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DISSERTATION

Effect of AT_1-antagonist (eprosartan) on the mechanical dysfunction ("atrial stunning") after DC shock of atrial strip of patients with and without atrial fibrillilation

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This thesis is dedicated to my dearest wife Vivian Okae and my son Nana Kofi Okae.

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1 Introduction

1.1 Background

Atrial fibrillation (AF) is the commonest sustained disorder of cardiac rhythm. When it is present, many prognostic and therapeutic implications exist as overall morbidity and mortality increase appreciably. Despite this, atrial fibrillation is often neglected, probably because many patients have few symptoms. In fact, some patients with chronic atrial fibrillation may require long-term treatment with potent antiarrhythmic and anticoagulant drugs, which may have important pharmacological interactions and adverse effects. In addition, Lip et al. (1995) showed that the treatment differs considerably for chronic and paroxysmal atrial fibrillation and for atrial flutter, and other forms of supraventricular arrhythmias.

Although the effective treatment for and mechanism of most other supraventricular tachyarrhythmias have been discovered, the pathophysiology of AF is not completely understood. In the last ten years a lot of studies about AF have been conducted, but only the simple understanding of the cause, the pathophysiology and groundbreakings have been made because there is no unification of the mechanism of AF. One major problem that arises after a treatment of AF is atrial stunning, another hurdle that has to be cleared in order to avoid complications after the treatment of AF.

1.2 History of atrial fibrillation

“Perhaps the earliest description of atrial fibrillation is in The Yellow Emperor’s classic of Internal Medicine (Huang Ti Nei Ching Su Wen). The legendary emperor physician is believed to have ruled China between 1696 and 2598 BC. The poor prognosis associated with chaotic irregularity of the pulse was clearly acknowledged by most of the ancient physicians, but in recorded history, William Harvey in 1628 was probably the first to describe “fibrillation of the auricles” in animals” (Gregory et al., 1995).

Because the diagnosis of atrial fibrillation requires measurement of the electrical activity of the heart, atrial fibrillation was not truly described until 1874, when Edmé Félix Alfred Vulpian observed the irregular atrial electrical behavior that he termed "fremissement fibrillaire" in dog hearts (Vulpian A, 1874). However, the irregular pulse and its association with disease have been recognized since antiquity. Direct visualization of cardiac fibrillation was first described in 1628 by William Harvey in dying

animals. In the mid-eighteenth century, Jean Baptiste de Sénac made note of dilated, irritated atria in people with mitral stenosis (McMichael J, 1982). The irregular pulse associated with AF was first recorded in 1876 by Carl Wilhelm Hermann Nothnagel and termed "delirium cordis", he stated, "In this form of arrhythmia the heartbeats follow each other in complete irregularity. At the same time the height and tension of the individual pulse waves are continuously changing" (Nothnagel H, 1876). Correlation of delirium cordis with the loss of atrial contraction as reflected in the loss of waves in the jugular venous pulse was established by Sir James MacKenzie in 1904 (MacKenzie J, 1904). Willem Einthoven published the first electrocardiogram showing AF in 1906 (Einthoven W, 1906). The connection between the anatomic and electrical manifestations of AF and the irregular pulse of delirium cordis was made in 1909 by Carl Julius Rothberger et al. and Lewis T. (Rothberger J. et al., 1909, Lewis T., 1909).

1.3 Epidemiology, prevalence and prognosis of atrial fibrillation and atrial stunning

Atrial fibrillation (AF) is currently the most common sustained clinical arrhythmia in adults and is responsible for the substantial proportion of hospital costs incurred in the treatment of cardiac rhythm disorders. AF becomes increasingly common with age, having an incidence averaging <0.5% in patients <50years of age and reaching >10% of the octogenarian population. On the average two percent (2%) of the adult population suffers from AF (Nattel et al., 2002). Thus AF is likely to become increasingly important with the ageing of the population.

AF can occur in paroxysms lasting less than 24 hours (but longer lasting paroxysms are not unusual) with intermittent sinus rhythm (SR). Paroxysmal AF converts either spontaneously or is terminated with an intravenously administered antiarrthythmic drug. In contrast, during persistent AF, the arrhythmia is continuously present until the moment of investigation, i.e. at least two consecutive electrocardiograms of AF more than 24 hours apart and without intercurrent sinus rhythm. Persistent AF does not convert spontaneously.

AF has the tendency to become more persistent over time. This is illustrated by the fact that about 30% of the patients with paroxysmal AF eventually will develop persistent AF. Also pharmacological and electrical cardioversion maintenance of sinus rhythm thereafter becomes more difficult the longer the arrhythmia exists (Brundel et al., 2004).

AF doubles mortality and increases the risk of stroke. Structural remodelling of the atrial tissue follows the early appearance of electrical remodelling. In the past decade scientist have been encouraged to investigate the etiology and pathophysiology of this type of frequent arrhythmia. These investigations have resulted in concepts on the initiation and perpetuation of this arrhythmia. New techniques are being developed for its treatment. However the majority of patients are candidates for the traditional treatment: antiarrhythmic drugs and/or electrical cardioversion. Direct current cardioversion of persistent AF is the most effective treatment, but it is hampered by a high percentage of recurrences (Brundel et al., 2004). Although more often than not AF is symptomatic, it may also be asymptomatic. Fainting, orthostatic hypotension, low blood pressure and angina are some of the symptoms of AF. Inability of the heart to pump enough blood to sustain normal bodily function may cause heart failure. Related to the formation of blood clots, stroke and transient ischaemic attacks (TIAs) may occur. AF is the single most important cause of ischaemic stroke in people older than 75 (Hart et al., 2001).

AF is an intermediate-phase disease, meaning that condition usually develops in response to damage to the heart, or by changes in the cardiovascular system. Causes may include untreated arterial hypertension, abnormal mitral valve function, e.g. due to rheumatic heart disease. Any surgical procedure involving the heart, coronary heart disease, excessive alcohol consumption and overactive thyroid hormones (hyperthyroidism) may cause AF. In turn, untreated AF can precipitate further damage to the heart by weakening the muscles of the ventricles.

Although atrial stunning (AS) is the term mostly used to describe the phenomenon of postischaemic dysfunction of the myocardium, it is also used to describe the condition of reduced contractility after a direct current (DC) has been applied to the heart muscle. The exact mechanism underlying the development of atrial stunning still remains unknown. There are evidences to suggest that DC shocks lead to considerable myocardial damage at both a biochemical and histological level and that the shock itself may contribute to AS (Fatkin et al., 1994; Grimm et al., 1993). AS can also be defined as a transient prolonged depression of atrial and atrial-appendage mechanical function after cardioversion of an atrial fibrillation. AS has been reported after all methods of cardioversion of AF, including transthoracic electrical, low-energy internal electrical, pharmacological and spontaneous. Tachycardia-induced atrial cardiomyopathy, cytosolic calcium accumulation and atrial hibernation are some of the suggested

mechanisms of AS. AS is at maximum immediately after cardioversion and improves progressively with a complete resolution within a few minutes to 4-6 weeks, depending on the duration of the preceding atrial fibrillation, atrial size and structural heart disease (Khan et al., 2003). The duration of the preceding atrial fibrillation, atrial size, and underlying structural heart disease are some of the determinants of atrial fibrillation and smaller atrial diameter are associated with a relatively less severe stunning, lasting for a shorter period. AS after cardioversion of atrial fibrillation of less than 1 week usually resolves within 24 hours, and AS after cardioversion of chronic AF usually resolves within 4 weeks (Khan et al., 2003).

AS determines also the duration of post cardioversion anticoagulation therapy, the recovery of atrial contribution to the ventricular function and the functional recovery of the patients after successful cardioversion of AF (Khan et al., 2003).

1.4 Pathophysiology of atrial fibrillation and atrial stunning and their effect on the myocardium

New clinical evidence shows that AF almost invariably occurs in a setting of atrial electrical dysfunction that promotes arrhythmia. Transmembrane ionic currents are major determinants of the arrhythmia mechanisms. I_{K1} is the background current responsible for the considerable resting K^+ conductance that sets the resting potential to between –70 and –80 mV. Cell firing is caused by rapid depolarization through a large Na^+ current (I_{Na}) that brings the cell from its resting potential to a value in the region of +40 mV, providing the electrical energy for cardiac conduction. The cell then partially repolarizes through a transient outward K^+ current (I_{to}), inactivation of which produces a notch in the action potential. The inward L-type Ca^{2+} current (I_{Ca}), maintain a relatively flat portion of the action potential (the ‘plateau’). A series of K+ currents that activate in a time-dependent way and show little inactivation, the so-called ‘delayed rectifiers’ (I_K), leads to cellular repolarization. In human atrium, I_K has three components: an ultra-rapid component (I_{Kur}), a rapid component (I_{Kr}) and a slow component (I_{Ks}). Automatic cells are spontaneously depolarized by an inward pacemaker current (I_f). Na^+–Ca^{2+} exchanger (NCX) also carries an inward current during terminal repolarization. The balance between plateau inward and outward currents determines the action potential duration (APD): increased inward current prolongs the action potential, and increased

outward current shortens it. APD governs the time from cellular depolarization to recovery of excitability at about –60 mV; the ionic current balance therefore determines the refractory period and the likelihood of re-entry. Alterations in ionic currents that increase APD and thereby the refractory period can be used to prevent AF. I_{Ks} and I_{Kur} are under strong adrenergic control (Li et al., 1996), and their stimulation might contribute to AF that occurs in situations of increased adrenergic tone. I_{Kur} is carried by K+ channel gene (Kv1.5) that are expressed functionally in human atrium but not ventricle. Inhibiting these channels may provide a means of preventing AF without the risk of ventricular pro arrhythmia (Nattel et al., 1993).

Progress in our understanding of AF was made with the recognition that AF, once initiated, alters atrial electrophysiological properties in a manner that favours the ease of inducing and maintaining the arrhythmia, a process called ‘electrical remodelling’ (Wijffels et al.,1995). The roughly tenfold atrial rate increase caused by AF is the primary stimulus to atrial remodelling, and similar changes are produced by any form of sufficiently rapid tachycardia (Wijffels et al.1997). Ca^{2+} enters the cells through I_{Ca} with each action potential, so a tenfold increase in atrial rate substantially increases cellular Ca^{2+} loading (Sun et al., 2001).

Progressive Ca^{2+} loading threatens cell viability, and the cells respond to minimize the impact of increased rate on intracellular Ca^{2+} load. Short-term defence mechanisms include voltage-dependent and intracellular Ca^{2+} concentration-dependent inactivation of I_{Ca}. Over the longer term, the concentration of messenger RNA encoding the pore-forming I_{Ca} subunit (Yue et al., 1999) decreases, which in turn decreases I_{Ca}. Both short- and long-term decreases in I_{Ca} reduce Ca^{2+} entry and help to prevent Ca^{2+} overload; however, because I_{Ca} is a key contributor to the action potential plateau (Fig.1.3a), reduced I_{Ca} decreases APD, reduces the refractory period, and promotes the induction and maintenance of AF by multiple circuit re-entry (Gaspo et al., 1997). AF that begins by any mechanism causes electrical remodelling, which by promoting multiple-circuit re-entry will make this a final common pathway of AF irrespective of the initial mechanism. In addition to down-regulating I_{Ca}, AF induces many other changes, consistent with a substantial cellular insult caused by excessively rapid activation. Cellular Ca^{2+} handling is altered, decreasing the release of systolic Ca^{2+} in association with altered concentrations of intracellular Ca^{2+} handling proteins, cellular myolysis occurs, along with changes that suggest a return to a more fetal phenotype. Decreased release of systolic Ca^{2+} and myolysis impair atrial contractility, contributing to the

occurrence of blood stasis and thromboembolic events after termination of AF (Ohkusa et al., 1999; Ausma et al., 1997).

The reversal of AS and the relationship between the chronicity of AF and the severity of atrial cardiomyopathy could be a potential cause of the transient atrial mechanical dysfunction manifesting after conversion to sinus rhythm (Gao et at., 1997). The tachycardia-induced atrial cardiomyopathy is well described in ventricles, and is a completely reversible phenomenon. The tachycardia-induced atrial cardiomyopathy would probably result from the electrical and mitochondrial changes taking place due to frequent atrial depolarisations during atrial fibrillation. The gradual return of atrial mechanical function to normal levels after conversion to sinus rhythm (SR) also supports the hypothesis that left AS may be a form of tachycardia-induced atrial cardiomyopathy, which translates into atrial mechanical dysfunction after conversion to SR (Gao et al., 1997).

The changes in cardiac function caused by calcium overload are reviewed. Intracellular Ca^{2+} may increase in different structures, for example sarcoplasmic reticulum, cytoplasm and mitochondria to an excessive level which induces electrical and mechanical abnormalities in cardiac tissues. The electrical manifestations of Ca^{2+} overload include arrhythmias caused by oscillatory (V_{os}) and non-oscillatory (V_{ex}) potentials. The mechanical manifestations include a decrease in FOC/A. The underlying mechanisms involve a role of Na^{+} in electrical abnormalities as a charge carrier in the Na^{+}-Ca^{2+} exchange and a role of Ca^{2+} in mechanical toxicity. Ca^{2+} overload may be induced by an increase in $[Na^{+}]_i$ through the inhibition of the Na^{+}-K^{+} pump or by an increase in Ca^{2+} load. The Ca^{2+} overload is enhanced by fast rates. If the SR is predominantly Ca^{2+} overloaded, V_{os} and fast discharge are induced through an oscillatory release of Ca^{2+} in diastole from the sarcoplasmic reticulum; if the cytoplasm is Ca^{2+} overloaded, the non-oscillatory V_{ex} tail is induced at negative potentials. The decrease in contractile force by Ca^{2+} overload appears to be associated with a decrease in high energy phosphates, since it is enhanced by metabolic inhibitors and reduced by metabolic substrates. The ionic currents I_{os} and I_{ex} underlie V_{os} and V_{ex}, respectively, both being due to an electrogenic extrusion of Ca^{2+} through the Na^{+}-Ca^{2+} exchange. I_{os} is an oscillatory current due to an oscillatory release of Ca^{2+} in early diastole from the Ca^{2+}-overloaded SR, and I_{ex} is a non-oscillatory current due to the extrusion of Ca^{2+} from the Ca^{2+}-overloaded cytoplasm. I_{os} and I_{ex} can be present singly or simultaneously. An increase in $[Ca^{2+}]_i$ appears to be involved in the short- and long-

term compensatory mechanisms that tend to maintain cardiac output in physiological and pathological conditions. Eventually, $[Ca^{2+}]_i$ may increase to overload levels and contribute to cardiac failure. Experimental evidence suggests that clinical concentrations of digitalis increase force in Ca^{2+}-overloaded cardiac cells by decreasing the inhibition of the Na^+/K^+ pump by Ca^{2+}, thereby leading to a reduction in Ca^{2+} overload and to an increase in force of contraction. Acute left contractile dysfunction may be related to the cytosolic calcium overload resulting from abnormal trans-sarcolemmal fluxes in the course of frequent and irregular depolarization of atrial myocytes during atrial fibrillation (Daoud et al., 1997; Leistad et al., 1997). Chronic calcium overload state may thus lead to desensitization or down-regulation of the calcium receptors resulting in an associated decrease in mechanical function which would be expected to normalize as calcium receptors return to their baseline state.

1.5 Atrial contractility

1.5.1 Excitation-contraction coupling in stunned myocardium

The site of the lesion in excitation-contraction coupling has recently been the focus of intensive investigation. Because electrical activation is normal (Hanish et al., 1993) the basis for stunned myocardium must lie in either of two broad mechanistic categories. First, the availability of activator calcium might be restricted; such an effect could be mediated by abnormal calcium entry into or removal from the cytosol due to lesions in one or more cellular calcium-handling pathways. Alternatively, the responsiveness of the contractile machinery to calcium might be blunted such that the myocardium generates less force for any given rise of $[Ca^{2+}]_i$.

The last 12 years of research have overwhelmingly implicated the myofilaments as the site of the critical lesion in stunning, at least in isolated perfused heart models. The first clue that myofilament function was abnormal came from Kusuoka et al., 1987, who found a depressed maximal calcium-activated pressure (the whole heart equivalent of maximal calcium-activated force) in stunned ferret hearts. Marban and co-workers (Marban et at., 1988; Marban et al., 1990) later developed and validated the methodology for measuring $[Ca^{2+}]_i$ in isolated perfused ferret hearts by NMR spectroscopy. Functional studies of the myofilaments during steady-state calcium activation revealed both a decrease in maximal force and a decrease in sensitivity. This

study further showed that simple changes of cross-bridge attachment and detachment rates in a quantitative model of myofilament interaction reproduced the salient features of the contractile dysfunction of stunned myocardium.

1.5.2 Intracellular calcium homeostasis in frequency-dependent force generation (Bowditch-staircase Effect)

More than a century ago it was recognized that cardiac contractility can be enhanced by an increase of the stimulation frequency (Bowditch H, 1871). This behaviour was termed the "treppe" (staircase) phenomenon or the force-frequency relationship (FFR). Although force is not measured as a primary parameter of contractility in most models (shortening in cardiac myocytes, force in papillary muscles, and pressure in isolated hearts), the terminology is still commonly used (Lewartowski et al., 1987).

The changes in cardiac mechanics that occur during an increase in frequency can be further divided. All mammals studied show an abbreviation of cardiac contraction and relaxation when stimulation frequency is increased, within the physiological frequency range (Bers et al., 1991; Buckley et al., 1972). This has been confirmed in vivo and in vitro (isolated cardiac myocytes, papillary muscles, and isolated hearts) and is also found in myocardium from diseased human hearts (Mulieri et al., 1992). This aspect of the force-frequency relation is determined by parameters, such as the maximum contraction velocity and maximum relaxation velocity.

Small rodents like mice display a decrease in contractility if the stimulation frequency is increased (negative force-frequency relationship), whereas an increase in contractility or positive force-frequency relationship has been described in other mammals (Bers et al. 1991; Buckley et al., 1972). A more negative force-frequency relationship occurs also in the diseased heart in both animals and humans (Mulieri et al., 1992).

1.6 Angiotensin II receptors, their subtypes and the effect of AT_1 receptor antagonist on the human myocardium

The effect of Ang II on cardiac tissue is related to the activation of 2 specific receptors, AT_1 and AT_2 (Baker et al., 1984; Rioux et al., 1975). The AT_1 receptor has 2 subtypes: AT_{1A} and AT_{1B} (Chiu et al., 1989). AT_{1A} receptors are major blood pressure regulators and potent growth stimulators in cardiomyocytes in vivo, whereas AT_{1B} receptors are

involved in the control of vascular tone when AT_{1A} receptors are absent (Hein et al., 1998). Eprosartan binds only to AT_1 subtype, whereas the compound CGP42112A and PG123177 both selectively recognize the subtype AT_2.

Ang II receptors are 7-transmembrane domain receptors whose primary structures have been established by molecular cloning (Sasaki et al., 1991, Murphy et al., 1991). The activation of the receptor is coupled to several intracellular proteins, starting with a G protein (Clauser et al., 1998). The receptor domains that couple to G proteins involve the second and third cytosolic loops and the proximal segment of the carboxyl-terminal domain (Inagami et al., 1994). In the rat, AT_{1A}, AT_{1B}, and AT_2 receptors are located on chromosomes 17, 2, and X, respectively. Samyn et al., 1998, have demonstrated that cardiac AT_1 receptor gene expression remains relatively unchanged during fetal and newborn life and that AT_2 receptor mRNA expression is high during fetal development and decreases rapidly after birth.

Exposure of AT_{1A} and AT_{1B} receptors to Ang II is followed by translocation of the receptor to intracellular vesicles (Sasamura et al., 1997). Internalization of the Ang II–AT_1 receptor complex occurs with a half-life of <2 minutes. Unlike AT_2 receptors, which are not internalized (Anderson et al., 1993). AT_1 receptors appear to cycle continuously between endosomal vesicles and the plasma cell membrane (Hein et al. 1997). In agreement with this contention, AT_1 receptors in rat myocytes have been localized in the sarcolemma, T tubules, and nuclei (Fu et al., 1998). Internalized Ang II is either degraded in the cell or exerts intracellular effects. In support of the latter, evidence is available that Ang II couples to a nuclear binding site, and that binding of Ang II to a chromatin high-affinity receptor leads to a conformational change in chromatin (Re et al. 1984). Intracellular dialysis of Ang II in adult rat myocytes reduces cell communication, an effect abolished by intracellular administration of losartan. (De Mello et al., 1994) Similarly, the intracellular actions of Ang II in vascular smooth muscle cells are suppressed by the AT_1 receptor antagonist candesartan (Haller et al., 1999). These findings indicate that there may be a functional intracellular Ang II receptor similar to the AT_1 receptor, although further studies are necessary to clarify this point.

The mechanism of Ang II receptors is quite well defined. Activation of AT_1 receptors results in the initiation of a variety of events, such as the stimulation of phospholipase C, with subsequent activation of protein kinase C (PKC) and release of Ca^{2+} from intracellular depots. In addition, tyrosine kinase and mitogen-activated protein kinase are phosphorylated. Interestingly, AT_2 receptor blockade increments the early signals of

AT_1 receptor–mediated cardiac growth responses in the hypertrophied rat heart (Thomas et al., 1996; Bartunek et al., 1999), suggesting that AT_2 receptors counteract the effects of AT_1 receptors. The establishment of left ventricular hypertrophy in spontaneously hypertensive rats is associated with increased expression of AT_{1A} and AT_{1B} receptors (Ijima et al., 1998). AT_2 receptors are upregulated by interleukin-1ß and insulin, whereas an increase in intracellular Ca^{2+}-activated PKC as well as in several growth factors (epidermal growth factor, nerve growth factor, and platelet-derived growth factor) induces a down-regulation of AT_2 receptors. AT_2 receptors activate the kinin/NO/cGMP system and stimulate protein tyrosine phosphatase and serine/threonine phosphatase. Protein tyrosine phosphatase stimulation inactivates AT_1 receptor activated mitogen-activated protein kinase, and this may explain the above interaction between AT_1 and AT_2 receptors. In addition, activation of extracellular signal regulated kinase, which underlies the mitogenic or hypertrophic response after AT_1 receptor stimulation, can be reversed by AT_2 receptor–mediated stimulation of serine/threonine phosphatase 2A (Nakajima et al., 1995; Huang et al., 1996).

1.7 Isoprenaline, its receptors and its effect on the heart.

Isoprenaline, a beta-adrenergic agonist, has a positive inotropic effect generally associated with a stimulation of the I_{Ca}. Beta-1 and beta-2 adrenergic receptors coexist in the heart of various animal species, including man. Both receptors are positively coupled to the adenylyl cyclase system and participate in the mediation of the positive chronotropic and inotropic effects of catecholamines (Stiles et al., 1984). However, the relative amount of each receptor subtype as well as the postreceptor cellular signalling pathways may differ significantly depending on the cardiac tissue, the animal species, the pathophysiological state, the age or the developmental stage (Stiles et al., 1984). Competitive radioligand binding studies performed in membranes from homogenized hearts have shown that only 20 to 30% of the total beta adrenergic receptors are of the beta-2 subtype in adult mammalian ventricular tissue.

