Chapter 5
The Role of Proinflammatory Cytokines in Regulation of Cardiac Bioelectrical Activity: Link to Mechanoelectrical Feedback

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Abstract In this review we cover key issues, concerned with the effects of cytokines in cardiac tissue and cardiac cells. We discuss the effects of proinflammatory cytokines under non-pathological conditions and their mechanisms dependent and independent of nitric oxide. The role of proinflammatory cytokines is considered in acute myocardial infarction and in heart failure. We also describe proinflammatory cytokines as inductors of arrhythmia. We discuss ionic current alternation as possible mechanisms of cytokines action in heart. We consider TNF-α as a possible player in this signaling cascade. It was shown that TNF-α induced alternation of transmembrane action potentials. Influence of TNF-α on transient outward current (I_{to}), I_{Kur}, I_{Kr}, I_{Ks}, I_{K1} is also reported. We discuss the interplay between TNF-α and Ca^{2+} current, influence of TNF-α on SERCA. Then we consider influence of IL-1 on action potentials, I_{Na}, I_{Ca}, I_K. We also address the role of IL-2, IL-6, and IL-11. Finally using TNF-α and IL-6 as an example we discuss the effects of cytokines on mechanoelectrical feedback. Perfusion of cardiac tissue with TNF-α containing solution leads to abnormalities in cardiac electrical activity, majorly to prolongation of APD90 and appearance of hump-like depolarization at APD90 level. After reaching E_c hump-like depolarization transforms into extra-AP, leading to sustained arrhythmias. TNF-α activates NO cardiomyocyte synthases and the rise of intracellular NO levels opens MGCs, which leads to sodium entry into the cell, which depolarizes cellular membrane, shifting resting potential towards E_C. We proposed and proved that TNF-α triggered arrhythmias can be mediated through activation of MGCs. Stretching of preparations removed TNF-α. Perfusion of preparation with IL-6 containing solution leads to fibrillation in response to low levels of stretch. IL-6 mechanisms of action are mediated by NO synthases

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in cardiomyocytes. The circulating levels of TNF-α and IL-6 were found to be significantly higher in patients suffering from atrial fibrillation. It suggests a positive feedback between inflammation and atrial fibrillation. Proinflammatory cytokines are believed to be markers of atrial fibrillation, and more over, the key element in this positive feedback system.

Keywords Heart · Arrhythmia · Proinflammatory cytokines · Cardiomyocytes · Mechanoelectrical feedback · Mechanically gated channels · TNF-α · IL-2 · IL-6 · IL-11 · Nitric oxide · Ionic currents

5.1 Introduction

First cytokine related investigations date back to 1950s, but till present a number of questions in this field remain unanswered. For example till late 2000s it was generally accepted that biological effects of cytokines are known. However recently it was found that their effects expand far beyond the immune system and affect many organs and tissues. Latest studies of cardiovascular pathology genesis lead to emergence of a new related field, which can be called "Cardio-immunology". The role of cytokines in regulation of cardiac function is well described in literature. Unfortunately much fewer studies investigated the cross talk between cytokines effects and effects of myocardium stretch of healthy and pathological tissue. First studies in this field addressed this topic on whole heart level. For example one lab studied the induction of myocardial stretch in an isolated perfused Langendorff preparation by inflation of an intraventricular balloon to an end-diastolic load, which according to their publication resulted in a nearly six fold increase in VEGF message level. They reported a possibility of stretch-induced induction of VEGF expression (Li et al., 1997). Later works focused primarily on cytokine production during stretching of cardiac cells in culture (For example Yokoyama et al., 1999). This field of research remains relatively new and to our knowledge there is only a few number of reviews addressing this topic (for example see Chapter 2 of this Volume). The effects of cytokines on stretched heart and their role in regulation of mechanoelectrical feedback remains unknown. Moreover the role of cytokines in regulation of ion channel activity, their single channel currents and action potentials remain unaddressed. For this reasons we focused our chapter on discussion of the role of main proinflammatory cytokines in regulation of heart bioelectrical activity. In conclusion of our review we analyze the role of a number of cytokines in regulation of mechanoelectrical feedback.

5.2 Physiological Role of Proinflammatory Cytokines

5.2.1 Effects of Proinflammatory Cytokines under Non-pathological Conditions and Their Mechanisms Dependent and Independent of Nitric Oxide

The main and the most obvious physiological function of proinflammatory cytokines is induction of inflammation in the case of tissue damage. However, some of them demonstrate pleiotropic nature, for example IFN-γ is considered a proinflammatory cytokine, but possesses antiviral activity and induces activation of cytotoxic T cells (Dinarello, 2000). The basic mechanism of proinflammatory cytokine-mediated promotion of inflammation is up-regulation of several genes encoding the mediators of inflammation, which are usually not produced in healthy persons. For example, some of these genes code for enzymes that increase the synthesis of leukotrienes, prostanoids and NO. Others are genes of chemokines, the small peptides that facilitate the passage of leukocytes from bloodstream into the tissues, and endothelial adhesion molecules, which are essential for leukocytes adhesion to endothelium (Dinarello, 2000). Of course, proinflammatory action of cytokines is expressed mostly in the damaged tissue, however, as far as proinflammatory cytokines can get to the bloodstream and induce fever and other types of systemic response, they may affect all other organs located far from the nidus of inflammation. Heart is one of their possible targets.

That is why cardiotropic effects of proinflammatory cytokines undoubtedly merit extensive investigation. In the last two decades various effects of proinflammatory cytokines, mostly TNF and IL-1, on contractile and electrical activity of myocardium were demonstrated in diverse in vivo and in vitro studies. These effects may be divided in two groups: the early ones begin to develop immediately after cytokine application and completely develop within the first 3 h, while the delayed response is lasting from hours to days (Prabhu, 2004a).

The early response may be either cardiostimulatory or inhibitory depending on the concentration of cytokine and prevailing cellular and biological milieu. For example, low concentrations of TNF-α (200–500 U/ml) cause transient decrease of AP duration and amplitude of peak Ca^{2+} transient without affecting $I_{Ca,L}$ in adult mammalian cardiomyocytes (Yokoyama et al., 1993; Sugishita et al., 1999; Stamm et al., 2001a, b). However, high concentrations (≥10,000 U/ml) were observed to produce either inhibition of $I_{Ca,L}$ accompanied with depression of peak Ca^{2+} transient (Krown et al., 1995) or no changes in Ca^{2+} turnover despite reduced contractility (Goldhaber et al., 1996). In other studies early positive inotropic effects of TNF-α mediated by increase of peak Ca^{2+} transient were observed in adult rat cardiomyocytes (Cailleret et al., 2004; Amadou et al., 2002). Other proinflammatory cytokines: IL-1β (Liu and Schreur, 1995; Schreur and Liu, 1997), IL-6 (Sugishita et al., 1999) and IL-2 (Cao et al., 2003a, b) were shown to suppress contractile activity in moderate concentrations (about 1–5 ng/ml). This effect is mediated by $I_{Ca,L}$ depression in the case of IL-1β.

Alteration of Ca^{2+} cycling is not the only mechanism of immediate cytokine effects. Stimulation of cytokine receptors also leads to rapid induction of NO production by cNOS as well as activation of sphingomyelinase and PLA2-dependent signaling cascades (Prabhu, 2004a; Hedayat et al., 2010). The role of cNOS activation in distribution of early negative inotropic effects was demonstrated for TNF-α (Finkel et al., 1992; Goldhaber et al., 1996), IL-1β (Cain et al., 1999; Kumar et al., 1999), IL-2 (Finkel et al., 1992; McGowan et al., 1994) and IL-6 (Finkel et al., 1992; Kinugawa et al., 1994; Sugishita et al., 1999) in various cardiac preparations and isolated cardiomyocytes. Some of these authors argue that NO-dependent negative inotropic effects of proinflammatory cytokines are produced via activation of soluble guanylate cyclase and subsequent elevation of intracellular cGMP level (Kinugawa et al., 1994; Kumar et al., 1999). On the contrary, several other groups presented serious argumentation against participation of NO-dependent pathway in mediation of acute cytokine effects (Yokoyama et al., 1993; Edmunds et al., 1999; Grandel et al., 2000). Thus, interrelations between cytokines and NO signaling cascade are rather controversial.

It is known that proinflammatory cytokines activate sphingomyelinases in several cell types (Liu et al., 1998; Kolesnick 2002). Sphingomyelinases hydrolyze membrane phospholipide sphingomyeline to form ceramide, which, in turn, can be deacylated by ceramidase to yield sphingosine. This amino alcohol substance and its metabolite sphingosine-1-phosphate act as a second messenger, producing suppression of Ca^{2+} turnover in cardiomyocytes (Dettbam et al., 1994; Friedrichs et al., 2002). Several studies have indicated the possible contribution of sphyngolipid signaling pathway to early negative inotropic effects of TNF-α in adult feline (Oral et al., 1997) and guinea pig cardiomyocytes (Sugishita et al., 1999), rat ventricular myocardium (Hofmann et al., 2003), isolated rat (Edmunds and Woodward, 1998) and rabbit hearts (Stamm et al., 2001a, b). The role of sphingomyelinase signaling cascade in the contractile effects of other proinflammatory cytokines is sparsely studied, although it was proposed for negative inotropic effect of IL-1β in human atrial trabeculae (Cain et al., 1999). Interestingly, activity of neutral sphingomyelinases is inhibited by glutathione, therefore negative inotropic effect of cytokines exerted via activation of sphingomyelinase signaling cascade depends on the intracellular redox state. In vivo administration of glutathione precursor *N*-acetylcysteine blocks early cardiodepressant effects of TNF-α, unmasking the positive inotropic effect of this cytokine (Cailleret et al., 2004).

Effects of proinflammatory cytokines attributed to activation of sphingolipid pathway are at least partly dependent on another second messenger – arachidonic acid. TNF-α and IL-1β stimulate phospholipase A2 via activation of their receptors and therefore increase the intracellular level of arachidonic acid (Jayadev et al., 1994; Liu and McHowat, 1998). This messenger activates neutral sphingomyelinases (Robinson et al., 1997), but also has own positive inotropic effect in cardiomyocytes, which is produced via increase of Ca^{2+} transients (Kang and Leaf, 1994; Damron and Summers, 1997). Therefore, small concentrations of arachidonic acid improve myocardial contractility, while higher concentrations suppress it due to

the activation of sphingolipid pathway (Amadou et al., 2002; Damron and Summers, 1997). That is why some researchers explain the dual inotropic effects of TNF-α by such dual action of arachidonic acid (Amadou et al., 2002). Arachidonic acid may also mediate the positive inotropic effect of IL-2 (Bracco et al., 1991).

Delayed effects of proinflammatory cytokines are substantially less controversial than the acute ones. It is generally accepted that sustained application of proinflammatory cytokines, in the first place TNF-α and IL-1β, induces marked decrease of basal myocardial contractility which is aggravated by suppressed responsiveness to stimulation of β-adrenoreceptors (Prabhu, 2004a, b). The latter effect for the first time was demonstrated in rat neonatal myocytes (Gulick et al., 1989; Chung et al., 1990). Authors explained this effect by loss of β-adrenoreceptors ability to activate adenylate cyclase, because cytokines didn't change the density of receptors and on the other hand direct stimulation of adenylate cyclase by forskolin induced normal cAMP accumulation and augmentation of contractile activity. Subsequent studies confirmed that impairment of signal transduction from receptor to adenylate cyclase, which is mediated by G_s protein, is the main reason of decreased β-adrenoreceptors responsiveness (Rozanski and Witt, 1994; Bick et al., 1997).

Several other groups of investigators believe that suppression of β-adrenoreceptors responsiveness is mediated by sustained upregulation of iNOS leading to increased cGMP intracellular content resulting in decrease of cAMP level due to the activation of PDE2 by cGMP (Balligand et al., 1993, 1994; Balligand, 1999; Ungureanu-Longrois et al., 1995, 1997; Joe et al., 1998). It seems likely that both NO-dependent and NO-independent mechanisms of β-adrenoreceptors responsiveness are decreased by proinflammatory cytokines which are present in the myocardium. Moreover, upregulation of iNOS, which is already shown for most of proinflammatory cytokines in the case of their prolonged application (Hedayat et al., 2010), exerts several other crucial consequences such as S-nitrosylation of thiol residues of important regulatory proteins and formation of peroxynitrite, leading together to prolonged depression of contractile function (Campbell et al., 1996; Massion et al., 2003; Prabhu, 2004a). Obviously, increase in generation of reactive oxygen species (ROS), which is also induced in myocardium by different proinflammatory cytokines (Oyama et al., 1998; Cheng et al., 1999; Panas et al., 1998; Ferdinandy et al., 2000), amplifies the latter effect greatly. For example, in isolated rat heart combination of IL-1β, TNF-α and IFN-γ provokes augmentation of xanthine oxidoreductase and NAD(P)H oxidase activity, which is accompanied with enhanced activity of iNOS and leads to sustained decline in contractility (Ferdinandy et al., 2000). It is important to note that oxidative modifications induced by ROS and peroxynitrite tend to be irreversible (Stamler et al., 2001) and are therefore attributed to serious pathological changes in the structure and function of myocardium.

Thus, the modulation of normal heart function by proinflammatory cytokines is extremely complex and is mediated by diverse signaling mechanisms. However, it becomes even more versatile in the case of cardiac disease. Here we briefly discuss the role of proinflammatory cytokines in pathogenesis of myocardial infarction, heart failure and arrhythmias.

5.2.2 *Proinflammatory Cytokines in Acute Myocardial Infarction*

The onset of myocardial infarction starts from ischemic and subsequent reperfusion injury of myocardium leading to cardiomyocytes necrosis. Proinflammatory cytokines have significant impact on heart function during all these stages. Although they promote myocardial malfunction during and after ischemic injury, cytokines are able to increase resistance of heart to such injury if added prior to ischemia.

