THE EFFECT OF GENDER DIFFERENCES AND PINACIDIL (ATP SENSITIVE POTASSIUM CHANNEL OPENER) ON THE ISCHEMIA – REPERFUSION INDUCED ARRHYTHMIAS IN RATS

by

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ABSTRACT

THE EFFECT OF GENDER DIFFERENCES AND PINACIDIL (ATP SENSITIVE POTASSIUM CHANNEL OPENER) ON THE ISCHEMIA – REPERFUSION INDUCED ARRHYTHMIAS IN RATS

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K_{ATP} channels play important roles in the generations of ischemia-reperfusion induced arrhythmias. There are still controversy about the opening or closing of the channels involve in the suppression of the arrhythmia. Since the number of K_{ATP} channel is more in female, arrhythmic response to the reperfusion may be different in females. There are a few study indicating the effect of K_{ATP} channel opening on the arrhythmias following coronary ligation in female. That is why, in this study the effect of gender differences on reperfusion induced arrhythmia and the role of the K_{ATP} channel opening was researched.

Seventeen male and eighteen female rats weighing 210-280 gr in 6-8 months old were used in this study. Myocardial ischemia was produced by ligation of LAD and reperfusion by releasing of this artery. Pinacidil was given

intravenously in a dose of 1mg / kg, 10 min. before coronary artery ligation. Electrocardiogram (ECG) and arterial blood pressure were recorded during ischemia and reperfusion.

The total length of arrhythmia, VF and VT observed following ligation and reperfusion were significantly low in female than males ($P < 0.05$). Pinacidil increased arrhythmia in female, but oppositely decreased arrhythmia in male. Arrhythmia score was significantly decreased in pinacidil treated male rats ($P < 0.05$) ; 3.7 in control and 2.0 in pinacidil treated group.

As a result, opening of K_{ATP} channel was not effective to decrease arrhythmia in female, but decreased arrhythmia in male. This opposite effect may be derived from more K_{ATP} channel found in female. Opening of the channel by pinacidil in male may decrease electrical inhomogenity between ischemic and nonischemic region and may lead to the decreased arrhythmia.

Key words: Coronary Ligation, Ischemia – Reperfusion, Arrhythmia, Gender, Pinacidil.

ÖZET

SIÇANLARDA İSKEMİ – REPERFÜZYONLA UYARILAN ARİTMİLER ÜZERİNE CİNSİYET FARKLILIĞININ VE PİNASİDİL' İN (ATP DUYARLI POTASYUM KANAL AÇICISI) ETKİSİ

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İskemi ve reperfüzyon ile uyarılan aritmilerin oluşumunda K_{ATP} kanalları önemli rol oynamaktadır. Bu aritmilerin baskılanmasında, kanalların açılması ya da kapanmasının etkisi halen tartışmalıdır. Dişilerde fazla sayıda K_{ATP} kanalı bulunduğundan reperfüzyonu takiben oluşan aritmiler dişilerde farklı olabilir. Dişilerde koroner ligasyonu takiben oluşan aritmiler üzerine K_{ATP} kanallarının açılmasının etkisini belirten az sayıda çalışmaya rastlanmıştır. Bu nedenle bu çalışmada cinsiyet farklılığının reperfüzyonla uyarılan aritmiler üzerine etkisi ve bu etkide K_{ATP} kanallarının açılmasının rolü araştırılmıştır.

Araştırmada 210-280 gr ağırlıkta, 6-8 aylık, 17 erkek ve 18 dişi sıçan kullanıldı. Miyokardial iskemi sol koroner arter bağlanarak, reperfüzyon ise bu arter gevşetilerek oluşturuldu. Pinasidil, 1 mg / kg dozda, ligasyondan 10 dakika önce intravenöz olarak verildi. İskemi ve reperfüzyon boyunca,

elektrokardiyogram ve kan basınçları kayıt edildi. Reperfüzyon boyunca oluşan toplam aritmi süreleri ve yoğunlukları belirlendi. Dişilerde koroner ligasyon ve reperfüzyonu takiben oluşan toplam aritmi süreleri, VT ve VF süreleri erkeklere göre istatistiksel olarak anlamlı bir azalma gösterdi ($P < 0,05$). Pinasidil dişi sıçanlarda aritmileri artırdı fakat erkek sıçanlarda azalttı. Aritmi skoru pinasidil verilen erkek sıçanlarda belirgin bir şekilde azaldı ($P < 0,05$) ; kontrol grubunda 3.7, pinasidil verilen grupta 2.

Sonuç olarak, K_{ATP} kanallarının açılması dişilerde aritmileri azaltmada etkili olmadı, ancak erkeklerde aritmileri azalttı. Bu zıt etki dişilerde K_{ATP} kanallarının daha fazla olmasından kaynaklanabilir. Erkek sıçanlarda pinasidil ile kanalların açılması iskemik ve iskemik olmayan bölgeler arasında elektriksel heterojenliği azaltabilir ve bu nedenle erkek sıçanlarda daha az aritmi oluşmasına neden olabilir.

Anahtar Kelimeler: Koroner Ligasyon, İskemi-Reperfüzyon, Aritmi, Cinsiyet, Pinasidil.

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1. INTRODUCTION

Cardiovascular disease is a major cause of death in both men and women in advancing age. The major reason of these death is the lethal arrhythmias that appear after the coronary occlusion or reperfusion. It has been known that potassium leakage from the ischemic cells cause the generation of these arrhythmias. On the other hand, the activation of K_{ATP} channels is an important part of endogenous cardioprotective signaling that promotes cellular survival under the ischemia. In recent years, research have been intensified on the role of potassium channel in the genesis of these arryhthmias. It is observed that oestrogen increase protection against ischemia-reperfusion induced arrhythmias (1) and the higher density of K_{ATP} channel was higher in female than male (2).These findings is thought that ischemia and reperfusion arrhythmias may be affected from gender differences. But experimental research have been usually performed on male animals. There are very few studies indicating different effect of gender on the arrhythmias induced by ischemia and reperfusion (3).

In this study, it is aimed to research the different effect of potassium channel opening by pinacidil on ischemia and reperfusion induced arrhythmias in both gender. Our hypothesis is that if the number of K_{ATP} channel in myocardial cell of female is higher than male, the effect of pinacidil on ischemia reperfusion induced arrhythmias will be different in both gender.

1. 1. ELECTROPHYSIOLOGY OF THE HEART

1.1.1. ANATOMY OF THE HEART

The mammalian heart has four chambers; two on the left side, left atrium and ventricle and two on the right side, right atrium and ventricle. The atria and ventricle are separated by atrioventricular valves which prevent blood flow backwards into the ventricle. The AV valve between right atria and ventricle is called as mitral valve and between the left atria and ventricle as tricuspid valve, **Figure 1.1**. Furthermore, there are semilunar valve between the aorta and left ventricle and between the pulmonary artery and right ventricle that prevents back flow to the ventricle during the diastole.

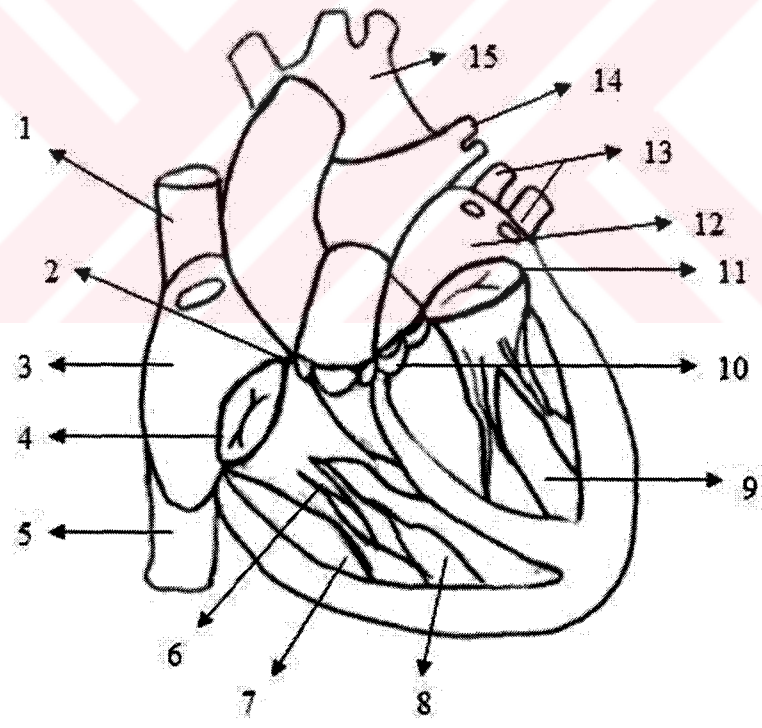

Figure 1.1. Anatomy of the heart. 1) Superior Vena Cava, 2) Pulmonary Valve, 3) Right Atrium, 4) Tricuspid Valve, 5) Inferior Vena Cava, 6) Chorda Tendinea, 7) Right Ventricle, 8) Papillary Muscle, 9) Left Ventricle, 10) Aortic

Valve,11) Mitral Valve, 12) Left Atrium, 13) Left Pulmonary Veins, 14) Left Pulmonary Arteries, 15) Arch of Aorta

The basic function of the heart is to pump blood from ventricles to the pulmonary and systemic circulation. The deoxygenated venous blood continuously flow to the right side of the heart by the superior and inferior vena cava. Then it is ejected from the right ventricle to the lungs. Oxygenated blood from lung return to the left atria. In every heart beats, ventricular contraction is triggered by a stimuli originated from sino-atrial nodes that is a collection of specialized cells in the right atrium.

The electrical impulses spreads rapidly to the right and left atria and then to the atrioventricular node by specialized conducting fibers, **Figure 1.2**. The impuls slows down in the AV node and then flows down through a specialized bundle of conducting fibers, that is called as His bundle. His bundle include two major branches, the left and right bundles that conduct the electrical impulses into the right and left side of ventricules. Finally, the electrical impulses are transmitted to the purkinje fibers that stimulate myocardial cells of the ventricles.

The majority of myocardial cells are contractile cells that mainly include contractile proteins. Some of the myocardial cells are specialized to generate automatic stimuli that are localized in sino-atrial and atrioventricular nodes. Some of the myocardial cells are only speciliazed to conduct electrical impulses.

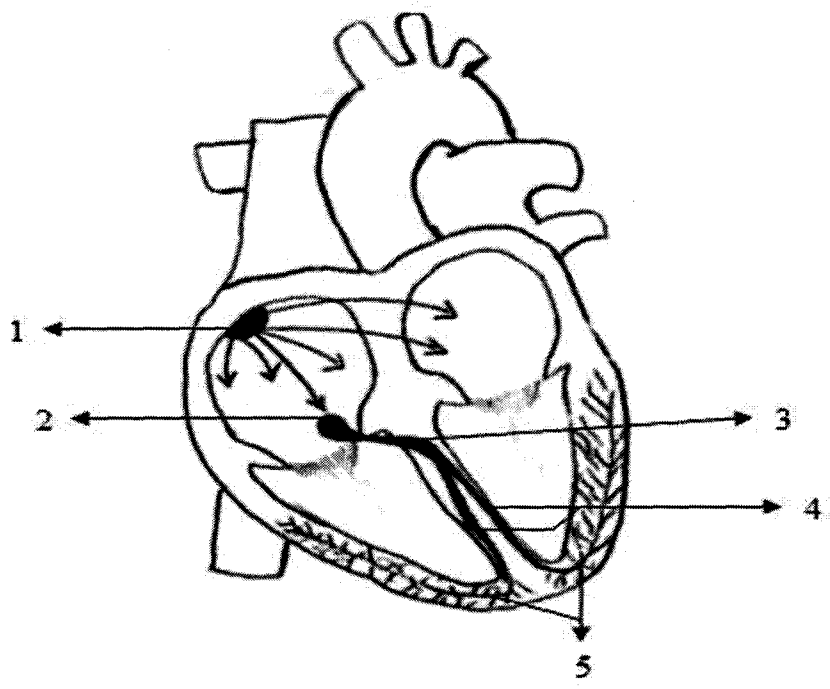

Figure 1.2. Conducting pathways of impulse in the heart. 1) SA node, 2) AV node, 3) His Bundle, 4) Left and right Bundle Branch, 5) Left and right Purkinje Fibers

Myocardial cells are supplied for nutrients by coronary arteries. Coronary arteries are originated from the base of aorta. There are two main coronary arteries, the right and the left. The right coronary arteries supply to the right side and left coronary artery to the left side of the heart.

1.1.2. ELECTROPHYSIOLOGIC PROPERTIES OF MYOCARDIAL CELLS

The automatic cells have some different electrophysiologic properties in respect to other cells, **Figure1. 3**. These cells can not maintain a stable resting potential and can not remain long in repolarized state. Automatic slow depolarization occur just following the repolarization in this type of cells. Contractile cells have rapid depolarization and long action potential in contrast to automatic cells. The resting potential of contractile cell is more

negative than the automatic cells. Rapid depolarization occur in contractile cells. There is a plateau phase following the peak of action potential. Conducting cells, purkinje and bundle cells, have rapid depolarization but short plateau phase and no automatic activity. The resting potential of these cells are similar to the contractile cells.

1. 1. 3. AUTONOMIC ACTIVITY OF THE HEART

The duration of action potentials are much longer in cardiac cells than nerve cells or other tissue cells. Two main types of action potentials can be recorded in heart. One type of action potential recorded in atrial, ventricular and purkinje fibers has rapid upstroke velocity that is called as fast response. The other type of action potential recorded from the sinoatrial node and atrioventricular node has slow upstroke velocity that is called as slow response.

