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Angiotensin II: a regulator of cardiomyocyte function and survival

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TABLE OF CONTENTS

1. ABSTRACT

Angiotensin II (Ang II) has been recognized as an important myocardial inotropic modulator, but the subtleties of the signalling pathways involved remain to be fully elucidated. The inotropic effect of Ang II reflects the net outcome of competing positive and negative signalling mechanisms. In pathophysiological states such as heart failure, characterized by chronic exposure to elevated Ang II, the balance of inotropic influences could be shifted to unmask negative inotropism linked with p38 MAPK activation. Coincident with loss of inotropic balance, Ang II-mediated morphologic remodelling of the heart and apoptosis are observed and accepted to play a crucial role in contractile dysfunction and transition to heart failure. Both Ca^{2+}-dependent and independent pathways appear to be important, and we highlight Ang II mediated CaMKII activation as a potential key integrator of these two pathways in apoptosis induction in the failing heart. To identify new therapeutic molecular targets in heart failure, further work is required to clearly establish the signalling events involved in Ang II inotropic and apoptotic signalling and the potential link between them.

2. INTRODUCTION

The octapeptide angiotensin II (Ang II) is the effector molecule of the renin-angiotensin system. The circulating renin-angiotensin system consists of angiotensinogen, which is cleaved by the proteolytic enzyme renin, to form angiotensin I, a decapeptide subsequently converted to the active peptide Ang II by the angiotensin-converting enzyme (ACE). This biologically active peptide, initially identified as a powerful vasopressor, was discovered simultaneously in 1940 by Braun Menéndez *et al.* (1) in Argentina who named the substance hypertensin and Page and Helmer (2), in the USA who termed it angiotonin. In 1958, the two groups settled to rename this mediator angiotensin. From these foundational studies, Ang II has become one of the most studied regulatory peptides in cardiovascular pathophysiology (3). It is well established that Ang II is implicated in volume and electrolyte homeostasis, vascular smooth muscle contraction (4), and the pathogenesis of hypertension (5). In the heart, Ang II is involved in vascular dysfunction and the progression of coronary heart disease (6).

The myocardial trophic actions of Ang II have also been extensively studied. There is evidence that Ang II exerts myocardial influence by endocrine, paracrine and intracrine action (7). *In vitro* and *in vivo* studies indicate that Ang II plays a crucial role in facilitating cardiac hypertrophy. Ang II initiates cardiomyocyte growth and stimulates protein synthesis in culture (5, 8-11). Ang II also stimulates fibroblast proliferation and secretion of various other growth factors, and when these cell types are in communication the hypertrophic effects of Ang II are reinforced. Experimental *in vivo* administration of Ang II induces expression of the so-called 'immediate early genes' (c-fos, c-jun, jun-B Egr-1) and the cardiac hypertrophic marker genes including β-myosin heavy chain, skeletal α-actin and atrial natriuretic peptide (5, 6). The well characterized hypertrophic signalling pathway operates through the Gαq/Gα11 family of G-proteins and involves activation of phospholipase C (PLC) (12-14). The PLC-mediated generation of 1,2,-diacylglycerol (DAG) promotes activation of the mitogen-activated-protein-kinases (MAPKs) including the extracellular receptor kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. The PLC-mediated generation of inositol 1,4,5-trisphospate (IP_3) facilitates IP_3 receptor – Ca^{2+}-calmodulin kinase II complex formation, transducing the hypertrophic signal to the nucleus in a process which has been termed 'excitation-transcription' coupling - highlighting the link between workload and growth in the cardiomyocyte (15).

Systemic infusion of Ang II experimentally induces cardiac hypertrophy which may or may not be independent of hemodynamic action (16-18). Clinically, an association between plasma Ang II levels and left ventricular size (independent of blood pressure and body size), suggests an endogenous cardiac trophic action of Ang II independent of hemodynamic status (19). An intracardiac renin-angiotensin system has been delineated (20), and cardiac Ang I and Ang II levels in the heart can be several-fold higher than levels detected in plasma (21). Whether renin is produced to an appreciable extent in the heart, or is sequestered from the circulation has been debated (22). Importantly, chronic local cardiac elevation of Ang II production achieved by genetic manipulation in a normotensive murine model is associated with cardiomyocyte hypertrophy, excitation-contraction coupling dysfunction and eventual progression to failure (23). This finding firstly demonstrates that Ang II induces hypertrophy in the absence of cardiac loading, and secondly confirms that Ang II has a crucial role in modelling cardiomyocyte excitation-contraction coupling independent of systemic hemodynamics. Although Ang II has long been recognized as a key regulator of myocardial inotropy, an understanding of how prolonged exposure to this peptide may be involved in the contractile demise and cardiomyocyte loss associated with the transition to failure is only recently emerging.

This review examines the evidence which characterizes Ang II as a mediator of both positive and negative inotropy in the heart. The complexity of the signalling pathways involved and the multiple cellular effectors of the inotropic responses are considered in detail. The role of Ang II as an apoptotic inducer is also evaluated – and two parallel signalling pathways contrasted. The Ang II-dependent links between Ca^{2+} dysregulation impacting contractile function and Ca^{2+} dysregulation as an apoptotic trigger are discussed. In particular the potential importance of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) in integrating both inotropic and apoptotic signalling pathways is examined.

3. ANG II AND CARDIAC CONTRACTILITY

Ang II is well characterized as a potent vasoconstrictor. Thus, by modulation of cardiac preload and afterload, systemically elevated Ang II can profoundly influence myocardial cardiac function. There is however extensive evidence indicating that Ang II has a role in direct modulation of myocardial contractility acting at the cardiomyocyte level, and these actions are the primary focus of the present review. Ang II has been shown to elicit a wide range of direct effects on cardiac contractility (24-35). Among these, Ang II has been reported to increase (24-30, 32), not change (28, 31) or even decrease (33, 36) cardiac inotropism. In part these diverse actions of Ang II could arise from interspecies variation (28) and/or the different experimental conditions used to assess the cardiac modulatory actions of this peptide. For instance, it has been shown that Ang II exerts a positive inotropic effect in rat myocardium at muscle lengths that are on the ascending limb of the Frank-Starling curve, but has negative inotropic actions at muscle lengths associated with maximum force (36). Binding studies have revealed the presence of a number of Ang II receptor subtypes in various tissues (37). In the heart at least two pharmacologically distinct Ang II receptors of the G-protein coupled receptor subtype have been described, namely, the AT_1 receptor, sensitive to inhibition by losartan (38), and the AT_2 receptor, inhibited by PD12377 (39). Although both receptor subtypes coexist in the heart, the relative proportion in which they are expressed varies among the different species. Rat and rabbit heart contain equal amounts of both receptor subtypes (40-42), whereas bovine and simian heart tissues contain mainly AT_1 receptors (40, 43). In the human myocardium AT_2 expression levels are reported to be at least comparable to AT_1 levels and in some pathological contexts AT_2 is the predominantly expressed subtype (43, 44), with fibroblast AT_2 expression particularly pronounced (45). A novel type of angiotensin receptor has also been reported and named the AT_4 receptor (46). This receptor has been identified in cardiac preparations (47), and has been shown to have high affinity for the hexapeptide metabolite of Ang II, commonly known as Angiotensin IV (Ang IV). However, the effects of the Ang IV/AT_4 receptor activation have not been delineated in the heart.

Ang II-induced inotropism mediated by the AT_1 receptor is well established. Selective blockade of the Ang II induced positive inotropic effect by losartan, but not by PD12377, has been observed in cardiac preparations from various mammalian species, suggesting that the AT_1 but not the AT_2-receptor subtype is involved in the Ang II-induced positive inotropic effect (48-50). In some settings (see below) Ang II elicits a negative inotropic response.

The notion of an AT_2-mediated negative inotropy in the myocardium is attractive (ie an influence to offset the AT_1-mediated positive inotropy), but to date experimental evidence to support a direct Ang-II-activated AT_2-mediated inotropic effect is lacking. In a murine model of AT_2 overexpression, Ang II has been observed to elicit a negative inotropic effect, but this effect was not blocked by PD12377 (51). In rabbit papillary muscles selective AT_2 stimulation associated with negative inotropy has also been observed, but this response was attributed to endocardial NO release (52). In our experiments with rat myocytes/myocardium, we have been unable to inhibit an Ang II-induced negative inotropic effect with the AT_2 receptor blocker, PD12377 (unpublished observations), indicating that AT_1-receptor activation is also implicated in the negative inotropic response induced by Ang II. These seemingly contradictory results, where both negative and positive inotropic actions of Ang II result from AT_1 receptor activation, suggests that the AT_1 receptor can couple to distinct signal transduction pathways with opposing effects on cardiac excitation and contraction coupling, and the final inotropic outcome reflects the result of a complex balance between opposing pathways.

The subcellular mechanisms involved in the Ang II-induced positive inotropic effect have been examined in detail (27, 28, 34, 35, 48, 49, 53). However, those involved in the negative contractile effect of the peptide, as observed in the rat and mouse heart (31, 33, 54-56), have received less attention. Understanding the signalling events underlying both positive and negative limbs of Ang II-inotropy is crucial for a more complete comprehension of the mechanisms that modulate cardiac contractility in health and disease. In the setting of progressive myocardial pathology, Ang II-induced positive inotropic effect could serve as an initial compensatory mechanism contributing to the maintenance of adequate cardiac output during the early stage of disorder. However, in more advanced disease states, an Ang II-mediated negative inotropic effect may contribute to the decline of cardiac function observed in the failing heart. Consistent with this hypothesis are observations that Ang II exacerbates contractile dysfunction in failing hearts of rats and dogs (25, 57). Numerous studies have reported that the inhibition of the angiotensin-converting enzyme or treatment with the AT_1-receptor blocker, losartan, attenuates cardiac dysfunction associated with heart failure (57, 58). Recent evidence showing that Ang II contributes to deterioration of cardiac function independently of hemodynamic changes (59) suggests that the beneficial effect provided by these inhibitors could be at least in part due to the inhibition of the negative inotropic effect elicited by Ang II.

