

**THE EFFECT OF THE SARCOLEMMA AND MITOCHONDRIAL
ATP DEPENDENT POTASSIUM CHANNELS ON
THE ISCHEMIA / REPERFUSION ARRHYTHMIAS
IN MALE AND FEMALE RATS
THE ROLE OF ENDOGENOUS ESTROGEN**

**by
ERSÖZ GONCA**

FEBRUARY 2008

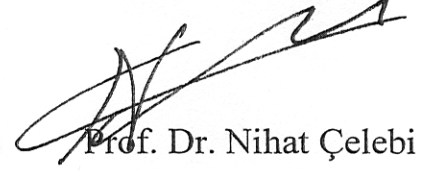
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by
ERSÖZ GONCA

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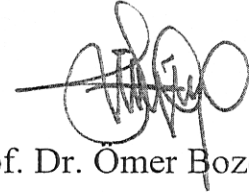
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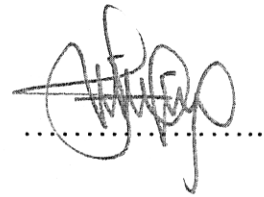


Prof. Dr. Ömer Bozdoğan

Head of Department

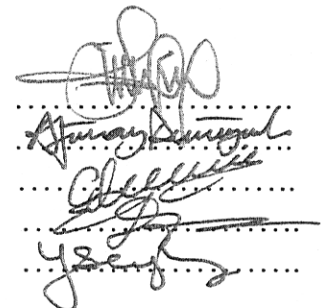
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Prof. Dr. Ömer Bozdoğan
(Supervisor)



Examining Committee Members

Prof. Dr. Ömer Bozdoğan
Prof. Dr. Tuncay Demiryürek
Prof. Dr. Cihan Demirci
Assoc. Prof. Dr. Ewa Doğru
Assist. Prof. Dr. Seyhun Yurdugül



ABSTRACT

THE EFFECT OF THE SARCOLEMMA AND MITOCHONDRIAL ATP DEPENDENT POTASSIUM CHANNELS ON THE ISCHEMIA / REPERFUSION ARRHYTHMIAS IN MALE AND FEMALE RATS THE ROLE OF ENDOGENOUS ESTROGEN

Gonca, Ersöz
PhD., Department of Biology
Supervisor: Prof. Dr. Ömer Bozdoğan
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The role of ATP dependent potassium channel in ischemia reperfusion induced injury and arrhythmias have been investigated intensively, however the effect of sarcolemmal and mitochondrial ATP dependent potassium channel modulation by drugs on these arrhythmias remains controversial. Therefore, the aim of the present study is to investigate the role of sarcolemmal and mitochondrial K_{ATP} channel modulation on ischemia and reperfusion induced arrhythmias.

In this study 143 Sprague-Dowley rats in male and female aged 7-8 months old were used. Six minutes ischemia and six minutes reperfusion was generated by the ligation and releasing of left coronary artery. The effect of pinacidil, HMR 1098, 5-HD and diazoxide on ischemia reperfusion induced arrhythmia have been examined.

The score of arrhythmia in reperfusion and total duration of arrhythmias in ligation was found to be lower in female and ovariectomized female than in male rats ($P<0.05$). The score of arrhythmias and total length of arrhythmias in males during

reperfusion was lower in pinacidil, HMR 1098, 5-HD and diazoxide groups compared to control ($P<0.05$). Pinacidil did not decrease the score of arrhythmia in males during reperfusion when it was combined with 5-HD ($P<0.05$). In females, the combination of Pinacidil and HMR 1098 decreased total length of arrhythmias during reperfusion ($P<0.05$). In ovariectomized females, HMR 1098 decreased the score of arrhythmias during reperfusion while 5-HD increased duration of total arrhythmias during ligation ($P>0.05$).

The lower arrhythmia scores observed in female did not depend on the circulating estrogen levels. The ovariectomy changed the response of females to HMR 1098 and 5-HD. It may indicate estrogen involvement in the modulation of K_{ATP} channel by selective drugs. Both activation and inhibition of mitochondrial or sarcolemmal K_{ATP} channel produced antiarrhythmia in males. The protective effect of pinacidil against arrhythmia was abolished by the combination of pinacidil with mitochondrial K_{ATP} channel blocker, That may suggest that pinacidil decreases arrhythmia through mitochondrial potassium channel opening.

As a conclusion, both the blockage of sarcolemmal K_{ATP} dependent channel and opening of mitochondrial K_{ATP} channel at the same time may be effective to decrease ischemia reperfusion induced arrhythmia.

Key Words: Ischemia reperfusion, Arrhythmias, Rat, Sex, ATP dependent potassium channels, Estrogen, Pinacidil, HMR 1098, 5-HD, Diazoxide.

ÖZET

SARKOLEMMAL VE MİTOKONDRIYAL ATP BAĞIMLI POTASYUM KANALLARININ ERKEK VE DIŞI SIÇANLARDA İSKEMİ VE REPERFÜZYON ARİTMİLERİNE ETKİSİ; ENDOJEN ÖSTROJENİN ROLÜ

Gonca, Ersöz
PhD., Biyoloji Bölümü
Danışman: Prof. Dr. Ömer Bozdoğan
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ATP bağımlı potasyum kanallarının iskemi ve reperfüzyon aritmileri ve reperfüzyon hasarı üzerine etkisi yoğun bir şekilde araştırılmaktadır, ancak sarkolemmal ve mitokondrial ATP bağımlı potasyum kanallarının aritmiler üzerindeki etkisinin ilaçlarla değişmesi tartışmalı bir konudur. Bu nedenle, bu çalışmanın amacı, sarkolemmal ve mitokondrial K_{ATP} kanallarının uyarılmasının, iskemi ve reperfüzyonla uyarılan aritmiler üzerindeki rolünü araştırmaktır.

Bu çalışmada 143 adet 7-8 aylık erkek ve dişi Sprague-Dowley cinsi sıçanlar kullanıldı. Sol koroner arter bağlanarak altı dakika iskemi ve gevşetilerek altı dakika reperfüzyon yapıldı. İskemi ve reperfüzyon ile uyarılan aritmiler üzerine pinacidil, HMR 1098, 5-HD ve diazoksit'in etkileri incelendi.

Reperfüzyon sırasındaki aritmi skoru ve ligasyondaki toplam aritmi süresi dişi ve ovaryumu çıkarılmış dişi sıçanlarda erkek sıçanlardakine göre daha az bulundu ($P<0.05$). Erkeklerde reperfüzyon sırasında saptanan aritmi skoru ve toplam aritmi süresi pinasidil, HMR 1098, 5-HD ve diazoksit gruplarında kontrole göre daha azdı

($P<0.05$). Erkeklerde, pinasidil 5-HD ile birlikte verildiğinde reperfüzyon sırasında saptanan aritmi skoru azaldı ($P<0.05$). Dişilerde Pinasidil, HMR 1098 ile birlikte verildiğinde reperfüzyon boyunca oluşan toplam aritmi süresi azaldı ($P<0.05$). Ovaryumu çıkartılmış dişilerde, 5-HD ligasyondaki toplam aritmi süresini arttırırken, HMR 1098 reperfüzyon sırasında saptanan aritmi skorunu azalttı ($P<0.05$).

Dişilerde gözlenen daha az aritmi skoru dolaşımdaki östrojenin seviyesine bağlı değildi. Ovaryumun çıkartılması dişilerin HMR 1098 ve 5-HD'ye verdiği cevabı değiştirdi. Bu K_{ATP} kanallarının özgül ilaçlara olan duyarlılığının östrojen ile değişebileceğini düşündürmektedir. Erkeklerde mitokondrial ya da sarkolemmal ATP bağımlı potasyum kanallarının hem açılması hem de kapanması aritmiyi azaltmaktadır. Pinasidil'in aritmilere karşı koruyucu etkisi, pinasidil, mitokondriyal K_{ATP} kanal blokeri ile kombine edildiğinde kaybolmuştur, bu sonuç pinasidil'in aritmileri azaltmadaki etkisinin mitokondriyal K_{ATP} kanallarını açarak olduğunu ortaya koymaktadır.

Sonuç olarak, aynı anda hem sarkoplazmik K_{ATP} kanallarının bloke edilmesi hem de mitokondriyal K_{ATP} kanallarının açılması iskemi ve reperfüzyonla uyarılan aritmilerin azaltılmasında etkili olabilir.

Anahtar Kelimeler: İskemi reperfüzyon, Aritmi, Sıçan, Cinsiyet, ATP bağımlı potasyum kanalları, Östrojen. Pinasidil, HMR 1098, 5-HD, Diazoksit.

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LIST OF ABBREVIATIONS

K_{ATP} channel: ATP dependent potassium channel.

SarcK_{ATP} channel: Sarcolemmal ATP dependent potassium channel.

MitoK_{ATP} channel: Mitochondrial ATP dependent potassium channel.

K_{Ca} channels: Ca⁺⁺ activated K⁺ channels.

APD: Action potential duration.

Kir 6.x: Inwardly rectifying K⁺ channel core.

SUR: Sulfonylurea receptor.

TMD: Transmembrane domains.

NBF: Nucleotide binding fold.

KCO: ATP dependent potassium channel opener.

SDH: Succinate dehydrogenase.

ANT: Adenine nucleotide translocator.

IPC: Inorganic phosphate carrier.

IMS: Intermembrane space.

MitoIM: Mitochondrial inner membrane.

ROS: Reactive oxygen specious.

MPTP: Mitochondrial permeability transition pore.

PPIase: Peptide prolyl cis trans isomerase.

CyP-D: Cyclophilin.

VDAC: Voltage dependent anion channel.

Mi-CK: Mitochondrial creatine kinase.

IPC: Ischemic preconditioning.

PKC: Protein kinase C.

NE: Norepinephrine.

NO: Nitric oxide.

NOS : Nitric oxide synthase.

L-NAME: L-nitro arginine methyl ester.

VF : Ventricular fibrillation.

VT: Ventricular tachycardia.

VPC: Ventricular premature contraction.

1. INTRODUCTION

More than 50% of the sudden death occurs as a result of the coronary artery failure in human (1). Since the K^+ ions play important role on the generation of arrhythmias after myocardial ischemia, the researchers have been intensified on the role of the cardiac ATP dependent potassium channel (K_{ATP} channel) (2). ATP dependent potassium channels are present both in sarcolemmal (sarc K_{ATP} channel) and mitochondrial membrane (mito K_{ATP} channel) (3, 4). The sarc K_{ATP} channels open due to the fall in intracellular ATP during myocardial ischemia and cause the extracellular K^+ accumulation (5). Although opening of the mito K_{ATP} channels does not affect the electrophysiological properties of the myocytes, they play a role in mitochondrial bioenergetics (6). On the other hand, the modulation of the sarc K_{ATP} channels alters the action potential duration of myocardial cells (7).

Previous studies on the effect of K_{ATP} channel modulators on cardiac arrhythmias are inconsistent. The blockage of the sarc K_{ATP} channels by drugs have reported to be antiarrhythmic (8-13). On the other hand, some other researchers have revealed that K_{ATP} channel blockers are proarrhythmic or they do not have any effect on arrhythmias (14-16). The controversial findings have also been found regarding the role of K_{ATP} channel openers in protection against malignant arrhythmias in a variety of experimental model. Some investigators have described K_{ATP} channel openers as antiarrhythmic (13, 17-19). Others have suggested that K_{ATP} channel openers as proarrhythmic (10, 20, 21). The reason of the discrepancy in these results may depend on the using of the nonselective drugs or the difference in experimental model used. Nonselective K_{ATP} channel modulators affect both sarcolemmal and the mitochondrial K_{ATP} channels. So underlying mechanism in the antiarrhythmic effect of nonselective K_{ATP} channel modulators in ischemia and reperfusion is not clear.

The mechanism of the K_{ATP} channel blockers and openers in the antiarrhythmic effect is not clear and the studies that research the effect of the selective K_{ATP} channel modulators are limited. The studies related with I/R arrhythmias have been only performed in male animals. But it has been reported that the number of the K_{ATP} channels is more in female than male (22). That is why, the effect of K_{ATP} channel modulators might be different in females. Likewise, we have previously demonstrated that K_{ATP} channel blockage with Glibenclamide (a nonselective K_{ATP} channel blocker) showed antiarrhythmic effect in male but it was not effective in female (23). Anyway, there are gender differences between male and female due to estrogen. These differences may be reason of lesser reperfusion induced arrhythmia observed in female than male rats (19, 24).

In a study it has been suggested that the antiarrhythmic and antiinfarct effects of the exogenous estrogen depend on the modulation of the sarcolemmal and mitochondrial K_{ATP} channels (25). Endogenous estrogen may have similar antiarrhythmic mechanism. In addition, estrogen changes the expression of the sarc K_{ATP} channels (26).

1.1. The Aim of the Study

In the present study, it is firstly aimed to research the roles of sarcolemmal and mitochondrial K_{ATP} channel modulation on arrhythmias observed during acute myocardial ischemia and reperfusion, and the relative effect of nonselective opener (Pinacidil) on the ischemia/reperfusion induced arrhythmia occur via sarc K_{ATP} or mito K_{ATP} channel have been researched. Secondly we aimed also to clarify the gender specific effect of ATP dependent potassium channel modulation (sarc K_{ATP} and mito K_{ATP} channels) on I/R arrhythmias and the role of the estrogen in this effect. As a result, this study has been expected to provide some evidence about which K_{ATP}

channels; sarcK_{ATP} or mitoK_{ATP} channels are effective in protection against ischemia reperfusion induced arrhythmias in rats.

1.2. The Role of Potassium (K⁺) Ions in Ischemia Reperfusion Arrhythmias

There are various channels in myocardial cell membrane. One of them is sarcolemmal ATP dependent potassium channel (sarcK_{ATP}) that is very important in generation of ischemia-reperfusion induced arrhythmias. Under normoxic condition, the sarcK_{ATP} channels are in closed state in myocardial cells. However during myocardial ischemia as the intracellular ATP concentration falls, sarcK_{ATP} channels open. The opening of these channels result in an outward potassium (K⁺) current and lead extracellular potassium accumulation (2, 5, 27). Maintenance of Na⁺ and K⁺ gradients across the cell membrane depends on Na-K ATPase pump activity. Inadequate production of ATP results in loss of Na-K ATPase pump activity and also causes the extracellular K⁺ accumulation (28). The increased extracellular potassium concentration is an arrhythmogenic stimulus (2, 29). K⁺ accumulation in the extracellular space led to the depolarization of normal myocardial cell in nonischemic zone (30, 31). K⁺ accumulation in extracellular space changes the action potential of the myocardial cells. The transmission of the impulses slow down or completely block depends on the shortening of the action potential duration. These factors create the conditions necessary for reentry type of arrhythmia (31, 32). Arrhythmia may be also induced by reperfusion after the ischemia. Reperfusion increase inhomogeneity in electrolyte balance in myocardial cells. Again leakage of potassium out of ischemic cells continue to generate severe arrhythmias. These arrhythmias are called as ischemia reperfusion induced arrhythmias and range in severity from ventricular premature contraction (VPC) to more serious rhythm disturbances such as ventricular tachycardia (VT) and ventricular fibrillation (VF) (33). Ischemia reperfusion induced arrhythmias have been observed in experimental species and also in humans (33, 34).

Several mechanisms have been proposed for reperfusion arrhythmias. Some researchers suggest that arrhythmia induced by myocardial ischemia is associated with an increase in free cytosolic calcium (35-37). Arrhythmias at reperfusion occur via excessive intracellular calcium cycling and delayed afterdepolarization (36). Beside this, the generation of oxyradicals play a role in the pathogenesis of reperfusion arrhythmias (38, 39). The reentrant mechanism caused by the marked dispersion of refractoriness and abnormal contraction also lead to reperfusion arrhythmias (40). The electrical inhomogeneity between the ischemic and nonischemic myocardium is another factor that leads to reperfusion arrhythmias after the ischemia via reentrant mechanism (40).

1.3. ATP Dependent Potassium Channels (K_{ATP})

Since the K^+ leakage from the ischemic cells plays a role in the generation of the arrhythmia, an extensive research have been carried out on the role of myocardial ATP dependent potassium channels (K_{ATP} channel) (41). Two K_{ATP} channel subtypes exist in the myocardium, with one subtype located in the sarcolemmal membrane of the myocytes that is called as sarcolemmal ATP dependent potassium channel (sarc K_{ATP} channel) and the other in the inner membrane of the mitochondria that is called mitochondrial ATP dependent potassium channel (mito K_{ATP} channel) (3, 4).

1.3.1. Sarcolemmal ATP Dependent Potassium Channel (Sarc K_{ATP})

Sarcolemmal ATP dependent potassium channels (sarc K_{ATP} channel) have been first discovered in cardiomyocytes by Noma (3). The sarc K_{ATP} channels are widely distributed in many tissues and cell types including heart (3), pancreatic β cells (42), brain (43), skeletal (44) smooth muscle (45) and kidney (46). They are activated by changes in intracellular adenine nucleotide concentration (47). They couple the metabolic state of the cell to membrane potential. So the sarc K_{ATP} channels play important roles in the cellular responses of various tissues under

various metabolic states including hyperglycemia, hypoglycemia, ischemia and hypoxia (48).

In the pancreatic β cells, they regulate the insulin secretion (49). In brain, they protect neuronal damage and neurodegeneration (50). In vascular system, they regulate the tonus of vascular smooth muscle (51, 52). In coronary artery, they involve in the vasodilatation response during ischemia (53, 54).

1.3.1.1. The Mechanism of the Protection in Myocardial Cells by SarcK_{ATP} channels

The sarcK_{ATP} channels play roles in metabolic stress in heart. It has been reported that the number of expressed sarcK_{ATP} channels regulate the resistance to metabolic stress in cardiomyocytes. An acute increase in the number of sarcK_{ATP} channels increase the cellular resistance to hypoxia (55). The presence of sarcK_{ATP} channels enhances cytoprotection and survival under stress condition (56-58). The sarcK_{ATP} channels are activated during ischemia or hypoxia. This activation leads to K⁺ loss in ischemic cells. The action potential duration (APD) shortens in myocardial cells. The shortening of the APD leads to a decrease in Ca⁺⁺ influx through the voltage gated Ca⁺⁺ channels that protect against cardiac damage during ischemia (59, 60).

1.3.1.2. The Molecular Structure of the SarcK_{ATP} channels

The sarcK_{ATP} channel is heterooctameric complex comprising two subunits; the pore forming subunit, inwardly rectifying K⁺ channel core (Kir 6.x). And the regulatory subunit, sulfonylurea receptor (SUR) (61) (Figure 1.1).

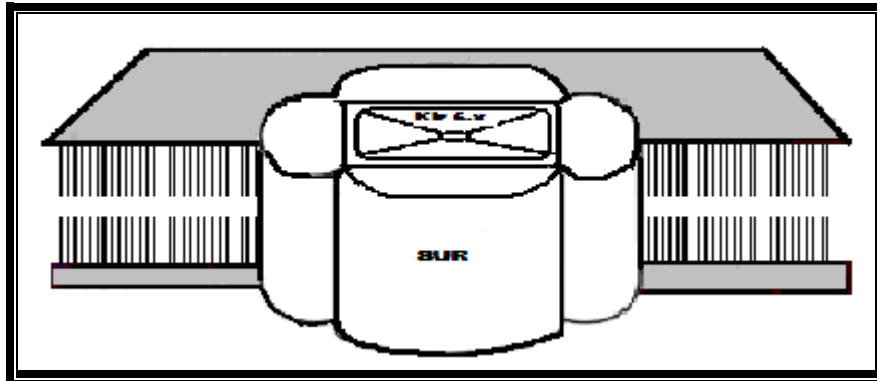


Figure 1.1. The sarcK_{ATP} channels in sarcolemmal membrane.

SUR: Sulfonylurea receptor, **Kir 6.x:** Inwardly rectifying channel core.

Kir 6.x belongs to the inward rectifier K⁺ channel family (62, 63). SUR belongs to the ATP binding cassette protein superfamily (64). There are three isoforms of the sulfonylurea receptors; SUR1, SUR2A, SUR 2B. There are two subunit of the K⁺ channel core; Kir 6.1 and Kir 6.2. The expression of different combinations of SUR and Kir.x types constitute different types of sarcKATP channels (41). SUR 2A and Kir 6.2 constitute sarcolemmal ATP dependent potassium channel in cardiomyocyte (65) ; SUR1, Kir6.2 in pancreatic β cells (62); SUR2B, Kir6.2 in smooth muscle cells (66) and SUR2B, Kir6.1 in vascular smooth muscle cell (67) (Table 1.1).

Table 1.1. The types of sarcK_{ATP} channels

Subunit Composition	Type
SUR2A/ Kir6.2	Cardiomyocyte
SUR1/ Kir6.2	Pancreatic β cells
SUR2B/ Kir6.2	Smooth muscle
SUR2B/ Kir6.1	Vascular smooth muscle

The SUR subunits has 17 transmembrane segments (TM) arranged in three transmembrane domains (TMD); TMD 0 (5TM), TMD1 (6TM), TMD2 (6TM) (63).

The sulfonylurea receptor has two nucleotide binding fold (NBF1 and NBF2) on the cytoplasmic side (68).

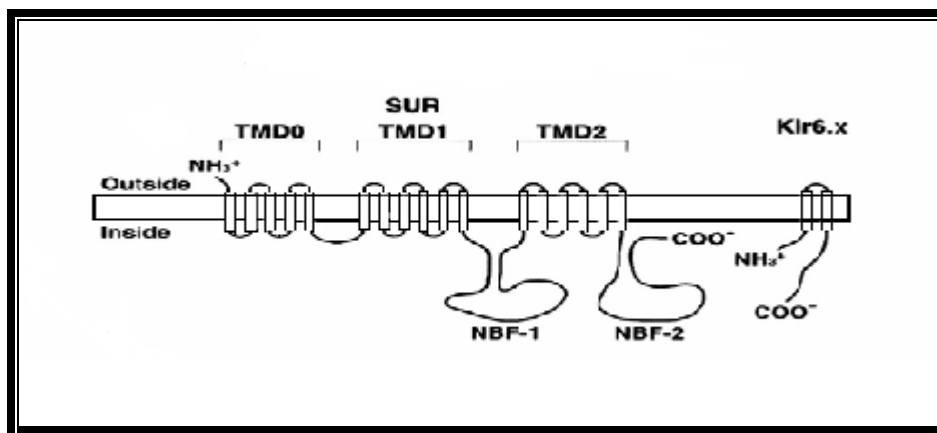


Figure 1.2. The parts of the SUR and Kir6.x subunits of sarcoK_{ATP} channels

1.3.1.3. The Binding of the Potassium Channel Opener to SUR Subunits

ATP dependent potassium channel openers (KCO) including diazoxide, pinacidil, cromacalim and nicorandil are a structurally diverse group of drugs that open K_{ATP} channels in various cell types (69). It has been shown that different types SUR of the K_{ATP} channels change their sensitivities to K_{ATP} channel openers. SUR1/Kir 6.2 channels are activated strongly by diazoxide but not by pinacidil. SUR2A/Kir 6.2 channels are activated by pinacidil and cromacalim but only weakly by diazoxide. SUR2B/Kir6.2 channels are activated by diazoxide, pinacidil and cromacalim (57, 60, 61, 70, 71, 72). However the sensitivity of SUR2A/Kir6.2 channels to diazoxide increases in the presence of Mg ADP (71).

The sensitivities of SUR2 to the specific KCO such as Pinacidil depend on different arrangements of TMD 2 of SUR2A (72, 73). However the precise location of the binding site for Diazoxide is still unknown.

1.3.1.4. The Regulation of the SarcK_{ATP} Channels by Nucleotides

Since SUR1 possesses two nucleotide binding fold (NBF), it has been thought that these subunits mediate the effects of nucleotides (68). However, in addition to SUR, Kir 6.2 subunit also related to the ATP sensitivity of the K_{ATP} channels (74). It has been suggested that N (NH₃⁺) and C (COO⁻) terminal regions of Kir 6.2 interact cooperatively and inhibit channel activity in the presence of the ATP (75). However, the ATP sensitivity of channel constituted only by Kir 6.2 subunits is much lower than that of the channel constituted from both Kir 6.2 and SUR1 (76).

The following model related with the modulation of the K_{ATP} channels by ATP and ADP has been proposed (76); When the ATP/ADP ratio is decreased, nucleotide binding fold-1 (NBF1) of the SUR binds ATP and nucleotide binding fold-2 (NBF2) of the SUR binds MgADP. In this conformational state the interaction of SUR1 with Kir 6.2 reduces the affinity of Kir 6.2 to ATP. The ATP molecules dissociate from the Kir6.2. This results in the opening of the K_{ATP} channels (76).

When the ATP/ADP ratio is increased, ATP dissociates from the nucleotide binding fold 1(NBF1) of the SUR and MgADP dissociates from the nucleotide binding fold 2 (NBF2) of the SUR. In this conformational state the interaction of SUR1 with Kir 6.2 increases the affinity of Kir 6.2 to ATP. Kir6.2 binds to the ATP molecules. This results in the closure of the K_{ATP} channels (76), (Figure 1.3).

