THE EFFECT OF ATP DEPENDENT POTASSIUM CHANNEL BLOCKAGE OR OPENING ON ARRHYTHMIAS OBSERVED FOLLOWING PARTIAL LAD OCCLUSION IN FEMALE RATS

DERYA KOYUNCU

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by

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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 BLOCKAGE OR OPENING ON ARRHYTHMIAS OBSERVED FOLLOWING PARTIAL LAD OCCLUSION IN FEMALE RATS

Koyuncu, Derya
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In this study, the effect of both ATP dependent potassium channel blocker (glibenclamide) and opener (pinacidil) on ischemia induced arrhythmia produced by ligation of smaller branch of left coronary arteries was researched.

In the research, 30 Female Spraque-Dawley rats weighing 215 - 245 g. in 7-8 month old were used. Glibenclamide was given in 0, 5 mg/kg/100ul dose intraperitoneally 20 minute before coronary ligation and pinacidil was given in 0, 01 mg/kg/100ul dose intravenously from cocygeal tail vein 10 minute before coronary ligation. Electrocardiogram (ECG) and arterial blood pressure was recorded during the 30 minute of coronary ligation.

Blood pressure decreased ($P<0.05$) immediately after the ligation in all group of animals. Glibenclamide decreased the incidence of ventricular tachycardia (VT) ($P<0.05$) but pinacidil did not in respect to control. The score of arrhythmia calculated by using the type and duration of arrhythmia

observed during 30 minutes of ligation was decreased by glibenclamide which was significantly different from both control and pinacidil group.

As a result, ATP dependent potassium channel blockage with glibenclamide is found to be effective to decrease arrhythmia occuring 30 minutes ischemia in rats. This effect of glibenclamide to decrease arrhythmia may depend on decreasing electrical heterogenity between nonischemic and ischemic area. This heterojenity may increase in pinacidil group because of pinacidil opens ATP dependent potassium channel in normal myocardial cells.

Key Words: Coronary ligation, Myocardial ischemia, Ischemia, Arrhythmia, Rats.

ÖZET

DİŞİ SIÇANLARDA ATP BAĞIMLI POTASYUM KANALLARININ BLOKE EDİLMESİ YADA AÇILMASININ SOL KORONER ARTERİN KISMİ TIKANMASINI TAKİBEN OLUŞAN ARİTMİLER ÜZERİNE ETKİSİ

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Tez Danışmanı: Prof. Dr. Ömer BOZDOĞAN
Şubat 2008, 54 sayfa

Bu çalışmada, sol koroner arterin küçük bir dalının bağlanması ile oluşturulan iskemi sırasında gözlenen aritmiler üzerine hem ATP bağımlı potasyum kanal blokeri (Glibenclamide), hem de ATP bağımlı potasyum kanal açıcısının (Pinacidil) etkisi araştırıldı.

Araştırmada 215-245 gr ağırlığında ve 7-8 aylık 30 Spraque-Dawley türü dişi sıçan kullanıldı. Glibenclamide 0, 5 mg/kg' lık dozda, koroner ligasyondan 20 dakika önce intraperitonel olarak, pinasidil 0, 01 mg/kg' lık dozda koroner ligasyondan 10 dakika önce intravenöz olarak kuyruk veninden verildi. Otuz dakikalık koroner ligasyon boyunca elektrokardiogram ve kan basınçları kaydedildi.

Ligasyondan hemen sonra kan basıncı bütün gruplarda düştü. Glibenclamide kontrole göre ventriküler taşikardi yoğunluğunu azalttı ancak pinacidil bu etkiyi göstermedi. Glibenclamide otuz dakikalık ligasyon süresince gözlenen aritmilerin süresi ve tipine gore hesaplanan aritmi skorunu kontrol ve pinasidil grubuna göre azalttı.

Sonuç olarak, ATP bağımlı potasyum kanal blokeri olan glibenclamide sıçanlarda 30 dakika iskemi sırasında oluşan aritmileri azaltmada etkili bulunmuştur. ATP bağımlı potasyum kanal blokerlerinin aritmiyi azaltmada etkili olması, iskemik olmayan alanla iskemik alan arasındaki elektriksel heterojenliğin azalmasına bağlı olabilir. Pinacidil normal miyokardiyal hücrelerde de potasyum kanallarının açılmasına neden olduğundan bu heterojenlik artabilir.

Anahtar Kelimeler: Koroner ligasyon, Miyokardial iskemi, İskemi, Aritmi, Sıçan.

TO MY FATHER,

MUHARREM KOYUNCU

AND

MY MOTHER,

NURSEN KOYUNCU

ACKNOWLEDGEMENTS

I would like to express my special thank to supervisor, Prof. Dr. Ömer Bozdoğan for patience, valuable guidance, encouragement and great help throughout the course of this work. I would like to thank MSc student, Selçuk Yaşar and Res. Asistant Ersöz Gonca for their help and support during the operation. I would like to express my special thanks to Res. Asistant Yasin Bakış for their technical support. And also, I want to thank all my family and friends for endless patience and support.

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1. INTRODUCTION

Coronary artery disease (CAD) is the most common cause of mortality in the developed world (1). It is responsible for about 50% of cardiovascular mortality, which itself accounts for 30-50% of all deaths in developed nations (2).

The term "coronary artery disease" comprises a range of diseases that result from atheromatous change in coronary vessels. CAD is thought to be a simple process of artery narrowing, eventually resulting in complete vessel blockage (1).

The major reason of death in CAD is the lethal arrhythmias that appear after the coronary occlusion. It has known that potassium leakage from the ischemic cells cause the generation of these arrhythmias. It is clear that arrhythmia and the rate of death in myocardial infarction have been seen more in men than premenopasual women (3). Arrhythmia and death following ischemia and reperfusion was observed more in male rats in respect to female was shown in previous studies (4, 5). Otherwise underlying mechanism of arrhythmia occurring following ischemia and reperfusion and different response in both sexes has not been clarified yet. Generation of arrhythmia is related with the size of infarct (6). There are many studies suggesting that arrhythmia appear following ischemia and reperfusion depends on potassium leakage from ischemic region to nonischemic area

(7, 8). It has been shown that blockage or opening of ATP dependent potassium channel affect the incidence of arrhythmia and survival rate following ischemia and reperfusion (9, 10). Some researches suggest that blockage of ATP dependent potassium channel decrease ischemia or reperfusion induced arrhythmia but others defend opposite (11-14). There are still controversies about ATP dependent potassium channel blockage or opening is effective to decrease these arrhythmia. Ischemia and reperfusion induced arrhythmia depends on electrical inhomogenity between ischemic and nonischemic region (15). Blockage of ATP dependent potassium channel provide ischemic cell to turn normal cell, but opener converts normal cell to ischemic cell. In both cases inhomogenity tend to be decrease. But it depends on infarct size. In the presence of larger infarct zone, opener may be more effective to decrease arrhythmia. Otherwise the presence of smaller infarct, glibenclamide may be more effective to decrease arrhythmia. There have not been found any research in literature that compare the effect of opener and blocker of ATP dependent potassium channel on arrhythmia in the presence of smaller infarct. That is why in this study; the effect of both ATP dependent potassium channel blocker (glibenclamide) and opener (pinacidil) on ischemia induced arrhythmia in the presence of smaller infarct was aimed to be researched.

1.1. PHYSIOLOGY OF THE HEART

1.1.1. Heart Anatomy

The heart is located near the anterior chest wall, directly posterior to the sternum. It lies slightly to the left of the midline of longitudinal axis of the body. Heart is surrounded by a double membrane, pericardium. Pericardium is subdivided into visceral, the outer layer of the heart, and parietal pericardium, the inner portion of the pericardial sac (16).

The heart wall is composed of three layers: a superficial epicardium, a middle myocardium, and a deep endocardium. All three layers are richly supplied with blood vessels (17).

The heart has four chambers; two atria and two ventricles. Right atrium receives deoxygenated blood from the systemic circuit and passes it to the right ventricle which discharges blood into the pulmonary circuit. Left atrium receives oxygenated blood from the pulmonary circuit and passes it to the left ventricle which discharges blood into the systemic circuit (18, 19) (**Figure 1.1.**).

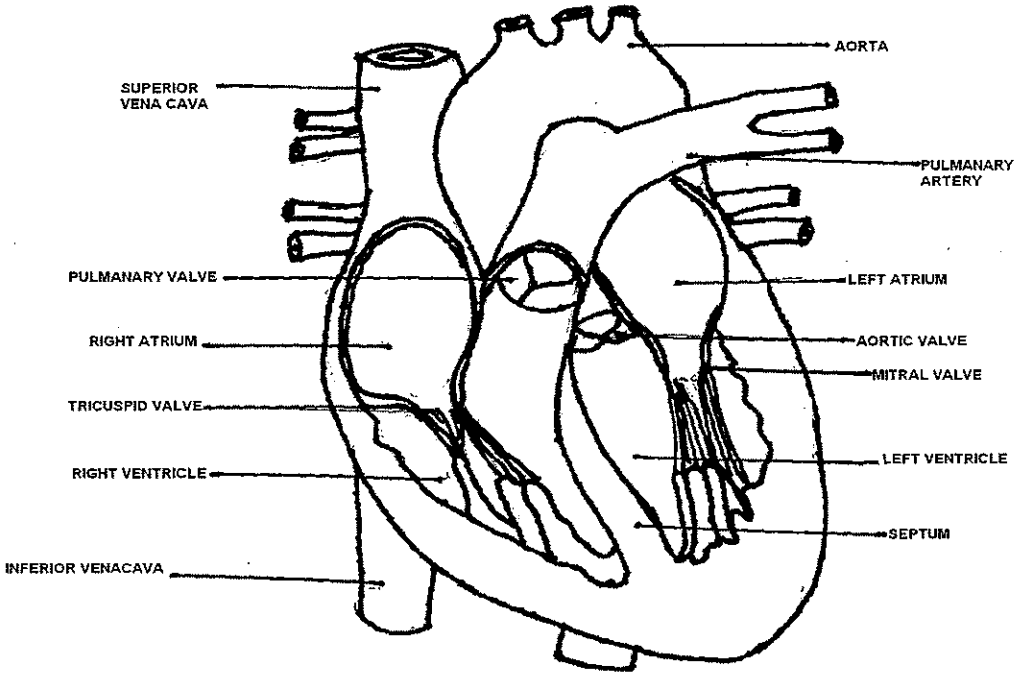


Figure 1.1. Anatomy of the heart.