It is accepted that application of TNF-α (Nakano et al., 1998; Eddy et al., 1992), IL-1β (Brown et al., 1990; Maulik et al., 1993) and IL-6 (Smart et al., 2006) prior to induction of ischemia-reperfusion injury exerts clear beneficial cardioprotective effects. The mechanisms of such cytoprotective preconditioning are diverse, but in the case of TNF-α and IL-1β they are undoubtedly associated with induction of manganous superoxide dismutase (MnSOD), which neutralizes the cytotoxic oxygen free radicals, which are generated in the myocardium during ischemia-reperfusion (Chen et al., 1998; Wong and Goeddel, 1988; Maulik et al., 1993). The second substantial protective mechanism is concerned with upregulation of various heat-shock proteins, which attenuate the excessive myocardial production of inflammatory cytokines, following the ischemic and reperfusion injury and having strong deleterious impact on cardiac function (Nakano et al., 1996). IL-1β preconditioning promotes accumulation of leukocytes and generation of H_2O_2 in myocardium, leading to moderate oxidative stress and induction of cytoprotective mechanisms, which increase resistance of myocardium to ischemic injury (Brown et al., 1990). IL-6 preconditioning is mediated mainly by inhibition of apoptotic signaling pathways in cardiomyocytes via PI3K/Akt-mediated activation of iNOS (Smart et al., 2006).

It is not surprising that during ischemia and subsequent reperfusion proinflammatory cytokines aggravate irreversible injury of myocardium (Schulz, 2008; Schulz and Heusch, 2009). In ischemic myocardium negative effects of TNF-α, such as induction of apoptosis (Haudek et al., 2007) are produced via activation of first type TNF receptors (Flaherty et al., 2008) and can be prevented by treatment with TNF-α antibodies (Belosjorow et al., 2003) or soluble TNF receptors of first type (Sugano et al., 2004), which are capable of binding and inactivation of TNF-α. IL-1 also enhances apoptosis during ischemia and reperfusion (Haudek et al., 2007). This effect is mediated by activation of NO production (Ing et al., 1999) via stimulation of IL-1 receptors and can be blocked by antagonists of these receptors (Abbate et al., 2008). Similarly to IL-1, IL-6 provokes contractile dysfunction in ischemic myocardium via activation of iNOS mediated by JAK2/STAT3 signaling pathway (Yu et al., 2003a, b). In the last years IL-18 was accepted as a new powerful proapoptotic factor, which plays very negative role during ischemia. This cytokine promotes apoptosis in cardiomyocytes via upregulation of death receptors leading to enhancement of extrinsic proapoptotic signaling (Marino and Cardier, 2003; Chandrasekar et al., 2004), activates caspases and suppresses antiapoptotic PI3K/Akt pathway via induction of phosphatase and tensin homolog expression (Chandrasekar et al., 2006).

After the ischemic lesion the myocardium itself becomes the source of proinflammatory cytokines. Obviously, the increased production of cytokines endangers nearby cells altering cardiomyocytes structure and function. However, infarction leads to increased level of proinflammatory cytokines in blood and, as a result, severe systemic inflammatory response syndrome known as the leading cause of death in patients with acute myocardial infarction. TNF-α, IL-6 and, especially, IL-1β play the key role in its pathogenesis (Debrunner et al., 2008). Nevertheless, we will focus on alteration of heart function caused by proinflammatory cytokines, produced in the heart itself after myocardial infarction.

Firstly, all acute cardiotropic effects of proinflammatory cytokines, described in Section 5.2.1, take place after infarction but in much bigger extent than in normal conditions, because concentration of cytokines in the heart are many times higher. Second, described proapoptotic action of cytokines aggravates after infarction. Moreover, other consequences of inflammation, such as infiltration of leukocytes and increase of ROS production take place in the heart and together with proper cytokines' effects lead to progressive loss of cardiac function. Finally, expression of proinflammatory cytokines provokes hypertrophy of cardiomyocytes and remodeling of extracellular cardiac matrix. These effects potentiate delayed cardiodepressant action of cytokines (see Section 5.2.1) and accelerate development of cardiac failure following acute myocardial infarction (Hedayat et al., 2010).

5.2.3 Role of Proinflammatory Cytokines in Heart Failure

Hypertrophic growth response is shown for all proinflammatory cytokines (Hedayat et al., 2010). Hypertrophic effects of TNF-α, measured in terms of cardiomyocytes size (both length and width), cardiac weight and left ventricular wall thickness (Janczewski et al., 2003) are at least partly mediated by upregulation of angiotensin-converting enzyme and resulting increase in angiotensin II protein level (Flesch et al., 2003). TNF-α stimulates expression of other proinflammatory cytokines in myocardium, which in turn amplify its own hypertrophic action. Hypertrophic effect of IL-1, which was demonstrated in several in vitro (Palmer et al., 1995; Thaik et al., 1995) and in vivo studies (Nishikawa et al., 2006), is NO-independent and appears to be mediated through a tyrosine kinase signaling pathway (Palmer et al., 1995; Thaik et al., 1995). IL-6 also contributes to hypertrophic growth response (Yamauchi-Takihara and Kishimoto, 2000), which is believed to be mediated mainly through JAK/STAT3 signaling pathway (Kunisada et al., 2000). On the contrary to IL-1 and IL-6, hypertrophy of cardiomyocytes induced by IL-18 is mediated through PI3K cascade (Chandrasekar et al., 2005; Colston et al., 2007). Thus, cytokines produced in the heart due to acute infarction induce hypertrophic growth of cardiomyocytes, which primarily helps to regain pumping function, but frequently transforms to maladaptive response leading to progressive heart failure.

Alterations of collagenous extracellular matrix (ECM) providing the physical scaffolding for the spatial organization of cardiomyocytes into cardiac tissue have been demonstrated to play a central role in cardiac remodeling and resulting

progressive heart failure (Fedak et al., 2005; Ju and Dixon, 1996). It is shown that TNF-α (Siwik and Colucci, 2004), IL-1β (Siwik et al., 2000) and other proinflammatory cytokines (Hedayat et al., 2010) cause imbalance between synthesis and degradation of ECM through disregulation of degradative enzymes and of matrix metalloproteinases (MMPs) activity. These effects are largely influenced by the duration of exposure, which ranges from increased fibrillar collagen degradation to excessive fibrillar collagen deposition. For example, in short term, activation of MMPs induced by TNF-α leads to enhanced degradation of ECM components which promotes progressive dilation of left ventricle (Sivasubramanian et al., 2001; Li et al., 2000). However, in long term, sustained action of TNF-α results in excessive collagen deposition and increased LV stiffness (Sivasubramanian et al., 2001).

Thus, increased expression of proinflammatory cytokines in the myocardium leads to sustained depression of cardiomyocytes contractile activity (see Section 5.2.1), which is accompanied with even more oppressive irreversible effects: cardiomyocytes hypertrophy and ECM remodeling and results in chronic heart failure.

5.2.4 Proinflammatory Cytokines as Inductors of Arrhythmia

The possibility of proinflammatory cytokines contribution to pathogenesis of cardiac arrhythmias is not so obvious as in the case of myocardial infarction and cardiac failure. Among cardiac arrhythmias, the most common and well-studied is atrial fibrillation (AF). However the specific mechanism of AF initiation remains unknown. It is generally accepted that AF is associated somehow with inflammation. The circulating levels of IL-6 (Marcus et al., 2008), TNF-α (Sata et al., 2004) and IL-18 (Luan et al., 2010) were found to be significantly higher in patients suffering from AF. However, it is unclear whether inflammation is a cause or an effect of AF and, in either case, what mechanistic pathways may be important. One of the hypothesis proposes that AF leads to myocyte calcium overload, promoting atrial myocyte apoptosis, which, in turn, induces local inflammation and complement activation. Tissue damage then ensues and fibrosis sets in (Avilles et al., 2003; Demelis and Panaretou, 2001). Locally released factors of inflammation, including cytokines, can further contribute to membrane dysfunction by inhibiting the calcium cycling machinery. This can eventually lead to the maintenance of AF (Avilles et al., 2003; Demelis and Panaretou, 2001, 2006).

Some experimental data sheds light on mechanism of arrhythmogenic action of proinflammatory cytokines in atrial myocardium. Administrating TNF-α in rats can induce arrhythmias (Krown et al., 1995) and mice with overexpression of TNF-α have a greater incidence of AF (London et al., 2003). The latter is associated with abnormalities in systolic and diastolic Ca^{2+} handling and may be due to them. In particular, enhanced expression of TNF-α is accompanied by increase in frequency and magnitude of spontaneous Ca^{2+} releases from the sarcoplasmic reticulum in working cardiomyocytes (Saba et al., 2004). Such releases may lead to induction

of delayed afterdepolarizations, which are well known triggers for AF, via activation of reverse Na^+/Ca^{2+} exchanger. In cardiomyocytes from pulmonary veins, which are believed to be important foci of ectopic beats initiating AF or tachycardia (Chen et al., 1999), TNF-α also markedly alters the calcium homeostasis in different ways, increases amplitudes and frequency of delayed afterdepolarizations and thereby increases pulmonary veins ability to initiate AF (Lee et al., 2007).

Thus, there seems to be a positive feedback between inflammation and AF and proinflammatory cytokines are believed to be not only markers of AF, but the key element in this positive feedback system. Moreover, various anti-inflammatory agents, such as statins or angiotensin-converting enzyme inhibitors, are able to suppress atrial remodeling associated with AF and, therefore decrease the possibility of new AF paroxysms (Issac et al., 2007).

The role of proinflammatory cytokines in ventricular arrhythmias induction was also studied just recently. In dogs, etanercept, which binds and inactivates TNF-α, decreased probability of ventricular tachyarrhythmias after coronary artery ligation (Yu et al., 2005a, b), indicating important role of this cytokine in sudden cardiac death. Mechanisms of proarrhythmic effects of cytokines in ventricles seem to be very similar to shown in atrial myocardium. TNF-α (Duncan et al., 2010; Saba et al., 2004) and IL-1β (Duncan et al., 2010) suppress total release of Ca^{2+} from the sarcoplasmic reticulum induced by AP, but promote local spontaneous Ca^{2+} releases during diastole. These releases lead to initiation of delayed afterdepolarizations via activation of depolarizing Na^+/Ca^{2+} exchanger current.

5.3 Ionic Current Alternation as a Possible Mechanisms of Cytokines Action in Heart

5.3.1 Tumor Necrosis Factor-α (TNF-α)

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that is implicated in a number of pathophysiological events. It was established that TNF-α affects myocardium through several pathways. TNF-α influences both mechanical and bioelectrical activity of myocardium by alternating membrane potassium and calcium currents and cytoplasmic calcium handling. It was also shown that tumor necrosis factor-α is able to induce rapid as well as slow effects in cardiac muscle.

A lot of studies were carried out to clarify mechanisms of TNF-α action in heart, but often, represented results are contradictory, especially in cases of investigation of cardiac bioelectrical activity. It is necessary to mention that in animal models or patients with heart failure serum TNF-α concentrations belong to pictogram range (up to 10 pg/ml) (Cugno et al., 2000; Testa et al., 1996). While, TNF-α concentration range, utilized to treat heart preparation and cells in electrophysiological studies extend from picogram to hundreds of nanogram per milliliter.

Here some aspects of TNF-α influence on action potentials and ionic currents in heart and TNF-α induced contraction reduction are discussed.

5.3.1.1 TNF-α Induced Alternation of Transmembrane Action Potentials

In isolated guinea pigs papillary muscle (Alloatti et al., 1999) TNF-α in concentration of 1–10 ng/ml induce APD decrease by 10–15% after short term (20–25 min) cytokine administration (Fig. 5.1a). Decreasing of TNF-α induced AP duration was concentration- and time-dependent. APD decreasing was accompanied by reduction of the contractile force. However, as Alloatti reports, the resting membrane potential, the overshoot and the maximum rate of depolarization of the APs were not affected by TNF-α. Recovery of mechanical activity and APs parameters was observes after 20 min of washout by solution free from TNF-α.

Effects of TNF-α on APs were completely abrogated by pretreatment of papillary muscle by the NOS inhibitor – L-NAME or PAF-receptor antagonists (PAF – platelet aggregation factor, CV 3988). Also TNF-α-induced APD decreasing was abolished by chemical (Triton X-100) endocardial endothelium removing. Alloatti et al. suggested that both the generation of NO and PAF production contributes to the TNF-induced alteration of APD. It was also speculated that the production of NO is consequent to the production of PAF.

TNF-α influence on APs of isolated canine ventricular cardiomyocytes was opposite to that, observed in guinea pigs papillary muscle. APD was significantly longer in canine cells pretreated with TNF-α (Fig. 5.1b) then in control cells. Alternation of APD occurred only after 10 h of incubation with 10 ng/ml TNF-α (Wang et al., 2004).

Effects of TNF-α were investigated in experiments with isolated rat ventricular cardiomyocytes. In this study it was shown that after 48 h of exposition of

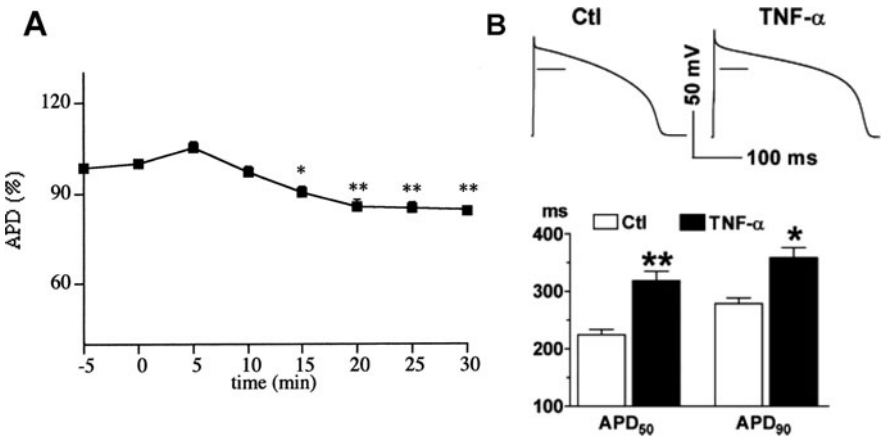

Fig. 5.1 TNF-α alternate action potential duration in heart. (**a**) Effects of TNF-α (10 ng/ml) on action potential duration (APD) in the isolated guinea pig papillary muscle (from Alloatti et al. (1999) with permission of Elsevier and British Cardiac Society from Copyright Clearance Center). (**b**) Effects of TNF-α (10 ng/ml, 6-h incubation at 4°C) on action potential duration in canine ventricular cells (from Wang et al. (2004) with permission of American Society for *Biochemistry and Molecular Biology* via Copyright Clearance Center)

cardiomyocytes to 5 ng/ml TNF-α APD increased more than two times (Fernandez-Velasco et al., 2007).