3.1. Signal transduction: from receptors to target proteins

At present, the general consensus is that the majority of the functional cardiac actions of Ang II are mediated by the activation of the β isoenzymic form of PLC (PLC_β) and the subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate to form two second messengers, IP_3 and DAG. IP_3 has been shown to release Ca^{2+} from intracellular stores in several tissues (28, 61) and could thus be a possible mediator underlying the Ang II-induced positive inotropic effect. However, IP_3-induced Ca^{2+} release remains a controversial mechanism in cardiac muscle (60-63). In addition to a PLC activation, there has been reports of an Ang II mediated inhibition of myocardial adenylyl cyclase activity (64), but this effect has not been associated with functional consequence. Thus at present DAG appears to be the most likely candidate involved in mediating the Ang II contractile responses. Indeed DAG has been shown to activate PKC, an enzyme that catalyzes the phosphorylation of several cellular proteins implicated in cardiac excitation-contraction coupling. Among these, PKC has been shown to phosphorylate and modulate the L-type Ca^{2+} channels (65), the Na^+/H^+ exchanger (66-68), the Na^+/Ca^{2+} exchanger (69), NAD(P)H oxidase (70), MAPK's (71) and selected contractile proteins (72, 73). Considering that these multiple effector molecules have the potential to induce both positive and negative inotropic actions, understanding how Ang II modulates each to impact excitation-contraction coupling is not straight forward. In the discussion to follow the subcellular mechanisms postulated for the opposing inotropic actions induced by Ang II are considered in detail.

3.2. Possible mechanisms underlying the Ang II-induced positive inotropic effect

The current available evidence indicates that the Ang II-induced positive inotropic effect may be mediated by two basic mechanisms: (1) An increase in cytosolic Ca^{2+} concentration (35, 53, 74, 75) and/or (2) an increase in myofilament responsiveness to Ca^{2+} (27). Which of these two mechanisms are responsible or if both are required for the Ang II-induced positive inotropic effect may be context specific, and the extent to which each of these mechanisms is operative appears to be species-dependent. An Ang II-induced increase in the intracellular Ca^{2+} transient amplitude has been reported both in the intact heart (76) as well as in isolated myocyte preparations of several species (35, 53, 74, 75). Different mechanisms have been proposed to account for the increase in intracellular Ca^{2+}: stimulation of the L-type Ca^{2+} channel (29, 35, 77, 78), the Na^+/Ca^{2+} exchanger (53, 75) and IP_3 receptor activation (25). We have shown in cat myocytes that the positive inotropic effect of Ang II is preserved in the presence a specific blocker of reverse mode Na^+/Ca^{2+} exchange (KB-R4739), and in the presence of an IP_3-receptor blocker (2APB), suggesting that Na^+/Ca^{2+} exchanger-mediated Ca^{2+} entry and IP_3-induced Ca^{2+} release from the SR are not the major sources of Ca^{2+} recruited by Ang II to enhance contractility (34, 35). In contrast, we and others have demonstrated consistent and reproducible increases in the Ca^{2+} current (I_{Ca}) evoked by Ang II, using conditions that preserve intracellular environment such as the perforated patch-clamp technique (35, 79, 80). In agreement with these results and underscoring the central role of I_{Ca}, Talukder and Endoh, using pharmacological tools, concluded that the influx of Ca^{2+} through the L-type Ca^{2+} channels was one of the main mechanisms by which Ang II increases cytosolic Ca^{2+} and thus, contractility (81) (Figure 1). Interestingly, the positive inotropic effect induced by low concentrations of Ang II (< 1 nM) seems to be mediated by a different mechanism, although also dependent on an increase in

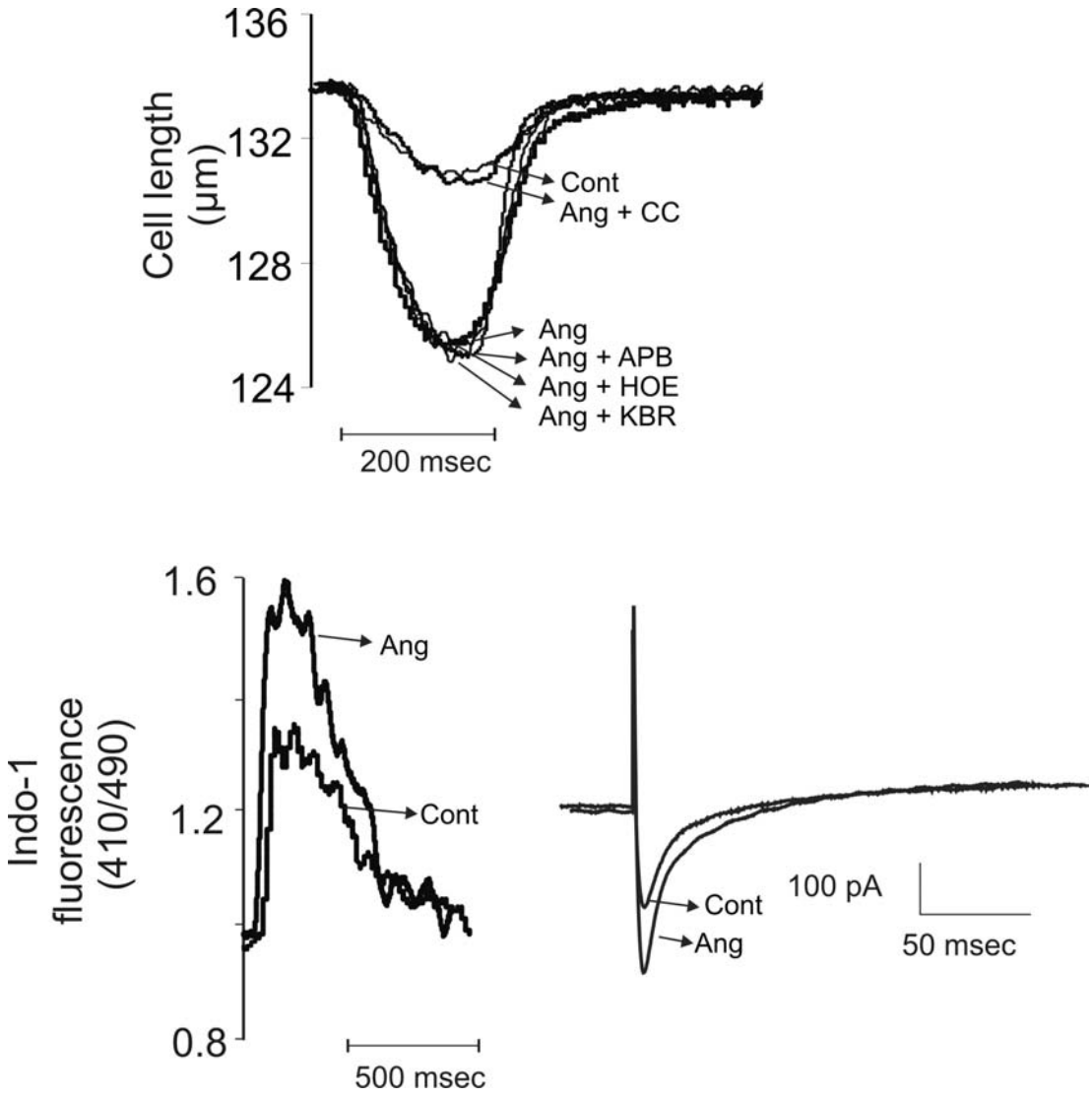

Figure 1. Effects of Ang II on contraction and Ca^{2+} handling in the cat, a species that responds with a positive inotropic effect. Top: Superimposed recordings of twitch contractions either in the absence or in the presence of Ang II, Ang II + HOE642 (HOE), Ang II + KB-R4739 (KBR), Ang II+ APB and Ang II + calphostin C (CC). Ang II produced, in cat myocytes, a large increase in contraction amplitude that was abolished by PKC inhibition (CC) but was not affected by inhibiting either, IP_3 release (APB), the Na^+/H^+ exchanger (HOE) or the reverse mode NCX (KBR). Bottom: Superimposed tracing of Ca^{2+} transients (left) and L-type Ca^{2+} currents (right) showing that the Ang II-induced positive inotropic effect was associated with a large increase in the Ca^{2+} transient and in the Ca^{2+} current.

intracellular Ca^{2+}. Aiello and coworkers (82) have shown that at low concentration Ang II induces a positive inotropic effect via the release of endothelin, which acts as an autocrine mediator to trigger an increase in reactive oxygen species (ROS) production. A ROS-associated stimulation of the Na^+/H^+ exchanger favors an increase in intracellular Na^+ which activates the reverse mode Na^+/Ca^{2+} exchanger and promotes the increase in intracellular Ca^{2+} (82).

The concept of an increase in myofilament responsiveness to Ca^{2+} as the mediator of the Ang II-induced positive inotropic effect was first proposed by Ikenouchi *et. al.* (27). These authors showed that in isolated rabbit myocytes, the Ang II-induced positive inotropic effect was associated with an increase in intracellular pH and concluded that an increase in myofilament responsiveness to Ca^{2+}, probably due to an intracellular alkalinisation mediated by the activation of the Na^+/H^+ exchanger, played a major role in the positive inotropic effect induced by Ang II. However, our work using isolated cat cardiac myocytes has shown that the positive inotropic effect of Ang II is preserved in the presence of Na^+/H^+ exchanger blockade with cariporide (HOE642) (35). Therefore, the conclusion that an increase in myofilament responsiveness to Ca^{2+} due to an increase in intracellular

pH plays a predominant role in determining the increase in contractility induced by Ang II cannot be generalized and does not apply for all mammalian species.

3.3. Possible mechanisms underlying the Ang II-induced negative inotropic effect

Although an Ang II-induced negative inotropic effect has been reported in rat isolated myocytes (31, 54, 83) as well as in mouse cells (56), the subcellular signalling events have only recently begun to be explored in detail (33). Considering that this Ang II-induced negative inotropic effect could, in principle, be one of the mechanisms underlying the decline in cardiac function of the failing heart, a full understanding of the subcellular signalling mechanisms involved is of critical importance.

As for the positive inotropic effect induced by Ang II, the Ang II-induced negative inotropic response can be mediated by alteration in intracellular Ca^{2+} and/or by changes in myofilament responsiveness to Ca^{2+}. We have shown that in the rat myocyte, Ang II elicits a decrease in cell shortening without a parallel decrease in the intracellular Ca^{2+} transient, suggesting that the mechanism underlying the negative inotropic effect of the peptide is a decrease in myofilament responsiveness to Ca^{2+} (33). Consistent with these results, Sakurai *et al.*, working with isolated mouse cardiomyocytes also failed to detect a significant decrease in the Ca^{2+} transient associated with the negative inotropic response induced by Ang II in this species (56). Several mechanisms can potentially decrease myofilament responsiveness to Ca^{2+}, including changes in intracellular pH and contractile protein phosphorylation. Ang II has been shown to produce an increase in myocyte cytosolic pH (27, 35). As described above, it has been postulated that intracellular alkalinization could, in some species, account for at least a component of the positive inotropic effect of Ang II produced by an increase in myofilament responsiveness to Ca^{2+} (27). This mechanism, obviously, cannot explain the negative inotropic effect observed in the rat myocyte. Moreover, we showed that Ang II fails to modify intracellular pH in isolated rat cardiac cells both in HEPES buffer, or when using the more physiological bicarbonate buffer (33). These results support the conclusion that pH-dependent mechanisms are not involved in the decrease in myofilament responsiveness to Ca^{2+} that mediates the Ang II-induced negative inotropic effect.

