THE EFFECT OF ATP DEPENDENT POTASSIUM CHANNEL BLOCKERS AND OPENERS ON ARRHYTHMIAS OBSERVED FOLLOWING PARTIAL LEFT CORONARY ARTERY OCCLUSION IN MALE RATS

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MARCH 2009

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by
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THESIS SUBMITTED TO
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE ABANT IZZET BAYSAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN
THE DEPARTMENT OF BIOLOGY

MARCH 2009

Approval of the Graduate of Natural and Applied Science.

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I certify that this thesis satisfies all the requirements as a thesis for the Degree of Master of Science.

Prof. Dr. Ömer BOZDOĞAN

Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Biology.

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ABSTRACT

THE EFFECT OF ATP DEPENDENT POTASSIUM CHANNEL BLOCKERS AND OPENERS ON ARRHYTHMIAS OBSERVED FOLLOWING PARTIAL LEFT CORONARY ARTERY OCCLUSION IN MALE RATS

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March 2009, 73 pages

More than 50 % of Sudden Death in human being occurs due to heart attack that is generated by occlusion of coronary arteries. The reason of these sudden death is ventricular arrhythmia that occurs due to the myocardial ischemia. That's why experimental studies have been done for the prevention or decreasing of these arrhythmias. Researches were concentrated on the ATP dependent potassium channels that have role in the generation of these arrhythmias. In this study, unlike previous studies; the effect of both ATP dependent potassium channel blocker and opener on ischemia induced arrhythmia were researched in rats having smaller infarct size.

In the present study, 280-377 g weight, 8-9 months old, 21 Spraque-Dawley male rats were used. Pinacidil was given intravenously and glibenclamide was given intraperitoneally before coronary ligation.

Electrocardiogram and blood pressure were recorded during 30 minutes of coronary ligation.

Blood pressure decreased in all animals after coronary ligation. Glibenclamide increased the incidence of arrhythmia during 30 minutes of coronary ligation but there was no statistical difference observed. Similar insignificant effect was seen in pinacidil treated groups. Similarly arrhythmia score calculated from duration and type of arrhythmia was not significantly different in pinacidil and glibenclamide treated group according to control rats.

As a result, the effect of pinacidil and glibenclamide on the arrhythmias induced by smaller ischemic area in male rats were different as it compared with larger ischemic model. This different effect may be due to the presence of less number of ATP dependent potassium channels and the appearance of less arrhythmia in males than females.

Key Words: Coronary ligation, Myocardial ischemia, Arrhythmia, ATP dependent potassium channels, Glibenclamide, Pinacidil, Rats.

ÖZET

ERKEK SIÇANLARDA KISMİ SOL KORONER ARTER TIKANMASINI TAKİBEN OLUŞAN ARİTMİLER ÜZERİNE ATP BAĞIMLI POTASYUM KANAL BLOKER VE AÇICILARININ ETKİSİ

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Mart 2009, 73 sayfa

İnsanlarda ani ölümlerin % 50 den fazlası koroner damar tıkanması sonucu meydana gelen kalp krizine bağlı olarak oluşmaktadır. Kalp krizi sonucu oluşan ani ölümlerde esas neden miyokardiyal iskemi sonucu oluşan aritmilerdir. Bu nedenle bu aritmilerin azaltılması ya da önlenmesi ile ilgili deneysel çalışmalar yapılmaktadır. Araştırmalar, bu aritmilerin oluşumunda rol alan ATP bağımlı potasyum kanalları üzerinde yoğunlaşmıştır. Bu çalışmada önceki çalışmalardan farklı olarak daha küçük bir alanda miyokardiyal iskemi oluşturularak, ATP bağımlı potasyum kanal açıcılarının ve blokerlerinin, iskemi ile uyarılan aritmiler üzerine etkisi araştırılmıştır.

Araştırmada 280–377 gr ağırlıkta, 8–9 aylık, 21 Spraque-Dawley erkek sıçan kullanıldı. Koroner ligasyondan önce pinacidil intravenöz olarak ve glibenclamide intraperitonel olarak verildi. 30 dakikalık koroner ligasyon süresince elektrokardiyogram ve kan basınçları kayıt edildi.

Koroner ligasyon yapıldıktan sonra bütün hayvanlarda kan basıncı düştü. Glibenclamide 30 dakikalık ligasyon boyunca aritmi yoğunluğunu arttırdı, fakat istatiksel olarak anlamlı bir farklılık oluşturmadı. Pinacidil grubunda da benzer etki görüldü. Aritmi tipi ve süresine göre hesaplanan aritmi skoru glibenclamide ve pinacidil gruplarında kontrol grubundaki sıçanlara göre anlamlı bir farklılık oluşturmadı.

Sonuç olarak, erkek sıçanlarda, pinacidil ve glibenclamide'in iskemik aritmiler üzerine etkisi büyük iskemik alan oluşturulan hayvan modellerine göre farklı bulundu. Bu farklı etki, erkeklerde ATP bağımlı potasyum kanal sayısı ve aritmi yoğunluğunun dişilere göre daha az olmasından kaynaklanabilir.

Anahtar Kelimeler: Koroner ligasyon, Miyokardiyal iskemi, Aritmi, ATP bağımlı potasyum kanalları, Glibenclamide, Pinacidil, Sıçan.

TO MY FAMILY

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to supervisor, Prof. Dr. Ömer Bozdoğan his unceasing interest, valuable guidance, encouragement and great help throughout the course of this work. I would like to thank my committee members, Prof. Dr. Cihan Demirci and Associate Prof. Dr. Ewa Doğru, for their critical reading of this manuscript.

I want to thank to Derya KOYUNCU since she has been helpful and friendly in the laboratory studies. I want to thank to Nevin KOÇ for technical support.

My deepest appreciation is given to my family, for their encouragement and support.

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1. INTRODUCTION

Cardiovascular disease is a major cause of sudden death in both men and women. Coronary artery disease (CAD) is responsible for about 50% of cardiovascular mortality, which itself accounts for 30-50% of all deaths in developed nations (1). The term "coronary artery disease" encompasses a range of diseases that result from atheromatous change in coronary vessels (2). CAD happens when the arteries that supply blood to heart muscle become hardened and narrowed. This is due to the build up of cholesterol and other material, called plaque, on their inner walls. As the buildup grows, less blood can flow through the arteries. As a result, the heart muscle can not get enough blood or oxygen. This can lead to chest pain (angina) or a heart attack. Most heart attacks happen when a blood clot suddenly cuts off blood supply to the heart, causing permanent heart damage.

The considerable reason of death in patients having heart attack is the lethal arrhythmias that appear after the coronary occlusion. It is clear that arrhythmia and the rate of death in myocardial infarction have seen more in men than premenopausal women (3). And coronary artery diseases occur usually in patients having diabetes mellitus (4). It has been known that the severity of arrhythmia occurring in heart attack is related with the infarct size (5, 6).

The heterogeneity is more in larger ischemic area that produces frequent ectopic stimuli and finally leading more frequent ventricular beats. There are various cellular causes of ischemia induced arrhythmia. It has been known that these arrhythmias are especially linked to an intracellular potassium ion (K^+) loss through adenosine tri-phosphate (ATP) - modulated K^+ channels. This channel opens in ischemic cell in response to decreasing ATP level. Opening of the channel decreases intracellular calcium ions and contractility of myocardial cells. Decreased contractility is protective mechanism against ischemia. Otherwise potassium efflux from ischemic cell is arrhythmogenic. That is why using ATP dependent potassium channel blocker decreases arrhythmia that is observed in some research (7). There are controversies related to the protection against ischemia induced arrhythmia mediated by ATP dependent potassium channel blocker and opener. In these studies, usually left branch of coronary artery were ligated (LAD) and larger ischemia were produced. Different result from these studies may be depending on decreased or increased electrical homogeneity between ischemic and nonischemic region (8). Blockage of ATP dependent potassium channel in ischemic cells by blockers may close these cells to non ischemic cells and decrease arrhythmia. But opening ATP dependent potassium channel closes the normal cells to ischemic cells and again may decrease arrhythmia. There are few studies comparing the effect of ATP dependent potassium channel modulators on ischemia reperfusion induced arrhythmia in smaller and larger ischemic conditions. In a recent study, it has been found that pinacidil (ATP dependent channel openers) and glibenclamide (ATP

dependent channel blockers) did not affect the severity of arrhythmia in respect to control animals in female rats having smaller infarct (9). The severity of arrhythmias following myocardial ischemia and reperfusion has been found different in male and female (10, 11). The incidence and severity of ischemia and reperfusion induced arrhythmia is more in male than female rats. Although the effect of both opener and blocker has been researched in female having smaller infarct, It has been found no studies indicating same result in male animals.

In this study, it is aimed to research the effect of K_{ATP} channel blocker (glibenclamide) and opener (pinacidil) on ischemia induced arrhythmias in the presence of smaller infarct size in male rats.

1.1. ANATOMY AND PHYSIOLOGY OF HEART

1.1.1. Anatomy

The heart is a muscular organ which is mechanically pump blood from ventricles to the pulmonary and systemic circulation. The heart is located between the right and left lungs that is near the anterior chest wall in mammals. The mammalian heart has four chambers. The heart is surrounded by a pericardium. The pericardium is composed of two layers: one lies on the outer surface of the heart is called visceral pericardium and the other is in contact with the surrounding lungs and other tissues is called parietal pericardium

The heart is actually two separate pumps: a right heart that pumps blood to the lungs and a left heart that pumps the blood to the peripheral organs. In turn, each of these hearts is a pulsatile two chamber pump

composed of an atrium and a ventricle. The atrium functions principally as a weak primary pump for the ventricle, helping to move the blood into the ventricle. The ventricle in turn supplies the main force that propels the blood either through the pulmonary circulation by the right ventricle or through the peripheral circulation by the left ventricle (12). The chambers are separated with septum and valves. Right and left atrium separated with atrial septum and right and left ventricle separated with interventricular septum. The atria and ventricle are separated by atrioventricular valves (A-V valves) which prevent backflow of blood from the ventricles to the atria during systole. The left A-V valve is called the bicuspid or mitral valve and the right A-V valve is called tricuspid valve. The aortic and pulmonary valves (semilunar valves) prevent backflow from the aorta and pulmonary arteries into the ventricles during diastole (12) (**Figure 1.1.**).

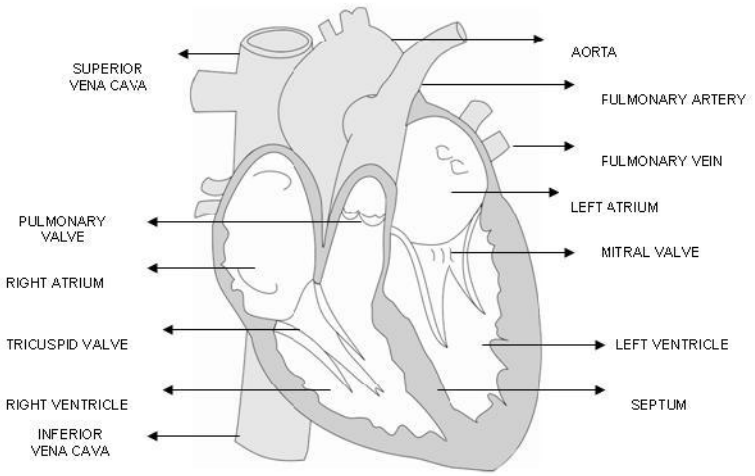


Figure 1.1. Anatomy of the heart.

The basic function of the heart is to provide the blood circulation. The deoxygenated venous blood continuously flows from the peripheral organs to the right side of the heart by the superior and inferior vena cava. Blood is ejected from the right ventricle to the lungs for gas exchange. Then, oxygenated blood turns back to the heart in to the left atrium. This circulation is named as pulmonary circulation. The ventricle pumps the blood to the aorta and aorta carries the oxygenated blood to all peripheral body tissues. This circulation is named as systemic circulation.

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 left and the right coronary arteries. The left coronary arteries supply to the left side and the right coronary arteries supply to the right side of the heart. The coronary circulation, that supplies blood to the heart, is the shortest circulation in the body.

1.1.2. Histology of Heart

Heart wall contain three layers: epicardium is a outer surface of the heart, myocardium is a main muscle layer of the heart and endocardium is a inner surface of the heart (13). The heart is composed of three major types of cardiac muscle. These are atrial muscle, ventricular muscle, and specialized excitatory and conductive muscle fibers. The atrial and ventricular muscle types contract in the same way as skeletal muscle. But the duration of contraction is much longer. The specialized excitatory and conductive fibers contract only feebly because they contain few contractile fibrils. They exhibit rhythmicity and varying rates of conduction (12, 13). Cardiac muscle fibers are made up of many individual cells connected in

series with one another. The electrical resistance of intercalated disc is much less than the outside membrane of the cardiac muscle fiber. The cell membranes fuse with one another in such a way that they form permeable communicating junctions are called gap junctions that allow almost totally free diffusion of ions (12, 13).