In ventricular cardiomyocytes, isolated from normal mice, which received chronically NTF-α significant alternation of ionic currents was observed, while AP duration remained unaltered (Grandy and Fiset, 2009). Only slight reduction of AP amplitude was observed. Authors speculate, that the lack of TNF-α-induced APs alterations could be due to counterbalanced alternation of repolarizating and depolarizating currents (resulting in APD preservation).

Transgenic mice that overexpress TNF-α selectively in the heart tissue have been studied as a model of congestive heart failure (Janczewski et al., 2003; Kubota et al., 1997; London et al., 2003; Sivasubramanian et al., 2001). TNF-α- overexpressing mice develop atrial and ventricular arrhythmias. In such mice heart level of TNF-α exceeds cytokine level usually reached in disease state more than ten times (Kubota et al., 1997). Several electrophysiological investigations were realized with such animals. AP duration in ventricles of perfused isolated hearts and in isolated ventricular transgenic mice cardiomyocytes was significantly lengthened (London et al., 2003; Petkova-Kirova et al., 2006) compared to APD in wild type mice.

Thereby, literature data, describing influence of TNF-α to action potential is contradictory. Some authors report decreasing of APs duration, on the contrary to other reports, TNF-α induce prolongation of APs. These discrepancies may occur due to different animals (mice, rat, dog), experimental protocol and wide range of TNF-α concentration used in experiments.

5.3.1.2 TNF-α and Ca^{2+} Current

Krown and coworkers has shown that in isolated rat ventricular cardiomyocytes TNF-α caused significant inhibition of I_{Ca} which mediated by TNF-α receptors TNFRI (Krown et al., 1995). TNF-α inhibition of the I_{Ca} was dose dependent (Fig. 5.2), but significant inhibition of I_{Ca} was observed in presence of very high concentration of TNF-α (>300 ng/ml), which 1000 times exceeds cytokine level observed in the pathophysiological condition (≈0.01–0.05 ng/ml) (Testa et al., 1996). As follows from *I–V* curves TNF-α exerts an inhibitory action at all tested potentials. Only partial recovery of I_{Ca} from inhibition was observed after TNF-α washout. Therefore, authors conclude involvement of intracellular second messengers in inhibitory effects of TNF-α.

Therefore, Krown and colleagues assume I_{Ca} suppression as a significant component of mechanism of TNF-α cardiodepressant action in different pathophysiological conditions.

On the contrary several studies reported lack of TNF-α effects on I_{Ca}. In isolated rat and cat ventricle myocytes treated by TNF-α peak I_{Ca} suppression and alternation of *I–V* relation were not detected (Yokoyama et al., 1993; Fernandez-Velasco et al., 2007).

Same observation was made in cardiomyocytes isolated from NTF-a overexpressing mice (Petkova-Kirova et al., 2006). Measurements of $I_{Ca,L}$ showed no difference in the peak current density between control and TNF-overexpressing

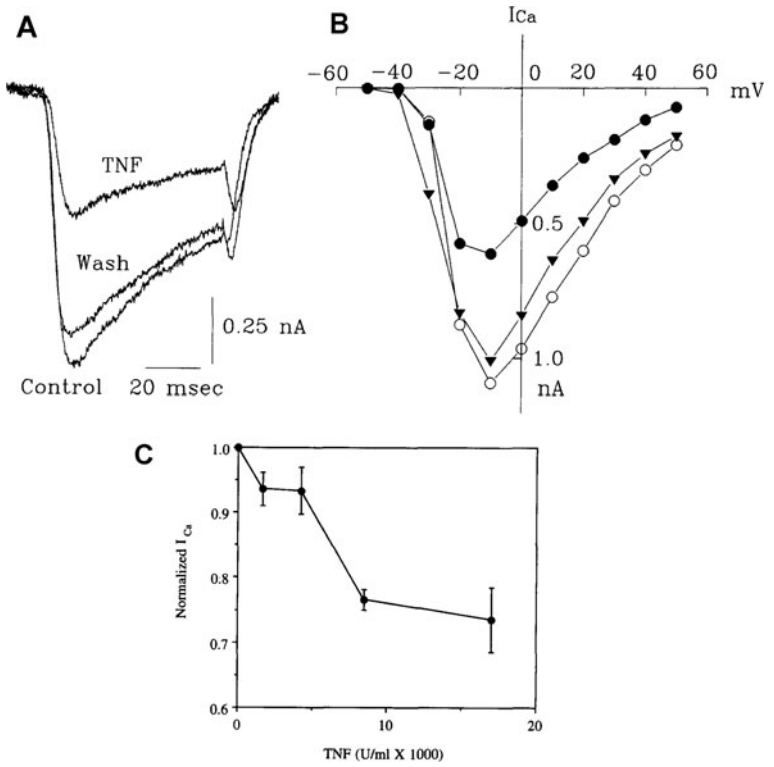

Fig. 5.2 TNF-α alternate cardiac $I_{Ca,L}$. (**a**) L-type channel recordings from a whole cell patch-clamped adult rat cardiac myocyte (5 min, 18,000 U/ml). There was a partial recovery after washout. Control experiments using fatty acid free BSA (data not shown) failed to show a non-specific protein effect on the channel in the range of protein used in the TNF-α experiments. (**b**) Current–voltage relationship for $I_{Ca,L}$ in the same cell prior to TNF-α application (*open circles*), 5 min after TNF-α application (*closed circles*) and after washout (*closed triangles*). (**c**) Average dose-dependent effect of TNF-α on L-type channel current in adult cardiac myocytes demonstrated half-maximal inhibition with 6400 U/ml. Holding potential –50 mV, pulses to 0 mV (from Krown et al. (1995) with permission of Elsevier Limited and *FEBS Letters* via Copyright Clearance Center)

myocytes over the range of potentials from –10 to +50 mV. At more negative potentials $I_{Ca,L}$ was slightly larger in TNF-α myocytes. No differences of inactivation and deactivation kinetics of $I_{Ca,L}$ were detected.

Hereby, ability of TNF-α to alternate I_{Ca} in cardiomyocytes remains questionable.

5.3.1.3 Influence of TNF-α on Transient Outward Current (I_{to})

Under various pathological conditions, including heart failure, prolongation of action potentials, which occurs due to downregulation or suppression of the

potassium currents (I_K) (Nuss et al., 1999; Beuckelmann et al., 1993) was observed. Depression of I_K and AP prolongation increases probability of arrhythmogenic events in heart (Nattel et al., 2007). Transient outward current (I_{to}) plays an essential role in early repolarization and regulation of duration of APs in myocardium (Hund and Rudy, 2004). In several studies it was shown that failing hearts (in human and in animals model) demonstrate significantly decreased I_{to} (Benitah et al., 1993; Kaab et al., 1998; Li et al., 2004). Isolated cardiomyocytes from transgenic mice overexpressing TNF-α demonstrate prolonged APs (London et al., 2003). Therefore, hypothesis accounting TNF-α as a mediator of I_{to} reduction was elaborated. In turn, TNF-α could modulate I_{to} through mechanisms involving upregulation of iNOS, NO and oxidant species (Fernandez-Velasco et al., 2007).

In study developed by Fernandez-Velasco isolated rat ventricular cardiomyocytes were exposed to TNF-α in concentration of 1–10 ng/ml for 48 h. After exposure to TNF-α amplitude of I_{to} was significantly reduced. Suppression of I_{to} was accompanied by AP prolongation. Inhibitory effect of TNF-α was concentration-dependent. TNF-α affect amplitude and density of current but not voltage dependence. Inactivating curve for I_{to} in cells treated with TNF-α was shifted to negative potentials. Half-maximal inactivation was –49.9 mV in control and –55.6 mV in cells treated with TNF-α. Authors also showed that exposure to TNF-α leads to lowering of I_{to} channel proteins level.

Thereby, depression of I_{to} is caused by several mechanisms – decreasing of current density, lowering level of channels proteins and shifting of inactivating curve to negative potentials.

The decrease of I_{to} induced by TNF-α was fully reversed in cells pretreated by iNOS inhibitors. Similarly, preliminary exposure of cardiomyocytes with antioxidants also reverses TNF-α induced I_{to} decreasing. Discussing these data, authors support the idea that highly reactive oxidant species, such a peroxynitrite, formed by NO and superoxide anions, play a central role in TNF-α-induced ionic currents suppression. This report was hypothesized that posttranslational modification of I_{to} channels by peroxynitrite (nitrosylation, nitration or oxidation) is the main mechanism of TNF-α action (Fernandez-Velasco et al., 2007).

Investigation provided by Kawada and colleagues was focused on TNF-α effect in cultured cardiomyocytes from neonatal (1-day-old) rats (Kawada et al., 2006). Cultured cells were incubated during 48 h with 50 ng/ml TNF-α. Peak I_{to} was significantly reduced in TNF-α – treated myocytes compared with the control cells (17.7 and 3.2 pA/pF respectively). Expression of I_{to}-related mRNA was also reduced.

Study of mice ventricular cardiomyocytes, similar to results obtained in rat and dog cardiomyocytes, demonstrated TNF-α – induced inhibition of I_{to} (by chronic TNF-α treatment) (Grandy and Fiset, 2009). However, depression of I_{to} was not accompanied by lowering of mRNA or protein expression level.

In transgenic mice overexpressing NTF-a, ventricle cardiomyocytes demonstrate significantly decreased I_{to} (Petkova-Kirova et al., 2006). Level of channels proteins responsible for I_{to} was measured in this study. As in rat ventricular cardiomyocytes, transgenic mice show lowered level of I_{to}-related proteins.

Thereby, in studies utilized different animals and experimental protocols it was shown that TNF-α causes reduction of I_{to}. Such convergent results make possible speculation that preferentially I_{to} depression is responsible for AP alternation and plays an important role in TNF-α-induced heart dysfunctions.

5.3.1.4 TNF-α Induced Alternation of I_{Kur}

In most species, including human, ultrarapid-activating K^+ current (I_{Kur}) was present only in atrial myocardium (Nerbonne and Kass, 2005). Studies focused on electrophysiological effects of NTF-α in heart predominantly deal with ventricular cardiomyocytes. This is, probably, the reason why there is insufficient information about NTF-α influence on the I_{Kur}. TNF-α-induced alternation of I_{Kur} was investigated only in mice. As shown previously, ultrarapid K^+ current (also called $I_{K,slow1}$) is present in adult mice ventricular myocardium (Liu et al., 2011; Brouillette et al., 2004).

In study provided by Grandy and Fiset I_{Kur} parameters were measured in isolated mice ventricular cardiomyocytes after NTF-a chronic (6 week in vivo) administration (Grandy and Fiset, 2009). In cardiomyocytes from TNF-α pretreated mice density of I_{Kur} was significantly smaller than in cell from control animals. *I–V* curves for I_{Kur} reveal significant difference, especially at positive potentials (Fig 5.3a). Inactivation of I_{Kur}.was similar in myocytes from all animals. Recovery from inactivation was significantly longer in the cells isolated from TNF-α-treated then from control animals. Authors suggested that the prolongation in the recovery from inactivation of I_{Kur} could result in a reduction in current. Also this study revealed that level of mRNA and expression of proteins related to I_{Kur} were not altered by chronic administration of TNFα. Posttranslation modification of channels as result of reactive oxygen species generation in cardiomyocytes is postulated as a main mechanism of TNF-α action.

$I_{K,slow1}$ was measured in transgenic mice, overexpressing TNF-α. In TNF-α cardiomyocytes at potentials positive than –10 mV the density of $I_{K,slow1}$ was significantly smaller than in cells from wild type mice (Fig. 5.3b). The mean $I_{K,slow1}$ density measured at +40 mV in TNF-α cardiomyocytes was suppressed approximately two times in comparison to controls (22.6 and 12.5 pA/pF respectively). In contrast with previous, level of channels proteins responsible for $I_{K,slow1}$ was reduced by more than 40% (Petkova-Kirova et al., 2006).

5.3.1.5 Role of TNF-α in Regulation of K^+ Currents – I_{Kr}, I_{Ks}

Down-regulation of K^+ current, including rapid component of delayed-rectifier current – I_{Kr} observed in congestive heart failure is accompanied by prolongation of APs (Nattel et al., 2007). Pore-forming α-subunit of I_{Kr} is encoded by human "ether-a-go-go"-related gene ("HERG channels"). In human ventricular myocardium I_{Kr} is the main contributor to AP repolarization among delayed currents (Virag et al., 2001). Excessive decreasing of I_{Kr} leads to early afterdepolarization and tachyarrhythmias (Rashmi and Hondeghem, 2005; Priebe and Beuckelmann, 1998).