The atria and ventricle are separated by atrioventricular valves which prevent backwards blood flow into the atria during ventricular contraction. The right atrioventricular (AV) valve, the tricuspid valve, has three flexible cusps. The left AV valve with two flaps is called the bicuspid, or mitral valve. Besides, 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 (20).

The basic function of heart is to pump blood from ventricles to the pulmonary and systemic circulation. The blood vessels carry blood to and from the lungs through the pulmonary circuit. This circuit strictly serves gas exchange. The blood vessels that carry blood to and from all body tissues

constitute the systemic circuit. Furthermore, the coronary circulation, that supplies blood to the heart, is the shortest circulation in the body (21). Myocardial cells are supplied for nutrients by coronary arteries. Coronary arteries are originated from the base of aorta. The arterial supply of the coronary circulation is provided by the right and left coronary arteries. The right coronary arteries supply to the right side and left coronary arteries to the left side of the heart (19) (**Figure 1.2.**).

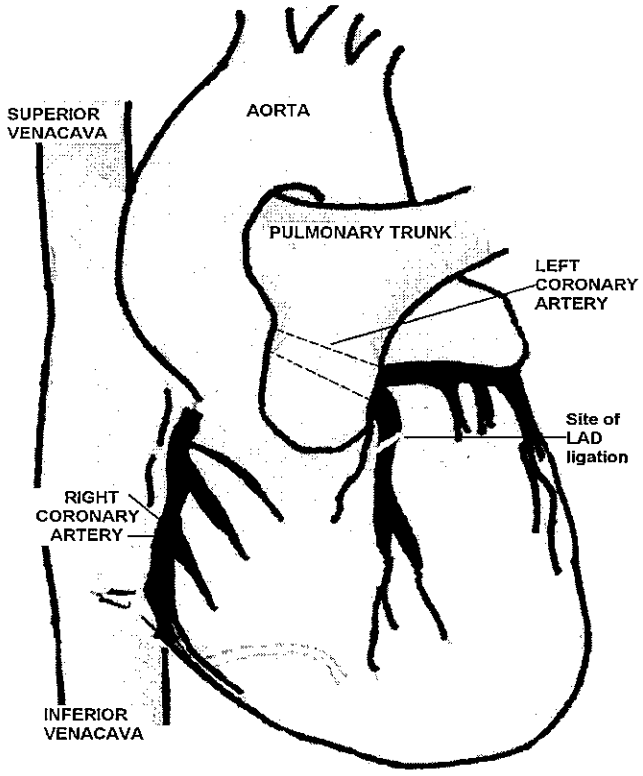


Figure 1.2. Coronary arteries in rats.

1.1.2. Cardiac Conduction System

1.1.2.1. Intrinsic Control of the Heart Rate

The independent activity of the heart is provided by (1) the presence of gap junctions, and (2) the autorhythmic specialized myocardial cells. Autorhythmic cardiac myocytes have the ability to depolarize spontaneously to threshold and fire action potentials. Autorhythmic cardiac cells are localized in the following areas: (1) sinoatrial (SA) node, (2) atrioventricular (AV) node, (3) atrioventricular (AV) bundle (bundle of His), (4) right and left bundle branches, and (5) ventricular walls as Purkinje fibers. Impulses pass across the heart in the same order.

The heartbeat originates at the SA node which is localized in the right arterial wall, just inferior to the entrance of the superior vena cava. SA node typically generates impulses about 75 times every minute. SA sets the pace for the heart as a whole. For this reason, it is known as the heart's pacemaker and its rhythm (sinus rhythm) determines heart rate. The depolarization begins in SA node cells and spreads via gap junctions throughout the atria and via the internodal pathway to the AV node, located in the inferior portion of the interarterial septum immediately above the tricuspid valve. At the AV node, the impulse is delayed momentarily to allow the atria to complete their contraction before the ventricular contraction. From the AV node, the impulse travels to the AV bundle. The AV bundle is the only electrical connection between the atria and the ventricles. The impulse travels on to the left and right bundle branches and onward to the Purkinje

fibers which begin at the heart apex and extend upward the ventricles (**Figure 1.3.**).

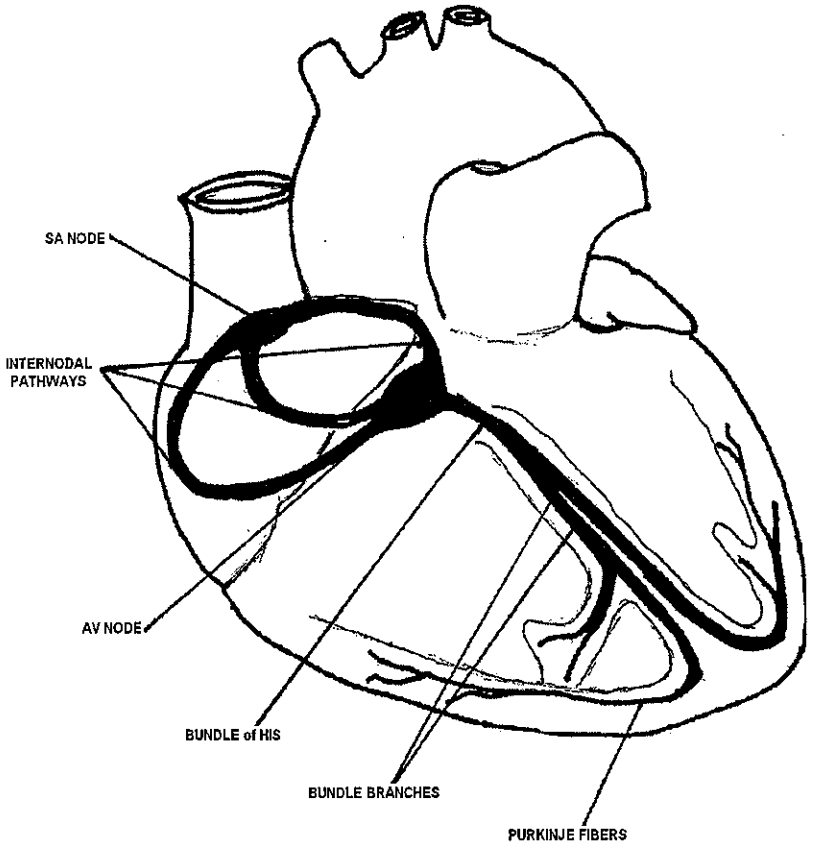


Figure 1.3. The intrinsic conduction system of the heart.

1.1.2.2. Extrinsic Control of Heart Rate

Sympathetic nerve input increases both the rate and the force of contraction while parasympathetic input decreases the heart rate. The medulla oblongata contains the sympathetic cardioacceleratory center and the parasympathetic cardioinhibitory center. Sympathetic nerve fibers project to the SA node, AV node, and the bulk of the ventricular myocardium.

Parasympathetic fibers project via the vagus nerve to the SA and AV nodes. Various hormones (epinephrine, thyroxin) also affect the heart's rhythm (16-18) (**Figure 1.4.**).