The atria are separated from the ventricles by fibrous tissue that surrounds the atrioventricular valvular openings between the atria and ventricles. Potentials are not conducted from the atrial syncytium into the ventricular syncytium directly through this fibrous tissue. They are conducted by way of a specialized conductive system called the A-V bundle (12).

1.1.3. Excitation and Impulse Conduction in Heart

The heart have a specialized excitatory and conductive system for generating rhythmical impulses to cause rhythmical contraction of the heart muscle and conducting these impulses rapidly throughout the heart (12). This activity occurs by the presence of the more gap junctions. The autorhythmic cardiac cells are; sinoatrial (SA) node, internodal pathways, atrioventricular (AV) node, atrioventricular bundle (bundle of His), right and left bundle branches and purkinje fibers. Impulses pass across the heart in the same order (**Figure 1.2.**).

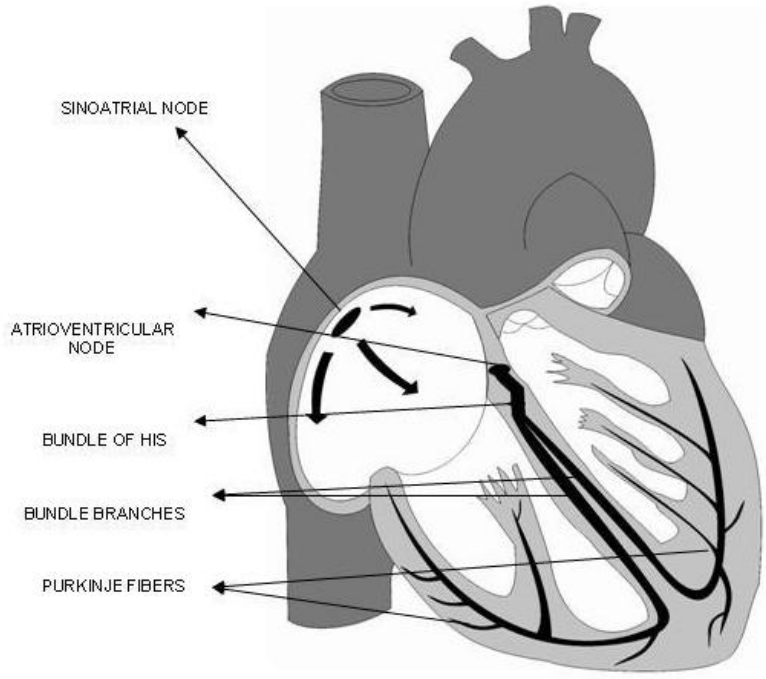


Figure 1.2. Impulse conduction system of the heart.

The SA node is located in the superior posterolateral wall of the right atrium immediately below and slightly lateral to the opening of the superior vena cava. The normal rhythmical impulse is generated by the SA node (12, 13). Inside of the sinus nodal fibers negative and extracellular fluid contain high sodium ions. Sinus nodal fibers are leaky to ions that depolarize the cells slowly to treshold and fire action potential. The sinus nodal fibers connect directly with the atrial muscle fibers, so that any action potentials that begins in the sinus node spreads directly into the atrial muscle wall. SA node controlled by both the sympathetic and parasympathetic system (13). Internodal pathways which contain more gap junctions , conduct the impulses to the AV node. AV node is located in the posterior wall of the right atrium immediately behind the tricuspid valve. Cardiac impulse does not move from the atria into the ventricles too

rapidly which is delayed by the AV node. This delay allows time for the atrial contraction before ventricular contraction begins (12). Impulse moves from the AV node to the AV bundle. AV bundle allowing only forward conduction from the atria to the ventricles. Then the impulse travels on the left and right bundle branches and spreads by the purkinje fibers. They contain more gap junctions. Bundle branches divides into smaller branches, this branches are purkinje fibers which in turn course around each ventricular chamber and back toward the base of the heart (**Figure 1.2.**).

1.1.4. Neural Innervation of Heart

Heart beat intervals, which are determined basically by regular excitations of the sinoatrial node as a cardiac pacemaker, shows significant variability due to influence of the activity of the central nervous system (CNS) through the sympathetic and parasympathetic (vagal) branches of the autonomic nervous system (ANS).

The heart is innervated by vagal and sympathetic fibers. Sympathetic stimulation increases both the heart rate and force of cardiac contraction. Parasympathetic stimulation decreases both the heart rate and strength of cardiac contraction.

Sympathetic nerves effect the SA node, AV node and also to the ventricular myocardium. Parasympathetic nerves affect the SA node and AV node. Sympathetic and parasympathetic stimulations of the heart are controlled by the medulla oblongata which located in the brainstem above the spinal cord.

1.1.5. Autonomic Activity of the Heart

Autonomic activity of the heart started in the SA node that contains excitatory cells. Autorhythmic cells have an unstable resting membrane potential that continuously depolarizes. When the pacemaker cells depolarize to threshold level, action potential fires. The reason for the firing of slow depolarization in autorhythmic cells is more K^+ efflux in repolarization phase. The more K^+ efflux cause the slow calcium entry in to the cell.

On the other hand action potential generation is different in contractile myocardial fibers. Action potentials in these cells start with the opening of Na^+ channels and rapid Na^+ influx in to the cells. As the cell reach to the peak of action potentials, small repolarization occur by the entrance of Cl^- ions then Ca^{2+} channels open and plato phase does occur. After the plateau phase, slow Ca^{2+} and Na^+ channels are inactivated and K^+ channels open. K^+ efflux from the cells cause rapid repolarization. Na^+/K^+ ATPase pump is activated after the repolarization to replace the Na^+ and K^+ ions in their right place (12).

1.2. CARDIAC ARRHYTHMIAS

Cardiac arrhythmia is a disturbance in the regular rhythm of the heart beat. The mechanisms responsible for cardiac arrhythmias are generally divided into categories of disorders of impulse formation, disorders of impulse conduction, or combination of both (14).

1.2.1. Types of Arrhythmias

1.2.1.1. Sinus Tachycardia

Tachycardia means fast heart rate. Sinus tachycardia occurs by increased automatic stimuli from SA node. The general causes of sinus tachycardia are increased body temperature, stimulation of the heart by the sympathetic nerves, and toxic conditions of the heart. Sinus tachycardia in rats occurs when the sinus rate exceeds to 450 beats/minute. In this condition P waves and ST segments are normal but PR interval is shortened.

1.2.1.2. Sinus Bradycardia

Bradycardia means slow heart rate. The high level of vagal tone on the SA node causes bradycardia. In the sinus bradycardia, heart rate lesser than 250 beats/min in rats. Normal and constant PR interval, normal ST segments appears (15).

1.2.1.3. Sinus Arrhythmia

Sinus arrhythmia can result from any one of many circulating reflexes that alters the strength of the sympathetic and parasympathetic nerve signals to the sinus node of the heart. Although the excitation of the heart occurs from SA node, the interval between beats is not equal. Sinusal arrhythmia sometimes appear in respiration that the heart rate increases during inspiration and decreases during expiration. This type of arrhythmia occurs in different phase of anesthesia in rats.

1.2.1.4. Atrial Premature Contraction (APC)

A premature contraction is a contraction of the heart before the time that normal contraction would have been expected. Sometimes impulse originated from a focus other than the sinus node. Atrial premature contraction arises from an ectopic focus in the atria. In the ECG, normal QRS complex and unusual shape of P wave are seen. This type of arrhythmia can not be differentiated in ECG recorded from rats.

1.2.1.5. Atrial Fibrillation (AF)

Atrial fibrillation is an abnormal irregular heart rhythm of the atria. Many electrical signals are generated randomly in the atria (16). Atrial and ventricular muscles are separated by the fibrous tissue (12). Because of this, atrial fibrillation occurs without ventricular fibrillation. AF is not fatal, because blood flows passively through the atria into the ventricles. AF includes irregular rhythm with no P waves (17).

1.2.1.6. Ventricular Premature Contraction (VPC)

Ventricular premature contraction arises from ectopic focus generated in the ventricles. When impulse conduction is slow in muscle of the ventricles, the ectopic impulses capture the heart for stimulation before sinusal rhythm. In this case the ventricules contract first and less blood pumped to the circulation. These types of arrhythmias occur frequently in myocardial ischemia and reperfusion in rats. More than 5 ventricular beats in sequence is called as ventricular paroxysmal tachycardia (**Figure 3.6.**).

1.2.1.7. Ventricular Tachycardia (VT)

Ventricular tachycardia occurs in a similar way to ventricular extra beat but the frequency and rate is higher. Ventricular tachycardia is long lasting, sustained continuous ventricular beat. As a result of this rhythm, blood pressure decreases more and if it is not recovered it may lead to death. In this condition, less blood is pumped to the systemic circulation and also to the coronaries. This produces ischemia in myocardium. Two reasons cause the ventricular tachycardia are ischemic cells and automatic impulse generation from ventricles (**Figure 3.6.**).

1.2.1.8. Ventricular Fibrillation (VF)

Ventricular fibrillation usually occurs following ventricular tachycardia. Long lasting ventricular tachycardia leads to global ischemia in heart that causes generation of many ectopic focus. There is no coordinated contraction of myocardial cells in heart. Many small portions of the ventricular muscle are contracted individually. Ventricular fibrillation is also lethal just like ventricular tachycardia. If ventricular fibrillation last more than 10 second, irreversible damage occurs in the heart. Blood pressure declines to very low level, about 10mmHg in rats having ventricular fibrillation. If ventricular fibrillation does not stop within 2 to 3 minutes, it is almost invariably fatal (18). During VF irregular ECG waves are seen and QRS complex is unidentified (**Figure 3.6.**).

1.2.2. Underlying Mechanism of Arrhythmia

1.2.2.1. Abnormal Automaticity

Abnormal automaticity is generated by the spontaneous abnormal depolarizing current of the cardiac cells. A pacemaker elsewhere than SA Node is called as ectopic pacemaker. Abnormal impulse is generated by the ectopic pacemaker. The ectopic pacemaker is produced by the ischemic cells or by increased automaticity other than SA cells. If the rate of impulse generation from the ectopic foci is higher than the rate of SA node, impulse from SA node is blocked. Rhythms resulting from increased automaticity may be atrial, junctional, and ventricular in origin (14).

1.2.2.2. Triggered Activity

The definition of the triggered activity is the abnormal action potential triggered by a preceding action potential. The abnormal impulse formation results from abnormal depolarization during diastole of the myocardial cells. Triggered activity is initiated by afterdepolarizations, which are depolarizing oscillations in membrane voltage induced by one or more preceding action potentials. There are two types afterdepolarizations; early and delayed afterdepolarizations. Depolarization can occur before or after full repolarization of the cardiac fiber and it is called early afterdepolarizations (19). They arise from a reduced level of membrane potential during phases 2 and 3 of the cardiac action potential. Early afterdepolarization may be responsible ventricular tachyarrhythmia in several clinical situations (14). When depolarization occur after completion of repolarization (phase 4), it is called delayed

afterdepolarization (20). The mechanism is poorly understood; however, delayed afterdepolarization is found to be associated with high intracellular Ca^{2+} concentrations that occur with digitalis toxicity or excessive catecholamine stimulation.

1.2.2.3. Re-entrant Circuit

This type of arrhythmia occurs due to the conduction block in myocardium. Conduction blocks may occur due to the ischemia or abnormal stimulation of myocardial cells. The impulse is blocked in one pathway and propagates slowly in the adjacent pathway. When the transmission is slowed down or blocked in this pathway, the normal stimuli pass around of the blocked region. Then, this region generates re-entrant stimuli when the normal myocardium returns the resting state. Most ventricular arrhythmias occurred by reentrant mechanism (21) (**Figure 1.3.**).

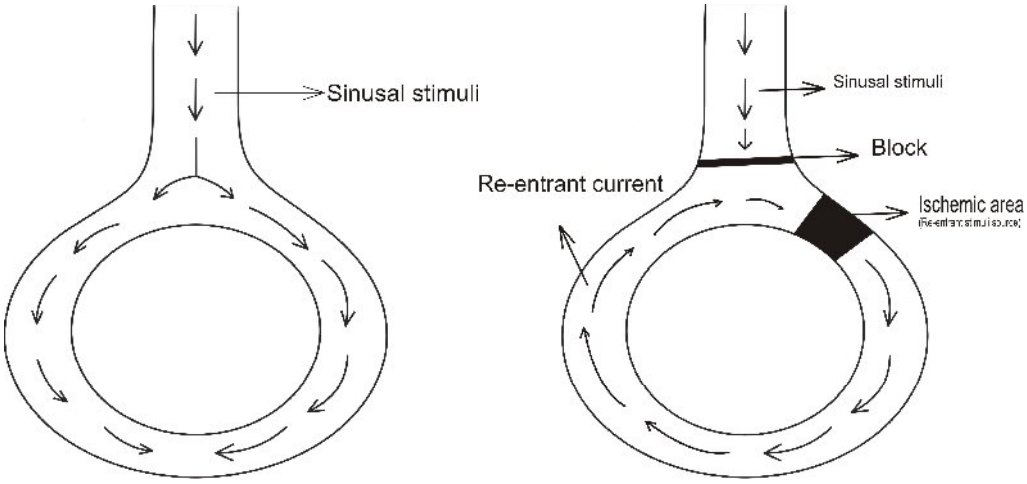


Figure 1.3. Re-entrant circuit

1.2.3. Ischemia Induced Arrhythmia

There are two periods of arrhythmia following ischemia in all experimental animals. These are early and delayed arrhythmic periods. In rats, early arrhythmic period occurs in 3-5 minutes and late arrhythmic period occurs in 6-12 minutes after the coronary artery ligation (22).