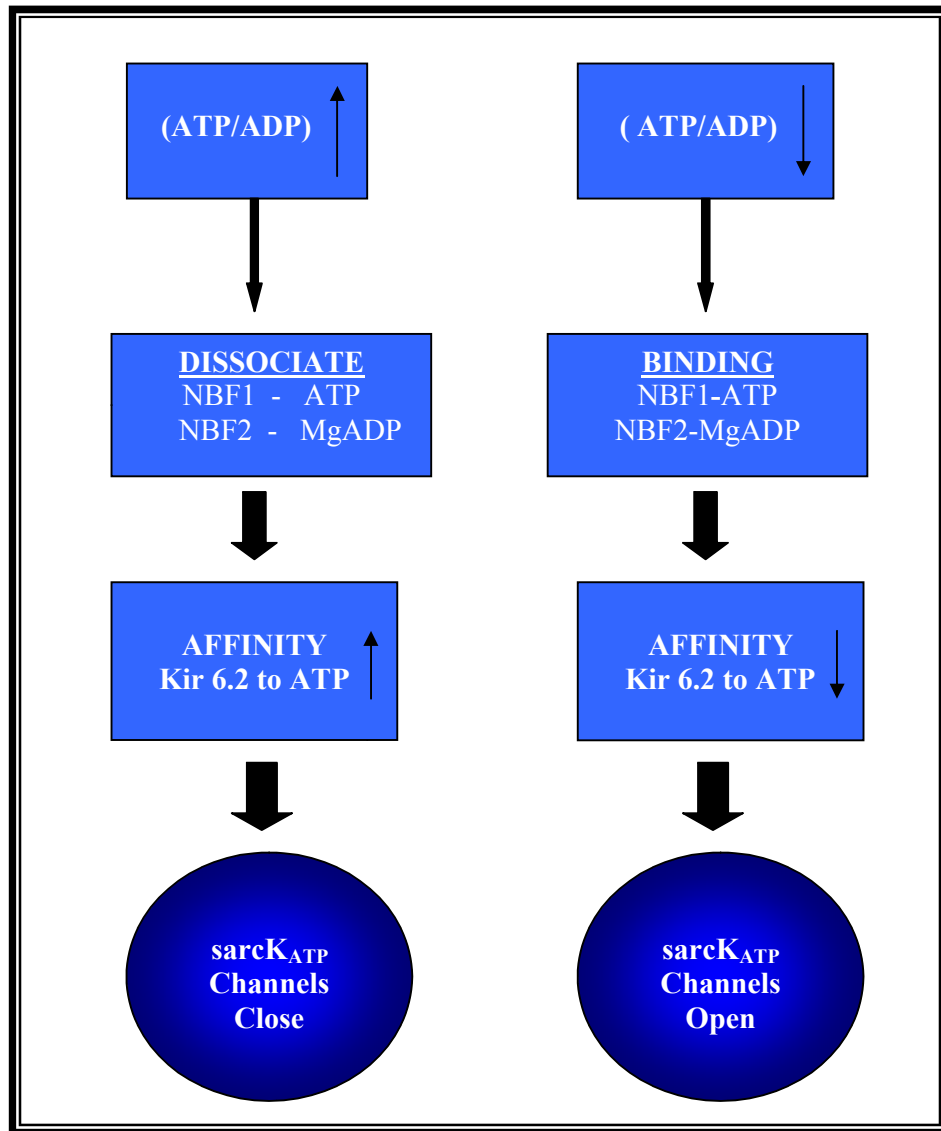


Figure 1.3. The regulation of the sarcK_{ATP} channel by change in ATP/ADP ratio.

1.3.2. Mitochondrial ATP Dependent Potassium Channels (MitoK_{ATP} channel)

Mitochondrial ATP dependent potassium channels (mitoK_{ATP} channel) was first discovered from rat liver in 1991 by Inoue I. (4). Then it was found in myocardial cells by Paucek P. (77). While the sarcK_{ATP} channels alter the myocardial action potential, the modulation of the mitoK_{ATP} channels does not affect the electrophysiological properties of the myocardial cells. The mitoK_{ATP} channels plays a role in mitochondrial bioenergetics (78).

1.3.2.1. The Molecular Structure of the MitoK_{ATP} channels

The molecular structure of mitoK_{ATP} channels is not completely clear. It has been suggested that the mitoK_{ATP} channels contains subunits similar to Kir and Sur proteins of the sarcK_{ATP} channels. The mitoK_{ATP} channels contains 55 kDa and 63 kDa proteins (79). It has been proposed that the 63kDa is the sulfonylurea binding subunit of channel; the 55 kDa is the pore forming unit of channel. Likewise, Suzuki M. et al. (80) suggested the existence of a Kir 6.1 like protein in the mitochondria. Krebs cycle enzyme, succinate dehydrogenase (SDH) is a target of the mitoK_{ATP} channel modulators (81, 82). It has been found that several pharmacological agents target SDH induced protection instead of the acting on mitoK_{ATP} channels (83, 84). It was identified at least four SDH associating proteins; these are mitochondrial ATP binding cassette protein1, adenine nucleotide translocator (ANT), ATP synthase, inorganic phosphate carrier (IPC) (85).

This protein complex is capable of transporting K⁺. SDH and mitoK_{ATP} channels interact both functionally and physically. It has been proposed that SDH influences mitoK_{ATP} channel activity through its physical and functional interaction with this complex (84) (Figure 1.4).

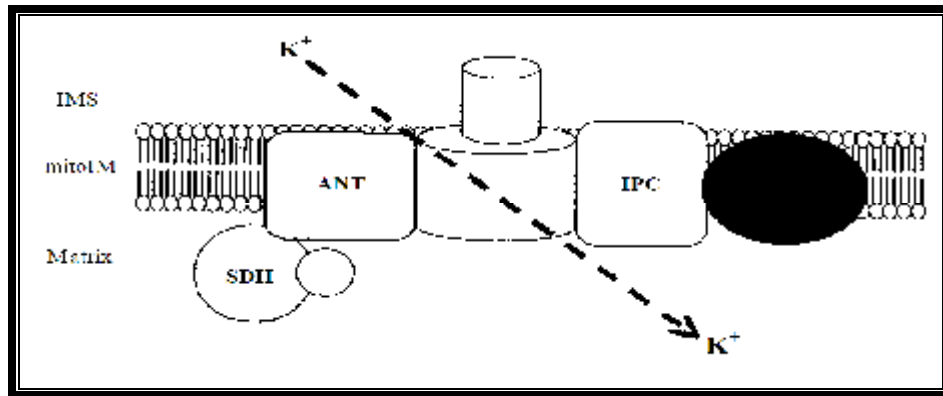


Figure 1.4. The structure of the mitoK_{ATP} channels.

IMS: Intermembrane space; **mitoIM:** Mitochondrial inner membrane.

ANT: Adenine nucleotide translocator; **SDH:** Succinate dehydrogenase.

1.3.2.2. The Role of Mitochondria in Ischemia Reperfusion (I/R) Injury

Cell death occurs by either necrosis or apoptosis in ischemia and reperfusion. Mitochondria are involved in both processes (86).

In myocardial ischemia energy production falls in mitochondria due to the inadequate supply of O₂ (87). This causes abnormal accumulation of ions and metabolites. In particular, lactic acid accumulates and intracellular pH decrease. Intracellular Na⁺ and Ca⁺⁺ ions increase due to the changes in activity of Na⁺-H⁺ and Na⁺-Ca⁺⁺ exchanger. Elevated level of Ca⁺⁺ lead to the activation of degradative enzymes (phospholipases, proteases and nucleases) that ultimately damage the tissue irreversibly (88, 89).

After the reperfusion abundant amount of reactive oxygen specious (ROS) are generated. The major two sources of ROS are the xanthine oxidase and the mitochondrial respiratory chain. 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 phospholipid (88). Although the majority of

reports suggest the ischemia itself causes damage to the mitochondria, others suggest that mitochondrial function is initially preserved by ischemia but is compromised by oxygen free radicals upon reperfusion (90).

Mitochondrial inner membrane have permeability barrier to protons. This barrier is required to maintain the membrane potential and pH gradient to provide the driving force for ATP synthesis through oxidative phosphorylation. Mitochondrial inner membrane have also specific transporter that maintains the gradient of metabolites between the cytosol and mitochondria. If the permeability barrier of inner membrane and specific transporter are disrupted, mitochondria become uncoupled and neither synthesize ATP by oxidative phosphorylation nor separate cytosolic and mitochondrial pools of metabolites (90).

Under physiologic condition, mitochondrial Ca^{++} entry occurs via Ca^{++} uniporter that is primary route for Ca^{++} entry into mitochondrial matrix and Ca^{++} efflux occurs via $\text{Na}^+/\text{Ca}^{++}$ exchanger in the inner membrane of the mitochondria (pumps 3 Na^+ ions inside, 1 Ca^{++} ion out of the mitochondria). Ca^{++} uniporter is sensitive to Ca^{++} uniporter inhibitor (Ruthenium red) and is highly dependent on the mitochondrial membrane potential (91, 92), (Figure 1.5).

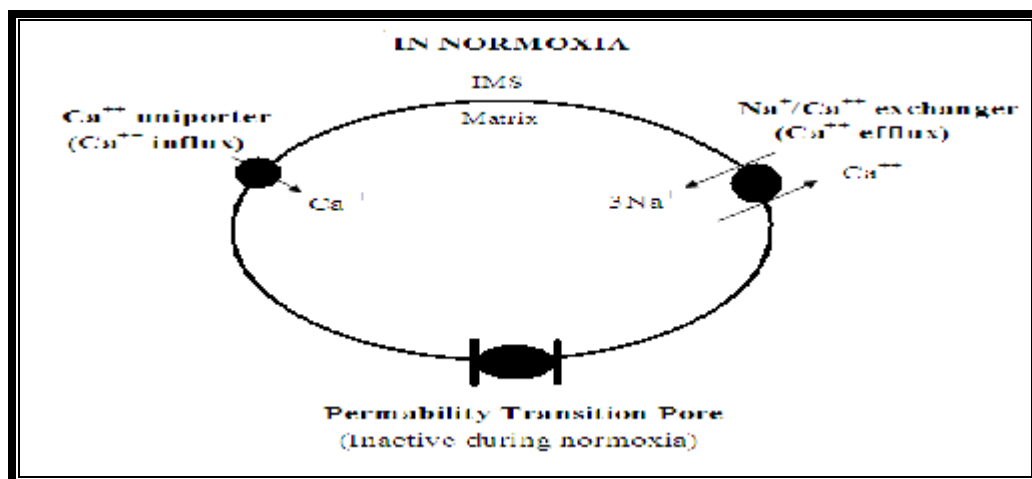


Figure 1.5. The mitoK_{ATP} ion channels in normoxia; IMS: Intermembrane space.

In normal cardiac function mitochondrial Ca^{++} transport pathway play significant role in the coordination of ATP supply and demand. In increased cardiac workload, cytosolic Ca^{++} increase due to enhanced release of Ca^{++} from the sarcoplasmic reticulum. Cytosolic Ca^{++} is relayed into the mitochondrial matrix by the mitochondrial Ca^{++} transporter. In mitochondria, Ca^{++} activates enzymes of the citric acid cycle. NADH supply increase and hence ATP production increase (93).

Mitochondria have the capacity to take up the extra amount of Ca^{++} ions and could potentially remove toxic levels of Ca^{++} from the cytosol (94). Mitochondrial membrane potential declines progressively during the ischemic period (95). During hypoxia, Ca^{++} uniporter become inactive due to decrement in mitochondrial membrane potential. In this case, $\text{Na}^+/\text{Ca}^{++}$ exchange works in opposite direction, Ca^{++} ions enters into matrix region and Na^+ ions are pumped out of the matrix. This results in Ca^{++} accumulation inside of the mitochondrial matrix. In ischemia huge amount of Ca^{++} ions can damage mitochondria both by competition for ATP production and by inducing the permeability transition pore. In reperfusion, large quantity of Ca^{++} produce ROS and mitochondria structurally alter (86, 91, 94), (Figure 1.6).

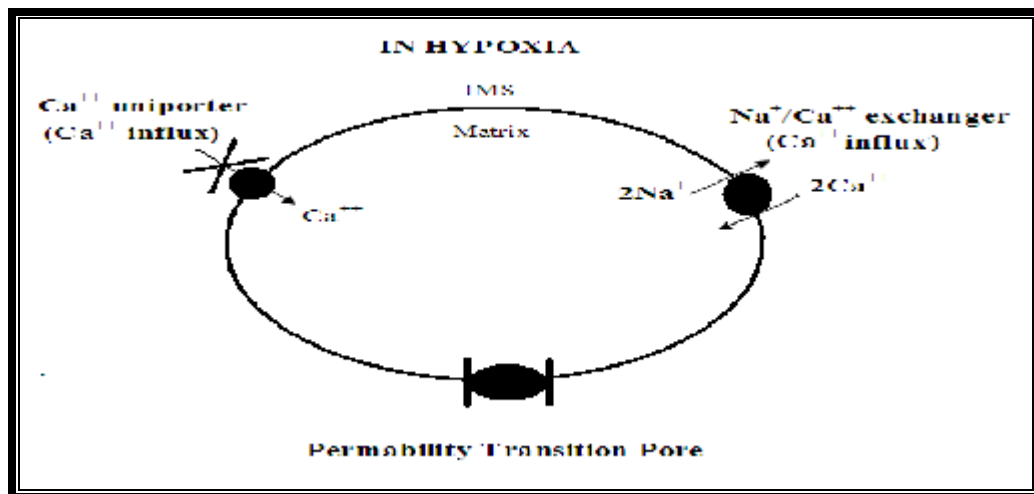


Figure 1.6. The mitoK_{ATP} ion channels in hypoxia. IMS: Intermembrane space.

When the mitochondria are exposed to high amount of Ca^{++} concentration, the pores called as MPTP (Mitochondrial permeability transition pore) are formed by the opening of a nonspecific pores on mitochondrial membrane (96). Peptide prolyl cis trans isomerase (PPIase), known as cyclophilin (CyP)-D is a mitochondrial enzyme that involved in the formation of the MPTP. The cyclophilin (CyP)-D bind to ANT (The adenine nucleotide translocase is a membrane protein whose normal function is to catalyze the transport of ADP in and ATP out of the mitochondria). The excess amount of Ca^{++} ions inside of the mitochondrial matrix causes a conformational change on the shape of proteins by altering the peptide bound around proline residues in specific pores. Thus the specific transporters are converted into a nonspecific pore (MPTP) (97, 98), (Figure 1.7).

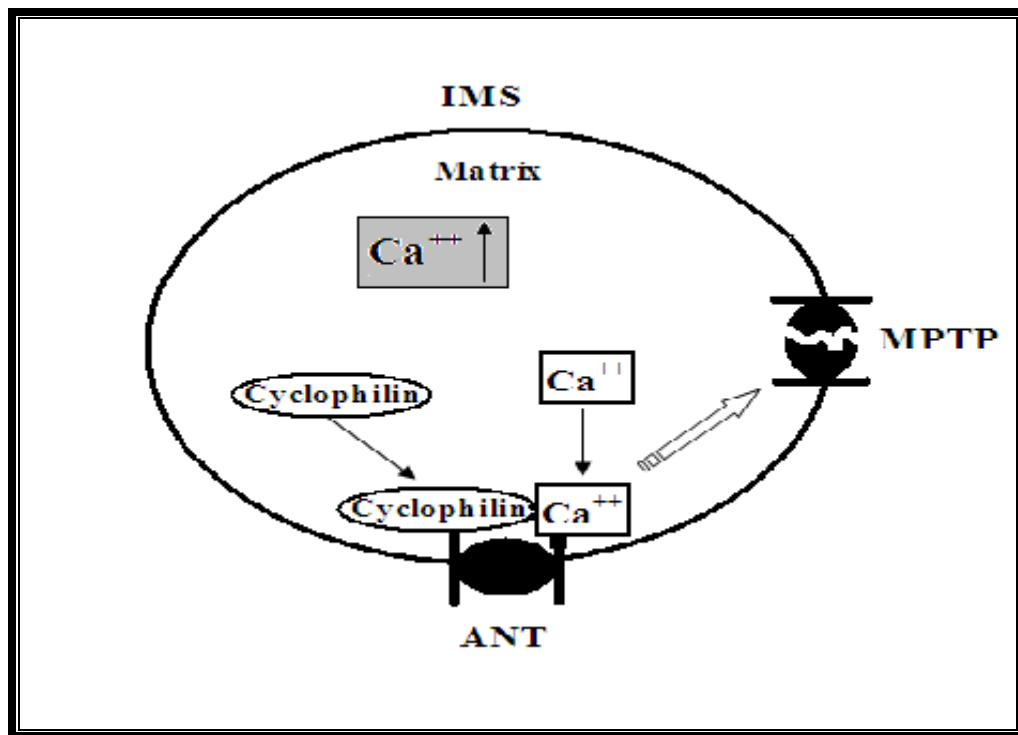


Figure 1.7. The opening of MPTP in mitochondria. IMS: Intermembrane space.

ANT: Adenine nucleotide translocator.

The opening of MPTP occurs upon reperfusion under high Ca^{++} and oxidative stress. During ischemia opening of MPTP is inhibited at $\text{pH} < 7$. However the pH returns to preischemic value during the reperfusion and MPTP open (99).

MPTP opening causes the inhibition of ATP synthesis and lead to the activation of the hydrolysis of glycolytically derived ATP. Since the MPTP produces swelling of mitochondria and rupture of the outer membrane it causes cytochrome c release that activates the apoptotic caspase (100).

1.3.2.3. The Role of MitoK_{ATP} channels in Cardioprotection

The mitoK_{ATP} channels play a role in cardioprotection both as a trigger and end effectors (101,102). In normoxia mitochondrial membrane potential is high. Since the membrane potential is high before the ischemia and reperfusion insult, the opening of the mitoK_{ATP} channels by drugs causes K^+ influx in to mitochondria. Increased K^+ influx drives H^+ ion ejection via K^+-H^+ exchanger in the inner membrane of the mitochondria that causes alkalinization in mitochondrial matrix. The matrix alkalinization causes mitochondrial ROS production (103).

ROS have dual effect deal with the cardioprotection according to the timing of ROS generation in I/R. The ROS generated before ischemia is thought to be protective (104). However the ROS that is produced during reperfusion is detrimental and causes cell death (105).

The opening of mitoK_{ATP} channel before ischemia is protective via the ROS generation that decreases detrimental ROS generation during reperfusion phase that prevents the MPTP opening (106).

The mitochondrial matrix volume is controlled by the activity of the specific transporter. Voltage dependent anion channel (VDAC) controls mitochondrial outer membrane permeability to ADP and ATP (107). In heart, VDAC is normally low

conductance state that is poorly permeable to nucleotides (108). Mitochondrial creatine kinase (Mi-CK) bridge the intermembrane space between VDAC in outer membrane and adenine nucleotide translocator (ANT) in inner membrane whose normal function is to catalyze the transport of ADP into and ATP out of the mitochondria (109,110).

In ischemia or high inotropy cardiomyocytes are undergoing high rates of ATP production and consumption. In this case the mitochondrial membrane potential decreases since the electron transport is high. This results in reduced K^+ and water uptake, contraction of the matrix and expansion of intermembrane space between the inner and outer membrane of the mitochondria. In this case, mitochondrial creatine kinase dissociate from VDAC that leads to higher outer membrane conductance to ATP and ADP (the ADP is lost from the permeable VDAC). The opening of the mitoK_{ATP} channels provide inward K^+ currents that counteracts the decrease in K^+ influx and matrix contraction in ischemia. So normal mitochondrial matrix and intermembrane space volume will be maintained. In this case the outer membrane (VDAC) will retain its low permeability to nucleosides. This preserve ADP for phosphorylation upon reperfusion (prevent the escape of ADP fromVDAC). Because there is no net change in volume, there is no increase in ROS production in high work state and ischemia (6, 78), (Figure 1.8).

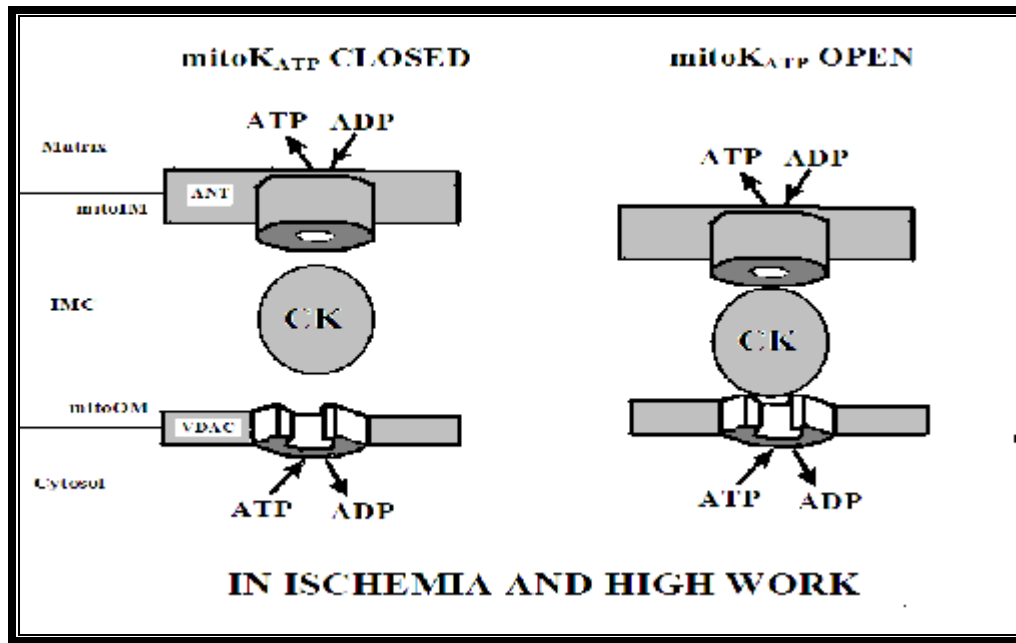


Figure 1.8. The mitoK_{ATP} channel regulation of VDAC permeability to nucleotides. IMS: Intermembrane space; **mitoIM**: Mitochondrial inner membrane; **mitoOM**: Mitochondrial outer membrane; **ANT**: Adenine nucleotide translocase **VDAC**: Voltage dependent anion channel.

The opening of mitoK_{ATP} channels with the influx of K⁺ ions result in an increase in the osmotic pressure and water movement into the mitochondrial matrix. These cause the mitochondrial matrix swelling (111-113). Mitochondrial matrix swelling caused by the opening of the mitoK_{ATP} channels activates the respiratory chain. This activation provides more ATP to support the recovery of the myocardium (114-116). It has been proposed that matrix swelling leads to a more optimal contact between membrane protein on the inner and outer membranes of the mitochondria. This lead to an increase in ADP transport and ATP synthesis in mitochondria (103).

The mitoK_{ATP} channel opening mediates the change in mitochondrial Ca⁺⁺ uptake by a partial depolarization of mitochondrial membrane potential. It has been shown that opening of mitoK_{ATP} channels resulted in the reduction of Ca⁺⁺ accumulation in the mitochondria during simulated ischemia reperfusion (117). The

mitoK_{ATP} channel opening attenuates the mitochondrial Ca⁺⁺ accumulation during ischemia in intact hearts (118). Diazoxide and Pinacidil have also been shown to reduce the magnitude of the mitochondrial Ca⁺⁺ uptake and the effect of both can be reversed by 5HD (119).

The mitoK_{ATP} channel opening during ischemia through its effect on mitochondrial intermembrane space volume reduce the rate of ATP loss, reduces the rate of adenine nucleotide degradation so that ADP is available for phosphorylation upon reperfusion and reduces mitochondrial membrane potential and Ca⁺⁺ accumulation. These effects preserve mitochondrial function. So they can return to their normal function upon reperfusion (120).

If the myocyte survives after ischemia, ATP is low, phosphocreatine is consumed and Na⁺ and Ca⁺⁺ ions are elevated in ischemic cells before reperfusion. In order to correct these imbalances, mitochondria must be able to supply ATP to the cytosol. If oxidative phosphorylation is not efficient, reintroduction of oxygen lead to high rates of potentially deleterious mitochondrial ROS production. When mitoK_{ATP} channels are open during reperfusion the association of mi-CK (mitochondrial creatine kinase) with voltage dependent anion channel (VDAC) is preserved. This provides less permeability of VDAC to ADP and ATP as in normoxic condition and permit direct energy transfer to creatine phosphocreatine system. This system is more efficient and it may be required to support energy transfers at high rates at the time of reperfusion (121,122).

1.3.2.4. Relation of MitoK_{ATP} channels with Preconditioning

Preconditioning is a pretreatment of heart with various factors that reduces myocardial damage from subsequent ischemia reperfusion. Preconditioning involves a series of cell signaling pathway that modify the cellular components which play a role in protection. Different modes of cardioprotection have been described;

Ischemic preconditioning (IPC) is the phenomenon in which brief period of ischemia protects the myocardium from damage induced by a subsequent longer period of ischemia (123). This protection includes a reduction in both infarct size and incidence of cardiac arrhythmias (124, 125).

Ischemic preconditioning has two distinct phase; an early phase appear in 1 hour after the IPC stimulus and lasts 3 hours and delayed phase of protection appears in 12-24 hours after the IPC stimulus and lasts 48 to 96 hours (126). The former involves modification of the cellular targets used as mediators and triggers for protection and latter involves modified gene expression that protects the myocardial cells (127).

A number of triggers and mediators associated with the IPC have been identified. Recent studies have implicated different protein kinases in the signaling cascade responsible for IPC, including Src tyrosine kinases, PI-3 kinase, p38 MAPK and the JAK/STAT pathway (127). There is convincing evidence that PKC is a central mediator of preconditioning (128). A variety of triggers have been identified which are activated and/or released during the preconditioning insult including adenosine, catecholamine, angiotensin 2, bradykinin, endothelin and opioids (128).

The cellular reaction in IPC is initiated by the released metabolites. These metabolites are adenosine, epinephrine, bradykinin and opioids. These metabolites cause the activation of the receptors include α_1 adrenoreceptor, adenosine A_1/A_2 , bradykinin and opioids receptors (59, 129). This leads to subsequent activation of downstream pathways including PKC, tyrosine kinase and mitogen activated protein kinase. In recent years many researches have focused on the central involvement of the $\text{sarcK}_{\text{ATP}}$ or $\text{mitoK}_{\text{ATP}}$ channels as both a trigger and distal effectors in IPC with equivocal results (130, 103). The role of the $\text{mitoK}_{\text{ATP}}$ channel has been suggested both in acute and delayed ischemic preconditioning (130, 131).

