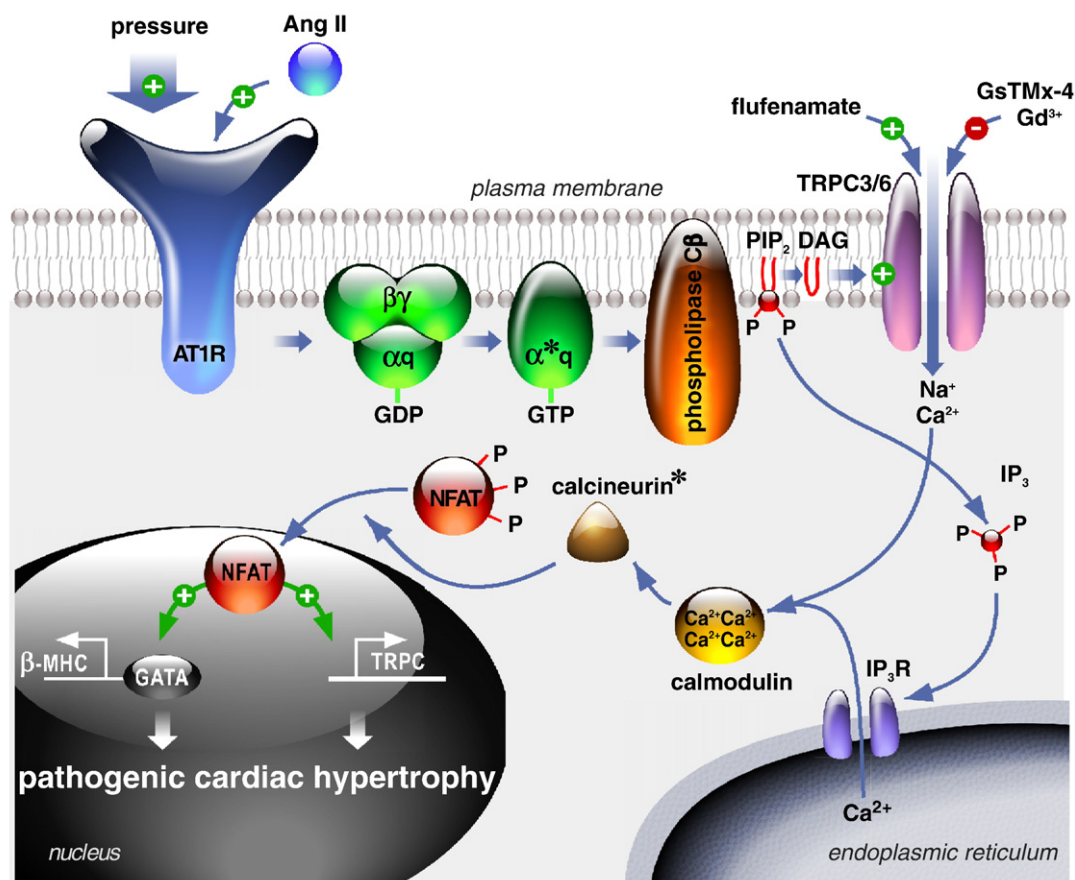
### 5. TRPC6 and ventricular hypertrophy

The heart responds to injury and chronic pressure overload by pathological growth and remodeling, which frequently result in heart failure and sudden death. TRPC6 is up-regulated in mouse hearts in response to activated calcineurin and pressure overload, as well as in

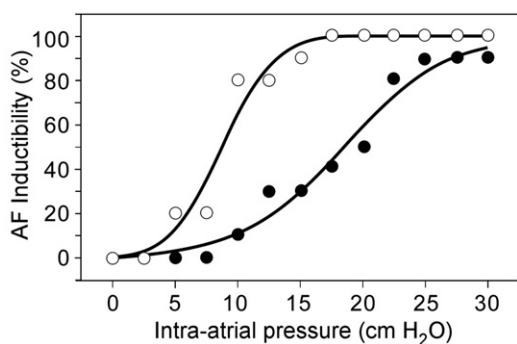
failing human hearts [45]. Two conserved NFAT consensus sites in the promoter of the *TRPC6* gene confer responsiveness to cardiac stress [45]. Moreover, cardiac-specific overexpression of *TRPC6* in transgenic mice results in heightened sensitivity to stress, a propensity for lethal cardiac growth and heart failure, and an increase in NFAT-dependent expression of beta-myosin heavy chain, a sensitive marker for pathologic hypertrophy [45] (Fig. 5). However, in this study the expression of other TRPC subunits, including TRPC3 which may possibly be altered in the TRPC6 transgenic hearts as observed in the knock-out TRPC6 mouse, has not been analyzed. The DAG-induced  $Ca^{2+}$  signaling pathway through TRPC6 is also essential for AngII-induced NFAT activation and cardiac hypertrophy [46]. All together, these findings implicate TRPC6 as a positive regulator of calcineurin-NFAT signaling and a key component of a  $Ca^{2+}$ -dependent regulatory loop that drives pathological cardiac remodeling [45,46] (Fig. 5).