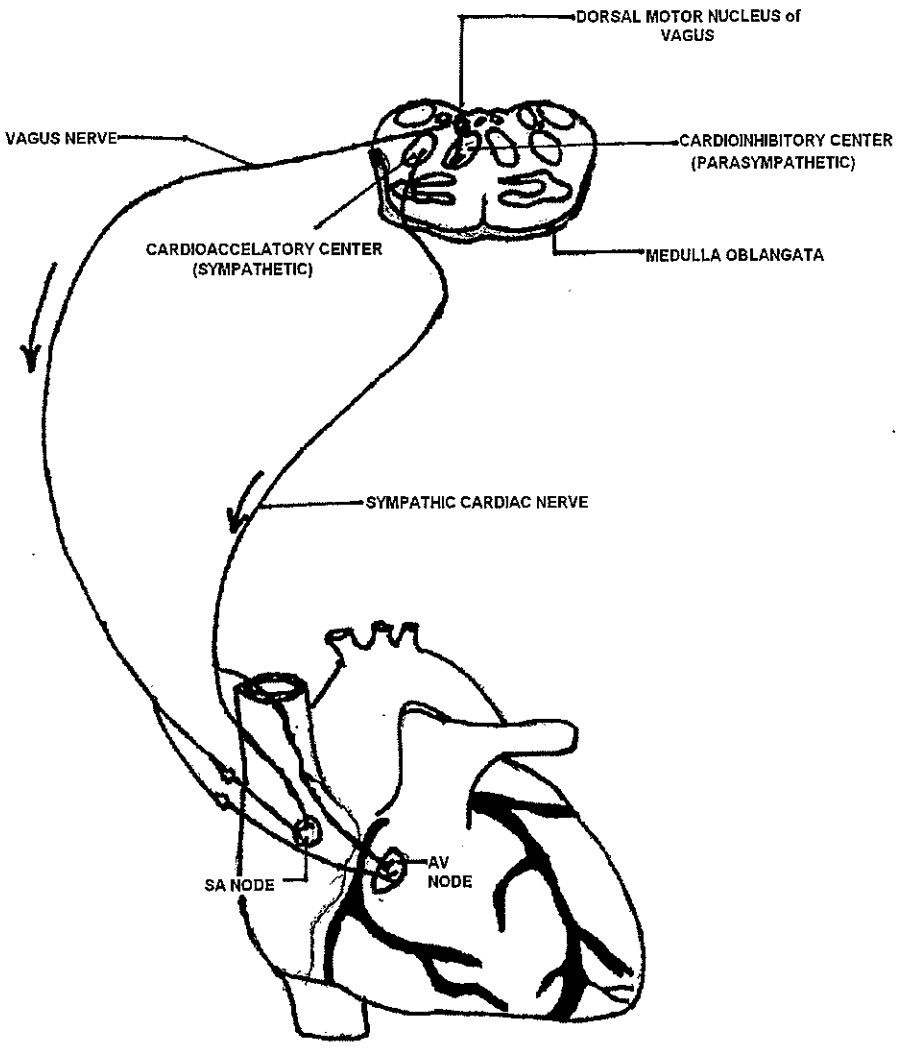


Figure 1.4. The extrinsic innervations of the heart.

1.1.3. Electrophysiologic Properties of Myocardial Cells

Autorhythmic cells in the intrinsic conduction system generate action potentials that spread throughout the heart, triggering contraction in the contractile cells. Autonomic cells have some different electrophysiologic properties in respect to other cells. Unlike unstimulated contractile cells of the heart (and neurons and skeletal muscle fibers), the autorhythmic cells do not maintain a stable resting membrane potential. Instead, they have an unstable resting potential that continuously depolarizes, drifting slowly toward threshold for firing. These spontaneously changing membrane potentials are called pacemaker potentials and they initiate the action potentials that spread throughout the heart to trigger its rhythmic contractions. The precise mechanism of the pacemaker potentials is still in doubt, but it is believed to result from gradually reduced membrane permeability to K^+. Then, the balance between K^+ loss and Na^+ entry is upset and the membrane interior becomes less and less negative. Ultimately when threshold is reached, fast Ca^{2+} channels open, allowing explosive entry of Ca^{2+} from the extracellular space. Thus, in this autorhythmic cells, an influx of calcium produces the rising phase of the action potential and reverses the membrane potential. As in other excitable cells, the falling phase of the action potential and repolarization reflect increased K^+ permeability and efflux from the cell. Once repolarization is complete, the K^+ channels are inactivated, and the process of slow depolarization to threshold begins again.

That is, these cells cannot maintain a stable resting potential and cannot remain long in repolarized state. Automatic slow depolarizations occur

but, contractile cells have rapid depolarization and long action potential in contrast to automatic cells. There is a plateau phase following the peak of action potential in non autorhythmic cells. Conducting cells, purkinje and bundle cells, have rapid depolarization but short plateau phase and no autonomic activity (16, 21).

The contractile myocardial cells have three electrical states in one cardiac cycle: Depolarization, repolarization and resting sate. The resting myocardial cell is negatively charged to -90mv. This negative charged is maintained until the cell is depolarized.

1.1.3.1. Depolarization

Depolarization is the process by which a resting cell becomes more positive. A myocardial cell is activated by an impulse from the sinus node. It switches from its resting negative charge of -90mv to about +40mV.

1.1.3.2. Repolarization

Repolarization is the process by which a depolarized cell is restored to its resting state. The repolarization process begins after rapid depolarization. Initially small repolarization occurs and is followed by plato phase having no potential change and then again rapid repolarization restores the membrane to its resting state of -90mv. The plateau permits a refractory period during which the cell can not be activated again.

1.1.3.3. Automaticity

Automaticity is the ability of a cell to depolarize itself (slow depolarization). The automatic cells differ from those of the rest of the myocardial cells in that they are capable of spontaneous depolarization; they posses the property of automaticity.

1.1.3.4. Action Potential

The action potential is a graph of the transmembrane potential of a single cell during the cardiac cycle. Autorhythmic cells in the myocardium generate action potentials that spread throughout the heart, triggering contraction in the contractile cells (**Figure 1.5.**). Action potential in autorhythmic cells and contractile myocardial cells ara different. Action potential of contractile myocardial cells include various phases that is designated as 0, 1, 2, 3, 4 phases.

Figure 1.5. Action Potential; on the right is the action potential of an autorhythmic cell; on the left, the action potential of a contractile cell.

Phase 0- Rapid depolarization, rapid influx of Na^+ ions via voltage dependent sodium channels.

Phase 1- Rapid, brief, incomplete beginning of repolarization, a transient outward K^+ current through fast-activating and transient potassium channels.

Phase 2- Slow repolarization, plateau (refractory period), a balance of inward and outward ion fluxes across the membrane to maintain equilibrium.

Phase 3- Late, rapid repolarization, the slow calcium channels close, accelerating the process of repolarization.

Phase 4- Electrical diastole when all the heart rests, except the pacemaker cells, at the end of the phase 3, pacemaker cells begin to process that is intended to bring them to threshold potential (17,22,25).

1.2. CORONARY CIRCULATION

1.2.1. Coronary Circulation

The heart itself needs to be supplied with blood. The myocardium receives its entire nutritional blood supply from the coronary arteries (23, 24). This is delivered by the two coronary arteries and their branches. Coronary arteries lie on the surface of the heart. Right and left arteries arise at the root of aorta and provide the blood supply to myocardium. The right coronary artery supplies the right ventricle; the left coronary artery supplies the left ventricle (25, 26).

Main coronary arteries give rise to several collaterals. Pigs, rats and rabbits do not develop collateral vessels. They have virtually no preexisting

collaterals, and infarcts develop rapidly and completely with acute coronary occlusion (27, 28). The cat, ferret and swine have low levels of collateral vessels (28, 29). In humans, collateral blood vessels are preexisting. The density of preexisting collateral channels in humans appears to be somewhat more modest than the dogs (24, 27).

1.2.2. Regulation of Coronary Blood Flow

Under physiological circumstances, coronary blood flow is sustained perfectly and answers the metabolic needs of the heart (24). The control of coronary flow depends on several factors (30).

Firstly, local regulation mechanisms are the primary controllers of coronary blood flow: adenosine and nitric oxide. Adenosine is the molecule that is the end product of ATP (24). Adenosine is a potent coronary vasodilator. It is produced under ischemic condition (30). It has a role in stimulating vasodilation when tissue becomes ischemic. Its concentration increase when myocardial metabolic activity is raised because it is a break down product of ATP and ADP and it may, therefore, play an important part in local control of coronary blood flow (24). However, NO is another coronary vasodilator. NO predominantly has its effect on basal flow rates, therefore, playes a part in matching the coronary blood flow. Acetylcholine, histamine, ATP increase NO production. Increment of intracellular calcium also increase the release of NO. The increase in shear stress exerted on the endothelial wall associated with increased blood flow is another important mechanisms involved in controlling NO synthesis.

Nervous control is another regulation mechanisms of coronary blood flow. N.Vagus has limited effect, because parasympathetic nerves do not significantly innervate ventricles. Sympathetic stimulation cause vasoconstricton via α-adrenergic receptor stimulation by norepinephrine and vasodilation via β-adrenergic receptor stimulation by epinephrine. Adrenergic receptor expression is non-uniform: α-adrenergic receptors predominantly expressed at the epicardium, while β-adrenergic receptor are expressed at the endocardium (24, 30).

1.3. RECORDING OF THE ELECTRICAL ACTIVITY OF HEART

The heart is an electrical field in which currents flow in a repetitive patterns with each cardiac cycle, consist of electrical systole (depolarization and repolarization) and electrical diastole (the resting phase). The electrical activity of heart can be detected by placing electrodes of opposite polarity on the skin at opposite poles of the heart's electrical fields.

The electrocardiogram (ECG) is a record of the electrical activity of the heart. The recording of cardiac electrical cycle is called as electrocardiography.

1.3.1. Recorded waves in ECG

The normal activation of the heart is initiated by sinus node (SA). Impulses from SA node spread first to atria and then ventricules via AV node. Various waves appear during one cycle of heart beat. P wave in this cycle represents atrial depolarization, QRS complex represents ventricular

depolarization. The ST segment is a segment between S wave and T wave. T wave represents ventricular repolarization (17, 22, 25) (**Figure 1.6.**).

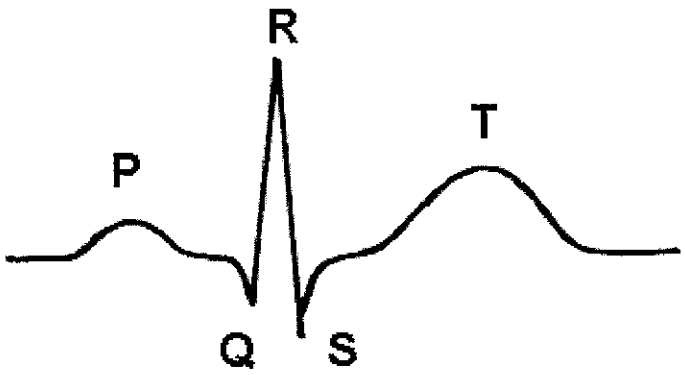


Figure 1.6. Normal electrocardiogram.