Pharmacologic preconditioning; is the phenomenon in which administration of the substances such as adenosine and epinephrine prior to ischemia protects the heart against reperfusion induced arrhythmias (132-134). Mitochondrial K_{ATP} channel was shown to be also involved in pharmacologic preconditioning (132).

Calcium preconditioning is the phenomenon in which transient increase in intracellular calcium protects the heart against subsequent global ischemia (135). Mitochondrial K_{ATP} channel was shown to be involved in calcium preconditioning (136).

Adaptive preconditioning is a long lasting cardioprotection arising from chronic hypoxia including high altitude (137). Mitochondrial K_{ATP} channel was shown to be also involved in adaptive preconditioning (138).

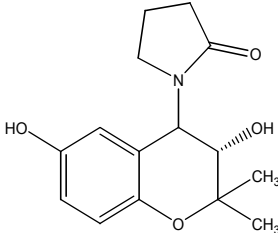
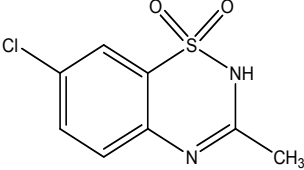
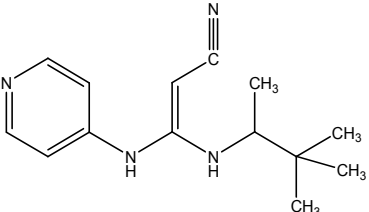
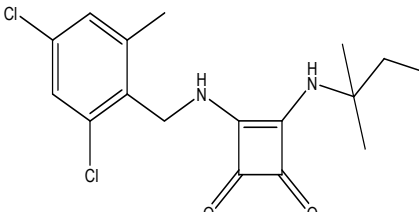
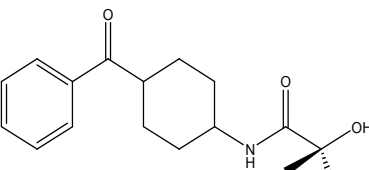
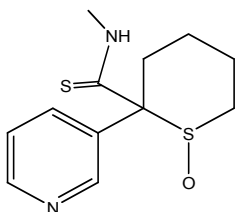
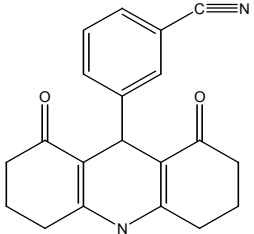
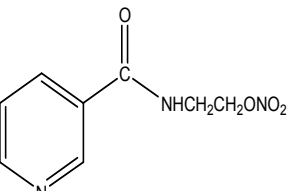
Preconditioning with K_{ATP} channel openers (KCO) are the phenomenon in which administration of a KCO prior to ischemia protects the heart (139). Early studies suggested that opening of sarc K_{ATP} channels may mediate the KCO induced cardioprotection.

The opening of the sarc K_{ATP} channel leads to shortening of the action potential and hyperpolarizing cell membrane. This would result in a reduction in the intracellular Ca^{++} levels that produce the cardioprotective effects (7). But it was shown that cardioprotection was preserved in conditions where there was no APD shortening (140, 141). So it was suggested that mito K_{ATP} channels rather than sarc K_{ATP} channels may be the key factor (142). Likewise in *in vivo* studies, Das B. and Sarkar C. (143, 144) reported that cardioprotective effect of K_{ATP} openers depend on the mito K_{ATP} channel opening in rabbit. Vegh A. and Parratt JR. (145) have also reported that the opening of mito K_{ATP} channels by Diazoxide suppress the arrhythmias like ischemic preconditioning in dogs.

1.3.3. The ATP Dependent Potassium Channel Openers (KCO)

ATP dependent potassium channel openers (KCO) are the family of drugs that activate the K_{ATP} channels. KCO stabilize membrane excitability and preserve metabolic expenditure (146). So that these class of therapeutics is used as cytoprotective agents under diverse conditions (147-149). The antihypertensive drugs, bronchodilators, bladder relaxants, islet cell protectors, antiepileptics and promoters of hair growth as KCO are used in clinical treatment (150).

They are chemically diverse and belong to a number of structural classes. 1. Benzopyrans (Cromakalim, Levromakalim, Bimakalim, Rimakalim), 2. Benzothiadiazines (Diazoxide), 3. Cyanoguanides (Pinacidil), 4. Cyclobutenediones (Way-151616), 5. Tertiary carbonoles (ZD-6169), 6. Thioformamides (Aprikalim) and 7. Dihydropyridine like structures (ZM-244085), 8. Nicotinamides (Nicorandil, KRN 2391), 9. Butenoic acid(ER 001533), 10. Pyrimidine (Minoxidil sulphate), 11. Other (BMS 182264, BMS 180448) (147,151) (Figure1.9).

<u>1. Benzopyrans</u> (Cromakalim, Levromakalim) 	<u>2. Benzothiadiazine</u> (Diazoxide) 
<u>3. Cyanoguanidines</u> (Pinacidil) 	<u>4. Cyclobutenediones</u> (WAY-151616) 
<u>5. Tertiary Carbinols</u> (ZD-6169) 	<u>6. Thiformamides</u> (Aprikalim) 
<u>7. DHP- related Structures</u> (ZM-244085) 	<u>8. Nicotinamides</u> (Nicorandil, KRN 2391) 

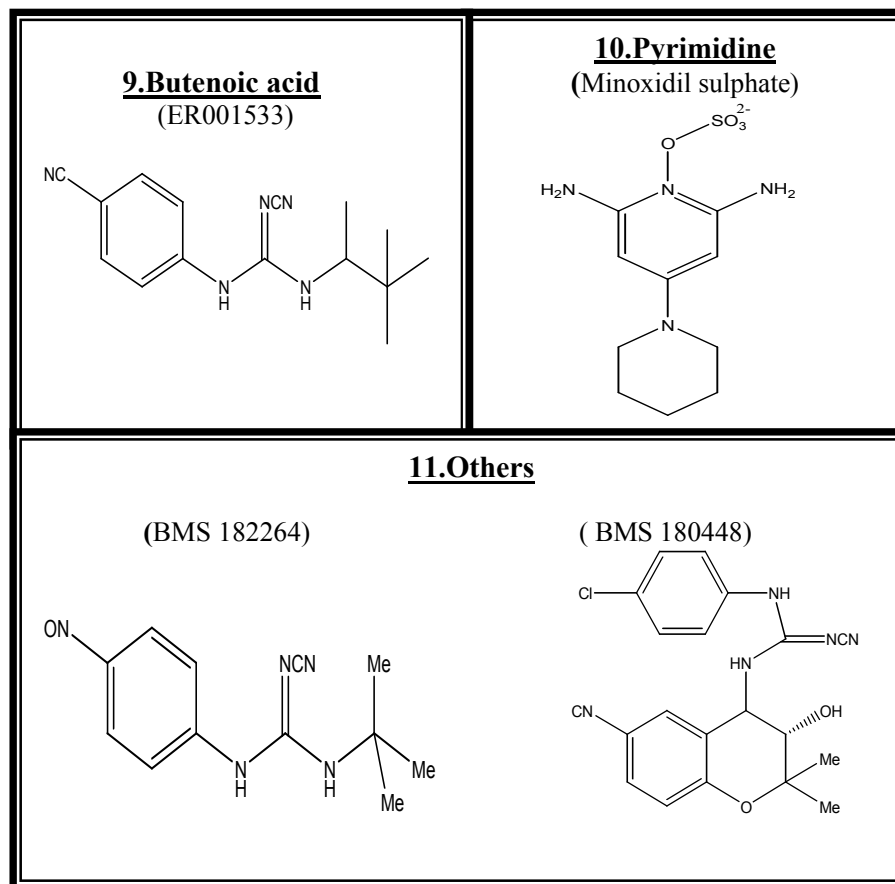


Figure 1.9. Classification of KCO based on chemical structure.

Potassium channel openers reduce the ATP induced channel inhibition so provide the opening of K_{ATP} channels. They promote binding of the Mg ADP to the nucleotide binding domain 2 of the SUR. So K_{ATP} channels are activated (152).

The K_{ATP} channel openers are used for therapy in a number of cardiac conditions; they protect myocardium in cardiopulmonary bypass (153, 154), preserve donor transplant heart (154, 155), treat ischemic heart disease (156-158), and decrease hypertension (159), prevent peripheral vascular disease (160) as well as arrhythmias related to abnormal depolarization (161, 162).

The K_{ATP} channel openers cause membrane hyperpolarization and so reduce Ca^{++} influx through the voltage gated Ca^{++} channels (163, 164). So potassium channel openers regulate the tone of the vascular smooth muscle. They are potent vasodilator (165, 166).

Potassium channel openers primarily vasodilate arteries and arterioles (159, 163). Nicorandil and KRN 2391 by means of a nitrate moiety also dilate venous capacitance in addition to arteries and arteriole vessels and reduce cardiac preload (157).

The $mitoK_{ATP}$ channels have approximately 2000 fold higher sensitivity to an opening by Diazoxide compared to $sarcK_{ATP}$ channels. Pinacidil opens both $mitoK_{ATP}$ and $sarcK_{ATP}$ channels (82, 142), (Table 1.2).

Table 1.2. Modulators of K_{ATP} channels and their sensitivity toward mitochondrial and sarcolemmal K_{ATP} channels

TYPE OF K_{ATP} MODULATORS	The site of action of the K_{ATP} modulators		
	$mitoK_{ATP}$	$mitoK_{ATP}$ and $sarcK_{ATP}$	$sarcK_{ATP}$
K_{ATP} Openers	Diazoxide Nicorandil BMS-180448 BMS-191095 Bepridil	Cromakalim Levcromakalim Bimakalim Pinacidil P-1060 Sildenafil Isoflurana EMD60480 Aprikalim BRL 34915 BRL 38227 RP 49356	P-1075 MCC-134
K_{ATP} Blockers	5-HD MCC-134	Glibenclamide Glimepride Tolbutamide Carbutamide	HMR1098 (HMR1883) HMR 1402 Bepridil

1.3.3.1. The Effects of the K_{ATP} Channel Openers in Ischemia and Reperfusion

1.3.3.1.1. The Effects of the K_{ATP} Channel Openers to Infarct Size

Several *in vivo* (139-141, 143, 144, 170, 173, 174, 179, 180) and *in vitro* (7, 167-172, 181-183) studies demonstrated that administration of K_{ATP} channel openers (KCO) preserve myocardial function and decrease infarct size. It was suggested that, the myocardial protection conferred by opening of the sarcK_{ATP} channels may be mediated by either decreased Ca⁺⁺ entry via L type calcium channel resulting from the shortening of the APD (Action potential duration) or inhibition of ischemic depolarization. The negative inotropy that occurs as a result of the decreased Ca⁺⁺ entry may protect the ischemic heart from more damage by preserving energy stores (4, 7, 181). Likewise it was demonstrated that calcium antagonist exert salutary effect on ischemia reperfusion injury (184, 185). On the other hand, some investigator suggests that cardioprotection may depend on the decrement in oxygen consumption of myocardium caused by the decreased afterload (168). In a limited number of *in vivo* studies it was reported that KCO failed to decrease the myocardial infarct size (175-178). Contradictory results were attributed to either the coronary steal as a result of the reflex tachycardia (176) or to surprising small infarct size in control animals that may depend on animal breed used in the studies or some other factors (178).

Yao Z. et al. (140) and Grover GJ. et al. (141) indicated that cardioprotective effects of Bimakalim are not correlated with the action potential shortening in the myocardial cells. In those studies authors reported that other unknown mechanism may be involved in the cardioprotective action of potassium channel openers rather than mechanisms mentioned above. McCullough JR. and his colleagues (186) demonstrated that antiischemic action of the cromakalim was blocked by 5-HD

(Nowadays, 5-HD is known as selective mitoK_{ATP} channel blocker). That study, however was performed before the discovery of mitoK_{ATP} channels in myocytes in 1992 (77). In line with these findings, in recent years Das B. et al. (143, 144) and Sato T. et al. (187) demonstrated that the cardioprotective effect of the KCO depend on the opening of the mitoK_{ATP} channels instead of the reduced cytosolic Ca⁺⁺ induced by the activation of the sarcK_{ATP} channels. Diazoxide (selective mitoK_{ATP} channel activator) that selectively activates the mitoK_{ATP} channels was reported to decrease the infarct size (101, 188). Diazoxide also decreased the apoptosis in a model of acute myocardial infarction (189). It is suggested that the cardioprotective actions of Diazoxide are mediated by generation of prooxidant environment (188, 189), as it was also demonstrated that K_{ATP} channel opener enhance the production of ROS in cardiac myocytes (190, 191).

1.3.3.1.2. The Effects of the K_{ATP} Channel Openers in I/R Arrhythmias

K_{ATP} channel openers were shown to suppress the arrhythmias in *in vitro* (192, 193) and *in vivo* studies (13, 17, 18, 19, 143, 144). It was suggested that antiarrhythmic activity of KCO is related to the vasodilator properties of the drug either through increasing blood flow to the ischemic myocardium or reducing arterial pressure and work load (17). It was also suggested that the reduction in action potential duration by KCO was thought to prevent intracellular Ca⁺⁺ overload which may be responsible for triggered activity and afterdepolarization (194, 195). KCO causes the action potential shortening of the myocardial cells even in the normally oxygenated myocardium (195,196). 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 (197). This inhomogeneity exists also within different areas of

the hypoxic tissues between less and more seriously injured myocardial fibers. It has been also suggested that KCO could further shorten the action potential duration of the slightly injured fibers in the heart during ischemia and reperfusion. This makes the injured myocardium more homogeneous with less ischemic myocardium and diminishes the development of life threatening ventricular arrhythmia (13, 18). In recent years, however, it was demonstrated that the antiarrhythmic effect of the KCO mainly depend on the opening of the mitoK_{ATP} channels instead of the shortening of APD (143, 144, 187).

There were reported the proarrhythmic effects of K_{ATP} channel openers both in *in vitro* and *in vivo* studies (10, 20, 21, 198-208). In some studies it was suggested that the proarrhythmic effect of the KCO was dependent on the activation of the cardiac sympathetic nerve and the release of noradrenalin induced by marked systemic hypotension (209). It has been also suggested that proarrhythmic effect of KCO may occur due to the reentrant type of arrhythmia generated by the shortening of the action potential (196). The rise in extracellular K⁺ concentration and reduction in action potential duration induced by KCO may mimic the reentrant circuit by local electrophysiological changes (210).

The reason of the discrepancy in the antiarrhythmic effect of KCO may also depend on different animal models and nonselective drugs used in the studies.

In recent year, in their *in vitro* studies Fiscbach PS. and his colleagues (207, 208) demonstrated that the proarrhythmic effect of the nonselective KCO depend on the activation of the sarcK_{ATP} channels. In the same line with this finding Bepridil that is an opener of mitoK_{ATP} channels and an inhibitor of sarcK_{ATP} channels was found to have cardioprotective effect on native cardiac myocytes (211).

1.3.4. The ATP Dependent Potassium Channel Blocker

Sulfonylurea derivatives are a class of antidiabetic drugs that are used in the management of diabetes mellitus type-2. They act by increasing insulin release from the beta cells in the pancreas.

Sulfonylureas bind to an ATP-dependent K^+ channel (K_{ATP} channel) on the cell membrane of pancreatic beta cells. This inhibits a tonic, hyperpolarizing outflux of potassium, which causes the electric potential over the membrane to become more positive. This depolarization opens voltage-gated Ca^{++} channels. The rise in intracellular calcium leads to increased fusion of insulin granulae with the cell membrane, and therefore increased secretion of (pro) insulin (212).

All sulfonylureas have a central phenyl ring with two branching chains. Drugs in this class are first generation; Acetohexamide, Chlorpropamide, Tolbutamide, Tolazamide, second generation; Glipizide, Gliclazide, Glibenclamide (glyburide) Glimepiride and Third generation; Glimepiride (213).

Cardioprotective effects of Tolbutamide, Carbutamide, Glibenclamide and Glimepiride were investigated in the animal experimental models (Figure.1.10).

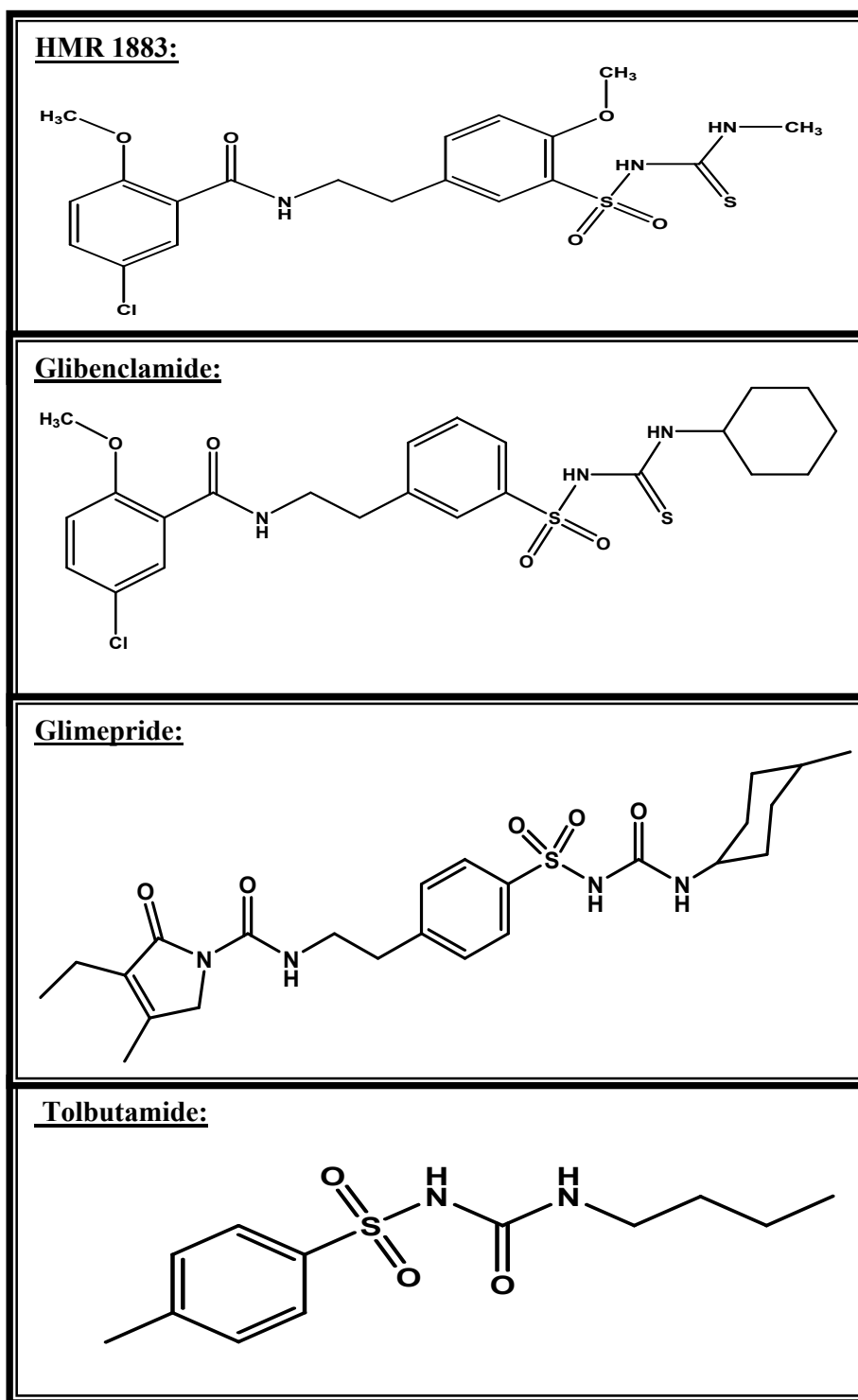


Figure 1.10. The chemical structures of some K_{ATP} channel blockers.

HMR 1883, (HMR 1098 is a sodium salt of the HMR 1883) is devoid of the vasoconstrictor action with no effect on blood glucose or insulin levels (214, 215). HMR 1098 is a selective sarcK_{ATP} channel blocker which does not block mitoK_{ATP} channel and 5-HD selectively blocks mitoK_{ATP} channel in ventricular myocytes (216, 217).

1.3.4.1. The Effects of the K_{ATP} Channel Blockers in Ischemia and Reperfusion

1.3.4.1.1. The Effects of the K_{ATP} Channel Blocker to Infarct Size

The blocking of the K_{ATP} channels during ischemia does not provide myocardial protection, even it may be also detrimental (218-220). It was suggested that the action potential lengthening as a consequence of sarcK_{ATP} channel inhibition leading to an increase in time of Ca⁺⁺ influx via L type Ca⁺⁺ channels and decrease time for extrusion of Ca⁺⁺ ions from the cell by the Na⁺-Ca⁺⁺ exchanger. Thus, the inhibition of the sarcK_{ATP} channel results in Ca⁺⁺ overload. The Ca⁺⁺ overload secondarily leads to oxyradical generation (86, 91). These result in injury of the myocardial cells (36, 218).

Recently, however, Pasdois P. et al. (221) reported that HMR 1098 (selective sarcK_{ATP} channel blocker) decrease the infarct size in isolated rat hearts. It is proposed that the mechanism of cardioprotection depends on extracellular calcium influx, the production of the ROS and mitoK_{ATP} channel activation. The data in this study suggests that a functional interaction between sarcK_{ATP} and mitoK_{ATP} channels exist in isolated rat heart ischemia model which is mediated by extracellular Ca⁺⁺ influx.

1.3.4.1.2. The Effects of the K_{ATP} Channel Blockers in I/R Arrhythmias

K_{ATP} channel blockers were shown to suppress the arrhythmias in *in vitro* (10, 20, 198, 220, 222, 223) and *in vivo* studies (9, 11, 12, 13, 23, 18, 214, 215, 224-229). In these studies the antiarrhythmic effect of the K_{ATP} channel blockers were attributed to the inhibition of the K⁺ loss from jeopardized cells and nonuniform shortening of the action potential duration during the progressive 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, antifibrillatory action. Apart from the inhibition of reentrant mechanisms based on the shortening of the action potential, K_{ATP} channel blockade may prevent extracellular potassium accumulation and thus the depolarizing effect of high extracellular potassium.

The proarrhythmic effect of K_{ATP} channel blockers was also reported in *in vitro* and *in vivo* studies (14, 15, 223, 224). The proarrhythmic effect of the K_{ATP} channel blockers was attributed to inhibitory effect of the K_{ATP} channel blocker on the coronary blood flow (10, 14, 232). This effect was also attributed to the worsening of the ischemia due to the myocardial Ca⁺⁺ overloading. The Ca⁺⁺ overload and oxyradicals generation induces ischemia and reperfusion arrhythmias (36-39).

It is unknown whether the controversial observations are due to differences in experimental protocols or to insufficient selectivity of the compounds. The unfavorable effects of nonselective K_{ATP} channel blocker on mitoK_{ATP} channel may oppose its antifibrillatory potential that arises from inhibition of sarcK_{ATP} channels.