1.8 Aims of the study

The reduced mechanical function after termination of AF which is termed atrial stunning has been described after electrical (DC shock, overdrive pacing, catheter ablation), pharmacological, and spontaneous cardioversion of AF. Pathophysiologically, the transient dysfunction of the atria appears to be due to abnormalities in cellular calcium handling and reduced calcium transient through L-type calcium channels (Schotten et al., 2003, Nattel et al., 2002). Furthermore, destruction of contractile proteins by calcium-dependent proteases such as calpains and functional alterations of the contractile proteins may contribute to prolonged mechanical alterations (Goette et al., 2002). Dagres et al., 2006, show that pre-treatment with AT_1 receptor antagonist, irbesartan, reduces atrial stunning in patients undergoing electrical cardioversion of persistent AF. The authors provide a new piece of information about the potential impact of the cardiac (atrial) angiotensin system in the process of mechanical dysfunction during AF. The results suggest that the use of irbesartan may be helpful to reduce AS, and thereby thrombo-embolic complications after cardioversion. Several reports have shown an activation of the atrial angiotensin II system in patients with AF (Schotten et al., 2003, Goette et al., 2002, Ehrlich et al., 2006). Angiotensin II can influence structural as well as electrophysiological atrial changes. Angiotensin II induces the cellular response by activation of two different receptors. Activation of AT_1 stimulates phospholipase C, leading to diacylglycerol-mediated activation of protein kinase C (PKC) and to inositol-1,4,5-trisphosphate-mediated release of calcium from intracellular stores. PKC can phosphorylate L-type calcium channels, which may alter calcium influx and can affect potassium channels, such as I_{to} and the delayed rectifier (Goette et al., 2002, Ehrlich et al., 2006). Furthermore, the AT_1 receptor is described to form complexes with Kv4.3, leading to internalization of the receptor-channel complex. Overall, angiotensin II has multiple effects on cardiac electrophysiology including ionic currents and gap junction expression. Irbesartan has been reported to inhibit currents carried by human potassium channel, hKv1.5 (I_{kur}), K+ channel gene, Kv4.3 (I_{to}), human ether-a-go-go-related gene (hERG (I_{kr}), and K+ channel gene, KvLQT1 (I_{ks}). These electrophysiological effects may be more relevant in remodelled/diseased atria (Schotten et al., 2003, Goette et al., 2002, Ehrlich et al., 2006). Thus, similar to antiarrhythmic drugs (dofetilide, AVE0118, verapamil), which have been reported to affect atrial contractility by their ability to block ion channels, eprosartan may also reduce atrial mechanical dysfunction by influencing the shape of the action potential,

and thereby affecting calcium transients, cellular calcium handling, and contractility. Furthermore, angiotensin II stimulates via AT_1 receptors the production of O_2^- by NADPH oxidases. Increased oxidative stress induced by NADPH oxidase activity has been shown to cause oxidative changes of structural proteins and contractile filaments in fibrillating atria (Dudley et al., 2005, Mihm et al. 2001). Thus, oxidative stress induced by AT_1 receptor activation may contribute to the development of atrial contractile dysfunction. Increased oxidative stress is also of relevance for pro-thrombogenic endocardial changes (Goette et al., 2002, Mihm et al. 2001).

Overall, the underlying pathophysiological mechanisms of how irbesartan affects atrial stunning need further investigations. Since eprosartan and irbesartan belongs to the same group, eprosartan may have a similar pathophysiology to that of irbasartan. We chose to experiment with eprosartan.

Many are the evidence to suggest that direct current (DC) shocks lead to considerable myocardial damage at both a biochemical and histologic level and that the shock itself may contribute to atrial stunning (Grimm et al., 1993, Manning et al., 1995, Yarbrough et al., 1964, Trouton et al., 1989). Previous studies of AS have been performed in patients with AF and atrial flutter and have demonstrated that both low energy internal and higher energy external defibrillation may be associated with depression of left atrial function (Grimm et al., 1997, Fatkin et al., 1994,Grimm et al., 1993). Fatkin et al., 1994, demonstrated an association between the number and energy level of DC shocks delivered in AF and the development of spontaneous echo contrast (SEC) with reversion to sinus rhythm. These investigators suggested that the DC shock contributed to the mechanism of atrial stunning. As to whether the shock itself is of importance in producing AS has not been studied in detail.

Some physiologic parameters with respect to the contractile function of the heart muscles change after DC shock. Through pharmacologic experimentation with eprosartan the functional examination of contraction recovery parameters such as force-frequency-relationship (FFR), force of contraction (FOC/A), maximum contraction velocity (dF/dt_{max}) and maximum relaxation velocity (dF/dt_{min}) were supposed to be verified, using atrial strips of patients with AF and SR. The changes of these parameters may indicate whether eprosartan has any cardioprotective properties during DC shock in human atrial strips.

1.9 Questions asked

1. AT_1-antagonist has been reported to attenuate atrial remodelling in AF, could these theoretically influence electrically injured atrial strips?
2. Has AT_1-antagonist (eprosartan) got any cardioprotective effect and can it improve the recovery rate of atrial strips after DC shock?
3. Are there any differences in the effect of AT_1-antagonist (eprosartan) on AF and SR?

2 Materials and Methods

2.1 Materials

Atrial myocardium used for the experiments were obtained during cardiac surgical operations with extracorporal circulation. Most of the patients underwent coronary artery bypass graft (CABG), mitral valve replacement (MVR) and aortic valve replacement (AVR). Written consents of the patients for the use of the tissue samples (usually discarded) were given.

2.1.1 Human atrial tissue samples

The study comprised 28 patients with atrial fibrillation or sinus rhythms. 11 patients were with atrial fibrillation (66.7 ± 2.8 years of age). To control for potential procedural effects on atrial mechanical function, 17 patients (65 ± 2.5 years of age) with sinus rhythms were evaluated. In this experiment, NYHA classifications of the patients were all of II-IV stage. At different stages of the experiment the samples underwent different experimental conditions.

In all 28 atrial strips the FOC/A, $dF/dt_{max,}$ and $dF/dt_{min,}$ which are some of the physiologic parameters that changes during atrial stunning were measured and recorded. The FOC/A at the various frequencies were compared to the baseline FOC/A (37℃, 1 Hz, and Tyrode`s solution) to get FOC/A (%). The control group was comprised of patients with normal heart rhythm, sinus rhythm and the AF group was comprised of patients with atrial fibrillation. The force-frequency relationship (Bowditch staircase effect), FFR, the percentage increment of FOC/A (FOC/A (%)) and the beta-adrenergic sensitivity of both groups to isoprenaline were also measured and compared. To facilitate comparison between preparations of different dimensions, force is expressed per unit of cross-sectional area.

The results obtained at the end of these experiments are shown in tab.3.1-8. The samples were immediately put into a cold cardioplegic BDM-solution (2,3-Butane-dione-monoxime) and stored in a fridge when not immediately used. The particulars of the patient (name, sex, age, NYHA-stage, premedication and heart related sickness) where recorded as can be seen in tab.2.1. Care was taking to ensure that atrial samples where used as early as possible. The LVEF (%) of the samples ranged from 25 to 78% (mean: 56 ± 2.90%).

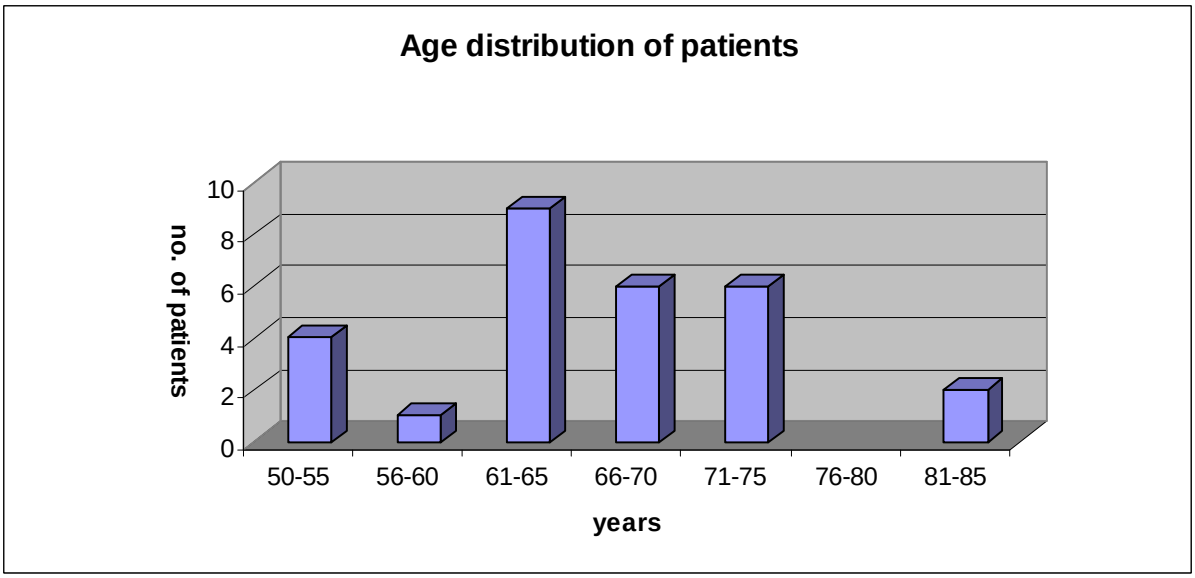


Fig. 2.1 Age distribution of the patients involved in the experiment

	AF group	SR group	P-value
Age (years)	66.7 ± 2.8	65 ± 2.5	ns
Male	6	10	ns
Female	5	7	ns
LVEF	56.3 ± 3.2	53.7 ± 4.3	ns
ACEI	2	5	ns
AT_1-antagonist	1	2	ns

Tab. 2.1 Characteristics of patients with atrial fibrillation (AF group) and sinus rhythm (SR group). Results are expressed as mean ± SEM. P<0.05. ns= not significant.

2.1.2 Solutions

Eprosartan mesylate: $C_{23}H_{24}N_2O_4\ SCH_4O_3S$

Molecular weight: 520.625g/mol

Eprosartan was dissolved in a small quantity of ethanol and Tyrode`s solution to get a stock solution with a concentration of $10^{-6}$ M. the solution was diluted with Tyrode`s solution to get a concentration of 10^{-7} M.

Isoprenaline: $C_{11}H_{17}NO_3HCl$

Molecular weight: 247.7g/mol

Isoprenaline (Sigma-Aldrich) was dissolved in normal Tyrode`s solution to get a stock solution with a final concentration of $10^{-6}$ M. The stock solution contained ascorbic acid (1mg/ml) as an antioxidant. The stock solution was then serially diluted with Tyrode`s solution to get a final isoprenaline concentr ation of 10^{-7} M, 10^{-8} M, and 10^{-9} M.

Tyrode`s solution

Tyrode`s solutions were modified to imitate the physiologic electrolyte concentration in human blood. The composition of the Tyrode`s solutions used is as follows:

Substances	Concentration	Manufacturer
NaCl	128 mMol	Merck, Darmstadt
KCl	2.3 mMol	Merck, Darmstadt
$NaHCO_3$	25 mMol	Merck, Darmstadt
$MgSO_4$	0.6 mMol	Merck, Darmstadt
$CaCl_2$	2.5 mMol	Merck, Darmstadt
KH_2PO_4	1.3(K) mMol	Merck, Darmstadt
Glucose	11.2 mMol	Merck, Darmstadt
Insulin	10 IE	Alt-Insulin, Darmstadt

Tab. 2.4 composition of Tyrode`s solution

Transport solution (cardioplegic BDM solution)

BDM: 2,3-Butane-dione-monoxime. Sigma.

Synonyms: diacetyl monoxime, biacetylmonoxime, diacetylmonoxime;

2,3-butanedionemonoxime.

Molecular weight: 101.11g/mol

Chemical formula: $C_4H_7NO_2$

BDM is a cholinesterase activator. BDM has been used as an inhibitor of myosins as it has been reported to inhibit actomyosin interaction in vitro (McKillop et al., 1994). It has been shown to possess cardioprotective properties related to the inhibition of cross-bridge force development, the reduction of myofilament Ca^{2+} sensitivity, and the attenuation of intracellular Ca^{2+} transients (McKillop et al., 1994).

2.1.3 Equipment for atrial strip preparation

1. Myocardial strip preparation place
2. Stereomicroscope: Olympus, Japan
3. Surgical instruments
4. Carbogen (95% oxygen and 5% carbon dioxide)

2.1.4 Equipment for atrial strip measurements

1. Balance BA 100 (0.00001-0.0001g): Sartorius, Germany
2. “Muscle Research System”, Scientific Instruments GmbH, Heidelberg, Germany
3. Steiert Organ Bad (Hugo Sachs Elektronik)
4. Stereomicroscope : Olympus, Japan
5. Oscilloscope HMEG HM 205, Germany
6. Computer, 166 MHz with AD-Transducer-Card NI
7. Software Labview® 5.1 Version. The New Data Line Company, Berlin

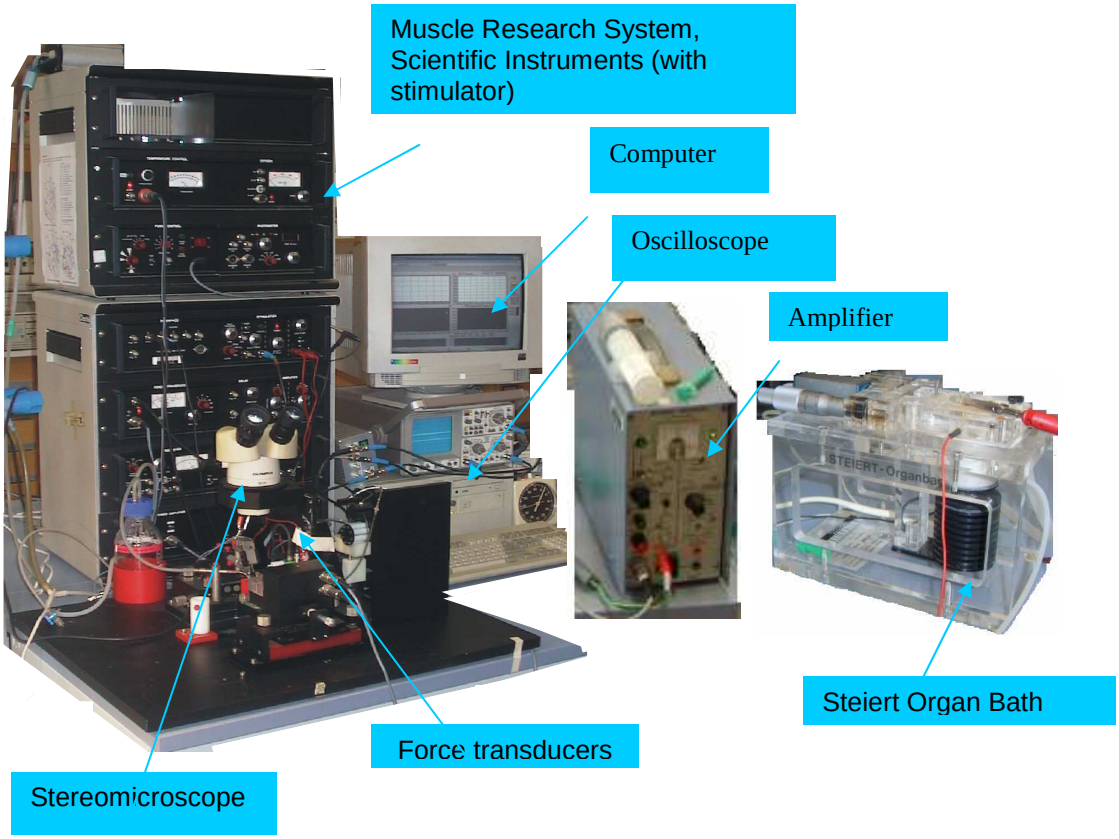


Fig. 2.2 Muscle Research System

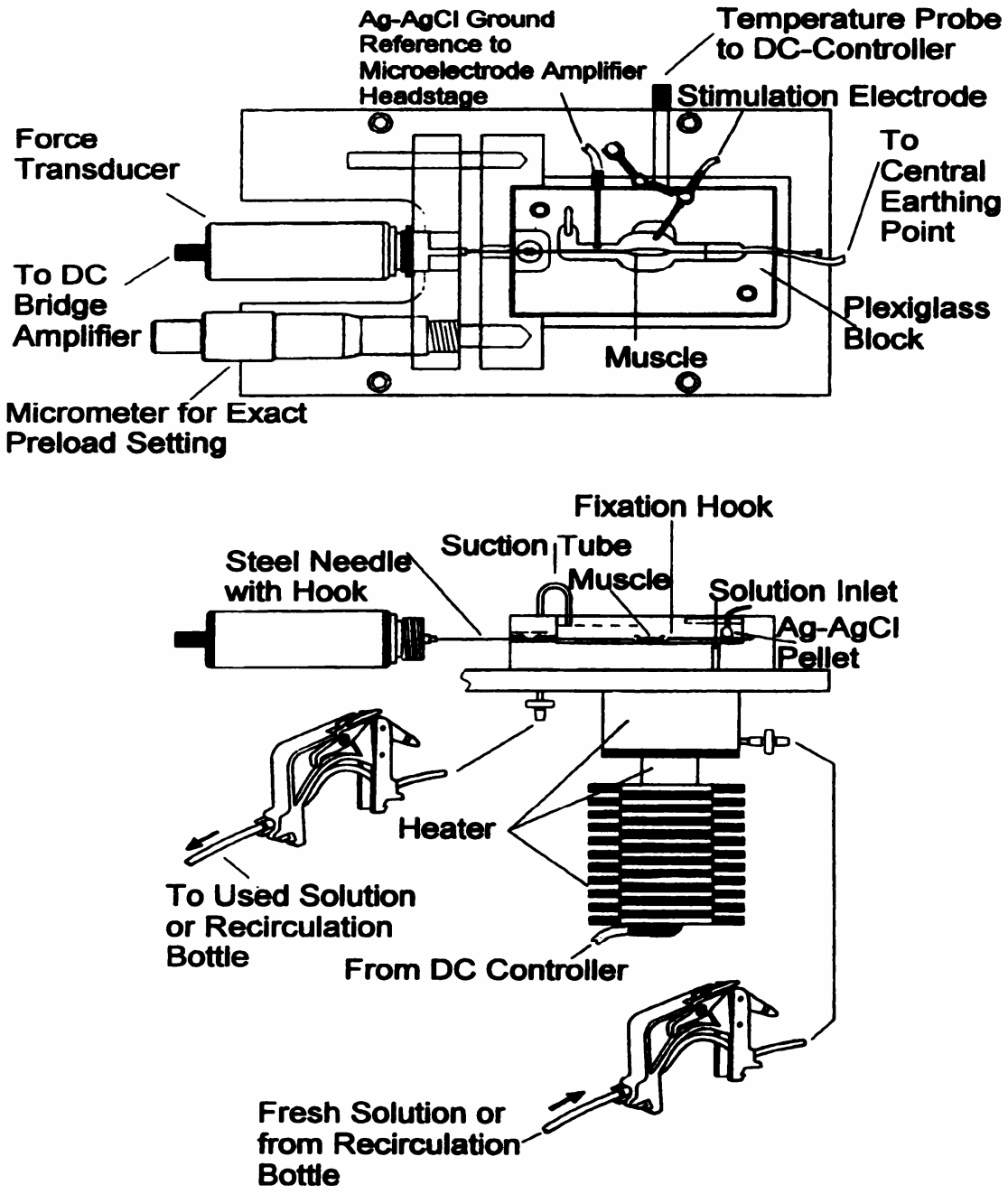


Fig. 2.3 Force measuring system with force transducer, slider for preload adjustment, and bath block with chamber of myocardial preparation. Various parts of apparatus are made mainly from stainless steel and are therefore largely inert and corrosion resistant. View from above (top). Lateral view (bottom). Source: Stefan M et al., the journal of nuclear medicine 2000.

2.2 Methods

2.2.1 Experimental procedure with human atrial strips

Atrial strips from the atrial muscle were carefully observed, and with the help of surgical tools only noninfarcted strips were taken and used for the experiment. The tissues are placed in a Tyrode`s's BDM solution (pH 7.4, gassed with 5% CO_2 and 95% O_2). Thin atrial muscle bundles were prepared parallel to the muscle fibre direction under stereomicroscopic control. The length of the bundles ranged between 6 and 11 mm, and the areas were 3.28 ± 0.31mm^2 (n=17) in SR patients and 3.09 ± 0.37 mm^2 (n=11) in AF patients (p= ns). In the organ bath (5ml capacity) schematically illustrated in Fig. 2.3, one end of the atrial strip was fixed to the chamber with a hanger and the opposite end was connected to a precalibrated force transducer via a stainless steel hook. They were connected to isometric force transducers with silk threads and placed in the organ bath filled with prewarmed (37℃) Tyro de`s solution (pH 7.4, gassed with 5% CO_2 and 95% O_2). After an equilibration period of 30 minutes, the muscles were stretched to a resting tension of 1.0 mN. External field stimulation was performed with rectangular pulses (5 ms, 5% to 10% above threshold) at a frequency of 1 Hz. Resting tension was increased in 0.1-mN steps until the muscle length providing maximal active force generation was reached, i.e. the preparation was gradually lengthened by micromanometric traction up to the apex of its length-tension curve (L_{max}).

An orthorhythmic stimulator stimulated the atrial strips. The chamber received incoming oxygenated fluid at about 5ml/min by a single-headed peristaltic pump. At L_{max}, resting tension zero-line was obtained.

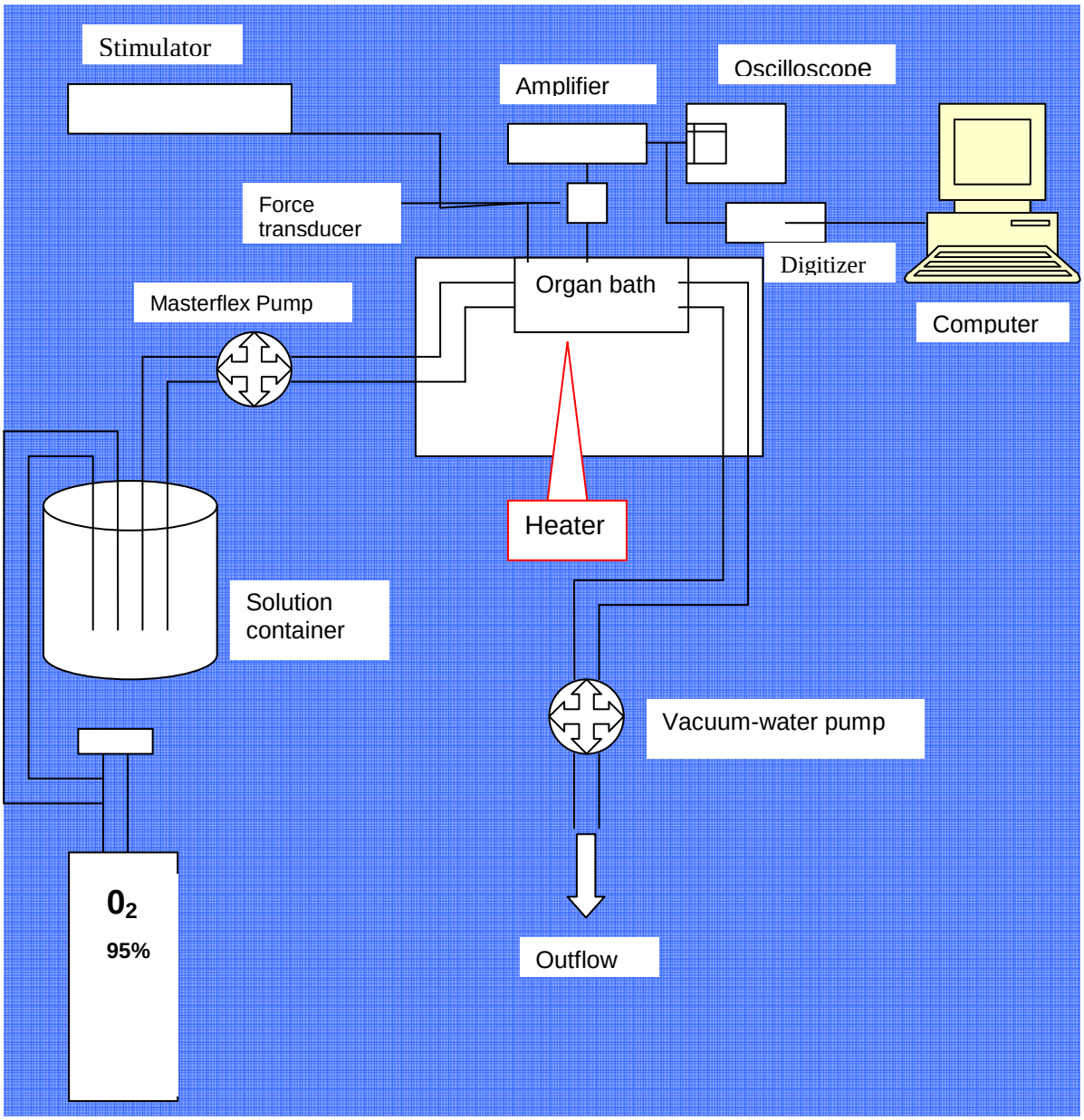


Fig. 2.4 Schematic drawing of the experimental set-up.