1.4. CARDIAC ARRHYTHMIAS

Arrhythmia is an abnormal cardiac rhythm (31). Many factors can cause the arrhythmia. Some of these are; (1) Abnormal rhytmicity of pacemaker, (2) Block at different points in the impulse, (3) Abnormal pathway of the impulse transmission, (4) Spontaneous ectopic impulse generation from any part of the heart (32, 33).

Various arrhythmias are created in response to ischemia. These arrhythmias may included sinusal tachycardia, bradycardia, bigeminal ventricular arrhythmia, ventricular premature contraction (VPC), ventricular tachycardia (VT), ventricular fibrillation (VF).

1.4.1. Sinus Rhythms

Sinus rhythm must originate in the Sino-atrial node. Regularly reoccurring sequences of P waves, QRS complexes and T waves. Heart rate in rats is between 300 and 450 beats per minute.

1.4.2. Sinus Tachycardia

Sinus tachycardia in rats occurs when the sinus rate exceeds to 450 beats/min. The mechanism of sinus tachycardia is enhanced automaticity that is due to sympathetic stimulation or vagal block and occurs in response to acute exercise or emotional stimuli. Also, some drugs or disease can cause a sinus tachycardia. In this rhythm, normal P waves and ST segments, normal or shortened PR interval appear.

1.4.3. Sinus Bradycardia

Sinus bradycardia in rats is the slow beating of the sinus node at rates of less than 250 beats/min. The mechanism of sinus bradycardia is caused by excessive vagal tone, decreased sympathetic tone. P waves are present and all are followed by a QRS complex. Normal and constant PR interval, normal ST segments appears (34).

1.4.4. Sinus Arrhythmia

Sinus arrhythmia is the variation in heart rate. It is observed to be synchronized with breathing, slowing with expiration and accelerating with inspiration. This physiologic variation is only an apparent irregularity.

1.4.5. Premature Beat

The terms “premature beat", "premature contraction” indicate that the atria, AV junction, or ventricle are stimulated prematurely.

1.4.5.1. Premature Atrial Contraction (PAC)

PAC is an atrial impulse that emerges earlier than the next expected sinus beat and originates from a focus other than sinus node. That is, PAC arises from an ectopic focus in the atria. It will have an identifiable P wave but the shape of the P wave may be altered. A normal QRS complex appears.

1.4.5.2. Premature Ventricular Contraction (PVC)

PVC arises from ectopic focus in ventricles. Early QRS is not preceded by a P wave. It will have an unusual shape and prolonged QRS.

1.4.6. Ventricular Tachycardia (VT)

Regularly occurring rhythm originating from a regular ventricular ectopic focus. QRS morphology is usually like PVC (35).

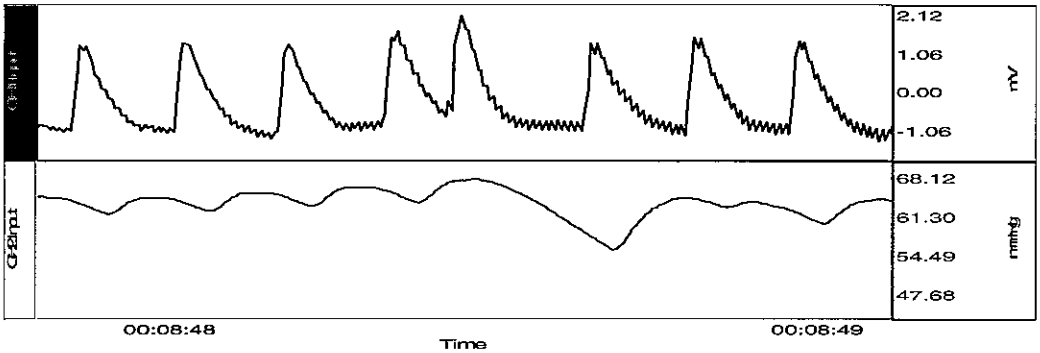
1.4.7. Atrial fibrillation

Atrial fibrillation is an abnormal irregular heart rhythm whereby electrical signals are generated randomly throughout the atria (36). It includes irregular rhythm with the absence of P waves (37, 38).

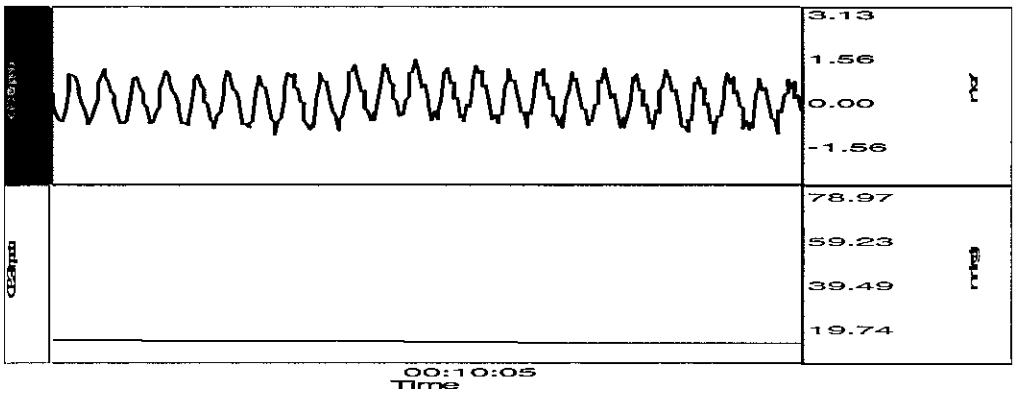
1.4.8. Ventricular Fibrillation (VF)

Thought to be a reentrant excitation of the ventricles; premature impulse may arise during vulnerable period. Ventricular fibrillation was defined as irregular ECG waves of inconsistent shape and unidentifiable QRS complexes. Usually the cause of "sudden death"(35) (**Figure 1.7.**).

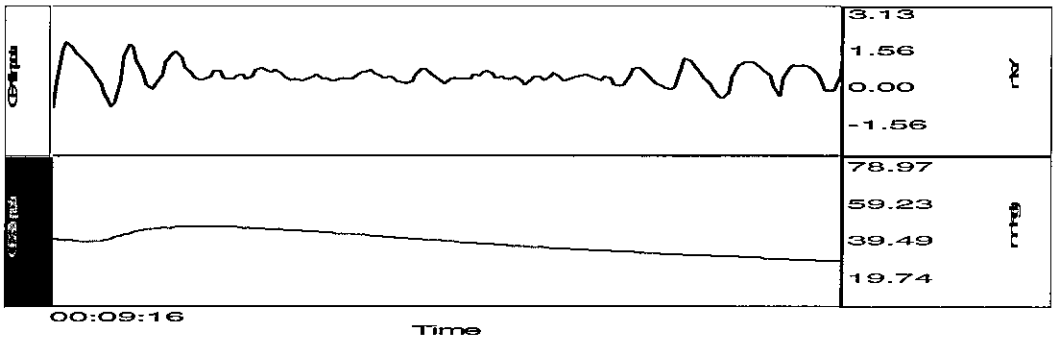
Normal Sinus Rhythm



Premature Ventricular Contraction (PVC)

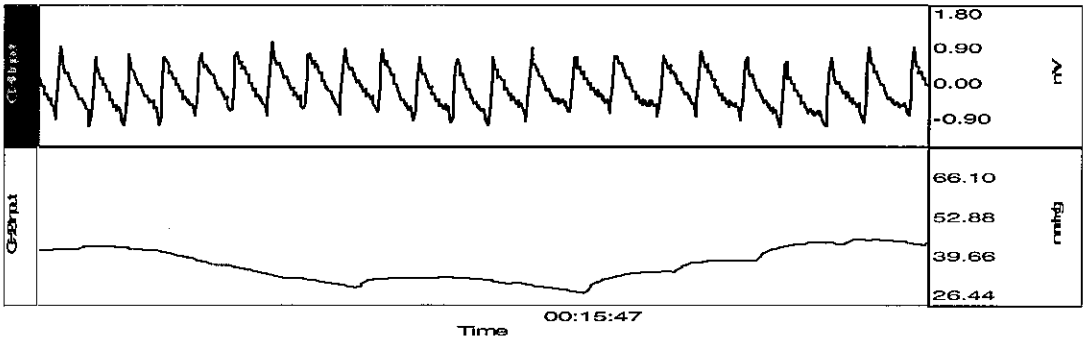


Ventricular Tachycardia (VT)

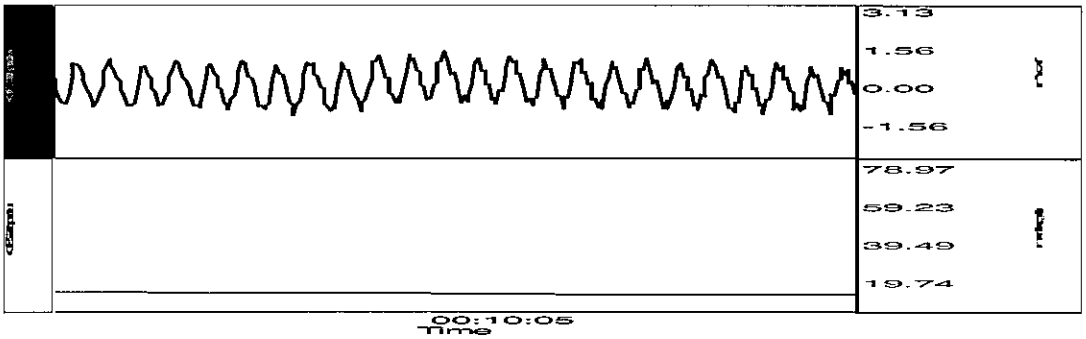


Ventricular Fibrillation (VF)

Figure 1.7. The types of arrhythmias and blood pressure observed before and after coronary ligation in rats.