In contrast, the Ang II-induced negative inotropic effect can be prevented using PKC inhibitors (33, 38, 56) suggesting that protein phosphorylation underlies the reduction in myofilament responsiveness elicited by Ang II. Indeed, in chick and rat isolated myocytes and in rat tissues, the activation of PKC using phorbol esters has been shown to produce a negative inotropic effect (84). In PKCβ-overexpressing mice, the activation of PKCβ has been shown to produce a decrease in contractility via a reduction in myofilament responsiveness to Ca^{2+} (85). The fact that both positive and negative inotropic effects of the peptide can be abrogated by inhibitors of PKC would suggest that distinct isoforms of PKC may be responsible for the multiple contractile actions of Ang II. The signalling events distal to the activation of PKC which reduce myofilament responsiveness to Ca^{2+} are currently under investigation. Relevantly, several groups have demonstrated that the activation of p38 MAPK can markedly attenuate cardiac contractility via decrease in myofilament responsiveness to Ca^{2+} (86, 87). We have recently shown that Ang II signals through PKC and tyrosine kinases to activate p38 MAPK linked with reduced myofilament Ca^{2+} responsiveness (33) (Figure 2). Interestingly a study using a murine model of constitutively active p38 MAPK showed a direct effect of this kinase on myofilament force development and ATPase activity. This effect was attributed to the activation of phosphatases that induce the dephosphorylation of tropomyosin and the reduction of myofilament responsiveness (88).

3.4. Pathophysiological relevance of the Ang II-induced inotropic effect

In relation to the human heart, it is interesting to speculate that even though a negative inotropic action of Ang II has not been observed in this species, this does not necessarily imply that a negative signalling pathway is not present. In the normal myocardium where modest positive inotropic responses to Ang II have been reported (31), this may simply be the predominant effect, which provides inotropic support for the healthy heart - but which is superimposed on an underlying negative inotropic response. In certain disease states when Ang II levels are elevated an unmasking of the negative inotropic influence may occur, with either accentuation of the negative inotropic signalling or diminution of the positive inotropic signalling. This hypothesis is consistent with observations that the usual positive inotropic response to Ang II observed in normal human atrial and ventricular muscle is diminished in the failing human heart (32).

Collectively, the findings considered above suggest that Ang II can differentially modulate contractile function depending on the balance between activation of two distinct signalling pathways: Ca^{2+} vs. p38 MAPK. Which of these pathways prevails during sustained Ang II exposure as occurs in the failing heart, and how each signalling pathway contributes to the pathological phenotype in failure is an important research focus. In failure Ca^{2+} handling is altered and p38 MAPK is overexpressed and both these phenomena are known to be associated with adverse remodelling of the failing heart (89, 90). Given the importance of altered 'balance' in relation to the inotropic responses of the heart to Ang II in the transition to failure, the question of Ang II involvement in the structural remodelling in failure arises. In particular, cellular loss by apoptosis characterizes the failing heart phenotype. Below we consider the evidence relating to Ang II induction of apoptosis, considering whether the identified inotropic signalling pathways (both positive and negative) are implicated in a parallel apoptosis-induction process.

4. ANG II AND CARDIAC CELL DEATH

Programmed cell death or apoptosis is an extremely complex process involving multiple signalling events and molecular mediators conserved across a wide

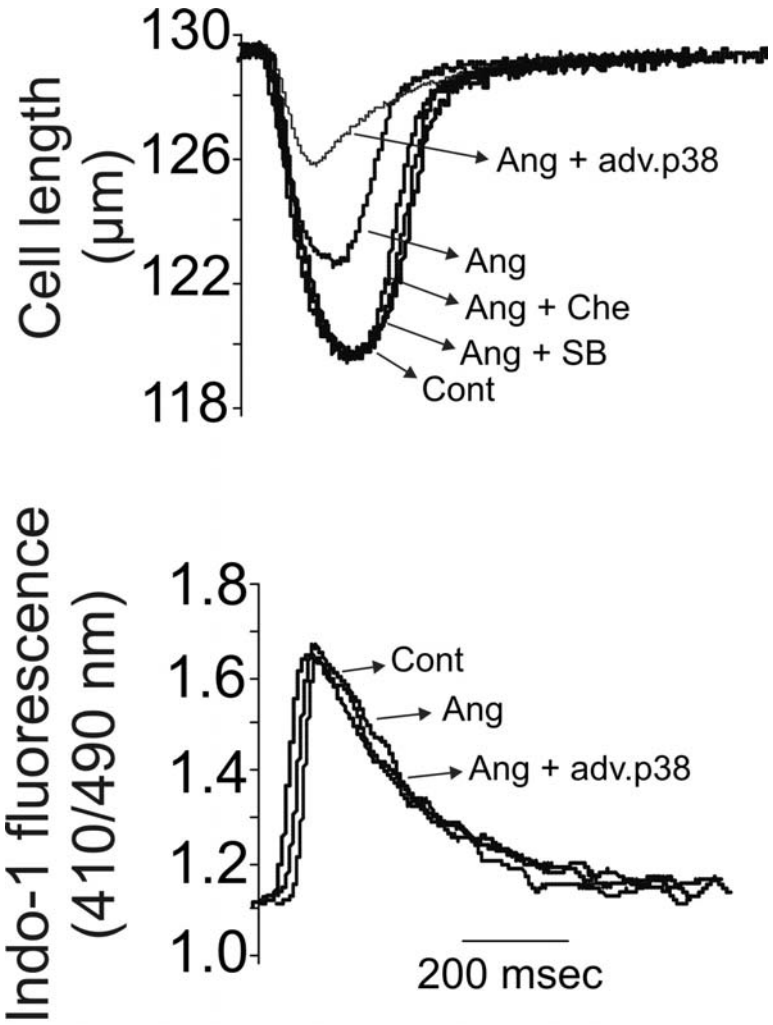

Figure 2. Effects of Ang II on contraction and Ca^{2+} transient in the rat, a species that responds with a negative inotropic effect. Top: Representative superimposed tracings of twitch contractions either in the absence or in the presence of Ang II, Ang II + PKC inhibitor Chelerethryne (Che), Ang II + p38 MAPK inhibitor SB202190 (SB) and Ang II in p38 MAPK overexpressing cells produced by adenoviral transfection (adv.p38). Ang II produced, in rat myocytes, a negative intropic effect that was abolished by PKC and p38 MAPK inhibition and was exacerbated by p38 MAPK overexpression. Bottom: Superimposed tracing of Ca^{2+} transients in the absence and presence of Ang II. Ang II did not affect the Ca^{2+} transient neither in control cells nor in cells overexpressing p38 MAPK.

range of species and tissues. The recent understanding of the pathological relevance of this process in cardiac muscle has led to concerted research efforts to identify the major intermediaries. Briefly, apoptosis is described as: 1) a pathway mediated by the activation of the so called death receptor, a receptor belonging to the tumor necrosis factor superfamily ("extrinsic pathway"), which may or may not involve mitochondria as an amplification loop, and 2) a cellular deprival and stress-mediated pathway ("intrinsic pathway"), which is regulated predominantly at the mitochondrial level. In both cases, the involvement of the mitochondria is reflected to the release of cytochrome c which is basically controlled by the balance between pro- and anti-apoptotic proteins (91). The final effectors of both these cascades, are the caspases, cysteine-proteases present in zymogene form which are activated in a chain reaction ending in the activation of the DNA fragmentation factor (92, 93). Given the high levels of endogenous caspase inhibitors present in cardiomyocytes, caspase-independent factors may be of particular regulatory importance in the induction of apoptosis in the myocardium (94). It has been previously reported that caspase-independent factors, such as apoptosis inducing factor (AIP), endonuclease G (Endo G) and high temperature requirement protein A2 (HtrA2/Omi) induce apoptosis in the heart without the intervention of caspases (95, 96). Regardless of the apoptotic pathway enacted, both caspase-dependent and -independent cascades culminate in DNA fragmentation and cell death (95, 96).

The emerging evidence of enhanced apoptosis in various myocardial disease states supports the conclusion

that the contractile dysfunction observed in the failing heart is not only due to altered Ca^{2+} handling, but also reflects a loss of contractile units by cell death (97-99). Because cardiac pump function is extremely dependent on optimal cell geometric and structural alignment, the death of individual cardiomyocytes (even when of modest extent), has the potential to disrupt mechanical and electrical communication with profound impact on cardiac function. Thus, limiting cardiac myocyte apoptosis could become an attractive therapeutic approach to prevent cardiac remodelling-mediated expansion of the interstitium involving collagen type I production, focal scar formation (100, 101) and re-alignment of adjacent cardiomyocytes (102).

Elevated circulating levels of Ang II and increased local cardiac Ang II production (103, 104) may play an important role in Ang II-induced cardiac apoptosis in heart failure. Studies of the beneficial effects of ACE inhibitors in reducing mortality and morbidity in congestive heart failure highlight the benefits of suppressed Ang II signalling in late stage disease (58). When interstitial levels of Ang II are persistently elevated (in the absence of Ang II receptor blockade) in parallel with long term effects on inotropic regulation a pro-apoptotic influence may be predicted. Below we review the evidence in support of this contention and examine the putative mechanisms involved in Ang II-induced apoptosis. In particular the links between the Ang II-dependent signalling mechanisms involved in inotropic regulation and apoptotic triggering are considered.

4.1. Subcellular mechanisms of apoptosis: receptors and signalling pathways

Ang II-induced apoptosis in cardiac cells was identified over a decade ago. The pioneering studies in this field were carried out by Anversa and colleagues, who described an Ang II-induced apoptotic response in ventricular myocytes of adult and neonatal rats (105, 106). Since these initial studies numerous reports have been published proposing multiple pathways and signalling molecules by which Ang II may induce apoptosis through receptor-mediated activation (Figure 3). Although most of the existing evidence indicates that the AT_1 receptor mediates the apoptotic process initiated by Ang II (106, 107), the AT_2 receptor has been shown to trigger the Ang II apoptotic pathway in a neural cell line (108). However, the observation that myocardial apoptosis is not increased in a murine model of AT_2- overexpression does not support the conclusion that AT_2 activation provides the primary pro-apoptotic signal in the myocardium (51). Interestingly, recent evidence suggests that the AT_2 receptor can be constitutively active in the absence of its ligand Ang II (109) and that these receptors can induce apoptosis in the absence of Ang II stimulation and in the presence of PD123319 (110). The extensive body of work which demonstrates inhibition of Ang II-induced apoptosis with AT_1 receptor blockade (106, 111), indicates that activation of the AT_1 receptor is the primary apoptotic signal in the myocardium.