In the early phase of ischemia the myocyte is not extremely impaired but the time of depolarization and repolarization varies in ischemic cells. In the ischemic region, impulse transmissions slow down. These factors induce the conditions necessary for reentrant type of arrhythmia (21). In the later phase of ischemia, ischemic myocardial cells remain in depolarized state and generate ectopic stimuli. In the very late phase of ischemia, cell death occurs and electrical instability decreases. Ventricular tachycardia, ventricular fibrillation and ventricular premature contractions are seen following the coronary artery ligation (23).

1.2.4. Reperfusion Induced Arrhythmia

Reperfusion injury causes more severe arrhythmias compared to ischemia. Ischemic cells in reperfusion try to recover back to normal situation, but inhomogeneities in action potential of myocardial cells in the region increase more. Irregular refractoriness in reperfused myocardial cells lead to generation of irregular ectopic stimuli. These changes overall cause the generation of reperfusion arrhythmias. Duration of ischemia is most important in severity of ischemia reperfusion induced arrhythmias because more ROS production occurs in reperfusion periods after prolonged ischemia. Free oxygen radical production (ROS) is a major causes in the generation of reperfusion arrhythmias. Free radicals are

produced in the cytoplasm of ischemic cells by the xanthine oxidase system; the mitochondria are considered the major source of free radical production during reperfusion. Free radicals produce damage in cell membrane and organic molecules inside of the cell and finally increase death of the cells in reperfusion area.

An important result of both myocardial ischemia and reperfusion is the occurrence of various disturbances in cardiac rhythm, including the potentially lethal arrhythmias (ventricular tachycardia and fibrillation) (24, 25).

1.3. EXPERIMENTAL MYOCARDIAL INFARCTION

1.3.1. Animal Model of Experimental Myocardial Infarction

Experimental myocardial infarction is studied both invivo and invitro conditions. In these studies, different animal models have been used such as; pig (26), sheep (27), canine (dogs and cats) (28, 29), rabbit (30), mice (31) and rat (7, 22).

Invitro studies, the heart is removed from the body of decapitated animals and is put in the solution and then settled in Langendorff perfusion assembly. Coronary artery is blocked by the ligature and the effects of some drugs are examined by adding to the perfusion solution. This technique is shown in **Figure 1.4.** and described in a reviewed in a reference numbered as 32.

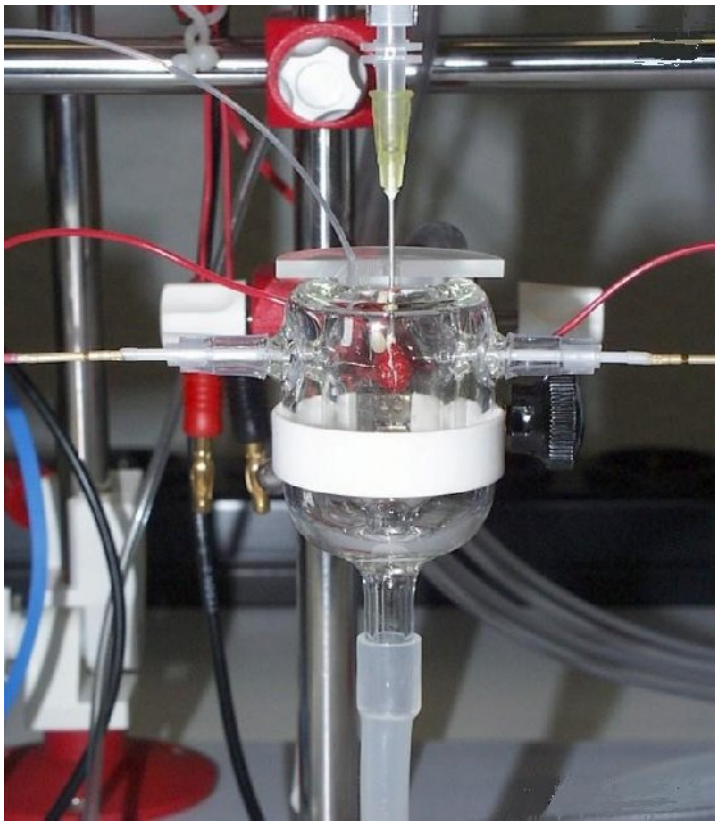


Figure 1.4. Isolated Langendorff Mouse Heart Preparation (getting from Föhr Medical Instruments GmbH. www.fmigmbh.de).

Invivo studies, the anesthetized or conscious animals are used. In these animals, first the chest cavity is opened and heart is not removed from the body. Coronary artery is blocked by the ligature and chest cavity is closed again. The effect of drugs is researched by given the drug intra-peritoneally or intravenously in anesthetized animal.

Rats have been commonly used as laboratory animals in cardiac studies. The advantage of using smaller animals in these studies are low financial cost, ease in manipulation of animals, less operation time, less usage of drug depending on small size of animals and availability in performing more experiment. Coronary artery ligation in the rat has been shown to be a useful technique for the assessment of interventions that

reduce infarct size (33). Disadvantage of using rats in the model of acute myocardial infarction techniques are; limitation in blood sampling and instrumentation possibilities and difficulties in handling of heart because of small size.

1.3.2. Methods to Produce Partial Infarction

There is a few study found in the literature that partial infarction was produced in rats (9). Partial infarction can be produced practically by both invivo and invitro studies. Branch of the main coronary artery is blocked by the ligature and small infarct area produced in this method. Since the branch of coronary arteries of rats is so small, a reference point for the ligation is used (**Figure 1.5.**). In this model, arrhythmia that occurs following ischemia and infarction are lesser than the larger ischemia and infarction. The arrhythmias occurred after the partial or smaller ischemia is short lasting and it is terminated in a short time after ligation (6).

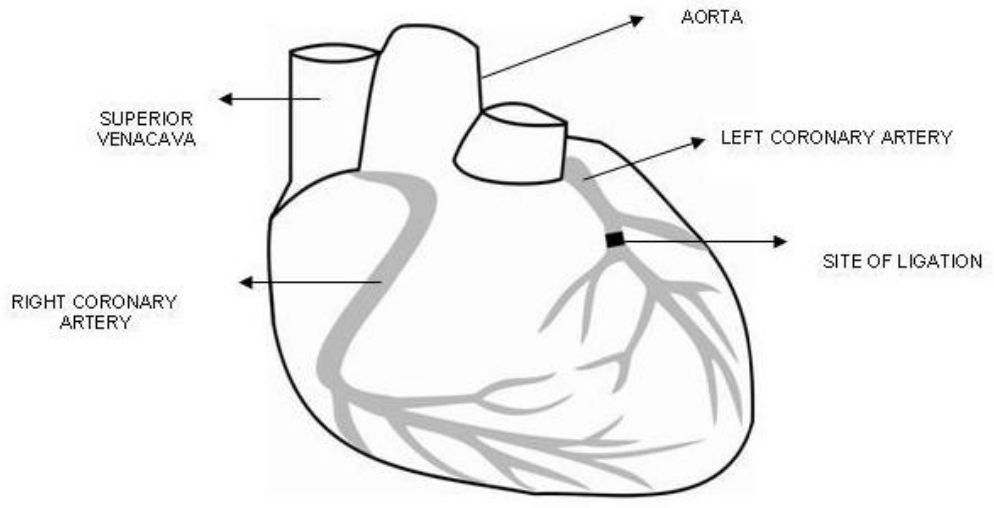


Figure 1.5. Site of coronary ligation and coronary arteries in rats.

1.4. ACUTE MYOCARDIAL INFARCTION

Acute myocardial infarction occurs by the sudden blockage of coronary arteries by thrombus or coronary spasm in man. Myocardial ischemia occurs when blood flow to the myocardial cell is obstructed by a blockage of a coronary artery (34, 35). Sustained ischemia leads to production of infarct in myocardial tissue. Infarct means death of myocardial cells. Ischemic myocardial cells can not get enough oxygen and substrate (nutrient, mineral) during ischemia. Acute myocardial infraction may cause a heart attack (36, 37). Generation of arrhythmias due to the ischemia is common cause of mortality. Ventricular arrhythmias appearing after myocardial infarction may be due to an alteration in the electrophysiological properties of cardiac fibers in the area of the heart deprived from normal blood supply. The elucidation of such alterations on a cellular level might enhance our understanding of these arrhythmias (38).

1.4.1. Cellular Changes after the Acute Myocardial Infarction

Following the myocardial ischemia, cellular changes and some abnormalities occur in ischemic cells. Tissue oxygen content falls rapidly and the contraction abnormalities appear in five seconds (39). Intracellular ATP concentration and creatine phosphate decrease. During ischemia, myocardial cells tend to provide energy by the way of anaerobic respiration (40). As a result of anaerobic respiration, intracellular pH decreases in a minute of ischemia. Adenosine released from ischemic cells increases during the ischemia by the hydrolysis of ATP, ADP, and AMP. Then the sarcolemmal K_{ATP} channels open and extracellular K^+

concentration increases. Na^+-K^+ pump impairs by the intracellular low ATP concentrations and Na^+ ions accumulate in the ischemic cells (39). Intracellular Na^+ accumulation activate Na^+/Ca^{2+} exchange channels, so intracellular Ca^{2+} increase (**Figure 1.7.**). The intracellular Ca^{2+} accumulations may be harmful because Ca^{2+} activates phospholipases and proteases that impair the integrity of the cell membrane. Then the ischemic damage occurs due to the catabolite accumulation including lactate and arachidonic acids.

Arachidonic acid is a one of the fatty acid that may produce reactive oxygen species. Free oxygen radicals damage the cell membranes and contribute to cell death (12, 14).

Energy production in mitochondria falls following myocardial ischemia due to the inadequate supply of O_2 (41). This causes abnormal accumulation of ions and metabolites. Calcium ion has important effect on the functions of mitochondria. Opening of the mitochondrial K_{ATP} channel reduce the mitochondrial membrane potential, thus suppressing Ca^{2+} influx into the mitochondria. The mitochondrial K^+ cycle, counterbalancing mitochondrial uniporter activity with K^+/H^+ exchange, is an important component in mitochondrial volume homeostasis. The opening of a mitochondrial K_{ATP} channel causes K^+ influx into mitochondria, accompanied by the movement of permeable anions and water. Matrix volume increase by influx of potassium and balanced with K^+/H^+ exchanger. K^+/H^+ exchanger prevent over swelling of matrix (42) (**Figure 1.7.**).

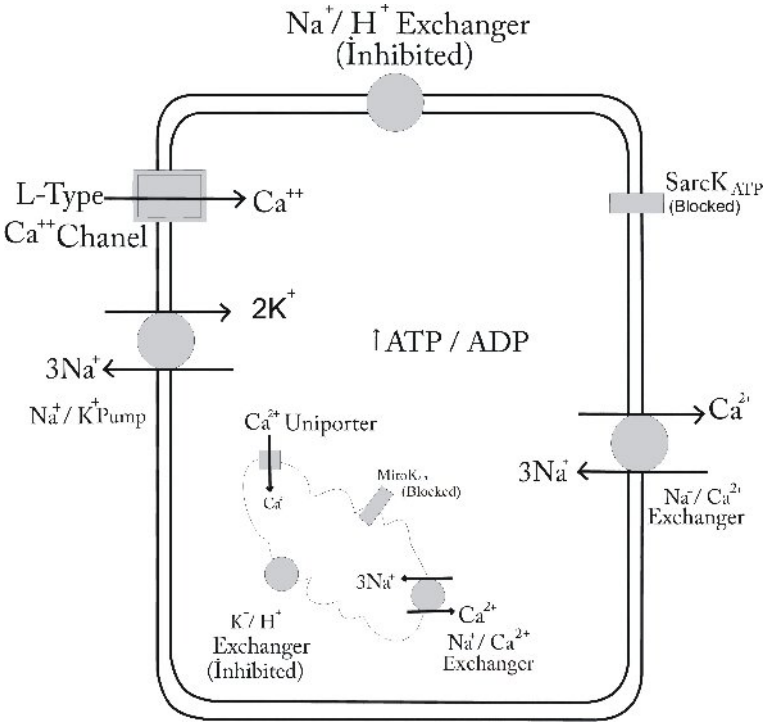


Figure 1.6. Channels and exchangers in normal condition.