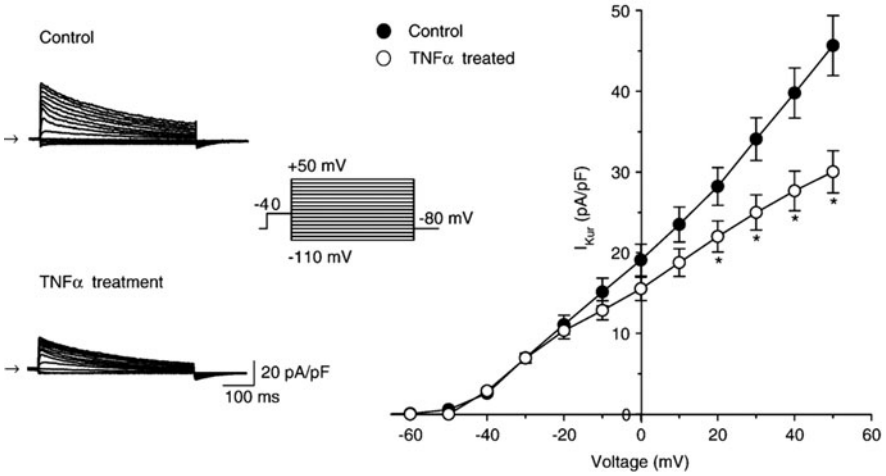

Fig. 5.3 Reduction of I_{Kur} by TNF-α. (**a**) I_{Kur} was markedly reduced in myocytes from TNF-α treated mice compared to myocytes from control mice. *Left*: Representative I_{Kur} traces for a control myocyte (*top*) and a myocyte from a TNF-α treated mouse (*bottom*). I_{Kur} was calculated by subtracting currents recorded in the presence of 100 μmol/l 4-AP from currents recorded in the absence of 4-AP (voltage protocol shown in *inset*). *Right*: The mean *I/V* curves show that I_{Kur} was significantly reduced in myocytes from TNF-α treated mice compared to myocytes from control animals at voltages of +20 mV to +50 mV. (**b**) Slowly inactivating delayed rectifier potassium current $I_{K,slow1}$ suppressed in TNF-α overexpressing mice. *Left*: Representative families of $I_{K,slow1}$ currents in control wild-type cells (*top*) and TNF cells (*bottom*). *Right*: Mean *I/V* relations for $I_{K,slow1}$ in cells from TNF mice (*squares*) and control wild-type mice (*triangles*) (from Grandy and Fiset (2009) with permission of Academic Press, International Society for Heart Research and *Journal of Molecular and Cellular Cardiology* via Copyright Clearance Center)

As was mentioned above serum level and tissue production of TNF-α in such pathological condition as heart failure is highly increased.

In study provided by Wang et al. (2004) it was shown that short time (15 min) exposure of isolated dog atrial and ventricular cardiomyocytes to TNF-α leads to concentration-dependent decreasing of I_{Kr} (Fig. 5.4a). Amplitude of HERG channels current was decreased by 9 and 35% after exposure to 1 and 100 ng/ml of TNF-α respectively. Effect of NTF-α was larger at more negative potentials. However, I_{Kr} kinetics was unaltered by TNF-α. Depression of I_{Kr} by TNF-α was accompanied by AP duration prolongation (Fig. 5.4b).

In this study it was shown that TNF-α -induced decreasing of I_{Kr} was mediated via TNFR1 receptors. Preincubation of cardiomyocytes with inhibitory anti-TNFR1 antibody for 1 h prevents suppression of I_{Kr} induced by short-time or prolonged TNF-α administration. As in case of I_{to}, author suggests intracellular reactive oxygen species generation as a main mechanism of TNF-α-mediated current suppression. Pretreatment of cardiomyocytes with antioxidants (vitamin E, MnTBAP) for 2 h prevented consequent I_{Kr} reduction induced by TNF-α.

Hatada and colleagues examined effects of NTF-α on delayed-rectifier potassium currents in cardiomyocytes isolated from guinea pig ventricle (Hatada et al., 2006).

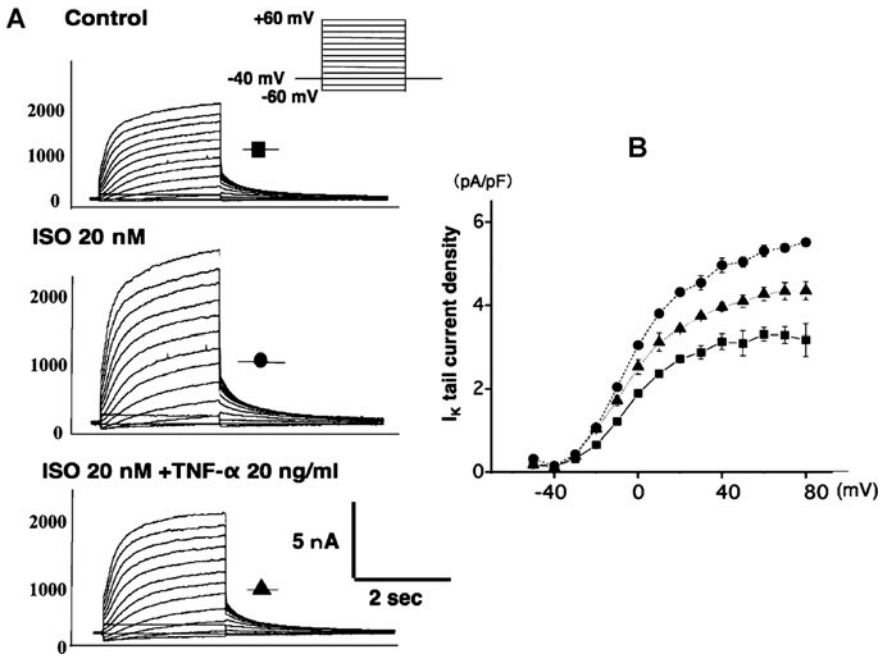

Fig. 5.4 Impairment of I_{Kr} function by TNF-α. (**a**) Three sets of current traces at test potentials between –60 and +60 mV in 10-mV increments. (**b**) Tail current amplitudes plotted against test potentials. *Solid square* indicates the control condition; *solid circle*, exposure to 20 nmol/l ISO; and *solid triangle*, after addition of 20 ng/ml TNF-α (from Hatada (2006) with permission of *Biochemical and Biophysical Research Communications* via Copyright Clearance Center)

In these experiments TNF-α suppressed I_K only after preliminary treatment of cells with isoproterenol, histamine or forskolin. Suppression of I_K in normal condition (at basal level) was not observed. Relating to pharmacological analysis, authors conclude that TNF-α preferentially affects slow component of delayed-rectifier (i.e. I_{Ks}) and that NTF-α inhibits potassium currents by reducing intracellular cAMP level.

5.3.1.6 TNF-α in Regulation of I_{K1}

Scarce data regarding influence of TNF-α on inwardly rectifying K^+ current (I_{K1}) is present to our knowledge. Only in Grandy and Fiset study (see above) analysis of I_{K1} after TNF-α administration was performed. In mice ventricular cardiomyocytes TNF-α has no effects on I_{K1}. The current–voltage (*I–V*) relationships show that the I_{K1} was comparable in cells from control and TNF-α pretreated mice (Fig. 5.5).

5.3.1.7 TNF-α Induced Stimulation of I_{KATP}

One study was reported on influence of TNF-α on ATP-sensitive potassium channels in cardiomyocytes (El-Ani and Zimlichman, 2003). In cardiac cell line H9c2

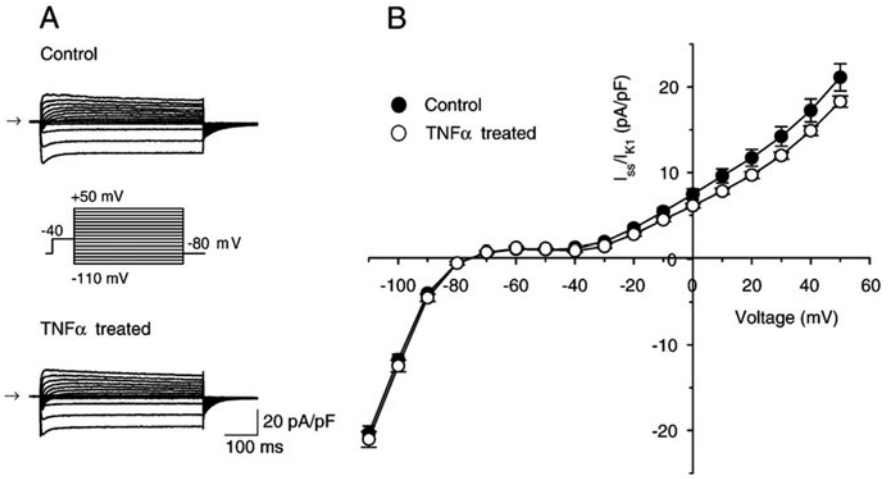

Fig. 5.5 I_{K1} after chronic TNF-α treatment in mice. The steady-state outward K^+ current (I_{ss}) and the inward rectifier K^+ current (I_{K1}) were comparable in ventricular myocytes from control and TNF-α treated mice. (**a**) Representative traces for I_{ss} and I_{K1} from a control myocyte (*top*) and a myocyte from a TNF-α treated mouse (*bottom*). (**b**) Mean *I/V* curves show that I_{ss} and I_{K1} density were similar in control myocytes and myocytes from TNF-α treated mice. The voltage protocol is shown in the inset. The current density for I_{K1} was measured between −110 and −40 mV. To record I_{ss}, the currents were initiated in the presence 100 μmol/l 4-AP and test steps were preceded with a 100 ms step to −40 mV (from Grandy and Fiset (2009) with permission of Academic Press, International Society for Heart Research and *Journal of Molecular and Cellular Cardiology* via Copyright Clearance Center)

pretreated with 50 ng/ml TNF-α for 30 min was caused activation of I_{KATP}. Preincubation of cardiomyocytes with glibenclamide, I_{KATP} blocker, inhibited the effects of TNF-α. Activation of I_{KATP} leads to depression of action potential and contractile force of cardiomyocytes (Zingman et al., 2007). Unfortunately, in this study ionic current was measured with use of radioactive isotope – ^{86}Rb and no electrophysiological data were obtained. Thereby, the role of TNF-α-induced I_{KATP} activation in APD regulating remains questionable.

5.3.1.8 TNF-α as a Regulator of Cytoplasmic Calcium Handling; Influence on SERCA

TNF-α affects contractility in the heart. Two main visions of TNF-α-induced negative inotropic action in cardiomyocytes exists. First one proposes that there is a reduced myofilament responsiveness to $[Ca^{2+}]_i$ which is mediated by nitric oxide or sphingosine production (Finkel et al., 1992; Goldhaber et al., 1996; Oral et al., 1997). Second one proposes that intracellular Ca^{2+} transients are mediated via down-regulation of structures (SERCA, NCX, RyR) relating to Ca^{2+} handling (Kao et al., 2010; Janczewski et al., 2003).

In study performed on isolated rabbit and guinea pig ventricular myocytes (Goldhaber et al., 1996) TNF-α affected only cell shortening, but not cytoplasmic calcium transients (amplitude or kinetics). Moreover, TNF-α caused a significant increase in diastolic cell length without any change in diastolic and systolic $[Ca^{2+}]_i$. In this investigation inhibition of NO production by L-NAME prevented TNF-α-induced suppression of myocytes contractile activity. The same observation was made in experiments with isolated hamster papillary muscles (Finkel et al., 1992). Contraction alternation without changing of cytoplasmic calcium level may occur due to a reduction in the responsiveness of the myofilaments to $[Ca^{2+}]_i$. Authors suggest that an NO-mediated increase in cGMP could account for decreased myofilament Ca^{2+} responsiveness.

TNF-α reduces $[Ca^{2+}]_i$ transients in isolated rat and feline cardiomyocytes (Krown et al., 1995; Yokoyama et al., 1993) (Fig. 5.6). TNF-α blocks spontaneous contractions of cultured murine cardiomyocytes (Weisensee et al., 1993). In heart of TNF-α overexpressing transgenic mice ("TNF1.6 mice") authors observed altered intracellular Ca^{2+} transients with decreased amplitude and peak systolic Ca^{2+}, elevated diastolic Ca^{2+}, and slower kinetics (Janczewski et al., 2003; London et al., 2003). Also in transgenic mice twitch contractions and response to the β-adrenergic stimulation of myocytes were decreased (Janczewski et al., 2003). However, mechanisms of cytoplasmic Ca^{2+} transients alternation remain unrevealed.

SERCA2a (sarcoplasmic reticulum Ca^{2+}-ATPase) plays an essential role in the cytoplasmic Ca^{2+} regulation in cardiomyocytes. Heart failure is accompanied with reduction of SERCA2a level (Meyer et al., 1995). Several studies suggested that key mechanisms of TNF-α-induced contraction dysfunction is the down-regulation of SERCA.

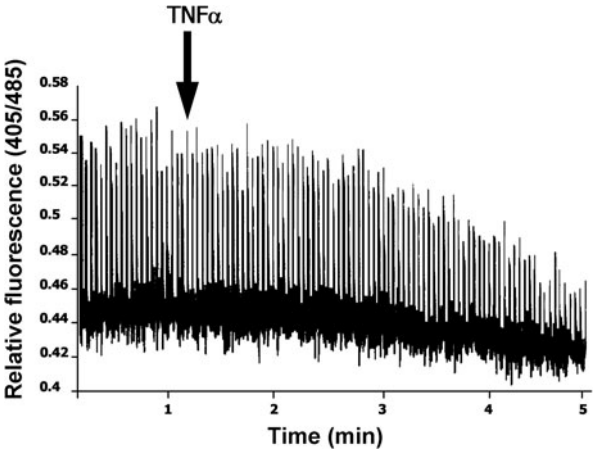

Fig. 5.6 Impairment of Ca^{2+} transients in cardiomyocites by TNF-α. Indo 1 Ca^{2+} transients of an adult rat ventricular myocyte are shown. Ca^{2+} transient measurements were made at room temperature from the cytoplasmic regions of individual cells electrically paced at 0.3 Hz. At the indicated times, 18,000 U/ml recombinant murine TNF-α was added (from Krown et al. (1995) with permission of Elsevier Limited and *FEBS Letters* via Copyright Clearance Center)

TNF-α has been shown to down-regulate the SERCA2a in the expressions of RNA and proteins level in isolated rabbit cardiomyocytes (Lee et al., 2007). Expression of SERCA mRNA and protein were markedly decreased in mouse cardiac muscle cell line – HL-1 treated by TNF-α (50 ng/ml for 24 h) (Kao et al., 2010). Down-regulation of SERCA was accompanied by reduction of $[Ca^{2+}]_i$ transients amplitude.