An AT_2-mediated apoptotic effect could be operative in the failing myocardium, where the AT_1/ AT_2 receptor balance is reduced (45, 112) and where AT_1 availability may be limited due to increased internalization/desensitization of this receptor subtype caused by high circulating levels of Ang II (113, 114). A cardioprotective role for AT_2 receptor activation (attributed to both an antiapoptotic and antihypertrophic action) has been described which could at least partially explain the beneficial effects of AT_1 receptor blockers in the treatment of chronic congestive failure (115-117). With prolonged AT_1 receptor blockade where AT_1 receptor-mediated signaling is suppressed, the AT_2 receptor response remains intact or may even be amplified by the increased plasma levels of Ang II associated with chronic AT_1 blocker treatment (118). Such an AT_2 effect could in part explain results obtained in clinical trials where AT_1 receptor blockade appears to be more effective than ACE inhibition (119). However, this mechanistic interpretation is controversial, given that recent reports have demonstratred that simultaneous administration of ACE inhibitors and AT_1 receptor blockers confers additional benefits beyond those achieved by the administration of either agent alone (see ref (120, 121) for review).

As noted above, apoptotic death may occur as a consequence of the activation of cell surface death receptors by extracellular ligands and/or by the activation of mitochondrial-dependent pro-apoptotic mechanisms. These pathways are not completely independent. Current evidence supports a role for Ang II in activation of the mitochondrial apoptotic cascade (122, 123). However, the death receptor pathway could contribute to cardiomyocyte apoptosis under certain pathophysiological settings, in the late phases of cardiac diseases when death receptors and their ligands are upregulated and the operation of protective pathways is attenuated (124, 125). Transactivation of the death receptor promoted by AT_1 may also occur and these possibilities remain to be explored.

The contribution of PKC to the proapoptotic effects of Ang II is well demonstrated. In addition to the inotropic and hypertrophic actions of Ang II mediated by PKC discussed above (33, 35), a specific PKC-dependent apoptotic action of Ang II has been identified. An increase in intracellular Ca^{2+} via a PKC-dependent mechanism has been shown to stimulate the Ca^{2+}-dependent endonuclease, DNase I, responsible for DNA cleavage and cell death (106). Moreover, phorbol myristate acetate and adenoviral PKCδ expression, but not PKCε or dominant negative PKCα, have been shown to increase transcription of Nix, a mitochondrial protein that causes cytochrome *c* release, activation of caspase-3 and apoptotic cell death (126). The pathways beyond PKC involved in the downstream enactment of apoptosis include both Ca^{2+}-dependant and Ca^{2+}-independent signalling processes, and are discussed further below.

4.2. Putative Ca^{2+} dependent Ang II-induced signalling pathways of apoptosis

Several Ca^{2+}-dependent processes have been described as Ang II-induced apoptotic signalling

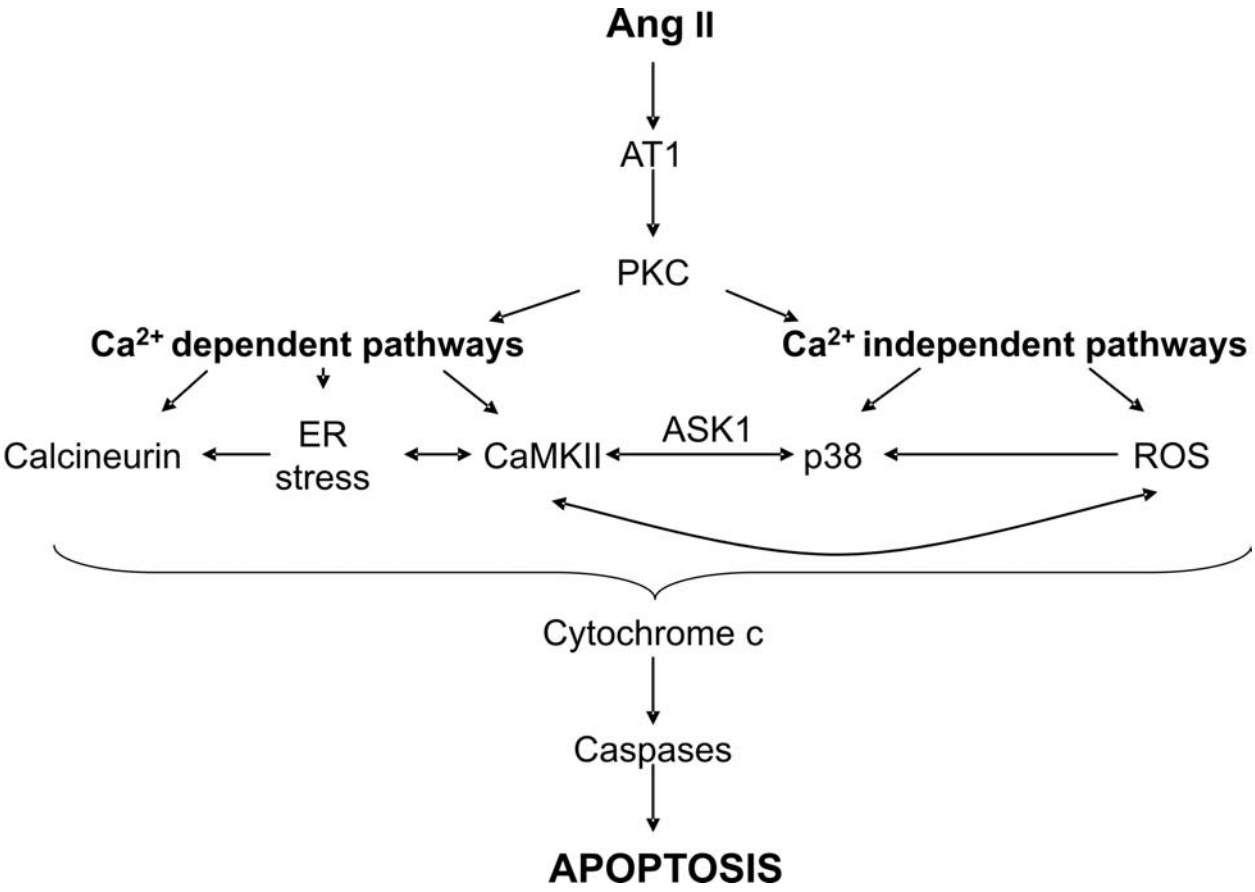

Figure 3. Putative signalling cascades involved in Ang II-induced apoptosis. The cartoon depicts the signalling cascades for Ang II-induced apoptosis described in the text emphasizing the possible Ca^{2+}-dependent and -independent pathways and the potential cross-talk between them.

mechanisms. In this regard, it is important to note that the disturbance of any endoplasmic/sarcoplasmic reticulum (ER/SR) function, i.e. Ca^{2+} storage and signalling, as well as the folding, modification and sorting of newly synthesized proteins, can lead to so-called ER stress (127). Both Ca^{2+} overload and depletion of the ER Ca^{2+} pool can produce this phenomenon. Prolonged ER stress stimulates the activation of pro-caspase-12, which acts as an activator of caspase-3 to induce apoptosis (128). In cardiomyocytes, the Ang II-induced increase in intracellular Ca^{2+} responsible for the acute positive inotropic effect could promote SR stress and induce apoptosis, if the stimulus is sufficiently prolonged (Figure 3). Indeed, an early report from Anversa *et al.* (106) concluded that Ang II-induced apoptosis was due to enhanced Ca^{2+} entry through the L-type Ca^{2+} channel, causing Ca^{2+} overload and mitochondrially-mediated cell death. Although the mitochondrial uniporter Ca^{2+} affinity is low, increased intracellular Ca^{2+} levels exceeding a certain threshold in pathological conditions have been shown in non-myocyte cell types to induce mitochondrial Ca^{2+} overload (129). This Ca^{2+} overload is amplified by the proximity of the mitochondria to the ER/SR and the creation of Ca^{2+} 'hotspots' at the mouth of Ca^{2+} release channels (130). Thus the local Ca^{2+} concentration at these sites can reach very high levels. Mitochondrial Ca^{2+} overload can then activate the permeability transition pore, through which pro-apoptotic proteins are released into the cytoplasm.

Other potential targets for Ca^{2+} signalling in cardiac apoptosis have been identified. Among these, a mitochondrial pro-apoptotic pathway triggered by Ang II causing increased intracellular Ca^{2+} has been shown to involve calcineurin activation. Calcineurin, a protein phosphatase, mediates translocation of Bad, a pro-apoptotic Bcl-2 family member which promotes cytochrome c release and caspase activation (131). Similarly, Ang II-induced Ca^{2+} elevation could activate CaMKII, described as a pro-apoptotic molecule (132-134). This mechanism seems plausible since CaMKII activity is increased, as are Ang II levels, in heart failure. We have recently demonstrated the participation of CaMKII in the induction of apoptosis by Ang II (135) *in vitro*. *In situ*ations where there is an Ang II-induced increase in intracellular Ca^{2+}, CaMKII activation would be coincident. CaMKII phosphorylation of the ryanodine receptors (RyR2) at the Ser^{2815} site has been shown to enhance the open probability of the receptor and lead to SR Ca^{2+} leak (136). This mechanism has been reported to be enhanced in heart failure by increased expression and activity of CaMKII in the RyR2 complex

(where associated phosphatases are also reduced) (137). SR Ca^{2+} leak 'hotspots' in failing myocytes could induce mitochondria Ca^{2+} overload and trigger apoptosis. CaMKII activation could also favour SR Ca^{2+} overload via phosphorylation of the Thr^{17} residue of phospholamban and the subsequent activation of SERCA2a, triggering apoptosis via a similar mitochondrial Ca^{2+} overload process. Such an increase in apoptotic activity in cardiomyocytes of transgenic mice overexpressing the L-type Ca^{2+} channel has been observed and involves CaMKII-dependent SR and mitochondrial Ca^{2+} overload (138). Despite the circumstantial evidence of a link between elevated Ang II and CaMKII activation, a causative association between these mediators and pathophysiological induction of apoptosis in heart failure has not yet been established.