1. 1. 3. 1. SLOW RESPONSE

The action potential in autorhythmic cells start with a slow continuous influx of Na^+ and Ca^+ ions and reduced efflux of K^+ ions. Due to the slow inward currents, the membrane gradually becomes more positive and the cell reach to the threshold level. When the threshold is reached, fast Ca^{+2} and Na^+ channels are open and action potential is produced, **Figure 1.3-B**. As the action potential reach to peak, K^+ channels are open, resulting in rapid efflux of K^+. This efflux of potassium repolarized the cell even later lead to hyperpolarization. During the repolarization, Na^+ / K^+ pump works and replaced the Na^+ and K^+ with each other. The hyperpolarization of the cell

triggers the slow sodium and calcium channel to open and again lead to the generation of another new automatic stimuli.

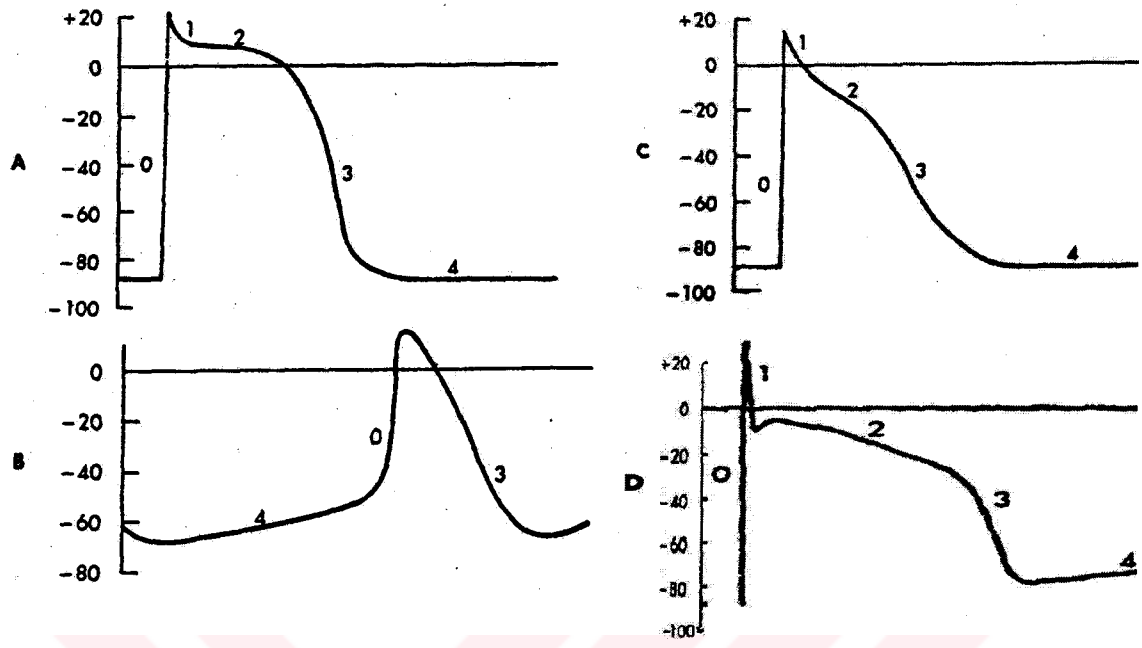

Figure 1. 3. Typical action potential (mV) recorded from cells in ventricle (A), sinoatrial node (B), atrium (C), purkinje fiber (D). From Mosby Berne and Lewy (4).

1. 1. 3. 2. FAST RESPONSE

In the fast response, the duration of action potential is longer than slow response. It is generated in myocardial muscle cell that is stimulated by a stimuli reaching from automatic or conducting cell. During the depolarization, Na^+ ions from the automotic cells move through the gap junctions to the adjacent contractile cells. The entrance of Na^+ ions into the neighboring cells creates a small voltage change that initiate depolarization. In the cell having fast response, the action potential starts with the opening of fast Na^+ channels. There are four phase of action potential in these cells. In phase 0 ; rapid Na^+ influx occur through rapid Na^+ channel and action potential reach

to peak. Just following the peak, there is a slow decline in action potential that occurs by influx of Cl^- ions. This phase is called as phase 1. Following phase 1, slow inward Ca^{+2} current occur and the cell remain long in depolarized state that is called as plateau phase. At the end of plato phase, slow Ca^{+2} channels start to close and K^+ channels to open that result in rapid repolarization. As the cell reach to the resting potential, Na^+ / K^+ pump works and replaced the ions with each other. These cells remain in repolarized state until the new stimuli reach to them, **Figure 1. 3 - A, C, D.**

1. 2. MYOCARDIAL ISCHEMIA AND INFARCTION

1. 2. 1. CELLULAR CHANGES AFTER THE MYOCARDIAL ISCHEMIA

Occlusion of the coronary artery restricts the coronary blood flow to the myocardium. Myocardial ischemia occurs when the oxygen and substrate supply to the myocardium is less than its demand. Myocardial ischemia causes, several specific biochemical and metabolic alterations in cardiac cells. Following the ischemia, tissue oxygen content rapidly falls and the contraction abnormalities appear in 5 second as a result of inhibition of mitochondrial oxidative metabolism. Then intracellular high energy phosphate especially creatine phosphate and ATP stores are depleted. In the ischemic cells, aerobic respiration change to the anaerobic respiration. Acidosis progressively develops in 1 min of ischemia as a consequence of several catabolic pathways including anaerobic glycolysis, lipolysis and ATP hydrolysis. Adenosine increases during the ischemia and reperfusion by hydrolysis of high energy phosphate such as ATP, ADP, AMP. Adenosine mediates a variety of response such as dilatation of vessel through its activity

on specific receptors in myocytes, vascular smooth muscle cells, endothelial cells and neutrophils. The Na^+ / K^+ pump impair and Na^+ ions accumulate in myocardial cells due to the fall in ATP levels. Accumulation of the Na^+ ions activate Na^+ / Ca^{+2} exchange channels, so intracellular Ca^{+2} ions increase. The entry of Ca^{+2} into the ischemic cells may be harmful because Ca^{+2} activates phospholipases and proteases that impair the integrity of the cell membrane. Then the ischemic injury occurs due to the deleterious effect of catabolite accumulation including lactate, arachidonic acids and purine nucleosides.

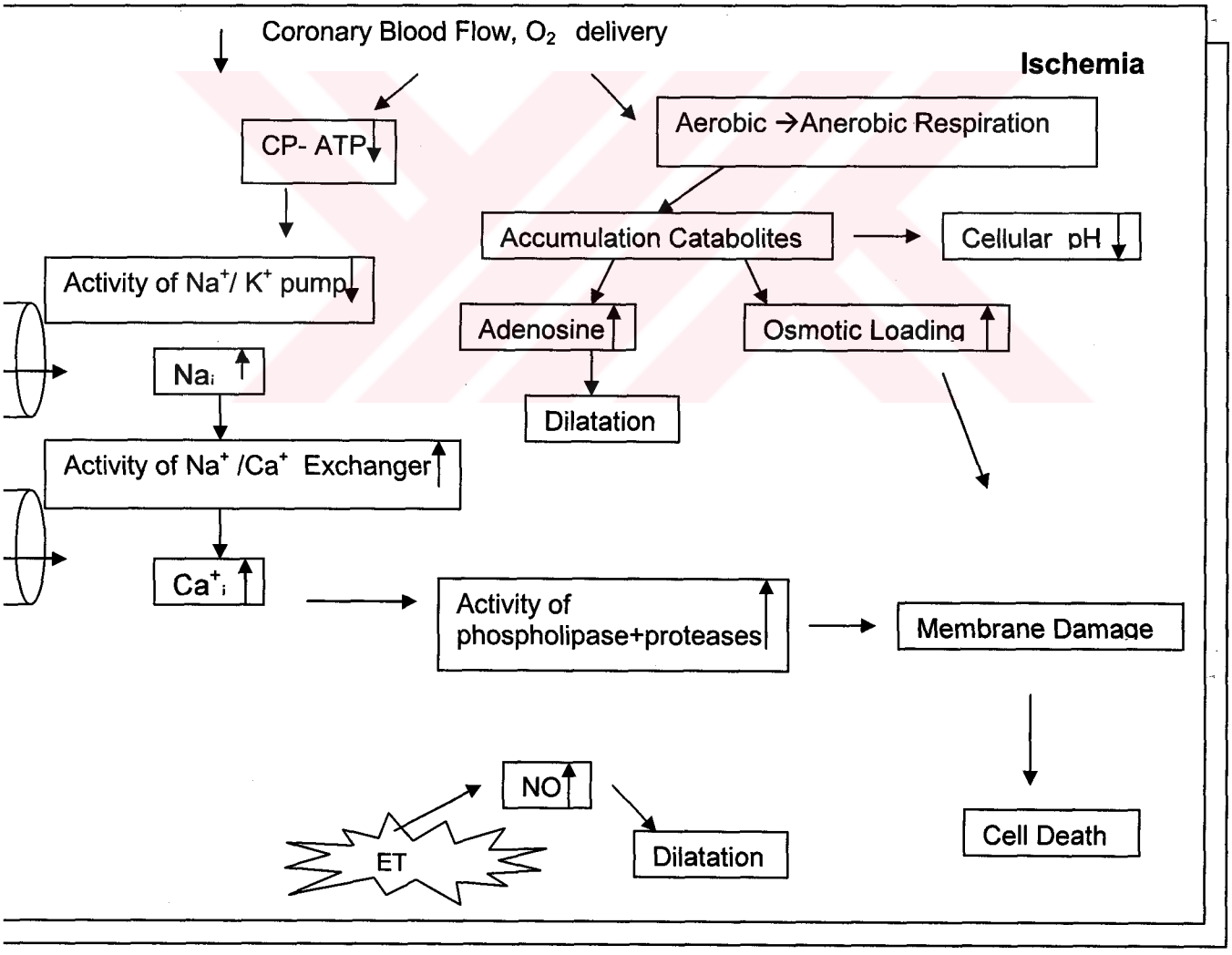

Figure 1. 4. Cellular changes after the myocardial ischemia. Creatine Phosphate (CP), ET (Endothelial), NO (Nitric Oxide).

Arachidonic acid is one of the fatty acid that may produce reactive oxygen species. Free oxygen radicals damage cell membranes and contribute to cell death (4), **Figure 1. 4**.

1. 2. 2. CELLULAR CHANGES AFTER THE REPERFUSION

The opening of occluded coronary artery that can be produced experimentally provide reperfusion. Reperfusion of myocardium cause more damage in ischemic myocardial cell. As a result, more severe ventricular arrhythmias can be observed. The washout of metabolites and electrolytes that have accumulated in the ischemic zone generates chemical and electrical gradients between ischemic and nonischemic zone. The electrophysiological derangement are probably responsible for reperfusion arrhythmias. Reperfusion is accompanied by changes in regional concentrations of K^+, Ca^{+2}, H^+, catecholamines and lysophosphoglycerides that are derived from degradation of membrane phospholipids (6). Reperfusion results with the osmotic cell swelling, intracellular Ca^{+2} overload, and especially the burst production of oxygen free radicals (7). Reoxygenation of the ischemic heart is associated with a burst of Reactive Oxygen Species (ROS) that occurs within a few seconds. The generation of ROS is a key process in development of reperfusion injury which result in arrhythmia, reversible contractile dysfunction (myocardial stunning), endothelial dysfunction and cell death (9). The burst of the ROS can be blocked by scavengers or antioxidants given at the time of reperfusion and provides rapid energy conversion (8). High amount of adenosine and its degradation products released during the reperfusion was particularly associated with ventricular fibrillation (9). Endothelial derived NO is a

powerful vasodilator that may improve blood flow during reperfusion (8), **Figure 1. 5.**

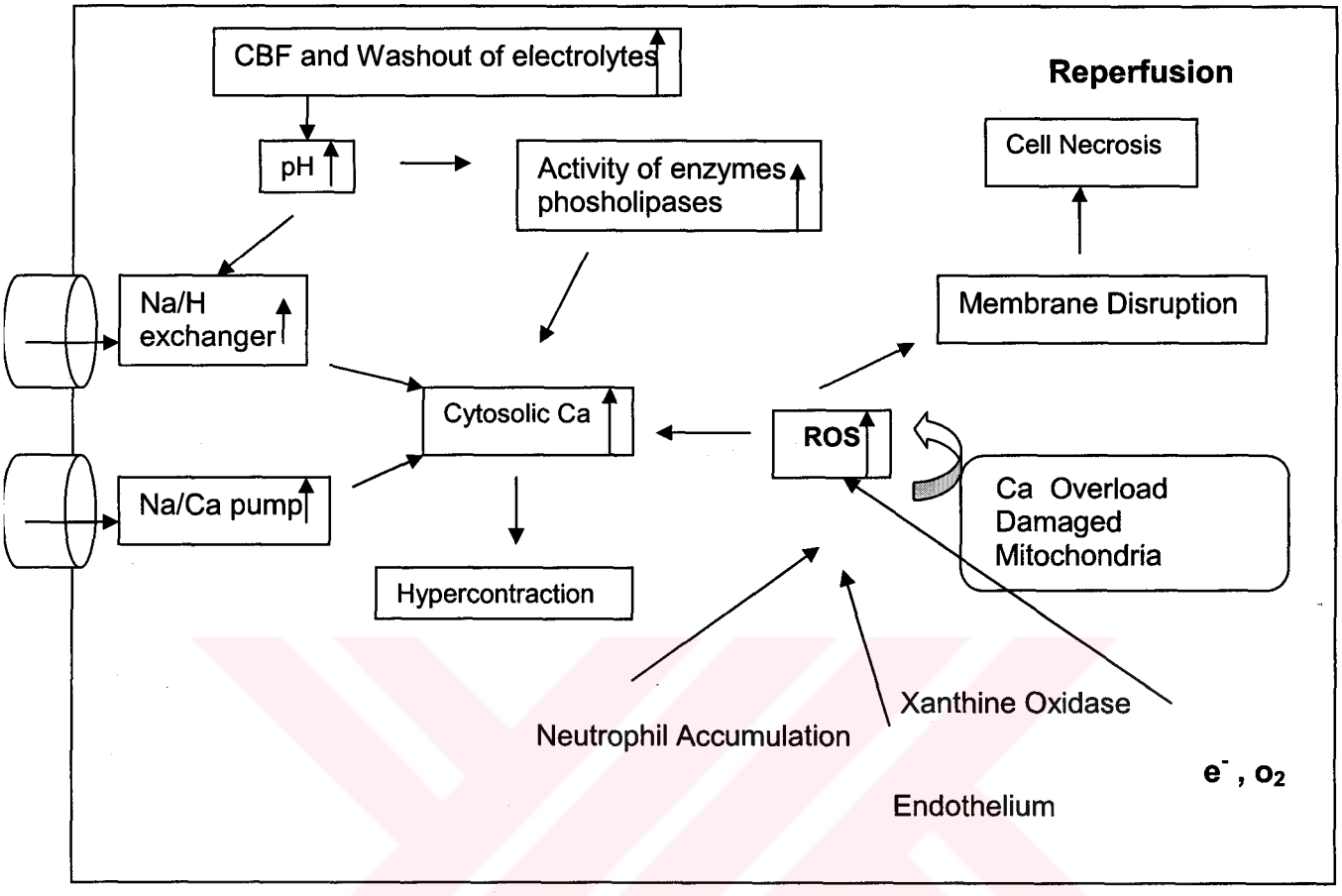

Figure 1.5. Cellular changes after the reperfusion. CBF (Coronary Blood Flow), ROS (Reactive Oxygen Species).