On the one hand in isolated ventricular myocytes from "TNF1.6 mice" transcripts encoding SERCA 2 and phospholamban (PLB) were significantly reduced in comparison with wild type mice (Kubota et al., 1997; Janczewski et al., 2003). Despite reduced mRNA expression SERCA and PLB protein levels were not different in transgenic and wild type mice. Similarly to previous, the molecules related to Ca^{2+}-handling, such as ryanodine receptor, SERCA and NCX (Na/Ca-exchanger) did not show any specific changes in rat cultured cardiomyocytes after treatment with 50 ng/ml TNF-α (Kawada et al., 2006). Thereby, mechanism of TNF-α-induced cytoplasmic calcium transients alternation remain unclear.

5.3.2 Interleukin IL-1

5.3.2.1 I_{Na}

Alterations of Na^+ channel function can contribute to rhythm disturbances (Grandy et al., 2010). Disease-mediated alterations in Na^+ current could be partially responsible for arrhythmias that have been observed HIV patients (Sani and Okeahialam, 2005). Similarly HIV patients and CD4C/HIV mice have elevated levels of the proinflammatory cytokine IL-1β (Monsuez et al., 2007; Grandy et al., 2010). In ventricular myocytes isolated from HIV mice Na^+ peak current was significantly reduced. It is possible that alterations in serum proinflammatory cytokines could mediate the reduction of Na^+ current and the corresponding changes in the AP waveform as observed in ventricular myocytes from CD4C/HIV mice. Overall, the results suggest that elevated levels of IL-1β may result in a reduction in Na^+ current, altering the action potential upstroke and increasing the risk of arrhythmias (Grandy et al., 2010).

5.3.2.2 $I_{Ca,L}$

IL-1β has been suggested to play a role in impaired cardiac performance under these conditions. This suggestion has been supported by in vitro studies showing that IL-1β decreases cardiac contractility (Evans et al., 1993; Hosenpud et al., 1989; Weisensee et al., 1993).

To determine whether cytokines alter the electrical properties of heart cells, the effects of human recombinant IL-1β were examined in excised tissues and dissociated myocytes from guinea pig ventricles (Li and Rozanski, 1993). In excised papillary muscles, IL-1β significantly prolonged action potential duration (measured at 90% repolarization) by 24.2 ± 2.2 (SEM) ms and effective refractory

period by 22.9 ± 2.3 ms. Other measured variables were not affected. Treatment of muscles with cyclo-oxygenase inhibitors, indomethacin (10^{-5} M) or acetyl salicylic acid (2×10^{-4} M), abolished the prolongation of action potential duration elicited by IL-1β. However, the effects of IL-1β were also blocked by the lipoxygenase inhibitor nordihydroguaiaretic acid (2×10^{-5} mol/l) or by treating tissues with the leukotriene receptor blocker, ICI 1986 I5 (10^{-8} mol/l). In isolated myocytes, 1 ng/ml IL-1β increased I_{Ca} density in 44 of 78 cells by 33.6 ± 7.5% during voltage steps from –40 to 0 mV. Thus IL-1β modifies electrical properties of cardiac cells via lipid second messengers generated by cyclo-oxygenase and lipoxygenase pathways. Voltage clamp analyses suggest that these effects are mediated, at least in part, by changes in the conductance of Ca^{2+} channels (Li and Rozanski, 1993). In guinea pig ventricular myocytes, IL-1 has been reported to increase $I_{Ca,L}$ (Li and Rozanski 1993). However, these authors in that study GTP was not included in the pipette solution, and only 56% of ventricular myocytes responded to IL-l exposure (Liu and Schreur, 1995). When GTP was included in the pipette solution, authors found that IL-1β inhibited I_{Ca} in all experiments. Therefore, in the presence of GTP, IL-1β suppressed the cardiac $I_{Ca,L}$ by a receptor-coupled G protein-mediated mechanism (Liu and Schreur, 1995). IL-1β attenuates cardiac L-type Ca^{2+} currents ($I_{Ca,L}$) via pertussis toxin-insensitive G protein rather than by stimulation of cGMP production. IL-1β caused a concentration-dependent decrease in the peak $I_{Ca,L}$ (Ba^{2+} as the charge carrier). IL-1β did not significantly alter the voltage dependence of the peak $I_{Ca,L}$ nor the steady-state inactivation and activation, but did slightly slow the rate of inactivation (Liu and Schreur, 1995).

Many studies have suggested that nitric oxide generation via IL-1β-mediated induction of nitric oxide synthase plays a role in the observed effects of IL-1β on cardiac contractility (for example, Evans et al., 1993; Harding et al., 1995). In ferret papillary muscles, IL-1β suppressed the contraction by increasing nitric oxide (NO) production via a dexamethasone-sensitive pathway that consequently increased cellular cGMP (9). However, the NO-mediated negative inotropic effect was not observed when hamster papillary muscles were exposed to IL-1α (Finkel et al., 1992). In cells internally dialyzed with pipette solutions without GTP, the hydrolysis of which is essential for cGMP production, the absence of GTP blocks the suppression of $I_{Ca,L}$ by IL-1β. These results could be explained by an increase in cell cGMP level stimulated by NO synthesis. The reduction of $I_{Ca,L}$ by IL-1β may account for the decreased myocardial contractility associated with pathophysiological conditions, such as immunologically mediated cardiac disorders and myocardial infarction.

Modulation of the β-adrenergic control of the cardiac L-type Ca^{2+} current (I_{Ca}) by human recombinant IL-1β was examined in guinea pig ventricular myocytes using the whole cell voltage clamp technique (Rozanski and Witt, 1994b). I_{Ca} was evoked in Cs^{+}-loaded myocytes by depolarizing pulses from a holding potential of –40 mV. In the presence of an acidic external solution (pH 5.8), the response of I_{Ca} to isoproterenol was markedly decreased compared with control myocytes studied at pH 7.4. However, when cells were pretreated with 1 ng/ml IL-lβ and then exposed to acid media, β-responsiveness was significantly increased compared

with untreated cells. Despite this effect of IL-lβ, maximum I_{Ca} density with 0.01 and 1 μM isoproterenol was still 51 and 58%, respectively, less than that measured at pH 7.4. The enhanced β-responsiveness produced by IL-1β was eliminated by adding amiloride to block Na^+/H^+ exchange or protein kinase C inhibitors staurosporine (10 nM) and calphostin C (50 nM). However, a direct activator of protein kinase C, phorbol 12-myristate 13-acetate, did not mimic the effects of the cytokine. These data demonstrate that IL-lβ partially restores the β-adrenergic control of cardiac Ca^{2+} channels suppressed under acidic conditions. Moreover, they suggest that IL-lβ acts by enhancing Na^+/H^+ exchange through a second messenger pathway that may involve protein kinase C. These cellular mechanisms may play a role in altering ventricular function during cytokine-mediated inflammatory processes that are initiated by myocardial ischemia (Rozanski and Witt, 1994b). Isoproterenol exposed to myocytes pretreated with 1 ng/ml IL-1β evoked a significantly smaller increase in I_{Ca} density compared with control cells. This IL-l-mediated decrease in β-responsiveness was usually observed with pretreatment periods of >l h and varied as a function of the L-arginine concentration of the pretreatment medium. It was prevented by (i) IL-1 receptor antagonist, (ii) substituting D-arginine for L-arginine, or (iii) incubating cells with the nitric oxide synthase inhibitor NG-monomethyl-L-arginine. Thus the present data illustrate that IL-1 significantly alters the β-adrenergic control of cardiac Ca^{2+} channels by cellular mechanisms that involve the activation of nitric oxide synthase. These mechanisms may play a role in altering ventricular function during cytokine-mediated inflammatory processes affecting the heart (Rozanski and Witt, 1994a).

The possible mechanism by which IL-1β affects β-adrenergic responsiveness of L-type Ca^{2+} current ($I_{Ca,L}$) was examined in adult rat ventricular myocytes by use of whole cell patch-clamp techniques (Liu et al., 1999). In the presence of isoproterenol, exposure for 3 min to IL-1β suppressed the isoproterenol-activated $I_{Ca,L}$. In the presence of IL-1β, the response of $I_{Ca,L}$ to isoproterenol was decreased, and the EC_{50} for isoproterenol stimulation was increased. However, IL-1β had no effect on basal and isoproterenol-enhanced cAMP content. When $I_{Ca,L}$ was activated by extracellular application of forskolin or 8-(4-chlorophenylthio)-cAMP, a membrane-permeable cAMP analog, or by intracellular dialysis with cAMP, IL-1β had little effect on $I_{Ca,L}$. In contrast, in the presence of cAMP, IL-1β still suppressed the Iso-enhanced $I_{Ca,L}$. These results show that the IL-1β -induced decrease in β-adrenergic responsiveness of $I_{Ca,L}$ does not result from inhibition of β-adrenoceptor binding, adenylyl cyclase activity, or cAMP-mediated pathways, suggesting a cAMP-independent mechanism (Liu et al., 1999).

5.3.2.3 Na^+–K^+ ATPase

Heart failure coupled with ischemia and myocardial hypertrophy were associated in turn with a decrease in the concentration of myocardial Na^+–K^+ ATPase (Bundgaard and Kjeldsen, 1996) an enzyme located in the T tubules and peripheral sarcolemma (McDonough et al., 1996) and responsible for the generation and maintenance of the electrochemical gradient for Na^+ and K^+. Many studies have shown that heart

diseases are always accompanied with high levels of IL-1β and a decrease in Na^+–K^+ ATPase concentrations (Harada et al., 1999; Kreydiyyeh et al., 2004). Because in heart failure, changes in the Na^+–K^+ ATPase activity and expression were noted to accompany increases in circulating levels of IL-1β, the presence of a cause effect relationship between the cytokine and the pump has been investigated (Kreydiyyeh et al., 2004). IL-1 β reduced the protein expression of the α1 subunit of the Na^+–K^+ ATPase in right and left ventricular homogenates and exerted a significant inhibition of the ATPase activity which correlated with its down-regulatory effect and could thus be ascribed to the decrease in the number of the catalytic a1 subunits. Because the ventricular homogenate is a mixture of different types of cells (endothelial cells, macrophages, fibroblasts), the observed change in the ATPase activity and expression may not reflect necessarily changes in cardiomyocytes. To clarify this point cardiomyocytes were isolated from rats treated with IL-1β, and the activity and protein expression of the ATPase was studied in a homogenate of these isolated cells. IL-1β caused down-regulation of the pump in isolated cardiomyocytes and inhibited its activity. The results suggest that the cytokine acts directly on the cardiomyocytes. Effect of IL-1β is mediated through mitogen-activated protein kinases (MAPK).

Such an inhibition of the Na^+–K^+ ATPase by IL-1β is expected to decrease the Na^+ gradient and Na^+-dependent electrolyte movements that regulate excitation–contraction coupling in cardiac myocytes especially calcium movements. Cytosolic Ca^{2+} increases during contractions and is restored back to normal levels upon relaxation via the Na^+–Ca^{2+} exchanger whose activity is dependent on the Na^+ gradient established by the Na^+–K^+ pump. An inhibition of the pump is thus expected to dissipate the Na^+ gradient, inhibit the Na^+–Ca^{2+} antiporter, increase cytosolic Ca^{2+} and by so doing alter the systolic–diastolic cycle and delay relaxation. In fact, inhibition of the Na^+–K^+ ATPase has been reported in some cases of heart failure (Paganelli et al., 2001; Long, 2001) and was associated with increases in the levels of pro-inflammatory cytokines. Similarly increases in cytosolic Ca^{2+} were reported to occur in association with increases in the levels of IL-1β (Kato et al., 1987). The demonstrated inhibitory effect of IL-1β on the pump may thus provide a possible explanation of the previously reported increase in cytosolic Ca^{2+} levels (Bick et al., 1997, 1999) that occur in myocytes following IL-1β treatment and whose cause remained controversial.

The inhibition of the pump is expected also to alter the function of the Na^+/H^+ exchanger which regulates intracellular pH and the activity of which is dependent on the Na^+ gradient generated by the Na^+–K^+ ATPase. IL-1β is thus expected to increase intracellular acidity, a process that has already been reported in the ischemic heart where pH_i and pH_o were noted to reach values as low as 6.0 and 6.5, respectively (Karmazym et al., 1999; Park et al., 1999).

5.3.3 Interleukin-2

IL-2 has been known as a mediator which plays one of the key roles in the immune system via stimulating and coordinating immune responses (Arai et al., 1990). IL-2

and its receptor have been intensively studied on the molecular level. Apart from the cells belonging to the immune system, IL-2 binding sites have been found in various tissues, including rat brain (Araujo et al., 1989), isolated human and rats muscular cells and some other (Smith et al., 1989). Moreover, physiological effects of IL-2, suppressing long-term potentiation of hippocampal neurons and prolonging the open time of extrajunctional acetylcholine receptors in muscle have been described (Trotter et al., 1988).

IL-2 occurs in increased amounts during inflammatory neuronal diseases, such as multiple sclerosis and polyradiculoneuritis. Since the latter is characterized by a severe paralysis, the question arose whether IL-2 may be responsible for a reduction of cellular excitability by blocking excitatory Na^+ channels. A preliminary account of some of the results has appeared (Brinkmeier et al., 1992).

Despite direct action as an immune system mediator some other indirect effects of IL-2 such as sodium current inhibition, sarcoplasmic reticulum activity mediation have been described. IL-2 may act either through own IL-2 receptor or through direct interaction with other receptors, including k-opioid receptor (Cao et al., 2003a, b) and sodium channels (Brinkmeier et al., 1992). It has been shown that treatment with IL-2 increasing in a dose-dependent manner the activity of sarcoplasmic reticulum Ca^{2+}-ATPase, but the sarcolemmal Ca^{2+}-ATPase activity is not changing (Cao et al., 2003a, b).

Molecular mechanisms of sarcoplasmic reticulum mediation include cAMP. Treatment with IL-2 at 200 U/ml decreasing the intracellular cAMP concentration in the isolated rat heart, which suggest that cAMP is a target component of IL-2 in the heart. It is also known that cardiac k-opioid receptor stimulation inhibits adenylyl cyclase via Gi/o proteins, leading to a decreased intracellular cAMP level (Zhang and Wong, 1998). Results of decreased sensitivity of SR Ca^{2+}-ATPase to free calcium by IL-2 action, indicates that the activation of k-opioid receptor by IL-2 results in a decreased intracellular cAMP level, which may be responsible for reduced sensitivity of SR Ca^{2+}-ATPase to calcium by affecting the degree of cAMP-dependent phosphorylation of phospholamban.