After completion of an experiment, the muscle was dried, the base used to fix it to the chamber floor cut off, and the actively contracting portion weighed on a precision balance (Sartorius model BA 110 S, Sartorius AG, Göttingen, Germany).

The length of the piece of atrial strip was obtained by measuring it under a stereomicroscope. The weight was also obtained by measuring it under Balance BA 100 (0.00001-0.0001g) Sartorius, Germany. The formula used to calculate the cross-sectional area (A) as the ratio of blotted muscle weight ($\rho = 1.0 mg/mm^3$) to muscle length (L_{max}) was: $A[mm^2] = weight[mg] / length[mm] \bullet \rho[mg/mm^3]$.

It was made sure that the strips prepared were uniform, i.e. the strips were carefully obtained in such a way that the diameter was almost the same throughout the whole length in order to avoid unwanted contraction and relaxation of the pieces of muscles along the length in order to get a meaningful result.

2.2.2 FFR, isoprenaline, DC shock with and without eprosartan

The atrial strip clamped in an organ bath was constantly supplied with oxygen and Tyrode`s solution or eprosartan or isoprenaline, depending on the stage of the experiment. On the PC the software labview, which displayed and saved the maximum force (F_{max}), effective force of contraction (FOC = F_{max}-F_{min}), maximum contraction velocity (dF/dt_{max}), maximum relaxation velocity (dF/dt_{min}), and the isometric contraction curve, was used. After a stable FOC had been attained and recorded for the first 1 Hz, the same process was repeated for the following frequencies: 2 Hz, 1.5 Hz, 0.75 Hz, 0.5 Hz, 0.33 Hz, and 0.25 Hz. In this work FOC is expressed as force of contraction per unit area (FOC/A).

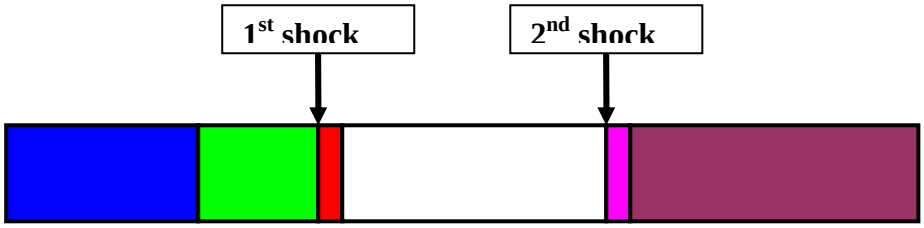
The response of the atrial strips to beta-adrenergic (isoprenaline) was also experimented at 1 Hz at isoprenaline concentrations of 10^{-9}, 10^{-8}, and 10^{-7} M. After the perfusion of isoprenaline, a baseline (1Hz, Tyrode`s solution) FOC/A, $dF/dt_{max}$ and $dF/dt_{min}$ was recorded for the preceding experiment. The atrial strip was than perfused with Tyrode`s solution for 5 minutes to washout isoprenaline.

Electrical injury was then induced on the atrial strip with a DC shock 15 volts for 60 seconds. The time and voltage chosen was acceptable due to results obtained in previous experiments.

The recovery of FOC/A, dF/dt_{max} and dF/dt_{min} was then recorded at the following time periods: 1^{st}, 3^{rd}, 5^{th}, 10^{th}, 15^{th}, 20^{th}, and 25^{th} minute after the DC shock. The maximum stable FOC/A was reached at the 25^{th} minute. This maximum stable FOC/A before the second shock served as the initial FOC/A for the proceeding part of the experiment with eprosartan (10^{-7} M) after the second shock. Previous experiments showed that the effect of second DC shock was more than that of the first shock, which is why we compared the effect of it on the two groups (SR and AF) in other to rule out precondition.

After perfusion of the atrial strips with eprosartan solution the recovery of all the above mentioned parameters were recorded.

The rate of recovery and the maximum attained FOC/A for the experiments before and after the “electrical injury with and without eprosartan were calculated and compared. Fig.2.4. shows schematically the various steps.



Step 1: Frequency stimulation (0.25 - 2 Hz)

Step 2: Inotropic stimulation (isoprenaline)

Step 3: First shock (15 volts for 60 sec)

Step 4: Perfusion with Tyrode`s solution after shock

Step 5: Second shock (15 volts for 60 sec)

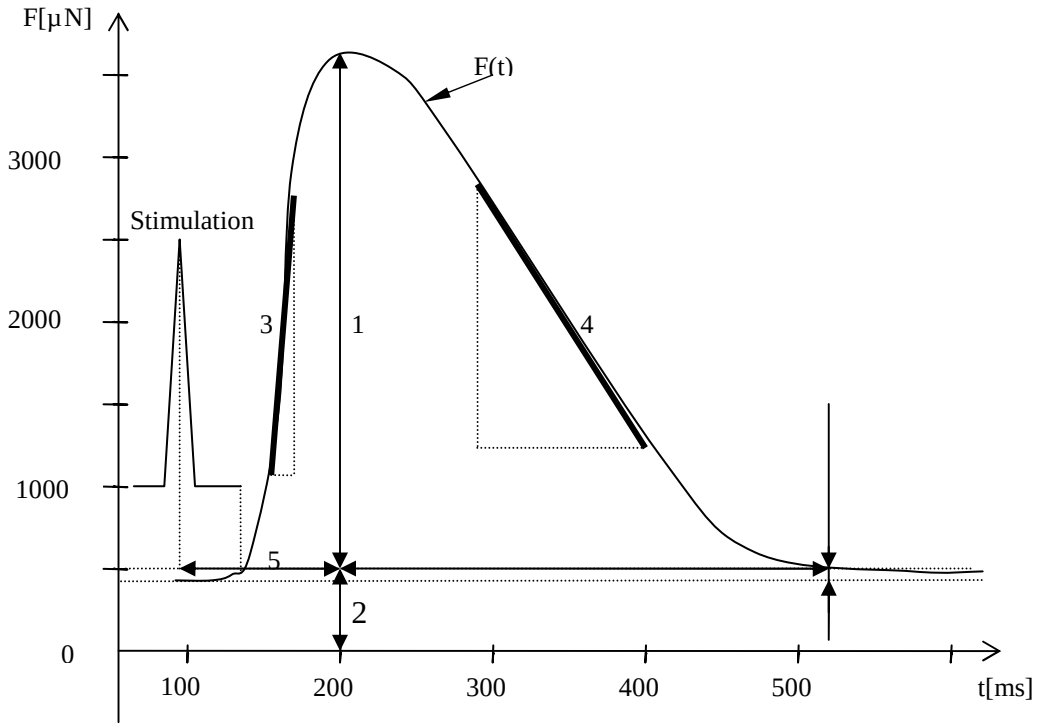
Step 6: Perfusion with Tyrode`s solution und eprosartan after shock

Fig. 2.5 Schema showing the various steps involved in the experimental process.

2.3 Contraction and relaxation curve shape

In this experiment, the atrial force of contraction was under the control of isometric contraction. Force is expressed as active stress, i.e., active force per unit cross-sectional area (mN/mm^2).The parameters of contraction and relaxation of the atrial strips were evaluated by a 2-component model. This isometric contraction and relaxation curve is shown in the standard schematic diagram in Fig. 2.6.

Isometric contraction was characterised by nearly a constant length of the muscle when tension was increased. The shape of isometric contraction and relaxation curve was evaluated by different contractive parameters that were obtained from measurement during experiments. The PC delivers more data, but because of clarity, only the following parameters in isometric contraction and relaxation were used: The effective force which is equal to maximum developed force (F_{max}) minus minimum developed force (F_{min}); maximal contraction velocity (dF/dt_{max}), time from stimulus to maximal developed force (TPT) and maximal relaxation velocity (dF/dt_{min}).



Parameter:

1) FOC (µN): Effective force of contraction (F_{max}-F_{min})

2) F_{min} (mN): Minimal developed force in relaxation

F_{max} (mN): Maximal developed force in contraction

3) dF/dt_{max} (mN/s): Rate of force development

4) dF/dt_{min} (mN/s): Rate of force relaxation

5) TPT (ms): Time to peak tension: time from stimulation to F_{max}

Fig. 2.6 The schematic of isometric contraction curve

2.4 Criticism of the method

Experimental conditions were to be kept constant in order to minimize changes in results caused by external factors such as temperature and oxygen supply. However such external factors could not be ruled out completely due to differences in the collection of the organ and the preparation for the experiment, so the preparation could not be totally standardised.

First of all, the muscle tissues should not have been kept in BDM solution in the refrigerator for more than 6 hours but at times this time limit was exceeded.

High intensity electrical fields generated near the shock electrodes can cause tissue injury and dysfunction.

The reduced mechanical function as a result of the DC shock may not be the same as that of atrial stunning that occurs as a result of cardioversion in vivo since the DC shocks were applied directly to the atrial strips.

The premedication of the patients before the operation makes the influence of eprosartan on the various atrial strips questionable. Also since eprosartan influences the function of other organs the dosage we used can be questioned.

Although most of the results obtained showed meaningful differences between the AF and the SR group, due to the small sample size, the statistical interpretation of the results made most of them insignificant.

The contraction parameters of the myocardium in vivo are results of complex interactions of myocardial and hemodynamic factors.

The investigation of inotropic effect of a medicine on a person in vivo is possible, however the judgment is difficult. Under clinical conditions, stroke volume, left ventricle ejection fraction, HZV, EF and dP/dt_{max} can be measured reliably as a parameter for the contractile function. However all of these parameters depend on the pre- and overload, that again could be affected through secondary changes of the inotropic and direct changes of the vessel tonus (Feldmann et al., 1988; Hasenfuss et al., 1994)

The in vitro experimentation on human atrial strips is more convincing than that of the use of animal tissue since there are some physiological and anatomical differences between animals and human beings.

2.5 Statistical analysis

SPSS 11 for Windows was used for statistical analysis. The statistical analysis included descriptive statistics (frequency and percentage of categorical variables and mean and standard deviation of continuous variables). Pearson's χ^2 with Fisher's exact test for categorical variables and the Mann-Whitney test for continuous variables for intergroup comparisons were performed to confirm significance. The significance tests were two-sided. Data are expressed as mean ± SEM, 95 % confidence intervals are given. A value $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 FFR and adrenergic effects in patients with atrial fibrillation and sinus rhythm

3.1.1 FFR in SR and AF

As shown in Figs.3.1 and 3.2, the FOC/A values of the SR group were slightly higher those that of the AF group at almost all stimulation frequencies. A decrease of the stimulating frequency from 2Hz to 0.25Hz caused an increase in the FOC/A from 4.27 ± 0.69 mN/mm2 to 11.41 ± 1.86 mN/mm^2 in the SR group and from 4.41 ± 0.79 mN/mm^2 to 9.75 ± 1.61 mN/mm^2 in the AF group. There was no significant difference between the two groups.

The boxplot, Fig.3.2 represents the force-frequency relationship, i.e. the FOC/A when the maximum amount of Ca^{2+} is released. In this work the maximum FOC/A during the frequency stimulation was reached in all samples. There was no significant differences between the FOC/A of the AF and the SR group. The 50th percentile was 11.41 ± 1.86 mN/mm^2 for the SR group and 9.75 ± 1.61 mN/mm^2 AF group.

Fig.3.3 shows the percentage changes, i.e. the change in FOC/A at different frequencies as compared to the FOC/A at basal conditions (37℃, 1 Hz, and Tyrode`s solution). There was insignificant difference between the groups during the frequency stimulation. Frequencies (2 and 1.5Hz) higher than the basal frequency (1 Hz) proved to have a lesser FOC/A than that of lower frequencies (0.25-0.75Hz). At 0.25Hz the maximum FOC/A of 178.76 ± 7.88 mN/mm^2 and 174.89 ± 12.26 mN/mm^2 was attained by the SR and AF, respectively.

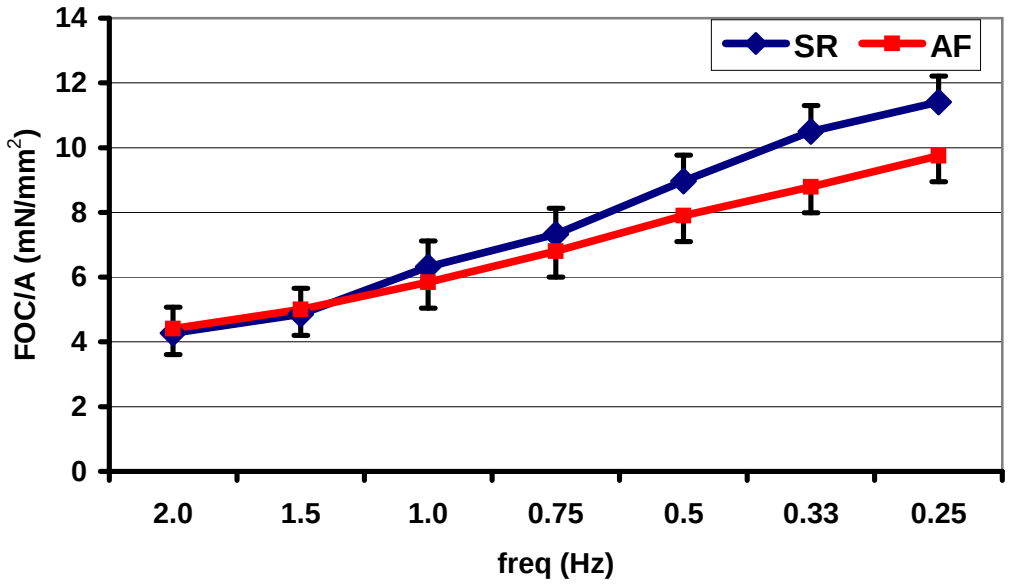


Fig. 3.1 Diagram of FOC/A of SR and AF plotted as a function of frequency of stimulation (2 - 0.25 Hz).

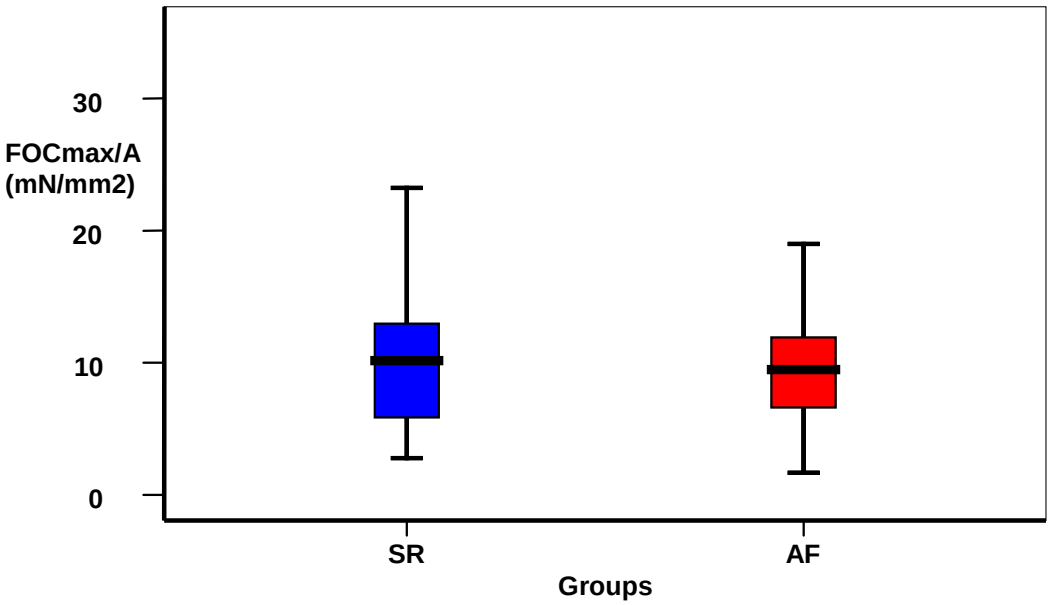


Fig. 3.2 Boxplot showing the force-frequency relationship (FFR) of SR and AF group after frequency stimulation with 0.25 Hz.

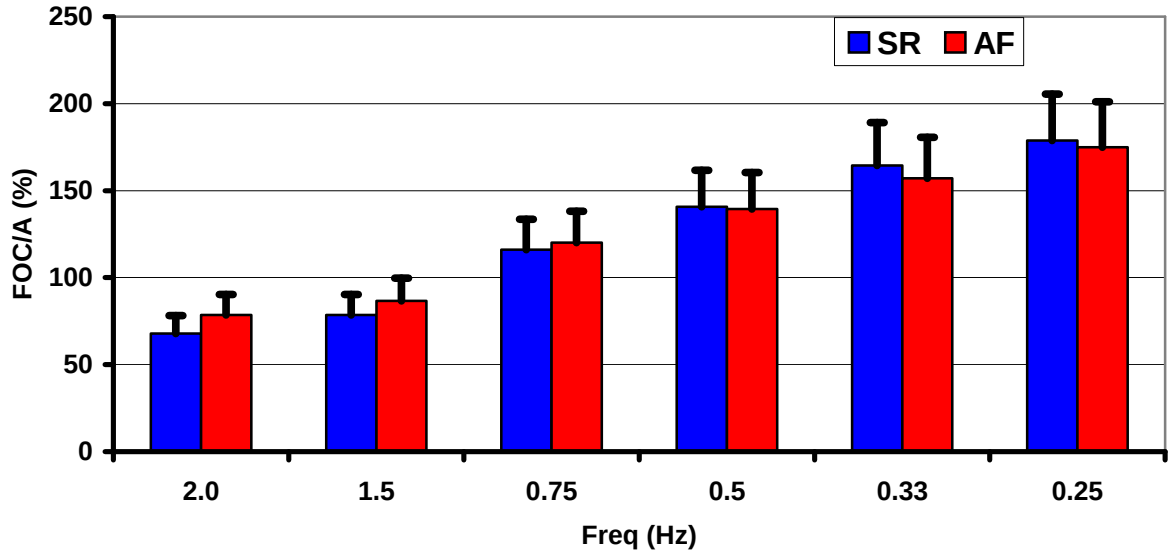


Fig. 3.3 FOC/A (%) of SR and AF at different stimulating frequencies (2 Hz - 0.25 Hz). The FOC/A at the various frequencies were compared to the baseline FOC/A (37℃, 1 Hz, and Tyrode`s solution) to get the FOC/A (%).

3.1.2 Inotropic effect of isoprenaline on FOC/A

Both groups responded positively to 10^{-9} M isoprenaline at 1Hz. After reaching a maximum of 10.34 ± 1,44mN/mm^2 (SR) and 9.34 ± 1.2 mN/mm^2 (AF) at 10^{-9}M, which was almost double the FOC/A at the baseline (37℃, 1 Hz, and Tyrode`s solution), an increase in the isoprenaline concentration from 10^{-9}M to 10^{-8}M and 10^{-7}M caused a slight decrease FOC/A in both the SR and AF groups as can be seen in the diagrams below.

There was no significant difference ($p>0.05$) between the FOC/A (%) of the SR and the AF samples perfused with an isoprenaline concentration of 10^{-9}M and the baseline FOC.

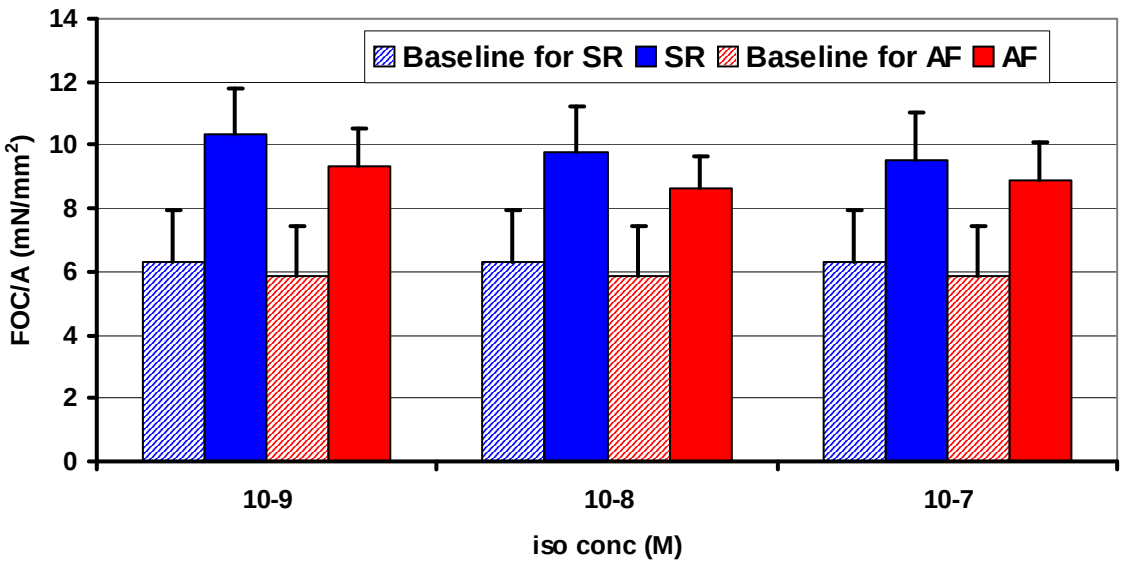


Fig. 3.4 A diagram of FOC/A showing the adrenergic response of the SR and AF after inotropic stimulation with isoprenaline (10^{-9} M, 10^{-8} M, 10^{-7} M) as compared to the baseline FOC/A (37℃, 1 Hz, and Tyrode`s solution).

3.2 Comparison of FOC/A (%) before and after DC shock in AF and SR with and without eprosartan perfusion.

3.2.1 Comparison of FOC/A (%) after DC shock in AF and SR without eprosartan perfusion.

Whilst after the 60 seconds of DC shock the FOC/A of the atrial strips with SR was decreased from its baseline FOC/A of 9.41 ± 1.39 mN/mm^2 to 1.71 ± 0.46 mN/mm^2 (about 15.58 ± 2.26% of its baseline FOC/A), the FOC/A of the AF muscle after the shock were 1.87 ± 0.52 mN/mm^2 (about 20.70 ± 4.03% of the baseline FOC/A, 8.67 ± 1.17 mN/mm^2). The FOC/A of the untreated muscles with AF and SR increased steadily at almost the same pace. There was however no significant differenced between the two groups. At the 25th minute the muscles with AF had attained about 72.02 ± 9.04% of the baseline FOC/A and the atrial strips with SR reached 67.62 ± 2.74% of its baseline FOC/A. The maximum FOC/A attained in the AF group was 6.06 ± 0.96 mN/mm^2 and that of the SR group was 6.34 ± 0.98 mN/mm^2. It can be seen from the graph that there

was a close match between the two after they had been subjected to different conditions. Here again there was no significant difference between the two groups.