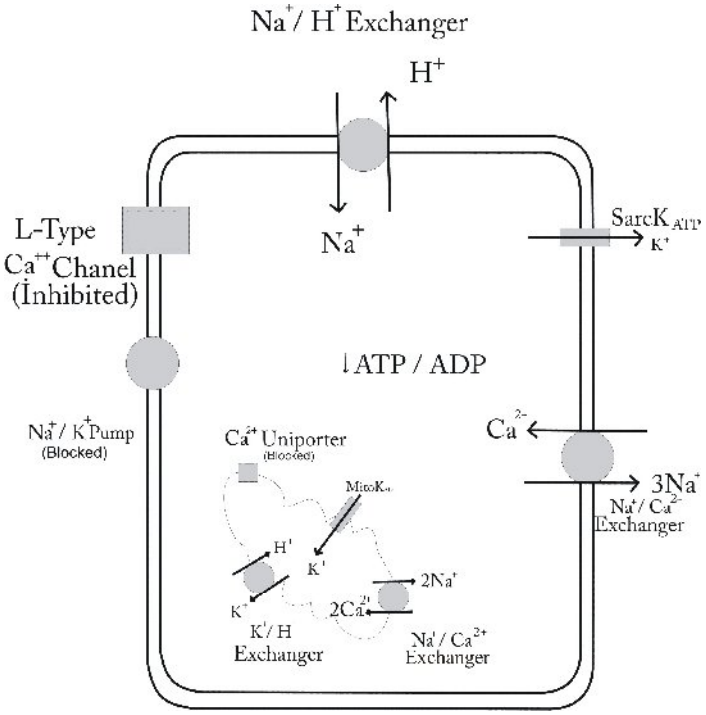


Figure 1.7. Channels and exchangers in ischemic condition.

1.4.2. Cellular Changes in Reperfusion

The opening of occluded coronary artery provides reperfusion. Reperfusion cause more damage in ischemic heart. Reperfusion result with the osmotic cell swelling, intracellular calcium overload, and especially the burst production of oxygen free radicals (43). The generation of reactive oxygen species (ROS) is a key process in development of reperfusion injury which results in arrhythmia, reversible contractile dysfunction, endothelial dysfunction and cell death (44). High amount of adenosine and its degradation products released during the reperfusion was particularly associated with ventricular fibrillation (44).

The major two sources of ROS are the xanthine oxidase and the mitochondrial respiratory chain in reperfusion. The generated ROS further impair the respiratory chain activity in mitochondria. ROS damage membrane ionic pumps and further exacerbate the cell ionic disregulation. ROS may also causes peroxidation of unsaturated fatty acid components of phospholipids (45).

1.5. FACTORS RELATED WITH THE ARRHYTHMIAS FOLLOWING CORONARY ARTERY LIGATION IN EXPERIMENTAL ANIMALS

The major clinical determinants of ischemia-associated arrhythmia are changes in heart rate, the size of the ischemic area, developments of collateral vessel, and changes in channel activity in myocardial cells (46).

1.5.1. Size of Ischemia

Size of ischemic area is more effective on the arrhythmias. Severity of arrhythmia increases in increasing infarct size (6). There are

considerable studies indicating direct relation of arrhythmias with infarct size (6, 47). Ectopic foci increases in the larger ischemic damage and more severe long lasting arrhythmias appear in this condition. Arrhythmias usually disappear after the all ischemic cell went to death. In the larger ischemia, more time required to reach the ischemic area to become more stable. In the larger ischemia, ventricular tachycardia produced are multifocal and do not be suppressed by increased heart rate. Otherwise arrhythmias occur in the presence of smaller infarct can be suppressed by increased heart rate (6).

After the ischemia, extracellular potassium increase by opening of K_{ATP} channels. Increasing of extracellular potassium causes lengthening of refractory period in normal cells in non-ischemic region that lead to slow transmission of stimuli. In the larger ischemia, potassium leakage is higher than smaller ischemia. Thus ischemic depolarization is higher and reentrant pathway is shortened.

1.5.2. Heart Rate

Sinusal tachycardia occurs by the sympathetic stimulation in the early phase of ischemia. Increasing sinusal beat causes suppression of ventricular extra systole. In the later stage since the effect of autonomic activity decrease and severity of ischemia increase ventricular arrhythmia in multifocal origin appear. Amount of catecholamine was found in higher concentration in the circulation during myocardial injury due to the reflex activation of sympathetic nerves (48). That is why not only re-entrant mechanism but also increased automatic activity in purkinje cells leads to more severe arrhythmia in later stage of ischemia. That is why, ventricular

arrhythmias produced in the later stage of myocardial infarction are not affected by changes in heart rate (6).

1.5.3. Channels

There are various channels in sarcolemmal and mitochondrial membrane which are very important in arhythmogenesis. These channels are; Sodium channels, voltage gated Ca^{2+} channels, Na^{+}/K^{+} Pump, Na^{+}/Ca^{2+} exchanger channels, Na^{+}/H^{+} exchanger channels and K_{ATP} channels.

1.5.3.1. Sodium Channels

The influx of Na^{+} ions plays an especially unique and critical role. The sodium current (I_{Na}) is not only responsible for initial cardiac action-potential depolarization, but also the influx of Na^{+} ions into the cytoplasm, which helps generate the prolonged depolarization characteristics of the action-potential plateau. The magnitude and kinetics of I_{Na} are controlled in a complex manner by both membrane potential and intracellular metabolites. Activation of Na^{+} channels is thought to result from a voltage-driven conformational change that opens a trans-membrane pore through the channel protein. Channel opening is triggered by membrane depolarization.

1.5.3.2. Calcium Channels

Calcium channels are membrane-spanning proteins that regulate the intracellular concentration of calcium ions (Ca^{2+}). After entering the cell, Ca^{2+}activates specific calcium receptor proteins, e.g., calmodulin,

troponin-C, or calcium-activated calcium, potassium, and chloride channels.

In the healthy heart, voltage activated calcium channels are essential for the generation of normal cardiac rhythm, for the propagation of excitation through the atrioventricular node, and for contraction in atrial and ventricular myocytes.

In diseased myocardium, calcium channels can contribute to abnormal impulse generation and cardiac arrhythmias.

Voltage-activated calcium channels are regulated by a change in the membrane potential, together with voltage-gated K^+ and Na^+ channels. Two types of voltage-activated calcium channels have been identified in cardiac muscle. They are T (transient)-type calcium channels (activated as low-voltage) and L (long-lasting)-type calcium channels (high-voltage activated).

Cardiac L-Type Calcium Channel provides an essential part of the Ca^{2+} necessary for contraction. In addition, the L-type Ca^{2+} current contributes to the plateau of the cardiac action potential (**Figure 1.6.**).

Low-voltage activated calcium channels are expressed predominantly in the cardiac atria and carry a tiny and transient (T-type) current. T-type channels may contribute to the generation of the action potential in the sinus node.

1.5.3.3. Cardiac Na^+/K^+ Pump

Na^+/K^+ pump is a pump which maintains Na^+ and K^+ at resting level on either side of myocardial cell membrane. The sodium current carried by Na^+ channels is fast and obviously responsible for the upstroke of the

cardiac action potential. The activity of the Na^+/K^+ pump, directly or indirectly, sets up and maintains the ionic gradients necessary for the proper generation of action potentials and contraction. Although the Na^+/K^+ pump exchanges Na^+ for K^+ during the transport cycle, the exchange is unequal. For every two K^+ ions moved into the cell, three Na ions are moved out of the cell (**Figure 1.6.**).

After the myocardial ischemia, Na^+/K^+ pump is inhibited (**Figure 1.7.**). There is interaction between Na^+/Ca^{2+} exchange mechanism and Na^+/K^+ pump activity. The inhibition of the Na^+/K^+ pump cause rising of intracellular Ca^{2+}. Activation of the Na^+/Ca^{2+} exchanger cause to release of Ca^{2+} into the cytosol, initiating a delayed after-depolarization. This is the most common arrhythmia resulting from digitalis intoxication in heart failure patients.

1.5.3.4. Cardiac Na^+/Ca^{2+} Exchanger

The calcium ion is essential in cardiac muscle contraction. Ca^{2+} ions enter cardiac myocytes through Ca^{2+} channels on each heart beat. The cardiac muscle relaxes when Ca^{2+} returns to SR by the Ca^{2+} pump or Ca^{2+}-ATPase. The amount of Ca^{2+} entered should be removed from the cell on each heart beat. A specific membrane transporter called the ‘‘Na^+–Ca^{2+} exchanger’’ is a main player of the Ca^{2+} extrusion in cardiac myocytes. The exchange ratio of Na^+–Ca^{2+} exchanger is $3Na^+$ inside $1Ca^{2+}$ outside of the cell in normal myocardial cells (**Figure 1.6.**). In ischemic cells, this channel works in reverse order (**Figure 1.7.**). The reversal potential of the exchange depends on intra and extracellular concentrations of Na^+ and Ca^{2+}.

1.5.3.5. Na^+/H^+ Exchanger

The Na^+/H^+ exchanger is in a closed state in normal myocardial cell (**Figure 1.6.**). When the intracellular acidosis develops, this exchanger starts to work. The Na^+/H^+ exchanger is an electro-neutral ion exchanger that pumps H^+ ions out of the cell when its activity is increased by an intracellular acidic load. The H^+ efflux is driven by the inwardly directed Na+ electrochemical gradient. The Na^+/H^+ exchanger is activated during ischemia and reperfusion and contribute to the intracellular accumulation of sodium, and consequently calcium, in myocardial cells. The Na^+/H^+ exchanger prevents the deleterious effects of ischemia/reperfusion injuries (**Figure 1.7.**).

1.5.3.6. K^+ Channels

There are different types of potassium channels in myocardial cells. ATP dependent potassium channel is most related with ischemia and reperfusion injury and arrhythmias. Another one is voltage-dependent K^+ channels that play an essential role in repolarization of cardiac myocytes.

1.6. ATP DEPENDENT POTASSIUM CHANNELS

The ATP-sensitive K^+ (K_{ATP}) channels were discovered by Noma in cardiac ventricular myocytes (49). Since then, these channels have been identified in various tissues, such as pancreatic β-cells (50), skeletal muscle (51), vascular and nonvascular smooth muscle (52), neuronal cells (53), and kidney (54).

The physiological function of K_{ATP} channels is not known because these channels are to be closed in the normal functioning heart (55)

(**Figure 1.6.**). They may play significant roles in pathological conditions, especially in myocardial ischemia, where cellular ATP levels fall substantially. The intracellular ATP concentration is more effective to the K_{ATP} channels.

K_{ATP} channels are inhibited by physiologic level of ATP, but they have been found to be modulated by various stimuli such as decreased pH, increased NO and fatty acids, activated G protein, released adenosine and acetylcholine (56).

Two K_{ATP} channel subtypes exist in the myocardium; one subtype located in the sarcolemmal membrane of the myocytes that is called as sarcolemmal ATP dependent potassium channel (sarcK_{ATP}) and the other in the inner membrane of the mitochondria that is called mitochondrial ATP dependent potassium channel (mitoK_{ATP}) (49) (**Figure 1.6.**).

K_{ATP} channels play an important role in the regulation of cardioprotective mechanism (57, 58). It has been shown that opening of K_{ATP} channels during ischemia leads to arrhythmias and blockage of these channels exert antiarrhythmic action (57, 7). On the other hand opening of the channel decrease ischemia but blockage of the channel in ischemia increase infarct size.

1.6.1. Effect of Channel Opening to the Arrhythmias

In the normal heart, the K_{ATP} channels are closed state in the presence of high intracellular ATP concentration. During ischemia, ATP concentration decrease and ADP concentration increase. Ischemic cells need energy to maintain metabolic activity and this energy is provided from anaerobic respiration. As a result of anaerobic respiration lactic acid

and H^+ ions increase in the ischemic cells. Then intracellular pH decrease and anaerobic respiration is inhibited.

Na^+-K^+ pump do not work in ischemic cells because of depletion of ATP. Since sodium accumulates inside the cells, Na^+-H^+ and Na^+-Ca^{2+} exchangers are activated. K_{ATP} channels open due to the fall in intracellular ATP during myocardial ischemia. High amount of potassium efflux occur from the ischemic myocardial cells. Outflux of potassium causes the hyperpolarization of the cell membrane and shortening of the action potential duration. This situation cause decreasing Ca^{2+} influx by the voltage gated Ca^{2+} channels (**Figure 1.7.**). This situation leads to a regional heterogeneity between ischemic and nonischemic regions (59) that is supposed to be one of the main causes of malignant arrhythmia formation (60). Selective opening of these channels caused marked inhomogeneities of refractory period that provoked extrasystoles (61). Opening of mitochondrial K_{ATP} channels cause the potassium influx into the mitochondrial matrix ragion and lead to activation of K^+/H^+ exchanger. K^+ efflux and H^+ influx occur by this exchanger in the mitochondrial inner membrane. Entering H^+ reduces ATP production by increased intramitochondrial acidity (62). MitoK_{ATP} channel opening buffer to Ca^{2+} entry (**Figure 1.7.**). Opening of mitochondrial K_{ATP} channels leads to membrane depolarization, matrix swelling, slowing of ATP synthesis (63).