1. 3. HORMONAL AND METABOLIC CHANGES IN ACUTE-CHRONIC MYOCARDIAL INFARCTION

1. 3. 1. HORMONAL CHANGES

Arterial adrenaline concentration rises progressively during coronary occlusion and reperfusion (10). Ischemia results in the release of norepinephrine from adrenergic nerve endings and increase tissue levels of cAMP (11). Within the few minutes after the coronary occlusion, plasma and local catecholamine concentration increase in ischemic region (11). This

increased adrenergic activity deteriorates the myocardial function and triggers the arrhythmias following coronary ligation. Catecholamine induces the elevation of cAMP that increases Ca^{+} influx, and finally led to necrosis (12). Furthermore, catecholamine increase myocardial oxygen consumption and impair the ability of coronary arteries to dilate that result in reduced collateral blood flow. Catecholamines also increase afterload by peripheral constriction (13).

In myocardial ischemia, the level of thyronine (T3) decrease (14). This may be protective mechanism against ischemia. Increased adrenergic activity during myocardial ischemia reduce insulin secretion (14). This hormonal changes stimulate lipase activity that result in an increase FFA levels in blood. Increased blood FFA induces serious arrhythmias (15).

1. 3. 2. METABOLIC CHANGES

There is a relationship between the electrical activity of heart and ATP production by glycolysis. ATP synthesis in myocardial cell decrease due to the less oxygen supply. The fall in ATP stores promotes electrical instability and alterations in conduction. The accumulation of end products due to anaerobic glycolysis; lactate and H^{+} lead to the inhibition of the enzyme phosphofructokinase which limits the rate of glycolysis and ATP production (16). Metabolic inhibition also results in an increase catecholamine and cAMP. The content of glucose in ischemic myocardial cells are depleted due to its consumptions by anaerobic glycolysis. Then the cells tend to use free fatty acid that produced by catecholamines induced lipolysis. Both long chain acyl CoA and acyl carnitine which accumulate as a consequence of the

failure of β oxidation of FFA inhibit glycolysis. Several inventions demonstrated that the level of FFA increase during the ischemia that culminates the arrhythmias (17). Cyclic AMP also increases during the ischemia and leads to the arrhythmia (18).

1. 4. CARDIAC ARRHYTHMIA

1. 4. 1. DEFINITION

Normal autonomic rhythm of the heart is initiated by spontaneous impulse generation from SA node in equal interval. This spontaneous autonomic stimuli provides synchronic contraction of the heart which is termed as a sinusal rhythm. SA node have an intrinsic firing rate that is modulated primarily by changes in autonomic nerve activity. If there is a high level of vagal tone on the SA node, the rhythm is slow down and called as bradycardia. On the other hand, an abnormally high level of sympathetic tone on the SA node results in sinus tachycardia. Abnormality in impulse initiation and propagation or conduction cause to the abnormalities in electrical activity of the heart. These electrophysiological abnormalities induce arrhythmias. Various arrhythmias are created in response to ischemia and reperfusion. These arrhythmias may include sinusal bradycardia, tachycardia, bigeminal ventricular arrhythmia, idioventricular rhythm (salvo), ventricular premature contraction (VPC), ventricular tachycardia (VT), ventricular fibrillation (VF), **Figure 1. 6.**

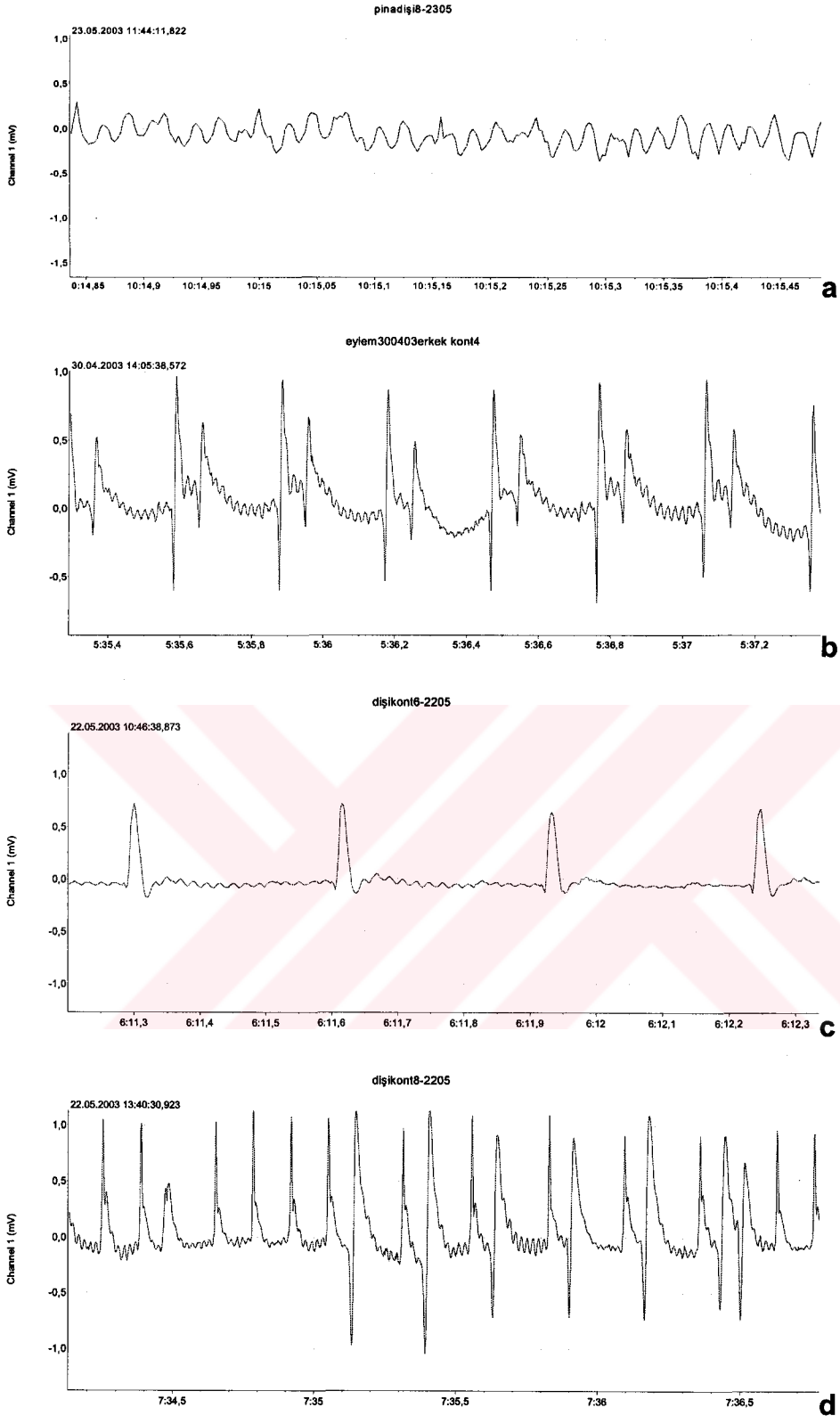

Figure 1. 6. a)Ventricular Fibrillation (VF), b)Ventricular Bigemini, c)Salvo d)Ventricular Premature Contraction (VPC).

1. 4. 2. MECHANISM OF THE ARRHYTHMIA

There are three mechanism of arrhythmias induced by ischemia and reperfusion. These are abnormal automaticity, triggered activity and reentrant circuits.

1. 4. 2. 1. ABNORMAL AUTOMATICITY

Abnormal impulse is generated by the ectopic pacemaker within the heart. The ectopic pacemaker is produced by the ischemic cell or increased automaticity in the conducting fibers. If the rate of impulse generation from ectopic foci is higher than the rate of normal pacemaker activity, normal pacing stimuli from SA node is blocked. The decreased pacing activity in SA node or delayed conduction through the myocardium also facilitate the formation of abnormal automaticity. Normally atrial and ventricular fibers do not exhibit automaticity. In some cases an abnormal automatic stimuli may develop. Abnormalities both of impulse conduction and generation induce the escape rhythms in non-automatic site.

1. 4. 2. 2. TRIGGERED ACTIVITY

Another type of abnormal impulse formation results from abnormal depolarization during diastole of the myocardial cells. Triggered activity means the abnormal action potentials triggered by a preceding action potential. The abnormal impulses are seen as spontaneous depolarizations that occur during either phase 3 or 4 of preceding action potential. There are two types of afterdepolarizations, early and delayed. Early afterdepolarizations that is series of rapid and prolonged series of depolarizations occur during

Phase 2 or 3. This form of triggered activity is more likely to occur when the action potential duration is increased. Therefore, drugs that decrease action potential duration (e.g., lidocaine) are often effective against tachyarrhythmias generated by this mechanism. Calcium channel blockers can also be effective in their treatment. Delayed afterdepolarizations occur after Phase 3 when the action potential is fully repolarized. The mechanism is poorly understood; however, it is found to be associated with high intracellular Ca^{++} concentrations as occurs with digitalis toxicity or excessive catecholamine stimulation. The triggered impulse can lead to a series of rapid depolarizations, leading to tachyarryhthmias, **Figure1. 7.**

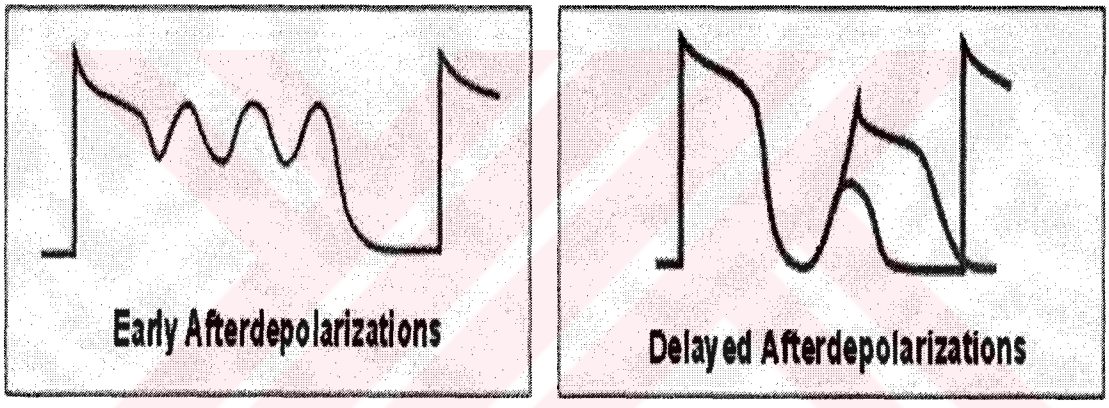

Figure 1. 7. Early and Delayed Afterdepolarization.

1. 4. 2. 3. REENTRANT CIRCUIT

These arrhythmias occur due to the conduction block in the myocardium. Conduction blocks may occur due the ischemia or abnormal stimulation of myocardial cell. It can cause either bradycardia or tachycardia. When the impulse propagation is slow down in a localized region of myocardium, this region remain long in depolarized state, even the other region of the heart is completely repolarized. This kind of long depolarization

may capture the surrounding myocardium in repolarized state and stimulate them before pacing stimuli. Different refractoriness that develops due to the ischemia may lead to the re-entry circuit. If the re-entry circuit continuously occur from a single region, it produce unifocal tachycardia. But if it is originated from various region, it leads to the various irregular localized contraction called as multifocal tachycardia. If the number of re-entrant circuit is many, fibrillation occurs, **Figure 1. 8.**

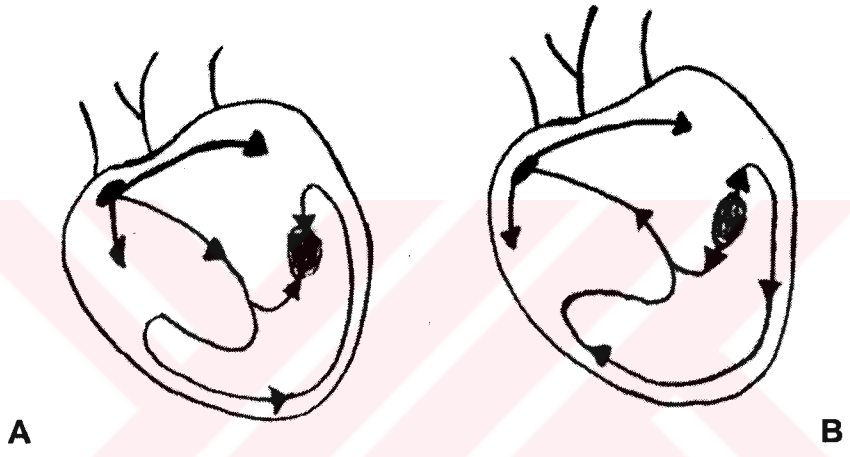

Figure1. 8. A) Blockage of sinusal impulse in ischemic region, B) Stimulation of heart with the reentry impulse from the ischemic region.