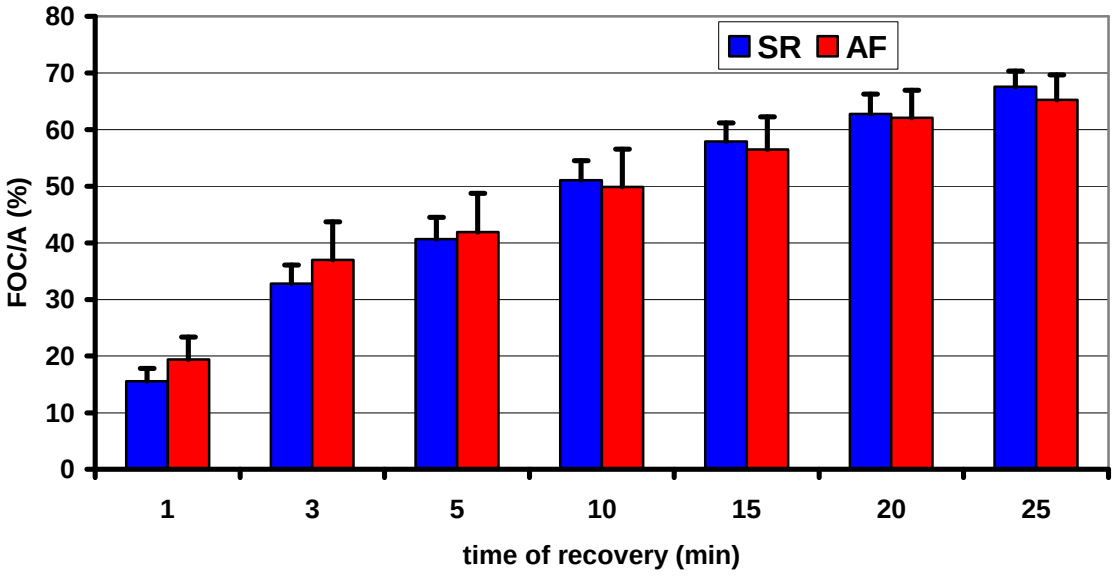


Fig. 3.5 Recovery of FOC/A (%) of untreated AF and SR atrial strips after DC shock (15 volts for 60 s). FOC/A is expressed as percentage of the baseline FOC/A (37℃, 1 Hz, and Tyrode`s solution)

3.2.2 Comparison of FOC/A (%) in AF and SR after DC shock with eprosartan perfusion

When the atrial myocardial strips with AF and SR were treated with the AT_1 antagonist eprosartan it was found that there was a significant difference in the FOC/A (%) after the muscles were stunned ($p<0.05$). The FOC/A of the muscles with AF dropped from the baseline FOC/A of 6,06 ± 0.96 mN/mm^2 to 1.80 ± 0.58 mN/mm^2, (i.e. about 81 ± 3.98% of the baseline FOC/A) after the muscles were stunned with 15 volts for 60 seconds whilst the muscles with SR attained just 1.26 ± 0.24 mN/mm^2, (18 ± 3.66% of its baseline FOC/A (6.34 ± 0.98 mN/mm^2)). The recovery of FOC/A of both groups increased steadily. A significant difference ($P<0.05$) was seen after the 3rd minute. At this time the FOC/A (%) of the control group was 31.06 ± 3.90% and that of the AF group was 52.29 ± 5.78%*. The FOC then increased gradually and significantly

(p<0.05), reaching its maximum and stable state at the 25th minute. At the 10th minute there was a significant difference of p<0.01. At the 25th minute a maximum value of 5.89 ± 1.18 mN/mm^2 was reached, that makes about 91.49 ± 5.56 (%)* of the initial FOC/A of the AF group before the muscles were stunned and a value of 5.18 ± 0.86 mN/mm^2, i.e. about 79.70 ± 3.14% was attained after the 25th minute by the SR group.

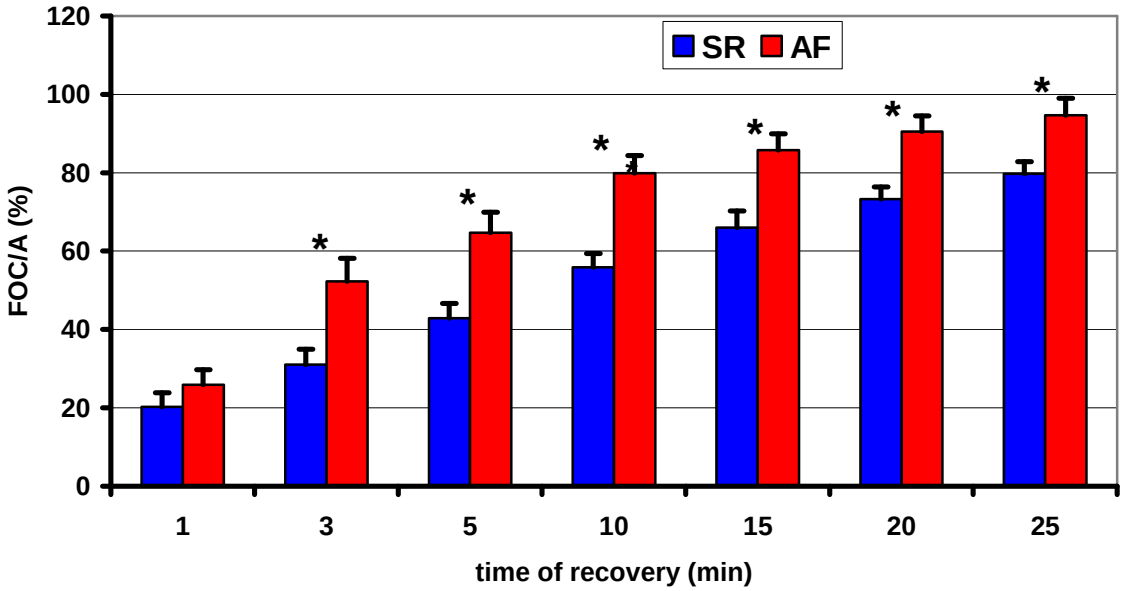


Fig. 3.6 Effect of eprosartan on the recovery of FOC/A after DC shock (15volts, 60sec) in AF and SR. FOC/A is expressed as percentage of the baseline FOC/A (FOC/A before DC shock at 37℃, 1 Hz, and Tyrode`s solution) *P < 0.05.

3.3 Comparison of FOC/A (%) of AF with and without eprosartan perfusion after DC shock

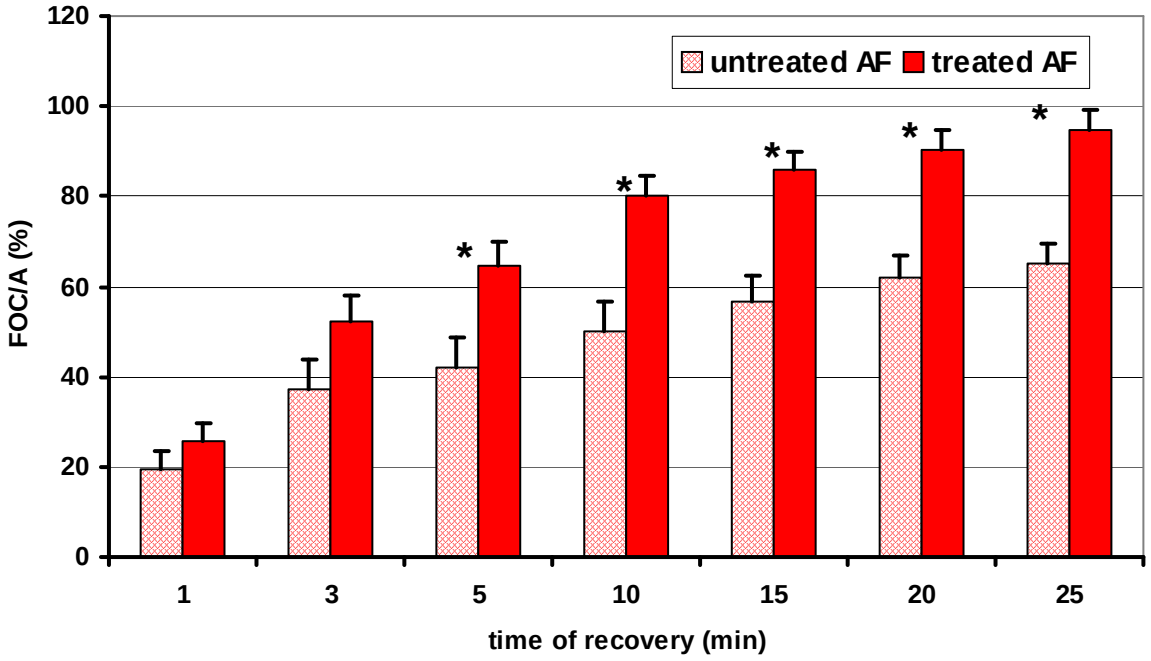


Fig. 3.7 Effect of eprosartan on the recovery of FOC/A after DC shock (15volts, 60sec) in treated and untreated AF. FOC/A is expressed as percentage of the baseline FOC/A (FOC/A before DC shock at 37℃, 1 Hz, and Tyrode`s solution) *P<0.05.

The diagram (Fig.3.7) above shows a comparison of recovery rate of the treated and untreated atrial strips with AF after DC shock. After the shock, the FOC/A of both groups decreased to about 20% of the baseline FOC/A. Immediately after the stunning, i.e. at 1st minute, there were no significant differences in FOC/A between the treated and the untreated samples. While immediately after the stunning the FOC/A of the untreated AF dropped to 20.18 ± 3.66%, FOC/A of the treated group was 25.81 ± 3.98%, i.e. about 22% greater than that of the untreated AF group**.** During the perfusion of eprosartan and the perfusion of Tyrode`s solution at the 3rd minute there were also no significant differences between the two. It was after the 5th minute that a significant difference ($p<0.05$) between the two groups occurred. While at the 5th minute the untreated samples had a value of 47.68 ± 10.34(%), and the treated samples had a

percentage of 64.76 ± 5.21(%)* of the baseline FOC/A. In the two groups, the FOC/A increased steadily reaching a maximum and stable FOC/A at the 25th minute. The maximum at this point as compared to the baseline FOC/A was 91.49 ± 5.56(%) for the samples perfused with eprosartan and 72.02 ± 9.04% for the nonperfused samples.

3.4 Comparison of FOC/A (%) of SR with and without eprosartan perfusion after DC shock

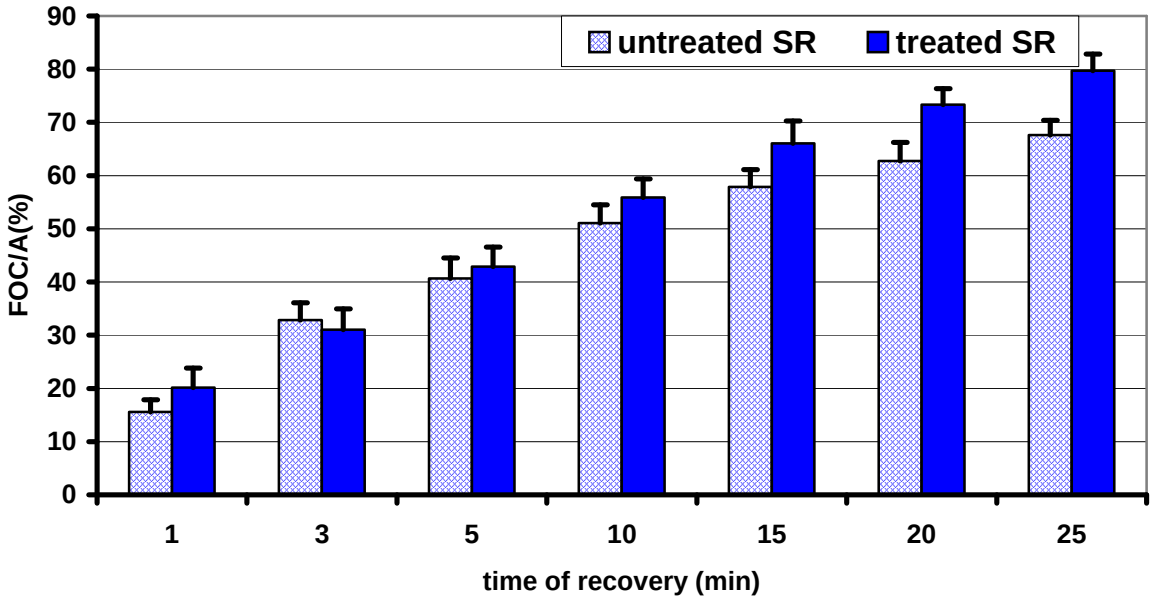


Fig. 3.8 Effect of eprosartan on the recovery of FOC/A after DC shock (15 volts, 60 sec) in treated and untreated SR muscles. FOC/A is expressed as percentage of the baseline FOC/A (FOC/A before DC shock at 37℃, 1 Hz, and Tyrode`s solution).

In Fig.3.8, the effect of eprosartan on the SR group was not as great as in the case of the AF group. FOC/A had decreased significantly during shocking in comparison to the baseline FOC/A (P<0.05). At the first minute, the FOC/A of the SR was just a little under 20%.The contraction force increased with time but there were no significant differences (p<0,05) as can be seen in the diagram above. The highest value of FOC/A (%) was 67.62 ± 2.74% at 25th minute, the lowest value was 15.58 ± 2.26% at 1st minute before the muscles were perfused with eprosartan; for the treated samples, the highest value

was 79.70 ± 3.14% at 25th minute, the lowest value of FOC(%) was 20.18 ± 3.66% at the 1st minute.

Characteristics	**SR group**	**P-value**	**AF group**
Number of samples	17	ns	11
Average area of atrial strips	3.28 ± 0.31	ns	3.09 ± 0.37
FOC/A at 2 Hz	4.27 ± 0.69	ns	4.41 ± 0.79
FOC/a at 1.5 Hz	4.86 ± 2.97	ns	5.00 ± 0.92
FOC/A at 1 Hz	6.32 ± 0.72	ns	5.84 ± 1.10
FOC/A at 0.75 Hz	7.33 ± 1.11	ns	6.80 ± 1.18
FOC/A at 0.5 Hz	8.96 ± 1.46	ns	7.90 ± 1.42
FOC/A at 0.33 Hz	10.50 ± 1.71	ns	8.79 ± 1.51
FOC/A at 0.25 Hz	11.41 ± 1.86	ns	9.75 ± 1.61
FOC/A (%) at 2 Hz	67.91 ± 3.57	ns	78.52 ± 1.61
FOC/A (%) at 1.5 Hz	78.60 ± 2.26	ns	86.69 ± 3.39
FOC/A (%) at 0.75 Hz	116.09 ± 3.52	ns	120.19 ± 4.34
FOC/A (%) at 0.5 Hz	140.67 ± 5.23	ns	139.42 ± 7.03
FOC/A (%) 0.33 Hz	164.44 ± 7.48	ns	157.04 ± 9.88
FOC/A (%) 0.25 Hz	178.76 ± 7.88	ns	174.89 ± 12.25
FOC/A at iso conc. of 10^{-9}M	10.34 ± 1.44	ns	9.34 ± 1.20
FOCA at iso conc. of 10^{-8}M	9.77 ± 1.45	ns	8.64 ± 1.03
FOC/A at iso conc. of 10^{-7}M	9.54 ± 1.51	ns	8.92 ± 1.20
FOC/A (%) at iso conc. of 10^{-9}M	115 ± 4.37	ns	113.4 ± 11,44
FOC/A (%) at iso conc. of 10^{-8}M	106.1 ± 2.91	ns	105.2 ± 9,94
FOC/A (%) at iso conc. of 10^{-7}M	102.3 ± 2.43	ns	105.3 ± 9.03

Tab. 3.1 Table showing FOC/A and percentage change of the FOC/A (FOC/A%) of the AF (n = 11) and SR (n = 17) after they have been subjected to different frequency and inotropic (isoprenaline) stimulation. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

Characteristics	SR group	P-value	AF group
FOC/A-baseline	9.41 ± 1.39	ns	8.67 ± 1.17
FOC/A at 1 min	1.71 ± 0.46	ns	1.87 ± 0.52
FOC/A at 3 min	349 ± 0.70	ns	3.58 ± 0.88
FOC/A at 5 min	4.14 ± 0.77	ns	4.04 ± 0.93
FOC/A at 10 min	4.80 ± 0.78	ns	4.65 ± 0.95
FOC/A at 15 min	5.36 ± 0.82	ns	5.19 ± 0.92
FOC/A at 20 min	5.88 ± 0.93	ns	5.73 ± 0.93
FOC/A at 25 min	6.34 ± 0.96	ns	6.06 ± 0.96
FOC/A (%) at 1 min	15.58 ± 2.26	ns	20.70 ± 4.02
FOC/A (%) at 3 min	32.84 ± 3.25	ns	43.02 ± 10.82
FOC/A (%) at 5 min	40.67 ± 3.83	ns	47.68 ± 10.35
FOC/A (%) at 10 min	51.07 ± 3.44	ns	56.24 ± 10.55
FOC/A (%) at 15 min	57.90 ± 3.25	ns	63.15 ± 10.10
FOC/A (%) at 20 min	62.74 ± 3.51	ns	68.84 ± 9.32
FOC/A (%) at 25 min	67.62 ± 2.74	ns	72.02 ± 9.03

Tab. 3.2 Table showing FOC/A and FOC/A% of the AF (n = 11) and SR groups (n = 17) after DC shock without eprosartan solution. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

Characteristics	SR group	P-value	AF group
FOC/A-baseline	6.34 ± 0.98	ns	6.06 ± 0.96
FOC/A at 1 min	1.26 ± 0.25	ns	1.80 ± 0.58
FOC/a at 3 min	2.06 ± 0.39	*	3.58 ± 0.84
FOC/A at 5 min	2.92 ± 0.53	*	4.29 ± 0.92
FOC/A at 10 min	3.70 ± 0.66	*	5.11 ± 1.00
FOC/A at 15 min	4.35 ± 0.80	*	5.44 ± 1.05
FOC/A at 20 min	4.79 ± 0.83	*	5.64 ± 1.15
FOC/A at 25 min	5.18 ± 0.88	*	5.89 ± 1.18
FOC/A (%) at 1 min	20.18 ± 3.66	ns	25.81 ± 3.98
FOC/A (%) at 3 min	31.06 ± 3.89	ns	52.29 ± 5.79
FOC/A (%) at 5 min	42.9 ± 3.69	ns	64.76 ± 5.20
FOC/A (%) at 10 min	55.86 ± 3.49	ns	79.93 ± 4.43
FOC/A (%) at 15 min	66.047 ± 4.25	ns	85.75 ± 4.16
FOC/A (%) at 20 min	73.31 ± 3.05	ns	87.29 ± 5.46
FOC/A (%) at 25 min	79.70 ± 3.14	ns	91.49 ± 5.55

Tab. 3.3 Table showing the FOC/A and FOC/A (%) of AF (n = 11) and SR (n = 17) after DC shock and perfusion with eprosartan solution. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

3.5 Maximum contraction velocity (dF/dt_{max}) and maximum relaxation velocity (dF/dt_{min}) in SR and AF

3.5.1 dF/dt_{max} and dF/dt_{min} of AF and SR after frequency and inotropic stimulation with isoprenaline

Fig.3.9 to 3.12 shows the dependency of the maximum contraction velocity (dF/dt_{max}) and maximum relaxation velocity (dF/dt_{min}) on frequency of stimulation and isoprenaline concentration. As can be seen in the diagram, though there were no significant differences between the two groups, the SR group attained a higher dF/dt_{min} during frequency stimulation and inotropic stimulation using isoprenaline at different concentrations (10^{-9} M, 10^{-8} M, and 10^{-7} M). The highest value of dF/dt_{max} during frequency stimulation was reached in both groups at 0.25Hz. The SR group reached a

value of 534.68 ± 75.12 mN/s and the AF group attained a maximum of 398.22 ± 64.31 mN/s at the said frequency. The highest value at the inotropic stimulation was 527.46 ± 53.87 mN/s and 460.58 ± 48.82 mN/s at isoprenaline concentration of 10^{-9}M in the SR and AF groups, respectively. An increase in the concentration of isoprenaline from 10^{-9} M to 10^{-8}M and 10^{-7}M caused a slight decrease in the dF/dt_{max} in both groups. The magnitude of dF/dt_{max} in the SR and AF groups was about 1.5 times higher than the dF/dt_{min} in both groups. The SR group showed dominance in dF/dt_{min} at all frequencies (2Hz-0.25Hz) just like in the dF/dt_{max}. The minimum values were attained at 2Hz and the maximum at 0.25Hz in both groups. The minimum Value was -141.88 ± 13.40 mN/s for the SR and –127.02 ± 14.40 mN/s for the AF group. During the inotropic stimulation using isoprenaline, an increase in concentration caused a slight decrease in dF/dt_{min}. While the dF/dt_{max} at 0.25Hz (534.68 ± 75.12 mN/s) was higher than that of isoprenaline concentration of 10^{-9}M (527.46 ± 53.87 mN/s), the dF/dt_{min} at isoprenaline concentration of 10^{-9}M was higher than at 0.25Hz.

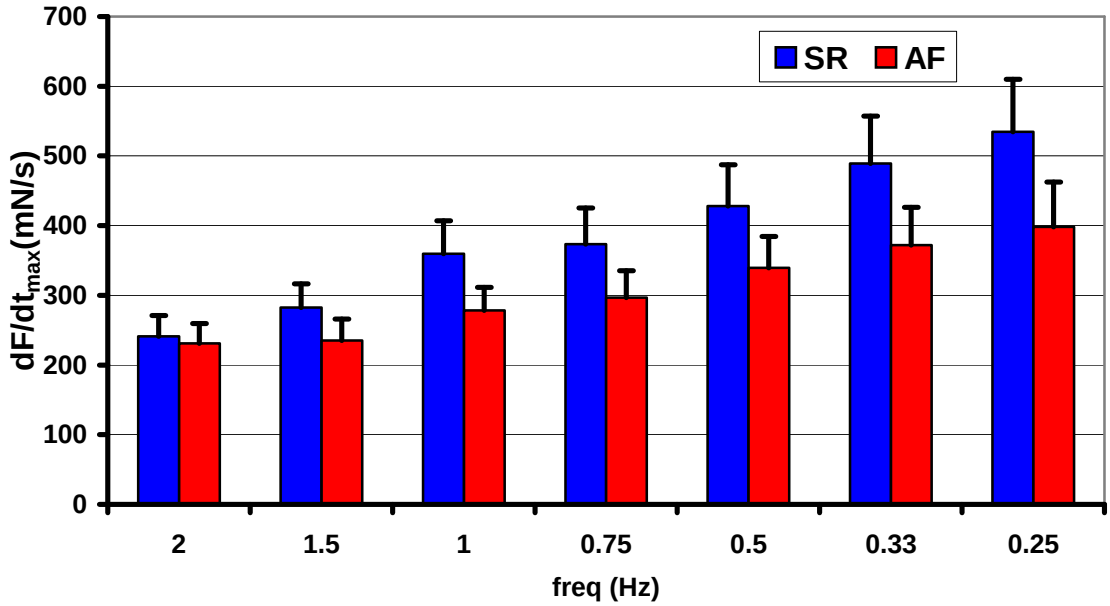


Fig. 3.9 Diagram of dF/dt_{max} plotted as a function of frequency of stimulation (0.25 - 2 Hz) under baseline conditions (37℃, 1 Hz, and Tyrode`s solution).