4.3. Putative Ca^{2+} independent Ang II-induced signalling pathways of apoptosis

Ca^{2+} is not involved in all forms of apoptosis. Many stimuli induce apoptosis in the absence of any detectable change in Ca^{2+} fluxes and where a direct role for Ca^{2+} signalling is not apparent (127). Interestingly, CaMKII can also be activated by Ca^{2+}-independent mechanisms such as PKC, ROS or p38 MAPK, all well known targets molecules of Ang II (33, 139). Consistently, we and others have shown that Ang II-induced apoptosis is at least in part, mediated by Ca^{2+}-independent ROS-dependent activation of CaMKII (140, 141), furthermore, this signalling cascade seems to require p38 MAPK activation. In addition p38 MAPK, the downstream effector of the Ang II-induced negative inotropic response (Ca^{2+}-independent), has been shown to participate in the apoptotic processes triggered by Ang II (142). We and others have shown that Ang II can activate apoptosis by other Ca^{2+} independent signalling processes. Among these, it has been clearly demonstrated that NADPH oxidase activity is increased under sustained Ang II stimulation, enhancing production of ROS, including superoxide ($O_2^{-\cdot}$) and hydrogen peroxide (H_2O_2) (33). Oxidative stress has also been reported to activate the mitochondrial permeability transition pore and promote apoptosis (143). The deleterious effects of oxidative stress induced by Ang II are not limited to induction of cardiac apoptosis, and also involve pro-fibrotic and pro-hypertrophic actions. Cardiac hypertrophy induced by short-term subpressor Ang II infusion is inhibited in $Nox2^{-/-}$ mice, deficient in one of the major cardiac NADPH oxidase isoenzymes (144). Increased interstitial cardiac fibrosis induced by Ang II involves Nox2-NADPH oxidase-dependent up-regulation of profibrotic genes with activation of the transcription factor NF-κB and matrix metalloproteinase-2 (145). In diabetic cardiomyopathy, Ang II receptor blockers improve diastolic function in association with normalization of oxidative stress linked with attenuation of myocyte hypertrophy and interstitial fibrosis. Similarly, overexpression of the antioxidant enzyme glutathione peroxidase improves diastolic function and also reduces myocyte hypertrophy, interstitial fibrosis and apoptosis (146).

Ang II-induced activation of p38 MAPK is also known to be mediated by H_2O_2. In adult ventricular myocytes the overexpression of catalase (147), an enzyme that catabolizes H_2O_2, or treatment with the ROS scavenger MPG (71) both inhibit Ang II-stimulated p38 MAPK activation. It has been recently reported that the ROS sensor responsible for p38 MAPK activation is the apoptotic signal regulating kinase 1 (ASK1) (148). This kinase is required for ROS-induced JNK and p38 MAPK activation. These latter two kinases are amongst the family of stress-induced kinases that influence cell survival, apoptosis, and differentiation. Activation of p38 MAPK has been show to increase p53 protein levels, which secondarily promotes apoptosis by inducing expression and mitochondrial translocation of Bax, another pro-apoptotic protein of the Bcl-2 family. Moreover, p38 MAPK activation has been associated with caspase-3 cleavage and activation. For schematic interpretation of these Ca^{2+}-independent Ang II-induced signalling pathways of apoptosis see Figure 3.

5. PERSPECTIVES

Ang II has long been recognized as an important myocardial inotropic modulator, but the subtleties of the signalling mechanisms involved remain to be fully elucidated. The net inotropic effect of Ang II stimulation in different contexts reflects a variable outcome of competing positive and negative inotropic signalling mechanisms. PKC mediation of both positive and negative inotropism is indicated and further work is required to delineate the specific PKC isoforms involved. In pathophysiological states such as heart failure, characterized by chronic exposure to elevated Ang II, the balance of inotropic influences could be shifted to unmask increased negative inotropism linked with Ca^{2+}-handling dysfunction and p38 MAPK activation. Coincident with loss of inotropic balance, an Ang II influence in mediating the morphologic remodelling of the heart in the transition to failure is also observed. Loss of myocardial structural integrity associated with Ang II induced myocyte apoptosis is now a generally accepted phenomenon, but the signalling mechanisms involved are incompletely understood. Both Ca^{2+}-dependent and Ca^{2+}-independent signalling pathways appear to be important, and we highlight Ang II mediated CaMKII activation as a potential key modulator in apoptosis induction in the failing heart. To identify new therapeutic molecular targets in heart failure, further work is required to establish a causative link between Ang II and CaMKII activation and the pathophysiological induction of cardiomyocyte apoptosis.

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7. REFERENCES

1. Braun-Menendez, E., J. C. Fasciolo, L. F. Leloir & J. M. Munoz: The substance causing renal hypertension. *J Physiol*, 98, 283-98 (1940)

2. Page IH, H. O.: Acrystalline pressor substance (angiotonin) resulting from the reaction between renin and renin activator. *J. Exp. Med.*, 15, 693-698 (1940)

3. Mattiazzi, A.: Positive inotropic effect of angiotensin II. Increases in intracellular Ca2+ or changes in myofilament Ca2+ responsiveness? *J Pharmacol Toxicol Methods*, 37, 205-14 (1997)

4. Timmermans, P. B., P. C. Wong, A. T. Chiu, W. F. Herblin, P. Benfield, D. J. Carini, R. J. Lee, R. R. Wexler, J. A. Saye & R. D. Smith: Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev*, 45, 205-51 (1993)

5. Sadoshima, J. & S. Izumo: Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. *Circ Res*, 73, 413-23 (1993)

6. Sadoshima, J. & S. Izumo: Signal transduction pathways of angiotensin II--induced c-fos gene expression in cardiac myocytes *in vitro*. Roles of phospholipid-derived second messengers. *Circ Res*, 73, 424-38 (1993)

7. De Mello, W. C. & A. H. Danser: Angiotensin II and the heart : on the intracrine renin-angiotensin system. *Hypertension*, 35, 1183-8 (2000)

8. Aceto, J. F. & K. M. Baker: [Sar1]angiotensin II receptor-mediated stimulation of protein synthesis in chick heart cells. *Am J Physiol*, 258, H806-13 (1990)

9. Kent, R. L. & P. J. McDermott: Passive load and angiotensin II evoke differential responses of gene expression and protein synthesis in cardiac myocytes. *Circ Res*, 78, 829-38 (1996)

10. Schunkert, H. & B. H. Lorell: Role of angiotensin II in the transition of left ventricular hypertrophy to cardiac failure. *Heart Failure* 10, 142 - 149 (1994)

11. Schunkert, H., J. Sadoshima, T. Cornelius, Y. Kagaya, E. O. Weinberg, S. Izumo, G. Riegger & B. H. Lorell: Angiotensin II-induced growth responses in isolated adult rat hearts. Evidence for load-independent induction of cardiac protein synthesis by angiotensin II. *Circ Res*, 76, 489-97 (1995)

12. Dinh, D. T., A. G. Frauman, C. I. Johnston & M. E. Fabiani: Angiotensin receptors: distribution, signalling and function. *Clin Sci (Lond)*, 100, 481-92 (2001)

13. Dorn, G. W., 2nd & T. Force: Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest*, 115, 527-37 (2005)

14. Mehta, P. K. & K. K. Griendling: Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol*, 292, C82-97 (2007)

15. Wu, L., M. Iwai, H. Nakagami, R. Chen, J. Suzuki, M. Akishita, M. de Gasparo & M. Horiuchi: Effect of angiotensin II type 1 receptor blockade on cardiac remodeling in angiotensin II type 2 receptor null mice. *Arterioscler Thromb Vasc Biol*, 22, 49-54 (2002)

16. Griffin, S. A., W. C. Brown, F. MacPherson, J. C. McGrath, V. G. Wilson, N. Korsgaard, M. J. Mulvany & A. F. Lever: Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. *Hypertension*, 17, 626-35 (1991)

17. Kim, S., K. Ohta, A. Hamaguchi, T. Yukimura, K. Miura & H. Iwao: Angiotensin II induces cardiac phenotypic modulation and remodeling *in vivo* in rats. *Hypertension*, 25, 1252-9 (1995)

18. Reudelhuber, T. L., K. E. Bernstein & P. Delafontaine: Is angiotensin II a direct mediator of left ventricular hypertrophy? Time for another look. *Hypertension*, 49, 1196-201 (2007)

19. Harrap, S. B., A. F. Dominiczak, R. Fraser, A. F. Lever, J. J. Morton, C. J. Foy & G. C. Watt: Plasma angiotensin II, predisposition to hypertension, and left ventricular size in healthy young adults. *Circulation*, 93, 1148-54 (1996)

20. Zhang, X., D. E. Dostal, K. Reiss, W. Cheng, J. Kajstura, P. Li, H. Huang, E. H. Sonnenblick, L. G. Meggs, K. M. Baker & *et al.*: Identification and activation of autocrine renin-angiotensin system in adult ventricular myocytes. *Am J Physiol*, 269, H1791-802 (1995)

21. Danser, A. H., J. P. van Kats, P. J. Admiraal, F. H. Derkx, J. M. Lamers, P. D. Verdouw, P. R. Saxena & M. A. Schalekamp: Cardiac renin and angiotensins. Uptake from plasma versus *in situ* synthesis. *Hypertension*, 24, 37-48 (1994)

22. Pieruzzi, F., Z. A. Abassi & H. R. Keiser: Expression of renin-angiotensin system components in the heart, kidneys, and lungs of rats with experimental heart failure. *Circulation*, 92, 3105-12 (1995)

23. Domenighetti, A. A., Q. Wang, M. Egger, S. M. Richards, T. Pedrazzini & L. M. Delbridge: Angiotensin II-mediated phenotypic cardiomyocyte remodeling leads to age-dependent cardiac dysfunction and failure. *Hypertension*, 46, 426-32 (2005)

24. Baker, K. M. & J. A. Aceto: Characterization of avian angiotensin II cardiac receptors: coupling to mechanical activity and phosphoinositide metabolism. *J Mol Cell Cardiol*, 21, 375-82 (1989)

25. Capasso, J. M., P. Li, X. Zhang, L. G. Meggs & P. Anversa: Alterations in ANG II responsiveness in left and

right myocardium after infarction-induced heart failure in rats. *Am J Physiol*, 264, H2056-67 (1993)
doi not found.

26. Drimal, J. & D. Boska: Effects of angiotensin-II on myocardial mechanics and contractile state of heart muscle. *Eur J Pharmacol*, 21, 130-8 (1973)

27. Ikenouchi, H., W. H. Barry, J. H. Bridge, E. O. Weinberg, C. S. Apstein & B. H. Lorell: Effects of angiotensin II on intracellular Ca2+ and pH in isolated beating rabbit hearts and myocytes loaded with the indicator indo-1. *J Physiol*, 480 (Pt 2), 203-15 (1994)

28. Ishihata, A. & M. Endoh: Species-related differences in inotropic effects of angiotensin II in mammalian ventricular muscle: receptors, subtypes and phosphoinositide hydrolysis. *Br J Pharmacol*, 114, 447-53 (1995)
29. Kass, R. S. & M. L. Blair: Effects of angiotensin II on membrane current in cardiac Purkinje fibers. *J Mol Cell Cardiol*, 13, 797-809 (1981)

30. Koch-Weser, J.: Nature of the Inotropic Action of Angiotensin on Ventricular Myocardium. *Circ Res*, 16, 230-7 (1965)

31. Lefroy, D. C., T. Crake, F. Del Monte, G. Vescovo, L. Dalla Libera, S. Harding & P. A. Poole-Wilson: Angiotensin II and contraction of isolated myocytes from human, guinea pig, and infarcted rat hearts. *Am J Physiol*, 270, H2060-9 (1996)
doi not found.