IL-2 may also act without involvement of second messenger system, directly to the sodium channels in human muscle cells. Clear results demonstrate an inhibitory effect of human IL-2 on the excitatory Na^+-channels in skeletal muscle. With IL-2 concentration as those found in body fluids of patients suffering from inflammatory neuronal diseases (Lorenzon et al., 1991) of those used to induce T-lymphocyte activation in vitro. Doubts in direct action of the IL-2 was solved in experiments with anti-IL-2 receptor antibody. This preincubation did not prevent the inhibitory effect of IL-2 on the Na^+-channels on the Na^+-currents. Furthermore, it is shown that the cytokine blocks the voltage-dependent muscular Na^+-channels by keeping the channels in the state of fast inactivation. An IL-2 receptor and a second messenger system are not likely to be involved in this reaction. Thus, the effect of IL-2 is comparable to the action of local anesthetics on Na^+-channels (Kaspar et al., 1994).

The same results of the IL-2 was shown on sodium channels in human cardiomyocytes. It is shown that IL-2 has class I antiarrhythmic-like effect with extracellular cytokine concentrations that are also usually reached when patients

are under high-dose rIL-2 therapy. According to (Vaughan Williams, 1984) class I antiarrhythmics block sodium channels, shift the steady state inactivation curve in the negative direction, delay recovery of the sodium channels from block and are use dependent. All these properties are possessed by rIL-2.

Human IL-2 consists of 133 amino acids with a molecular weight of 15,000 Da in its active form. Its three-dimensional structure is known. Carboxy-peptidase removes the amino acids one after the other, starting at the C-terminal. Only arginine, proline and hydroxyproline are resistant to its action. The sequence of IL-2 contains an arginine at location 120, so only the last 13 amino acids can be removed. Unless removal of these amino acids changes the structure of the molecule, the sodium channel-binding site of rIL-2 is presumably located within the last 13 amino acids of the molecule. The receptor binding site of IL-2 is located within the first 54 amino acids, near the N-terminus, because blocking of amino acids 59–133 with monoclonal antibodies does not inhibit receptor binding (Proebstle et al., 1995).

Affinity quantitative comparison with established antiarrhythmic drugs shows that the blocking potency of rIL-2 on sodium channels is quite high.

5.3.4 Interleukin-6

IL-6 along with TNF-α is known as one of the main pro-inflammatory cytokines, especially during acute myocardial infarction (AMI). IL-6 exerts acute negative inotropic action without detectable reduction in I_{ca} under basal conditions. Expression of IL-6 and IL-8 genes is increasing in patients assigned to take calcium blockers.

IL-6 exerts an acute negative inotropic action without detectable reduction in I_{Ca} under basal conditions. This sounds as if IL-6 directly inhibited EC-coupling instead of blocking I_{Ca}. However, inhibition of NOS (by L-NMMA) abolished the negative inotropic action of IL-6 (Sugishita et al., 1999). An acute inhibitory effect of NO on I_{Ca} has been shown to be mediated through cGMP-dependent protein kinase in mammalian hearts (Thakkar et al., 1988; Levi et al., 1989; Mery et al., 1991; Ono et al., 1991). The effects of cGMP have been shown to be mediated by phosphodiesterase-dependent mechanisms in lower species such as frogs while cGMP-dependent protein kinase (cGMP-PK) may play a key role in the action of cGMP in rat hearts. Thus, IL-6 may act on L-type calcium channels mediated by activation of cGMP-PK in the ventricular myocytes of the guinea-pig, as used in this study. The NO/cGMP pathway appears to be involved in the reversal of cAMP-stimulated I_{Ca} in guinea-pig ventricular myocytes (Ono et al., 1991; Levi et al., 1994; Wahler et al., 1995). IL-1 also decreased α-adrenergic control of I_{Ca} through NO production in adult guinea-pig ventricular myocytes (Rozanski et al., 1994).

Four different Ca^{2+}-channel blocker, amlodipine, felodipine, isradipine and manidipine, are capable of inducing the activation of genes coding for IL-6 and EL-8 in cultured human VSMC and fibroblasts. The effect occurs at nanomolar concentrations which can be achieved in vivo during therapy. The novel activity of the dihydropyridines cannot be substituted for by two other blood pressure lowering

agents such as β-receptor antagonists or diuretics. This suggests an apparently specific activity of the class of drugs affecting inflammatory mechanisms. However, the exact mechanism by which the drugs stimulate remains unclear. Two possible modes of action can be hypothesized; (i) the binding site of the potential operated Ca^{2+}-channels contain an unknown signal transducing element which can be activated by interaction with Ca^{2+}-channel blockers, or (ii) the induction of transcription of IL genes by Ca^{2+}-channel blockers is mediated via an unknown membranous binding site for Ca^{2+}-channel blockers which is linked to a signaling pathway. Further studies should unravel the mechanisms of Ca^{2+}-channel blockers on the interaction of the two ILs with their various receptors (Roedler et al., 1994).

Concentration of pro-inflammatory cytokines (IL-6, TNF-α and hs-CRP) and anti-inflammatory cytokine IL-10 were elevated in AMI patients in comparison with the group with stable angina pectoris, but statistically significant difference was found in IL-6 and hs-CRP level. IL-6:IL-10 ratio is higher in AMI which emphasis the hypothesis of pro-inflammatory to anti-inflammatory cytokine misbalance in AMI. IL-10 may be useful as a marker of myocardial reperfusion in AMI. There is positive linear correlation between IL-6 and IL-10 in acute myocardial infarction and negative linear correlation between HDL and IL-6 (Dizdarević-Hudić et al., 2009).

It is also well known that the cardiac effects of NO are complex and somewhat controversial, probably due to the distinct concentration-dependent sensitivities of its targets and distinguishable signaling mechanisms. NO is known to target the L-type Ca^{2+} channel and SR in cardiac myocytes through signaling pathways such as cGMP, and/or by directly modifying the proteins (such as by S-nitrosylation/oxidation) (Campbell et al., 1996; Hare, 2003; Massion et al., 2003). Studies in adult ferret ventricular myocytes showed that SIN-1, a NO donor, induces biphasic and bimodal changes in basal $I_{Ca,L}$ (Campbell et al., 1996), which were not observed in frog (Mery et al., 1993), guinea-pig (Wahler and Dollinger, 1995) or rat (Abi-Gerges et al., 2001) ventricular myocytes. We also observed no significant change in basal $I_{Ca,L}$ in ARVM upon acute exposure to 0.1 mM SIN-1 (S.J. Liu, unpublished data: In Yu et al., 2005a, b). Similarly, SIN-1 (up to 0.1 mM) has no significant effect on contractile function of papillary muscle isolated from adult rat (Wyeth et al., 1996). Although we found that cumulative NO production in cell lysates and in culture medium is 2.5- to 3-fold of the basal level after 2 h of IL-6 treatment, it is not surprising that $I_{Ca,L}$ is not significantly altered in our experimental conditions. However, the possibility for alteration in $I_{Ca,L}$ during longer exposure (e.g. >24 h) to IL-6 cannot be excluded.

In summary, exposure to IL-6 for 2 or more hours results in a negative inotropy probably by inhibiting SR Ca^{2+} reuptake in concomitance with increases in NO in ARVM. The IL-6-induced decrease in PLB phosphorylation is also in accord with its reduction of SR function. The increase in NO production results primarily from expression/activation of iNOS that is mediated via IL-6-induced JAK2/STAT3 activation as shown previously (Yu et al., 2003a, b). Inhibition of iNOS/NO abolishes the IL-6-induced decrease in SR function. Therefore, this leads to our conclusion that IL-6 suppresses SR function via iNOS after chronic exposure. Moreover, such

cardiac effects of IL-6 are sustained even after removal of IL-6. This suggests that the transient elevation of IL-6 observed in plasma or ventricular muscle of patients with many cardiac diseases can cause prolonged effects on contractility. An in vivo study in transgenic mice showed that cardiac-specific overexpression of iNOS causes an increase in NO_x in hearts (2.5-fold above the wild-type controls) but no severe cardiac dysfunction (Heger et al., 2002). One interpretation for this seeming inconsistency is that the increase in plasma and heart NO_x in the transgenic models is moderate so the associated systemic adaptation during development in these transgenic mice results in less detrimental functional consequences. A recent in vivo study reported that IL-6 administration causes heart failure in a dose-dependent manner (Janssen et al., 2005). Thus, IL-6 might play an important role in the pathogenesis of associated chronic heart diseases. Treatment to antagonize the induction and activation of iNOS in the early stage of IL-6-associated heart dysfunction might be beneficial (Yu et al., 2005a, b).

5.3.5 Interleukin-11

There are clinical reports of sudden cardiac fibrillation in cancer patients who were treated with recombinant human IL-11. The question was either that effect is a direct arrhythmogenic action of IL-11 or that was indirect system side effect. The results from (Sartiani et al., 2002) which was performed on single human atrial myocytes using standard patch-clamp technique clearly show the lack of effect on $I_{Ca,L}$, I_K, I_{to}, in agreement with the observation that rhIL-11 does not influence the atrial action potential configuration and particularly it's duration.

Shortening of atrial action potential is generally associated with shortening of atrial refractory period and hence facilitates induction of atrial flutter/fibrillation (Narayan et al., 1997). Conversely, lengthening of action potential duration is a perquisite for the appearance of early after depolarization, which may trigger arrhythmias (Janse, 1992). Calcium handling by sarcoplasmic reticulum and Na/Ca exchanger is crucial for the changes in atrial action potential duration and the appearance of delayed afterdepolarizations (Tavi et al., 1996, 1998; Benardeau et al., 1996). To avoid any perturbation by the patch pipette of the intracellular milieu and calcium homeostasis, which could have hindered the development of Ca-dependent electrical alterations, action potentials were measured with the perforated-patch technique. Thus, our results allow inferring that superfusion with rhIL-11 does not have direct, acute electrophysiological effects on action potential parameters measured in atrial myocytes. Furthermore, acute exposure of atrial myocytes to rhIL-11 does not affect the pacemaker current I_f, nor did it modify the resting potential in these cells. Taken together, these data suggest that it is unlikely that rhIL-11, at least by its direct effects, may cause afterdepolarizations, thought to be the trigger for atrial fibrillation, which is maintained by re-entry (Janse, 1997). We did not find a single direct electrophysiological alteration indicative that rhIL-11 may acutely influence atrial electrical activity. The only electrophysiologic effect of rhIL-11, however not statistically significant, was a small reduction in the I_f amplitude.

I_f is an inward current that is responsible for the diastolic depolarization phase and automatic activity in pacemaker cells (Difrancesco, 1993). This current has been recently described and characterized in human atrial myocytes (Hoppe et al., 1998; Pino et al., 1998; Porciatti et al., 1997). An increase of I_f amplitude in association with a reduction in the inward rectifier I_{K1} or in association with the presence of delayed afterdepolarizations due to a cellular calcium overload has been suggested to potentially trigger premature depolarizations able to generate atrial fibrillation if an appropriate substrate for re-entry is present (Opthof, 1988; Pino et al., 1998). A reduction in I_f amplitude should reduce the likelihood for generation of such premature depolarizations.

Thus, it is possible that acute atrial dilatation and consequent atrial stretch rather than direct action on the myocytes by rhIL-11 could be the mechanism by which rhIL-11 might affect cellular atrial electrophysiology and induce atrial arrhythmias. The experimental study in rats presented in the accompanying paper supports this hypothesis. In summary, it seems reasonable to conclude that the lack of effect of rhIL-11 on AP and ionic currents during acute exposure of human atrial myocytes seems to exclude a direct effect of rhIL-11 in the genesis of atrial arrhythmias in patients.

In conclusion, it is very likely that effect of IL-11 is mediated via blockade of sodium channels, water accumulation, edema, atrium dilatation and further mechanically induced fibrillation.

5.4 Cytokines and Mechanosensitivity of the Heart

In recent years many groups investigated the role of cytokines in generation of cardiac stretch effects. This studies employed one of two approaches. Firstly – investigation of cytokine production during stretch of issue or single cells. Those studies are discussed in review of Kovalchuk et al. (Chapter 2, this volume). The second approach – investigation of cytokine effects on cells and tissues during their stretching. We will discuss their findings in this section.

However there is a number of studies addressing cytokine production during tissue stretching (for review see Chapters 2 and 10 of this Volume), investigation of the effects of exogenous cytokines on cells, for example in heart, are limited to those, which were described in previous sections of this chapter. On the other hand there are practically no studies of the regulation of mechanoelectrical feedback in heart by cytokines. Therefore their effects on stretched cardiac tissue remain unknown. For the purpose of investigation of the role of cytokines in regulation of mechanoelectrical feedback we used microelectrode registration of bioelectrical activity of the fragment of the right atria during discrete tissue stretching (Kamkin et al., 2000a).

Several intracellular electrophysiological alterations in the healthy and diseased heart, which were ascribed to mechanoelectrical feedback were reported for the first time about 10 years ago (Kiseleva et al., 2000; Kamkin et al., 2000a). Later it was showed that stretch of isolated cardiomyocytes induced mechanosensitive

whole-cell currents which lead to membrane depolarization (Kamkin et al., 2000b, 2003a, b; Zeng et al., 2000; Zhang et al., 2000; Isenberg et al., 2003; Kondratev and Gallitelli, 2003) and elicited stretch-induced depolarizations (SID), observed as prolongation of APD90 (stretch-induced depolarizations: SID) or hump-like SID, that appeared in repolarization phase at APD50 or APD90 level (Kamkin et al., 2003b). Membrane depolarization and SID in action potentials provoke extra-action potentials when these hump-like SID reach a threshold potential. This finding correlated with previous studies of step-wise application of stretch to atrial and ventricular tissue from healthy animals and animals, suffering from infarct, which reported SIDs, emerging as prolongation of APD90 and as typical hump-like SIDs, leading to extra-action potentials (extra-AP), paroxysmal tachycardia and even fibrillation (Kiseleva et al., 2000; Kamkin et al., 2000a). Later they were studied in detail (Lozinsky and Kamkin, 2010; Ward et al., 2010). Thus, activation of mechanically gated channels (MGCs) and the increase of mechanosensitive whole-cell currents, which during cardiomyocyte stretching leads to ion entry carrying the positive charge together compose the cellular mechanisms underlying different mechanically induced alterations and abnormalities.