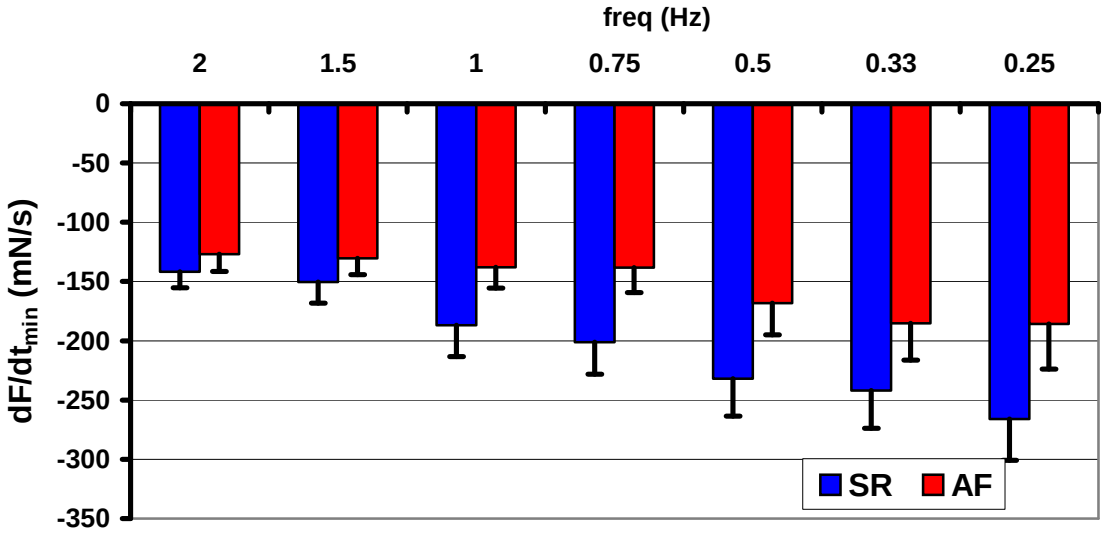


Fig. 3.10 Diagram of dF/dt_{min} plotted as a function of frequency of stimulation (0.25 - 2 Hz) under baseline conditions.

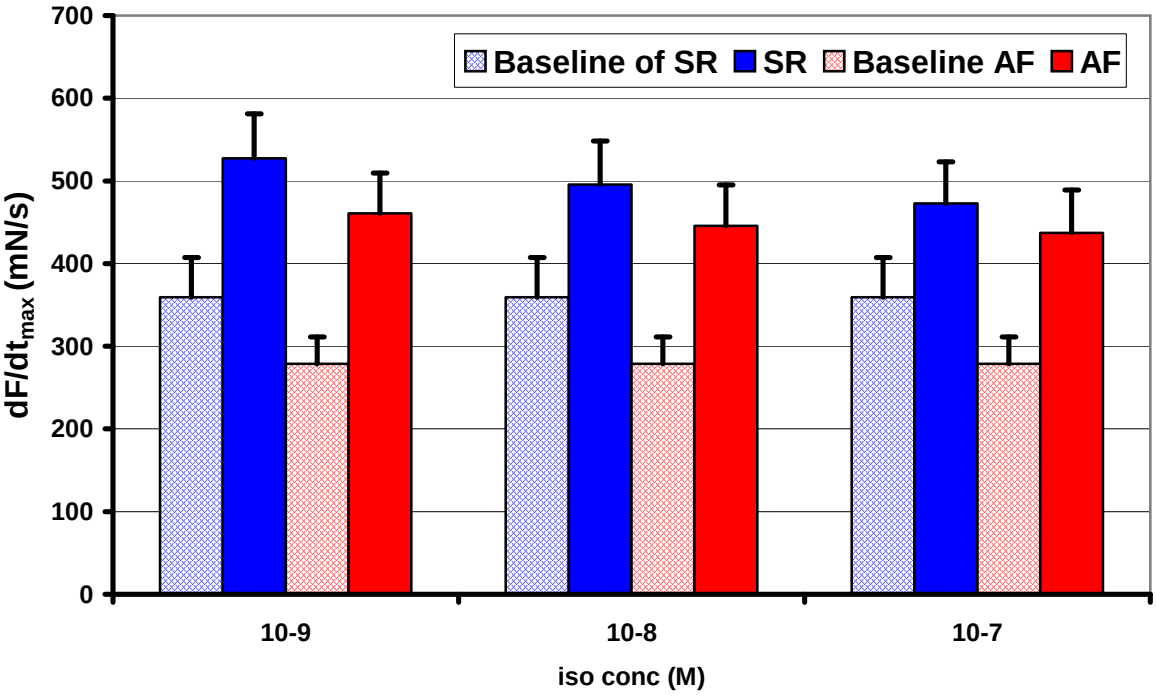


Fig. 3.11 A diagram of dF/dt_{max} (%) showing the adrenergic response of the SR and AF after inotropic stimulation with isoprenaline (10^{-9} M, 10^{-8} M, 10^{-7} M) as compared to the baseline dF/dt_{max}.

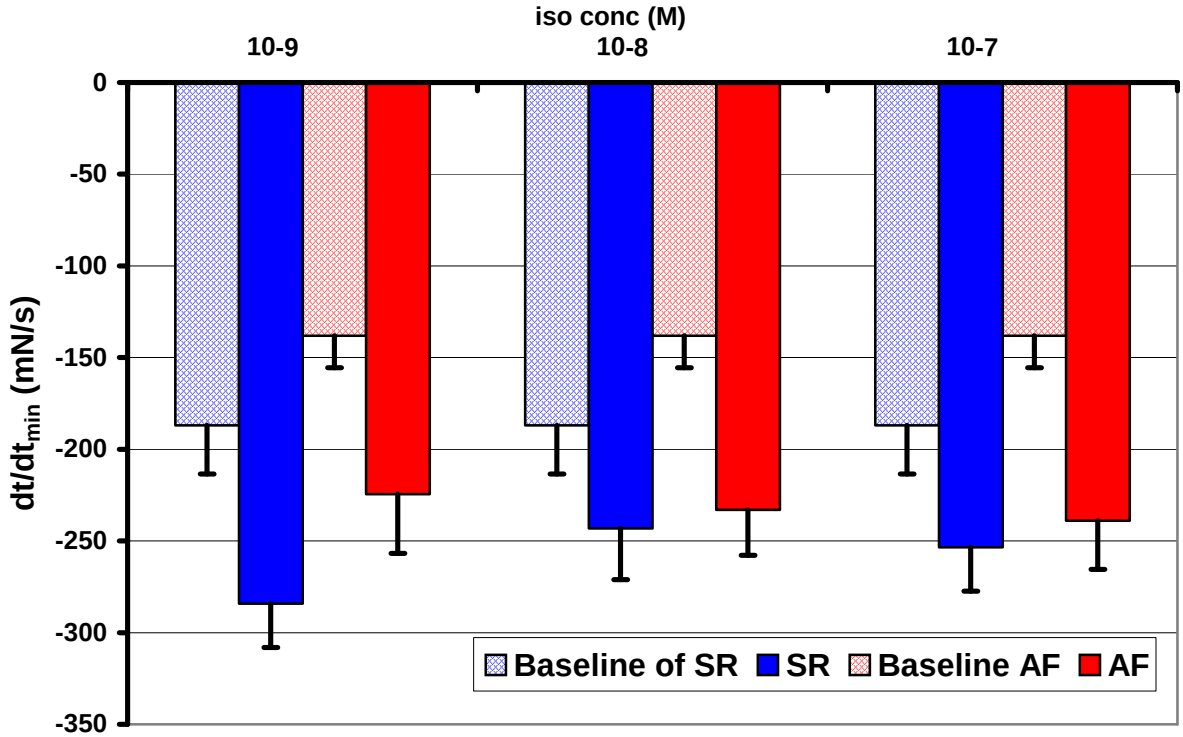


Fig. 3.12 A diagram of dF/dt_{min} showing the adrenergic response of SR and AF after inotropic stimulation with isoprenaline (10^{-9} M, 10^{-8} M, 10^{-7} M) as compared to the baseline dF/dt_{min}.

3.5.2 dF/dt_{max} and dF/dt_{min} in AF with and without eprosartan perfusion after DC shock

It can be seen from fig.3.13 and fig.3.15 that there was no dominance from any group and also no significant differences between the perfused AF samples and nonperfused samples in either dF/dt_{max} or dF/dt_{min}. From the 1st to the 3rd minute after the shock it was the untreated AF samples that dominated in dF/dt_{max} and from 5th to 15th minute the treated AF samples dominated in dF/dt_{max} value. From the 20th to the 25th minute the untreated AF then took over the SR group in dF/dt_{max} again. At 1st minute the dF/dt_{max} were 154.95 ± 30.14 mN/s and 99.6 ± 14.60 mN/s for nonperfused and perfused AF samples respectively. The maximum values were 304.66 ± 47.39 mN/s for the perfused and 314.04 ± 39.84 mN/s for the nonperfused muscles at the 25th minute.

The dF/dt_{min} for the AF samples perfused and nonperfused with eprosartan at 1st minute were -87.71 ± 13.12 mN/s and -91.31 ± 9.79 mN/s, respectively. There were no significant differences between the two groups. The average dF/dt_{min} at different measuring times showed a change between the two groups. The maximum and stable dF/dt_{max} was reached at the 25th minute. The value for the AF treated with eprosartan was -187.46 ± 27.29 mN/s and that of the untreated AF samples was -190.44 ± 17.34 mN/s. The FFR was negative in all stages.

The values of dF/dt_{max} (%) and dF/dt_{min} (%) as can be seen in fig.3.14 and fig.3.16 of the treated and untreated AF showed some significant differences ($p<0.05$). The minimum value was in the first minute after the shock in the two groups. The recovery rate of the dF/dt_{max} was the same in the 1st minute. From the 3rd minute to the 25th minute there was a significant difference between the two with reference the baseline dF/dt_{max} (%). The value of at the 3rd minute was 62.73 ± 7.05 (%) * for treated AF whilst the untreated AF reached 48.62 ± 9.43 (%). The recovery rate increased steadily in both groups but the recovery rate of the treated AF was significantly faster than that of the untreated AF. At the 25th minute, the value of dF/dt_{max} (%) for the treated AF was 97.01 ± 6.96 (%)* and that of the untreated AF 74.95 ± 9.50 (%).

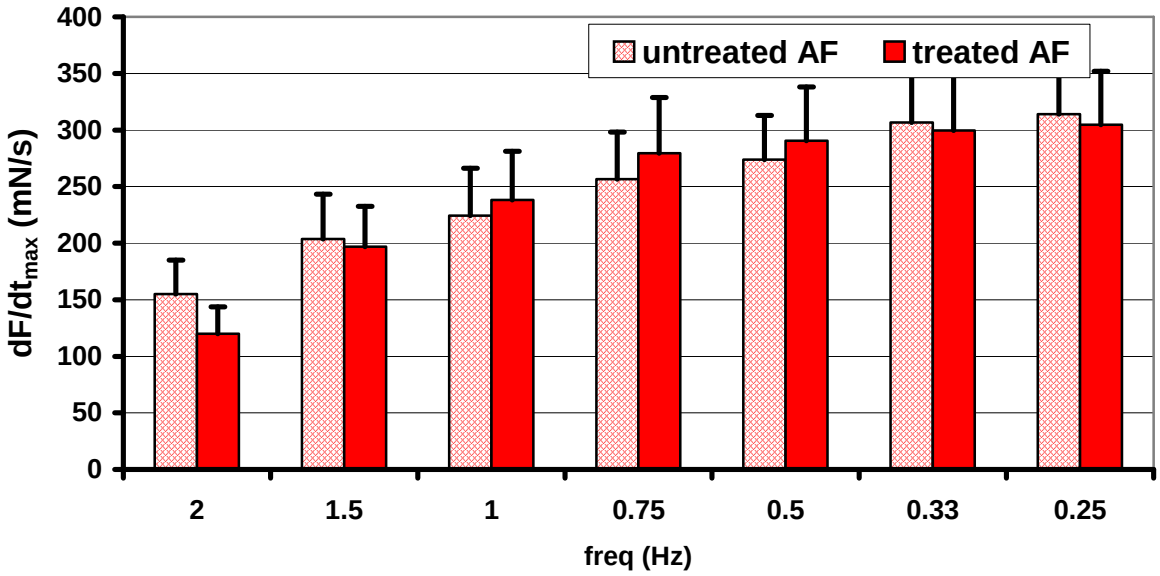


Fig. 3.13 Diagram of dF/dt_{max} plotted as a function of time of recovery in AF with and without eprosartan perfusion after DC shock.

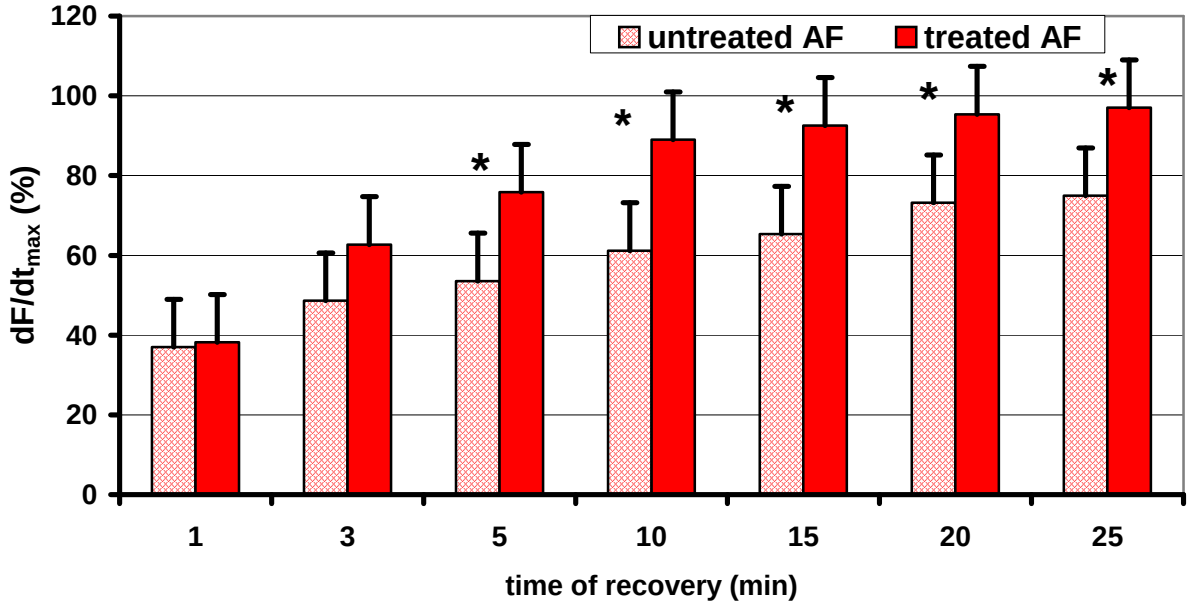


Fig. 3.14 Diagram of dF/dt_{max} (%) plotted as a function of time of recovery in AF with and without eprosartan perfusion after DC shock.

The recovery rate of dF/dt_{min} (%) of the untreated and treated AF showed a similar pattern to that of the dF/dt_{max} (%). As can be seen in fig.3.16 and fig.3.17 there was no significant difference between the two in the first 3 minutes. At the 5th minute the dF/dt_{min} (%) of the treated AF was 71.40 ± 5.97 (%)* and that of the untreated was 60.58 ± 8.82 (%). The recovery rate for the treated AF was also significantly higher that that of the untreated in the 10th, 15th, 20th, and 25th minute.

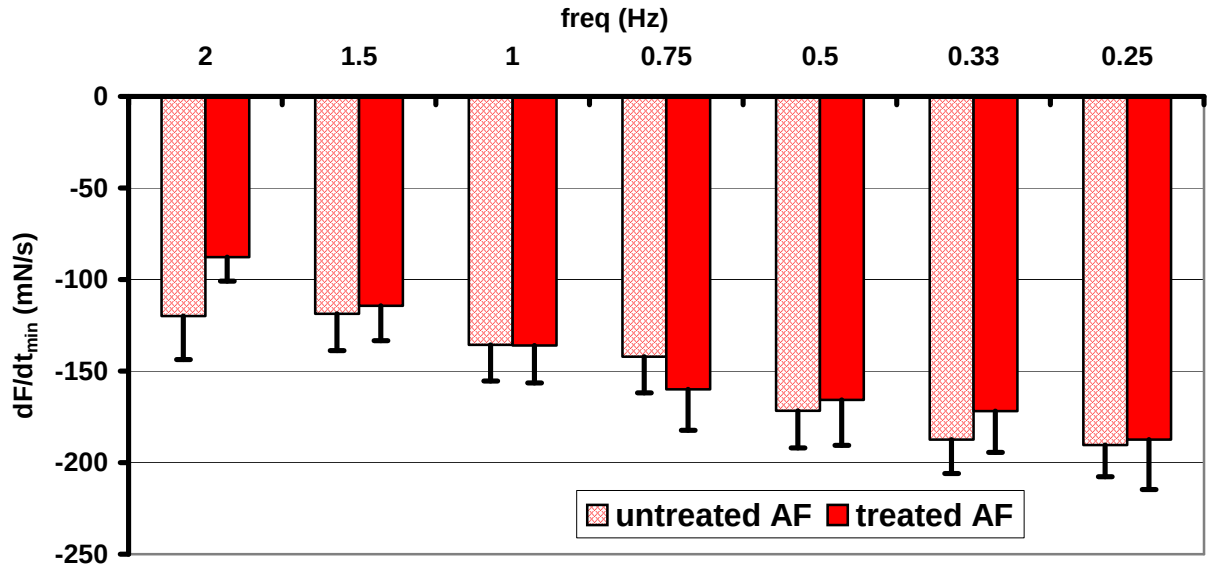


Fig. 3.15 Diagram of dF/dt_{min} plotted as a function of time of recovery in AF with and without eprosartan perfusion after DC shock.

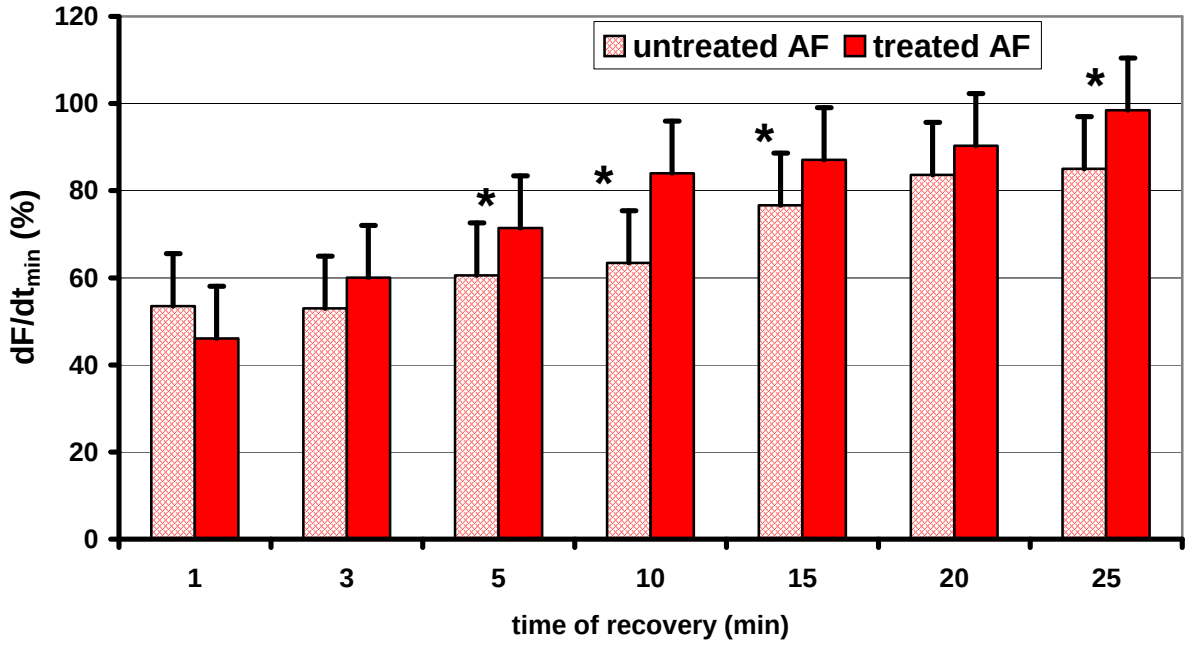


Fig. 3.16 Diagram of dF/dt_{min} (%) plotted as a function of time of recovery in AF with and without eprosartan perfusion after DC shock.

3.5.3 dF/dt_{max} and dF/dt_{min} in SR with and without eprosartan perfusion after DC shock

In fig.3.17 and fig.3.19 it can be seen that there were no significant differences between with eprosartan treated SR samples and untreated SR samples in either dF/dt_{max} or dF/dt_{min}. At 1st minute the dF/dt_{max} was 89.79 ± 14.44 mN/s and 99.6 ± 11.73 mN/s for treated and untreated SR, respectively. The maximum value was 258.52 ± 31.88 mN/s for the treated SR samples and 308.71 ± 34.11mN/s for the untreated ones at the 25th minute. The dF/dt_{min} for the treated and untreated AF samples at 1st minute was -69.6 ± 4.96 mN/s and -68.94 ± 7.63 mN/s, respectively. A maximum value of -156.14 ± 14.16 mN/s was reached by the treated SR samples and a value of -168.28 ± 13.72 mN/s was reached by the untreated SR.

The values of dF/dt_{max} (%) as shown in fig.3.18 of the treated and untreated SR showed no significant differences after treatment with eprosartan unlike that of the treated AF from the 15th minute to the 25th minute with the values risen from 71.00 ± 3.73 (%)* at the 15th minute to 83.74 ± 3.18 (%)* at the 25th minute for the treated SR and from 61.71 ± 6.56 (%) at the 15th minute to 67.70 ± 7.48 (%) at the 25th minute. The minimum value was in the first minute after the shock in the two groups.