Table 1.3. The selected studies that research the effect of the modulation of K_{ATP} channels on ischemia and reperfusion arrhythmias

Study	Animal	Method	Drug	Result
Kerr MJ. (1985)	Dog	Coronary artery ligation (<i>in vivo</i>)	Pinacidil	Antiarrhythmic
Pogatsa G. (1988)	Rat and Rabbit	Drug induced arrhythmias (<i>in vivo</i>)	Glibenclamide	Antiarrhythmic
			Tolbutamide	Proarrhythmic
			Carbutamide	Proarrhythmic
Wolleben CD. (1989)	Rat	Langendorff (30 min. low flow ischemia)	Glyburide	Antiarrhythmic
			Tolbutamide	Antiarrhythmic
			Pinacidil	Proarrhythmic
			BRL34915	Proarrhythmic
Chi L. (1989)	Dog	Coronary artery ligation (<i>in vivo</i>)	Pinacidil	Proarrhythmic
Chi L. (1990)	Dog	Coronary artery ligation (<i>in vivo</i>)	Pinacidil	Proarrhythmic
Kantor PF. (1990)	Rat	Langendorff (Global and regional ischemia)	Glyburide	Antiarrhythmic
Fish A. (1990)	Rabbit	Drug induced (<i>in vivo</i> anaesthetized rabbit)	Pinacidil	antiarrhythmic
	Dog	Drug induced arrhythmic activity in cardiac purkinje fibers (<i>in vitro</i>)		

Study	Animal	Method	Drug	Result
Spinelli W. (1991)	Dog	Drug induced abnormal automaticity in single ventricular myocytes (<i>in vitro</i>)	Pinacidil	Antiarrhythmic
Brill A. (1992)	Rat	Langendorff (Regional ischemia)	Glibenclamide	Proarrhythmic
Di'Diego JM. (1993)	Dog	Standard microelectrode techniques (<i>in vitro</i>)	Pinacidil	Proarrhythmic
			Glibenclamide	Antiarrhythmic
Delacoussaye JE. (1993)	Dog	Coronary artery ligation (<i>in vivo</i>)	BRL 38227, (the active enantiomer of cromakalim)	Proarrhythmic
Billman GE. (1993)	Dog	Coronary artery ligation (<i>in vivo</i>)	Glyburide	Antiarrhythmic
Tosaki A. (1993)	Rat	Langendorff (Normothermic Global ischemia)	Cromakalim	Proarrhythmic
			Glibenclamide	Antiarrhythmic
Smallwood JK. (1993)	Dog	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (90min. I/ 300 min. R)	Pinacidil	Proarrhythmic
			3-Pyridyl Pinacidil	Proarrhythmic
Fagbemi SO. (1993)	Rabbit	Langendorff (Global ischemia)	Pinacidil	Proarrhythmic
			Glibenclamide	Antiarrhythmic
Chi L. (1993)	Rabbit	Langendorff (Global hypoxia /Reoxygenation 12 min-40 min.)	Pinacidil	Proarrhythmic
			Glibenclamide	Antiarrhythmic

Study	Animal	Method	Drug	Result
Yao Z. (1994)	Dog	Coronary ligation 60 min. (<i>in vivo</i>)	Bimakalim	Proarrhythmic
Tosaki A. (1994)	Pig	Langendorff (Normothermic Global ischemia)	Glibenclamide	Antiarrhythmic
			Cromakalim	Proarrhythmic
Rees SA. and Curtis MJ. (1995)	Rat	Langendorff (Regional ischemia)	Glibenclamide	Antiarrhythmic
			RP 49356	No effect
Ferdinandy P. (1995)	Rat	Langendorff (Regional ischemia)	Cromakalim	Antiarrhythmic
			Glibenclamide	No effect
Friedrich GS. (1996)	Dog	Coronary ligation and programmed electrical stimulation (<i>in vivo</i>)	5-HD	No effect
Lepran I. (1996)	Rat	Conscious Coronary ligation (<i>in vivo</i>)	Glibenclamide	Antiarrhythmic
			Pinacidil	Antiarrhythmic
Bernauer W. (1997)	Rat	Langendorff (Regional ischemia)	Glibenclamide	Proarrhythmic
Vegh A. (1997)	Dog	Ischemia Reperfusion (I/R) arrhythmia model (<i>in vivo</i>) (25min. I/ 25 min. R)	Levcromakalim	No effect
Baczko I. (1997)	Rat	Ischemia Reperfusion (I/R) arrhythmia model (<i>in vivo</i>) (6min. I/ 5min. R)	Glibenclamide	Antiarrhythmic
			Pinacidil	Antiarrhythmic
			Cromakalim	Antiarrhythmic
Billman GE. (1998)	Dog	Exercise plus ischemia test (<i>in vivo</i>)	Glibenclamide	Antiarrhythmic
			HMR 1883	Antiarrhythmic

Study	Animal	Method	Drug	Result
El Reyani NE. (1999)	Rat	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (6min. I/ 5min. R)	Glibenclamide	Antiarrhythmic
			Glimepride	Antiarrhythmic
Wirth KJ. (1999)	Rat	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (6min. I/ 5min. R)	HMR1883	Antiarrhythmic
			Glibenclamide	Antiarrhythmic
Harbinson MT. (2000)	Pig	Endocardial stimulation induced fibrillation (<i>in vivo</i>)	Levcromakalim	No effect
			Glibenclamide	No effect
Fryer RM. (2000)	Rat	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (30min. I/ 30min. R)	HMR 1098	Antiarrhythmic
Bozdoğan Ö. (2000)	Rat	Conscious Coronary ligation (<i>in vivo</i>) (15 min.)	Glibenclamide	No effect
Vale HF. (2001)	Sheep	Conscious Coronary ligation (<i>in vivo</i>) (12min. I/ 120 min. R)	Glibenclamide	Proarrhythmic
Zhu BM. (2003)	Dog (Selected in both sex)	Programmed electrical stimulation (<i>in vivo</i>)	HMR 1098	Antiarrhythmic
			Glibenclamide	No effect

Study	Animal	Method	Drug	Result
Das B. (2003)	Rabbit	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (20min. I/ 30 min. R)	Cromakalim	Antiarrhythmic
			Nicorandil	Antiarrhythmic
			5-HD	No effect
Fiscbach PS. (2003)	Rabbit	Langendorff (Global hypoxia /Reoxygenation 12 min-40 min	Pinacidil	Proarrhythmic
Fiscbach PS. (2004)	Rabbit	Langendorff (Global hypoxia /Reoxygenation 12 min-40 min.)	Pinacidil	Proarrhythmic
			Cromakalim	Proarrhythmic
Billman GE (2004)	Dog	Exercise+Ischemia test (<i>in vivo</i>)	HMR 1402	Antiarrhythmic
Gonca E. (2004)	Rat (Respectively in male and female)	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (6min. I/ 6 min. R)	Glibenclamide (In males)	Antiarrhythmic
			Glibenclamide (In female)	No effect
Bozdogan Ö. (2005)	Rat	Ischemia Reperfusion arrhythmia model (<i>in vivo</i>) (6min. I/ 6 min. R)	Pinacidil	Antiarrhythmic
Gok S. (2006)	Rat	Langendorff (Regional ischemia)	5-HD	No effect
			HMR 1098	No effect
Vajda S. (2007)	Rat	Ischemia Reperfusion arrhythmia model and Conscious Coronary ligation (<i>in vivo</i>) (6min. I/ 6 min. R) (15 min. I)	5-HD	No effect
			HMR 1098	Antiarrhythmic
			Glibenclamide	Antiarrhythmic

1.4 Sex Dependent Biochemical and Cellular Differences in Heart Structure and Its Relation to Ischemia and Reperfusion

1.4.1 Sex Dependent Biochemical and Cellular Differences in Heart Structure

Some cellular structures and biochemical characteristics of heart are affected by gonadal hormones. It has been shown that the density of the sarcolemmal K_{ATP} channels was higher in females than males (22). In this study the potassium current that is evoked by K_{ATP} channel opener Pinacidil was found to be higher in females than males. This means that there are more functional cardiac sarc K_{ATP} channel in females than males. The activation of K_{ATP} channels is an important part of endogenous cardioprotective mechanism that promotes cellular survival under the metabolic stress condition (22). The numbers of sarc K_{ATP} channels in myocardial cells decrease with aging in females, but not in males (233). The loss of sarc K_{ATP} channel with aging makes female heart more sensitive to ischemia. This may dependent on decreasing in concentration of circulating estrogen with aging. Estrogen specifically increase expression of SUR 2A, thereby upregulate the number of sarc K_{ATP} channels (26). it has been demonstrated that female hearts are more resistant to I/R injury than males and it has been proposed that sex differences in the susceptibility to infarct size may be related to increased expression of sarc K_{ATP} channels (234). The numbers of these channels are also altered in diabetes, and hence; preconditioning does not develop in diabetic hearts (235).

There are several reports showing that both estrogen and androgen rapidly inhibit various cardiac ionic currents discussed by Farukawa T. et al. (236) and causes the gender related differences in ventricular depolarization (237).

It was reported that the release of endothelial NO is greater in female than in male and ovariectomy eliminate this difference (238). The increase in basal nitric

oxide synthase (NOS) levels can lead to an increase in baseline NO generation in females (239). In the absence of estrogen, plasma levels of NO metabolites such as nitrites and nitrates reduced (240). The protective effect of the NO during ischemia and reperfusion has been shown in several studies (241-243). NO reduce myocardial oxygen consumption and produce negative inotropic effect (243). Pabla R. et al. (243) showed that NO improve myocardial perfusion by mediating rapid recovery of coronary blood flow following coronary ligation. There are also reported gender differences in the effects of estradiol on basal and angiotensin-2 induced protein kinase C (PKC) activity (244). It has been found that the lowering of estrogen produce an increase in the number of cardiac L-type Ca^{++} channel and lead to the abnormalities in cardiac excitability and increased risk of arrhythmia (245). An increased in NOS activity and NO in females causes increased S-nitrosylation of the L-type Ca^{++} channels which result in less Ca^{++} loading during ischemia (246).

An increase in NO involve in cardioprotection via activation of protein kinase G which leads to opening of $\text{mitoK}_{\text{ATP}}$ channels (247). An increase in NO also results in increased S-nitrosylation of complex I of mitochondria (248). It is suggested that this might alter ROS generation during ischemia and reperfusion. Likewise NO is also reported to reduce mitochondrial ROS generation (249). The decrement in ROS production and Ca^{++} overload may decrease MPTP opening in reperfusion.

Inhibition of the norepinephrine (NE) release from the sympathetic nerve via presynaptic alpha two adrenoreceptors is more potent in females than males under both normal and acute ischemic condition (250). This study suggests that female hearts give less pronounced response to sympathetic nerve stimulation than the males (250). Estrogen deficiency caused by ovariectomy produced a significant increase in NE release in the heart (251). In addition to this it was reported that estrogen specifically potentiates parasympathetic activity on the heart (252). In another study

that was compatible with the finding mentioned above, heart rate significantly increases in male but decrease in female during the coronary ligation. In later phase of the ligation, arterial pressure showed significant recovery in males. After the vagotomy, this difference abolished and blood pressure and heart rate was found higher in females as in males (253).

1.4.2. Sex Dependent Response to Ischemia and Reperfusion

The rate of coronary heart disease in women before menopause is much lower than that in the same age of men (254). After the menopause the risk of the heart disease increases rapidly to levels comparable to that of their male counterparts. It has been thought that the female sex hormones may play a role in this cardioprotection. The major mechanism responsible for the protective effect in female is believed to be due to the antiatherogenic action of female sex hormone (255). Beside the antiatherogenic action, investigators have been suggested the myocardial cells are target for direct effects of estrogen (246).

There are various studies that investigate the effect of sex on myocardial ischemia-reperfusion injury and arrhythmias in different species and experimental model. Some researchers found no effect of sex difference (256-258) on infarct size but others found decrease in infarct size in female (234, 259). Some investigators have shown a reduction in ischemia and ischemia-reperfusion induced arrhythmia in female compared to male (19, 251, 252). Others found no sex difference in ischemia reperfusion induced ventricular arrhythmias (257, 258, 261, 262). This gender difference has frequently been attributed to cardioprotective effect of the ovarian hormones (263). Endogenous estrogen plays cardioprotective role in ischemia reperfusion injury in female (264, 265). This protection is mediated by the improving Nitric oxide (NO) release. Likewise the release of endothelial NO is greater in female than in male and ovariectomy eliminate this difference (266-268). The protective

effects of the NO during ischemia and reperfusion has been shown in several studies (269, 270). Others also suggest that this protection is mediated by attenuating myocardial Ca^{++} accumulation and preserving mitochondrial function (264, 271). The lowering of estrogen produce an increase in the number of cardiac L-type Ca^{++} channels and leads to the abnormalities in cardiac excitability and increased risk of arrhythmia (272). In some other research, however, it has been also reported that ovariectomy did not change the reperfusion arrhythmias (261, 273).

The exogenous estrogen has been shown to be protective against ischemia and reperfusion injury and arrhythmias in ovariectomized female hearts (263-265, 274). Only one study reported that neither estrogen withdrawal nor replacement affects the ischemia reperfusion arrhythmias and infarct size in rats (258). In a recent study, there was not found significant effect of ovariectomy on ischemia reperfusion arrhythmias in conscious ischemia reperfusion rat models (261)

The exogenous estrogen has been also shown to be protective against ischemia and reperfusion injury when it is given to unovariectomized females (25, 242, 259, 262, 276-278). This protection was attributed to NO and opening of Ca^{++} activated K^{+} channels (K_{Ca} channels) (242). Estrogen specifically has antiarrhythmic action possibly by inhibiting L-type Ca^{++} channel activity (279). This research suggests that, underlying antiarrhythmic mechanism of the estrogen appears to be due to the inhibitory effect of estrogen on the afterdepolarization. It has been also suggested that estrogen might attenuate reperfusion induced arrhythmias by virtue of its antioxidant properties (280).

In recent years the researchers have been investigated the relation of the protective effect of exogenously given estrogen with K_{ATP} channel subtypes. The studies in dogs reported that the antiarrhythmic and antiischemic effect of estrogen is mediated respectively by the opening of the $\text{sarcK}_{\text{ATP}}$ channels and the $\text{mitoK}_{\text{ATP}}$

channels (25, 259). Das B. et al. (277) suggested that both antiischemic and antiarrhythmic effects of the estrogen are mediated by the activation of the mitoK_{ATP} channel in rabbits. Testosterone was also found antiarrhythmic as like estrogen (262). Orchidectomy increases the ischemia reperfusion arrhythmias (261). The mitoK_{ATP} channel may also play a role in the protective effects of testosterone in ischemia reperfusion induced myocardial injuries (281).

2. MATERIAL AND METHOD

2.1. Animals

143 Male Sprague-Dawley, 7-8 months old rats in both sex that male weighing between 250-310 grams and female weighing between 200-250 grams were used. Animals were fed a standard laboratory rat feed pellet and allowed to drink tap water ad libitum. All animals were treated in adherence to all of the guiding principles in the care and use of animals together with the recommendation from the Declaration of Helsinki.

2.2. Coronary Artery Ligation and Reperfusion

Rats were anaesthetized with thiopental sodium (100 mg/kg) and tracheotomy was applied for artificial respiration. The left carotid artery was cannulated to measure blood pressure with a blood pressure transducer (Power Lab, AD Instruments, United Kingdom). Carotid arteries was cannulated by catheter that was filled with saline including heparin (100 IU/ml). Systolic and diastolic oscillations in arterial blood pressure were monitored from the left carotid artery and displayed on computer screen with a standard lead-1 electrocardiogram. The chest was opened by left thoracotomy, followed by sectioning of the fourth and fifth ribs. After the pericardium was incised, the heart eased out of the chest by gentle pressure on the rib cage. A 5/0 silk suture was placed around the arteria descendence branch of the left coronary artery (LAD), approximately 2 mm from its origin. The heart was replaced in the chest and artificial respiration was applied using a ventilator (Ugo Basile Rodent Ventilator, Italy), that set in air 0.9 ml/100 grams body weight at a rate of 60 strokes/minute. The animals were allowed to stabilize for five minutes before coronary artery ligation was applied. Animals having arrhythmias during this surgical procedure or having sustained decrease in mean arterial blood pressure

below 70 mmHg was discarded. Ligation was made by tightening the silk by producing a bowknot and reperfusion was produced by pulling the silk through the bowknot. The six minutes of ischemia was followed by six minutes of reperfusion.

Two ml. of intracardiac blood sample for biochemical analysis was taken at the end of the reperfusion from the survived females and ovariectomized females. Centrifugation was applied at 10 000 rpm for 30 minutes at 4 °C. Plasma sample was separated from the blood cells and stored in deep freeze (-80 °C) for later biochemical analysis.

The heart was excised out. The left coronary artery was retightened and the heart was first perfused with ten milliliters isotonic sodium chloride (NaCl) solution retrogradely, then two milliliters ethanol for demarcation of the occluded and nonoccluded myocardium. The nonperfused area that remained red was separated from the well perfused area that seemed white in color. The occluded and non-occluded myocardium were cut, separated from each other and weighed. The percentage of occluded ventricular myocardium at risk of infarction to the whole ventricular myocardium was calculated.

2.3. Ovariectomy

One group of animals was ovariectomized to remove the source of estrogen first in this operation before coronary artery ligation (n: 48). Firstly, the rats were anesthetized with ether. The skin was prepared, and a lateral abdominal incision was made. The ovaries were isolated and removed along with the oviduct. The abdomen and skin were then closed with continuous suture. The animals were monitored in the laboratory until full recovery from anesthesia and were then put back to the animal care, where they were observed daily. Ovariectomized animals were used for coronary ligation after two to four weeks of operation.

One group of female rats was sham operated to see only the effect of operation on ischemia-reperfusion induced arrhythmias (n: 7). The anesthesia and the operation procedure were the same as for the ovariectomized group except that the ovaries were exposed but not removed.

2.4. Hormone Measurement

Plasma estradiol hormone levels were measured in both control and operated females using commercial ELISA kits (Caymen, USA) according to the manufacturer's instructions.

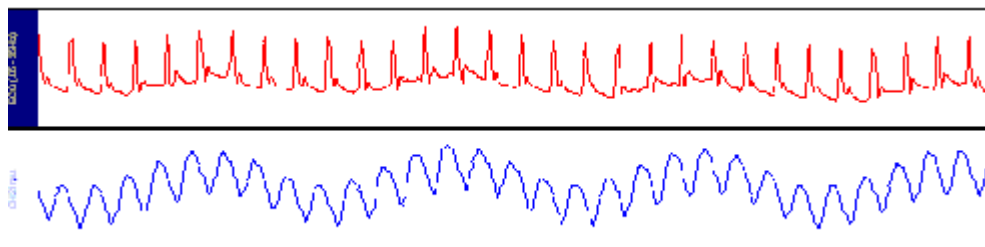
2.5. Drug Applications

The doses of drugs used in the present study were those reported to produce satisfactory inhibition or activation of mitoK_{ATP} and sarcK_{ATP} channels in rats (131, 138, 18, 230, 282, 283). Drugs were kept in dark containers to protect from light induced decomposition. Drug containing solutions were prepared daily. All drugs were given intravenously from coccygeal tail vein. Pinacidil (Nonselective K_{ATP} channel opener) was first dissolved in ethanol and then mixed with saline in 1/1 ratio. It was given in a dose of 0.1mg /kg/100 µl, 5 min. before coronary artery ligation. Diazoxide (selective mitoK_{ATP} channel opener) was first dissolved in DMSO (Dimethyl sulfoxide) and then mixed with saline in 1/1 ratio. It was given in a dose of 10 mg /kg/100 µl, 10 min. before coronary ligation. HMR 1098 (the sodium salt, 1-[5-[2-(5-chloro-*o*-anisomido) ethyl]-2-methoxyphenyl] sulfonyl-3-methylthiourea), (Selective sarcK_{ATP} channel blocker) (generous gift from Sanofi Aventis, Deutschland) and 5-HD (5-hydroxydecanoate), (Selective mitoK_{ATP} channel blocker) were dissolved in saline solution. HMR 1098 was given in a dose of 3 mg /kg/100 µl and 5-HD was given in a dose of 10 mg /kg/100 µl, 5 min. before coronary artery ligation.

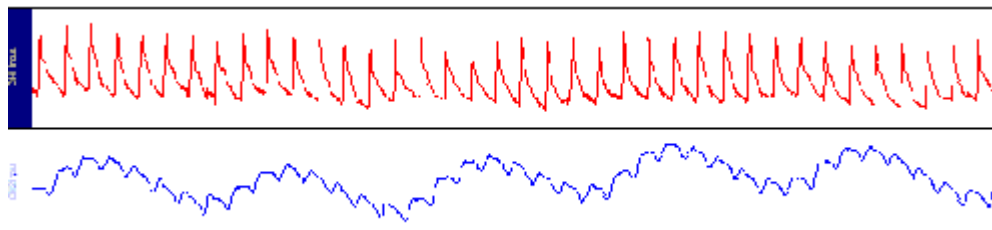
2.6. Recording and Evaluation

Bipolar electrocardiogram (ECG) was recorded using needle electrodes, and blood pressure measured from the carotid artery by a blood pressure transducer (AD Instruments, United Kingdom) and stored in a computer using the Biopac System. Arrhythmia duration and heart rate were determined by the ECG.

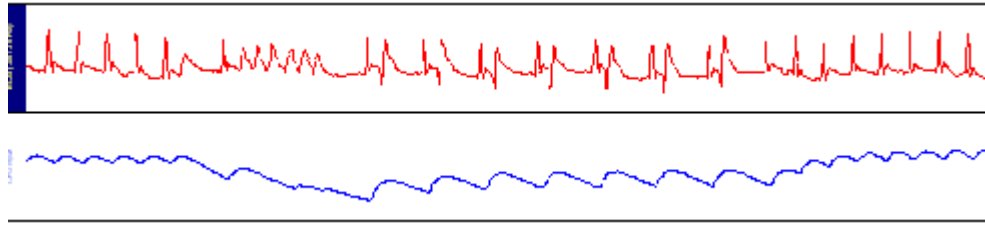
In recorded ECG, the heart rate and the blood pressure at 1, 3, 5 min. of the ischemia and reperfusion were determined. The incidence and the total length of the arrhythmias (sec) were recorded during six min. of the ischemia and reperfusion. The time of the beginning and the end of arrhythmias were determined. The arrhythmias were classified as ventricular fibrillation (VF), ventricular tachycardia (VT), ventricular premature contraction (VPC) according to Lambeth convention (284). The arrhythmias are designated as sinus tachycardia in the heart rate of 500 to 600, as ventricular tachycardia in the heart rate of 600/min and abrupt decrease in pressure to very low value below 40 mmHg and as ventricular fibrillation in an abrupt decrease in pressure to value below 20 mmHg, Figure 2.1.



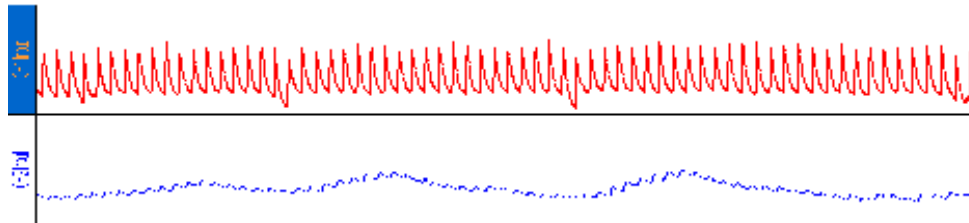
A.) Sinus rhythm



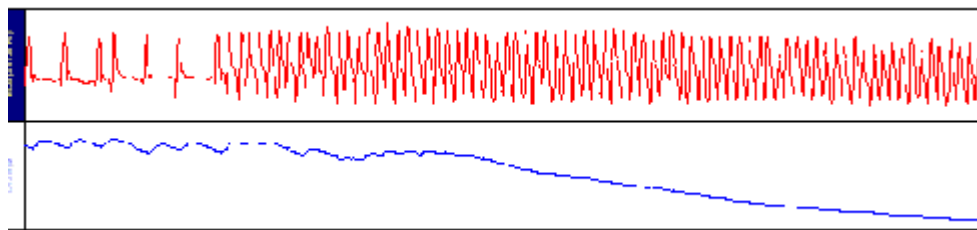
B) ST segment elevation



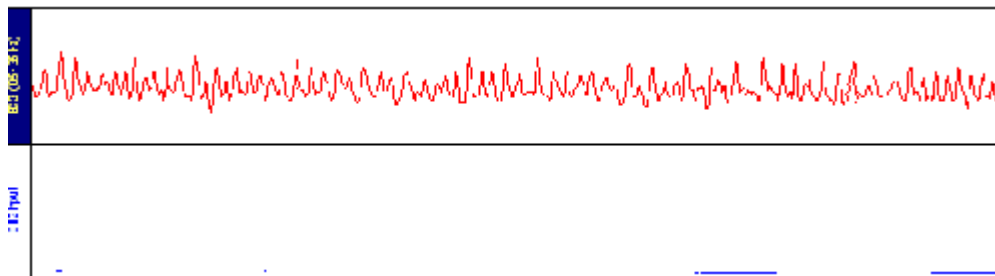
C) Ventricular premature contraction



D) Sinusal tachycardia



E) Ventricular tachycardia



F) Ventricular fibrillation

Figure 2.1. ECG recorded from rats: a) Sinusal rhythm, b) ST segment elevation, c) Ventricular premature contraction, d) Sinusal tachycardia, e) Ventricular tachycardia, f) Ventricular fibrillation

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

2.7. Statistical Analyses

Data were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., USA, and Ver. 11.0). The mean and standard errors were determined for all parameters including heart rate, blood pressure and hormone levels. The duration of arrhythmias and hormone levels were compared by the one way analysis of variance (One way ANOVA combined with the LSD post hoc. test). The survival rate and the incidence of arrhythmia were compared by the chi-square test (Fisher exact test, two tailed). Values were considered statistically significant at $P < 0.05$. Data were presented as mean $\pm$ standard error ($M \pm SE$).

2.8. Groups of Studies

Studies have been made in three groups; male, female and sham operated ovariectomized female rats. But comparison of groups has been done between controls and between drug treated animals. The effect of each drug in female, male and ovariectomized female groups of animals has been evaluated individually.

2.8.1. The Effect of the Estrogen and Sex on Ischemia Reperfusion Induced Arrhythmias

The effect of estrogen and sex differences on arrhythmias was evaluated by the comparison with control animals. These controls are as follows.

- a. Sham operated female (n: 7)

- b. Female (n: 7)
- c. Ovariectomized female (n: 9)
- d. Male(n: 9)

2.8.2. The Effect of K_{ATP} Channel Modulators on Ischemia Reperfusion Induced Arrhythmias in Male

These subgroups of animals were designed to clarify the effect sarc K_{ATP} and mito K_{ATP} channel modulation on ischemia reperfusion induced arrhythmias in males. 44 male Sprague-Dawley rats were used in this group. The groups of animals in this comparison are as follows.

- a. Control (n: 6)
- b. Pinacidil (n: 6)
- c. Pinacidil + HMR 1098 (n: 6)
- d. HMR 1098 (n: 6)
- e. 5-HD (n: 8)
- f. Pinacidil + 5-HD (n: 6)
- g. Diazoxide (n: 6)

2.8.3. The Effect of K_{ATP} Modulators on Ischemia Reperfusion Induced Arrhythmias in Female

These subgroups of animals were designed to clarify the effect of sarc K_{ATP} and mito K_{ATP} channel modulation on ischemia reperfusion induced arrhythmias in female. 44 female Sprague-Dawley rats were used in this group. The groups of animals in this comparison are as follows.

- a. Control (n: 7)
- b. Pinacidil (n: 6)
- c. Pinacidil + HMR 1098 (n: 6)
- d. HMR 1098 (n: 6)
- e. 5-HD (n: 6)

- f. Pinacidil + 5-HD (n: 7)
- g. Diazoxide(n: 6)

2.8.4. The Effect of K_{ATP} Modulators on Ischemia Reperfusion Induced

Arrhythmias in Ovariectomized Female

These subgroups of animals were designed to clarify the effect sarc K_{ATP} and mito K_{ATP} channel modulation on ischemia reperfusion induced arrhythmias in ovariectomized female. 55 female Sprague-Dawley rats were used in this group. The groups of animals in this comparison are as follows.