Normal Sinusal Tachycardia



Ventricular Tachycardia (VT)

Figure 1.8. Comparison of sinusal tachycardia and ventricular tachycardia in rats.

1.5. UNDERLYING MECHANISM OF ARRHYTHMIA

1.5.1. Abnormal Automaticity

Abnormal automaticity is the spontaneous beating of cardiac cells with abnormally depolarized resting membrane potentials. A pacemaker elsewhere than SA Node is called ectopic pacemaker. Ectopic pacemaker causes an abnormal sequence of contraction of the different part of the heart. The ectopic pacemaker is produced by the ischemic cells (**Figure 1.9.**).

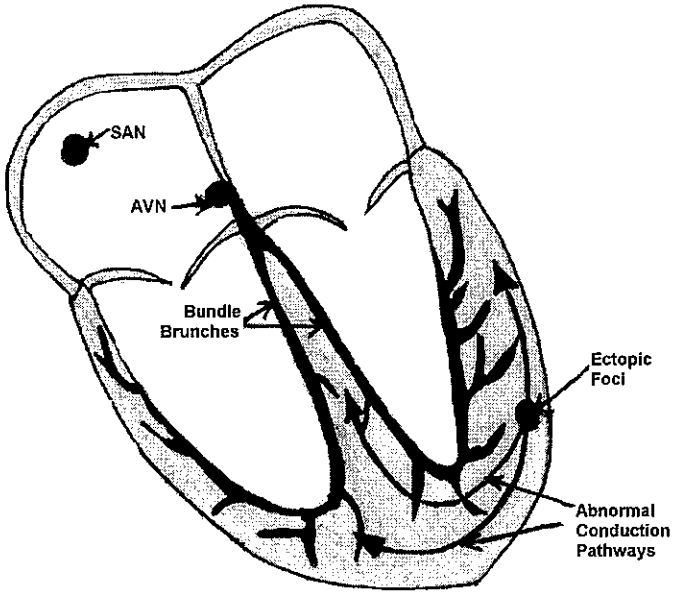


Figure 1.9. Abnormal electrical conduction due to ventricular ectopic foci.

1.5.2. Reentrant Circuit

The localized reentry involves the presence of a unidirectional block within a conducting pathway. When the transsmission slow down or block in

this pathway, the normal stimuli pass around the blocked region. Then this region generates re-entrant stimuli. Most ventricular arrhythmias occurred by reentrant mechanism (25, 33).

1.5.3. Triggered Activity

Triggered activity means the abnormal action potentials triggered by a preceding action potential. Triggered activity can occur due to the presence of afterdepolarizations. These can occur before or after full repolarization of the myocardial cells.Triggered activity is the result of afterdepolarization; there are two types early and delayed. Early afterdepolarizations are the result of prolonged QR intervals; delayed afterdepolarizations are the result of excess intracellular calcium. Early afterdepolarizations occur during phase 2 or 3. Delayed afterdepolarizations occur during phase 4 (39, 40).

1.6. MYOCARDIAL ISCHEMIA AND INFARCTION

Cardiac ischemia occurs when blood flow to the heart muscle is obstructed by a partial or complete blockage of a coronary artery (28, 41). A sudden, blockage of coronary artery may lead to a heart attack (42, 43). Myocardial ischemia produced by coronary occlusion cause a serious abnormal heart rhythm (arrhythmia), which can cause fainting or even sudden death (41, 43, 44).

Atherosclerotic plaque and coronary artery spasm are the cause of myocardial infarction (45). Atherosclerotic plaque is a deposit or degenerative accumulation of lipid-containing plaques on the innermost layer of an artery (41, 42). It damages arterial walls and produce a thrombus, or blood clot at

the site of the plaque. Thrombus can cause occlusion of the epicardial coronary artery, so that coronary blood flow is interrupted and delivery of nutrients to myocardium is blocked (45, 46).

A coronary artery spasm is a brief, temporary contraction of the smooth muscles around artery. The spasm can slow or stop blood flow through the artery. This can reduce or even stop the blood flow to any part of the heart (45).

1.6.1. Cellular Changes After The Myocardial Ischemia

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. Probably, the three most important are (1) generation of oxygen derived free radicals, (2) calcium overload, and (3) reduced sensitivity of myofilaments to calcium.

After the myocardial ischemia, tissue oxygen tension rapidly falls and contraction abnormalities appear within 5 second (29). Then intracellular high energy phosphate levels including creatine phosphate and ATP decrease. In ischemic cells aerobic respiration change to the anaerobic respiration (45, 47). Intracellular and extracellular acidosis progressively develops in 1 minute of ischemia. Adenosine increases during the ischemia (42, 48). The Na-K pump impairs and Na^+ ions accumulate in the myocardial cells due to the falls in ATP level (45, 49, 50). Accumulation of Na+ ions activate Na^+/ Ca^+ exchange channels, so intracellular Ca^+ ions increase. Accumulation of intracellular Ca^+ ions increase myocardial contraction and stimulate Ca-

ATPase. This event causes consumption ATP and O_2 (47, 51). Besides, Ca^+ ions activate phospholipases and proteases that impair the integrity of the cell membrane. Ischemia induced Ca^+ ions influx activates arachidonic acid metabolism which in turn may produce free oxygen radicals. Oxygen radicals damage cell membranes and contribute to cell death (22, 52-54) (**Figure 1.10.**).

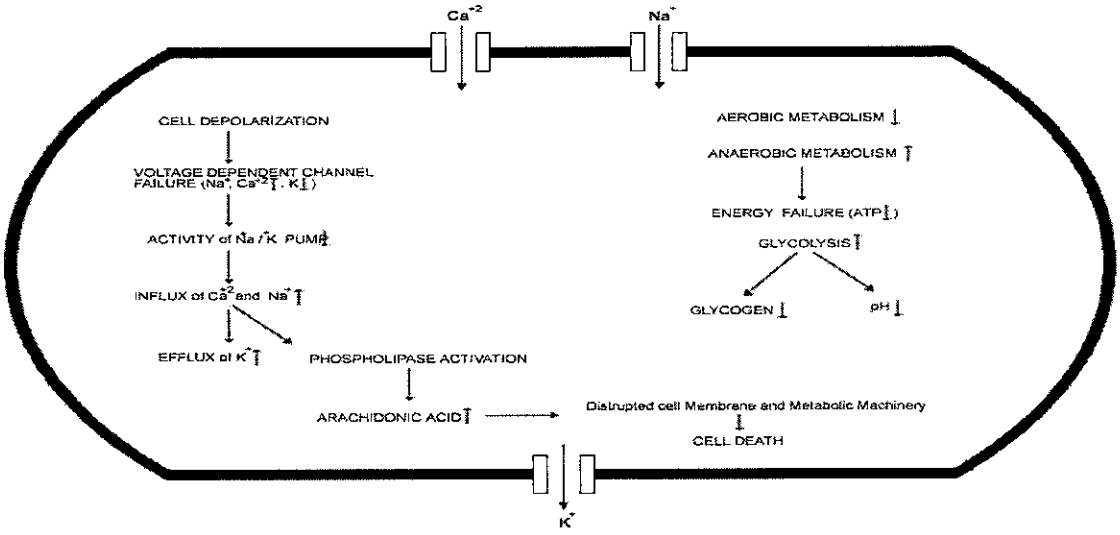


Figure 1.10. Cellular changes after the myocardial ischemia.

1.7. ARRHYTHMIAS INDUCED BY ISCHEMIA

There are two period of arrhythmia in all experimental animal. These are early and delayed arrhythmic periods. Early phase of the arrhythmia occurs during the 30 minute of coronary occlusion in big animals such as pigs, dogs; this phase frequently culminates in ventricular fibrillation (VF). This period is followed by a phase which does not have any arrhythmia that

is named as quescenet peroid. Delayed phase of the arrhythmia start about 4-8 hours after coronary ligation and last two to four days (55). In smaller animals such as the rats, first arrhythmia starts 3-4 min and late is in 6-12 of the ligation (56, 57). In acute myocardial infraction, frequently observed arrhythmias are ventricular premature contraction (VPC), ventricular tachycardia (VT), ventricular fibrillation (VF) (58).

1.8. ATP SENSITIVE POTASSIUM CHANNEL (K_{ATP} CHANNEL)

K_{ATP} channels were first described by Noma in cardiac ventricular myocytes (11, 59) and found in various tissues, such as pancreatic β cells, pituitary tissue, skeletal muscle, brain, and vascular and nonvascular smooth muscle (60-62). K_{ATP} channels play an important role in the regulation of cardioprotective mechanism (6, 7, 59, 63). Activation of ATP-sensitive K^+ channels (K_{ATP}) during ischemia leads to arrhythmias and blockage of these channels exert antiarrhythmic action (7, 8, 11). These channels are very sensitive to intracellular ATP concentration (64). 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 (9, 62, 65). K_{ATP} channels are closed state in normally oxygenated heart (66). These channels open, when ADP, lactate and H^+ ions increase inside of the cell (66, 67). If efflux of K^+ ions increases, action potential duration reduces and influx of Ca^{+2} ions decrease into the cardiomyocytes (68). When intracellular Ca^{+2} ions decrease, myocardial contractility decreases. A decreased myocardial

contractility leads to preservation of ATP during the ischemia (69). There are two types of ATP-sensitive K_{ATP} channels: sarcolemmal and mitochondrial (9, 70). Openers of the sarcolemmal K_{ATP} channels activate K^+ efflux through the plasma membrane, thus inducing action potential shortening (9, 12, 65).