Reentrant circuit may occur between the atria and ventricles or locally within a small region of the ventricle or atrium. The localized reentry involves the presence of a unidirectional block within a conducting pathway. When the transmission slow down or block in this pathway, the normal pacing stimuli pass around the blocked region. Then this region generates re-entrant stimuli

1. 4. 3. ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS

There are two period of arrhythmia in all experimental animals; early and delayed arrhythmic periods. In big animals, early phase of arrhythmia occurs during the 30 min of coronary occlusion, this phase frequently culminate in ventricular fibrillation. This period is followed by a phase which do not have any arrhythmia that is named quiescent period. In delayed phase, the arrhythmia starts about 4-8 hours after ligation and last 2 to 4 days (19). In smaller animals, first arryhthmia starts in 3-4 min and late is in 6-12 min of the ligation (20,21).

In the early phase of ischemia, the myocyte is not extremely impaired but the time of depolarization and repolarization in ischemic cells varies. In these regions, transmission of the impulses slow down or completely blocked. This factors induce the conditions necessary for reentrant type of arrhythmia (22). However the K^+ leakage out of the ischemic cell increase and K^+ accumulates in the extracellular space that leads to the depolarization of normal myocardial cell in nonischemic zone (23). In the later phase of ischemia, energy requiring process of depolarization and repolarization extremely slow down and ischemic myocardial cell remain in depolarized state. These cells generate ectopic stimuli for normal myocardium. Integrity of the cell membrane completely impair and ischemic depolarization disappear. In the very late phase of ischemia, cell death occurs and ischemic cells replaced with necrotic tissue that is associated with progressively decreasing arrhythmia (24).

Reperfusion arrhythmias can occur within the seconds of reperfusion. There is a direct relationship between the incidence of reperfusion arrhythmia

and the ischemic period. There are two main theories for underlying mechanism of these arrhythmias, excess cytosolic Ca^{+2} and formation of ROS. Increased cytosolic Ca^{+2} may contribute to the delayed afterdepolarization. On the other hand generation of ROS plays a pivotal role in the reperfusion arrhythmias (25). All of these events cause the heterogeneity in ischemic cells that lead to more severe arrhythmias (26) .

1. 5. ATP SENSITIVE POTASSIUM CHANNEL (K_{ATP} CHANNEL)

K_{ATP} channels were first described by Noma in cardiac ventricular myocytes (27). Several investigators have shown that K_{ATP} channels play a key role in the regulation of cardioprotective mechanism. The mechanism underlying the protective effect of K_{ATP} channel activation is not completely understood. These channels are very sensitive to intracellular ATP concentration. The activity of the K_{ATP} channels are normally suppressed by physiological levels of intracellular ATP. But its activation is modulated by various stimuli such as decreased intracellular pH, increased free fatty acids and NO, activated G protein, released adenosine, and acetylcholine (28). K_{ATP} channels are closed state in normally oxygenated heart, however the open probability of these channels increases as a result of accumulation of ADP, lactate and H^+ ions inside the cell. An increased efflux of K^+ leads to reduction of action potential duration and decreasing of Ca influx into the cardiomyocytes (29). Decreased intracellular calcium that is associated with decreased myocardial contractility leads to preservation of ATP during ischemia. Such a mechanism generates natural protections against ischemia

that preserves the function of the myocardium (30). Cardiomyocyte contains two different types of K_{ATP} channels. One type is located in the sarcolemma ($sarK_{ATP}$) and the other type is located in the inner membrane of mitochondria ($mitK_{ATP}$) and that each had distinct pharmacological profile (31,32). Mitochondrial K_{ATP} and $sarK_{ATP}$ channels appear to exhibit minor differences in structure but the function of them are different. Mitochondrial K_{ATP} channels appear to be intimately involved in the control of matrix volume (33), but $sarK_{ATP}$ channels in the control of electrical activity of the cell. In this instance, opening of $mitK_{ATP}$ channels leads to membrane depolarization, matrix swelling, slowing of ATP synthesis and accelerated respiration. Studies showed that mtK_{ATP} channels rather than $sarK_{ATP}$ channels is more sensitive to the openers and blockers (34). And there are selective openers for only $mitK_{ATP}$ such as diazoxide. Several reports have suggested that $mitK_{ATP}$ and $sarK_{ATP}$ channels function separetely in the modulation of infarct size and functional recovery and the $mitK_{ATP}$ channel but not $sarK_{ATP}$ modulate cell viability (35,36,37).

1. 5. 1. K_{ATP} CHANNEL OPENERS

K_{ATP} channel opening is more protective in the ischemic myocardial cells that can be mimicked by the K_{ATP} openers. K_{ATP} channel openers open K^+ channels in normal and ischemic myocardial cells. Opening of K_{ATP} channels, led to decreased Ca^{+2} entry during the plateau phase of action potential and subsequent shortening of action potential duration (APD) and cell membrane hyperpolarization. ATP preservation occurs with the

prevention of intracellular Ca^{+2} accumulation (38,39). Therefore, many researchers named K_{ATP} openers as indirect Ca antagonist. Protective effects of openers are abolished by K_{ATP} blockers. Although openers have been indicated to provide protection against the arrhythmia, some investigator suggest openers to be proarrhythmic (40,41). There are several reason for the proarrhythmicity of openers. Since openers shorten the APD, it may contribute to generation of reentrant arrhythmias observed in several in vivo (40) and in vitro (41) studies. The other possible mechanism may include beta adrenergic stimulation. D'alonzo et al have shown that the proarryhthmic effects of pinacidil are not mediated directly through activation of K_{ATP} channel, but may be indirectly mediated through an enhancement of catecholamine release. One hypothesis is that increased K concentration around the adrenergic nerve terminals might lower threshold for transmitter release by causing depolarization of membrane and enhancing catecholamine release during the ischemia (42). There are several possible mechanism for the protection of openers that are suggested by many studies. First, APD shorthening induced by openers may inhibit the abnormal impulse generation (43). Second, it may increase recovery of postischemic function, improved energy status and reduced necrosis (38,44, 45). Several studies performed in various species such as dog, rabbit, rat, pig showed that K_{ATP} openers reduce infarct size (46,47,48). K_{ATP} channel openers were more potent as vasodilators which reduced reperfusion contracture and improved reperfusion flow. There have been numerous in vivo studies showing these agents causes hypotension (49). Lower blood pressure causes increase in heart rate due to the enhanced sympathetic tone. The proarrhythmic effects

of K_{ATP} opener in global ischemia were associated with enhancement of catecholamine release in isolated heart (42). One possible mechanism for their protection was coronary vasodilatation. Several investigators showed that cardioprotective doses of K_{ATP} channel openers had no effect on coronary collateral blood flow, although they did enhance reperfusion blood flow (46-48). A number of ligands such as adenosine, acetylcholine interact with G protein that increase K_{ATP} channel open probability. Adenosine may also activate protein kinase C through the A_1 receptor and it is thought that PKC activates K_{ATP} channels by reducing channel sensitivity to ATP. Recent studies shown that extracellular ATP can enhance K_{ATP} current via a P_2 (purinergic) receptor, through activation of adenyly cyclase (50). In addition, some of the studies showed that K_{ATP} openers mimic ischemic preconditioning (IP). Yao and Gross (51) showed that K_{ATP} channel openers, significantly lowered the threshold for PC in a canine model. The effect of IP is observed to be abolished by K_{ATP} channel blockers (52). It has been suggested that K_{ATP} channel openers protect ischemic myocardium by increasing adenosine production secondary to activation of ectosolic 5'nucleotidase (53). Adenosine A_1 receptor stimulation inhibits PKA and can activate PKC, both of which are known to be involved in modulation of K_{ATP} channels. PKC activation involves in the PC that observed in several species (54).

Recently Liu et al found that the opening of K_{ATP} channel is modulated by the long chain fatty acids which are the major metabolic substrate of the heart (55). During the ischemia, oxidative phosphorylation is impaired and

affect the cytosolic concentration of FFA and corresponding acyl-CoA esters that change the activity of K_{ATP} channels.

1. 5. 2. K_{ATP} CHANNEL BLOCKERS

Sulfonylurea derivatives glibenclamide, glimepride are selective blockers of K_{ATP} channels which are used clinically as an antidiabetic drug (56). K_{ATP} channel blockers close the channels only in ischemic cell because these channel are closed state in normal myocardial cell. K_{ATP} channel blockers inhibits K^+ loss from the jeopardized cells and inhibit nonuniform shortening action potential duration during the ischemia and hypoxia (57).This effect decrease the development of electrical inhomogeneity between the ischemic and nonischemic myocardium and might suppress the reentrant pathways resulting in antiarrhythmic, antifibrillatory action (58). Another possible antiarrhythmic mechanism of the blockers may be hypoglycemia induced adrenergic activity (59,60) that may involve in ischemic preconditioning. This suggestion is supported by several in vivo and in vitro studies (60,61). On the other hand, K_{ATP} channel blockers increase severity of ischemia that is caused by Ca^{+2} loading into the myocardial cell. Furthermore, K_{ATP} channel blocker diminish coronary blood flow and this also potentiates ischemia (62).

1. 6. GENDER DIFFERENCES

The risk of heart disease in men increases with age. Premenopausal women have a significantly lower risk for coronary disease that it increases rapidly after menopause to levels comparable to that of their male counterparts. This protective effect in premenoposal women is believed to be due to an antiatherogenic action of female sex hormones on the lipid profile (63,64). Several studies have shown that estrogen protects the myocardium against myocardial infarction (1,65,66). Acute administration of estrogen has been shown to produce coronary dilation and enhanced the electrical stability of ischemia and reperfusion myocardium so the incidence of ventricular arrhythmias decrease (67). Chronic administration of estrogen restrict the oxidation of low density lipoprotein (68). The antioxidant activity of estrogen is thought to provide inhibition of membrane phospholipid peroxidation. Estrogen decreases the production of OH^- radicals in response to reperfusion (69). In clinical studies it is observed that estrogen therapy may be associated with antagonistic effect of Ca^{+2} because estrogen inhibits inward Ca^{+2} current (70). Estrogen causes an induction of NO synthase in myocardium which may produce vasodilation. The endogenous NO release during ischemia and reperfusion reduce myocardial injury (71). Several possible mechanisms may involve in benefical effects of NO on infarct size. First, NO may reduce the severity of ischemia by inhibiting the cytosolic accumulation of Ca^{+2} (72). Second, NO may reduce ROS generation by decreasing lipolysis, thereby limiting the generation of ROS through lipid peroxidation (73). NO may also protect the heart against VF that occur during reperfusion. Nitric oxide increase collateral blood flow, reduce oxygen

consumption, produce negative inotropic effect, inhibition of platelet aggregation and catecholamine release (70). Limiting of infarct size by estrogen was abolished by 5HD suggest that the cardioprotective effect of estrogen may result from activation of mitK_{ATP} channels (74). Estrogen activates PKC and G protein, which may link to the mitK_{ATP} channels.

Sympathetic affect is predominant in male than females in response to acute myocardial ischemia in rats (75). Higher sensitivity and density of α-adrenoreceptors were indicated to be found in females compared with males (76). Ischemia reduces neural norepinephrine release to greater extent in females than males. 17-β estradiol decrease myocardial necrosis, lower incidence of ventricular arrhythmias, preserve ventricular function (68). Recent studies have shown that female gender have more functional cardiac K_{ATP} channels than male (2). Activation of these channels in female under the metabolic stress condition may be reason of more endogenous protection that promote cellular survival .

2. MATERIALS AND METHOD

2. 1. ANIMALS

This study was performed on female and male Sprague-Dowley rats weighing 210-280 g and in 6-8 month old. The six animals were housed in one cage and allowed to have tap water ad *libitum* and animals were fed with commercial rat food. The animals were handled according to a protocol reviewed and approved by the ethical committe for the protection of animal research of the Hacettepe University-Ankara/Turkey.

2. 2. CORONARY ARTERY LIGATION AND REPERFUSION

Animals were anaesthetized with thiopental sodium (65mg/kg). Trachea was cannulated for artifical respiration. The catheter filled with saline including heparin inserted into the left carotid artery for measuring the blood pressure. The chest was opened in the fourth intercostal space and the heart was exposed. A loose loop of 5-0 atraumatic silk which had been passed through a cylinder shaped polyethylene tube was placed around the left main coronary artery, approximately 2 mm from its origin. Then the silk was led out through the tube. The heart was set back in its place and artificial respiration started using an animal respirator (Ugo Basile Rodent Ventilator, Italy) in 60 strokes/min. Subcutaneous needle electrodes were placed under the skin to record standart electrocardiogram. Then the animals were allowed to stabilize for 5 min. The ligature was tightened by clamping on the silk to produce the regional myocardial ischemia for 6 min and then the clamp was released 6 min. to produce reperfusion **Figure 2. 1**. In the survived rats the heart was perfused through the aorta with the solutions first NaCl and then ethanol to determine area at risk. After the perfusion of heart, the

nonperfused area that remained red was separated from the well perfused area that seemed white color. The non-perfused myocardium was cut and the percentage of this area to the total weight of ventricle was determined. Bipolar ECG and arterial blood pressure were continuously recorded during coronary ligation and reperfusion and stored in a computer for later analysis.