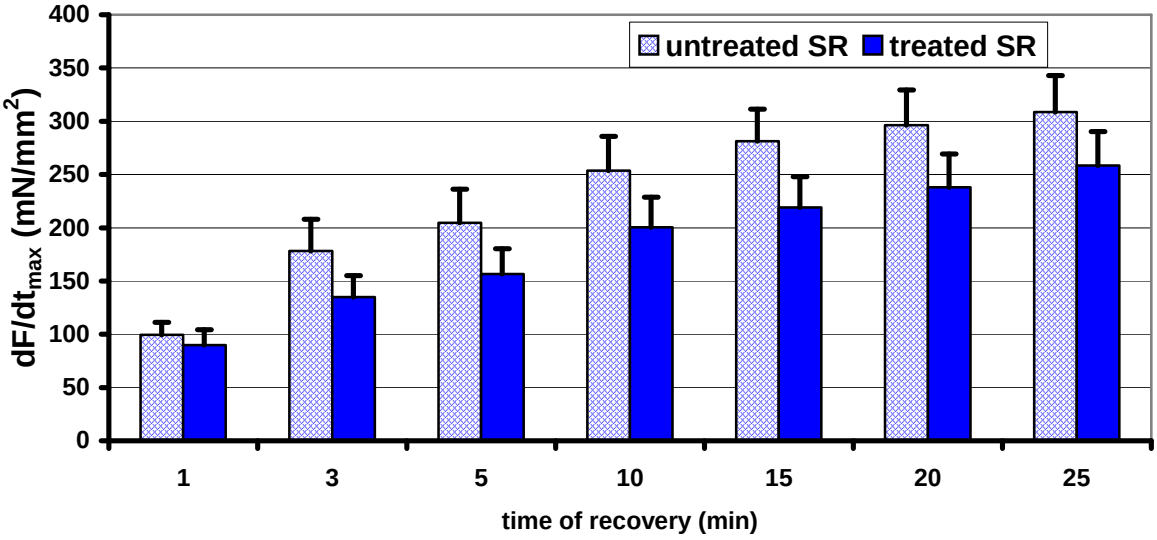


Fig. 3.17 Diagram of dF/dt_{max} plotted as a function of time of recovery (1-25 min) in SR with and without eprosartan perfusion after DC shock.

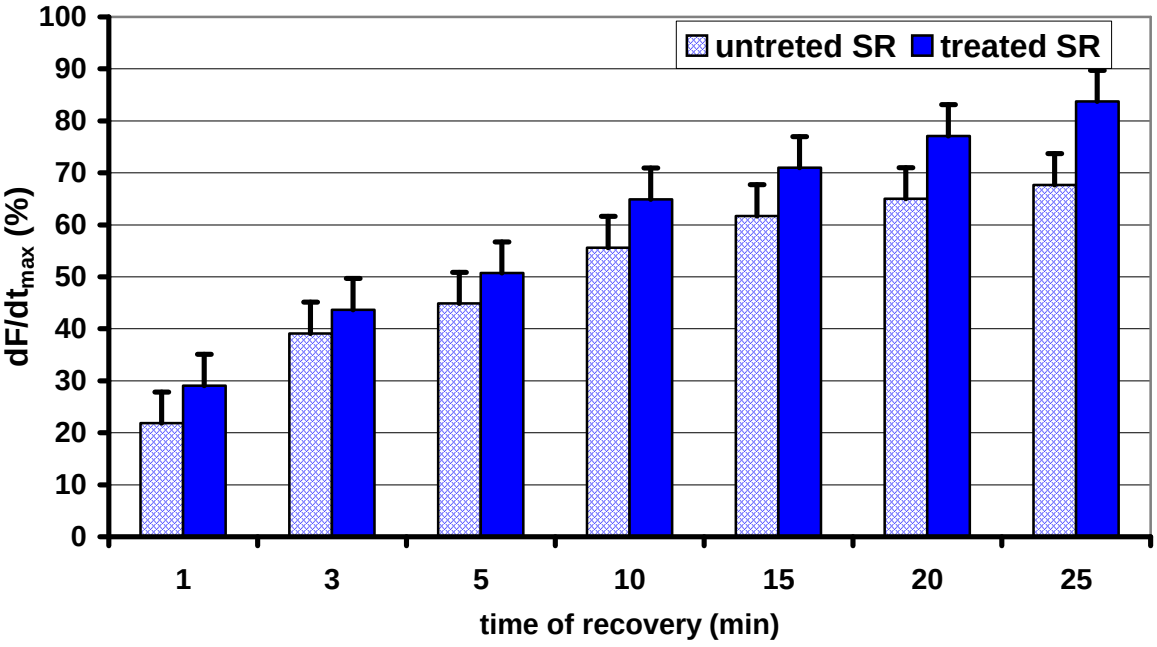


Fig. 3.18 Diagram of dF/dt_{max} (%) plotted as a function of time of recovery (1 – 25 min) in SR with and without eprosartan perfusion after DC shock.

The recovery rate of dF/dt_{min} (%) of the untreated and treated SR was similar to that of the dF/dt_{max} (%). As can be seen in fig.3.20 there was no significant difference between the two in the first 15 minutes. It was after the 15th minute that the dF/dt_{min} (%) of the treated SR increased significantly from76.17 ± 735 (%)* at the 15th minute to 92.79 ± 8.03 (%)* at the 25th minute. The untreated recovered from 29.12 ± 2.07 (%) at the 1st minute to 70.41 ± 5.74 (%) at the 25th minute. The recovery rate for the treated AF was also significantly higher than that of the untreated in the 10th, 15th, 20th, and 25th minute.

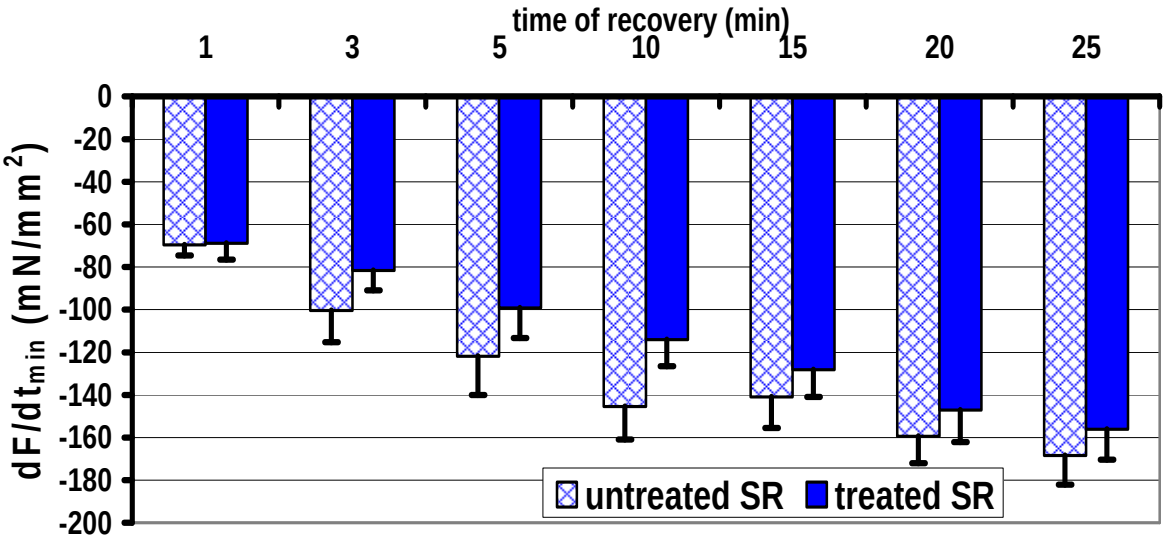


Fig. 3.19 Diagram of dF/dt_{min} plotted as a function of time of recovery (1-25 min) in SR with and without eprosartan perfusion after DC shock.

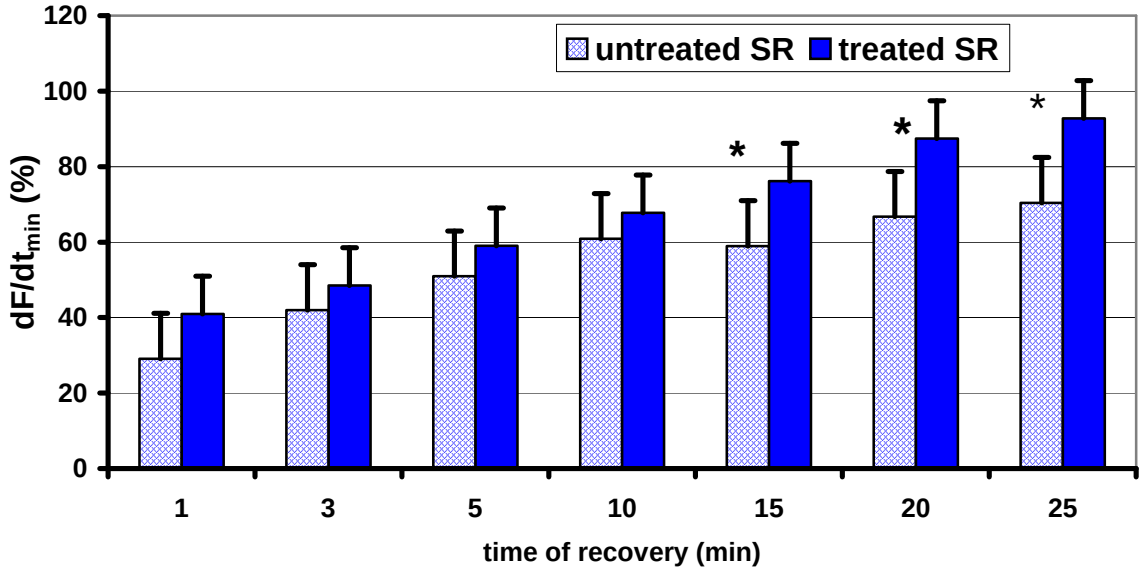


Fig. 3.20 Diagram of dF/dt_{min} (%) plotted as a function of time of recovery (1-25 min) in SR with and without eprosartan perfusion after DC shock.

3.5.4 Comparison of dF/dt_{max} and dF/dt_{min} in DC shocked AF and SR after eprosartan perfusion

The dF/dt_{min} and dF/dt_{max} of the atrial samples with AF and SR were recorded and compared. Below in fig.3.21 and fig.3.23, the dF/dt_{max} and dF/dt_{min} of two groups are compared. Although the dF/dt_{max} and dF/dt_{min} of the AF group were much higher after the muscles were shocked for 60s, there was no significant difference between the two groups.

Fig.3.23 shows that the dF/dt_{min} of both groups increased continuously after the shock until the 25th minute, where it reached a stable dF/dt_{min}. The minimum value of dF/dt_{min} for the two groups was in the 1st minute. At the 1st minute the AF group had a dF/dt_{min} of -87.71 ± 13.12 mN/s and a value of -68.94 ± 7.73 mN/s for the SR. The dF/dt_{min} of both groups had decreased significantly as compared to the baseline dF/dt_{min}. Here the dF/dt_{min} of the AF group was about 21% greater than that of the SR group. The baseline dF/dt_{min} of the AF group was -190.44 ± 17.33 mN/s and that of the SR group was –168.28 ± 13.72 mN/s. At the end of the 25th minute, the dF/dt_{min} of the AF muscles had reached about 98% of the initial dF/dt_{min} and the SR group reached 92% of the initial dF/dt_{min}.

In Fig.3.21, the comparison of the dF/dt_{max} of the AF to the SR can be seen. Similar to the $dF/dt_{min,}$ the recovery of the dF/dt_{max} rose in both groups steadily from the 1st minute to the 10th minute, after which the recovery rate slowed down. Here again there were no significant differences between the groups. The AF group dominated throughout. The initial value before the DC shock was 314.04 ± 39.84 mN/s for the AF and 308.71 ± 34.11 mN/s for the SR. After shocking the dF/dt_{max} of the SR and AF group dropped to 89.79 ± 14.44 mN/s and 119.89 ± 23.81 mN/s, respectively. A maximum value of 304.66 ± 47.39 mN/s was reached by the AF and 258.52 ± 31.88 mN/s by the SR.

In the dF/dt_{max} (%) and the dF/dt_{min} (%) however there were some significant changes. Right from the 3rd minute after shock to the 25th minute, there were significant differences between the dF/dt_{max} (%) of the SR and AF. At the 1st minute the recovery rate of the AF was higher than that of the SR but was not significant. At the 1st minute the value for the AF was 38.18 ± 5.65 (%) whilst that of the SR was 29.09 ± 5.23 (%). At the 3rd minute a significant difference was seen between the two with the SR reaching 43.67 ± 4.28 (%) and AF 62.73 ± 7.05 (%). The recovery rate of the AF dominated significantly until the 25th minute reaching about 97% of the baseline dF/dt_{max} (%). At the 25th minute the SR recovered about 83% of the baseline dF/dt_{min} (%).

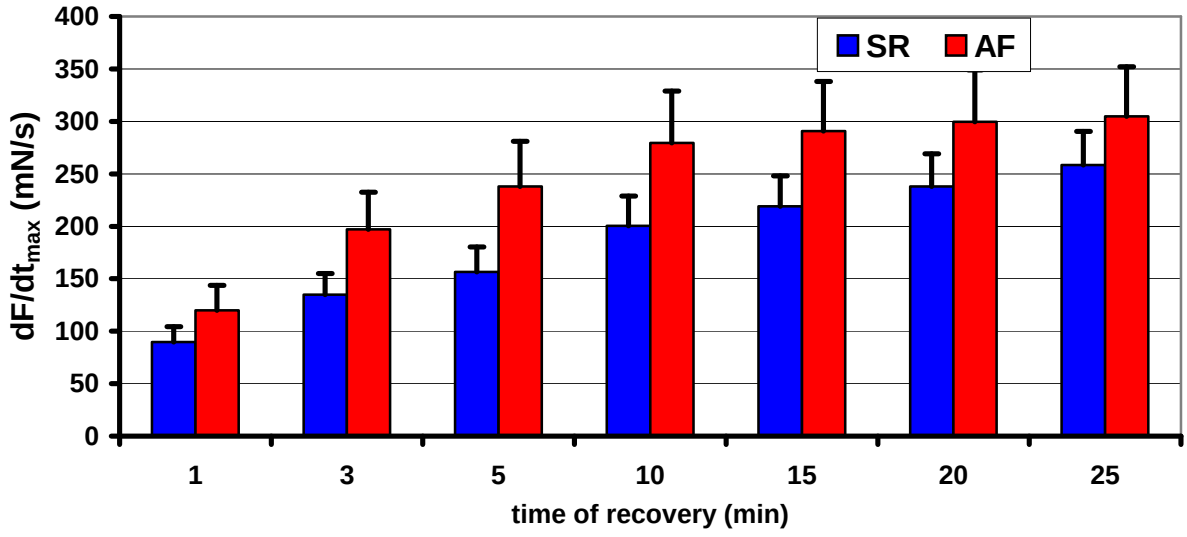


Fig. 3.21 Diagram showing the effect of eprosartan on dF/dt_{max} of DC shocked AF and SR plotted as a function of time of recovery (1 - 25 min) after eprosartan perfusion.

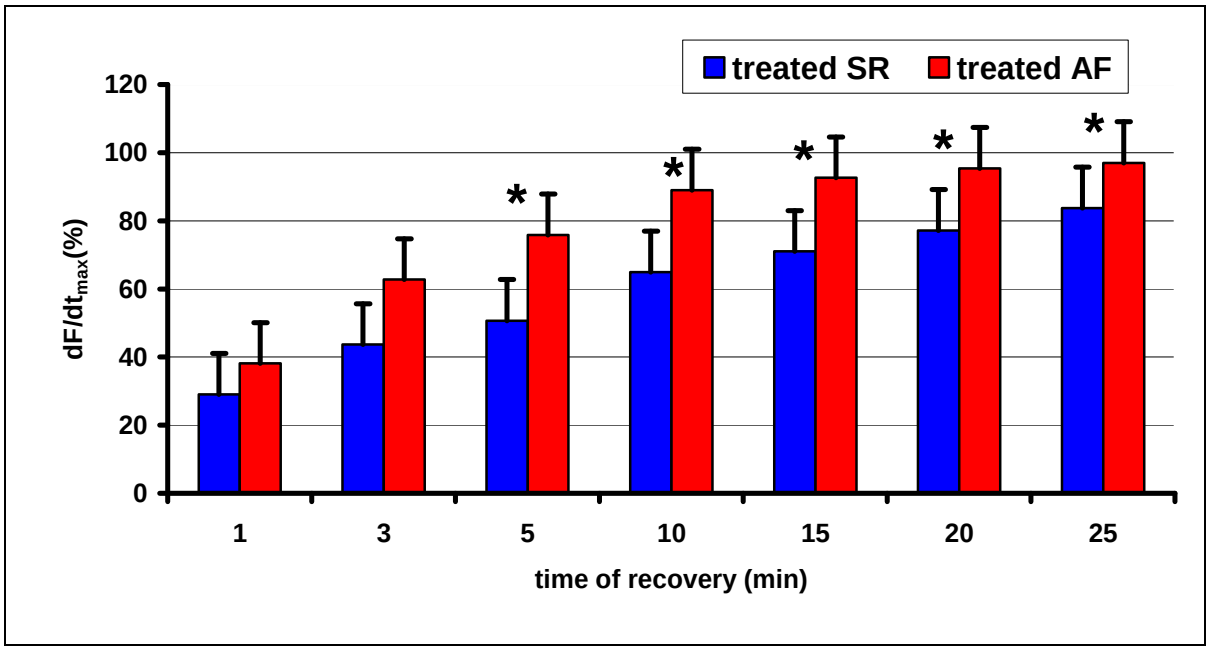


Fig. 3.22 Diagram showing the effect of eprosartan on dF/dt_{max} (%) of DC shocked AF and SR plotted as a function of time of recovery (1 - 25 min) after eprosartan perfusion.

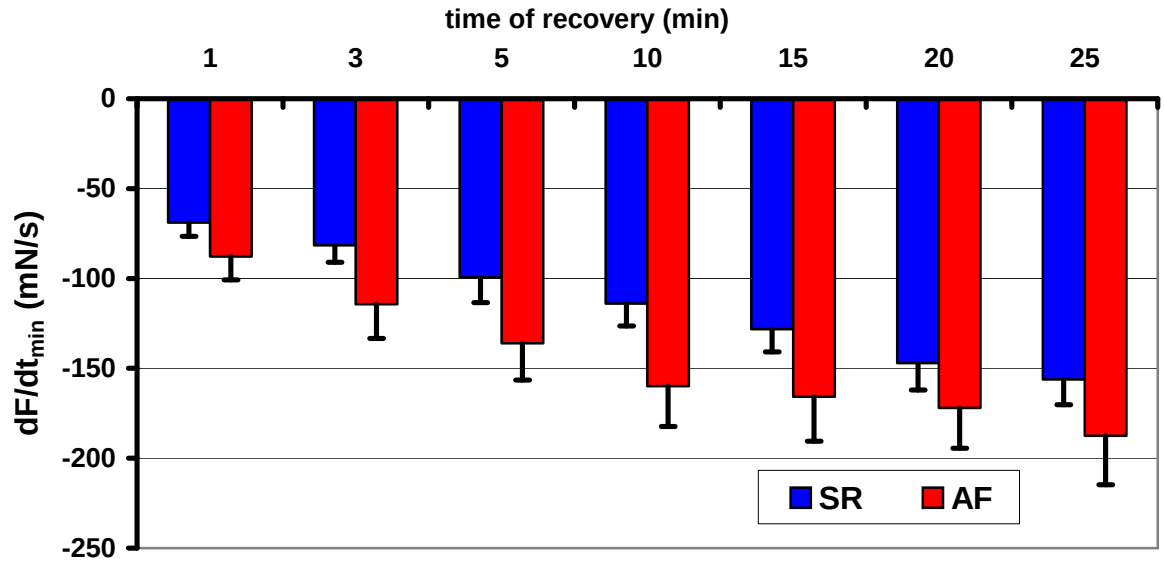


Fig. 3.23 Diagram showing the effect of eprosartan on dF/dt_{min} of DC shocked AF and SR plotted as a function of time of recovery (1 - 25 min) after eprosartan perfusion.

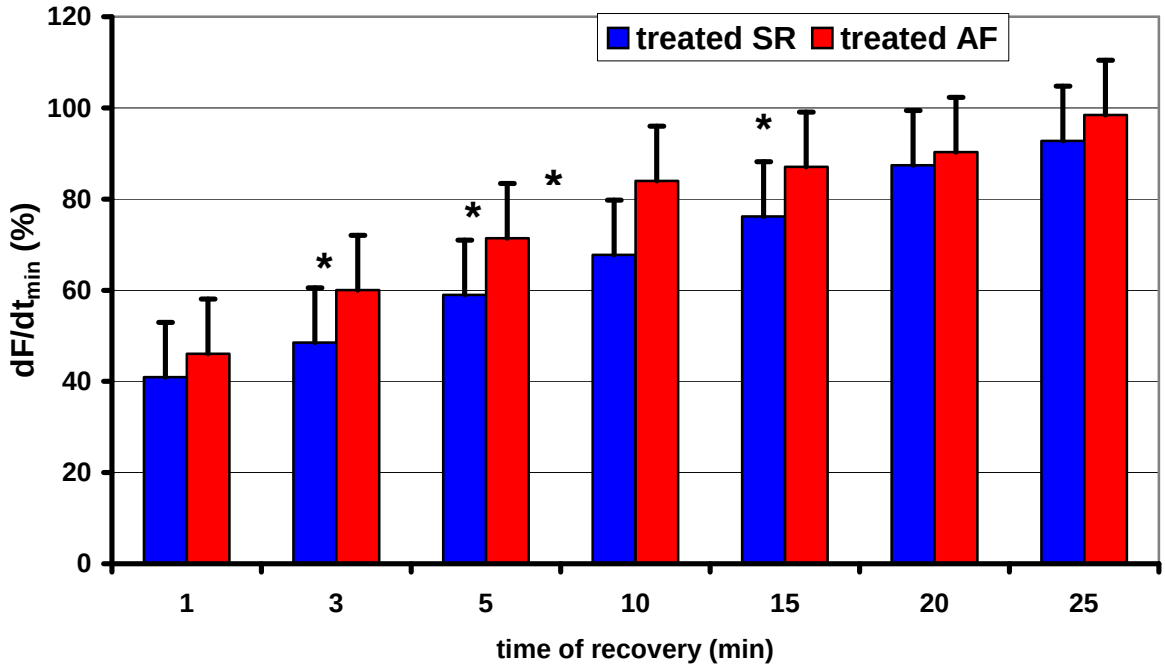


Fig. 3.24 Diagram showing the effect of eprosartan on dF/dt_{min} (%) of DC shocked AF and SR plotted as a function of time of recovery (1 - 25 min) after eprosartan perfusion.