Although the relative roles of the sarcK_{ATP} and mitoK_{ATP} channels remain elusive, it has been suggested that openings of the sarcK_{ATP} channels are arrhythmogenic (64), whereas openings of the mitoK_{ATP} channels are cardioprotective (65).

1.6.2. Effect of Openers on the Channel in the Ischemia Induced Arrhythmia

K_{ATP} channel openers (KCO) effect on cardiac and vascular smooth muscle (66). In some studies reported that proarrhythmic effect of K_{ATP} channel openers in vitro and in vivo studies (67). The proarrhythmic effect of the KCO is dependent on the reflex activation of the cardiac sympathetic nerve and the release of noradrenaline induced by marked systemic hypotension (68). When the K_{ATP} channels open, the influx of Ca^{2+} ions into the cell decreases during the plateau phase of action potential and shorten action potential duration. ATP preservation occurs with the prevention of intracellular Ca^{2+} accumulations (66). The action potential duration shortening effect of K_{ATP} channel opener can be proarrhythmic (69, 70).

Some researcher reported the anti arrhythmic effect of K_{ATP} channel openers (71, 72). KCO causes the action potential shortening of the myocardial cells even in the normally oxygenated myocardium (70) and vasodilates coronary arterioles and arteries. One of the electrophysiological consequences of regional myocardial ischemia is the shortening of the action potential duration in hypoxic myocytes that leads to the marked electrical inhomogeneity between normoxic and ischemic tissues, increasing the possibility of the development of severe arrhythmias (60). This inhomogeneity exists also within different areas of the hypoxic tissues between severely and less seriously injured myocardial fibers. KCO could further shorten the action potential duration of the slightly injured and normal fibers in the heart during ischemia. This

decrease inhomogeneity between nonischemic and ischemic myocardial tissues, because opening of K_{ATP} channels cause to change electrical properties of normal cells to ischemic cells (73).

1.6.3. Effect of Blockage of the Channel on the Ischemia Induced Arrhythmia

The K^+ leakage from the ischemic cells plays a role in the generation of the arrhythmia. The researches have been intensified on the role of myocardial K_{ATP} channels. K_{ATP} channels are blocked by the K_{ATP} channel blockers such as glibenclamide. K_{ATP} channel blocker closes the channels in ischemic cells (74), because these channels are in the closed state in normal conditions. The antiarrhythmic effects of K_{ATP} channel blockers were shown in some studies (7, 67, 75, 76). The antiarrhythmic effect of the K_{ATP} channel blockers was attributed to the inhibition of the K^+ loss from ischemic cells and the prevention of nonuniform shortening of the action potential duration during the myocardial ischemia. This effect may decrease the development of electrical inhomogeneity between ischemic and nonischemic myocardium and might suppress the substrate for reentrant pathways, resulting in antiarrhythmic action. K_{ATP} channel blockage may prevent extracellular potassium accumulation and prevents flow of depolarizing current from ischemic region to normal myocardium.

Some studies reported that the K_{ATP} channel blockers show proarrhythmic effect (77, 78). The proarrhythmic effect of the K_{ATP} channel blockers was attributed to diminished effect of the used K_{ATP} blocker on the coronary blood flow (77). This effect was also cause to the worsening of the ischemia due to the myocardial Ca^{2+} overloading. The Ca^{2+} overload

and oxyradicals formation generates ischemia and reperfusion induces arrhythmias (79).

1.7. EFFECT OF GENDER DIFFERENCES ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS

Cardiovascular mortality has seen observed lesser in premenopausal women than man in similar age (3). The risk of coronary disease in women rapidly increases after menopause and reach to level as like in men of the same age. The major cardio protective mechanism of the premenopausal female is believed to be an anti-atherogenic action of female sex hormones (80, 81). Some researches show that the number of the sarcolemmal ATP- dependent potassium channel was higher in females than in males (82). The presences of more sarcolemmal ATP-dependent K^+ channels contribute to more resistance against ischemia/reperfusion induced injury and arrhythmia. After myocardial ischemia, the opening of the ATP-dependent K+ channel shortens the myocardial action potential during repolarization. Estrogen inhibits inward Ca^{2+} current. As a result, the voltage dependent Ca^{2+} influx into the myocardial cell decreases during ischemia. Previous studies showed that the incidence of VF and total ventricular arrhythmias decreased in female rats with respect to males (75). This protective effect appears to be mediated by estrogen. And some studies demonstrated that exogenous estrogen significantly attenuates ischemia and reperfusion induced arrhythmias (83) and reduces infarct size (80). Estrogen enhances nitric oxide (NO) synthase in myocardial cells which may produce vasodilatation (84). Nitric oxide increases collateral blood flow, reduces oxygen

consumption, produces inhibition of platelet aggregation (83). NO may reduce the severity of ischemia by inhibition of the cytosolic accumulation of Ca^{2+} and NO may reduce ROS generation by decreasing lipolysis (85). Activation of PKC and G protein by estrogen may link to the mitochondrial ATP-dependent channels. Opening of mitochondrial ATP-dependent channels shows cardio-protective effect. Another difference of female from male is different autonomic activity to the hearts. Vagal activation is more common in women than in men during coronary artery occlusion; bradycardia and decreased blood pressure are usually seen in females rather than in males (75).

1.8. SYNDROMES OCCURRED ACUTE MYOCARDIAL INFARCTION

The prolonged periods of myocardial ischemia lead to irreversible damage of the myocardial cells. However, more recently, five additional ischemic syndromes have been described between transient ischemia and myocardial infarction in various clinical conditions.

1.8.1. Myocardial Stunning

Stunning can be attributed to the reperfusion following a brief bout of ischemia. In this condition ischemic myocardial cells remain in quiescent state but not goes to death. It has been considered that the underlying mechanisms of stunning are directly related to the detrimental effects of reperfusion injury. Stunning is completely reversible, and therefore there is no permanent damage to the myocardium. There are two theories for explaining the pathologic mechanism underlying myocardial stunning. Due to the formation of free-radical reactive species

or alterations in intracellular calcium, during ischemia intracellular acidosis can potentially generate intracellular calcium oscillations and calcium overload on reperfusion. The calcium sensitivity of the contractile myocardial cell decreases in the stunned myocardium and is considered primarily responsible for systolic dysfunction; for example, the stunned myocardium is still responsive to inotropic stimulation. During the early stage of stunning, it is considered beneficial to prevent calcium oscillations and thus attenuate significant injury caused by reperfusion.

1.8.2. Hibernating Myocardium

Hibernation is related in term of a depressed state of contractility and have a potential for dysfunctional myocardium to return to normal. Hibernating myocardium is in a chronic hypocontractile state because of a decreased oxygen supply and thus may only recover full function with revascularization. Although there is an absence of necrosis with myocardial hibernation, morphological changes to the myocardial architecture, such as loss of myofibrils and increased interstitial fibrosis, may occur if this state persists.

1.8.3. Maimed Myocardium

The maimed myocardium is a classic myocardial infarction in that ischemia-induced necrosis leads to loss of contractile performance. The duration of ischemia is long enough to result in necrosis; however, there can be partial recovery of function to this ischemic region following reperfusion. The maimed myocardium syndromes exhibit an incomplete recovery of myocardial function following drug-induced or mechanical

reperfusion of an occluded coronary artery and then demonstrate regions of viable myocardium in the ischemic area.

1.8.4. Ischemic Preconditioning

Ischemic preconditioning occurs by the short ischemic period before long lasting occlusion. It provides protection against arrhythmia produced in later stage of reperfusion. But the factors providing protection produced by ischemic preconditioning are still controversial and not clarified completely.

In the myocardium, ischemic preconditioning has been shown to be potentially infarct limiting as well as antiarrhythmic, although the latter of these effects has been disputed. The clinical applicability of ischemic preconditioning may be limited to situations in which the ischemic event can be anticipated e.g., bypass cardiac surgery, percutaneous trans-luminal coronary angioplasty.

1.8.5. Silent Ischemia

Silent ischemia refers to single or multiple asymptomatic episodes of transient ischemia. Silent ischemia can occur in the weeks following an acute myocardial infarction in patients with a history of coronary artery disease.

Silent ischemia is postulated to be related to a lack of oxygen supply rather than an increase in oxygen demand; however, controversy remains concerning the mechanism by which silent ischemia can proceed unnoticed.

1.9. METABOLIC FACTORS IN ISCHEMIA AND REPERFUSION

1.9.1. Adenosine

Adenosine is present in low concentrations in the normal myocardium but increases during ischemia and reperfusion by hydrolysis of ATP, ADP and AMP. Adenosine mediates a variety of responses via specific receptors on myocytes, vascular smooth muscle cells, endothelial cells and neutrophils.

ATP↔ADP→AMP→ADENOSINE→INOSINE

The movement of adenosine into or out of cardio-myocytes occurs by facilitated diffusion via nucleoside transporters. Adenosine improve myocardial energy metabolism. Adenosine reduces myocardial oxygen demand (86) which could result in preservation of high energy phosphate stores under ischemic conditions. Intracoronary adenosine given at the onset of reperfusion to dogs following coronary artery ligation preserves endothelial structure, attenuates neutrophil accumulation, improves regional ventricular function and reduces infarct size (87).

Adenosine plays a key role to activation of K_{ATP} channels. There is an adenosine A_1 receptor that is associated with K_{ATP} channel in the membrane. Adenosine A_1 receptor stimulation opens the K_{ATP} channels (88).

1.9.2. Reactive Oxygen Species (ROS)

Free radicals contain an unpaired electron and are accordingly highly reactive. Molecules involved in free radical reactions include superoxide anion (O^-_2), hydroxyl radical ($-OH^-$), hydrogen peroxide (H_2O_2),

peroxynitrite and hypochlorous acid. They are believed to be generated during ischemia and reperfusion in mitochondria.

During ischemia, ATP hydrolysed to ADP, then AMP, adenosine, inosine, and finally, hypoxanthine. In addition, the enzyme, xanthine dehydrogenase, is converted to xanthine oxidase by calcium-dependent proteases. When the ischemic tissue is reperfused, hypoxanthine reacts with molecular oxygen in the presence of xanthine oxidase to form xanthine; xanthine is then converted to uric acid by xanthine oxidase, and superoxide anions are formed. The superoxide anion also may combine with H_2O_2 in the presence of a transition metal catalyst such as iron or copper to form the hydroxyl radical (89).

These reactive oxygen species can be cytotoxic to cells by attacking fatty acids, which leads to lipid peroxidation of membranes, and reacting with proteins, including destruction and oxidation of amino acids, oxidation of sulfhydryl groups, and polypeptide chain (90).

1.9.3 Alpha and Beta Adrenergic Stimulation

During the early hours of acute coronary occlusion, intense adrenergic activation of the ischemic myocardium may contribute to the evolution of abnormal cellular electrical behavior and, ultimately, result in lethal ventricular arrhythmias, ventricular tachycardia (VT) or ventricular fibrillation (VF) (91).

Ischemia-reperfusion is known to cause sympathetic activation, resulting in elevated systemic catecholamine levels (6). The resultant activation of cardiac β-adrenoceptors by neuronal and humoral catecholamine mediates an increase in heart rate and myocardial inotropic

state, thereby increasing myocardial oxygen demand. The direct effect of the sympathetic neurotransmitter norepinephrine on coronary vascular smooth muscle cells is vasoconstriction through activation of α - adrenoceptors (92).

1.9.4. Activation Protein Kinase

Protein kinase C (PKC) is known to be a critical component in many intracellular signal transduction pathways (93), and a hypothesis has recently been proposed in which PKC plays a central role. Endogenous ligands such as adenosine and noradrenaline initiate an intracellular pathway by acting on G-protein-linked receptors, which eventually leads to the activation of PKC. Activated PKC then phosphorylates a secondary effector protein, which in some way induces protection (94). Protein kinase C has also been shown to be activated in the heart during ischemia (95). Several possibilities exist with respect to the mechanism by which PKC prevents diastolic dysfunction during the reperfusion period (96). These include decreased cytosolic calcium accumulation during ischemia by inhibiting L-type calcium channel opening. PKC activity is important for the opening of the mitoK_{ATP} channel.

2. MATERIALS AND METHODS

2.1. ANIMALS

In the present study, 280-377 g weight, 8-9 months old, 21 Spraque-Dawley male rats were used. Animals were allowed to drink tap water ad libitum and fed with commercial rat food pellet. All animals were treated in adherence to the guiding principles in the care and use of animals together with the protocol stipulated by the declaration of Helsinki.