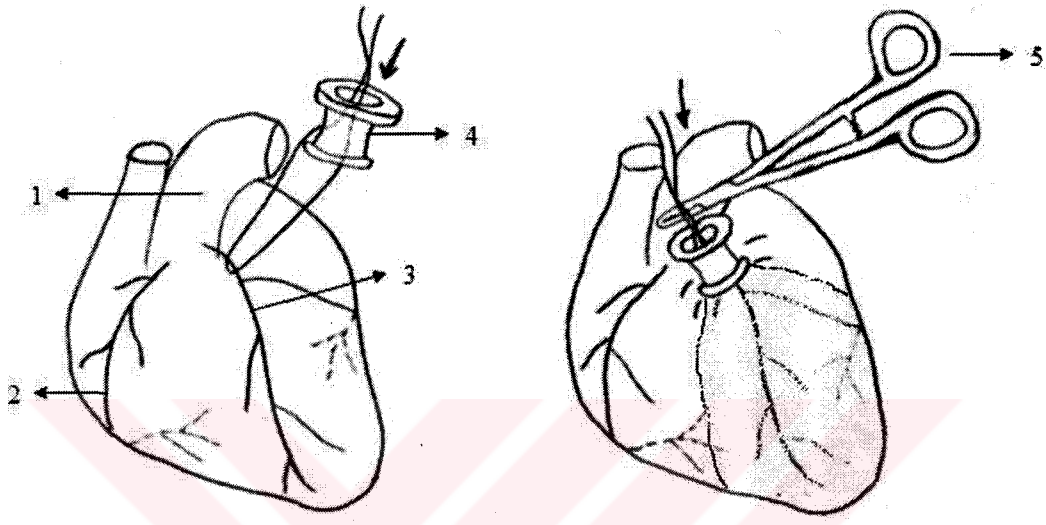

Figure 2. 1. A schematic drawing of the implanted loose coronary ligature.1) Aorta, 2) Right coronary artery (RAD), 3) Left coronary artery (LAD), 4) Polyethylene tube, 5) Clamp

2. 3. DRUG ADMINISTRATION PROTOCOL

Pinacidil was dissolved in isotonic saline solution and given in 1 mg/100μ/kg dose intravenously from cocygeal tail vein 10 min before coronary ligation. In control group, instead of the pinacidil, same value of isotonic saline was applied.

2. 4. THE EVALUATION OF THE ARRHYTHMIAS

Heart rate and blood pressure were recorded at 1, 3, 5 min during the ischemia and reperfusion. The incidence and the total length of arrhythmias

during six min of ischemia and reperfusion were calculated from recorded ECG. The onset and the offset of the arrhythmias were determined. Arrhythmic period was calculated by measuring the time interval between onset and offset of arrhythmias during reperfusion. The arrhythmias were classified as VT, VF and other type of arrhythmia including single ventricular extra beat, bigemini and salvos in accordance with lambeth convention. The sinusal tachycardia is differentiated from ventricular tachycardia according to the heart rate and blood pressure, **Figure 2. 2**. The arrhythmias were designated as sinusal tachycardia if HR was between 500 and 600 /min and the BP was higher than 70 mmHg and as ventricular tachycardia when the HR was above 600/min and BP was lower than 50mmHg.

An arrhythmia score was used to evaluate the incidence and duration of arrhythmias for each animals as follows; 0 = no arrhythmias; 1 = < 10 sec VT or other arrhythmias, 2 = 11- 30 sec VT or other arrhythmias, no VF; 3 = 31-90 sec VT or 10 sec reversible VF; 5 = > 180 sec VT or other arrhythmias and / or >10 sec reversible VF; 6 = irreversible VF.

2. 5. STATISTICAL ANALYSIS

The incidence of arrhythmia were compared by the chi-square test (Fisher's exact test, two tailed). The mean and standard errors were determined for all parameters including heart rate and blood pressure. The duration of arrhythmias and arrhythmia scores were compared by multiway analysis of variance (MANOVA combined with the LSD *post hoc* Test)

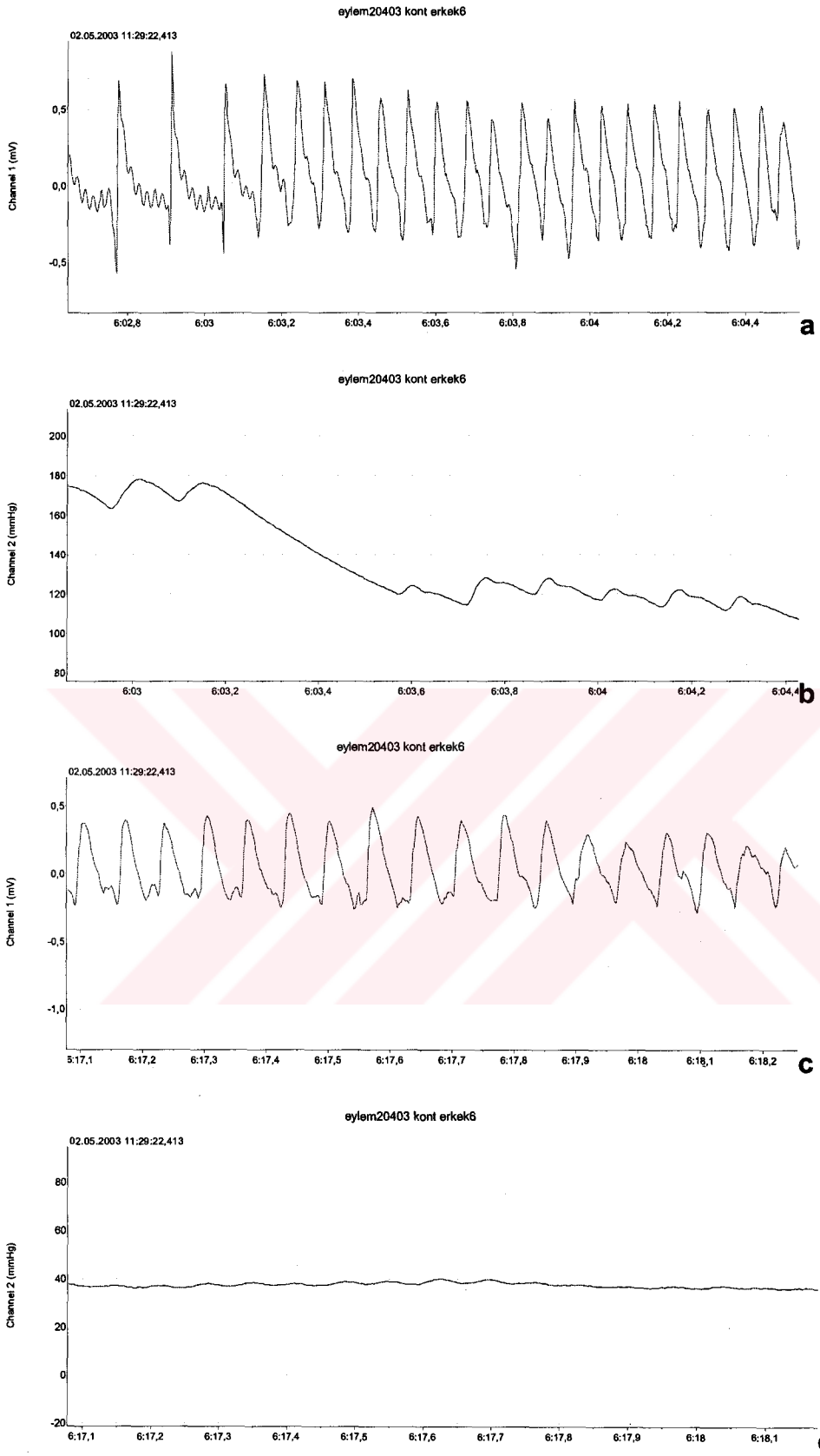

Figure 2. 2. Blood pressure (b), during sinusal tachycardia (a) and blood pressure(d),during ventricular tachycardia (c).

3. RESULTS

ST segment elevation or changes in the form of QRS complex were observed in all animals following the coronary ligation. ST segment elevation was evaluated as an electrocardiographic sign of ischemia. There was no infarct determined in unsuccessfully ligated rats. ST segment elevation was not also observed in these rats, **Figure 3. 1.** Reperfusion was not performed in rats when the blood pressure decreased below 70 mmHg during coronary ligation. All animals survived after 6 min of reperfusion.

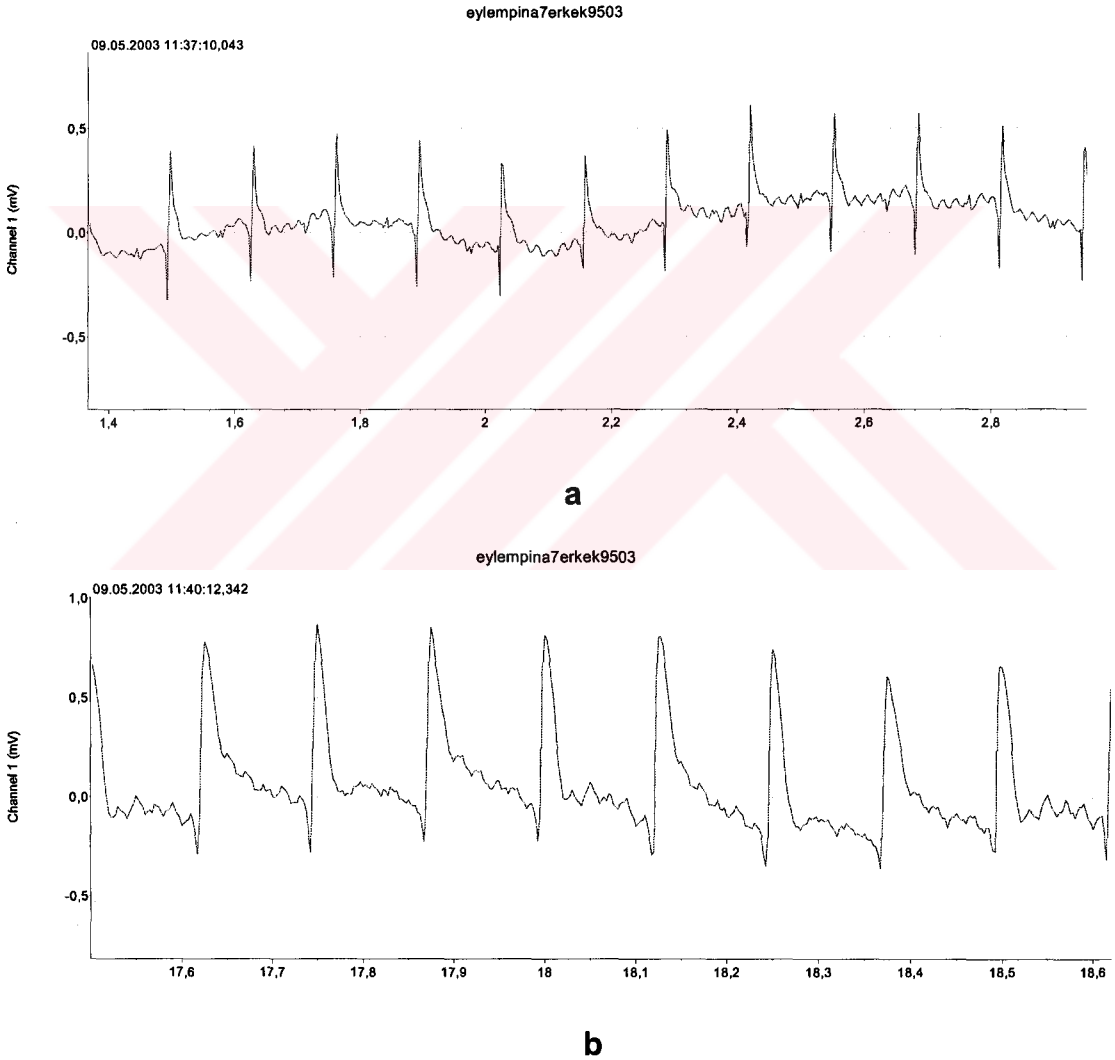

Figure 3. 1. ST segment elevation; (a) before and (b) after the ligation

Generally, arrhythmias started 3-5 minutes following ligation, and continued intermittently until reperfusion. Following the reperfusion, arrhythmias usually started early in male that was around 5-15 s, **Table 3. 3,** but it is delayed significantly in female (P < 0,055), **Figure 3. 2,** Although the arrhythmic period in reperfusion decreased in female and male pinacidil group in respect to their controls, they were not significant, **Figure 3. 3.**