- a. Sham operated (n: 7)
- b. Ovariectomized control (n: 9)
- c. Pinacidil (n: 7)
- d. Pinacidil+ HMR 1098 (n: 6)
- e. HMR 1098 (n: 7)
- f. 5-HD (n: 6)
- g. Pinacidil + 5-HD (n: 6)
- h. Diazoxide (n: 7)

3. RESULTS

ST segment elevation and QRS changes were observed in all animals following the ligation, Figure 2.1. These changes were not seen in animals having unsuccessful ligation. Blood pressure decreased immediately after the ligation in all groups. Following the reperfusion, the blood pressure gradually increased and was closed to basal value observed before ligation. The size of myocardium perfused by occluded coronary artery to total ventricular size was not different among groups.

3.1. THE EFFECT OF THE ESTROGEN AND SEX ON ISCHEMIA

REPERFUSION INDUCED ARRHYTHMIAS:

Plasma estrogen level:

The plasma level of the estrogen in ovariectomized control group was significantly lesser than female control and sham operated control groups (Table 3.1.1).

Table 3.1.1. The Plasma Estrogen Levels on the Day of Study

Groups	N	Plasma estrogen level (pg/ml)
Female Control	8	47±6
Sham Operated Female	10	59±12
Ovariectomized Control	11	22±4*

Data are means±SE

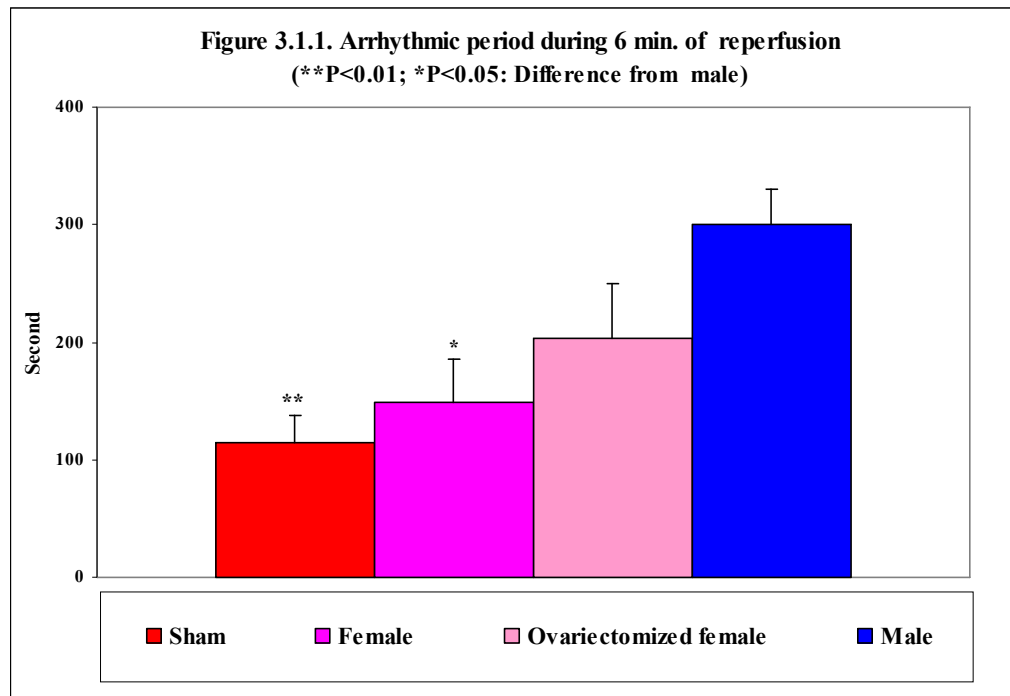
N: The number of the plasma sample.

***P<0.05:** Compared to female control and sham operated female group.

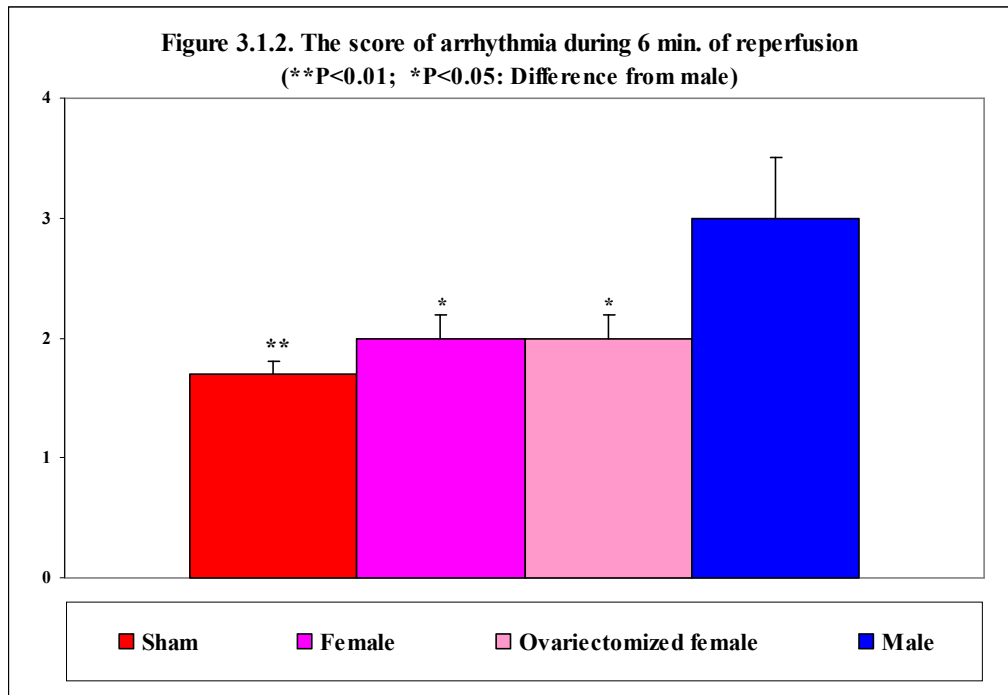
Blood pressure decreased immediately after the ligation in all groups. There was no difference between control groups in blood pressure and heart rate after coronary ligation (Table 3.1.2). No such differences were observed following the reperfusion too (Table 3.1.3).

Arrhythmias usually appeared in between 0-5 sec. following the reperfusion, only exception was unoperated female control group ($P<0.05$), (Table 3.1.4). The incidence of arrhythmias did not differ among groups (Table 3.1.5).

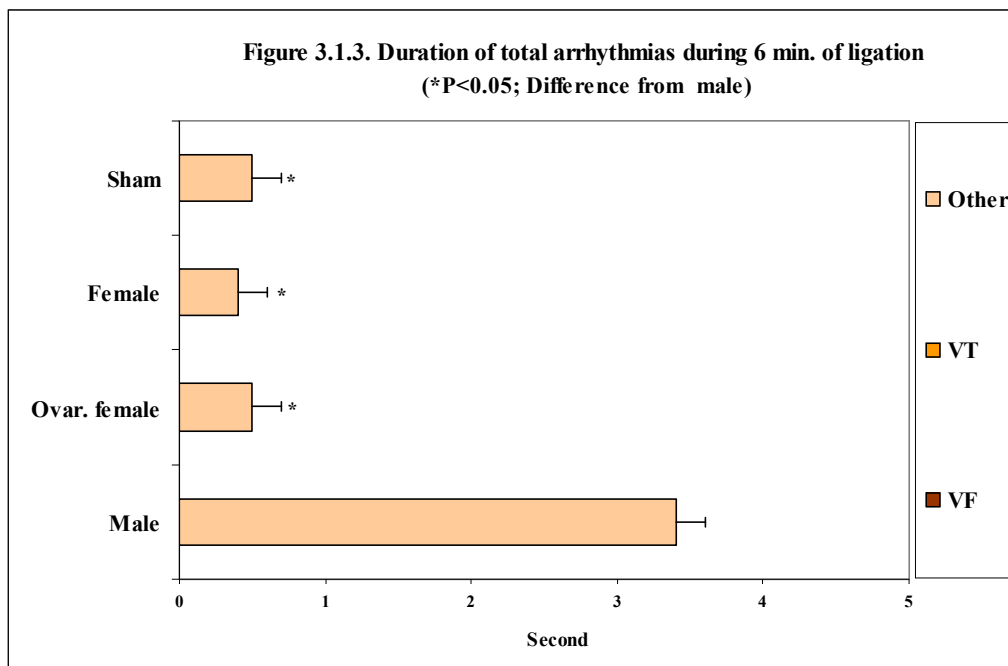
The length of arrhythmic period significantly decreased in sham operated female ($P<0.01$) and female group ($P<0.05$) as it compared to male group (Table 3.1.4), (Figure 3.1.1).



Arrhythmia scores was significantly lower in sham operated female ($P<0.01$), female and ovariectomized female groups ($P<0.05$) as it compared to male group during 6 min. of reperfusion (Table 3.1.5), (Figure 3.1.2).

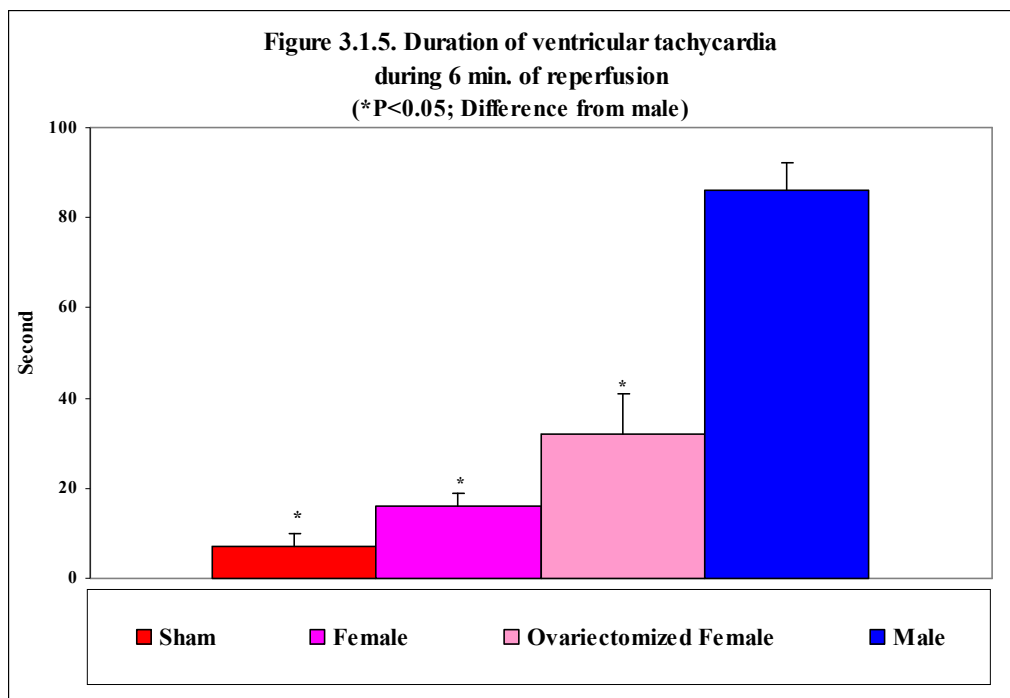
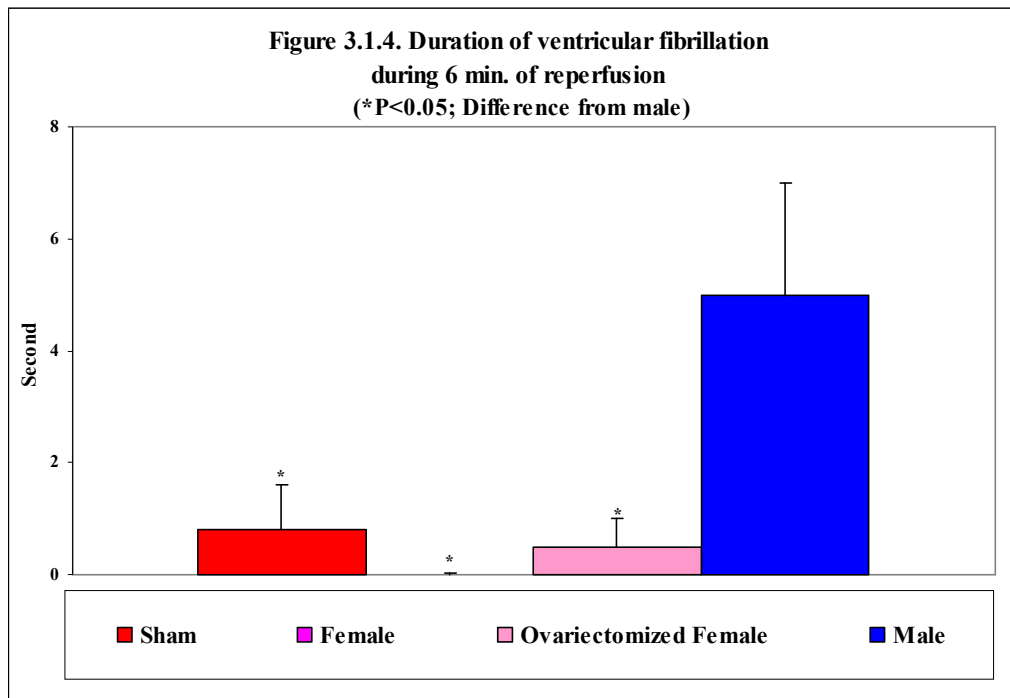


The duration of total and other type of arrhythmias during the ligation was lower in sham operated female, female and ovariectomized female groups as it compared to male group (*P<0.05), (Table 3.1.6), (Figure 3.1.3).



The durations of VF (Figure 3.1.4), VT (Figure 3.1.5) and total length of arrhythmias (Figure 3.1.6) during 6 min of reperfusion are lower in sham operated

female, female and ovariectomized female groups as it compared to male group ($P<0.05$), (Table 3.1.4).



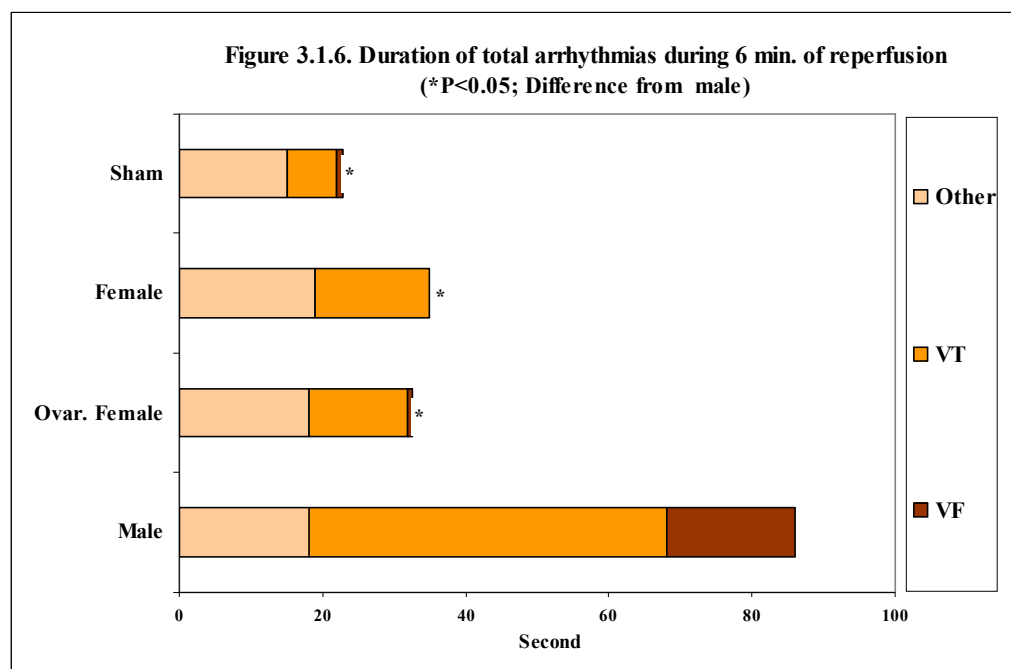


Table 3.1.2. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of coronary artery ligation (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat / minutes)				Blood pressure (mmHg)			
		Basal	01	03	05	Basal	01	03	05
Sham operated female	7	432 $\pm$ 16	422 $\pm$ 20	413 $\pm$ 11	407 $\pm$ 19	116 $\pm$ 10	94 $\pm$ 7	93 $\pm$ 9	98 $\pm$ 8
Female	7	407 $\pm$ 17	420 $\pm$ 14	419 $\pm$ 15	419 $\pm$ 18	116 $\pm$ 8	107 $\pm$ 27	113 $\pm$ 10	117 $\pm$ 9
Ovariectomized female	9	410 $\pm$ 22	399 $\pm$ 16	373 $\pm$ 26	369 $\pm$ 26	124 $\pm$ 8	107 $\pm$ 8	106 $\pm$ 10	106 $\pm$ 10
Male	6	387 $\pm$ 17	388 $\pm$ 16	381 $\pm$ 25	375 $\pm$ 26	108 $\pm$ 5	97 $\pm$ 4	102 $\pm$ 5	104 $\pm$ 5

N: The number of survived animals after 6 min. of coronary artery ligation.

Basal: Before coronary ligation

01, 03, 05: Respectively 1, 3 and 5 minutes after the coronary ligation and reperfusion.

Table 3.1.3. The heart Rate (HR) and mean arterial blood pressure (MBP) during 6 min. of reperfusion (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat / min.)			Blood pressure (mmHg)		
		01	03	05	01	03	05
Sham operated female	7	388 $\pm$ 26	414 $\pm$ 14	419 $\pm$ 10	86 $\pm$ 10	104 $\pm$ 6	112 $\pm$ 8
Female	7	444 $\pm$ 30	403 $\pm$ 16	419 $\pm$ 16	98 $\pm$ 12	116 $\pm$ 8	117 $\pm$ 7
Ovariectomized female	9	377 $\pm$ 25	405 $\pm$ 16	398 $\pm$ 17	99 $\pm$ 11	108 $\pm$ 11	99 $\pm$ 3
Male	6	370 $\pm$ 22	390 $\pm$ 34	422 $\pm$ 43	78 $\pm$ 14	97 $\pm$ 9	94 $\pm$ 6

N: The number of survived animals after 6 min. of reperfusion.

Table 3.1.4. The Duration of Arrhythmias during 6 min. of Reperfusion (Results: Mean $\pm$ SE).

Groups	N	Onset of arrhythmia (second)	Arrhythmic period (second)	Length of arrhythmia (second)				
				VF	VT	Other	Total	Bradycardia
Sham operated female	7	2 $\pm$ 2 [♥]	114 $\pm$ 25**	0.8 $\pm$ 0.8*	7 $\pm$ 3*	15 $\pm$ 2	23 $\pm$ 5*	0
Female	7	27 $\pm$ 13	149 $\pm$ 36*	0*	16 $\pm$ 3*	18 $\pm$ 4	35 $\pm$ 6*	0
Ovariectomized female	9	0 $\pm$ 0 [♥]	204 $\pm$ 46	0.5 $\pm$ 0.5*	14 $\pm$ 10*	18 $\pm$ 4	32 $\pm$ 12*	0
Male	6	2 $\pm$ 2 [♥]	300 $\pm$ 31	18 $\pm$ 14	50 $\pm$ 22	18 $\pm$ 2	86 $\pm$ 34	0

N: The number of survived animals after 6 min. of reperfusion.

VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Ventricular extrasystole, Bigemini and salvo.

*P<0.05; **P<0.01: Compared to male

[♥]P<0.05: Compared to female.

Table 3.1.5. The Incidence of arrhythmias during 6 min. of reperfusion (Results: Mean $\pm$ SE).

Groups	N	Survival (n / %)	Incidence of arrhythmia (N / %)				Arrhythmia score
			VF	VT	Other	Bradycardia	
Sham operated female	7	7/100	1/14	5/71	7/100	0	1.7 $\pm$ 0.1**
Female	7	7/100	0/0	7/100	7/100	0	2 $\pm$ 0.2*
Ovariectomized female	9	9/100	1/11	6/66	7/100	0	2 $\pm$ 0.2*
Male	6	6/100	3/50	6/100	6/100	0	3 $\pm$ 0.5

N: The number of animals at the onset of ligation.

n: The number of survived animals after 6 min. of reperfusion.

N: The number of animals in which arrhythmias observed.

*P<0.05; **P<0.01: Compared to male.

Table 3.1.6. The duration of arrhythmias during 6 min. of coronary ligation (Results: Mean $\pm$ SE).

Groups	N	Length of arrhythmia (s)				
		VF	VT	Other	Total	Bradycardia
Sham operated female	7	0	0	0.5 $\pm$ 0.2*	0.5 $\pm$ 0.3*	0
Female	7	0	0	0.4 $\pm$ 0.1*	0.4 $\pm$ 0.1*	0
Ovariectomized female	9	0	0	0.5 $\pm$ 0.4*	0.5 $\pm$ 0.4*	0
Male	6	0	0	3.4 $\pm$ 1.8	3.4 $\pm$ 1.8	0

N: The number of survived animals after 6 min. of ligation.

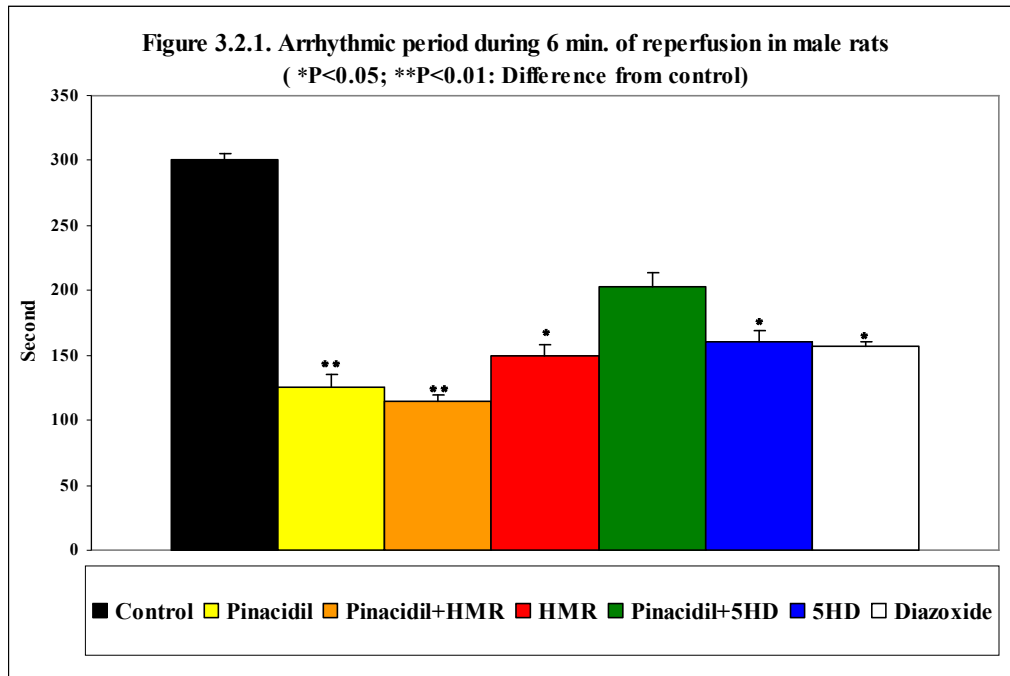
*P<0.05: Compared to male.

3.2. THE EFFECT OF K_{ATP} CHANNEL MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN MALES

No difference in blood pressure was noted before and during 6 min. of coronary ligation in these groups of animals (Table 3.2.1). Heart rate in Pinacidil and Pinacidil+HMR groups was higher than that in control before and during ligation. (Table 3.2.1). The blood pressure at 5th min. of reperfusion was significantly higher in Pinacidil+HMR as it compared to control group (Table 3.2.2). Heart rate was higher in Pinacidil+5-HD and Pinacidil+HMR groups according to control at 1st min. of reperfusion (Table 3.2.2).

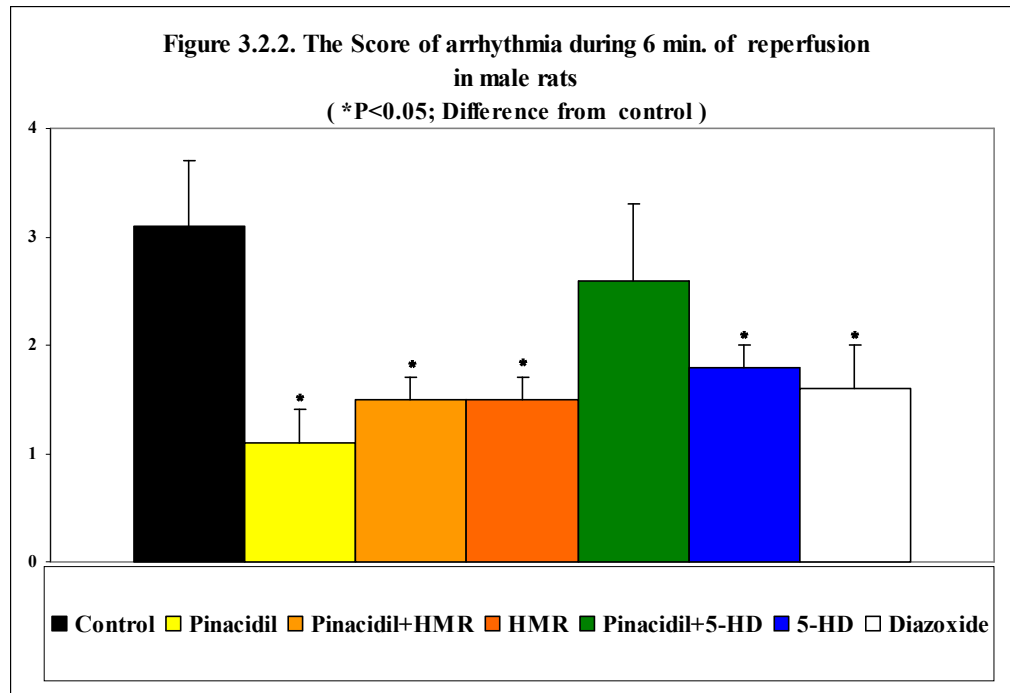
Arrhythmias usually appeared in between 5-10 sec. following the reperfusion. But it was delayed in Pinacidil group ($P<0.05$), (Table 3.2.3).