2.2. CORONARY ARTERY LIGATION AND PERFUSION

Animals were anesthetized with thiopental sodium (100 mg/kg). Trachea was cannulated for artificial respiration. The catheter for carotid cannulation was filled with heparin (25000UI/100ml) diluted 1/10 with saline and inserted into the left carotid artery for measuring the blood pressure. The chest was opened by cutting the fourth and fifth intercostals ribs. Pericardium was removed from heart, and the heart was exposed by using ecarteur. A loose loop of 5-0 autramatic silk was placed around the small branch of left main coronary artery. Then, the heart was set back in its place and artificial respiration was started using an animal respirator (Ugo Basile Rodent Ventilator, Italy) in 60 –strokes/minute. Subcutaneous needle electrodes were placed under the skin to record standard electrocardiogram. Then, the animals were allowed to stabilize for 5

minute. The ligation was made by tightening the silk thread and producing a bowknot resulting the regional myocardial ischemia for 30 minute. A ligation technique was shown in Figure 2.1. The arterial blood pressure, bipolar ECG and body temperature were recorded during the 30 minute of coronary ligation and stored in computer for later analysis.

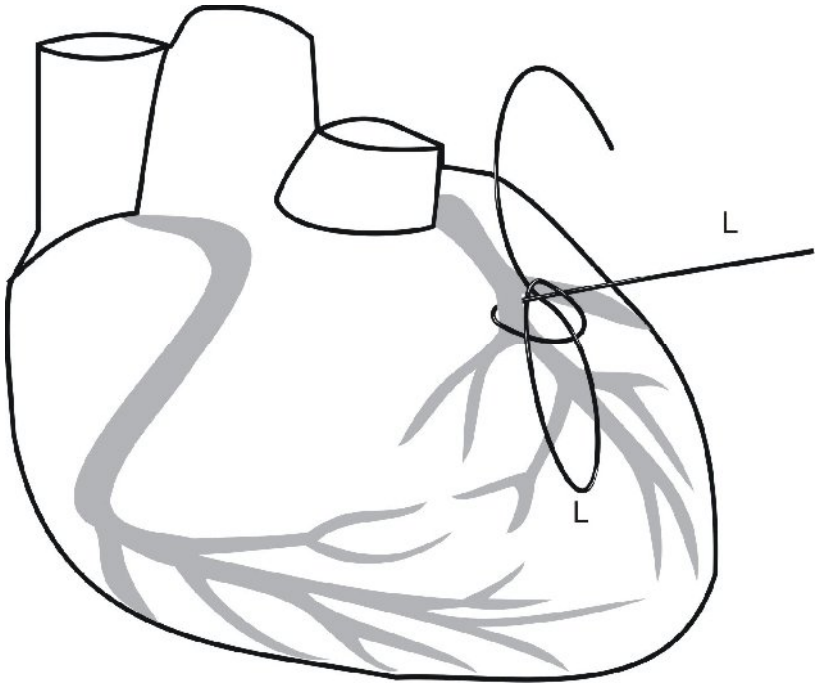


Figure 2.1. Producing partial coronary artery ligation in rat heart.

In the survived rats, the heart was perfused through the aorta with the solutions, first NaCl and then ethanol, to determine the risk of infarct area. **Figure 2.2.** shows the heart after perfusion. After the perfusion of heart, the non-perfused area that remained red was separated from the well perfused area that seemed white color. The non-perfuse myocardium was cut and weighed. The percentage of this area in respect to the total weight of ventricle was calculated. After that, non-perfused myocardium

was cut about six segments by razor blade. These segments were put in the solution prepared by nitrobluetetrazolium (NBT) about 3 minute for staining. Non Ischemic region was stained with NBT but ischemic area was not stained. Color of nonischemic region was seen red, infracted region was seen light pallor. Infarct region was separated from noninfarcted region by razor blade and weighed.

Finally, percentage of the risk of infarct area (Non-perfuse Area / All of the heart) and infarct area (Infarct Area / All of the heart) were calculated.

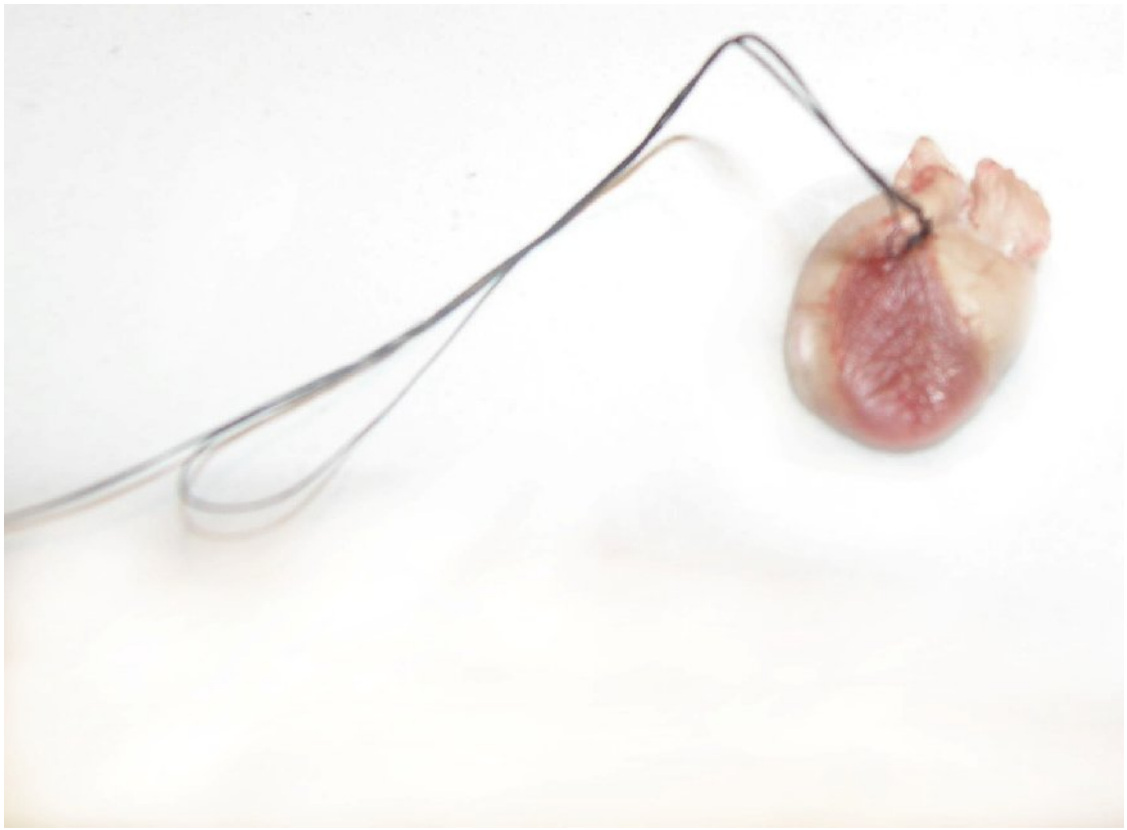


Figure 2.2. Perfused heart.

2.3. DRUG ADMINISTRATION PROTOCOL

Pinacidil was dissolved in ethyl alcohol and then mixed with isotonic solution. Stock solution was included 0, 2 mg pinacidil dissolved in 200µl

ethyl alcohol and 200µl isotonic solution. It was given in 0, 1 mg/kg dose intravenously from coccygeal tail vein 10 minute before coronary ligation.

Glibenclamide was dissolved in dimethylsulfoxide and then mixed with ethanol in same ratio. Stock solution was included 5 mg glibenclamide dissolved in 100µl DMSO and 100µl ethanol. It was given in 5 mg/kg dose intraperitoneally 20 minute before coronary ligation.

In control group, instead of the pinacidil and glibenclamide, same value of solvent was applied in both regions.

2.4. PREPERATION OF STAIN

Preparation of NBT; 0, 5 mg NBT was dissolved in a solution including 0, 9 g KH_2PO_4 substances in 100 ml distilled water. Then, 4, 68 g $NaHPO_4x2H_2O$ substances were dissolved in 400 ml distilled water. Finally, both these solutions were mixed.

2.5. THE EVALUATION OF THE ARRHYTHMIAS

The heart rate and blood pressure were determined from recorded ECG at 1, 3, 5, 10, 15, 20, 30 minute during the ischemia. The incidence and total length of arrhythmias during 30 minute of ischemia was calculated from recorded ECG. The onset and the offset of the arrhythmias were determined. The arrhythmias were identified as ventricular tachycardia (VT), ventricular fibrillation (VF), and other type of arrhythmia including single ventricular extra beat, bigemini and salvos. The sinusal tachycardia is differentiated from ventricular tachycardia according to heart rate and blood pressure. The arrhythmias are designated as sinusal tachycardia when the heart rate was 500/min to

600/min and the blood pressure was higher than 70 mmHg and as ventricular tachycardia when the heart rate was above 600/min and blood pressure was lower than 50 mmHg.

An arrhythmia score was given according to the incidence and duration of arrhythmias for each animal according to Lambeth conventions as follows; 0 = No arrhythmias; 1 = < 10 sec VT or other arrhythmias; 2 = 11–30 sec VT or other arrhythmias; 3 = 31–90 sec VT or other arrhythmias; 4 = 91–180 sec VT or other arrhythmias and / or < 10 sec reversible VF; 5 = > 180 sec VT or other arrhythmias and / or > 10 sec reversible VF; 6 = irreversible VF.

2.6. STATISTICAL ANALYSES

The mean and standard errors were determined for all parameters including heart rate and blood pressure. The duration of arrhythmias were compared by the analysis of variance (One – way ANOVA combined with the LSD post hoc. test). The survival rate and the incidence of arrhythmia were compared by chi-square test (Fisher exact test, two tailed).

3. RESULTS

Changes in the form of QRS complex and/or ST segment elevation were observed in all groups following the coronary artery ligation. All animals survived after 30 minute of coronary ligation. Arrhythmias started at 2-5 minutes following coronary ligation and terminated at about 12 minutes of coronary ligation in control animals **(Table 1)**. The arrhythmia started during later period of ischemia in pinacidil treated group. Pinacidil effect was yielded significant **(Figure 3.1.)**.

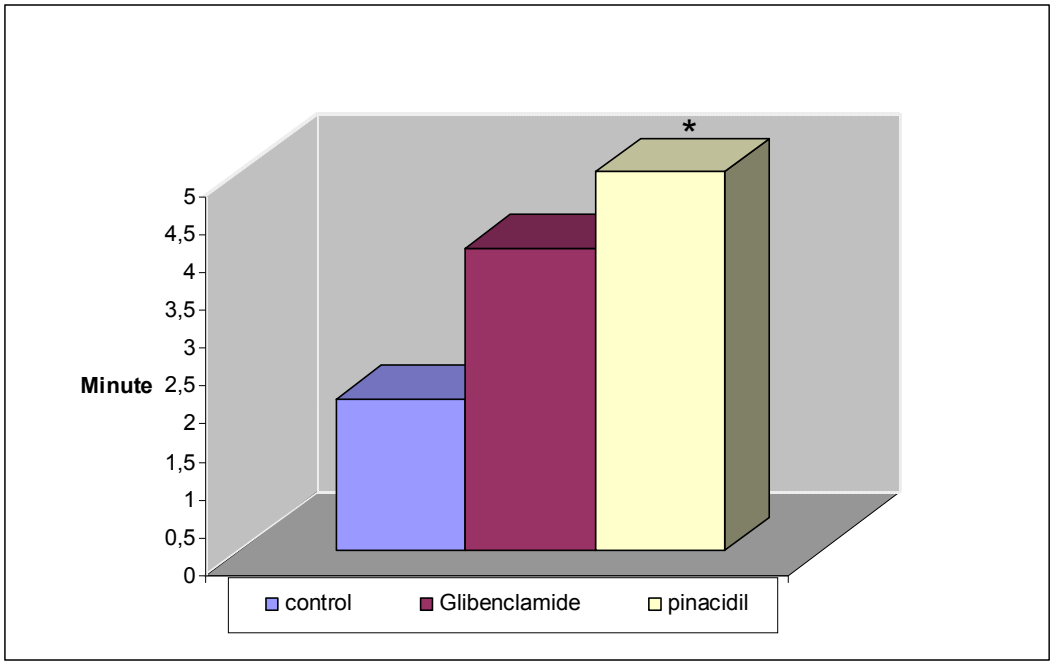


Figure 3.1. The Start of Arrhythmia after coronary artery ligation (P<0,05 According to Control).