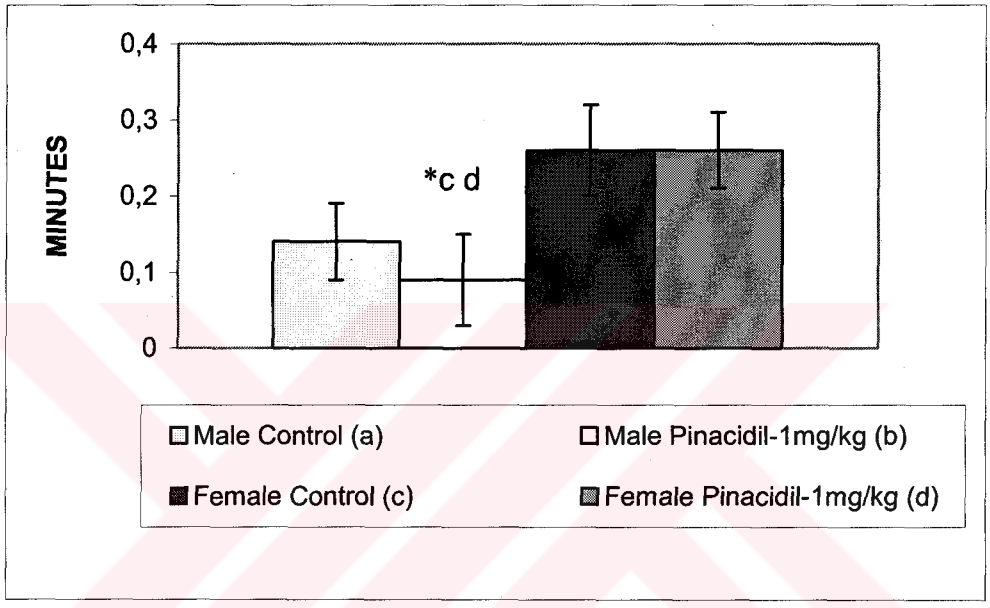

Figure 3. 2. Onset of arrhythmia during reperfusion

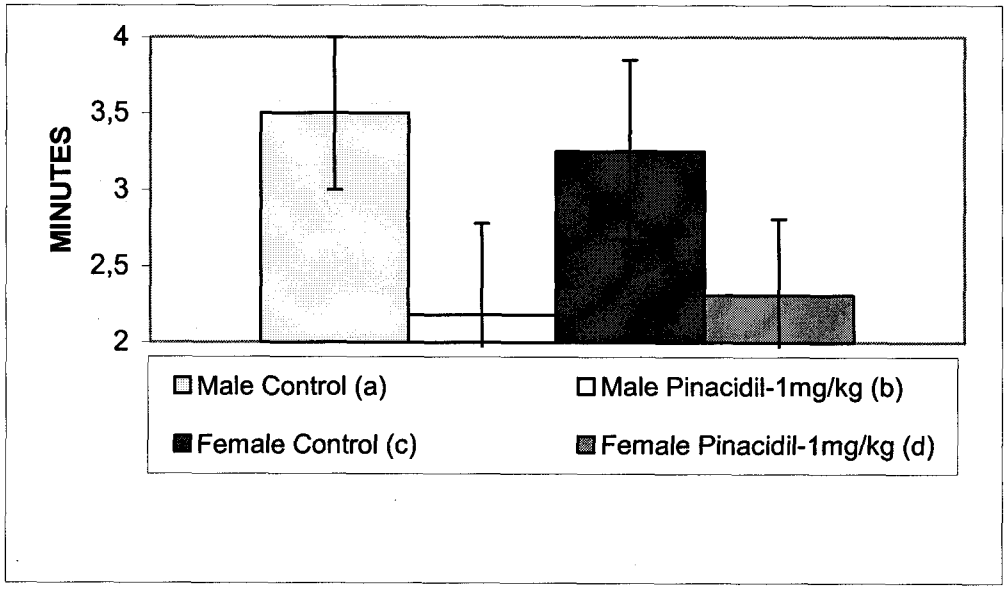

Figure 3.3. Arrhythmic period during reperfusion

Pinacidil decreased the blood pressure before ligation in both gender **Figure 3. 4.** Blood pressure decreased in all groups of animals following ligation and recovered earlier in pinacidil treated male toward the end of ligation, **Table 3.1**.

The heart rate in male was increased significantly after the treatment of pinacidil and it was sustained in high level at 1 min of coronary ligation. On the other hand there was no change in heart rate after the treatment of pinacidil in female, **Figure 3. 5**. The blood pressure gradually increased in all group of animals following reperfusion, **Figure 3. 6, Table 3. 2,** but this increase was more significant in the male pinacidil group at 1 min of reperfusion similarly to ischemia. Heart rate did not increase during reperfusion, **Figure 3. 7.**

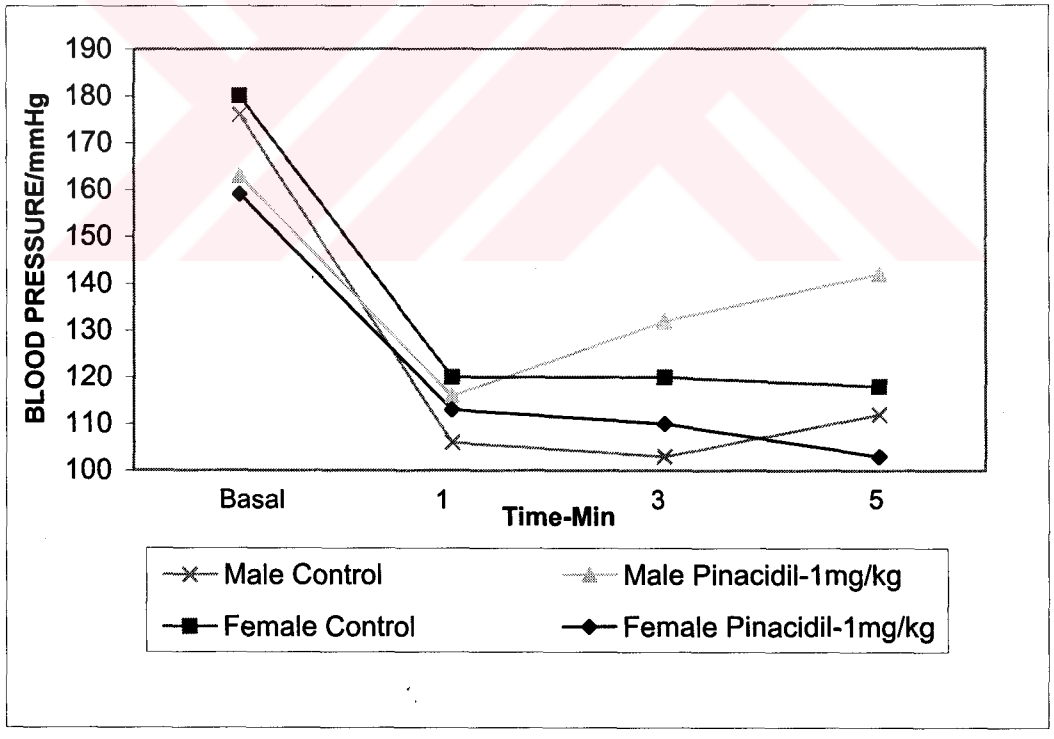

Figure 3. 4. Blood pressure during ligation

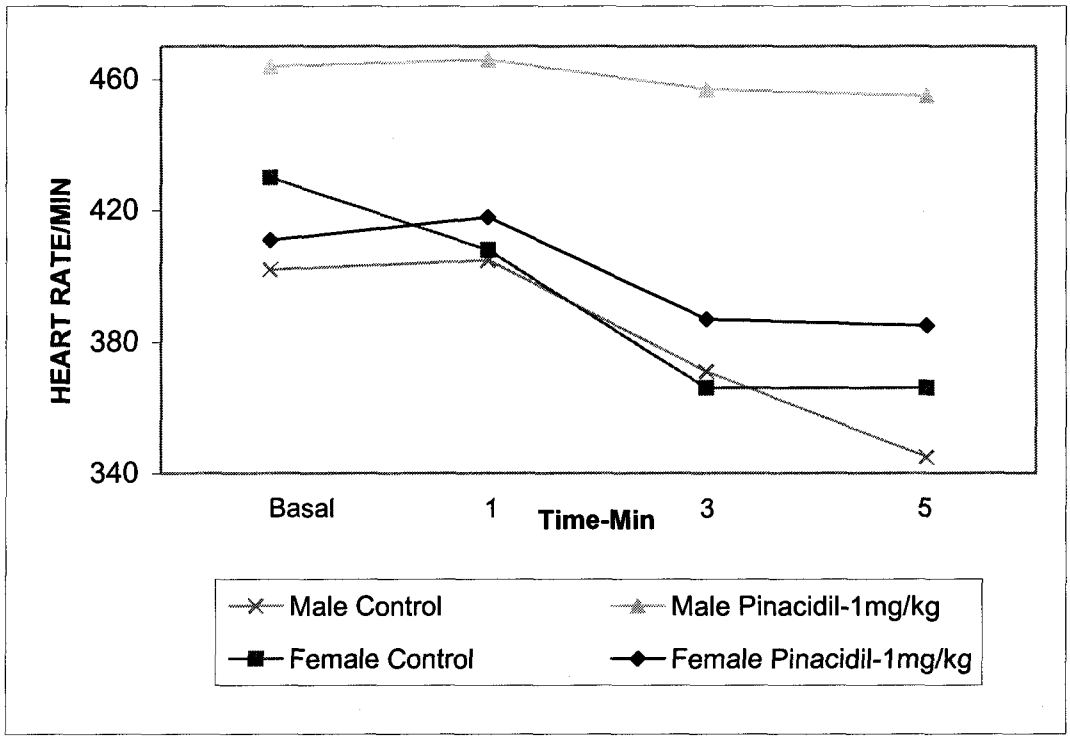

Figure 3. 5. Heart rate during ligation

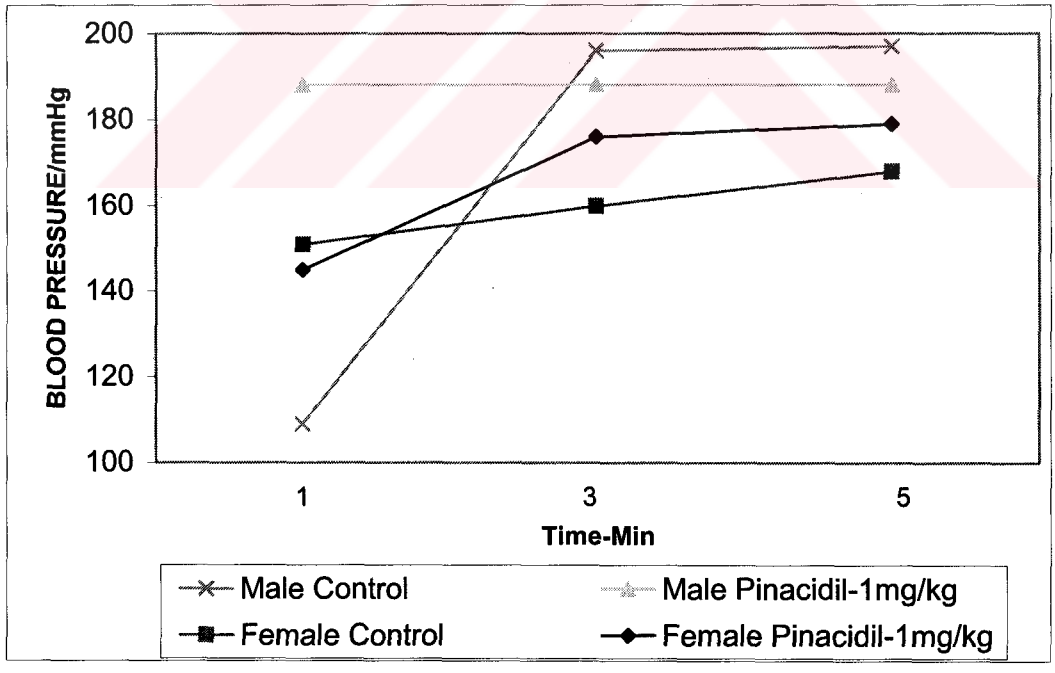

Figure 3.6. Blood pressure during reperfusion

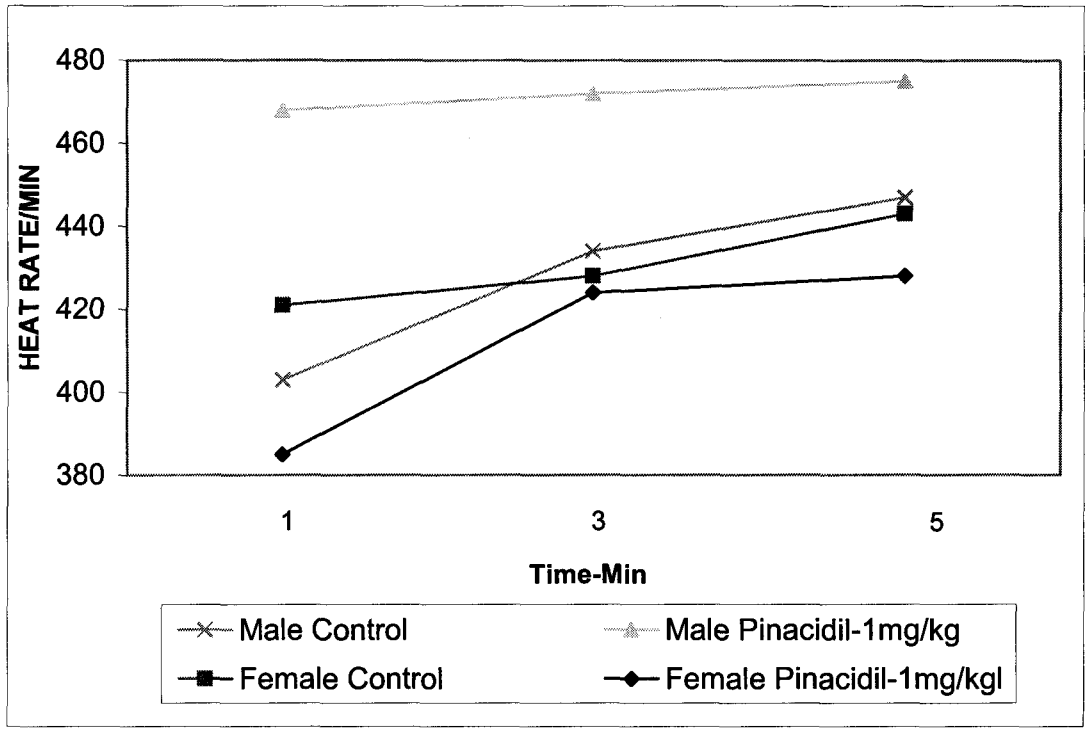

Figure 3. 7. Heart rate during reperfusion

The ventricular fibrillation was observed only in the male control and the female pinacidil group. Pinacidil increased the incidence of ventricular fibrillation in female but decreased the incidence of ventricular fibrillation in male, **Table 3.4.** The incidence of ventricular tachycardia was significantly lower in the pinacidil treated male in respect to its control, but it was not significantly different in the pinacidil treated female than its control **Table 3. 4.** The incidence of other arrhythmias was found to be significantly lower in male pinacidil group than both the male control and the female pinacidil group. The incidence of the bradycardia was not different among groups. Pinacidil decreased total length of arrhythmia in male, but not in female, **Table 3. 3.**

The length of VF was not significantly different among groups during coronary ligation, **Table 3. 5**. But the length of VT significantly was higher in male pinacidil group in respect to others. Total length of arrhythmia during coronary ligation was found low in pinacidil treated female and high in male control, **Table 3. 5**.