Parameters	SR group	P-value	AF group
Number of samples	17	ns	11
Average area of atrial strips	3.28 ± 0.31	ns	3.09 ± 0.37
dF/dt_{max} at 2 Hz	241.11 ± 29.95	ns	230.87 ± 28.63
dF/dt_{max} at 1.5 Hz	282.38 ± 33.96	ns	235.20 ± 30.63
dF/dt_{max} at 1 Hz	359.45 ± 47.69	ns	278.57 ± 32.63
dF/dt_{max} at 0.75 Hz	373.25 ± 52.29	ns	296.58 ± 38.53
dF/dt_{max} at 0.5 Hz	428.14 ± 59.23	ns	339.38 ± 45.20
dF/dt_{max} at 0.33 Hz	489.22 ± 67.97	ns	372.00 ± 54.19
dF/dt_{max} at 0.25 Hz	534.68 ± 75.13	ns	398.22 ± 64.34
dF/dt_{min} at 2 Hz	-141.88 ± 13.40	ns	-127.02 ± 14.37
dF/dt_{min} at 1.5 Hz	-150.31 ± 17.92	ns	-130.33 ± 13.86
dF/dt_{min} at 1 Hz	-186.91 ± 26.51	ns	-138.02 ± 17.56
dF/dt_{min} at 0.75 Hz	-201.22 ± 27.02	ns	-138.36 ± 21.08
dF/dt_{min} at 0.5 Hz	-232 ± 31.57	ns	-168.18 ± 26.72
dF/dt_{min} at 0.33 Hz	-241.86 ± 31.88	ns	-185.20 ± 31.18
dF/dt_{min} at 0.25 Hz	-265.98 ± 34.87	ns	-185.89 ± 38.11
dF/dt_{max} at iso conc of 10^{-9}M	527.46 ± 53.87	ns	460.58 ± 48.77
dF/dt_{max} at iso conc of 10^{-8}M	495.67 ± 52.60	ns	445.67 ± 49.55
dF/dt_{max} at iso conc of 10^{-7}M	472.59 ± 50.55	ns	437.16 ± 51.20
dF/dt_{min} at iso conc of 10^{-9}M	-284.26 ± 23,86	ns	-224.55 ± 32.30
dF/dt_{min} at iso conc of 10^{-9}M	-243.18 ± 27.97	ns	-233.13 ± 24.76
dF/dt_{min} at iso conc of 10^{-9}M	-253.53 ± 23.78	ns	-238.95 ± 26.55

Tab. 3.4 Table showing the maximum contraction velocity (dF/dt_{max}) and maximum relaxation velocity (dF/dt_{min}) of the AF (n = 11) and SR (n = 17) after being subjected to different frequency and inotropic (isoprenaline) stimulation. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

Parameters (without treatment)	SR group	P-value	AF group
dF/dt_{max} at 1 min	99.6 ± 11.73	ns	154.95 ± 30.11
dF/dt_{max} at 3 min	178.35 ± 29.67	ns	203.71 ± 31.15
dF/dt_{max} at 5 min	204.59 ± 31.53	ns	224.36 ± 39.50
dF/dt_{max} at 10 min	253.62 ± 32.29	ns	256.44 ± 41.85
dF/dt_{max} at 15 min	281.39 ± 29.91	ns	273.75 ± 39.17
dF/dt_{max} at 20 min	296.45 ± 33.02	ns	306.66 ± 42.70
dF/dt_{max} at 25 min	308.71 ± 34.11	ns	314.04 ± 32.11
dF/dt_{min} at 1 min	-69.6 ± 4.96	ns	-91.31 ± 39.79
dF/dt_{min} at 3 min	-100.38 ± 14.88	ns	-118.69 ± 20.19
dF/dt_{min} at 5 min	-121.78 ± 18.21	ns	-135.71 ± 19.77
dF/dt_{min} at 10 min	-145.46 ± 15.45	ns	-142.07 ± 19.83
dF/dt_{min} at 15 min	-140.91 ± 14.57	ns	-171.67 ± 20.24
dF/dt_{min} at 20 min	-159.44 ± 12.64	ns	-187.38 ± 18.60
dF/dt_{min} at 25 min	-168.28 ± 13.72	ns	-190.44 ± 17.32

Tab. 3.5 Table showing the maximum contraction velocity (dF/dt_{max}) and maximum relaxation velocity (dF/dt_{min}) of the AF (n = 11) and SR group (n = 17) after DC shock and without eprosartan perfusion. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

Parameters (without treatment)	SR group	P-value	AF group
dF/dt_{max} (%) at 1 min	21.84 ± 2.57	ns	36.98 ± 7.19
dF/dt_{max} (%) at 3 min	39.11 ± 6.51	ns	48.62 ± 9.43
dF/dt_{max} (%) at 5 min	44.87 ± 6.96	ns	53.55 ± 9.96
dF/dt_{max} (%) at 10 min	55.62 ± 7.08	ns	61.20 ± 9.99
dF/dt_{max} (%) at 15 min	61.71 ± 6.56	ns	65.33 ± 9.35
dF/dt_{max} (%) at 20 min	65.01 ± 7.24	ns	73.19 ± 10.19
dF/dt_{max} (%) at 25 min	67.70 ± 7.48	ns	74.95 ± 9.50
dF/dt_{min} (%) at 1 min	29.12 ± 2.07	ns	53,52 ± 10.62
dF/dt_{min} (%) at 3 min	42.00 ± 6.23	ns	52,99 ± 9.01
dF/dt_{min} (%) at 5 min	50.95 ± 7.62	ns	60,58 ± 8.82
dF/dt_{min} (%) at 10 min	60.86 ± 6.46	ns	63,43 ± 8.85
dF/dt_{min} (%) at 15 min	58.96 ± 6.10	ns	76,64 ± 9.04
dF/dt_{min} (%) at 20 min	66.71± 5.29	ns	83,65 ± 8.30
dF/dt_{min} (%) at 25 min	70.41 ± 5.74	ns	85,02 ± 7.73

Tab. 3.6 Table showing the dF/dt_{max} (%) and maximum relaxation velocity dF/dt_{min} (%) of the AF (n = 11) and SR group (n = 17) after DC shock and without eprosartan perfusion. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

Parameters (with treatment)	SR group	P-value	AF group
dF/dt_{max} at 1 min	89.79 ± 14.44	ns	119.89 ± 23.79
dF/dt_{max} at 3 min	134.8 ± 20.22	ns	196.98 ± 35.44
dF/dt_{max} at 5 min	156.59 ± 23.85	ns	238.11 ± 43.05
dF/dt_{max} at 10 min	200.45 ± 28.28	ns	279.46 ± 49.33
dF/dt_{max} at 15 min	219.18 ± 28.74	ns	290.66 ± 47.52
dF/dt_{max} at 20 min	238.07 ± 31.10	ns	299.56 ± 49.47
dF/dt_{max} at 25 min	258.52 ± 31.88	ns	304.66 ± 47.33
dF/dt_{min} at 1 min	-68.94 ± 7.63	ns	-87.71 ± 13.11
dF/dt_{min} at 3 min	-81.62 ± 9.30	ns	-114.29 ± 19.06
dF/dt_{min} at 5 min	-99.3 ± 14.11	ns	-135.96 ± 20.57
dF/dt_{min} at 10 min	-114 ± 12.43	ns	-159.93 ± 22.35
dF/dt_{min} at 15 min	-128.19 ± 12.68	ns	-165.78 ± 24.69
dF/dt_{min} at 20 min	-147.08 ± 15.01	ns	-171.93 ± 22.50
dF/dt_{min} at 25 min	-156.14 ± 14.16	ns	-187.46 ± 2726

Tab. 3.7 Table showing the maximum contraction velocity (dF/dt_{max}) and maximum relaxation velocity (dF/dt_{min}) of AF (n = 11) and SR (n = 17) after DC shock and eprosartan perfusion. Results are expressed as mean ± SEM. $^{*}P < 0.05$. ns = not significant.

Parameters (with treatment)	SR group	P-value	AF group
dF/dt_{max} (%) at 1 min	29.09 ± 5.23	ns	38.18 ± 5.65
dF/dt_{max} (%) at 3 min	43.67 ± 4.28	ns	62.73 ± 7.05
dF/dt_{max} (%) at 5 min	50.72 ± 3.70	*	75.82 ± 7.26
dF/dt_{max} (%) at 10 min	64.93 ± 4.02	*	88.99 ± 7.76
dF/dt_{max} (%) at 15 min	71.00 ± 3.73	*	92.55 ± 6.85
dF/dt_{max} (%) at 20 min	77.12 ± 3.25	*	95.39 ± 7.86
dF/dt_{max} (%) at 25 min	83.74 ± 3.18	*	97.01 ± 6.96
dF/dt_{min} (%) at 1 min	40.97 ± 6.57	ns	46.06 ± 5.87
dF/dt_{min} (%) at 3 min	48.50 ± 7.03	*	60.02 ± 6.32
dF/dt_{min} (%) at 5 min	59.01 ± 11.05	*	71.40 ± 5.97
dF/dt_{min} (%) at 10 min	67.74 ± 4.88	*	83.98 ± 6.25
dF/dt_{min} (%) at 15 min	76.17 ± 735	*	87.05 ± 8.35
dF/dt_{min} (%) at 20 min	87.40 ± 5.41	ns	90.28 ± 6.61
dF/dt_{min} (%) at 25 min	92.79 ± 8.03	ns	98.43 ± 8.05

Tab. 3.8 Table showing dF/dt_{max} (%) and dF/dt_{min} (%) of AF (n = 11) and SR (n = 17) after DC shock with eprosartan perfusion. Results are expressed as mean ± SEM. *P < 0.05. ns = not significant.

4 Discussion

A transient decrease in atrial mechanical function, referred to as atrial stunning, is a well documented phenomenon after cardioversion and DC shock of atrial fibrillation. Atrial mechanical stunning is thought to be one of the mechanisms responsible for the increased risk of thromboembolic complications after cardioversion of AF. A number of transthoracic and transoesophageal echocardiography studies have shown that after cardioversion the mechanical function of the left atrium and left atrial appendage (LAA) is reduced, resulting in lower LAA blood flow velocities, which in turn predisposes patients to the formation of new thrombi (Feldmann et al., 1988). Metabolic analysis shows the slow recovery of ATP concentrations while histological analyses show normal histological appearance of the stunned myocardium (Hasenfuss et al. 1994). Since no permanent damage occurred to the myocardial cells, this contractile dysfunction will gradually recover with time (Hasenfuss et al., 1994).

Although the exact mechanism of myocardial stunning remains unknown, a variety of possible mechanisms, not necessarily mutually exclusive, have been hypothesised (Schwinger et al., 1993). These include insufficient energy production by mitochondria, impaired energy use by myofibrils, reduced myofilament responsiveness to calcium, calcium overload, and reduced contractile protein activation due to sarcoplasmic reticulum dysfunction and generation of oxygen-derived free radicals (Mulieri et al. 1992). The underlying reasons for the remarkable growth of interest in atrial stunning since the 1980s have been due to the recognition that it is a common phenomenon in patients with coronary artery disease, and its significance in delaying the benefits of reperfusion therapy (Schwinger et al., 1993). It has also been shown that myocardial stunning occurs in various clinical settings (Brixius et at., 1999).

This work discusses the possible effect of DC shock on the mechanisms of atrial stunning and to some extent its clinical relevance.

4.1 Method used for the experimentation with the atrial strips

Physiological and pharmacological attempts to investigate the myocardial contractility in stunned heart muscles are central subjects in cardiology and heart insufficiency research. Which experimental method is best depends on the advantages and the disadvantages and its transferability. The investigation of the inotropic effect of a drug on a person in vivo is possible, however the evaluation is difficult.

The advantage of studying isolated human atrial strips is therefore that the direct effect of eprosartan on isolated muscle preparation can be grasped more simply and therefore more precisely evaluated. The uses of human myocardium for muscle physiological and pharmacological investigations are subject to several restrictions: Logistical problem could be solved extensively by use of Tyrode`s solution with the special substance 2,3-Butanedione Monoxime (BDM) (Schwinger et al 1993).

BDM prevents the forming of contractures probably through blockage of the calcium binding site at the troponin C and the calcium-canals. These effects enter quickly and are completely reversible within a short time through washing out of the BDM (Schwinger et al., 1993). Experimental investigations on the myocardium in vitro could either be done on an atrial strip-preparation or directly on isolated cardiomyocytes.

The isometric contractile force of an intact atrial strip can be measured. The experiments must if possible be under physiological conditions (temperature: 37℃ and stimulation frequency: 1Hz). Moreover it must be guaranteed that the oxygen in the innermost part of the strip preparations is sufficient. In addition, the preparation should show only a small cross-sectional area. Any mechanical injury that occurs leads to massive release of calcium which causes contractures. BDM also reduces the problem of the massive calcium release through mechanical injury of the myocardium is reduced.

Furthermore, not only cardiomyocytes exist in multicellular muscle preparation, but also a large amount of endothelial cells and fibroblasts. So fibrotic tissue and cellular necrosis within a strip preparation can affect the contractile properties. Cardioactive substances of endothelial cells, nerve ends and fibrocytes can evoke an inotropic effect on the muscle when released during preparation. Moreover, the interpretation of the muscle physiological and pharmacological results of the multicellular muscle preparations can be affected by the micro anatomic and biochemical changes that occur during the preparation and the experimentation. For example, long diffusion ways and narrow intercellular columns can lead to an inhomogeneous and insufficient distribution, of oxygen, extra cellular ions, nutrients and pharmacologic substances.

4.2 Force-frequency relationship of atrial myocardium (Bowditch-staircase phenomenon)

That an increase in the heart rate can lead to an increase in the force of contraction (inotropic effect of rate) has been well described for ventricular myocardium in normal hearts and has been termed the Bowditch-staircase phenomenon or force-frequency relationship (FFR). In the failing heart, this inotropic effect of rate is either not found or is reversed. In this work, failing atrial myocardium showed a negative frequency dependency of the force of contraction (FOC/A). As can be seen in diagram fig. 3.1 and fig. 3.2, the FFR was reversed when the frequency of stimulation was decreased from 2Hz stepwise to 0.25 Hz: the maximal force occurred during the minimal frequency. A decrease of the stimulating frequency from 2Hz to 0.25Hz caused an increase in the FOC/A from 4.27 ± 0.69 mN/mm^2 to 11.41 ± 1.86 mN/mm^2 in SR and from 4.41 ± 0.79 mN/mm^2 to 9.75 ± 1.61 mN/mm^2 in the AF. In all samples used the maximum FOC/A was attained at 0.25Hz, i.e. the frequency at which the maximum calcium ions are released. The FOC/A at 0.25Hz was more than 2 times the FOC/A at 2Hz. This negative FFR is in line with that of other researchers. Schwinger et al. were able to prove a positive power-frequency relationship in isolated right atrial trabeculae of healthy human hearts. In comparison with these results, the FOC/A of the insufficient atria development with increasing frequency was negative (Schwinger et al., 1993). They did not indicate further contraction parameters.

Evidence implicates intracellular calcium homeostasis in frequency-dependent force generation (Brixius et al., 1999), in particular the sarcoplasmic storage and release of the failing heart, sarcoplasmic reticulum calcium-ATPase has been demonstrated to be abnormal in function and is implicated in the observed negative force-frequency relationship. The efficiency of this storage and release of calcium from the sarcoplasmic reticulum is also implicated in the negative FFR seen at higher rates (Bers et al., 1985). After cardioversion of AF, Fatkin et al., found that worsening of the heart condition was accompanied by a variable degree of impairment of atrial contraction (Fatkin et al., 1994).

4.3 Effect of isoprenaline on atrial contractility

A further essential physiological mechanism to the regulation of atrial contractility is the sympathetic mediation of catecholamine stimulation of the heart (adrenergic

responsibility). The well examined positive inotropic effect of the beta-adrenergic agonist isoprenaline was also observed in this work in both atrial strips with atrial fibrillation (AF group) and sinus rhythm (SR group). Isoprenaline is an activator of the β-adrenoceptor, but in chronic heart failure, cardiac β-adrenoceptor function decreased due to endogenous beta-receptor downregulation by the elevated catecholamines, and this decrease is related to the severity (Khamssi et al., 1990).

In the SR and AF groups, as can be seen in Fig.3.4, 3.11 and 3.12, the FOC/A, dF/dt_{min} and dF/dt_{max} responded positively to an isoprenaline concentration of 10^{-9} M at 1Hz. After reaching a maximum of 10.34 ± 1.44 mN/mm^2 (SR) and 9.34 ± 1.2 mN/mm^2 (AF) at 10^{-9} M, which was almost twice the FOC/A at the baseline (37℃, 1 Hz, and Tyrode`s solution). An increase in the isoprenaline concentration from 10^{-9} M to 10^{-8} M and 10^{-7} M caused a slight decrease in FOC/A, dF/dt_{min} and dF/dt_{max} in both SR and AF. It may be due to down regulation but missing dose dependency.

Through the reinforced development of cAMP via stimulation of the adenylate cyclase, phosphorylation of different intracellular proteins occurs. The phosphorylation of the L-type calcium canals leads to an increase of the opening probability and so to an increased calcium inflow into the cells during the depolarisation (Wohlfahrt et al. 1982). In addition, through phosphorylation of phospholamban, an inhibitory protein of the SERCA, an inhibition of the pump activity and an increase of the calcium reception capacity of the sarcoplasmic reticulums occurs (Movesian et al., 1998). It has been shown that positive inotropic and lusitropic effects decreased significantly through β-adrenoceptor down regulation at the isolated single cell out of terminal insufficiency myocardium in comparison with control myocardium. Further investigations showed that the terminal insufficient myocardium positive inotropic effect was disproportionately increased (in comparison with the isometric contraction force) intracellular calcium concentration (Blinks et al., 1986; Kretschmann et al., 1995).

An isoprenaline concentration of 10^{-9} M increased the FOC/A (%) to about 166% and 159% in SR and AF, respectively. The insignificant increase of the FOC/A in atrial myocardium with SR as compared to that with AF could be due to the more increased calcium overload that occurs during atrial fibrillation or a decrease in the amount of beta-adrenergic receptors.The parameters of the maximum contraction and relaxation time decreased and the maximum contractions and relaxation velocity increased accordingly. The results are in line with those of other authors (Pieske et al., 1992).

These results indicate that the contractility of the myocardium which depends on cAMP may be influenced by cAMP-independent stimulation.

4.4 Effect of AT_1-antagonist (eprosartan) on electrical, contractile and structural remodelling

Cardiac myocytes become depolarised at about -80mV by an inward rush of positively charged ions and the action potential begins. Inward Ca^{2+} current causes the membrane potential to plateau, which contributes significantly to the action potential duration. The inward calcium current significantly increases myocyte calcium load at rapid atrial rates such as those during periods of fibrillation. Adaptive mechanisms rapidly reduce the load to protect the cell, because high intracellular calcium concentration can be toxic. The membrane channel responsible for calcium entry becomes less active early in the remodelling process. The production of that channel is eventually down regulated. The changes ultimately reduce the inward calcium current, and this in turn reduces the action potential duration. If the action potential duration shortens, the refractory period shortens too, and the cell can be ready for reactivation earlier. Hence, adaptive mechanisms that respond to intracellular calcium loads ultimately shorten the atrial refractory period, promoting functional re-entry and perpetuation of AF. Even in the case of prolonged AF, atrial electric remodelling reverses quickly and completely once sinus rhythm is restored.

Atrial electrophysiological changes induced by AF may have other, more widespread effects on myocyte function. In myofilament sliding for example, the cellular action responsible for muscle contraction is intimately dependent upon intracellular Ca^{2+} concentrations. Studies conducted on animals and humans have demonstrated AF-related reductions in atrial contractile function that were lessened by treatment with a calcium-channel antagonist or exaggerated by treatment with a calcium-channel agonist.AF also results in differentiation of myocytes to fetal forms, with cell architectures displaying reduced contractile elements and higher resistance to calcium-induced cell death. Thus, changes associated with AF contribute to the development of atrial cardiomyopathy.

These results also support the work of other experiments showing that AT_1 receptor antagonists may prevent electrical remodelling (Kumagai et al., 2003). The beneficial effects on atrial electrical and structural remodelling of angiotensin II inhibition after

stunning are independent of intra-atrial pressures (Nakashima et al., 2000). In a canine model, oral candesartan administered 1 week before, and 5 weeks during rapid atrial pacing prevented to some extent the shortening of the atrial effective refractory period and slowing of intra-atrial conduction, and decreased the inducibility and duration of AF (Nakashima et al., 2000).

Previous investigations have reported increased activity of the atrial angiotensin II system and angiotensin receptor changes in patients with AF (Goette et al 2000). Several studies have demonstrated that angiotensin II contributes to the development of the remodelling in AF; inhibition of RAS can lead to regression of the changes described earlier. Thus, angiotensin receptor antagonists have been reported to prevent the electrical remodelling in AF, especially in the short-term (Nakashima et al., 2000, Folkeringa et al., 2004).This effect is thought to be in part mediated by interference with ion currents, stabilization of electrolyte concentrations, a reduction of calcium overload, and a decrease of atrial stretch (Nakashima et al., 2000). These agents also attenuate the structural remodelling in AF; this is not surprising, as angiotensin II is a stimulator of collagen synthesis and a strong promoter of fibrosis (Sadoshima et al., 1993; Li 2001).

Inhibition of the remodelling process by blockade of angiotensin could possibly explain the attenuation of atrial stunning observed in this work, although the exact mechanism cannot be defined.

4.5 Cardioprotective effect of AT_1-antagonist (eprosartan) on atrial strips undergoing DC shock

The cardioprotective effect of eprosartan on atrial muscle undergoing electrical injury was studied by comparing the force of contraction of the atrial strips between the two conditions (with and without eprosartan). The main finding of this work as can be seen in fig.3.6, fig.3.22 and fig.3.24 is that treatment with eprosartan may attenuate atrial stunning in patients with AF. Here significant differences are observed in the recovery of FOC/A (%), dF/dt_{max} and dF/dt_{min}. As can also be seen in fig3.7, figs.3.14 and 3.16 there were also some significant differences between the dF/dt_{max} and dF/dt_{min} of the treated and untreated AF samples. Significant ($p>0,05$) difference was seen in FOC/A (%) after the 5^{th} minute when the FOC/A (%) reached 64.76 ± 5.21(%)* of the baseline FOC/A in the treated AF samples, while only 47.68 ± 10.34% was reached by the untreated AF samples, indicating that AT_1 antagonist, eprosartan may play a major role in the cardioprotection of the myocardium. Thus, an AT_1 antagonist like eprosartan may

represent an important pharmacological supplementation in patients after cardioversion. Several factors are believed to be involved in the development of atrial stunning. In this part of the essay we are going to examine the possible mechanism leading to the results obtained and the protective aspect of eprosartan on the heart during electrical injury.

Recent studies emphasize the role of intracellular calcium homeostasis (Sanders et al., 2003). It is also proposed that the structural remodelling that takes place during AF and which is characterized by progressive structural changes of the atria, resulting in atrial dilation and interstitial fibrosis (Ausma et al., 1997) might be a potential mechanism leading to atrial stunning, especially in the case of longer duration of the arrhythmia (Sanders et al., 2003).

This work indicates that AT_1 receptor antagonist (eprosartan) may promote the recovery of the heart muscle after stunning has been induced through the application of electrical current in patients with AF. Furthermore, experimental evidence suggests that the beneficial effects of AT_1 receptor antagonists are not confined to their haemodynamic effects. AT_1 receptor antagonists may also act by reducing the activation of AT_1 dependent MAP kinases, thus preventing cellular hypertrophy and fibrosis. Importantly, they do not block and can upregulate “protective” AT_2 receptors, the activation of which inhibits MAP kinases through activation of different phosphatases thus exerting antiproliferative effects (Rogg et al., 1992). Indeed, the altered expression of angiotensin II receptors with down-regulation of AT_1 and up-regulation of AT_2 receptors has been observed in tissue samples obtained from the right atrium in patients with AF, suggesting an adaptive mechanism aimed at preventing further accumulation of collagen. Immunohistological analysis of left atrial tissue has shown AT_1 up-regulation consistent with the pathophysiological mechanism of atrial remodelling. AT_1 receptor antagonists also prevent increased bradykinin degradation mediated by the angiotensin-converting enzyme in fibrillating atrial tissue which may contribute to degenerative changes associated with AF (Goette et al. 2000; Boldt et al., 2003).

4.5.1 Cardioprotective effect of eprosartan on calcium overload

Though intracellular calcium is vital for excitation-contraction coupling, it has been suggested that an increase in transient $[Ca^{2+}]_i$ during ischemia and early reperfusion could be a mechanism for myocardial stunning.

It has been shown that exposure of isolated ferret hearts to a transient calcium overload mimics several features of stunning, even in the absence of prior ischemia. Features such as decreased maximal Ca^{2+} activated force and sensitivity to calcium, ATP depletion, and absence of histological evidence of irreversible injury were observed (Kitakaze et al., 1988).