32. Moravec, C. S., M. D. Schluchter, L. Paranandi, B. Czerska, R. W. Stewart, E. Rosenkranz & M. Bond: Inotropic effects of angiotensin II on human cardiac muscle *in vitro*. *Circulation*, 82, 1973-84 (1990)

33. Palomeque, J., L. Sapia, R. J. Hajjar, A. Mattiazzi & M. Vila Petroff: Angiotensin II-induced negative inotropy in rat ventricular myocytes: role of reactive oxygen species and p38 MAPK. *Am J Physiol Heart Circ Physiol*, 290, H96-106 (2006)

34. Salas, M. A., M. G. Vila-Petroff, J. Palomeque, E. A. Aiello & A. Mattiazzi: Positive inotropic and negative lusitropic effect of angiotensin II: intracellular mechanisms and second messengers. *J Mol Cell Cardiol*, 33, 1957-71 (2001)

35. Vila Petroff, M. G., E. A. Aiello, J. Palomeque, M. A. Salas & A. Mattiazzi: Subcellular mechanisms of the positive inotropic effect of angiotensin II in cat myocardium. *J Physiol*, 529 Pt 1, 189-203 (2000)

36. Li, P., E. H. Sonnenblick, P. Anversa & J. M. Capasso: Length-dependent modulation of ANG II inotropism in rat myocardium: effects of myocardial infarction. *Am J Physiol*, 266, H779-86 (1994)

37. Peach, M. J.: Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol Rev*, 57, 313-70 (1977)

38. Dudley, D. T., R. L. Panek, T. C. Major, G. H. Lu, R. F. Bruns, B. A. Klinkefus, J. C. Hodges & R. E. Weishaar: Subclasses of angiotensin II binding sites and their functional significance. *Mol Pharmacol*, 38, 370-7 (1990)

39. Whitebread S, M. M., Kember B, de Gasparo M.: Preliminary biochemcal characterization of two angiotensin II reseptor subtypes. *Biochem. Biophis. Res. Commun.*, 163, 286-291 (1989)

40. Chang RS, L. V.: Angiotensin reseptor subtypes in rat, rabbit and monkey tissues: relative distribution an speies dependency. *Life Sci.*, 49, 1485-1490 (1991)

41. Rogg H, S. A., de Gasparo M.: Identification and characterization of angiotensin II reseptor subtypes in rabbit ventricular myocardium. *Biochem. Biophis. Res. Commun.*, 173, 416-422 (1990)

42. Sechi LA, G. C., Grady EF, Kalinyak, Schambelan M.: Characterization of angiotensin II reseptor subtypes in rat heart. *Circ Res*, 71, 1482-1489 (1992)

43. Nozawa Y, H. A., Oda N, Yamasaki Y, Matsuura N, Yamada S., Inabe K, Kimura R, Suzuki H, Hoshino T.: Angiotensin II reseptor subtypes in bovine and human ventricular myocardium. *J. Pharmacol. Exp. Ther.*, 270, 566-571 (1994)

44. Brink M, E. P., de Gasparo M, Rogg H, Schmid A, Stulz P, Bullock G.: Localization of the angiotensin II reseptor subtypes in human atrium. *J. Mol. Cell. Cardiol.*, 28, 1789-1799 (1996)

45. Tsutsumi, Y., H. Matsubara, N. Ohkubo, Y. Mori, Y. Nozawa, S. Murasawa, K. Kijima, K. Maruyama, H. Masaki, Y. Moriguchi, Y. Shibasaki, H. Kamihata, M. Inada & T. Iwasaka: Angiotensin II type 2 receptor is upregulated in human heart with interstitial fibrosis, and cardiac fibroblasts are the major cell type for its expression. *Circ Res*, 83, 1035-46 (1998)

46. Wang, L., M. Eberhard, E. Kohler & P. Erne: A specific binding site for angiotensin II(3-8), angiotensin IV, in rabbit cardiac fibroblasts. *J Recept Signal Transduct Res*, 15, 517-27 (1995)

47. Hanesworth, J. M., M. F. Sardinia, L. T. Krebs, K. L. Hall & J. W. Harding: Elucidation of a specific binding site for angiotensin II(3-8), angiotensin IV, in mammalian heart membranes. *J Pharmacol Exp Ther*, 266, 1036-42 (1993)

48. Ishihata, A. & M. Endoh: Pharmacological characteristics of the positive inotropic effect of angiotensin II in the rabbit ventricular myocardium. *Br J Pharmacol*, 108, 999-1005 (1993)

49. Mattiazzi, A., N. G. Perez, M. G. Vila-Petroff, B. Alvarez, M. C. Camilion de Hurtado & H. E. Cingolani: Dissociation between positive inotropic and alkalinizing effects of angiotensin II in feline myocardium. *Am J Physiol*, 272, H1131-6 (1997)

50. Scott, A. L., R. S. Chang, V. J. Lotti & P. K. Siegl: Cardiac angiotensin receptors: effects of selective angiotensin II receptor antagonists, DUP 753 and PD 121981, in rabbit heart. *J Pharmacol Exp Ther*, 261, 931-5 (1992)

51. Nakayama, M., X. Yan, R. L. Price, T. K. Borg, K. Ito, A. Sanbe, J. Robbins & B. H. Lorell: Chronic ventricular myocyte-specific overexpression of angiotensin II type 2 receptor results in intrinsic myocyte contractile dysfunction. *Am J Physiol Heart Circ Physiol*, 288, H317-27 (2005)

52. Castro-Chaves, P., S. Soares, R. Fontes-Carvalho & A. F. Leite-Moreira: Negative inotropic effect of selective AT2 receptor stimulation and its modulation by the endocardial endothelium. *Eur J Pharmacol*, 578, 261-9 (2008)

53. Fujita, S. & M. Endoh: Influence of a Na+-H+ exchange inhibitor ethylisopropylamiloride, a Na+-Ca2+ exchange inhibitor KB-R7943 and their combination on the increases in contractility and Ca2+ transient induced by angiotensin II in isolated adult rabbit ventricular myocytes. *Naunyn Schmiedebergs Arch Pharmacol*, 360, 575-84 (1999)

54. Meissner, A., J. Y. Min & R. Simon: Effects of angiotensin II on inotropy and intracellular Ca2+ handling in normal and hypertrophied rat myocardium. *J Mol Cell Cardiol*, 30, 2507-18 (1998)

55. Rosenkranz, S., G. Nickenig, M. Flesch, B. Cremers, P. Schnabel, O. Lenz, T. Krause, D. Ganten, S. Hoffmann & M. Bohm: Cardiac angiotensin II receptors: studies on functional coupling in Sprague-Dawley rats and TGR(alphaMHC-hAT1) transgenic rats. *Eur J Pharmacol*, 330, 35-46 (1997)

56. Sakurai, K., I. Norota, H. Tanaka, I. Kubota, H. Tomoike & M. Endo: Negative inotropic effects of angiotensin II, endothelin-1 and phenylephrine in indo-1 loaded adult mouse ventricular myocytes. *Life Sci*, 70, 1173-84 (2002)

57. Coats, A. J. & S. Adamopoulos: Neurohormonal mechanisms and the role of angiotensin-converting enzyme (ACE) inhibitors in heart failure. *Cardiovasc Drugs Ther*, 8, 685-92 (1994)

58. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) The CONSENSUS Trial Study Group. *N Engl J Med*, 316, 1429-35 (1987)

59. Mufti, S., S. Wenzel, G. Euler, H. M. Piper & K. D. Schluter: Angiotensin II-dependent loss of cardiac function: mechanisms and pharmacological targets attenuating this effect. *J Cell Physiol*, 217, 242-9 (2008)

60. Fabiato, A.: Two kinds of calcium-induced release of calcium from the sarcoplasmic reticulum of skinned cardiac cells. *Adv Exp Med Biol*, 311, 245-62 (1992)

61. Kentish, J. C., R. J. Barsotti, T. J. Lea, I. P. Mulligan, J. R. Patel & M. A. Ferenczi: Calcium release from cardiac sarcoplasmic reticulum induced by photorelease of calcium or Ins(1,4,5)P3. *Am J Physiol*, 258, H610-5 (1990)
doi not found.

62. Movsesian, M. A., A. P. Thomas, M. Selak & J. R. Williamson: Inositol trisphosphate does not release Ca2+ from permeabilized cardiac myocytes and sarcoplasmic reticulum. *FEBS Lett*, 185, 328-32 (1985)

63. Vites, A. M. & A. Pappano: Inositol 1,4,5-trisphosphate releases intracellular Ca2+ in permeabilized chick atria. *Am J Physiol*, 258, H1745-52 (1990)

64. Anand-Srivastava, M. B.: Angiotensin II receptors negatively coupled to adenylate cyclase in rat myocardial sarcolemma. Involvement of inhibitory guanine nucleotide regulatory protein. *Biochem Pharmacol*, 38, 489-96 (1989)

65. Kamp TJ, H. J.: Regulation of cardiac L-type calcium cahnnels by protein kinase A and protein kinase C. . *C. Circ Res.* , 87, 1095-1002 (2000)

66. Kandasamy, R. A., F. H. Yu, R. Harris, A. Boucher, J. W. Hanrahan & J. Orlowski: Plasma membrane Na+/H+ exchanger isoforms (NHE-1, -2, and -3) are differentially responsive to second messenger agonists of the protein kinase A and C pathways. *J Biol Chem*, 270, 29209-16 (1995)

67. Moolenaar, W. H., L. G. Tertoolen & S. W. de Laat: Phorbol ester and diacylglycerol mimic growth factors in raising cytoplasmic pH. *Nature*, 312, 371-4 (1984)

68. Wiederkehr, M. R., H. Zhao & O. W. Moe: Acute regulation of Na/H exchanger NHE3 activity by protein kinase C: role of NHE3 phosphorylation. *Am J Physiol*, 276, C1205-17 (1999)