During the reperfusion, the length of ventricular fibrillation decreased in pinacidil treated male. On the other hand it increased in pinacidil treated female, **Figure 3. 8**. The length of VT and total arrhythmia was significantly higher in male control group in respect to others, **Figure 3. 9**. Total arrhythmia was not significantly different between male and female pinacidil treated groups, **Figure 3. 10.** Pinacidil decreased the duration of total arrhythmia in male but not in female, **Table 3. 3.**

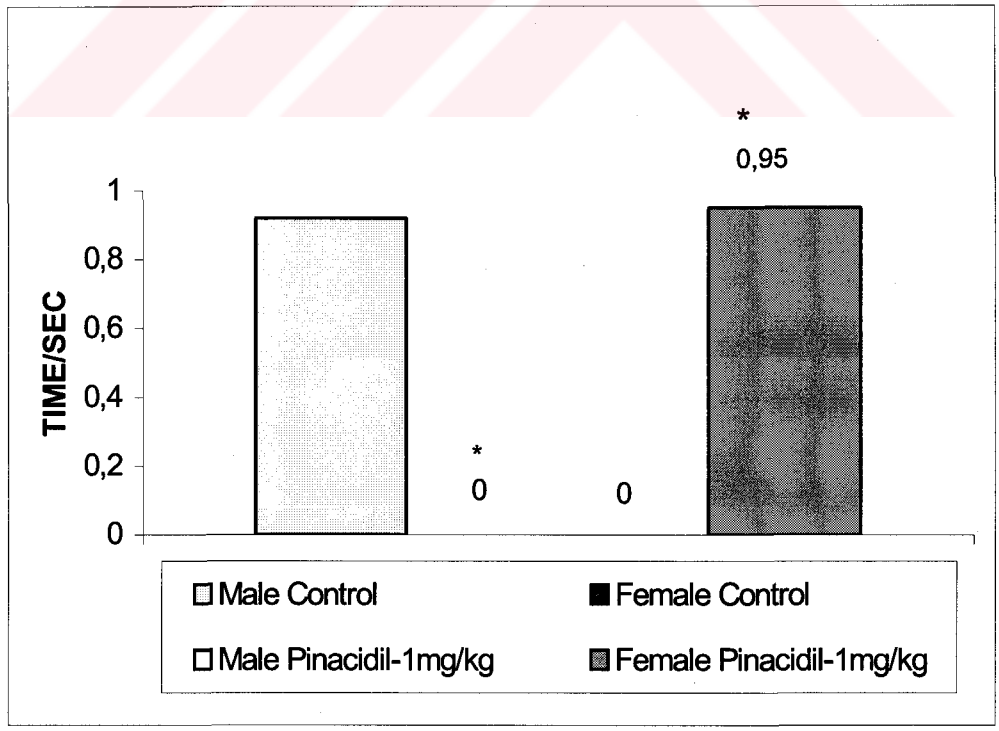

Figure 3. 8. Length of Ventricular Fibrillation during reperfusion

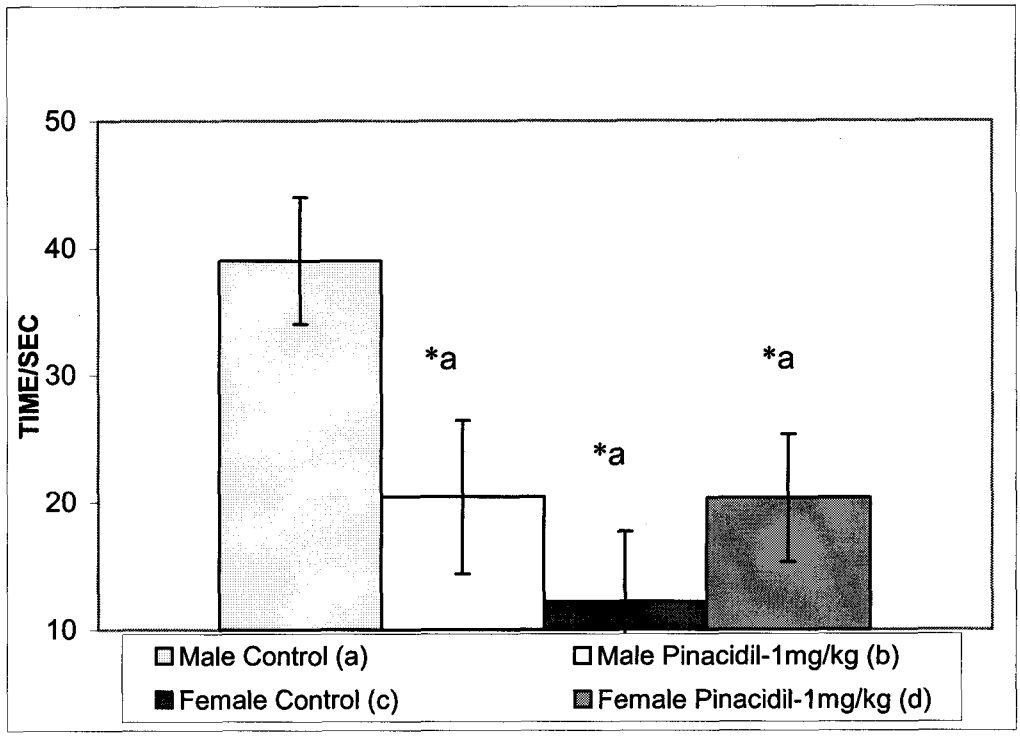

Figure 3. 9. Length of Ventricular Tachycardia during the reperfusion

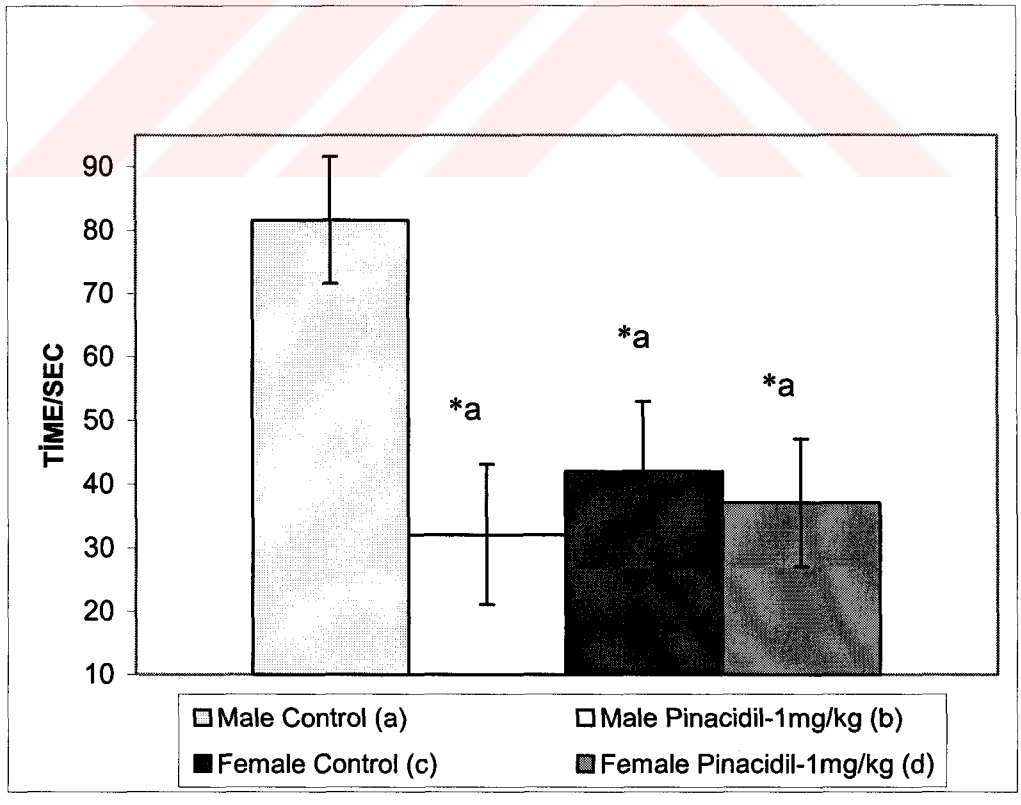

Figure 3.10.Total length of arrhythmias during reperfusion

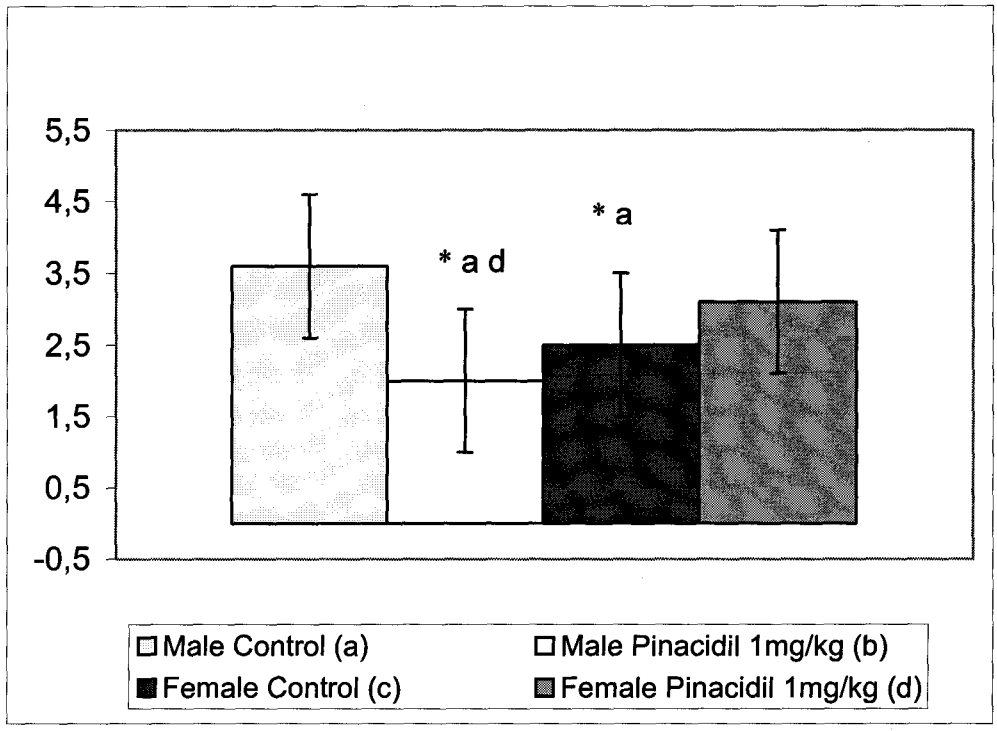

Figure 3. 11. Arrhythmia scores during reperfusion

Arrhythmia score in the male pinacidil group was significantly lower than its control **Figure 3. 11.** But there was not found any significance between female pinacidil and its control. Pinacidil decreased arrhythmia score more in male than female. Otherwise the arrhythmia score in control groups was significantly different with each other; low in female, high in male ($P<0,05$), **Table 3. 4.**

The area at risk was low in male pinacidil group according to its control and female pinacidil group so that less arrhythmia was observed in this group, **Figure 3.12**. The severe arrhythmia was usually associated with large area at risk that was observed in both pinacidil treated female and male control.