The length of arrhythmic period decreased in all groups according to control; Pinacidil ($P<0.01$), Pinacidil+HMR ($P<0.01$), HMR ($P<0.05$), 5-HD ($P<0.05$) and Diazoxide group ($P<0.05$). Only exception was the Pinacidil+5-HD group that arrhythmic period was not different with respect to control (Figure 3.2.1), (Table 3.2.3).



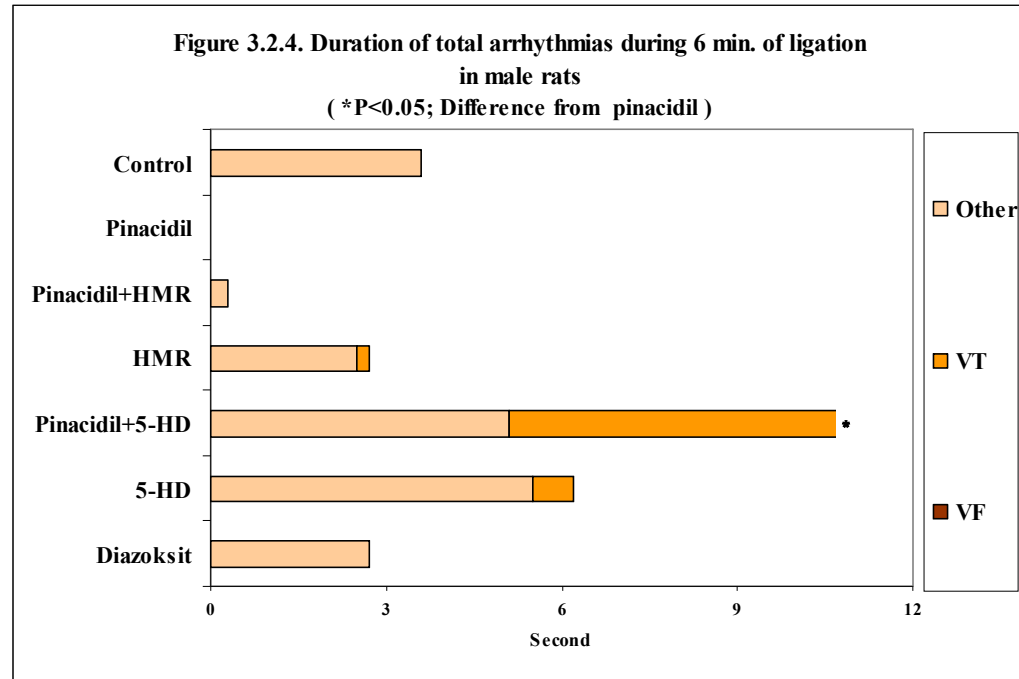
The incidence of VT was lower in Pinacidil group than that in control (Table 3.2.4).

Arrhythmia scores in reperfusion were significantly lower in Pinacidil, Pinacidil+HMR, HMR, 5-HD and Diazoxide groups according to control. On the other hand, combination of 5-HD with Pinacidil decreased the antiarrhythmic effect of Pinacidil ($P<0.05$), (Figure 3.2.2), (Table 3.2.4).

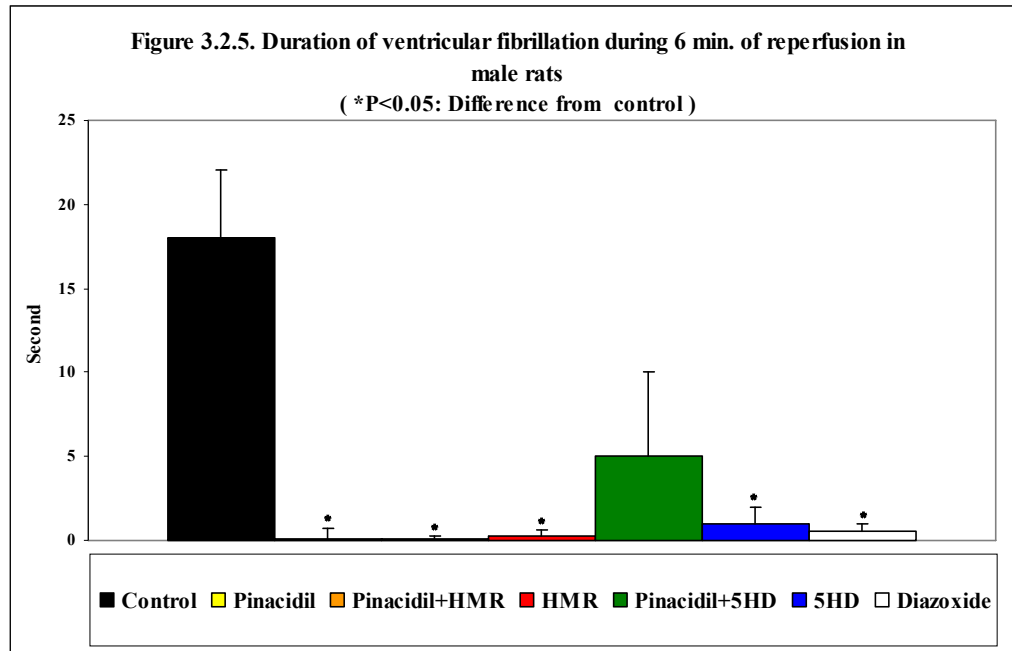


The duration of the VPC during the coronary artery ligation was not different among groups. The duration of VT during the coronary artery ligation was found higher in Pinacidil+5-HD group according to control group ($P<0.05$), (Figure 3.2.3), (Table 3.2.5).

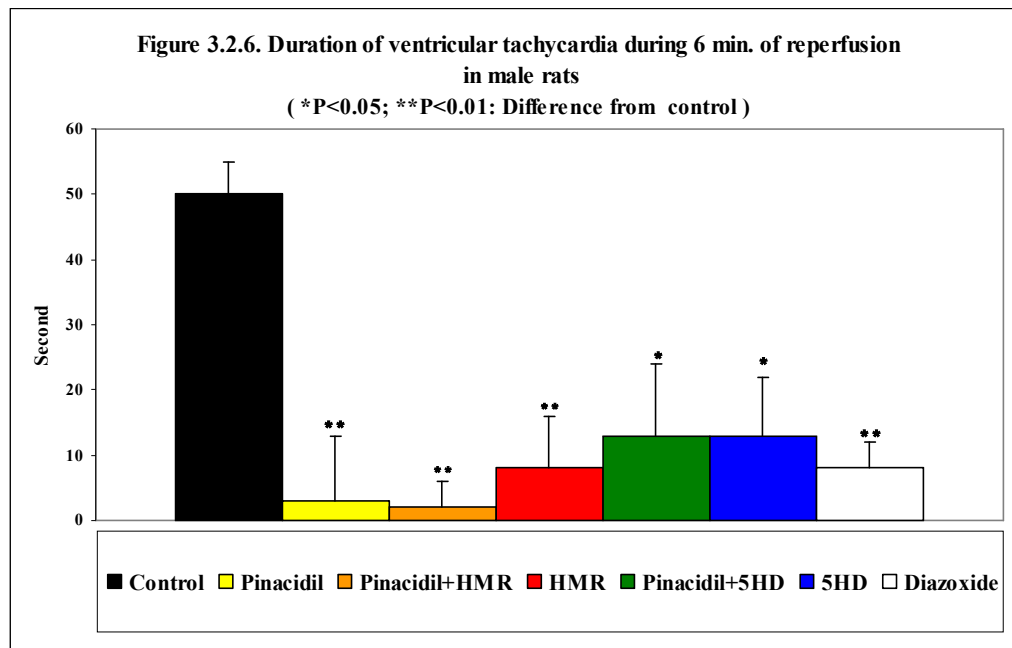
Total length of arrhythmias during ligation increased also in the combination of Pinacidil with 5-HD group according to Pinacidil ($P<0.05$), (Figure 3.2.4), (Table 3.2.5).



The duration of VF during reperfusion was lower in all groups in respect to control ($P<0.05$), only exception was Pinacidil+5-HD group that duration of the VF was not different according to control (Figure 3.2.5), (Table 3.2.3).



The duration of VT was lower in all groups according to control during reperfusion period, Pinacidil, Pinacidil+HMR, HMR, Diazoxide ($P<0.01$), Pinacidil+5-HD, 5-HD groups ($P<0.05$), (Figure 3.2.6), (Table 3.2.3).



The total length of arrhythmia was lower in all groups according to control during reperfusion period, Pinacidil, Pinacidil+HMR, HMR, 5-HD, Diazoxide ($P<0.01$) and Pinacidil+5-HD groups ($P<0.05$) (Figure 3.2.7), (Table 3.2.3).

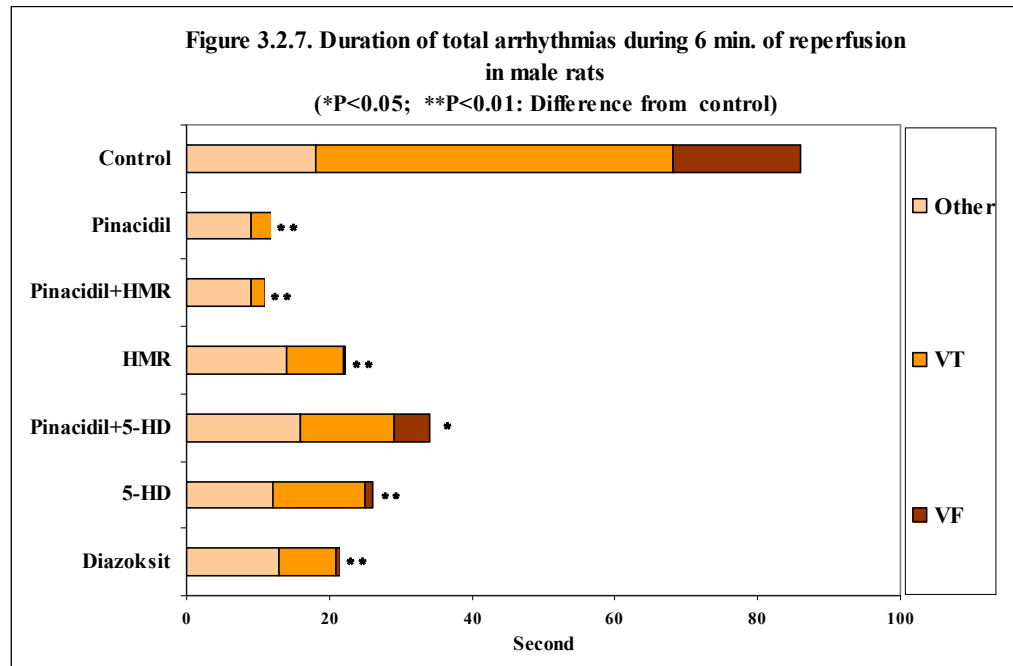


Table 3.2.1. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of ligation in male rats (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat/ min.)				Blood pressure (mmHg)			
		Basal	01	03	05	Basal	01	03	05
Control	6	387 $\pm$ 17	388 $\pm$ 16	381 $\pm$ 25	375 $\pm$ 26	108 $\pm$ 5	97 $\pm$ 4	102 $\pm$ 5	104 $\pm$ 5
Pinacidil	6	453 $\pm$ 22*	434 $\pm$ 17*	432 $\pm$ 29*	425 $\pm$ 20*	108 $\pm$ 10	83 $\pm$ 15	94 $\pm$ 14	94 $\pm$ 15
Pinacidil+HMR	6	458 $\pm$ 15*	454 $\pm$ 19*	445 $\pm$ 18*	442 $\pm$ 16*	114 $\pm$ 10	95 $\pm$ 6	102 $\pm$ 8	109 $\pm$ 3
HMR	6	412 $\pm$ 10	408 $\pm$ 11	409 $\pm$ 15	381 $\pm$ 25	123 $\pm$ 10	83 $\pm$ 13	86 $\pm$ 11	76 $\pm$ 15
Pinacidil+5-HD	6	378 $\pm$ 22	373 $\pm$ 21	396 $\pm$ 33	383 $\pm$ 31	100 $\pm$ 19	98 $\pm$ 9	106 $\pm$ 8	76,4 $\pm$ 8
5-HD	7	386 $\pm$ 15	377 $\pm$ 14	384 $\pm$ 9	380 $\pm$ 15	127 $\pm$ 7	82 $\pm$ 8	87 $\pm$ 9	102 $\pm$ 11
Diazoxide	6	405 $\pm$ 15	408 $\pm$ 17	398 $\pm$ 16	424 $\pm$ 23	98 $\pm$ 7	73 $\pm$ 10	77 $\pm$ 19	91 $\pm$ 13

N: The number of survived animals after 6 min. of ligation.

*P<0.05: Compared to control.

Table 3.2.2. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of reperfusion in male rats (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat/ min.)			Blood pressure (mmHg)		
		01	03	05	01	03	05
Control	6	370 $\pm$ 22	390 $\pm$ 34	421 $\pm$ 43	78 $\pm$ 14	93 $\pm$ 7	94 $\pm$ 6
Pinacidil	6	424 $\pm$ 12	462 $\pm$ 27	447 $\pm$ 17	85 $\pm$ 12	99 $\pm$ 11	99 $\pm$ 10
Pinacidil+HMR	6	443 $\pm$ 19*	434 $\pm$ 18	436 $\pm$ 16	104 $\pm$ 5	121 $\pm$ 2	121 $\pm$ 2*
HMR	6	409 $\pm$ 26	442 $\pm$ 34	396 $\pm$ 8	80 $\pm$ 13	102 $\pm$ 11	104 $\pm$ 10
Pinacidil+5-HD	6	480 $\pm$ 19*	423 $\pm$ 26	409 $\pm$ 28	89 $\pm$ 1	100 $\pm$ 10	96 $\pm$ 11
5-HD	7	398 $\pm$ 26	393 $\pm$ 23	394 $\pm$ 19	104 $\pm$ 13	115 $\pm$ 12	110 $\pm$ 12
Diazoxide	6	350 $\pm$ 17	377 $\pm$ 24	380 $\pm$ 27	95 $\pm$ 14	108 $\pm$ 9	111 $\pm$ 6

N: The number of the survived animals after 6 min. of reperfusion.

***P<0.05:** Compared to control.

Table 3.2.3. The duration of arrhythmias during 6 min. of reperfusion in male rats (Results: Mean $\pm$ SE).

Groups	N	Onset of arrhythmia (second)	Arrhythmic period (second)	Length of arrhythmia (second)				
				VF	VT	Other	Total	Bradycardia
Control	7	2 $\pm$ 2	300 $\pm$ 31	18 $\pm$ 14	50 $\pm$ 22	18 $\pm$ 2	87 $\pm$ 34	0
Pinacidil	6	30 $\pm$ 12**	125 $\pm$ 55**	0*	3 $\pm$ 2**	9 $\pm$ 4	12 $\pm$ 4**	0
Pinacidil+HMR	6	0 $\pm$ 0	115 $\pm$ 27**	0*	2 $\pm$ 1**	9 $\pm$ 2	11 $\pm$ 2**	0
HMR	6	0	150 $\pm$ 36*	0.3 $\pm$ 0.3*	8 $\pm$ 3**	14 $\pm$ 4	22 $\pm$ 7**	0
Pinacidil+5-HD	6	0	203 $\pm$ 40	5 $\pm$ 5	13 $\pm$ 11*	16 $\pm$ 4	34 $\pm$ 14*	0
5-HD	7	4 $\pm$ 4	160 $\pm$ 40*	1 $\pm$ 1*	13 $\pm$ 5*	12 $\pm$ 2	27 $\pm$ 6**	0
Diazoxide	6	9 $\pm$ 9	157 $\pm$ 35*	0.5 $\pm$ 0.5*	8 $\pm$ 3**	13 $\pm$ 2	22 $\pm$ 6**	0

N: The number of the survived animals after 6 min. of reperfusion.

***P<0.05; **P<0.01:** Compared to control.

Table 3.2.4. The incidence of arrhythmias during 6 min. of reperfusion in male rats (Results: Mean $\pm$ SE).

Groups	N	Survival (n / %)	Incidence of Arrhythmia (N / %)				Arrhythmia scores
			VF	VT	Other	Bradycardia	
Control	6	6 / 100	3 / 50	6 / 100	6 / 100	0 / 0	3.1 $\pm$ 0.6
Pinacidil	6	6 / 100	0 / 0	2 / 33*	5 / 83	0 / 0	1.1 $\pm$ 0.3*
Pinacidil+HMR	6	6 / 100	0 / 0	5 / 83	6 / 100	0 / 0	1.5 $\pm$ 0.2*
HMR	6	6 / 100	0 / 0	5 / 83	6 / 100	0 / 0	1.5 $\pm$ 0.2*
Pinacidil+5-HD	6	6 / 100	1 / 16	3 / 50	6 / 100	0 / 0	2.6 $\pm$ 0.7 [♥]
5-HD	8	7 / 87	2 / 28	6 / 85	7 / 100	0 / 0	1.8 $\pm$ 0.2*
Diazoxide	6	6 / 100	1 / 17	5 / 83	5 / 83	0 / 0	1.6 $\pm$ 0.4*

N: The number of animals at the onset of ligation, **n:** The number of survived animals after 6 min. of reperfusion.

N: The number of animals in which arrhythmias observed. * **P<0.05:** Compared to control. [♥]**P<0.05:** Compared to pinacidil.

Table 3.2.5. The duration of arrhythmias during 6 min. of coronary ligation in male rats (Results: Mean $\pm$ SE).

Groups	N	Length of arrhythmia (second)				
		VF	VT	Other	Total	Bradycardia
Control	6	0	0	3.6 $\pm$ 1.7	3.6 $\pm$ 1.7	0
Pinacidil	6	0	0	0	0	0
Pinacidil+HMR	6	0	0	0.3 $\pm$ 0.3	0.3 $\pm$ 0.3	0
HMR	6	0	0.2 $\pm$ 0.2	2.5 $\pm$ 2.5	2.6 $\pm$ 2.6	0
Pinacidil+5-HD	6	0	5.6 $\pm$ 3.8*	5.1 $\pm$ 5.1	10.7 $\pm$ 6.7 [♥]	4.1 $\pm$ 4.1
5-HD	7	0.1	0.7 $\pm$ 0.7	5.5 $\pm$ 1.3	6.3 $\pm$ 1.5	0
Diazoxide	6	0	0	2.7 $\pm$ 1.7	2.7 $\pm$ 1.1	0

N: The number of survived animals after 6 min. of ligation.

***P<0.05:** Compared to control. **♥P<0.05:** Compared to pinacidil.

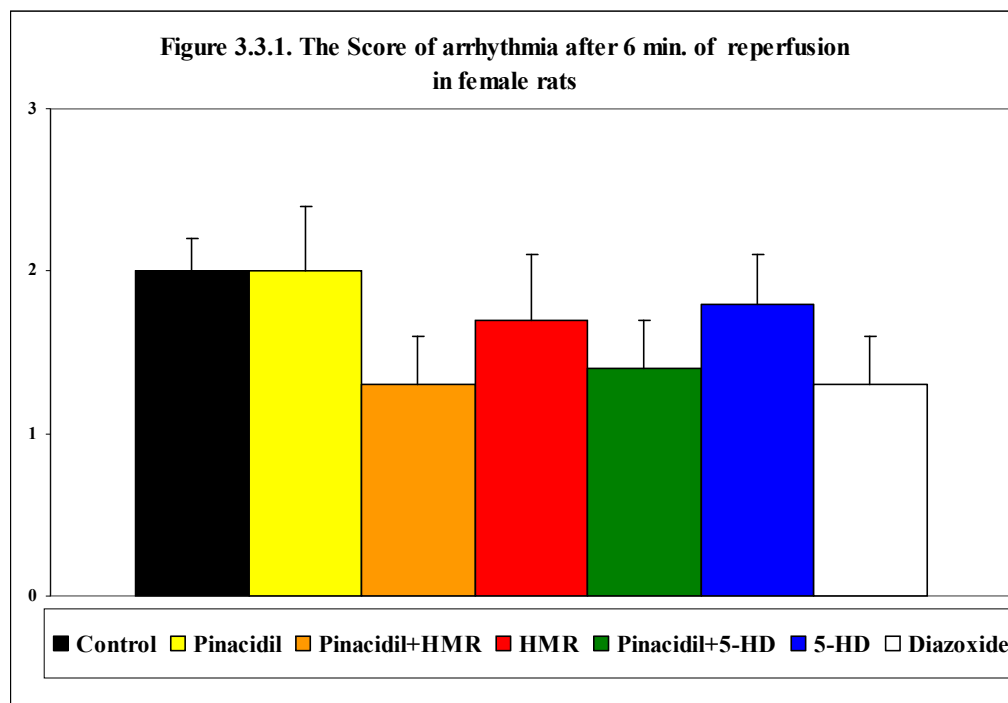
3.3. THE EFFECT OF K_{ATP} CHANNEL MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN FEMALES:

The blood pressure in 5-HD and Diazoxide treated groups at 5th min. of ligation was lower as it compared to control (Table 3.3.1). Heart rate at 1st, and 5th min. of coronary ligation in 5-HD treated group was low according to control. (Table 3.3.1). On the other hand the blood pressure and heart rate during the reperfusion period in drug treated group was not different from control (Table 3.3.2).

Arrhythmias in female groups usually appeared in between 0-40 sec. following the reperfusion (Table 3.3.3).

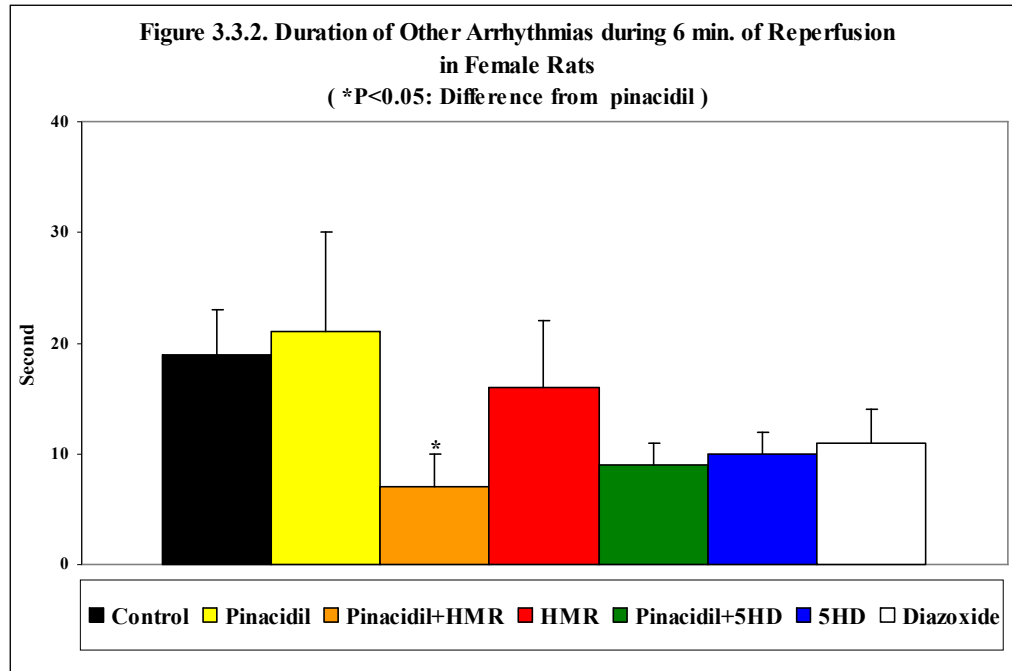
Arrhythmic period and the incidence of arrhythmias were not different among groups during reperfusion (Table 3.3.3), (Table 3.3.4).

The score of arrhythmia in reperfusion period was also not different among groups (Figure 3.3.1), (Table 3.3.4).



The duration of VF, VT, other and total length of arrhythmias was not different among groups during the ligation (Table 3.3.5).

The duration of other type of arrhythmias during reperfusion was lower in Pinacidil+HMR group according to Pinacidil group ($P<0.05$), (Figure 3.3.2), (Table 3.3.3).



The duration of total length of arrhythmias during reperfusion was lower in Pinacidil+HMR group than that in control group ($P<0.05$), (Figure 3.3.3), (Table 3.3.3).