69. Iwamoto, T., Y. Pan, S. Wakabayashi, T. Imagawa, H. I. Yamanaka & M. Shigekawa: Phosphorylation-dependent regulation of cardiac Na+/Ca2+ exchanger via protein kinase C. *J Biol Chem*, 271, 13609-15 (1996)

70. Wang, G., J. Anrather, M. J. Glass, M. J. Tarsitano, P. Zhou, K. A. Frys, V. M. Pickel & C. Iadecola: Nox2, Ca2+, and protein kinase C play a role in angiotensin II-induced free radical production in nucleus tractus solitarius. *Hypertension*, 48, 482-9 (2006)

71. Wenzel, S., G. Taimor, H. M. Piper & K. D. Schluter: Redox-sensitive intermediates mediate angiotensin II-induced p38 MAP kinase activation, AP-1 binding activity, and TGF-beta expression in adult ventricular cardiomyocytes. *Faseb J*, 15, 2291-3 (2001)

72. Clement, O., M. Puceat, M. P. Walsh & G. Vassort: Protein kinase C enhances myosin light-chain kinase effects on force development and ATPase activity in rat single skinned cardiac cells. *Biochem J*, 285 (Pt 1), 311-7 (1992)

73. Venema, R. C. & J. F. Kuo: Protein kinase C-mediated phosphorylation of troponin I and C-protein in isolated myocardial cells is associated with inhibition of myofibrillar actomyosin MgATPase. *J Biol Chem*, 268, 2705-11 (1993)

74. Barry, W. H., H. Matsui, J. H. Bridge & K. W. Spitzer: Excitation-contraction coupling in ventricular myocytes: effects of angiotensin II. *Adv Exp Med Biol*, 382, 31-9 (1995)

75. Skolnick, R. L., S. E. Litwin, W. H. Barry & K. W. Spitzer: Effect of ANG II on pHi, [Ca2+]i, and contraction in rabbit ventricular myocytes from infarcted hearts. *Am J Physiol*, 275, H1788-97 (1998)

76. Watanabe, A. & M. Endoh: Relationship between the increase in Ca2+ transient and contractile force induced by angiotensin II in aequorin-loaded rabbit ventricular myocardium. *Cardiovasc Res*, 37, 524-31 (1998)

77. Allen, I. S., N. M. Cohen, R. S. Dhallan, S. T. Gaa, W. J. Lederer & T. B. Rogers: Angiotensin II increases spontaneous contractile frequency and stimulates calcium current in cultured neonatal rat heart myocytes: insights into the underlying biochemical mechanisms. *Circ Res*, 62, 524-34 (1988)

78. Kaibara, M., S. Mitarai, K. Yano & M. Kameyama: Involvement of Na(+)-H+ antiporter in regulation of L-type Ca2+ channel current by angiotensin II in rabbit ventricular myocytes. *Circ Res*, 75, 1121-5 (1994)

79. Aiello, E. A. & H. E. Cingolani: Angiotensin II stimulates cardiac L-type Ca(2+) current by a Ca(2+)- and protein kinase C-dependent mechanism. *Am J Physiol Heart Circ Physiol*, 280, H1528-36 (2001)

80. Ichiyanagi, O., K. Ishii & M. Endoh: Angiotensin II increases L-type Ca2+ current in gramicidin D-perforated adult rabbit ventricular myocytes: comparison with conventional patch-clamp method. *Pflugers Arch*, 444, 107-16 (2002)

81. Talukder, M. A. & M. Endoh: Pharmacological differentiation of synergistic contribution of L-type Ca2+ channels and Na+/H+ exchange to the positive inotropic effect of phenylephrine, endothelin-3 and angiotensin II in rabbit ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol*, 355, 87-96 (1997)

82. Cingolani, H. E., M. C. Villa-Abrille, M. Cornelli, A. Nolly, I. L. Ennis, C. Garciarena, A. M. Suburo, V. Torbidoni, M. V. Correa, M. C. Camilionde Hurtado & E. A. Aiello: The positive inotropic effect of angiotensin II: role of endothelin-1 and reactive oxygen species. *Hypertension*, 47, 727-34 (2006)

83. Trongtorsak, P., T. O. Morgan & L. M. Delbridge: Combined renin-angiotensin system blockade and dietary sodium restriction impairs cardiomyocyte contractility. *J Renin Angiotensin Aldosterone Syst*, 4, 213-9 (2003)

84. Capogrossi, M. C., T. Kaku, C. R. Filburn, D. J. Pelto, R. G. Hansford, H. A. Spurgeon & E. G. Lakatta: Phorbol ester and dioctanoylglycerol stimulate membrane association of protein kinase C and have a negative inotropic effect mediated by changes in cytosolic Ca2+ in adult rat cardiac myocytes. *Circ Res*, 66, 1143-55 (1990)

85. Takeishi, Y., G. Chu, D. M. Kirkpatrick, Z. Li, H. Wakasaki, E. G. Kranias, G. L. King & R. A. Walsh: *In vivo* phosphorylation of cardiac troponin I by protein kinase Cbeta2 decreases cardiomyocyte calcium responsiveness and contractility in transgenic mouse hearts. *J Clin Invest*, 102, 72-8 (1998)

86. Chen, Y., R. Rajashree, Q. Liu & P. Hofmann: Acute p38 MAPK activation decreases force development in ventricular myocytes. *Am J Physiol Heart Circ Physiol*, 285, H2578-86 (2003)

87. Liao, P., S. Q. Wang, S. Wang, M. Zheng, M. Zheng, S. J. Zhang, H. Cheng, Y. Wang & R. P. Xiao: p38 Mitogen-activated protein kinase mediates a negative inotropic effect in cardiac myocytes. *Circ Res*, 90, 190-6 (2002)

88. Vahebi, S., A. Ota, M. Li, C. M. Warren, P. P. de Tombe, Y. Wang & R. J. Solaro: p38-MAPK induced dephosphorylation of alpha-tropomyosin is associated with depression of myocardial sarcomeric tension and ATPase activity. *Circ Res*, 100, 408-15 (2007)

89. Kranias, E. G. & D. M. Bers: Calcium and cardiomyopathies. *Subcell Biochem*, 45, 523-37 (2007)

90. Liu, Y. H., D. Wang, N. E. Rhaleb, X. P. Yang, J. Xu, S. S. Sankey, A. E. Rudolph & O. A. Carretero: Inhibition of p38 mitogen-activated protein kinase protects the heart against cardiac remodeling in mice with heart failure resulting from myocardial infarction. *J Card Fail*, 11, 74-81 (2005)

91. Petit, F., D. Arnoult, L. Viollet & J. Estaquier: Intrinsic and extrinsic pathways signaling during HIV-1 mediated cell death. *Biochimie*, 85, 795-811 (2003)

92. Porter, A. G., P. Ng & R. U. Janicke: Death substrates come alive. *Bioessays*, 19, 501-7 (1997)

93. Thornberry, N. A. & Y. Lazebnik: Caspases: enemies within. *Science*, 281, 1312-6 (1998)

94. Lorenzo, H. K., S. A. Susin, J. Penninger & G. Kroemer: Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. *Cell Death Differ*, 6, 516-24 (1999)

95. Cregan, S. P., V. L. Dawson & R. S. Slack: Role of AIF in caspase-dependent and caspase-independent cell death. *Oncogene*, 23, 2785-96 (2004)

96. Lockshin, R. A. & Z. Zakeri: Caspase-independent cell death? *Oncogene*, 23, 2766-73 (2004)

97. Anversa, P., A. Leri, C. A. Beltrami, S. Guerra & J. Kajstura: Myocyte death and growth in the failing heart. *Lab Invest*, 78, 767-86 (1998)

98. Feuerstein, G. Z. & P. R. Young: Apoptosis in cardiac diseases: stress- and mitogen-activated signaling pathways. *Cardiovasc Res*, 45, 560-9 (2000)

99. Narula, J., N. Haider, R. Virmani, T. G. DiSalvo, F. D. Kolodgie, R. J. Hajjar, U. Schmidt, M. J. Semigran, G. W. Dec & B. A. Khaw: Apoptosis in myocytes in end-stage heart failure. *N Engl J Med*, 335, 1182-9 (1996)

100. Anversa, P., J. Kajstura & G. Olivetti: Myocyte death in heart failure. *Curr Opin Cardiol*, 11, 245-51 (1996)

101. Li, Y., G. Takemura, H. Okada, S. Miyata, H. Kanamori, R. Maruyama, M. Esaki, L. Li, A. Ogino, T. Ohno, T. Kondo, M. Nakagawa, S. Minatoguchi, T. Fujiwara & H. Fujiwara: ANG II type 1A receptor signaling causes unfavorable scar dynamics in the postinfarct heart. *Am J Physiol Heart Circ Physiol*, 292, H946-53 (2007)

102. Feuerstein, G. Z.: Apoptosis in cardiac diseases--new opportunities for novel therapeutics for heart diseases. *Cardiovasc Drugs Ther*, 13, 289-94 (1999)

103. Dzau, V. J.: Multiple pathways of angiotensin production in the blood vessel wall: evidence, possibilities and hypotheses. *J Hypertens*, 7, 933-6 (1989)

104. Zheng, X., T. Zhang, H. Ding & C. Wang: Plasma levels of beta-endorphin, leucine enkephalin and arginine vasopressin in patients with essential hypertension and the effects of clonidine. *Int J Cardiol*, 51, 233-44 (1995)

105. Cigola, E., J. Kajstura, B. Li, L. G. Meggs & P. Anversa: Angiotensin II activates programmed myocyte cell death *in vitro*. *Exp Cell Res*, 231, 363-71 (1997)

106. Kajstura, J., E. Cigola, A. Malhotra, P. Li, W. Cheng, L. G. Meggs & P. Anversa: Angiotensin II induces apoptosis of adult ventricular myocytes *in vitro*. *J Mol Cell Cardiol*, 29, 859-70 (1997)

107. Masaki, H., T. Kurihara, A. Yamaki, N. Inomata, Y. Nozawa, Y. Mori, S. Murasawa, K. Kizima, K. Maruyama, M. Horiuchi, V. J. Dzau, H. Takahashi, T. Iwasaka, M. Inada & H. Matsubara: Cardiac-specific overexpression of angiotensin II AT2 receptor causes attenuated response to AT1 receptor-mediated pressor and chronotropic effects. *J Clin Invest*, 101, 527-35 (1998)

108. Yamada, T., M. Horiuchi & V. J. Dzau: Angiotensin II type 2 receptor mediates programmed cell death. *Proc Natl Acad Sci U S A*, 93, 156-60 (1996)

109. Porrello ER, D. L., Thomas WG. : The Angiotensin II type 2 (AT2) receptor: an enigmatic seven transmembrane receptor. *Frontiers in Bioscience,*, in press, (2008)