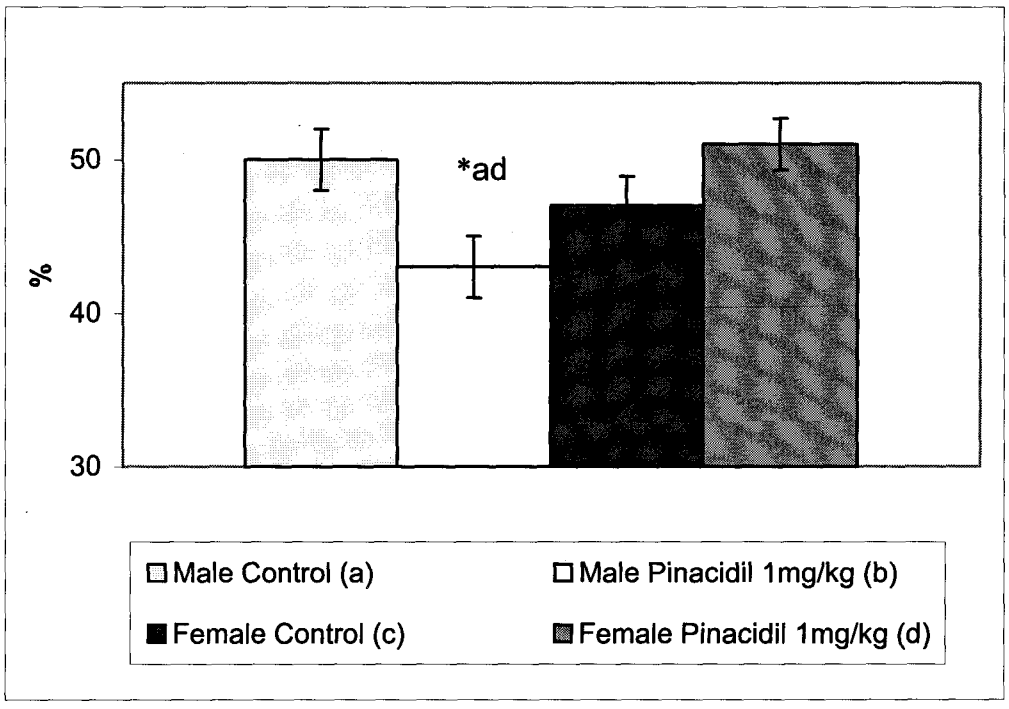

Figure 3.12. Area at risk

TABLE 3. 1. The heart rate (HR) and mean arterial pressure (BP) during 6 min. of coronary artery ligation.

Group	N	Heart Rate / min.				Blood pressure (mmHg)			
		Basal	1 min	3 min	5 min	Basal	1 min	3 min	5 min
Male Control(a)	10	402 ±13	405 ± 17	371 ± 27	345 ± 31	176 ±8	106 ± 9	103 ± 12	112 ± 12
Male Pinacidil(b)	7	464 ± 15*ad	466 ± 20*ac	457 ± 32	455 ± 36	163 ± 9	116 ± 11	132 ± 15	142 ± 15*a
Female Control(c)	8	430 ± 14	408 ± 19	366 ± 30	366 ± 34	180± 9	120 ± 10	120 ± 14	118 ± 14
Female Pinacidil(d)	10	411 ± 13	418 ± 17	387± 27	385 ± 31	159 ± 8	113 ± 9	110 ± 12	103 ± 12

Data are mean ± SE. * $P<0.05$, compared to corresponding male control value.

N: The number of the animals. Basal: Before coronary artery ligation and 1, 3, 5 min. after the coronary ligation.

TABLE 3. 2. The heart rate (HR) and mean arterial pressure (BP) during 6 min. of reperfusion

Group	N	Heart Rate / min.			Blood Pressure (mmHg)		
		1 min	3 min	5 min	1 min	3 min	5 min
Male Control(a)	10	403 ± 57	434 ± 32	447± 27	109 ± 26	196 ± 15	197 ± 13
Male Pinacidil(b)	7	468 ± 47	472 ± 26	475 ± 22	188± 21*a	188 ± 12	188 ±11
Female Control(c)	8	421 ± 46	428 ± 26	443 ± 22	151 ± 21	160 ±12	168 ± 11
Female Pinacidil(d)	10	385 ± 36	424 ± 20	428 ± 17	145 ± 16	176 ± 10	179 ± 8

Data are mean ± SE.

* P< 0. 05 ;

N: The number of the animals. 1, 3, 5 min. after the reperfusion.

TABLE 3. 3. The duration of the arrhythmias during 6 min of reperfusion.

Groups	N	Onset of arrhythmia (min)	Arrhythmic period (min)	Length of Arrhythmia (s)				
				VF	VT	Other	Total	Bradycardia
Male Control(a)	10	0,14 ± 0,05	3,50 ± 0,57	0,92 ± 0,35	39 ± 5,6*bcd	42 ± 6*bd	81,6 ± 10*bcd	29 ± 10,4
Male Pinacidil(b)	7	0,09 ± 0,06φcd	2,18 ± 0,68	0 ± 0,4	20,4 ± 6,7	14,5 ± 7	32,1 ± 11,8	0
Female Control(c)	8	0,26 ± 0,06	3,25 ± 0,64	0 ± 0,4	12,2 ± 6,3	30 ± 6,7	42 ± 11	0
Female Pinacidil(d)	10	0,26 ± 0,05	2,31 ± 0,58	0,95 ± 0,35	20,3 ± 5,6	16 ± 6	37,1 ± 10	0

Data are mean ± SE .

*P < 0. 05 ; $^{\varphi}$P = 0,05

Total = ventricular fibrillation (VF) + ventricular tachycardia (VT) + other types of arrhythmias including VPC, ventricular gemini, AV nodal arrhythmia and salvo.

TABLE 3. 4. The incidence of arrhythmias during 6 min of reperfusion.

Group		Survival	Incidence of arrhythmia				Arrhythmia Score
			VF	VT	Other	Bradycardia	
Male Control(a)	10	10 / (100%)	4 / (40%)	10 / (100%)	10 / (100%)	2 / (20%)	3,7 ± 0,3
Male Pinacidil(b)	7	7 / (100%)	0 / (0%)*ad	5 / (71,4%)	6 / (85,7%)	0	2 ± 0.36*a,d
Female Control(c)	8	8 / (100%)	1 / (12,5 %)	7 / (87,5%)	8 / (100%)*a	0	2,5 ± 0,34*a
Female Pinacidil(d)	10	10 / (100 %)	5 / (50%)	9 / (90%)	10 / (100%)*c	0	3,1± 0,3

Data are mean ± SE . *P< 0,05

N :Total number of animals at the beginning of reperfusion.

TABLE 3. 5 Duration of arrhythmias during 6 min. of coronary ligation

Group	N	Duration of Arrhytmia (s)				
		VF	VT	Other	Total	Bradycardia
Male Control(a)	10	0	0	8,4 ± 1,3	8,5 ± 1,4	34 ± 30
Male Pinacidil(b)	7	0	0,4 ± 0,9*acd	2,2 ± 1,7*a	2,6 ± 1,7*a	0
Female Control(c)	8	0	0	5,6 ± 1,6	5,6 ± 1,6	84 ± 34
Female Pinacidil(d)	10	0	0	0,7 ± 1,4*ac	0,7 ± 1,4*ac	26 ± 30

*P < 0. 05 ; N: The number of animals at the beginning of the ligation.

4. DISCUSSION

Heart rate in response to coronary ligation was different in males and females. Although a small but significant early increase in HR was observed in males, HR decreased significantly in females following ligation (77). Decline in blood pressure following ligation was similar in both gender. The recovery of blood pressure occured earlier in male than female (75). There was no studies that was comparable with the result of our study showing the effect of pinacidil on the hemodynamic parameters and heart rate in both sex. Lepran et al found that the heart rate measured in male before coronary ligation between control animals and pinacidil treated group was not significantly different (78). But, in our study, heart rate was significantly higher in pinacidil treated group both before and after the ligation. This discrepancy may be due to the usage of different dose and different time of drug application before coronary ligation. We used 1mg/kg/100µl dose of pinacidil 10 min before ligation instead of 0.1 or 0.3 mg/kg/100µl and 5 min. Although basal pressure was different before coronary ligation in both study, the recovery of pressure after reperfusion in pinacidil treated group was similar. Otherwise pinacidil pretreatment was observed not influencing heart rate response during acute phase of myocardial infarction in another study performed in conscious rat that used same dose of pinacidil as like in the present study (79). Generally, pinacidil decrease blood pressure and increase heart rate observed in most of the studies is also supported by the result of this study (80). Even other K_{ATP} channel openers used in different studies, show similar results, pinacidil did not effect neither heart rate nor blood pressure before and after ligation and also reperfusion in females.

These different reponses in female may be due to the relative symphatethic predominance in males and a parasymphatethic predominance in females (75).

In a previous study, less arrhythmia observed in female than male during reperfusion was supported by our findings (3). But unfortunately there were no more studies related with the role of gender in ischemia-reperfusion induced arrhythmia. There are several studies that researched the effect of estrogen on these arrhythmia in male. In numerous studies it has been shown that estrogen increase resistance against reperfusion induced arrhythmias (63,65,67).

Although there is no study found that compares the effect of pinacidil on reperfusion induced arrhythmias in both gender, there are several studies related with the effect of pinacidil in males (78,79,80). The incidence of ventricular fibrillation during reperfusion significantly decreased in males treated with pinacidil that shown in conscious (78) and anesthetized rats (79). These result was coincident with our findings that the incidence of ventricular fibrillation decreased significantly in pinacidil treated male. Similarly, the antiarrhythmic effect of pinacidil was observed in dogs 22-24 after coronary ligation (49).

Several factors may play important roles in the protection produced by pinacidil in male than female. Recent studies have shown that female gender is higher level of cardiac K_{ATP} channel (2). The presence of more cardiac K_{ATP} channels in female would contribute to the higher resistance of female to ischemia. Activation of this channel is known as endogenous cardioprotective mechanism that attenuates the ischemia. Opening of

channel decrease oxygen consumption of myocardium and leads to preservation of ATP during ischemia (39).

On the other hand, another protective effect of female gender is believed to be an antiatherogenic action of female sex hormones on the lipid profile which appears to be mediated by the vasoactive estrogen (63,64). The previous studies demonstrated that exogenous estrogen significantly reduce infarct size and attenuates ischemia-reperfusion induced arrhythmias (70). This protective effect has been attributed to increased nitric oxide production, Ca^{2+}-dependent K^+ channel activation or antioxidant activity (70). This protective effect abolished in ovarioectomized female rats (66). Female gender has greater capacity to produce NO than males, since the estrogen stimulates NO production (71). In an in vitro study, more severe myocardial damage and cardiac dysfunction was observed after the ischemia and reperfusion in ovarioectomized rats (66). In above study, NO production was attributed for the protection against ischemia and reperfusion injury. In another in vivo study, it has been suggested that augmentation of endogenous NO release and the opening of calcium dependent potassium channel (K_{Ca}) induced by 17β-estradiol contribute to the alleviation of irreversible ischemia and reperfusion injury and reduce infarct size (70). Antioxidant properties of estrogen, may prolong the half life of NO and reduce reperfusion injury by attenuating the generation of oxygen derived free radicals (73). Lee et al, found that 17β estradiol administration provided cardioprotection produced by activation of K_{ATP} channels in canine hearts. Estrogen posses direct cardioprotective effect that modulate expression of K_{ATP} channels due to the estrogen levels (74). In addition other

cardioprotective effect of estrogen may occur with the Ca antagonism. Estrogen reduces the number of cardiac L-type Ca^{+2} channel through the estrogen receptors and it produces antiarrhythmic action (81).

Preconditioning induced by estrogen was shown to enhance the reperfusion – induced restoration of myocardial high energy phosphate and attenuate the ischemia induced increase in tissue lactate content (82). In our study less arrhythmia was observed in female which may be depend on the increased resistance against ischemia generated by estrogen-induced glycogen storage.

Several studies have suggested that administration of openers of K_{ATP} channels during the myocardial infarction can preserve myocardial function and reduce infarct size (45,46,47). This result was also supported by our findings that area at risk decreased significantly in pinacidil treated male.

Coronary collateral blood flow may significantly limit the extent of myocardial damage. The vasodilatatory effect of nicorandil increase in collateral blood flow in both acute and chronic coronary occlusion models that may limit the infarct size and decrease incidence of arrhythmias (83). During the ischemia, intracellular ATP concentration falls and resulting improved repolarization due to the opening of K_{ATP} channels. Thereby contractile activity in ischemic zone decrease due to the inhibition of voltage dependent Ca^{+2} influx into the myocardial cells. This effect may preserve energy and improve tolerance to ischemia.

Several researchers suggest that electrical inhomogeneity contribute to the development of ventricular arrhythmias and VF. Since K_{ATP} channel in male is less than those in females, more severe arrhythmia might be

appeared in pinacidil treated females. Pinacidil increase shortening of action potential in non-ischemic myocardial cells and approximate them to refractor period of ischemic cells, so may lead to less arrhythmia. Since the number of K_{ATP} channel is less in male, pinacidil might decrease heterogeneity between ischemic and non-ischemic zone in male than female. This suggestion is also supported by other researchers showed that pinacidil decrease the refractory period of purkinje fibers and ventricular myocardium (43).

The mechanism by which pinacidil exerts its pharmacologic actions is also believed to involve more channels associated with K^+ ions efflux from the cell. Pinacidil may modify the gating of other channels. It might inhibits the Ca^{+2} flow into the cell and decreased Ca^{+2} overload in male in respect to female. That is why less arrhythmia and area at risk might be shown in pinacidil treated male.

Antiarrhythmic activity of pinacidil may be attributed to its vasodilator effects. Pinacidil dilate collateral coronary vessels to increase blood supply to the ischemic myocardium and thus may eliminate anoxic ventricular ectopic foci. Less area at risk and less arrhythmia observed in male in the present study might be explained with the presence of more collateral vessel and more blood flow in male(83).

5. CONCLUSION

As a conclusion, it has been shown in present study that 1) the incidence and the duration of the ischemia and reperfusion-induced arrhythmias in female was less than that in male. This findings suggest that experimental research related with the arrhythmias following coronary ligation should be performed in both sex because of this different response. 2) The opening of the K_{ATP} channel is more protective against arrhythmias in male than female. Since less K_{ATP} channel are found in males than females, opening of K_{ATP} channel might be less in males. This may decrease the electrical inhomogeneity between ischemic and nonischemic region of myocardium and may led to less arrhythmia in male.

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