Blood pressure decreased immediately after the coronary ligation in all group of animals. In pinacidil group, blood pressure was higher as compared to control group during 30 minute period of ischemia **(Figure 3.2)**. At 30th minute of coronary ligation, in pinacidil and glibenclamide group, the blood pressure was significantly higher than in control group (P<0, 05) **(Table 2).**

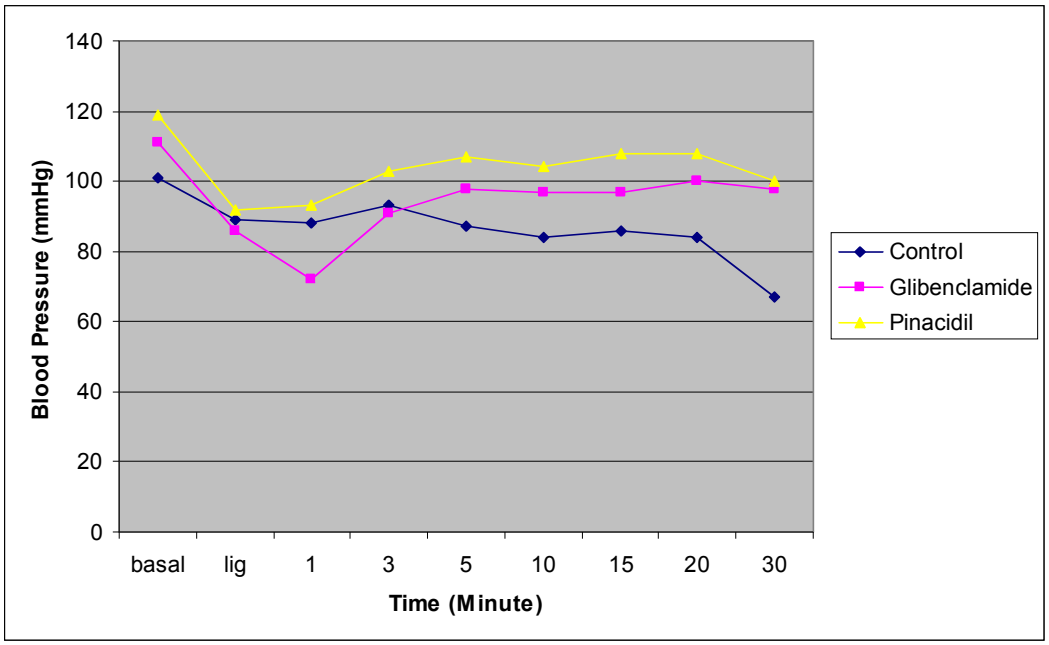


Figure 3.2. Blood pressure during coronary artery ligation.

Heart rate did not change during the 30 minutes ligation period in all three groups. However, at the end of 30 minutes of ligation the fall in heart rate was more significant in control group in respect to others (P<0,05) **(Figure 3.3) (Table 3).**

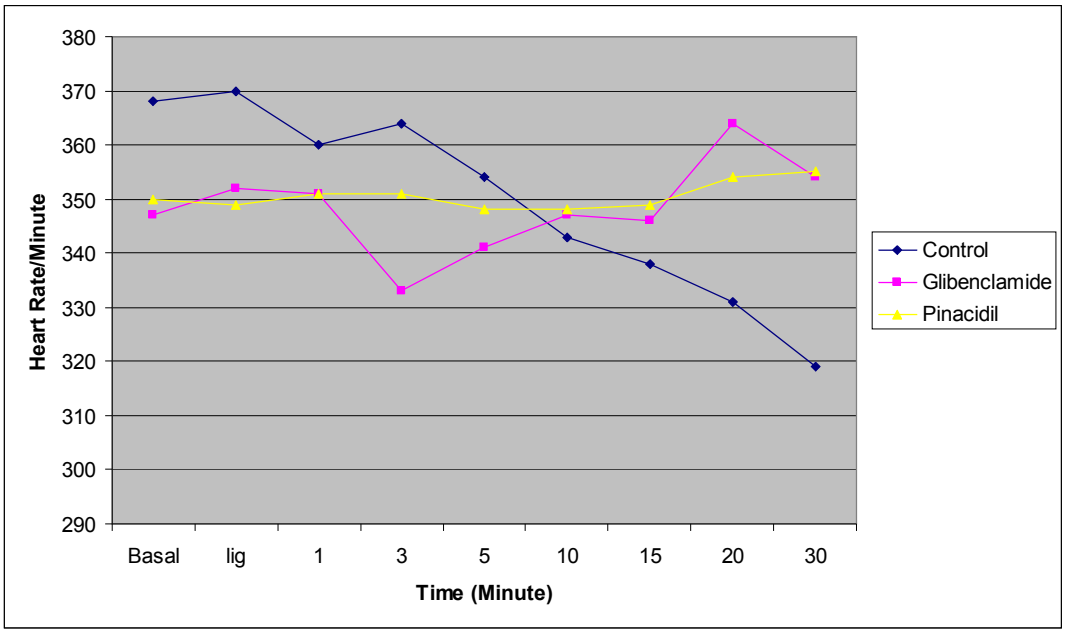


Figure 3.3. Heart rate during coronary ligation.

The length of the arrhythmic period was not significantly different in all groups of animals during ligation **(Figure 3.4.)**.

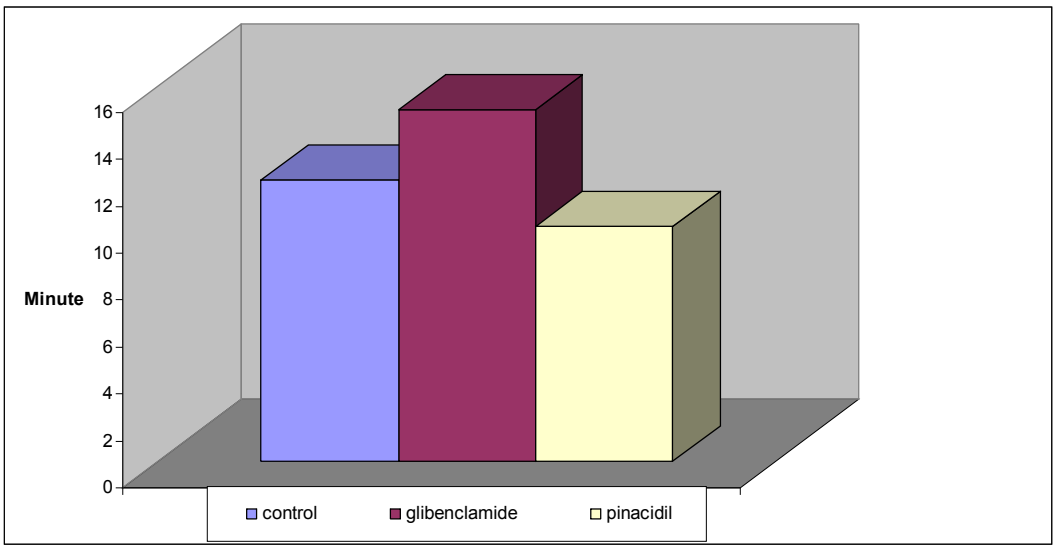


Figure 3.4. The arrhythmic period during 30 minutes of coronary ligation.

The total arrhythmia was not significantly different in all groups of animals during coronary ligation **(Figure 3.5)**.

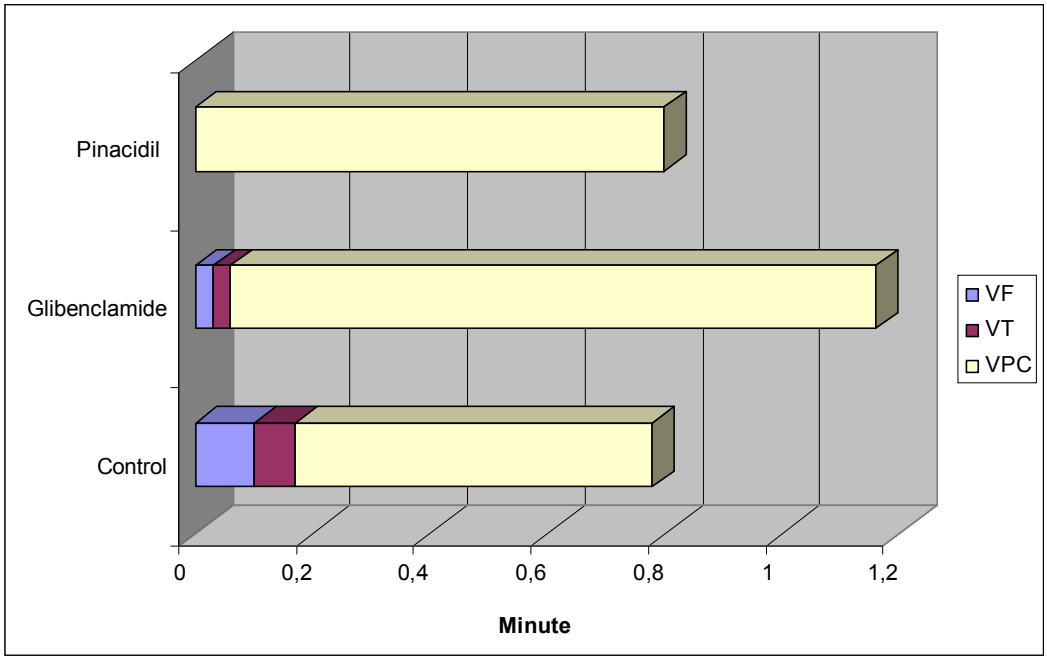
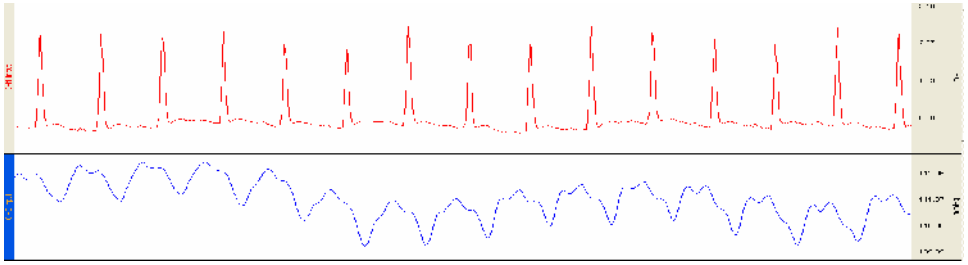
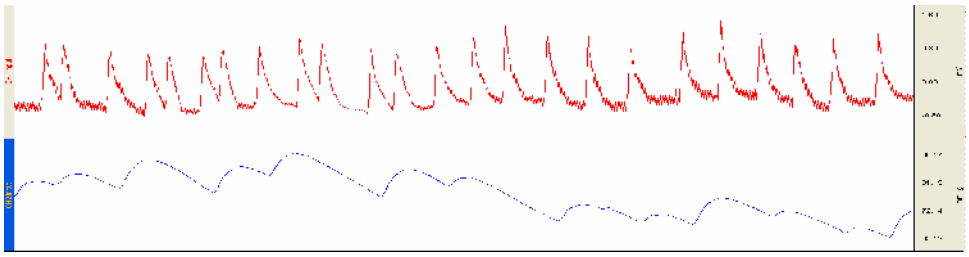


Figure 3.5. The type and duration of total arrhythmia during 30 minutes of coronary ligation.

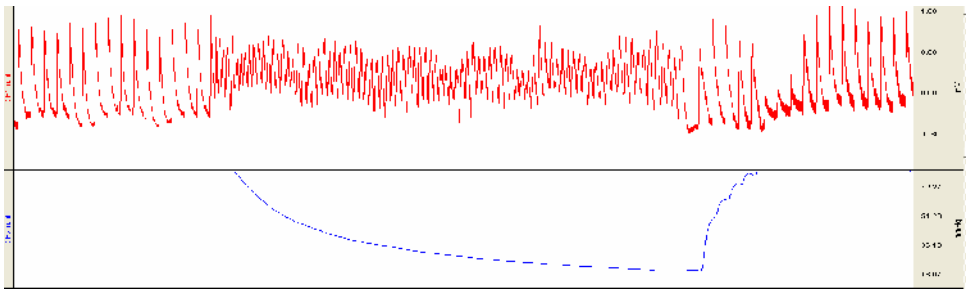
Incidence of arrhythmias was not significantly different among groups. Ventricular tachycardia and fibrillation were not observed in pinacidil treated animals. In other groups including control and glybenclamide group 1 animals out of 7 animals have ventricular tachycardia and fibrillation **(Table 1)**. All type of arrhythmias observed following coronary ligation is shown in **figure 3.6.**



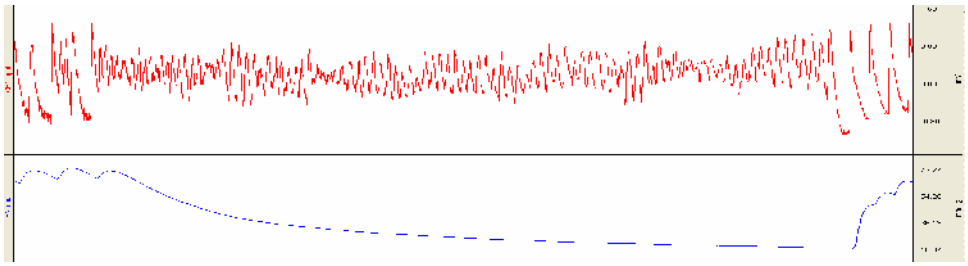
A) Sinusal rhythm



B) Premature Ventricular Contraction (PVC)



C) Ventricular Tachycardia (VT)



D) Ventricular Fibrillation (VF)

Figure 3.6. Types of arrhythmias and blood pressure observed after the coronary ligation in rats.