Additionally, a gain-of-function phenotype of TRPC3 similarly induces increased NFAT activation *in vivo*, cardiomyopathy and increased hypertrophy after pressure overload, which could be blocked by disruption of the *calcineurin*  $\beta$  gene [47]. Since both TRPC3 and TRPC6 associate, a heteromeric TRPC3/6 channel might therefore be at play in cardiac hypertrophy (Fig. 5). Other TRP subunits, including TRPC1, may additionally be involved in the cardiac hypertrophic response (for a recent review see [20]).

Interestingly, mechanical stress can induce cardiac hypertrophy *in vivo* through the AT1R in the absence of AngII, because pressure overload induced cardiac hypertrophy in ATG-deficient mice, as well as in wild-type mice, an effect that was significantly inhibited by candesartan [37] (Fig. 4). Therefore, mechanical activation of the



**Fig. 5.** Cartoon illustrating the role of TRPC3 and TRPC6 in cardiac hypertrophy. The AT1R stimulated by mechanical stress (i.e. tension in the membrane) or by its ligand AngII, leads to  $PIP_2$  hydrolysis. DAG directly activates TRPC channels and  $IP_3$  releases  $Ca^{2+}$  from the internal stores. Activation of calcineurin by  $Ca^{2+}$ -calmodulin dephosphorylates NFAT which then translocates into the nucleus and turns on pro-hypertrophic genes including *TRPC6*.



**Fig. 6.** GsTMx-4 inhibits pressure-induced atrial fibrillation. Using perfused rabbit hearts, fibrillation was initiated with a burst of high-frequency stimulation at different right-atrial pressures. GsTMx4 (black dots) reduces the inducibility (and duration) of pressure-induced AF. The figure is modified from [51, 52].

AT1R, independently of AngII, is anticipated to result in TRPC3/6 opening, NFAT activation and cardiac hypertrophy [36,37,45,46].

### 6. SACs and atrial fibrillation: a role for TRPC6?

AF is the most common type of cardiac arrhythmia and is a major risk factor in cardiovascular disease and stroke [48]. It occurs when contractile waves do not propagate correctly through the atria, and instead of exerting a concerted contraction, the muscle cells quiver. The disrupting impulses, termed simultaneous reentrant wavelets, lead to occasional (paroxysmal) or chronic alteration of atrial contraction [48,49]. During fibrillation, the atria do not fully clear the blood from the heart, allowing coagulation and the formation of clots and other health problems including stroke. Using multielectrode catheters to track the spatial and temporal progress of wavelets after the onset of fibrillation, several foci have been identified as the starting points of wavelets [50].

The study by Haissanguerre and colleagues [50] the pulmonary vein (PV), the vessel which carries oxygenated blood from the lungs to the heart, as the source of the majority of the wavelets, with some even originating from several centimeters up in the vein. Indeed, electrical isolation of the PV can inhibit the arrhythmia, further underscoring the importance of the PV in the generation of AF [50].

Several clues have recently emerged pointing to a role for SACs in AF. First, known inhibitors of SACs, the tarantula toxin GsMTx-4 as well as  $Gd^{3+}$ , inhibit AF (Fig. 6) [51,52]. Second, an increase in intra-atrial pressure leads to an increase in wavelet activity emerging from the PV towards the atrium [53]. Third, stretch applied to isolated PV increases the rate of spontaneous electrical activity, an effect reduced by  $Gd^{3+}$  [54]. Fourth, mechanically stretching PV single cardiomyocytes induces an immediate  $Gd^{3+}$  and streptomycin-sensitive depolarizing non-selective current [55].

All together, these findings led to the suggestion that the following scheme may be considered in the etiology of AF: atrial dilation of the PVs → activation of the  $Gd^{3+}$ - and GsTMx4-sensitive TRPC6-like stretch-activated cation channels → depolarization of PV cardiomyocytes → ectopic activity in PVs → paroxysmal AF → ionic remodeling of the atrium → chronic AF [39–41,55].

Although the role of SACs in AF is substantiated by these findings, the specific implication of TRPC6 remains to be established.

### 7. Conclusions

These recent findings indicate that Gq-coupled receptors, independently of their ligands, act as sensors of mechanical stress, leading to  $PIP_2$  hydrolysis, DAG production and opening of TRPC6. This mechanosensitive molecular pathway may be implicated in the myogenic arterial response to intraluminal pressure. Moreover, in the heart, stretch-induced opening of TRPC6 is involved in load-

induced hypertrophy and possibly AF. TRPC6 may act in concert with other DAG-sensitive TRPC subunits such as TRPC3 or TRPC7. The respective role of homomultimers or heteromultimers in the mechanical response of the heart has to be determined.

Multiple questions remain to be answered: is there linearity between AT1R expression and mechanosensitivity? Is there an allosteric response, such that two “types” of mechanosensitive responses are observed, one expected for the ligand-bound receptor, and another for the unbound receptor? Is there a single class of non-selective SACs? Are there non-selective channels directly activated by force? Is the activating force coming from the bilayer, from the cytoskeleton or both?

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