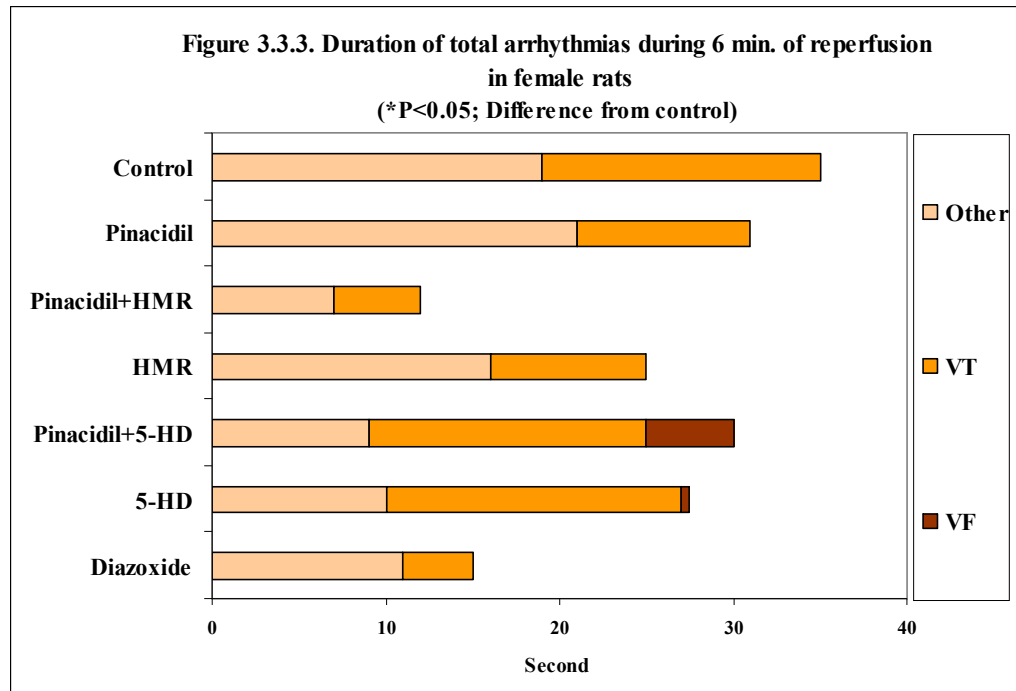


Table 3.3.1. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of ligation in female rats (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat / min.)				Blood pressure (mmHg)			
		Basal	01	03	05	Basal	01	03	05
Control	7	407 $\pm$ 17	420 $\pm$ 14	419 $\pm$ 15	419 $\pm$ 18	116 $\pm$ 8	107 $\pm$ 27	113 $\pm$ 10	117 $\pm$ 9
Pinacidil	6	398 $\pm$ 38	404 $\pm$ 17	408 $\pm$ 21	409 $\pm$ 22	109 $\pm$ 11	95 $\pm$ 27	101 $\pm$ 12	108 $\pm$ 10
Pinacidil+HMR	6	444 $\pm$ 42	438 $\pm$ 12	383 $\pm$ 43	441 $\pm$ 15	117 $\pm$ 7	83 $\pm$ 44	86 $\pm$ 20	110 $\pm$ 12
HMR	6	425 $\pm$ 35	416 $\pm$ 14	373 $\pm$ 29	376 $\pm$ 29	127 $\pm$ 6	98 $\pm$ 25	97 $\pm$ 13	95 $\pm$ 14
Pinacidil+5-HD	7	419 $\pm$ 32	433 $\pm$ 18	423 $\pm$ 9	428 $\pm$ 12	126 $\pm$ 2	110 $\pm$ 12	116 $\pm$ 4	115 $\pm$ 4
5-HD	6	366 $\pm$ 65	365 $\pm$ 30*	353 $\pm$ 29	355 $\pm$ 27*	112 $\pm$ 11	90 $\pm$ 25	93 $\pm$ 13	79 $\pm$ 14*
Diazoxide	6	416 $\pm$ 35	398 $\pm$ 16	401 $\pm$ 12	404 $\pm$ 19	112 $\pm$ 7	86 $\pm$ 26	86 $\pm$ 12	84 $\pm$ 13*

N: The number of survived animals after 6 min. of ligation.

***P<0.05:** Compared to control.

Table 3.3.2. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of reperfusion in female rats (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat / min.)			Blood pressure (mmHg)		
		01	03	05	01	03	05
Control	7	444 $\pm$ 30	403 $\pm$ 16	419 $\pm$ 16	98 $\pm$ 12	116 $\pm$ 8	117 $\pm$ 7
Pinacidil	6	450 $\pm$ 66	388 $\pm$ 29	406 $\pm$ 14	78 $\pm$ 40	112 $\pm$ 9	111 $\pm$ 11
Pinacidil+HMR	6	385 $\pm$ 41	423 $\pm$ 13	472 $\pm$ 40	99 $\pm$ 58	101 $\pm$ 15	96 $\pm$ 18
HMR	6	355 $\pm$ 34	370 $\pm$ 32	376 $\pm$ 31	100 $\pm$ 80	111 $\pm$ 9	103 $\pm$ 16
Pinacidil+5-HD	7	466 $\pm$ 35	408 $\pm$ 12	420 $\pm$ 26	86 $\pm$ 51	106 $\pm$ 10	93 $\pm$ 10
5-HD	6	363 $\pm$ 31	352 $\pm$ 30	374 $\pm$ 33	87 $\pm$ 60	100 $\pm$ 13	102 $\pm$ 13
Diazoxide	6	395 $\pm$ 13	399 $\pm$ 20	400 $\pm$ 16	82 $\pm$ 51	90 $\pm$ 9	95 $\pm$ 10

N: The number of the survived animals after 6 min. of reperfusion.

Table 3.3.3. The duration of arrhythmias during 6 min. of reperfusion in female rats (Results: Mean $\pm$ SE).

Groups	N	Onset of arrhythmia (second)	Arrhythmic period (second)	Length of arrhythmia (second)				
				VF	VT	Other	Total	Bradycardia
Control	7	27 $\pm$ 13	149 $\pm$ 36	0	16 $\pm$ 3	19 $\pm$ 4	35 $\pm$ 6	0
Pinacidil	6	35 $\pm$ 33	166 $\pm$ 42	0	10 $\pm$ 4	21 $\pm$ 9	31 $\pm$ 9	0
Pinacidil+HMR	6	3 $\pm$ 1	96 $\pm$ 34	0	5 $\pm$ 2	7 $\pm$ 3 [▼]	12 $\pm$ 4*	0
HMR	6	11 $\pm$ 8	181 $\pm$ 50	0	9 $\pm$ 4	16 $\pm$ 6	24 $\pm$ 8	0
Pinacidil+5-HD	7	40 $\pm$ 25	140 $\pm$ 41	0	16 $\pm$ 10	9 $\pm$ 2	25 $\pm$ 11	0
5-HD	6	15 $\pm$ 13	197 $\pm$ 37	0.5 $\pm$ 1	17 $\pm$ 10	10 $\pm$ 2	26 $\pm$ 10	0
Diazoxide	6	86 $\pm$ 38	125 $\pm$ 81	0	4 $\pm$ 1	11 $\pm$ 3	15 $\pm$ 4	0

N: The number of the survived animals after 6 min. of reperfusion. *P<0.05: Compared to control. [▼]P<0.05: Compared to pinacidil.

Table 3.3.4. The incidence of arrhythmias during 6 min. of reperfusion in female rats (Results: Mean $\pm$ SE).

Groups	N	Survival (n / %)	Incidence of arrhythmia (N / %)				Arrhythmia scores
			VF	VT	Other	Bradycardia	
Control	7	7 / 100	0 / 0	7 / 100	7 / 100	0 / 0	2.0 $\pm$ 0.2
Pinacidil	6	6 / 100	0 / 0	5 / 83	5 / 100	0 / 0	2.0 $\pm$ 0.4
Pinacidil+HMR	6	6 / 100	0 / 0	4 / 66	5 / 83	0 / 0	1.3 $\pm$ 0.3
HMR	6	6 / 100	0 / 0	5 / 83	6 / 100	0 / 0	1.7 $\pm$ 0.4
Pinacidil+5-HD	7	7 / 100	0 / 0	5 / 71	7 / 100	0 / 0	1.4 $\pm$ 0.3
5-HD	6	6 / 100	16 / 1	4 / 66	6 / 100	0 / 0	1.8 $\pm$ 0.3
Diazoxide	6	6 / 100	0 / 0	4 / 66	6 / 100	0 / 0	1.3 $\pm$ 0.3

N: The number of animals at the onset of ligation. **n:** The number of survived animals after 6 min. of reperfusion.

N: The number of animals which arrhythmias observed.

Table 3.3.5. The duration of arrhythmias during 6 min. of coronary ligation in female rats (Results: Mean $\pm$ SE).

Groups	N	Length of arrhythmia (second)				
		VF	VT	Other	Total	Bradycardia
Control	7	0	0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0
Pinacidil	6	0	0	2.0 $\pm$ 1.4	2.4 $\pm$ 1.4	0
Pinacidil+HMR	6	0	0	0.8 $\pm$ 0.4	0.8 $\pm$ 0.4	0
HMR	6	0	0	1.0 $\pm$ 0.4	1.0 $\pm$ 0.4	0
Pinacidil+5-HD	7	0	0	2.1 $\pm$ 0.8	2.1 $\pm$ 0.8	0
5-HD	6	0	0.3 $\pm$ 0.3	1.1 $\pm$ 0.4	1.5 $\pm$ 0.5	0
Diazoxide	6	0	0	1.5 $\pm$ 0.8	1.5 $\pm$ 0.8	0

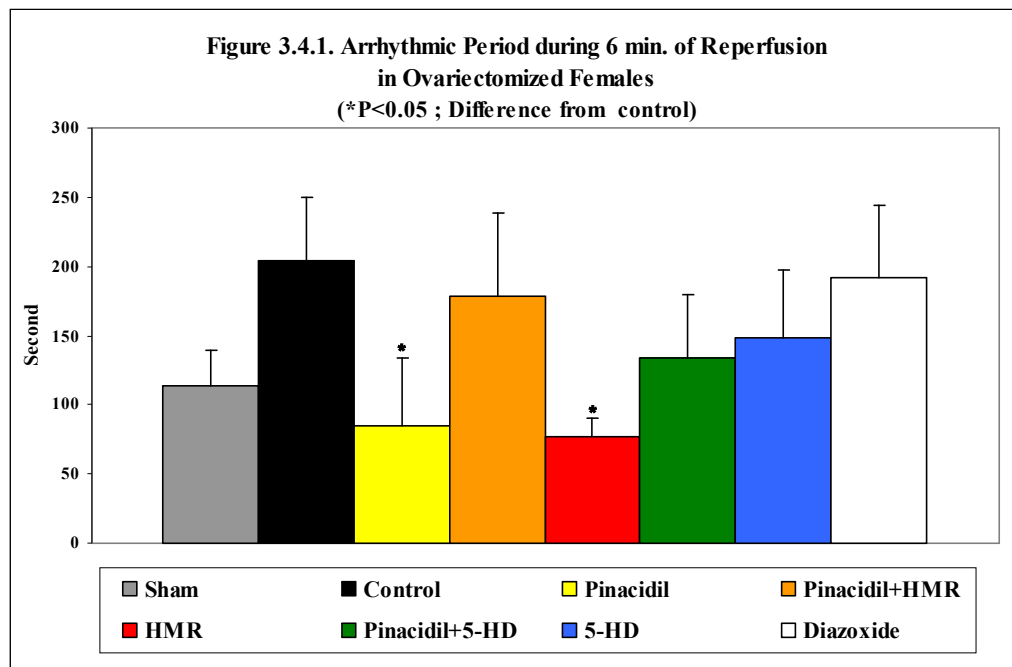
N: The number of survived animals after 6 min. of ligation

3.4. THE EFFECT OF K_{ATP} CHANNEL MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN OVARECTOMIZED FEMALES

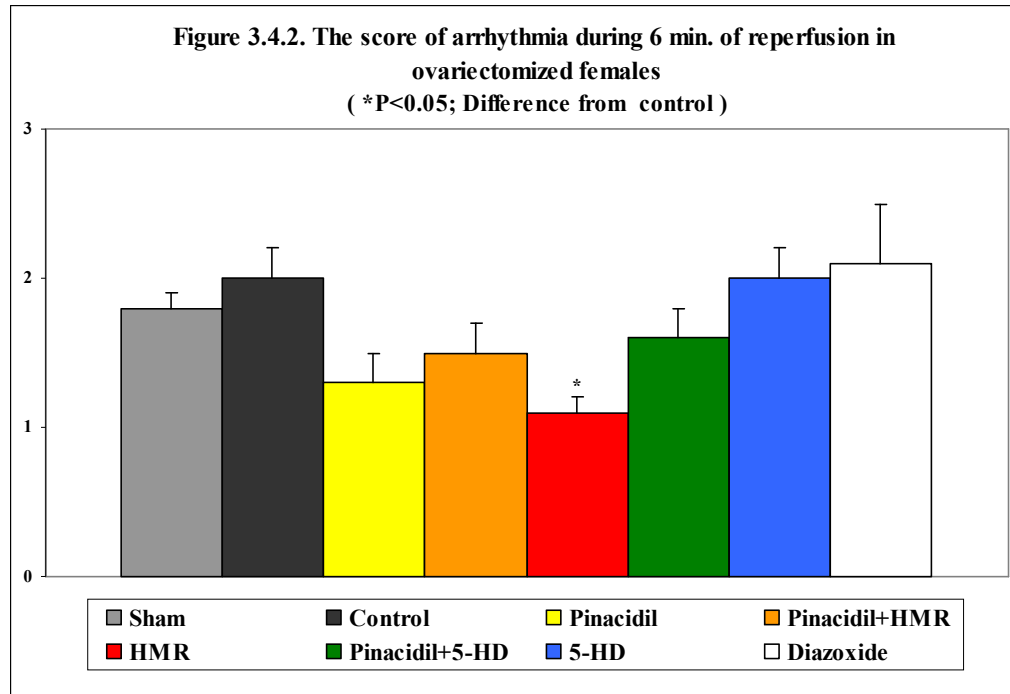
In drug treated ovariectomized groups, the blood pressure and heart rate during 6 min. of the ligation and reperfusion did not significantly differ from those of the control (Table 3.4.1), (Table 3.4.2). Only exception was the heart rate in 5-HD group at 3rd min. of the coronary ligation was higher than control (Table 3.4.1).

Arrhythmias usually appeared in between 0-14 sec. following the reperfusion. But it was delayed in Pinacidil+HMR group ($P<0.05$), (Table 3.4.3).

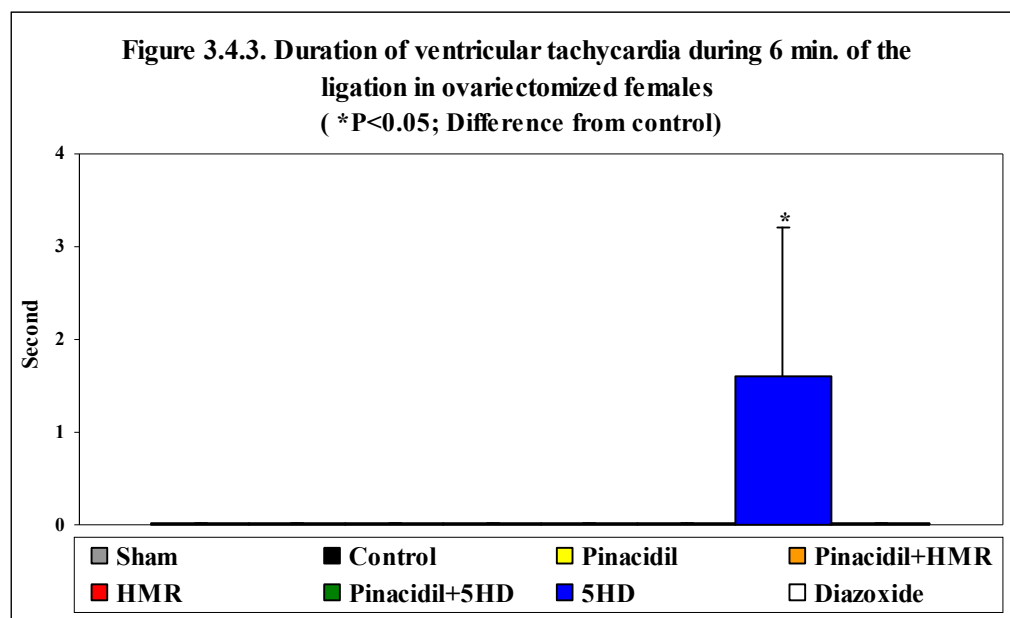
Arrhythmic period was lower in Pinacidil and HMR treated groups according to control group ($P<0.05$), (Figure 3.4.1), (Table 3.4.3). The incidence of arrhythmias was not different among groups (Table 3.4.4).



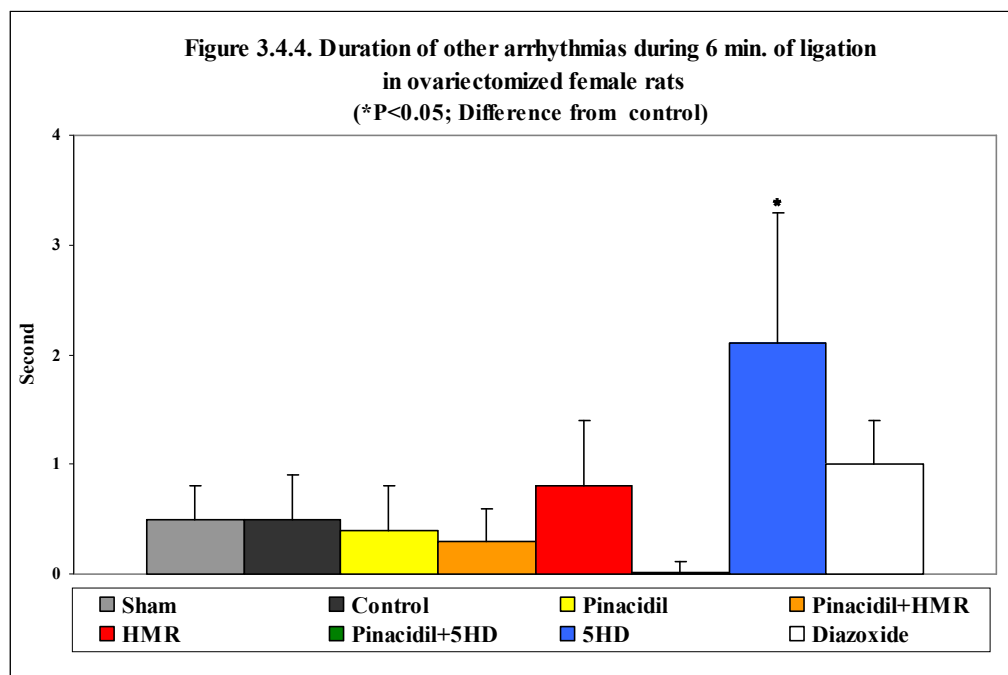
Arrhythmia score during 6 min. of the reperfusion was significantly lower in HMR treated group than that in control and sham operated groups ($P<0.05$), (Figure 3.4.2), (Table 3.4.4).



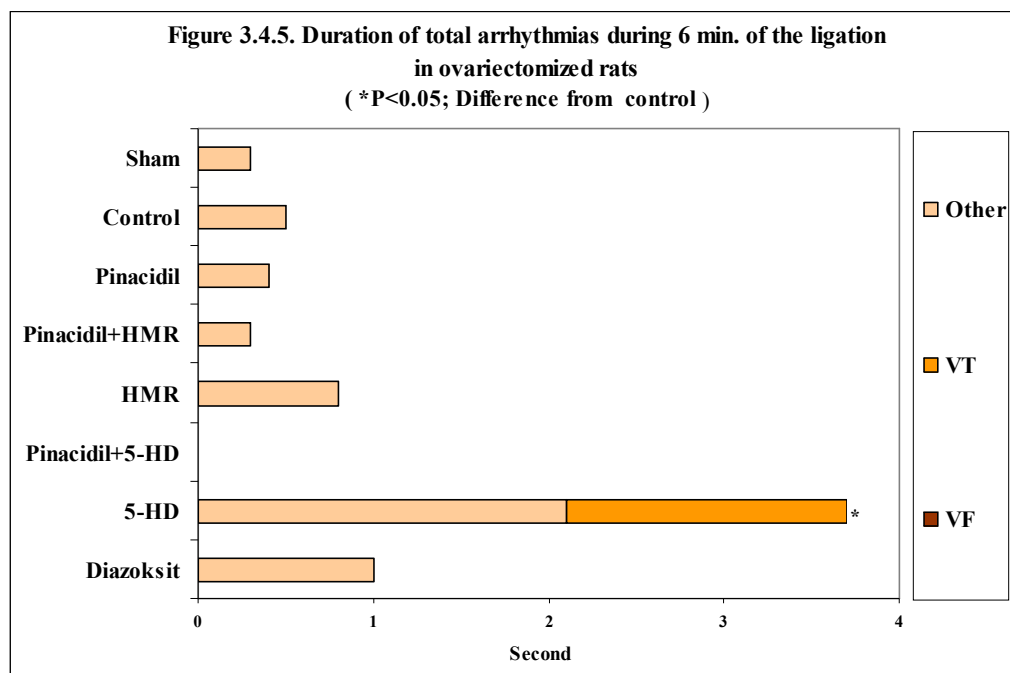
The duration of VT during coronary artery ligation was found higher in 5-HD treated group according to control ($P<0.05$), (Figure 3.4.3), (Table 3.4.5).



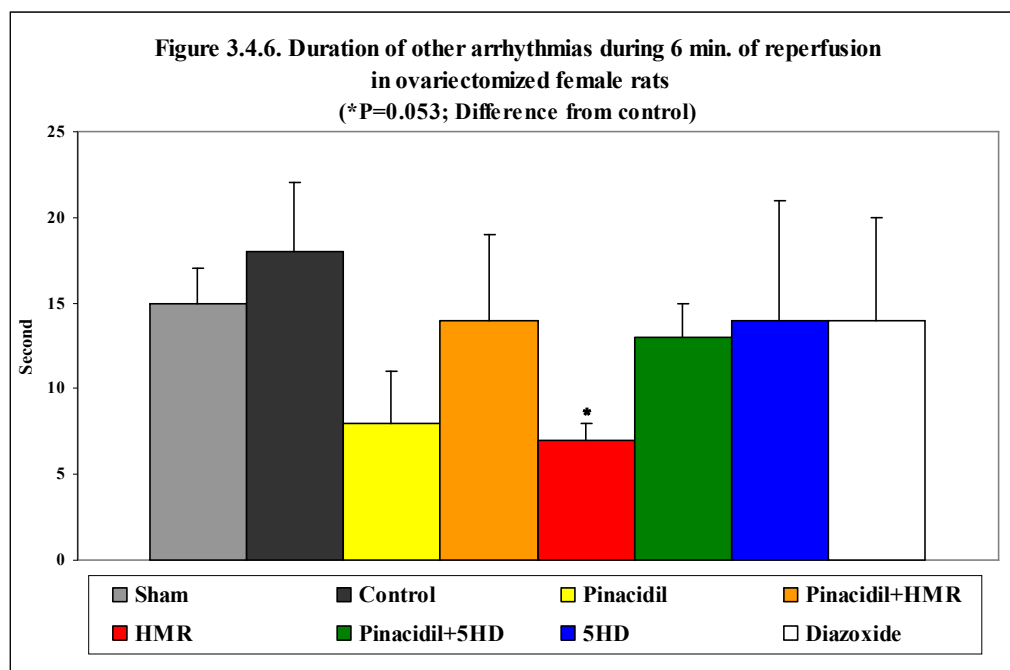
The length of other arrhythmias during coronary artery ligation was found higher in 5-HD group when it is compared to control ($P<0.05$), (Figure 3.4.4), (Table 3.4.5).



During 6 min. of coronary artery ligation, the length of total arrhythmias was found higher in 5-HD group according to other groups ($P<0.05$), (Figure 3.4.5), (Table 3.4.5).



During 6 min. of the coronary artery ligation, the length of other arrhythmias was lower in HMR group than that in control group ($P = 0.053$), (Figure 3.4.6), (Table 3.4.3).



The length of total arrhythmias in reperfusion was not different from control, (Figure 3.4.7), (Table 3.4.3).

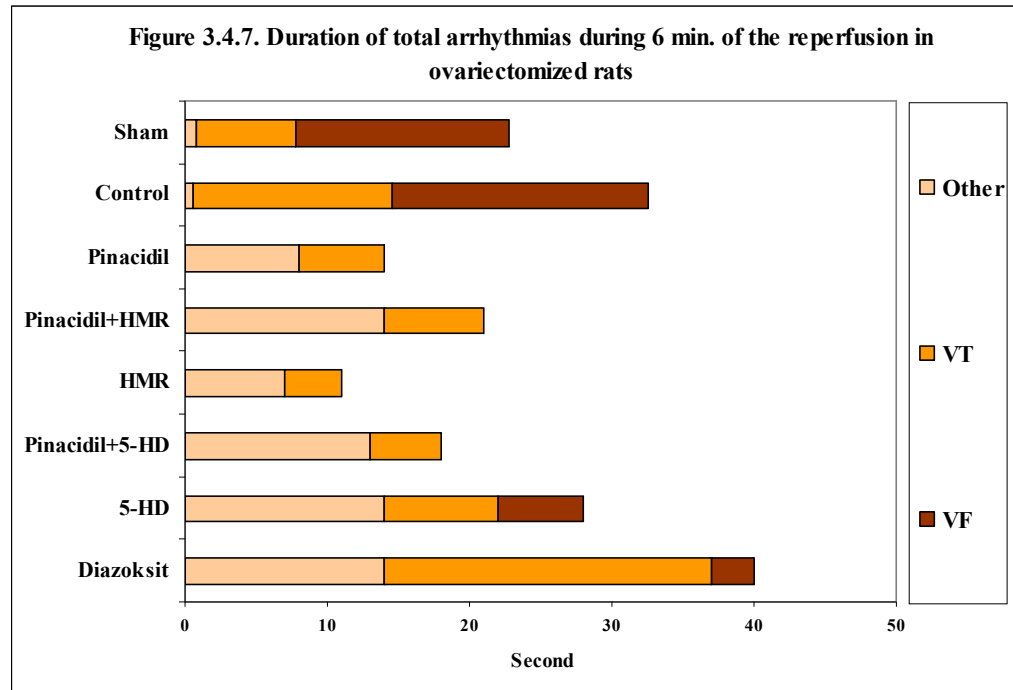


Table 3.4.1. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of ligation in ovariectomized female rats (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat/ min.)				Blood pressure (mmHg)			
		Basal	01	03	05	Basal	01	03	05
Sham	7	432 $\pm$ 16	422 $\pm$ 20	413 $\pm$ 11	407 $\pm$ 19	116 $\pm$ 10	94 $\pm$ 7	93 $\pm$ 9	98 $\pm$ 8
Control	9	410 $\pm$ 22	396 $\pm$ 16	373 $\pm$ 26	369 $\pm$ 26	124 $\pm$ 8	107 $\pm$ 8	106 $\pm$ 10	106 $\pm$ 10
Pinacidil	6	426 $\pm$ 14	437 $\pm$ 20	421 $\pm$ 21	407 $\pm$ 16	117 $\pm$ 12	98 $\pm$ 10	98 $\pm$ 12	97 $\pm$ 13
Pinacidil+HMR	6	417 $\pm$ 10	409 $\pm$ 14	407 $\pm$ 16	410 $\pm$ 18	122 $\pm$ 7	85 $\pm$ 9	95 $\pm$ 10	100 $\pm$ 10
HMR	7	422 $\pm$ 14	419 $\pm$ 17	416 $\pm$ 18	429 $\pm$ 16	128 $\pm$ 7	112 $\pm$ 7	118 $\pm$ 5	117 $\pm$ 5
Pinacidil +5HD	6	433 $\pm$ 23	434 $\pm$ 22	418 $\pm$ 25	398 $\pm$ 24	102 $\pm$ 5	90 $\pm$ 4	91 $\pm$ 4	95 $\pm$ 4
5HD	6	441 $\pm$ 34	434 $\pm$ 22	480 $\pm$ 33*	440 $\pm$ 18	131 $\pm$ 8	105 $\pm$ 9	112 $\pm$ 10	109 $\pm$ 12
Diazoxide	7	391 $\pm$ 20	399 $\pm$ 20	422 $\pm$ 17	400 $\pm$ 50	114 $\pm$ 11	86 $\pm$ 8	86 $\pm$ 28	82 $\pm$ 12

N: The number of survived animals after 6 min. of ligation. *P<0.05: Compared to control

Table 3.4.2. The heart rate (HR) and mean arterial blood pressure (MBP) during 6 min. of reperfusion in ovariectomized female rats (Results: Mean $\pm$ SE).