It is known that certain cytokines induce signaling, which includes for example, transducing signals within intracellular compartments, such as in the case of modification of transcription factors in mitochondria and the nucleus (see for review Calabrese et al., 2009). One of those signaling cascades includes NO-dependent pathway, which is well described in many publications. In the heart NO-synthases play the key role in NO level regulation.

Besides, in response to stretch in cardiac cells NO-synthase activity significantly increases (reviewed in Shah and MacCarthy, 2000; Casadei and Sears, 2003; Seddon et al., 2007). Recently it was shown that NO and NO-synthases as NO source directly regulates mechanically gated channels (MGCs) conductivity. Experimental data in this study was obtained from the isolated ventricular myocytes of mouse, rat and guinea pig by means of patch-clamp method in whole-cell configuration. The data demonstrated that NO donors lead to MGCs activation and appearance of MG-like currents in undeformed ventricular myocytes, whereas, it can also lead to the inactivation of the stretched cells with activation MGCs and inhibit the conductivity of these channels (Kazanski et al., 2010a, 2011). NO scavenger PTIO causes inactivation of all MGCs. In undeformed cells conductance through MGCs is blocked and their activation in the controlled, and on the background of stretch cell the PTIO causes complete MG-current inhibition. Non-selective inhibitors of NO-synthases L-NAME or LNMMA resulted in complete blocking of MGCs. In ventricular myocytes of wild-type mice, NOS1–/– and NOS2–/– the stretching of the cells triggered typical MG-current. On the contrary, in cells from NOS3–/– mice stretch does not cause MG-current. The results discussed on the channel level testify to the NO role in activation and inactivation of MGCs in cardiomyocytes and demonstrate that NOS3 dominates as NO source (Kazanski et al., 2010b, 2011). Therefore the appearance of hump-like SIDs and extra-AP during stretching of atrial tissue stripes is determined by MGCs functioning, which are modulated by NO and NO-synthases.

5.4.1 Tumor Necrosis Factor-α

TNF-α has been implicated in the pathogenesis of cardiovascular diseases, including heart failure, myocarditis, acute myocardial infarction and sepsis-related cardiac dysfunction (Levine et al., 1990; Low-Friedrich et al., 1992; Yokoyama et al., 1993; Latini et al., 1994; Packer, 1995; Kumar et al., 1996). TNF-α also has been shown to be related to the occurrence of cardiac arrhythmias. Mice with a higher TNF-α expression have a greater incidence of atrial and ventricular arrhythmias (London et al., 2003). Administrating TNF-α in rats can induce arrhythmias with a loss of the myocyte inotropy (Krown et al., 1995). TNF-α changes the L-type calcium currents ($I_{Ca,L}$), and calcium transient in ventricular myocytes (Krown et al., 1995; Goldhaber et al., 1996; Cailleret et al., 2004).

The direct effects of proinflammatory cytokine TNF-α on the contractility of mammalian heart were studied a lot of years ago (Finkel et al., 1992). It was shown, that TNF-α inhibited contractility of isolated hamster papillary muscles in a concentration-dependent, reversible manner. The authors demonstrated, that the nitric oxide synthase inhibitor NG-monomethyl-L-arginine (L-NMMA) blocked these negative inotropic effects. L-Arginine reversed the inhibition by L-NMMA. Removal of the endocardial endothelium did not alter these responses. These findings demonstrate that TNF-α regulated the contractility and the direct negative inotropic effect of cytokines is mediated through a myocardial nitric oxide synthase. The negative inotropic effect of TNF-α was observed in isolated rabbit cells (Goldhaber et al., 1996). This effect was apparent within 20 min of exposure to the cytokine, was similar to that described by Finkel et al. (1992) in a multicellular preparation.

Previous studies have shown that nitric oxide (NO) has important regulatory effects on the cardiovascular system (Moncada et al., 1991; Kelly et al., 1996). NO has been shown to have a role in the development of triggered arrhythmias generated by Ca^{2+}overload (Kubota et al., 2000). Previous study in vivo also showed that NO could suppress trigged activity induced ventricular tachycardia (Chen et al., 2001). It is known that pulmonary veins contain endothelium and smooth muscle which may produce NO through the enzyme of eNOS or iNOS. In addition, cardiac myocytes also express eNOS activity (Massion and Balligand, 2003; Balligand et al., 1994). NO has been shown to regulate PV arrhythmogenesis through mechanoelectrical feedback (Hu et al., 2009). Because PVs was known to induce atrial arrhythmia through the enhancement of triggered activity, it is possible that NO may play a critical role in the pulmonary veins arrhythmogenic activity.

It was shown that cardiac cells themselves produce different quantities of TNF-α during prolonged stretch or during cyclic application of mechanical stretch. Acute stretch caused by volume overload of aorto-caval fistula induces a variety of myocardial responses. These responses can be part of myocardial inflammation dictated by TNF-α, which is elevated after acute aorto-caval fistula. Western blot demonstrated increased local production of TNF-α in the left ventricle and cardiomyocytes. TNF-α produced by cardiomyocytes mediates a predominant inflammatory response to stretch in the early volume overload in the aorto-caval fistula rat, suggesting an

important role of TNF-α in initiating pathophysiological response of myocardium to volume overload (Chen et al., 2010). In addition to production of TNF-α by cardiomyocytes during their stretching, cardiac fibroblasts also produce TNF-α during stretch. To determine whether mechanical stress affect the production of TNF-α in the heart, neonatal rat cardiac myocytes and fibroblasts were cultured separately and treated for 6 h with cyclic mechanical stretch. Mechanical stretch stimulated the production of TNF-α in cardiac fibroblasts. Thus mechanical stress, can stimulate production of TNF in cardiac fibroblasts (Yokoyama et al., 1999).

Thus, TNF-α has been shown to be related to the occurrence of cardiac arrhythmias. Moreover, TNF-α regulated the contractility and the direct negative inotropic effect of TNF-α is mediated through a myocardial nitric oxide synthase. This data and studies reporting that NO directly regulates mechanically gated channels (MGCs) (Kazanski et al., 2010a, b, 2011) allowed us to propose that in heart TNF-α would be an effective regulator of MGCs operating and therefore will regulate mechanoelectrical feedback.

In order to test this hypothesis we used the standard microelectrode technique in combination with stretching of the tissue stripe of the right rat atria. Tissue was perfused with standard physiological solution, containing 50 ng of TNF-α per milliliter. Perfusion of tissue with TNF-α lead to appearance of abnormalities in development of bioelectrical activity observed as prolongation of APD90 and appearance of hump-like depolarization at APD90 level. After reaching E_c hump-like depolarization transformed into single extra-APs, with following development of sustained arrhythmia. Abnormalities at APD50 level were also observed.

Since TNF-α activates NO synthases of cardiomyocytes (Finkel et al., 1992; Goldhaber et al., 1996; Nakayama et al., 2009; Bougaki et al., 2010) and rise of the concentration of the intracellular NO activates MGCs (Kazanski et al., 2010a, b, 2011), which allows Na ions entry into the cell leading to cellular depolarization and shift of resting potential to E_C we proposed, that TNF-α induced arrhythmias can be linked with MGCs activation. This is the most simple mechanism, explaining the appearance of arrhythmias after TNF-α application.

Since the blocker of MGCs Gd^{3+} at 40 μmol/l eliminates not only hump-like depolarization, but arrhythmias, triggered by TNF-α, as well, it was proposed that TNF-α triggered abnormalities are linked with MGCs activity, which was triggered by increasing of intracellular NO concentration, caused by TNF-α application. In this case they would be similar to stretch induced depolarizations (SID). For testing the hypothesis about the role of NO in regulation of MGCs we used patch-clamp method in whole-cell configuration in combination with stretching of isolated cells (Kamkin et al., 2000b, 2003a; Lozinsky and Kamkin, 2010). It was shown that NO donor SNAP and DEO-NO open MGCs in unstretched cells, and closes them in stretched cells. Therefore MGCs activity is determined by NO concentration. It is known that stretching of cells and tissues stimulates NO synthases activity, which rises intracellular NO concentration (Shah and MacCarthy, 2000; Seddon et al., 2007). Besides that under control conditions tissue stretch induces hump-like depolarizations, which are originating from SIDs (Kamkin et al., 2000a). That's why we conducted a series of experiments in which we stretched atrial

tissue preparations during registration of TNF-α induced hump-like depolarizations or arrhythmias. Tissue stretch completely eliminated mechanoinduced electrical abnormalities, originating in cardiomyocytes in response to TNF-α application. Therefore, TNF-α induces electrical abnormalities in cardiomyocytes, while tissue stretching eliminates them. This effects is likely to be mediated via NO signaling cascades.

If TNF-α acts via an increase in NO concentration after activation of NO-synthase then application of NO donor, for example SNAP sold lead to similar changes in APD90 with following generation of arrhythmias. In order to prove that in absence of any changes in preload we perfused tissue with standard saline solution, containing 3×10^{-4} mol/l of SNAP. Already after 10 min of perfusion we observed appearance of hump-like depolarizations, which later transformed into sustained paroxysmal arrhythmic events, mediated by altered or even normal action potentials. Therefore SNAP caused abnormalities such as hump-like depolarizations, similar to those induced by TNF-α. In order to answer the question whether abnormalities and arrhythmias, induced by SNAP, are similar to those stretch-induced depolarizations (SID), we conducted two series of experiments with 40 μmol/l of Gd^{3+}. In the first series we added Gd^{3+} to perfusion solution after appearance of SNAP induced hump-like depolarizations or SNAP induced arrhythmias. It was shown that hump-like depolarization in abnormal patterns is completely blocked by 40 μmol/l of Gd^{3+} within 10 min after its application. SNAP induced arrhythmias were also blocked by 40 μmol/l of Gd^{3+}. In another series Gd^{3+} was added to the perfusion solution together with SNAP. In this case we did not observe any abnormal action potentials. Since discrete tissue stretching during TNF-α perfusion led to complete elimination of arrhythmias and then blocked electrical abnormalities and taking into consideration that TNF-α effects can be mediated via NO concentration increase we conducted a series of experiments in which we triggered SNAP induced SIDs and arrhythmias, and then stretched cells for further activation of NO-synthases. After appearance of SNAP induced abnormalities or arrhythmias the tissue was stretched. This stretch completely arrhythmias and SI-like depolarization triggered by SNAP application.

In conclusion it was shown that application of SNAP during observation of TNF-α induced abnormalities completely abolishes those abnormalities. For investigation of the functional role of NO-synthases in mediation of TNF-α effects we used their non-selective inhibitor L-NAME, with which we preincubated tissue during 30 min. After that we applied TNF-α for 30 more minutes in combination with L-NAME. Pre incubation of tissue with L-NAME containing solution did not lead to any abnormalities in action potentials even after 30 min of perfusion. More importantly after such pre-incubation TNF-α, applied in combination with L-NAME did not trigger appearance or development of hump-like depolarizations. Neither stretch lead to any hump-like depolarizations.

Therefore arrhythmogenic effect of TNF-α is mediated via MGCs activity and is NO dependent. TNF-α activates NO synthases, which leads to NO concentration increase triggering MGCs activation and Na^+ influx to the cell which depolarizes cellular membrane triggering arrhythmias. Tissue stretch completely abolishes

mechanoinduced electrical abnormalities, triggered by application of TNF-α to cardiomyocytes probably via additional activation of NO synthases and rise of intracellular NO concentration. Such NO concentration rise would lead to MGCs closing. This data revealing TNF-α NO mediated effects completely correlate with data from patch clamp experiments conducted in combination with single cardiomyocyte stretch. In unstretched cardiomyocytes NO donors trigger MGCs activation, which is abolished by cardiomyocyte stretch.

Thus, these experimental data sheds light on mechanism of arrhythmogenic action of TNF-α in atrial myocardium. We are trying to reveal the link between arrhythmogenic effect of TNF-α and its underlying mechanisms, which we described earlier, data about TNF-α application triggered arrhythmias in animals and clinical studies reporting levels of TNF-α from patients with cardiac arrhythmias and fibrillation. Administrating TNF-α in rats can induce arrhythmias (Krown et al., 1995) and mice with overexpression of TNF-α have a greater incidence of AF (London et al., 2003). The circulating levels of TNF-α (Sata et al., 2004) was found to be significantly higher in patients suffering from AF. In our study we were working with patients' congestive heart failure (New York Heart Association: Class II, Class III and Class IV). According to NYHA, at congestive heart failure arrhythmias and fibrillation are not considered to be symptoms of the decease. Data from Table 5.1 demonstrates the link between concentration levels of TNF-α and observation of ventricular arrhythmias. Table shows that elevation of TNF-α concentration correlates with increasing percentage of patients with clinical arrhythmic events.

5.4.2 Interleukin-6

IL-6 has been shown to be related to the occurrence of cardiac arrhythmias. IL-6 has been shown to significantly correlate with increased left atrial size (Psychari et al., 2005), an important risk factor for developing atrial fibrillation. In addition, polymorphisms in the promoter region of the *IL-6* gene have been associated with post-operative atrial fibrillation (Bittar et al., 2005; Gaudino et al., 2003). IL-6 has not previously been shown to be independently associated with spontaneous (non-post-operative) atrial fibrillation. Polymorphisms of the IL-6 gene were not studied

Table 5.1 Correlation of concentration of TNF-α with frequency of occurrence of ventricular arrhythmias

	Control $n = 55$	NYHA Class II, $n = 56$	NYHA Class III, $n = 62$	NYHA Class IV, $n = 42$	P
TNF-α, pg/ml	4.1±2.6	12.1±7.6	24.1±10.1	38.5±12.4	<0.01
Ventricular arrhythmia, *n*; %	4 7.3%	8 14.3%	23 37%	32 76%	<0.01

in these patients. Recent studies showed that atrial fibrillation is associated with elevated IL-6 levels and the IL-6 -174CC genotype (Marcus et al., 2008).