K_{ATP} channels are a complex of regulatory protein containing the sulfonylurea receptor (SUR subunit) and an inward-rectifying K^+ channel (Kir) serving as a pore-forming subunit (59, 60, 63, 71). The K_{ATP} channels present in heart cells consist of the specific combination of Kir6.2 and SUR2A subunits, whereas the other K_{ATP} channels contain Kir6.1 and SUR2B subunits in smooth muscle cells (13, 60, 72–74).

Mitochondrial K_{ATP} is different from the sarcolemmal K_{ATP} channels (75, 76). Sarcolemmal K_{ATP} channels appear to be involved in the control of electrical activity of the cell, but mitochondrial K_{ATP} channels in the control of matrix volume (77). Also, opening of mitochondrial K_{ATP} channels leads to membrane depolarization, matrix swelling, slowing of ATP synthesis (75, 78). K_{ATP} channels are activated by K^+ channel openers such as pinacidil and inhibited by K^+ channel blocker such as glibenclamide (10, 65, 79).

K_{ATP} channel openers affect on cardiac and vascular smooth muscle (80). Potassium channel openers vasodilate arterioles and arteries. When the K_{ATP} channels openers open, Ca^{+2} ions decrease during the plateau phase of action potential and following shortening of action potential duration cell membrane hyperpolarized. ATP preservation occurs with the prevention of intracellular Ca^{+2} accumulation (80, 81). Potassium channel openers have established useful in limiting myocardial dysfunction under ischemia and heart failure through direct actions on the myocardium (73, 79).

K_{ATP} channel blocker closes the channels in ischemic cell (10), because these channel are closed state in normal myocardial cell. They inhibit K^+ loss and nonuniform shortening action potential duration during the ischemia (11). Besides, K_{ATP} channel blockers increase severity of ischemia that is caused by Ca^{+2} loading into the myocardial cell. They diminish coronary blood flow (82).

1.9. EFFECT OF SEX DIFFERENCES ON ISCHEMIA INDUCED ARRHYTHMIAS

There are differences in the severity of arrhythmia produced by experimentally coronary ligation in male and female animals. Death or mycordial infarction have been seen less in premenoposal women than men. This results shown that female are more resist than men against ischemia or reperfusion induced arrhythmia. The level of coronary disease and death in women rapidly increases after menopause and reach to level as like in men. The major mechanism responsible for the protective effect of female gender is believed to be due to an anti-atherogenic action of female sex hormones (4, 61, 83).

Estrogens and number of K_{ATP} channels and resistance to ischemia/reperfusion injury in the heart is well established. More sarcolemmal K_{ATP} channels are more resistant to ischemia/reperfusion. That is, estrogens have numerous diverse effects on the cardiovascular system (61, 84).

Estrogen causes an activation of NO synthase in myocardium which may produce vasodilation (85). NO increase colleteral blood flow, reduce

oxygen consumption, produce inhibition of platelet aggregation. Hovewer, estrogen inhibits inward Ca^{+2} current. Therefore, estrogen is Ca^{+2} antagonist (86). The incidence of postischemic ventricular arrhythmias is reduced by estrogen in myocardium with the decreasing of intracellular Ca^{+2}, thus it protects heart from injuries (5).

2. MATERIAL AND METHODS

2.1. ANIMALS

In this study, 30 female Spraque-Dawley rats weighing 215 - 245 g and in 7-8 month old were used. Animals were fed with commercial rat food pellet and allowed to drink tap water ad libitum.

2.2. CORONARY ARTERY LIGATION

Animals were anaesthetized with thio pental sodium (100 mg/kg) and additional dose was given during operation when animal recover eye reflex and respiration. Trachea was cannulated for artificial respiration. The left carotid artery was cannulated for measuring the blood pressure. The catheter used for carotid cannulation was filled with saline that contain heparin. The chest was opened by cutting the fourth and fifth intercostal ribs. Then, pericardium was removed from heart, and the heart was exposed. A loose loop of 5-0 autramatic silk was placed around the 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 ligature was tightened by clamping on the silk to produce the regional myocardial ischemia for 30 minute. In the survived rats, the heart was perfused through the aorta with the solutions first with NaCl and then ethanols to determine the risk of infract

area. After the perfusion of heart, the nonperfused area that remained red was separated from the well perfused area that seemed white color. The nonperfused myocardium was cut by scissors, and weighed. The percentage of this area in respect to the total weight of ventricle was calculated. After that, nonperfused myocardium was cut about five segments by razor blade. These segments were put in the solution prepared by nitrobluetetrazolium (NBT) about 2-3 minute for staining. Nonischemic region was stained with NBT but ischemic region 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 Infact Area (Nonperfused Area / All of the heart) and Infact Area (Infarct Area / All of the heart) were calculated.

Bipolar ECG and arterial blood pressure was recorded during the 30 minute of coronary ligation and stored in computer for later analysis.

2.3. DRUG ADMINISTRATION PROTOCOL

Glibenclamide was dissolved in dimethylsulfoxide and then mixed with ethanol in 1/1 ratio. Stock solution was included 5 mg glibenclamide dissolved in 100ul DMSO + 100ul ethanol. It was given in 5 mg/kg/100ul dose intraperitoneally 20 minute before coronary ligation. In control group, instead of the glibenclamide, same value of solvent was applied.

Pinacidil was dissolved in ethyl alcohol and then mixed with isotonic solution. Stock solution was included 0,2 mg pinacidil dissolved in 200ul ethyl alcohol + 200ul isotonic solution. It was given in 0,1 mg/kg/100ul dose

intravenously from cocygeal tail vein 10 minute before coronary ligation. In control group, instead of the pinacidil, same value of solvent was applied.

2.4. STAINING METHOD

Firstly, 0, 9 gr KH_2PO_4 substances were dissolved in 100 ml distile water and 0, 5 mg NBT was dissolved in this solution. Then, 4, 68 gr $NaHPO_4x2H_2O$ substances were dissolved in 400 ml distile water. Finally, this mixture was added first solution, containing NBT.

2.5. THE EVALUATION OF THE ARRHYTHMIAS

In this study, heart rate and blood pressure were recorded at 1, 3, 5, 10, 15, 30 minute during the ischemia. The incidence and total length of arrhythmias during 30 minute of ischemia was calculated from recorded ECG. The appearance and the end 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 (VEB), bigemini and salvos. The sinusal tachycardia is different from ventricular tachycardia according to heart rate and blood pressure. The arrhythmias are designated as sinusal tachycardia when the heart rate was 500 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 (**Figure 1.8.**).

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

ST segment elevation or QRS changes was observed following coronary ligation in all groups. All animals survived after 30 minute of coranary ligation. Generally, arrhythmias started at about 2 minutes following ligation (**Table 1**). But arrhythmias appeared earlier in pinacidil group than control (**Figure 3.1.**).

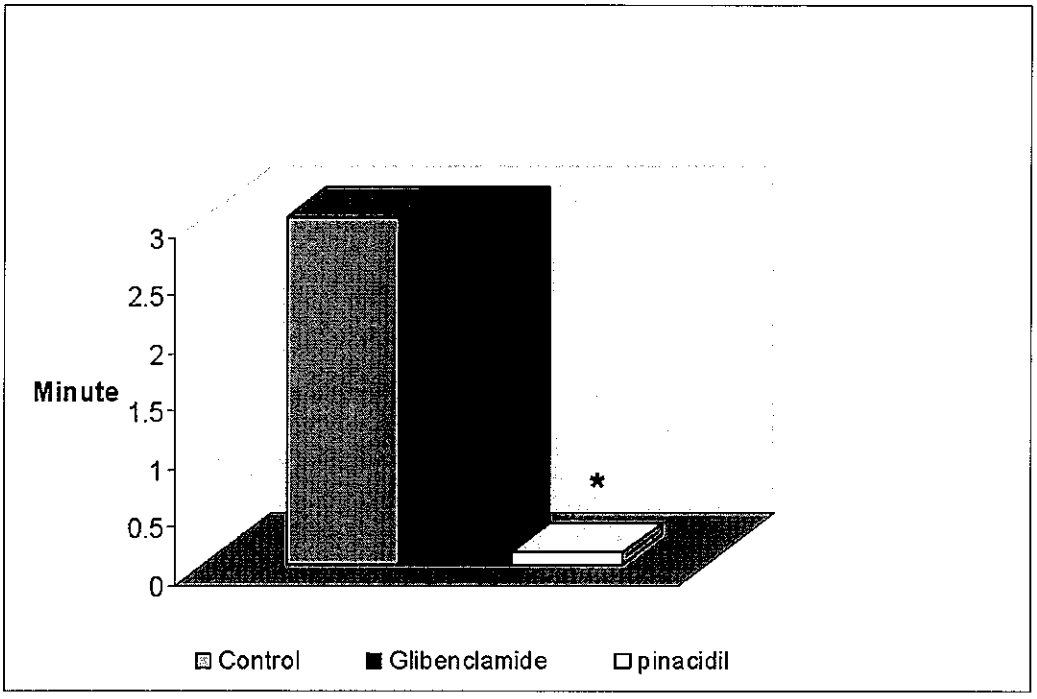


Figure 3.1. The start of arrhythmia after ligation (P<0,05; According to control).