110. Miura, S. & S. S. Karnik: Ligand-independent signals from angiotensin II type 2 receptor induce apoptosis. *Embo J*, 19, 4026-35 (2000)

111. Zhang, D., L. Yang, Z. Liu & S. Mi: Study on the effects of losartan on cardiomyocyte apoptosis and gene expression after ischemia and reperfusion *in vivo* in rats. *J Tongji Med Univ*, 20, 49-52 (2000)

112. Nio, Y., H. Matsubara, S. Murasawa, M. Kanasaki & M. Inada: Regulation of gene transcription of angiotensin II receptor subtypes in myocardial infarction. *J Clin Invest*, 95, 46-54 (1995)

113. Griendling, K. K., P. Delafontaine, S. E. Rittenhouse, M. A. Gimbrone, Jr. & R. W. Alexander: Correlation of receptor sequestration with sustained diacylglycerol accumulation in angiotensin II-stimulated cultured vascular smooth muscle cells. *J Biol Chem*, 262, 14555-62 (1987)

114. Lassegue, B., R. W. Alexander, G. Nickenig, M. Clark, T. J. Murphy & K. K. Griendling: Angiotensin II down-regulates the vascular smooth muscle AT1 receptor by transcriptional and post-transcriptional mechanisms: evidence for homologous and heterologous regulation. *Mol Pharmacol*, 48, 601-9 (1995)

115. Adachi, Y., Y. Saito, I. Kishimoto, M. Harada, K. Kuwahara, N. Takahashi, R. Kawakami, M. Nakanishi, Y. Nakagawa, K. Tanimoto, Y. Saitoh, S. Yasuno, S. Usami, M. Iwai, M. Horiuchi & K. Nakao: Angiotensin II type 2 receptor deficiency exacerbates heart failure and reduces survival after acute myocardial infarction in mice. *Circulation*, 107, 2406-8 (2003)

116. Holtz, J.: Role of ACE inhibition or AT1 blockade in the remodeling following myocardial infarction. *Basic Res Cardiol*, 93 Suppl 2, 92-100 (1998)

117. Oishi, Y., R. Ozono, Y. Yano, Y. Teranishi, M. Akishita, M. Horiuchi, T. Oshima & M. Kambe: Cardioprotective role of AT2 receptor in postinfarction left ventricular remodeling. *Hypertension*, 41, 814-8 (2003)

118. Jugdutt, B. I. & V. Menon: AT1 receptor blockade limits myocardial injury and upregulates AT2 receptors during reperfused myocardial infarction. *Mol Cell Biochem*, 260, 111-8 (2004)

119. Nickenig, G., J. Ostergren & H. Struijker-Boudier: Clinical evidence for the cardiovascular benefits of angiotensin receptor blockers. *J Renin Angiotensin Aldosterone Syst*, 7 Suppl 1, S1-7 (2006)

120. Anavekar, N. S. & S. D. Solomon: Angiotensin II receptor blockade and ventricular remodelling. *J Renin Angiotensin Aldosterone Syst*, 6, 43-8 (2005)

121. Bohm, M.: Angiotensin receptor blockers versus angiotensin-converting enzyme inhibitors: where do we stand now? *Am J Cardiol*, 100, 38J-44J (2007)

122. Adams, J. W., A. L. Pagel, C. K. Means, D. Oksenberg, R. C. Armstrong & J. H. Brown: Cardiomyocyte apoptosis induced by Galphaq signaling is mediated by permeability transition pore formation and activation of the mitochondrial death pathway. *Circ Res*, 87, 1180-7 (2000)

123. Kanamori, H., G. Takemura, Y. Li, H. Okada, R. Maruyama, T. Aoyama, S. Miyata, M. Esaki, A. Ogino, M. Nakagawa, H. Ushikoshi, M. Kawasaki, S. Minatoguchi & H. Fujiwara: Inhibition of Fas-associated apoptosis in granulation tissue cells accompanies attenuation of postinfarction left ventricular remodeling by olmesartan. *Am J Physiol Heart Circ Physiol*, 292, H2184-94 (2007)

124. Clerk, A., S. M. Cole, T. E. Cullingford, J. G. Harrison, M. Jormakka & D. M. Valks: Regulation of cardiac myocyte cell death. *Pharmacol Ther*, 97, 223-61 (2003) doi not found.

125. Scheubel, R. J., B. Bartling, A. Simm, R. E. Silber, K. Drogaris, D. Darmer & J. Holtz: Apoptotic pathway activation from mitochondria and death receptors without caspase-3 cleavage in failing human myocardium: fragile balance of myocyte survival? *J Am Coll Cardiol*, 39, 481-8 (2002)

126. Heidkamp, M. C., A. L. Bayer, J. L. Martin & A. M. Samarel: Differential activation of mitogen-activated protein kinase cascades and apoptosis by protein kinase C epsilon and delta in neonatal rat ventricular myocytes. *Circ Res*, 89, 882-90 (2001)

127. Orrenius, S., B. Zhivotovsky & P. Nicotera: Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol*, 4, 552-65 (2003)

128. Yoneda, T., K. Imaizumi, K. Oono, D. Yui, F. Gomi, T. Katayama & M. Tohyama: Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem*, 276, 13935-40 (2001)

129. Thor, H., P. Hartzell & S. Orrenius: Potentiation of oxidative cell injury in hepatocytes which have accumulated Ca2+. *J Biol Chem*, 259, 6612-5 (1984)

130. Hajnoczky, G., L. D. Robb-Gaspers, M. B. Seitz & A. P. Thomas: Decoding of cytosolic calcium oscillations in the mitochondria. *Cell*, 82, 415-24 (1995)

131. Zou, Y., H. Takano, H. Akazawa, T. Nagai, M. Mizukami & I. Komuro: Molecular and cellular mechanisms of mechanical stress-induced cardiac hypertrophy. *Endocr J*, 49, 1-13 (2002)

132. Vila-Petroff, M., M. A. Salas, M. Said, C. A. Valverde, L. Sapia, E. Portiansky, R. J. Hajjar, E. G. Kranias, C. Mundina-Weilenmann & A. Mattiazzi: CaMKII inhibition protects against necrosis and apoptosis in irreversible ischemia-reperfusion injury. *Cardiovasc Res*, 73, 689-98 (2007)

133. Zhang, T. & J. H. Brown: Role of Ca2+/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure. *Cardiovasc Res*, 63, 476-86 (2004)

134. Zhu, W. Z., S. Q. Wang, K. Chakir, D. Yang, T. Zhang, J. H. Brown, E. Devic, B. K. Kobilka, H. Cheng & R. P. Xiao: Linkage of beta1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. *J Clin Invest*, 111, 617-25 (2003)

135. Palomeque J, V. C., Salas MA, Mattiazzi A: CaMKII Activation is Involved in Angiotensin II-Mediated Cell Death. *Circulation*, 116, II_114-II_115 (2007)

136. Curran, J., M. J. Hinton, E. Rios, D. M. Bers & T. R. Shannon: Beta-adrenergic enhancement of sarcoplasmic reticulum calcium leak in cardiac myocytes is mediated by calcium/calmodulin-dependent protein kinase. *Circ Res*, 100, 391-8 (2007)

137. Ai, X., J. W. Curran, T. R. Shannon, D. M. Bers & S. M. Pogwizd: Ca2+/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca2+ leak in heart failure. *Circ Res*, 97, 1314-22 (2005)

138. Chen, X., X. Zhang, H. Kubo, D. M. Harris, G. D. Mills, J. Moyer, R. Berretta, S. T. Potts, J. D. Marsh & S. R. Houser: Ca2+ influx-induced sarcoplasmic reticulum Ca2+ overload causes mitochondrial-dependent apoptosis in ventricular myocytes. *Circ Res*, 97, 1009-17 (2005)

139. Caldiz, C. I., C. D. Garciarena, R. A. Dulce, L. P. Novaretto, A. M. Yeves, I. L. Ennis, H. E. Cingolani, G. Chiappe de Cingolani & N. G. Perez: Mitochondrial reactive oxygen species activate the slow force response to stretch in feline myocardium. *J Physiol*, 584, 895-905 (2007)

140. Erickson, J. R., M. L. Joiner, X. Guan, W. Kutschke, J. Yang, C. V. Oddis, R. K. Bartlett, J. S. Lowe, S. E. O'Donnell, N. Aykin-Burns, M. C. Zimmerman, K. Zimmerman, A. J. Ham, R. M. Weiss, D. R. Spitz, M. A. Shea, R. J. Colbran, P. J. Mohler & M. E. Anderson: A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell*, 133, 462-74 (2008)

141. Palomeque J, S. L., Valverde CA, Velez Rueda JO, Salas MA, Mattiazzi A, Vila Petroff, M.: CaMKII mediates angiotensin II-induced cardiomyocytes apoptosis: Role of Ca2+, ROS and p38 MAPK. *Journal of Molecular and Cellular Cardiology*, 44, 764 (2008)

142. Li, Z., J. Y. Ma, I. Kerr, S. Chakravarty, S. Dugar, G. Schreiner & A. A. Protter: Selective inhibition of p38alpha MAPK improves cardiac function and reduces myocardial apoptosis in rat model of myocardial injury. *Am J Physiol Heart Circ Physiol*, 291, H1972-7 (2006)

143. Griffiths, E. J. & A. P. Halestrap: Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J*, 307 (Pt 1), 93-8 (1995)

144. Bendall, J. K., A. C. Cave, C. Heymes, N. Gall & A. M. Shah: Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. *Circulation*, 105, 293-6 (2002)

145. Johar, S., A. C. Cave, A. Narayanapanicker, D. J. Grieve & A. M. Shah: Aldosterone mediates angiotensin II-induced interstitial cardiac fibrosis via a Nox2-containing NADPH oxidase. *Faseb J*, 20, 1546-8 (2006)

146. Matsushima, S., S. Kinugawa, T. Ide, H. Matsusaka, N. Inoue, Y. Ohta, T. Yokota, K. Sunagawa & H. Tsutsui: Overexpression of glutathione peroxidase attenuates myocardial remodeling and preserves diastolic function in diabetic heart. *Am J Physiol Heart Circ Physiol*, 291, H2237-45 (2006)

147. Ushio-Fukai, M., R. W. Alexander, M. Akers & K. K. Griendling: p38 Mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. *J Biol Chem*, 273, 15022-9 (1998)

148. Takeda, K., A. Matsuzawa, H. Nishitoh, K. Tobiume, S. Kishida, J. Ninomiya-Tsuji, K. Matsumoto & H. Ichijo: Involvement of ASK1 in Ca2+-induced p38 MAP kinase activation. *EMBO Rep*, 5, 161-6 (2004)

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