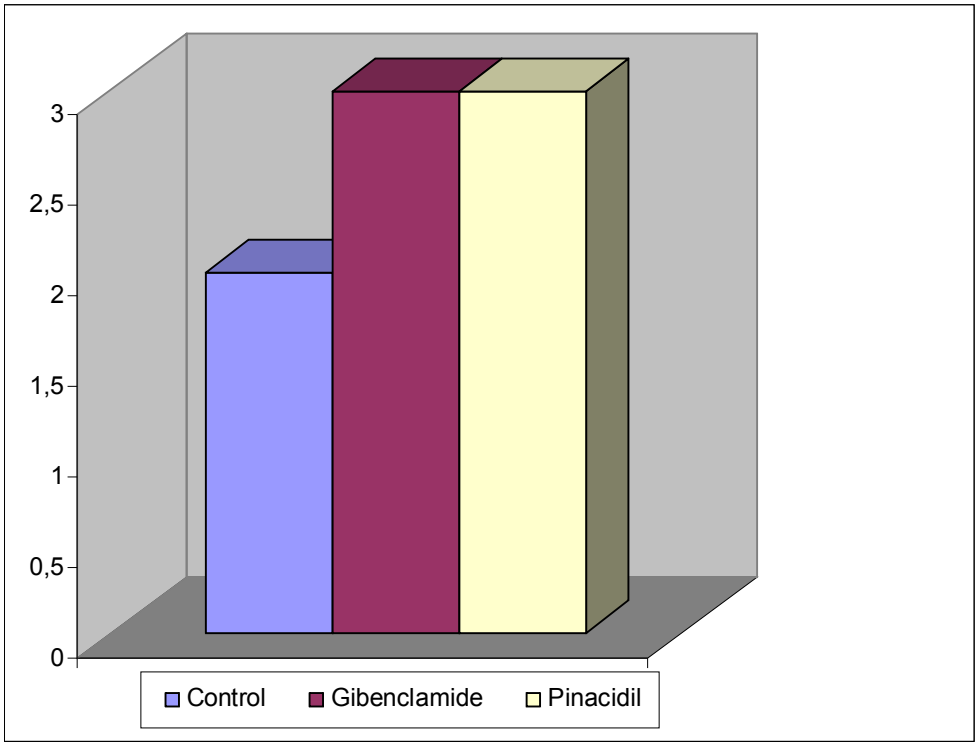


Figure 3.7. The score of arrhythmia.

Arrhythmia score calculated from duration and type of arrhythmia was not significantly different among groups **(Table 4) (Figure 3.7.)**.

The risk of the infarct area was not different among groups **(Table 5)**. But infarct area was significantly higher in the glibenclamide and pinacidil groups, according to control group ($P < 0, 05$) **(Figure 3.8.)**.

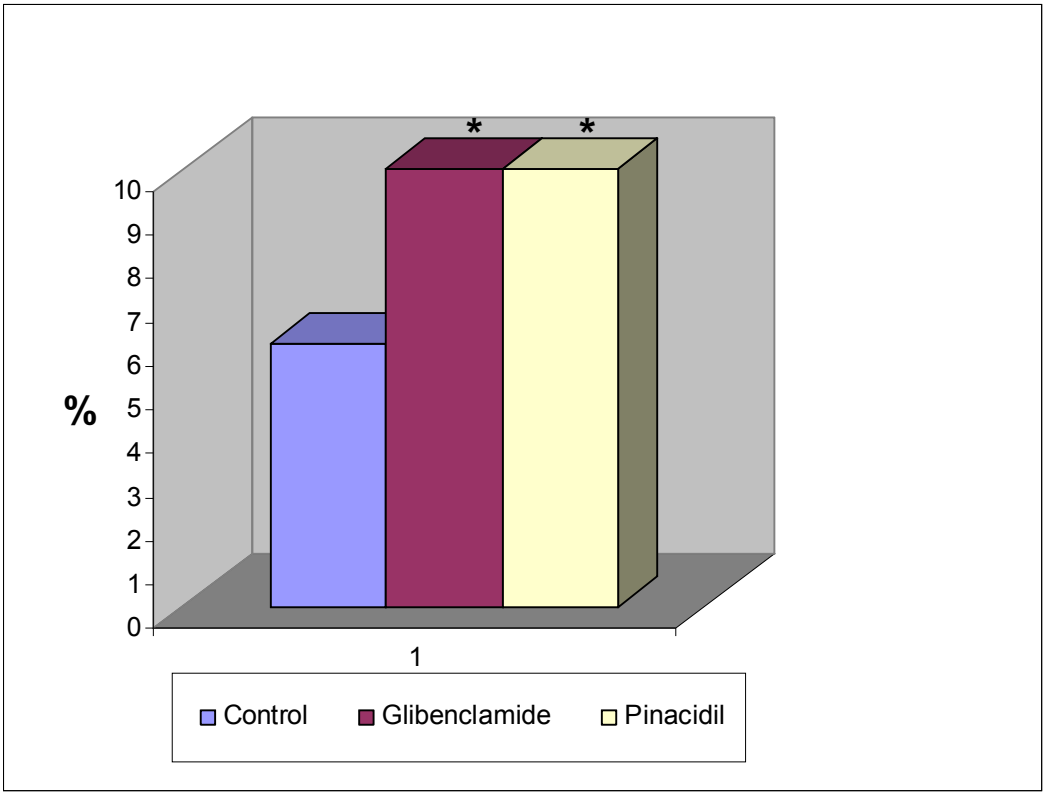


Figure 3.8. Infarct area (P < 0, 05; According to control).

4. DISCUSSION

There are various studies performed in animals related with the effect of K_{ATP} channel blockers and openers (KCO) in ischemia and reperfusion. In some studies, pro-arrhythmic effects of K_{ATP} channel blockers (73) and openers were observed. In another studies, K_{ATP} channel blockers and openers shows antiarrhythmic effects.

In contrast to the studies repeating glybenclamide dependent decrease arrhythmia occurred during 30 minutes of ischemia in male animals having larger ischemia and infarction, in the present study with smaller infarct, similar effect was not observed (76). There were found few studies related with ischemia induced arrhythmia. In most of the research, reperfusion induced arrhythmia have been studied and the effects of drugs on these arrhythmias were evaluated. In some earlier studies, glibenclamide was found proarrhythmic in male animals during reperfusion (77, 78). Glibenclamide and pinacidil was not found antiarrhythmic in female animals having larger infarct (77, 78). But in male animals having larger infarct, both drugs were found to decrease the score of arrhythmia (10, 11). In these studies however reperfusion induced arrhythmia was observed and evaluated. That is why these studies are not comparable directly with the result of the present study.

Earlier studies suggested that pinacidil decreased blood pressure with vasodilatation but, we did not observe this effect of pinacidil. This may be due to smaller dose of pinacidil used in this study.

Only one research that is similar to this study was carried out on female rats (9). In that study, glibenclamide was found to decrease incidence of arrhythmia in 30 minute of ischemia in female rats. Different effect of glibenclamide in male rats may be related to lower number of ATP dependent potassium channel in myocardial cells (75). Since the severity and incidence of arrhythmia is bigger in animals having larger ischemia than smaller one, glibenclamide and pinacidil may be found more effective in animals having larger infarct size. In this study the severity of arrhythmia anyway was not different from control animals. That is why, no effect produced by glibenclamide and pinacidil in this study. K^+ leakage from ischemic region to nonischemic region is responsible in producing arrhythmia. On the other hand electrical inhomogeneity in both regions depends on the number of opening ATP dependent potassium channel in ischemic cells. In the present study, since ischemic area is smaller and ATP dependent potassium channel density is lower in male, electrical inhomogeneity between ischemic and non ischemic region may be smaller. Since there are less ATP dependent potassium channel in male and more in female, K^+ leakage from ischemic cells to non ischemic region may be lesser in male than female. This may be reason of less arrhythmia observed in male in respect to female. In previous identical study, since the gender of animals used in the study are female, the arrhythmia score is more in control animals and the effect of glibenclamide and pinacidil

was observed clearly (9). In the present study, the infarct size significantly increased in glibenclamide and pinacidil treated group in respect to control animals. However the arrhythmia in 30 minutes of ischemia was not increased significantly in glibenclamide and pinacidil treated groups in respect to control. It is known that glibenclamide has ischemic effect (97) and increase infarct size. This may support the result of higher infarct size observed in glibenclamide group. On the other hand no difference between control and pinacidil groups in the infarct size was expected in this study. In earlier study that performed in females, pinacidil increased infarct size nonsignificantly. There are no other studies suggesting that pinacidil increases infarct size. Possible explanation may be at cellular level. Pinacidil is affecting both sarcolemmal and mitochondrial ATP dependent potassium channel. The blockage of sarcolemmal and opening of mitochondrial ATP dependent potassium channel may be found protective against arrhythmia (96) and infarct size. Possible explanation of more infarct size in pinacidil treated animals in this study may be related with the effect of pinacidil on mitochondrial ATP dependent potassium channel. But this result may require further studies.

5. CONCLUSION

Glibenclamide and pinacil did not decrease but nonsignificantly increase ischemia induced arrhythmia in 30 min of ligation period in rats. But both drugs increased infarct size in respect to control rats. The hypothesis that electrical inhomogeneity and the incidence of arrhythmia may be decreased in glibenclamide treated rats having smaller infarct, are not proved by the result of this study. Since glibenclamide and pinacidil are effective for the opening of both sarcolemmal and mitochondrial ATP dependent potassium channel. That is why both drugs might not be effective to decrease or increase arrhythmia and to change electrical inhomogeneity between ischemic and non ischemic region. In later study, if selective sarcolemmal channel opener (P-1075) or blocker (HMR1098) used in the study, this hypothesis may be clarified.

Table 1. The duration of arrhythmias during 30 minute of coronary ligation in male rats.

Groups	N	Onset of arrhythmia	Arrhythmic Period	Length of Arrhythmia (minute)				
				VF	VT	Other	Total	Bradycardia
Control	7	2±0,8	12±3	0,1±0,1	0,07±0,04	0,61±0,33	0,78±0,47	0
Glibenclamide	7	4± 1	15± 1	0,03±0,02	0,03±0,02	1,1±0,25	1,16±0,26	0
Pinacidil	7	5±0,9*	10±1	0	0	0,80±0,14	0,80±0,14	0

* P<0.05, Different from Control

Data are mean ± SE

Total= VF+ VT + Other arrhythmias including VPC, ventricular gemini, AV nodal arrhythmia and salvo.

Table 2. Mean arterial blood pressure (BP) during 30 minute of coronary artery ligation.

Groups	N	Blood Pressure (mmHg)								
		basal	ligation	01	03	05	10	15	20	30
Control	7	101±5	89±6	88±8	93±8	87±7	84±8	86±6	84±6	67±5
Glibenclamide	7	111±5	86±6	72±9	91±7	98±6	97±8	97±9	100±7	98±6*
Pinacidil	7	119±4*	92±5	93±5	103±6	107±5*	104±6	108±6*	108±6*	100±7*

* P<0.05, Different from Control

N: The number of animals.

Basal: Before coronary artery ligation; and 01, 03, 05, 10, 15, 20, 30 minutes after the coronary ligation.

Table 3. The heart rate (HR) during 30 minute of coronary artery ligation.

Groups	N	Heart Rate / minute								
		Basal	ligation	01	03	05	10	15	20	30
Control	7	368±20	370±19	360±21	364±23	354±20	343±18	338±14	331±13	319±12
Glibenclamide	7	347±15	352±13	351±18	333±12	341±12	347±16	346±16	364±18	354±16
Pinacidil	7	350±9	349±9	351±6	351±5	348±8	348±10	349±8	354±7	355±7*

* P<0.05, Different from Control

N: The number of animals.

Basal: Before coronary artery ligation; and 01, 03, 05, 10, 15, 20, 30 minutes after the coronary ligation.

Table 4. The incidence of arrhythmias during 30 minute of coronary ligation in male rats.

Group	N	Survival (N / %)	Incidence of Arrhythmias (n / %)				Arrhythmia Score
			VF	VT	Other	Bradycardia	
Control	7	7 / 100	1 / 14	3 / 43	7 / 100	0 / 0	2 ± 0,6
Glibenclamide	7	7 / 100	2 / 29	2 / 29	7 / 100	0 / 0	3 ± 0,4
Pinacidil	7	7 / 100	0 / 0	0 / 0	7 / 100	0 / 0	3 ± 0,2

N: The number of animals at the beginning of the ligation; VF: Ventricular fibrillation; VT: Ventricular Tachycardia; Other: Ventricular extra beat, bigemini and salvos.

Data are mean ± SE

Table 5. The Risk of Infarct Zone % and Infarct Area % during 30 minute of coronary artery ligation.

Groups	N	Risk of Infarct Area %	Infarct Area %
Control	7	29±2	6±1
Glibenclamide	7	33± 2	10± 2*
Pinacidil	7	30±2	10±1*

* Different from control

N: The number of animals.

Risk of Infarct Area %: Risk of infarct Area / All of the heart

Infarct Area %: Infarct Area / All of the heart

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