Blood pressure decreased (P<0.05) immediately after the ligation in all group of animals.

In pinacidil group, blood pressure during 30 min ischemia was higher than control. Similarly blood pressure was higher in glibenclamide group in respect to control.

The heart rate was not different after ligation in all group of animals. In pinacidil and glibenclamide, the heart rate was higher than control at 1st, 3rd, 10th, 15th, 30th minute of coronary ligation (**Table 2**).

The length of the arrhythmic period in pinacidil and glibenclamide was not different from control during ligation, but the length of arrhythmic period of pinacidil was different from glibenclamide (**Figure 3.2.**).

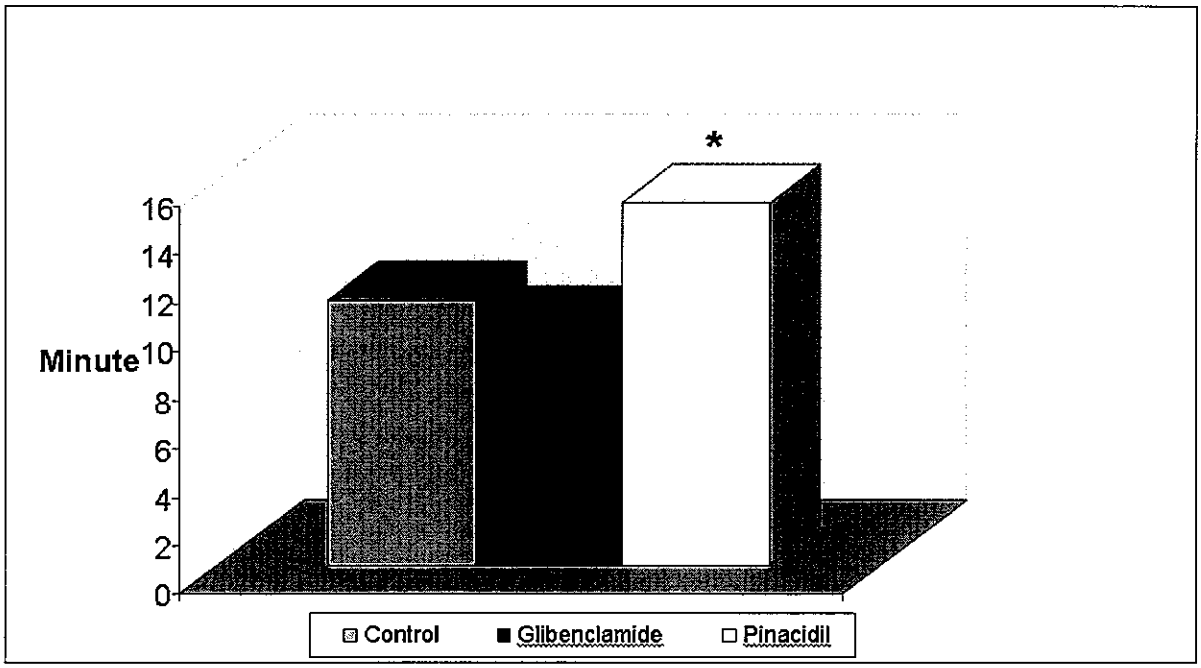


Figure 3.2. The arrhythmic period during 30 minutes of coronary ligation (P<0,05; According to glibenclamide).

The total arrhythmia was found higher in pinacidil group than glibenclamide (P<0.05) (**Figure 3.3.**). But it was not different from control.

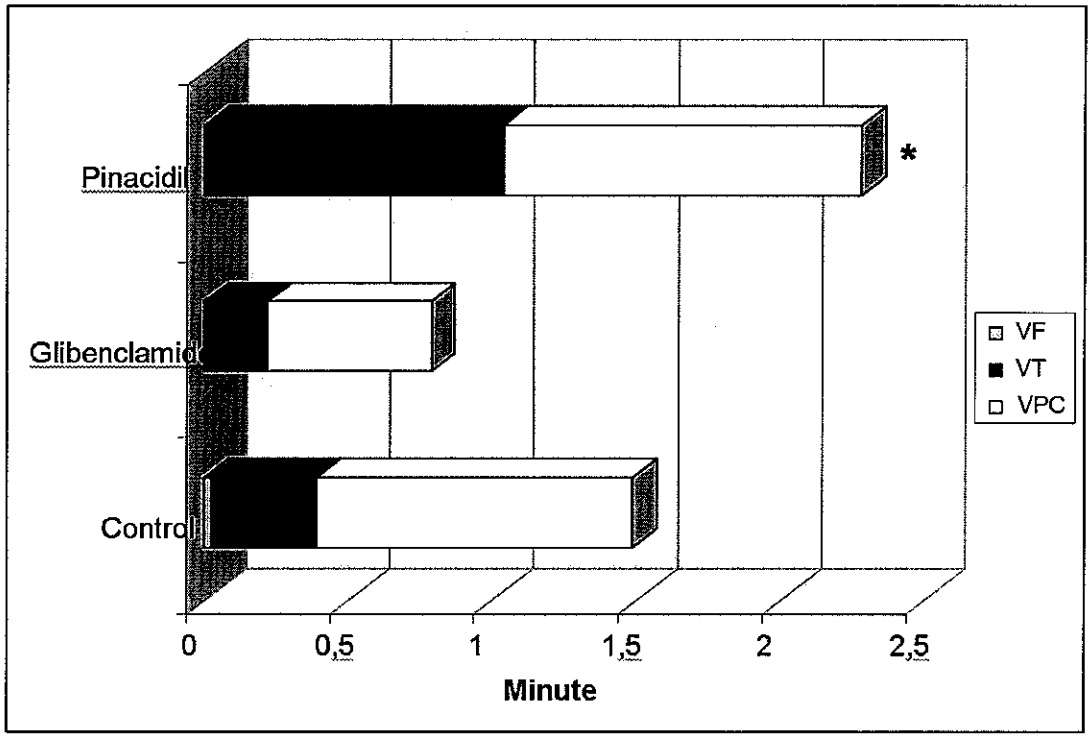


Figure 3.3. The type and duration of total arrhythmia during 30 minutes of coronary ligation (P< 0,05; According to glibenclamide).

Incidence of ventricular fibrillation and other type of arrhythmias was not different among groups but incidence of ventricular tachycardia significantly lower in glibenclamide group than control and pinacidil (P<0.05). Ventricular fibrillation appeared only in control group. Bradycardia appeared only in one animal in glibenclamide group (**Table 3**).

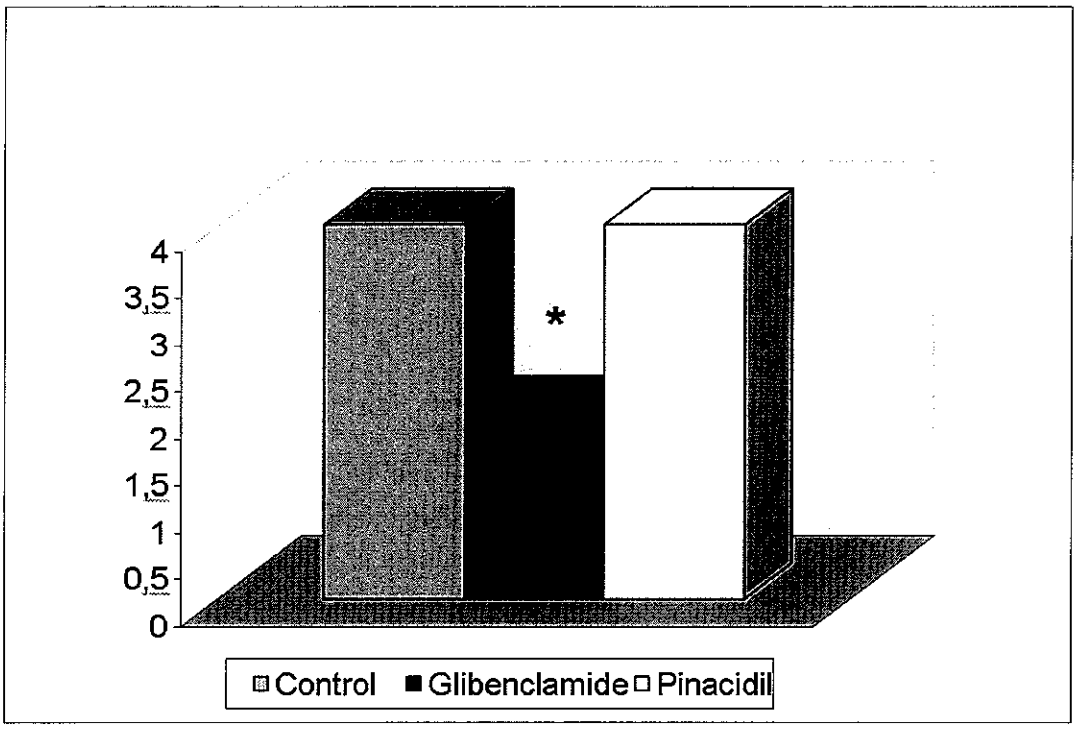


Figure 3.4. The score of arrhythmia (P< 0,05; According to control and pinacidil).

Arrhythmia score which calculated from duration and type of arrhythmia was lower in glibenclamide group in respect to control and pinacidil (**Figure 3.4.**).Otherwise arrhythmia score in pinacidil group was not different from control (**Table 3**).