In vivo study also showed that elevated IL-6 levels were observed 1–2 h after aortic declamping in cardiopulmonary bypass (Wan et al., 1996). The direct cardiac effect of IL-6 during this period of time remains undefined. De novo synthesis and activation of iNOS induced by IL-6 can be detected in adult rat ventricular myocytes as early as 2 h after exposure (Yu et al., 2003a, b). This study also demonstrated that the IL-6-elicited iNOS activation and decrease in postrest potentiation of contraction in adult rat ventricular myocytes are mediated by activation of Janus kinase (JAK)2/signal transducer and activator of transcription (STAT)3, the upstream mediators of IL-6 signaling (Yu et al., 2003a, b). However, whether iNOS is the downstream mediator of IL-6-induced negative inotropy remains undefined.

In vitro studies on cardiac contractile function have shown that exposure to IL-6 for 2–3 min decreased contractility in papillary muscle isolated from hamster heart (Finkel et al., 1992). Studies with single myocytes also showed that IL-6 suppressed peak systolic $[Ca^{2+}]_i$ and cell shortening within 5 min in chick embryonic cardiac myocytes (Kinugawa et al., 1994) and in adult guinea-pig ventricular myocytes (Sugishita et al., 1999). This acute negative inotropic effect of IL-6, accompanied by an increase in cell cGMP production (Kinugawa et al., 1994), was blocked by NG-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthase (NOS) (Finkel et al., 1992; Kinugawa et al., 1994; Sugishita et al., 1999). Thus, acute IL-6-induced suppression of cardiac contractility and $[Ca^{2+}]_i$ have been suggested to be mediated by a NO-dependent pathway via activation of NOS (Finkel et al., 1992; Kinugawa et al., 1994; Sugishita et al., 1999), probably a constitutive endothelial isoform (eNOS) (Kinugawa et al., 1994). After 2 h incubation IL-6 decreases contractility and $Ca^{2+}{}_o$ responsiveness with no significant effect on $I_{Ca,L}$ in ARVM. (IL-6 reduces PRP, caffeine responsiveness and the phosphorylation of PLB. The negative inotropic effect is sustained for at least 1 h after removal of IL-6 and IL-6 induces production of NO via activation of iNOS, with inhibition of iNOS abolishing IL-6-induced changes in PRP, caffeine- and $Ca^{2+}{}_o$-responsiveness, and PLB phosphorylation (Yu et al., 2005a, b).

In general, IL-6 has been shown to be related to the occurrence of cardiac arrhythmias. Moreover, IL-6 regulated the contractility and the direct negative inotropic effect of IL-6 is mediated through a myocardial nitric oxide synthase. This data and studies reporting that NO directly regulates activity of mechanically gated channels (MGCs) (Kazanski et al., 2010a, b, 2011) allowed us to suppose that in heart IL-6 would be an effective regulator of MGCs activity and therefore will regulate mechanoelectrical feedback.

For testing this hypothesis we used standard microelectrode technique in combination with stretching of right rat atria cardiac tissue preparation. Under control conditions (Kamkin et al., 2000a), during perfusion with standard saline solution stretching of tissue by 1.7 mN caused prolongation of APD90 and appearance of hump-like depolarizations at APD90 level (Fig. 5.7a). Perfusion of tissue with IL-6

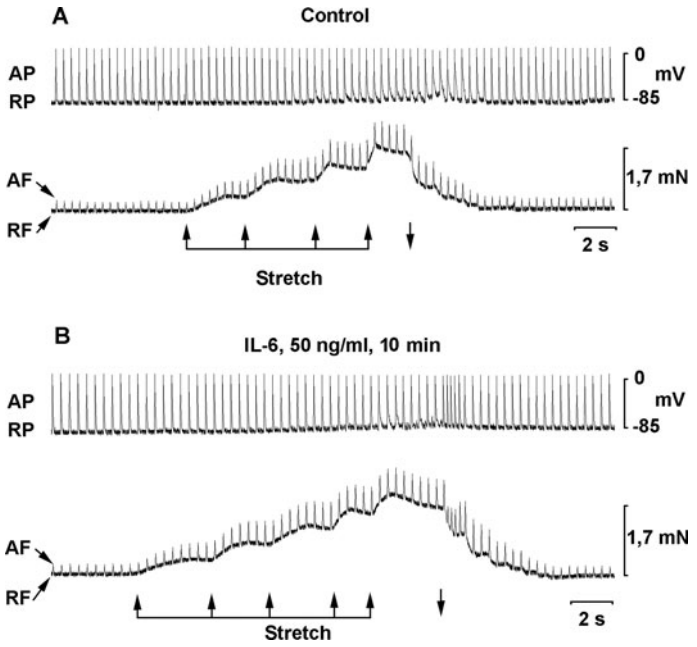

Fig. 5.7 Response of atrial cardiomyocyte membrane potential to stretch applied by the digital micromanipulator. (**a**) Control conditions. Stepwise increases in resting force due to stretch (indicated by ↑) up to 1.7 mN significantly increased APD90. Removal of stretch (indicated by ↓) reversed these effects. (**b**) IL-6 in concentration of 50 ng/ml, 10 min of perfusion. Stepwise increases in resting force due to stretch (indicated by ↑) up to 1.7 mN produced stretch-induced depolarization and tachyarrhythmia. Note: resting force (RF), active force (AF), resting potential (RF), active potential (AP) (the data obtained from the Department of Professor Andre Kamkin)

containing solution (50 ng/ml) lead to appearance of abnormalities in bioelectrical activity, majorly observed as APD90 prolongation. After 7–10 min of perfusion tissue stretch by 1.5–1.7 mN trigged hump-like depolarizations at APD90 level. After reaching E_c hump-like depolarization transformed into single extra-action potential later leading to typical tachycardia, which could be abolished by stretch (Fig. 5.7b).

Continuous perfusion of the preparation with IL-6 containing solution for periods of time longer than 20 min leads to fibrillation triggered by extremely low levels of stretch. Figure 5.8a shows a trace of registration of the resting force (RF), active force (AF), resting potential (RF) and active potential (AP) in control conditions during perfusion of the fragment of right rat atria with standard saline solution. Stretching of tissue by 1.7 mN triggered APD90 prolongation. Figure 5.8b shows effects of tissue stretch after 23 min of perfusion with IL-6 containing solution (50 ng/ml). Even small stretch by 0.3 mN triggered fibrillation, observed as typical bioelectrical activity and absence of AF. Stretch elimination stopped fibrillation. IL-6 effects and their underlying mechanisms are similar to those of TNF-α.

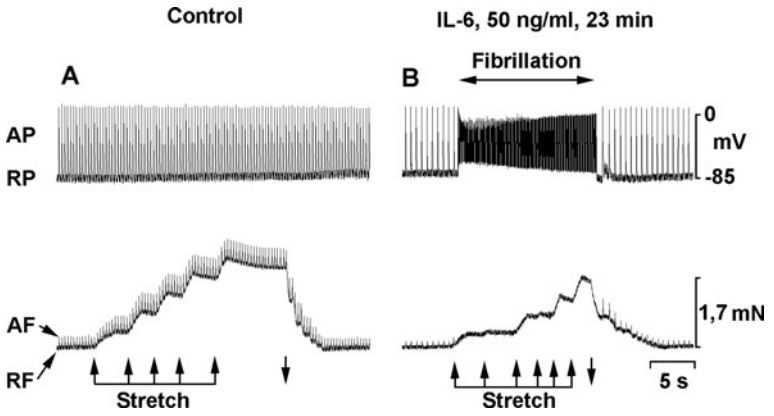

Fig. 5.8 Example of response of cardiomyocyte membrane potential to stretch applied by the digital micromanipulator of right atrial tissue. (**a**) Control conditions. Stepwise increases in resting force due to stretch (indicated by ↑) up to 1.7 mN produced stretch-induced depolarization (SID) near APD90. (**b**) IL-6 in concentration of 50 ng/ml, 10 min of perfusion. Stepwise increases in resting force due to stretch (indicated by ↑) only up to 0.3 mN produced fibrillation. Release of the stretch (↓) resulted in complete reversibility of stretch-induced effects. Note: resting force (RF), active force (AF), resting potential (RF), active potential (AP) (the data obtained from the Department of Professor Andre Kamkin)

5.5 Conclusion and Perspectives

All together the data from literature testifies towards hypothesis that studied cytokines regulate action potentials of cardiac cells influencing a number of ion fluxes including I_{Na}, I_{Ca}, I_{to}, I_{Kur}, I_{Kr}, I_{Ks}, I_{K1}. Besides that and possibly even more importantly those compounds regulate mechanoelectrical feedback in heart via activation of NO synthases in cardiomyocytes, primarily NOS3 as is shown on Fig. 5.9 (Prabhu, 2004a). NO imparts cardiac effects via two general signaling modalities: (i) the activation of soluble guanylate cyclase and the formation of cGMP, which in turn activates protein kinase G (PKG) and PKG-dependent phosphorylation events; and (2) the direct oxidation of thiol residues on critical regulatory proteins (S-nitrosylation) (Kojda and Kottenberg, 1999; Massion et al., 2003; Prabhu, 2004a). Rise of intracellular NO concentration opens MGCs, which allows sodium entry into the cell which in turn depolarizes them, shifting resting membrane potential towards E_C (Kazanski et al., 2011). This can directly or indirectly lead to arrhythmias triggered by elevated levels of cytokines. A large number of studies, performed in a variety of experimental models, have examined whether NO, derived from NOS3, contributes to early cytokine-induced dysfunction. We already highlighted that cytokine levels go up in different pathological states, including infarct and hypertrophy of cardiac tissue. In combination with data regarding correlation between such electrophysiological manifestations of cardiac pathology as arrhythmias and fibrillation and cytokine blood levels it is possible to propose cytokines as triggers of cardiac arrhythmias and fibrillation. Besides pure theoretical

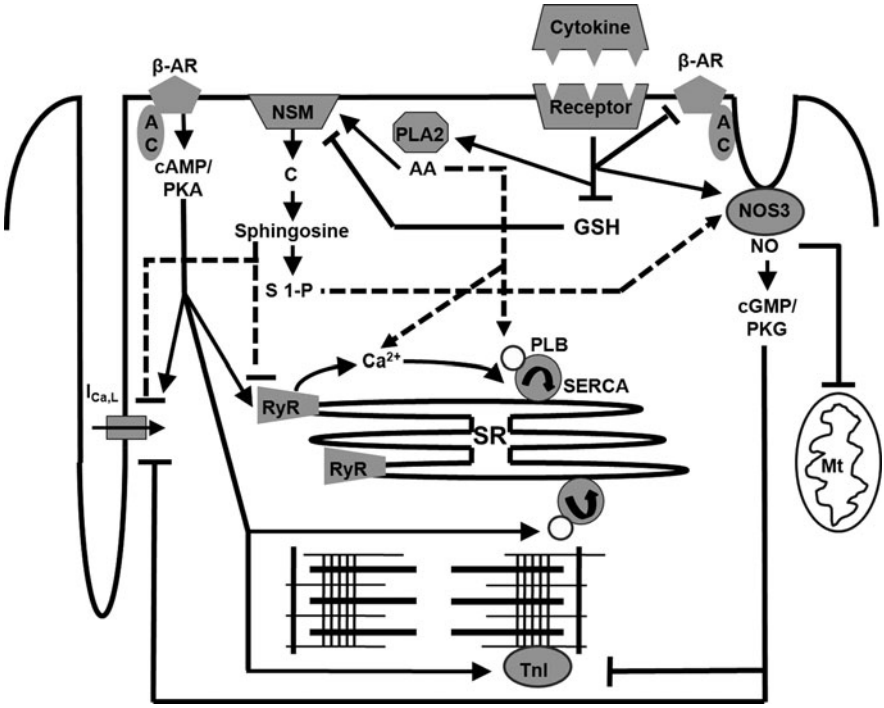

Fig. 5.9 Cellular mechanisms underlying immediate cytokine-induced contractile effects. Pro-inflammatory cytokines modulate several signaling pathways that can ultimately impact contractile function. Rapid activation of NOS3 generates NO, with depression of Ca^{2+} transients and myofilament Ca^{2+} sensitivity via the production of cGMP and cGMP-dependent activation of PKG. cGMP/PKG also blunts cAMP/PKA-mediated effects, and NO can also directly depress mitochondrial (Mt) respiration, thus altering mechanoenergetics. In low concentrations, NO can also exert a positive inotropic effect. Cytokines such as TNF-α also deplete GSH and activate membrane-associated neutral sphingomyelinase (NSM), which in turn increases ceramide (C) and sphingosine levels. Sphingosine blunts the inward Ca^{2+} current ($I_{Ca,L}$) and Ca^{2+} release from the ryanodine receptor (RyR) of the SR. Ceramide also blunts $I_{Ca,L}$. Cytokines also activate cytosolic PLA2 to generate AA, which can, under appropriate conditions, augment the Ca^{2+} transient and contractility. Cytokines may also acutely impair β-AR coupling to AC via unclear mechanisms. Cross-talk likely occurs between these various pathways. Free fatty acids such as AA have been shown to activate NSM, and sphingosine 1-phosphate (S 1-P) can phosphorylate and activate cNOS. GSH, in turn, inhibits NSM activation. Thus, the ambient oxidant stress and metabolic state, along with the cytokine dose and applicable reflex effects, influences the relative activation of these pathways, resulting in responses that are either cardiostimulatory (with low concentrations and short exposure duration) or cardiodepressant (with higher concentrations and longer exposure time). PLB indicates phospholamban; SERCA, SR Ca^{2+} ATPase; TnI, troponin I (from Prabhu (2004a) with permission of Lippincott Williams & Wilkins, *Circulation Research* and American Heart Association via Copyright Clearance Center)

importance such point of view can have clinical application. It would be a valuable approach for creation of test systems for early prediction of cardiac arrhythmias and fibrillation.

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