In this work and during DC shocking because high electrical energy is involved to induce electrical injury, the atrial muscles undergo a similar condition like ischemia. It is proposed that during ischemia H+ are produced excessively and accumulated (Tani et al. 1989). These are then exchanged for extracellular Na^+, slowly during ischemia and rapidly during early reperfusion, by H^+/Na^+ exchange. Increased intracellular Na+ are then exchanged for Ca^{2+} by the Na^+/Ca^{2+} exchange, and this causes calcium overload (Tani et al., 1989). Based on previous findings, an increase in $[Ca^{2+}]_i$ during ischemia and early reperfusion could activate Ca^{2+} depended protein kinases, which could then cause changes in myofilaments to decrease Ca^{2+} sensitivity and/or maximal Ca^{2+} -activated force through phosphorylation of contractile proteins. Recent studies by (Gao et al., 1997), have concluded that an increase in $[Ca^{2+}]_i$ during ischemia and early reperfusion causes Ca^{2+}-activated protease to partially degrade troponin I in the stunned myocardium. This in turn causes myofilament responsiveness to calcium to decrease. An increase in $[Ca^{2+}]_i$ could generate oxygen radicals via xanthine oxidase, which is in itself a mechanism of stunning (Przyklenk et al., 1986).

4.5.2 Cardioprotective effect of eprosartan on atria stretch

The pathophysiology of AF is largely attributed to stretch caused by increased atrial pressure and volume (Satoh et al., 1996). Activation of stretch-mediated channels in the fibrillating atria enhances calcium binding to cellular myofilaments, generating calcium currents. In experimental models of cardiomyopathy-induced AF, the activity of the Na^+/Ca^{2+}-exchange current (NCX) is increased. The NCX exchanges intracellular Ca^{2+}

accumulated in the cell during the action potential for extracellular Na^+ ions at a rate 1:3. Thus, the NCX carries one net positive charge into the cell with each cycle, leading to delayed depolarisations and triggered ectopic activity when NCX activity is enhanced. Because NCX regulates intracellular concentrations of free Ca^{2+} and Na^+, it also modulates the action potential indirectly by way of influencing the activity of other Ca^{2+}- and/or Na^+-sensitive currents. Atrial stretch is associated with slowing of conduction and increased dispersion of refractoriness, both of which favour re-entry. A blockade of stretch-activated channels by gadolinium impeded the initiation and maintenance of electrically induced AF and suppressed the occurrence of spontaneous arrhythmia (Bode et al. 2000). Furthermore, atrial dilatation in the presence of CHF contributes to the formation of a substrate for AF by creating “a critical atrial mass” capable of supporting the sufficient number of re-entry wavelets necessary for maintenance of the arrhythmia.

It was recently shown that 85–95% of asymptomatic adults have extensions (“sleeves”) of atrial myocardium into the pulmonary veins (Saito et al. 2000). These remnants of myocardial tissue may become active as a result of stretch and dilatation of the pulmonary veins, and produce spontaneous, rapid discharge, triggering AF leading to atrial remodelling. Studies in patients with “focal” AF have shown significant dilatation of the ostial and proximal portions of the pulmonary veins compared with control subjects. Extensions of atrial myocardium in patients with AF also exhibit a significantly higher degree of hypertrophy, discontinuity, and fibrosis (Goette et al 2000).

Chronic stretch and calcium overload during rapid atrial activity are likely to induce sustained proteolysis and a loss of contractile elements leading to atrial myopathy. CHF therefore favours AF by both creating a structural substrate for atrial re-entry and producing a functional basis for atrial ectopic activity that can trigger re-entry. Atrial stretch also results in an increased local synthesis of angiotensin II. Angiotensin-converting enzyme and angiotensin II levels are elevated in fibrillating atrial tissue. Effects of angiotensin-converting enzyme inhibition on the development of atrial fibrillation substrate in dogs with ventricular tachycardia-induced congestive heart failure with subsequent stimulation of AT_1 receptors initiate a cascade of phosphorylation processes that activate a family of mitogen-activated protein kinases (MAP kinases). MAP kinases promote atrial myocyte hypertrophy, fibroblast proliferation, accumulation of collagen, and apoptosis, further contributing to structural remodelling (Goette et al 2002). The three best-defined angiotensin II-dependent MAP kinases include

extracellular signal-regulated protein kinases 1 and 2, c-Jun N-terminal kinase, and p38 MAP kinase. The latter two are also activated by atrial stretch. When occurring in the presence of pre-existing atrial fibrosis, AF itself increases the amount of collagen accumulation, thus closing the vicious circle.

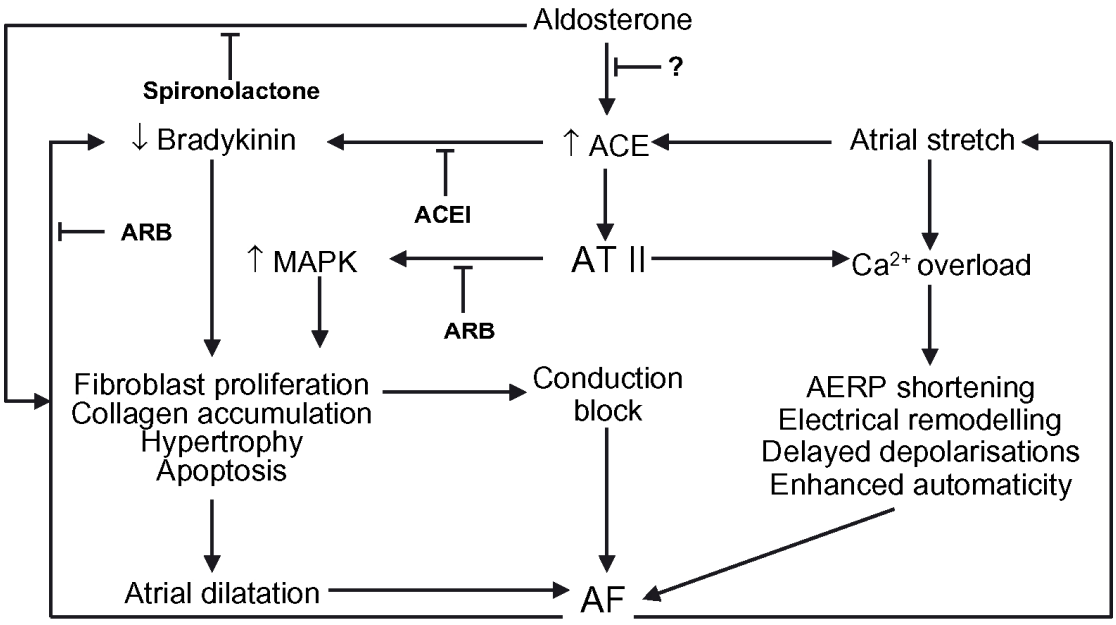


Fig.4.1: Angiotensin II in the pathogenesis of AF. Source: Goette et al. 2002.

Angiotensin II also modifies atrial electrophysiology by indirect effects on ion channels. Stimulation of AT_1 receptors activates erapamillic C leading to inositol-1,4,5-triphosphate (IP_3) mediated release of calcium from the sarcoplasmic reticulum Protein kinase C phosphorylates L-type calcium channels, which results in increased calcium influx, and is also implicated in the reduction of the transient outward potassium current (I_{to}) and the delayed rectifier potassium current (I_K) promoting increased dispersion of refractoriness and in that sense promoting atrial stunning. The application of eprosartan may therefore block the changes which promote atrial stunning and thereby speed up the recovery of the force of contraction (Berk et al., 1998).

4.5.3 Cardioprotective effect of eprosartan on myofilament responsiveness to calcium

Myofilaments Ca^{2+} sensitivity and maximal Ca^{2+} activated force is reduced in the stunned myocardium (Kusuoka et al., 1990). Thus, it is hypothesised that a reduced responsiveness of myofilaments to Ca^{2+} is a mechanism for atrial stunning. Either a decreased intracellular free Ca^{2+} concentration ($[Ca]_i$) transient or a decreased

sensitivity of myofilaments to calcium could cause a reduced myofilament responsiveness to extracellular calcium in the stunned heart. Since Kusuoka et al., 1990 have observed an increase in Ca^{2+} transients in the stunned heart; it is proposed that a decreased sensitivity of myofilaments to calcium is one of the mechanisms for atrial stunning. This decreased sensitivity could be a result of a shift in myofilament Ca^{2+} sensitivity and/or a reduction in the maximal Ca^{2+} activated force (Gao et al., 1992).

Murphy et al., 1989, found that $[Mg^{2+}]_i$ rises during shocking and remains elevated during early reflow. It has been observed that as $[Mg^{2+}]_i$ rises the $[Ca^{2+}]$ activated force relation is shifted to the right. These observations of magnesium transients however do not explain the reduced maximal Ca^{2+} activated force. Furthermore, increased $[Mg^{2+}]_i$ reducing the sensitivity of myofilaments to calcium, do not explain the ability of the stunned myocardium to respond to inotropic stimuli with a normal contractile reserve (Ito et al., 1987)

Recent studies by Gao et al., 1997, have found that troponin I is partially degraded in the stunned heart. Usually, the troponin-tropomyosin complex inhibits the binding of myosin and actin. A structural alteration occurs within tropomyosin when Ca^{2+} binds to troponin C. This exposes the myosin binding site on actin. The free energy needed to cause this structural change in the troponin-tropomyosin complex is obtained from the energy of Ca^{2+} binding to troponin C. This energy is then transduced by troponin T and troponin. Since troponin I is partially degraded in the stunned myocardium by Ca^{2+} activated protease, the energy obtain from Ca^{2+} binding to troponin C cannot be effectively transduced to cause the necessary structural change in the troponin-tropomyosin complex. Thus, a greater $[Ca^{2+}]_i$ is required to bring about muscle contraction, or in other words to reduce myofilament responsiveness to calcium. These studies have also found a reduction of both myofilament Ca^{2+} sensitivity and maximal Ca^{2+} activated force. Since only limited proteolysis of troponin I occurs (by calpain I), this would not affect the upstream mechanisms controlling $[Ca^{2+}]_i$ (Gao et al., 1995). Thus, the ability of the stunned myocardium to respond to inotropic stimuli with a normal contractile reserve is explained (Gao et al., 1997). Turnover of troponin I takes several days and this could explain the usual time of recovery of the stunned myocardium. Perhaps eprosartan helps the turnover of troponin I, hence the fast recovery of the myocardium contractility after perfusion of eprosartan.

4.5.4 Cardioprotective effect of eprosartan on the contractile protein activation due to sarcoplasmic reticulum dysfunction

The normal myocardial contraction-relaxation cycle depends on the proper functioning calcium release-uptake cycle. Release of Ca^{2+} from the sarcoplasmic reticulum stores causes the intracellular free Ca^{2+} concentration to rise. Through Ca^{2+} binding to troponin C contraction is generated. Relaxation is then achieved by sequestration of Ca^{2+} by the sarcoplasmic reticulum through Ca^{2+}-ATPase activity (Krause et al., 1989; Limbruno et al 1989). Krause et al., 1989, found that the sarcoplasmic reticulum isolated from stunned myocardium had decreased Ca^{2+} uptake ability and Ca^{2+}, Mg^{2+}-ATPase activity. This decrease in calcium uptake ability would result in less sequestration of Ca^{2+} and in turn less subsequent release from the sarcoplasmic reticulum stores (Krause et al 1989). Attenuated calcium release in turn would cause reduced contractile protein activation (Krause et al 1989). Thus, a dysfunction of the sarcoplasmic reticulum uptake ability could be a possible mechanism of atrial stunning. The effect of eprosartan on the release of Ca^{2+} from the sarcoplasmic reticulum stores through the blockage of angiotensin II receptor could attenuate stunning.

Since this hypothesis implies a decrease in $[Ca^{2+}]_i$ it may explain the ability of the stunned myocardium to achieve contractile function comparable to prestunning levels with the addition of the exogenous calcium or other inotropic agents (Ambrosio et al., 1989). The addition of inotropic agents like AT_1 antagonist (eprosartan) may therefore increase intracellular calcium concentration and contractile protein activation.

4.6 Clinical implications and limitations of the study

Atrial stunning has been implicated as an important pathogenic factor for cardioembolic stroke after cardioversion of chronic atrial arrhythmias. This process underscores the importance of postcardioversion anticoagulation. Incidence of AS after cardioversion of atrial fibrillation ranges from 36% to 80%. AS has been reported with all modes of conversion of AF or atrial flutter including transthoracic electrical cardioversion, low energy internal electrical cardioversion, pharmacological cardioversion, spontaneous conversion, conversion by overdrive pacing of atrial flutter, and radiofrequency ablation of atrial flutter. Even in patients with long standing AF, most of the AS resolves in about 46–72 h after cardioversion, which correlates with the clinical observation that most episodes of postcardioversion thromboembolism occurs

within this time frame after cardioversion of atrial fibrillation. This may have implications for determining the duration of anticoagulation therapy to prevent postcardioversion risk of thromboembolism in patients with atrial fibrillation of >48 h who successfully convert to sinus rhythm. Currently, the duration of such an anticoagulation therapy is of 4 weeks, although the atrial stunning is not of 4 weeks´ duration in all the patients who successfully convert to sinus rhythm.

Furthermore, atrial stunning due to lack of atrial contribution to cardiac output is attributed to the lack of improvement in cardiac output and, therefore, to the functional recovery of the patients immediately after cardioversion of atrial fibrillation, both of which improve as left atrial mechanical function improves over time. Although isoprenaline, acetylstrophenathidine, and dofetilide have shown to attenuate AS or to protect from its development, these studies are mostly done on animals.

The development of AS after the cardioversion of AF and AFL has been well documented. We have demonstrated that eprosartan may improve atrial mechanical function after DC shock. This may suggest that the atrial contractile apparatus is intact and functional in patients with AS after termination of AF. Although the present work has demonstrated that in atrial strips from AF patients, the recovery of the contractility of the muscle after DC shock may be improved with the help of eprosartan, a causative relationship between AF and the atrial contractile dysfunction has not been studied. We cannot rule out the possibility that a common underlying factor causes both the atrial contractile dysfunction and AS. However, the L-type Ca^{2+} current is the main determinant of contractile force and becomes down-regulated by AF. Therefore, it is reasonable to assume that electrical remodelling goes hand in hand with the atrial contractile dysfunction.

Although it has been demonstrated that eprosartan has a protective effect on electrically injured atrial strips, these measures were taken within few mitutes after DC shock. Whether the improvement in atrial mechanical function could be sustained with continued stimulation or the effects of these measures in the course of recovery of atrial mechanical function are important issues that have not been addressed in the present work.

4.7 Conclusion

In this in vitro experiment on atrial strips of patients with and without atrial fibrillation the recovery rate of the atrial strips with AF after DC shock was faster after the treatment with eprosartan (10^{-7}M). The results obtained also show that there are some differences in the effect of AT_1-antagonist (eprosartan) on AF and SR atrial strips. To date, the focus has been on AT_1 receptor antagonism because of demonstrations that long-term treatment with AT_1 antagonists acts cardioprotectively. The results suggest that short-term AT_1 receptor antagonism may be cardioprotective to human myocardium. One could therefore say that eprosartan may attenuate atrial stunning by preventing atrial remodelling in atrial myocardium of patients with AF after electrical injury. For this reasons the use of eprosartan for the improvement of atrial stunning in patients with AF after cardioversion should be examined clinically.

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6 Selected Abbreviations and Acronyms

ACEI: angiotensin converting enzyme

AERP: atrial effective refractory period

AF: atrial fibrillation

AFL: chronic atrial flutter

AI: aortic insufficiency

AR: adrenergic receptor

ASD: atrial septal defect

ATP: adenosine triphosphate

AS: atrial stunning

AVR: aortic valve replace

AVS: aortic valve stenosis

AVI: aortic valve insufficiency

AT I: angiotensin II receptor

ATPase: adenosinetriphosphatase

BDM: 2,3-butane-dione-monoxime

CABG: coronary arteries bypass graft

CHD: coronary heart disease

CP: creatine phosphate

dF/dt_{max}: maximum contraction velocity

dF/dt_{max} (%): $\frac{dF/dt_{max} * 100}{\text{Baseline } dF/dt_{max}}$

dF/dt_{min}: maximum relaxation velocity

dF/dt_{min} (%): $\frac{dF/dt_{min} * 100}{\text{Baseline } dF/dt_{min}}$

EF: ejection fraction

FFR: force-frequency relationship

G-protein: guanine nucleotide binding regulatory protein

FOC: force of contraction

FOC/A: force per unit area (tension)

FOC/A (%): $\frac{FOC/A * 100}{\text{Baseline FOC/A}}$

HIT: hibernation induction trigger

hKv1.5 (I_{kur}): human potassium channel

hERG (I_{kr}): human ether-a-go-go-related gene

IP_3: inositol 1,4,5-triphosphate

$I_{Ca:}$ L-type Ca2+ current

ISO-6: 10^{-6} Mol concentration of isoprenaline

ISO-7: 10^{-7} Mol concentration of isoprenaline

ISO-8: 10^{-8} Mol concentration of isoprenaline

ISO-9: 10^{-9} Mol concentration of isoprenaline l

K_{ATP} channel: ATP dependent potassium channel

KvLQT1 (I_{ks}): K+ channel gene

Kv4.3 (I_{to}): K+ channel gene

LA: left atrial

LAA: lA appendage

LAAS: LA appendage stunning

LVEF: left ventricular ejection fraction

L_{max}: muscle length

MAPK: mitogen activated protein kinase

MVR: mitral valve replace

NCX: Na^{+}/Ca^{++}-exchange current

NYHA: New york heart association

V_{os}: oscillatory potential

V_{ex}: non-oscillatory potentials

PKC: protein kinase C

PKA: protein kinase A

RA: right atial

SERCA-2: sarcoplasmic reticulum Ca^{2+}

SEC: spontaneous echo contrast

SR: sarcoplasmic reticulum

TI: tricuspidal insufficiency

Ical: L-type calcium channels

Itol: 4-aminopyridine-sensitive component

Icl(ca): Ca^{2+} activated and verapamil blocked Cl^{-}current

Iks: K+ channels

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8 Curriculum vitae

Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

9 Statutory declaration (Eidesstattliche Erklärung)

Hiermit erkläre ich, Samuel Oduro Okae an Eides statt, dass die vorliegende Arbeit von mir selbst und ohne unzulässige Hilfe Dritter verfasst wurde, dass sie auch in Teilen keine Kopie anderer Arbeiten darstellt und dass die benutzten Hilfsmittel und herangezogenen literarischen Quellen vollständig angegeben sind.

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Datum Okae, Samuel Oduro

10. Abstract

Objectives: The reduced mechanical function after termination of atrial fibrillation (AF) which is termed atrial stunning has been described after electrical (DC shock, overdrive pacing, catheter ablation), pharmacological, and spontaneous cardioversion of AF. This condition is responsible for an increased thrombo-embolic risk after cardioversion of atrial fibrillation. AT_1-antagonists have been reported to attenuate atrial remodelling in AF. Could these theoretically influence remodelling in electrically injured atrial strips? In the present work the cardioprotective effect of AT_1-antagonist, eprosartan on electrically injured (atrial stunning) atrial strips of patients with AF and SR was also studied and compared.

Methods and results: Atrial strips (n=28) from patients, who underwent cardiac surgical operations were used for this experiment. These samples were classified into two groups. The AF group (n=11) comprised of samples from patients with atrial fibrillation and the SR group (n=17), patients with sinus rhythm. The AF and the SR groups were studied under similar conditions. During the five stages of the experiment the samples went through the following stages: (1) Frequency stimulation (0.25Hz-2Hz), (2) Inotropic stimulation with Isoprenaline (3) Induction of electrical injury on the muscles with a direct current (DC) of 15 volts for 60 seconds (4) Recovery of the muscle after the electrical injury without eprosartan perfusion and (5) Recovery of the muscle after a repeated induction of electrical injury with eprosartan perfusion.

The FOC/A, maximum contraction velocity (dF/dt_{max}), and maximum relaxation velocity (dF/dt_{min}) were measured.

The FOC/A of the AF group treated with eprosartan recovered significantly ($p<0.05$) after DC shock by reaching 91.49 ± 5.56(%) of the baseline FOC/A (%) as compared to the untreated AF which attained 72.02 ± 9.04%. The dF/dt_{max} and dF/dt_{min} also showed some significant changes before and after treatment with eprosartan in the AF group.

Conclusion: Eprosartan significantly improved the recovery of atrial strips of patients with atrial fibrillation after DC shock indicating that eprosartan may have a protective effect on DC shocked atrial strips. For this reason the use of eprosartan for the improvement of atrial stunning in patients with AF after cardioversion should be examined clinically.

Key Words: Atrial stunning, atrial fibrillation, electrical cardioversion, angiotensin receptor antagonists, Eprosartan.

11 Zusammenfassung

Hintergrund: „Atrial stunning“ ist eine verzögerte Wiederherstellung der Vorhofmuskelfunktion nach einer elektrischen („DC shock“, „overdrive pacing“ Kather ablatio), Pharmakologischen und spontanen Kardioversion von Vorhofflimmern. Das thromboembolische Risiko ist dabei erhöht. Es ist berichtet worden, dass AT_1-antagonist, „atrial remodelling“ bei Vorhofflimmern abschwächen. Könnte diese Eigenschaft Einflüsse auf elektrisch verletzte Vorhofmuskelstreifen haben? Des Weiteren wurde in den vorliegenden Experimenten die protektive Wirkung von Eprosartan auf eine elektrische Schädigung an Vorhofmuskelstreifen von Patienten mit und ohne Vorhofflimmern untersucht.

Methode und Ergebnisse: Die verwendeten Vorhofmuskelstreifen (n=28), wurden von Patienten im Rahmen einer Herzoperation gewonnen wurden. Die Proben wurden entsprechend dem Vorhandensein bzw. nichtvorhandensein von in zwei Gruppen eingeteilt: AF-Gruppe (n=11) und SR-Gruppe (n=17). Folgende Bedingungen wurden untersucht: (1) Kraft-Frequenz-Beziehung (0,25Hz-2Hz), (2) inotrope Stimulation mit Isoprenalin, (3) elektrische Schädigung der Muskeln durch Anlegen einer hohen Gleichspannung von 15 Volt für 60 Sekunden, (4) Erholung der Muskelfunktion und (5) Genesung der Muskeln nach wiederholter elektrischer Schädigung unter Einfluß von Eprosartan. Die Kontraktionskraft (FOC/A), die maximale Kontraktionsgeschwindigkeit (dF/dt_{max}) und die maximale Relaxationsgeschwindigkeit (dF/dt_{min}) wurden fortlaufend gemessen.

Die Kontraktionskraft der mit Eprosartan behandelten AF-Gruppe erreichte nach der Schädigung in 25 Minuten durchschnittlich 91,49 ± 5,56 % von der Ausgangslage und damit signifikant mehr als die unbehandelte AF-Gruppe 72,02 ± 9,04%. Die dF/dt_{max} und dF/dt_{min} bei der AF-Gruppe zeigte ebenfalls eine signifikante Änderung durch Eprosartan. Die Vorhofmuskelstreifen SR-Gruppe zeigten durch Eprosartan keine signifikante Änderung, ebenso auch nicht die Parameter dF/dt_{max} und dF/dt_{min}.

Zusammenfassung: Eprosartan verbessert signifikant die Genesung von atrialen Vorhofmuskelstreifen von Patienten mit Vorhofflimmern nach elektrischer Schädigung. Daher sollte die Verwendung von Eprosartan zur Verbesserung des „atrialen stunning“ bei Patienten mit Vorhofflimmern nach Kardioversion klinisch untersucht werden.

Stichworte: Atrial stunning, Vorhofflimmern, Kardioversion, AT_1-Antagonist, Eprosartan.