The risk of the infarct area was not different among groups (**Table 4**). But the infarct area was smaller in glibenclamide group in respect to pinacidil (**Figure 3.5.**).

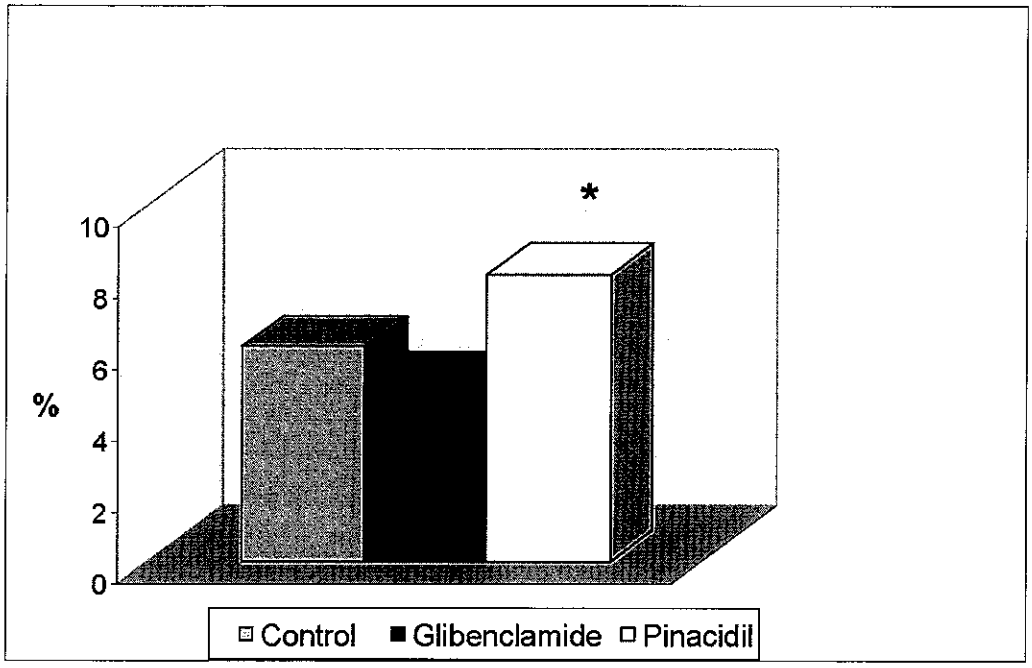


Figure 3.5. Infarct area (P< 0,05; According to glibenclamide).

In coincedent with this result, the severity of arrhythmia in pinacidil group was higher than glibenclamide group.

4. DISCUSSION

In pinacidil and glibenclamide, the blood pressure and the heart rate in response to coronary ligation was different from control. Although pinacidil usually decrease heart rate which was observed in various studies (13), pinacidil did not decrease blood pressure and heart rate in this study even blood pressure and heart rate was higher in respect to control animals. This may be due to lower dose of pinacidil used in this study. Since pinacidil decreased blood pressure just after the intravenous application, but it does not sustained long. There were not found any comparable studies related with the dose dependent effect of pinacidil on blood pressure.

There are various studies performed usually in male animals that indicate antiarrhythmic effect of K_{ATP} channel blocker and opener in ischemia and reperfusion. In most of the studies, glibenclamide has been observed to decrease ischemia reperfusion induced arrhythmia in male were observed (11, 12, 14), but it has not been found any research indicating the antiarrhythmic effect of glibenclamide in female. In present study, it was observed that glibenclamide was effective on ischemia induced arrhythmia in female. Since the experimental research related with ischemia and reperfusion arrhythmias has been done usually in male animals, there was not found more comparable study with the result of present study. Glibenclamide and pinacidil was not found antiarrhythmic in female animals having larger infarct (87, 88). In the present study, glibenclamide was more

effective to decrease arrhythmia in female having smaller infarct. Usually previous findings related with the effect of ATP dependent potassium channel blockage on ischemia or reperfusion induced arrhythmias was researched in male animals having larger infarct (11–14, 88, 89). The effect of ATP dependent potassium channel openers on ischemia and reperfusion induced arrhythmia was also researched in male animals having larger infarct (9, 13, 14, 79, 87). In these studies there are opposite result related with the effect of ATP dependent potassium channel openers and blockers. Several research suggest that blockage of ATP dependent potassium channel decrease arrhythmia (11–14, 56), but some group of researcher suggest opposite result (44). There are also opposite result related with the effect of ATP dependent potassium channel openers. Some researcher suggest that opening of the channel decrease arrhythmia (13, 14, 79), but others suggest that it increase arrhythmia (89). In a study, it is indicated that the severity of arrhythmia decreased when smaller infarct produced by partial coronary artery ligation (55). In that study it was found that the arrhythmias disappear in shorter time in animals having smaller infarct.

Our studies suggest that blockage instead of opening of ATP dependent potassium channel is effective to decrease arrhythmia. But there is no study found supporting this result in literature. In the present study, the reason of antiarrhythmia produced by blockage of K_{ATP} channel with glibenclamide and without antiarrhythmic effect produced by K_{ATP} channel openers with pinacidil may be related with heterogenity between ischemic and nonischemic region. It is suggested that decreasing heterogenity between ischemic and nonischemic region may decrease arrhythmia (7, 8,

91). This hypothesis are supported by the findings of present study. Since glibenclamide closes the ischemic cell to the nonischemic cell, electrical inhomogenities decrease between ischemic and nonischemic region and arrhythmia decreased. On the other hand, pinacidil closes nonischemic cell to ischemic cell and so produce more electrical inhomogenity between ischemic and nonischemic region. In larger infarct, half of the whole myocardium almost are ischemics and half of the myocardium is nonischemic. That is why using of ATP dependent potassium channel blockage and opening may be effective to decrease arrhythmia, because both drug in this case decrease electrical inhomogenity.

5. CONCLUSION

As a conclusion, glibenclamide decrease electrical heterogenity and decrease arrhythmia in smaller infarct. Decreasing in electrical inhomogenity between nonischemic and ischemic region may be important factor to decrease arrhythmia. ATP dependent potassium channel blockage with glibenclamide is effective to decrease ischemia induced arrhythmia in rats having smaller infarct. But opening of ATP dependent potassium channels by pinacidil in animals having smaller infarct do not decrease arrhythmia.

Table 1. The duration of arrhythmias during 30 min. of coronary ligation in female rats.

Groups	N	Onset of arrhythmia	Arrhythmic Period	Length of Arrhythmia (min)				
				VF	VT	Other	Total	Bradycardia
Control	10	3±0,9	11±1	0,02	0,37±0,10	1,1±0,16	1,50±0,15	0
Glibenclamide	10	3± 1	10± 2	0	0,22±0,17	0,57±0,21	0,79±0,33	0,03
Pinacidil	10	0,1±0,03* β	15±0,7 β	0	1,04±0,36 β	1,24±0,27 β	2,28±0,38 β	0

* P<0.05; Different from Control
β P<0.05; Different from Glibenclamide
Data are mean ± SE
Total= VF+ VT + Other arrhythmias including VPC, ventricular gemini, AV nodal arrhythmia and salvo.

Table 2. The heart rate (HR) and mean arterial pressure (BP) during 30 min. of coronary artery ligation.

Groups	N	Heart Rate / min.							Blood Pressure (mmHg)						
		Basal	01	03	05	10	15	30	Basal	01	03	05	10	15	30
Control	10	373±12	360±12	357±17	371±9	365±15	363±15	340±15	104±5	85±5	85±7	86±10	83±14	84±9	81±10
Glibenclamide	10	401±8	393±4*	395±4*	392±6	394±7*	394±6*	380±6*	113±6	104±6*	110±7*	113±9*	114±7*	108±8	100±9
Pinacidil	10	391±13	401±13*	404±12*	405±10*	396±7*	397±9*	394±8*	124±4*	114±5*	119±4*	122±3*	120±6*	100±10	106±1

* P<0.05; Different from Control
N: The number of animals.
Basal: Before coronary artery ligation and 01, 03, 05, 10, 15, 30 minutes after the coronary ligation.

Table 3. The incidence of arrhythmias during 30 min. of coronary ligation in female rats.

Group	N	Survival (N / %)	Incidence of Arrhythmias(n / %)				Arrhythmia Score
			VF	VT	Other	Bradycardia	
Control	10	10 / 100	2 / 20	8 / 80	10 / 100	0 / 0	4 ± 0,1
Glibenclamide	10	10 / 100	0 / 0	2 / 20*	10 / 100	1 / 10	2 ± 0,3*
Pinacidil	10	10 / 100	0 / 0	8 / 80	9 / 90	0 / 0	4 ± 0,4

N: The number of animals at the beginning of the ligation; VF: Ventricular fibrillation; VT: Ventricular Tachycardia; Other: Ventricular extra beat, bigemini ve salvos.
* P<0.05; Different from Control and Pinacidil
Data are mean ± SE
Total= VF+ VT + Other arrhythmias including VPC, ventricular gemini, AV nodal arrhythmia and salvo.

Table 4. The Risk of Infact Zone % and Infact Area % during 30 min. of coronary artery ligation.

Groups	N	Risk of Infact Area %	Infact Area %
Control	10	27±2	6±0,7
Glibenclamide	10	25± 1	5± 0,7
Pinacidil	10	29±1	8±0,9*

* Different from Glibenclamide
N: The number of animals.
Risk of Infact Area %: Risk of infarct area / All of the heart
Infact Area %: Infarct area / All of the heart

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