Groups	N	Heart rate (Beat/ min.)			Blood pressure (mmHg)		
		01	03	05	01	03	05
Sham	7	388 $\pm$ 26	414 $\pm$ 14	419 $\pm$ 10	86 $\pm$ 10	104 $\pm$ 6	112 $\pm$ 8
Control	9	377 $\pm$ 25	405 $\pm$ 16	398 $\pm$ 17	99 $\pm$ 11	107 $\pm$ 11	98 $\pm$ 13
Pinacidil	6	350 $\pm$ 52	408 $\pm$ 17	404 $\pm$ 21	117 $\pm$ 12	112 $\pm$ 12	111 $\pm$ 14
Pinacidil+HMR	6	445 $\pm$ 35	404 $\pm$ 13	414 $\pm$ 23	76 $\pm$ 11	106 $\pm$ 11	107 $\pm$ 12
HMR	7	409 $\pm$ 13	433 $\pm$ 19	419 $\pm$ 17	114 $\pm$ 6	120 $\pm$ 3	120 $\pm$ 3
Pinacidil +5HD	6	432 $\pm$ 27	414 $\pm$ 28	432 $\pm$ 29	95 $\pm$ 5	105 $\pm$ 8	101 $\pm$ 8
5HD	6	435 $\pm$ 20	411 $\pm$ 15	404 $\pm$ 20	83 $\pm$ 14	116 $\pm$ 6	113 $\pm$ 8
Diazoxide	7	388 $\pm$ 45	354 $\pm$ 34	349 $\pm$ 49	88 $\pm$ 11	96 $\pm$ 11	93 $\pm$ 12

N: The number of the survived animals after 6 min. of reperfusion.

Table 3.4.3. The duration of arrhythmias during 6 min. of reperfusion in ovariectomized female rats (Results: Mean $\pm$ SE).

Groups	N	Onset of arrhythmia (second)	Arrhythmic period (second)	Length of arrhythmia (second)				
				VF	VT	Other	Total	Bradycardia
Sham	7	2 $\pm$ 2	114 $\pm$ 25	0.8 $\pm$ 0.8	7 $\pm$ 3	15 $\pm$ 2	23 $\pm$ 5	0
Control	9	0 $\pm$ 0	204 $\pm$ 46	0.5 $\pm$ 0.5	14 $\pm$ 10	18 $\pm$ 4	32 $\pm$ 12	0
Pinacidil	6	0 $\pm$ 0	85 $\pm$ 49*	0	6 $\pm$ 3	8 $\pm$ 3	13 $\pm$ 5	0
Pinacidil+HMR	6	42 $\pm$ 12*	178 $\pm$ 61	0	7 $\pm$ 4	14 $\pm$ 5	22 $\pm$ 6	0
HMR	7	0 $\pm$ 0	77 $\pm$ 13*	0	4 $\pm$ 1	7 $\pm$ 1 [♦]	11 $\pm$ 6	0
Pinacidil +5HD	6	14 $\pm$ 12	134 $\pm$ 46	0	5 $\pm$ 2	13 $\pm$ 2	18 $\pm$ 3	0
5HD	6	2 $\pm$ 2	148 $\pm$ 19	5.8 $\pm$ 5.8	8 $\pm$ 2	14 $\pm$ 7	29 $\pm$ 8	0
Diazoxide	7	11 $\pm$ 6	192 $\pm$ 52	3 $\pm$ 3	23 $\pm$ 16	14 $\pm$ 6	40 $\pm$ 18	0

N: The number of the survived animals after 6 min. of reperfusion. ***P<0.05:** Compared to control. **♦P=0.053:** Compared to control.

Table 3.4.4. The incidence of arrhythmias during 6 min. of reperfusion in ovariectomized female rats (Results: Mean $\pm$ SE).

Groups	N	Survival (n / %)	Incidence of arrhythmia (N / %)				Arrhythmia scores
			VF	VT	Other	Bradycardia	
Sham	7	7 / 100	1/14	5/71	7/100	0	1.8 $\pm$ 0.1
Control	9	9 / 100	1/11	6/66	7/100	0	2.0 $\pm$ 0.2
Pinacidil	7	6 / 85	0/0	3/42	6/100	0	1.3 $\pm$ 0.2
Pinacidil+HMR	6	6 / 100	0/0	3/50	6/100	0	1.5 $\pm$ 0.2
HMR	7	7 / 100	0/0	7/86	7/100	0	1.1 $\pm$ 0.1*
Pinacidil +5HD	6	6 / 100	0/0	4/67	5/100	0	1.6 $\pm$ 0.2
5HD	6	6 / 100	1/16	5/83	6/100	0	2.0 $\pm$ 0.2
Diazoxide	7	7 / 100	1/14	5/71	6/100	0	2.1 $\pm$ 0.4

N: The number of animals at the onset of ligation. **n:** The number of survived animals after 6 min. of reperfusion. **N:** The number of animals which arrhythmias observed. ***P<0.05:** Compared to control and sham operated group.

Table 3.4.5. The duration of arrhythmias during 6 min. of coronary ligation in ovariectomized female rats (Results: Mean $\pm$ SE).

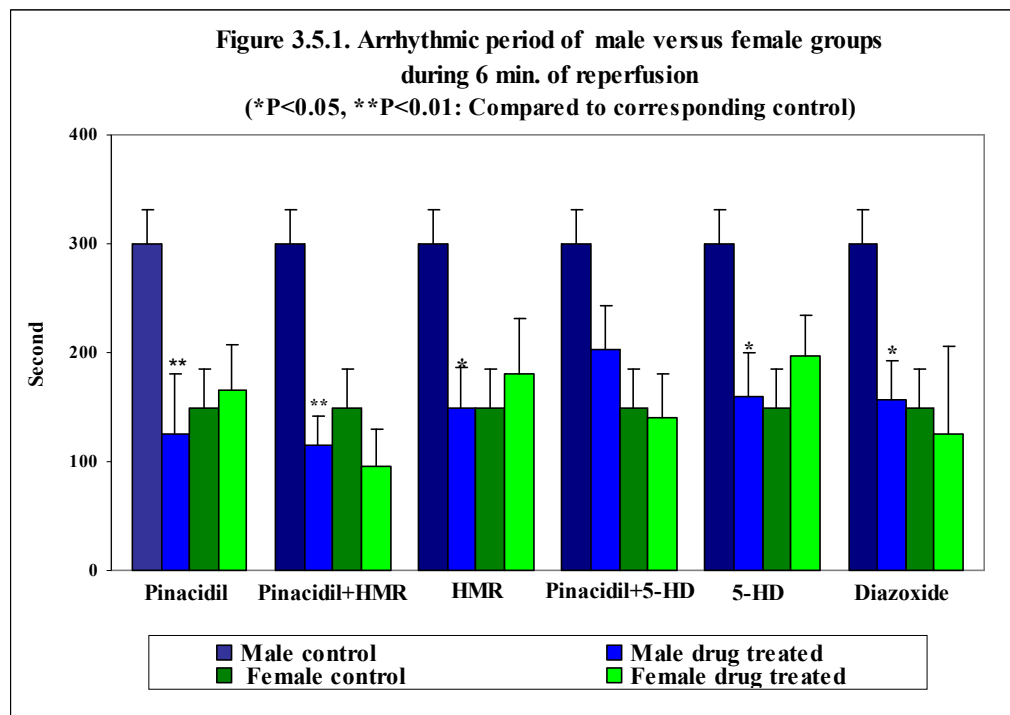
Groups	N	Length of arrhythmia (second)				
		VF	VT	Other	Total	Bradycardia
Sham	7	0	0	0.5 $\pm$ 0.3	0.3 $\pm$ 0.3	0
Control	9	0	0	0.5 $\pm$ 0.4	0.5 $\pm$ 0.4	0
Pinacidil	7	0	0	0.4 $\pm$ 0.4	0.4 $\pm$ 0.4	0
Pinacidil+HMR	6	0	0	0.3 $\pm$ 0.3	0.3 $\pm$ 0.3	0
HMR	7	0	0	0.8 $\pm$ 0.6	0.8 $\pm$ 0.6	0
Pinacidil +5HD	6	0	0	0	0	0
5HD	6	0	1.6 $\pm$ 1.6*	2.1 $\pm$ 1.2*	3.8 $\pm$ 2.8*	0
Diazoxide	7	0	0	1.0 $\pm$ 0.4	1.1 $\pm$ 0.4	0

***P<0.05:** Compared to control.

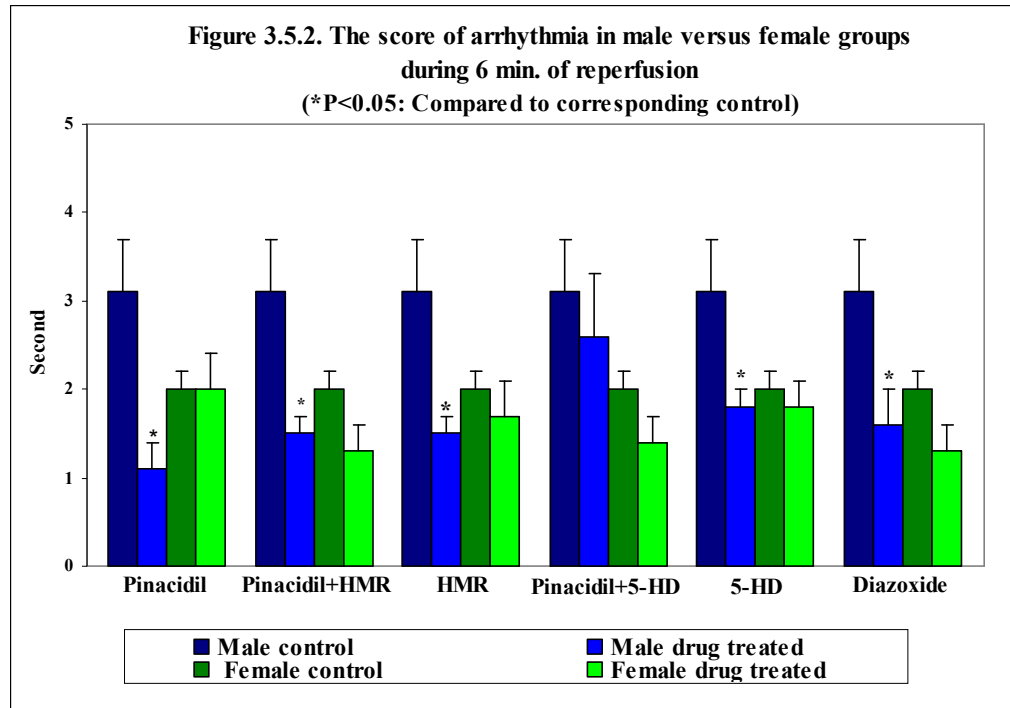
3.5. THE EFFECT OF K_{ATP} CHANNEL MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN MALE VERSUS FEMALES

In males, the combination of the Pinacidil with 5-HD increased the duration of VT during 6 min. of the ligation when it is compared to control group ($P<0.05$) (Table 3.2.5). But in female, drug treatment did not affect the duration of arrhythmias during ligation (Table 3.3.5).

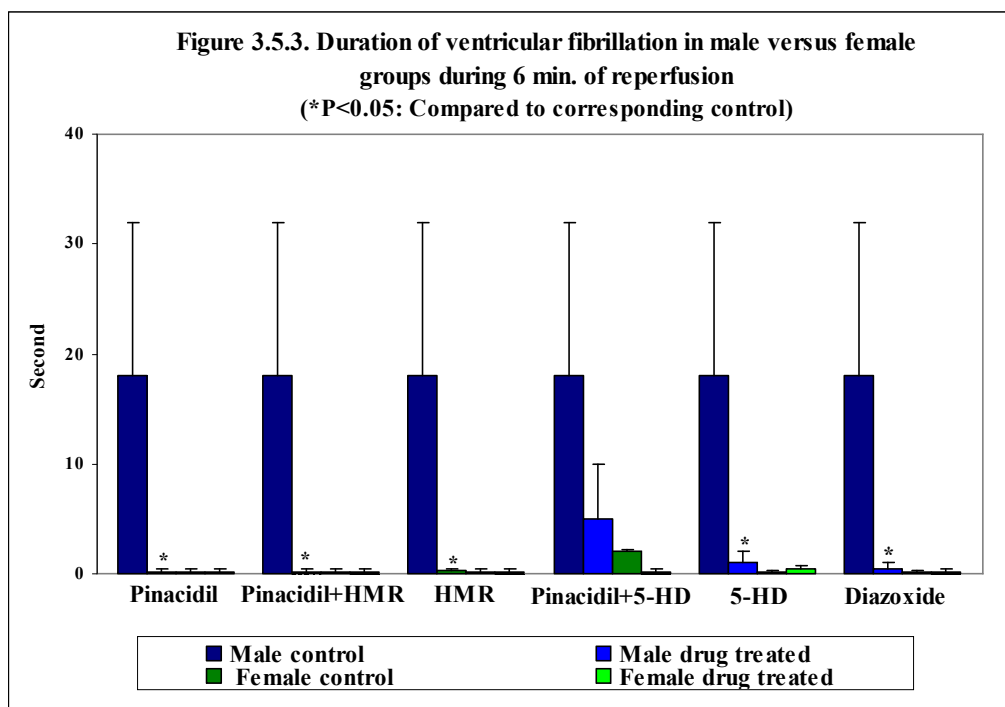
Drug treatments decreased the length of arrhythmic period during 6 min. of reperfusion in male groups according to control ($P<0.05$), ($P<0.01$) with only exception of the Pinacidil+5-HD group. On the other hand, in females, drugs effect on the length of arrhythmic period was nonsignificant (Figure 3.5.1), (Table 3.2.3), (Table 3.3.3).



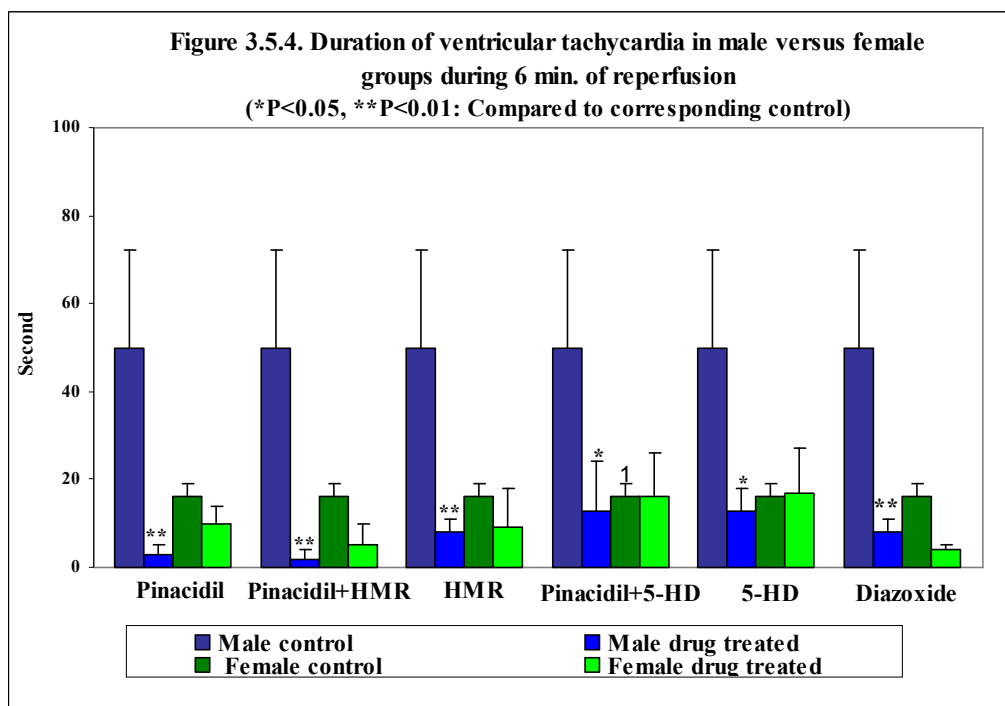
With exception of Pin+5HD group, the score of arrhythmia in all others drug treated male groups decreased during 6 min. of reperfusion ($P<0.05$). No drug effect was noted in female subject (Figure 3.5.2), (Table 3.2.4), (Table 3.3.4).



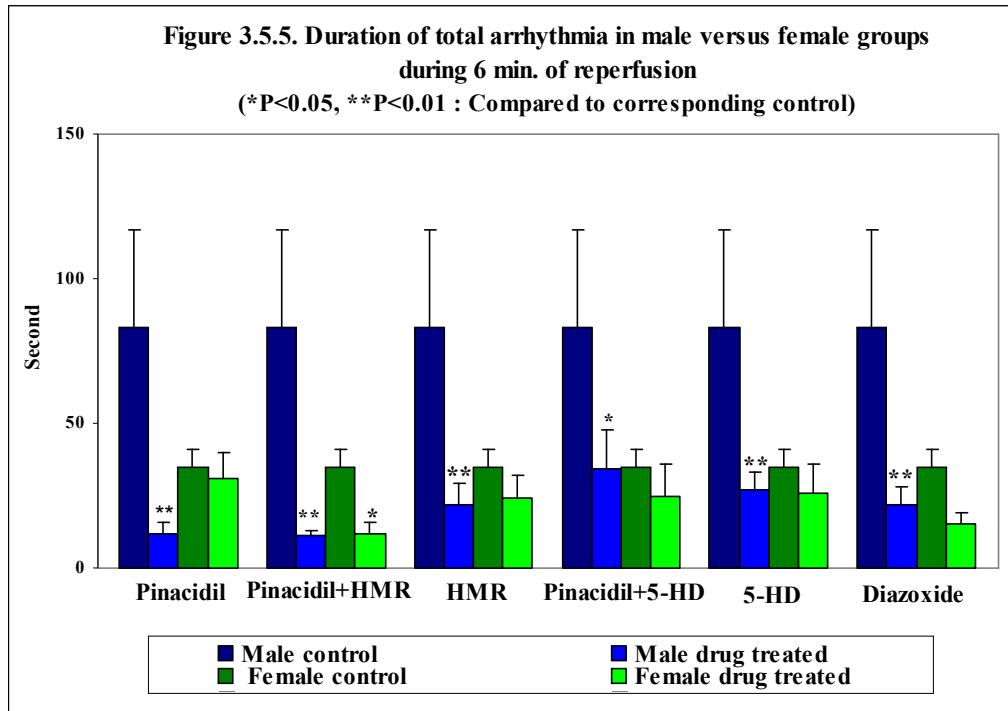
The damping effect of drug treatment on the duration of ventricular fibrillation during reperfusion was observed in all groups of males except Pinacidil+5-HD group ($P<0.05$). In females the duration of VF was not affected by drugs (Figure 3.5.3), (Table 3.2.3), (Table 3.3.3).



The duration of ventricular tachycardia decreased in all drug groups of males during 6 min. of reperfusion ($P<0.05$), ($P<0.01$). But in females it didn't change according to control (Figure 3.5.4), (Table 3.2.3), (Table 3.3.3).



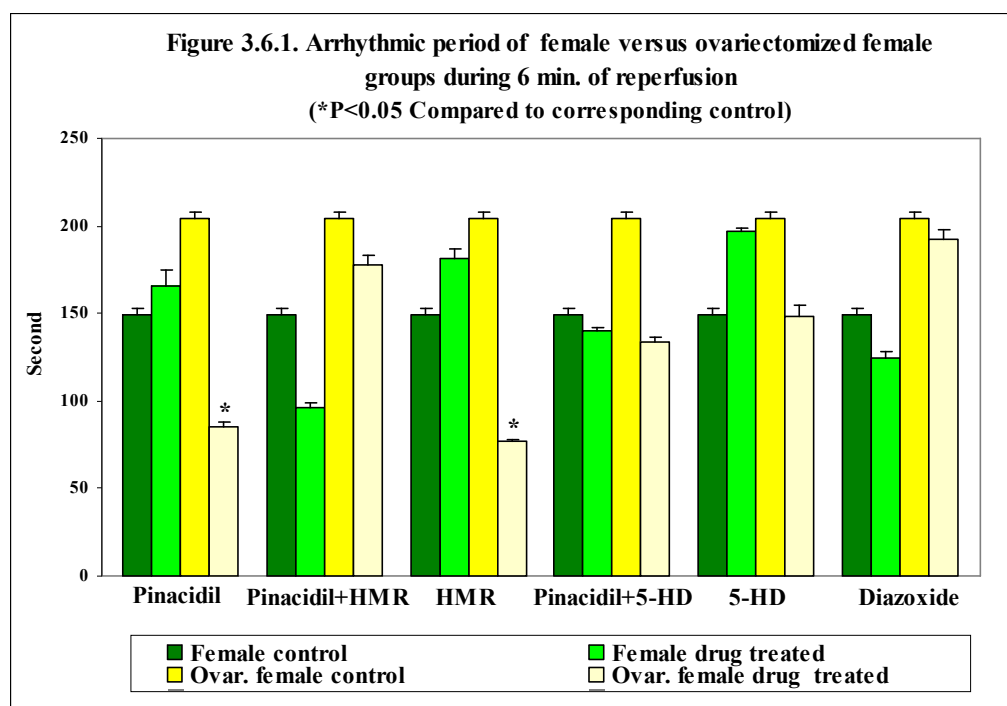
The total length of arrhythmias decreased in all male groups receiving drug treatment. In females during 6 min. of reperfusion only treatment with pinacidil and HMR combination decreased total length of arrhythmias ($P<0.05$). (Figure 3.5.5), (Table 3.2.3), (Table 3.3.3).



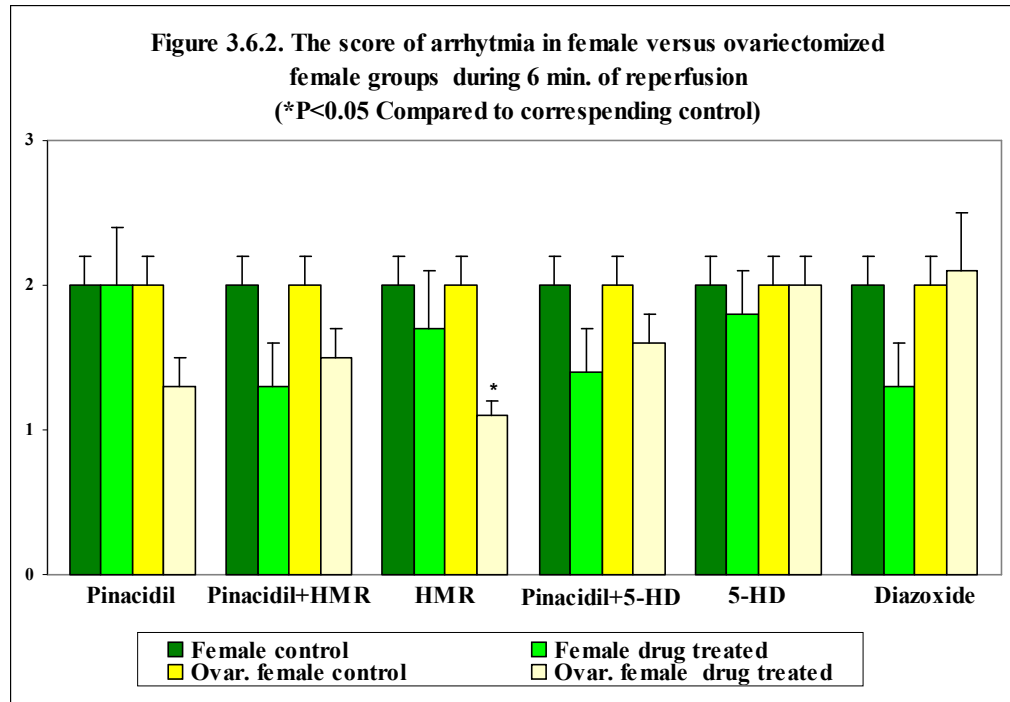
3.6. THE EFFECT OF K_{ATP} CHANNEL MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN FEMALE VERSUS OVARECTOMIZED FEMALES

In ovariectomized females, 5-HD treatment increased VT, as well as other and total length of arrhythmias during 6 min. of the ligation when it is compared to control group ($P<0.05$) (Table 3.4.5). But in unoveriectomized female, 5-HD treatment was not effective during the ligation (Table 3.3.5).

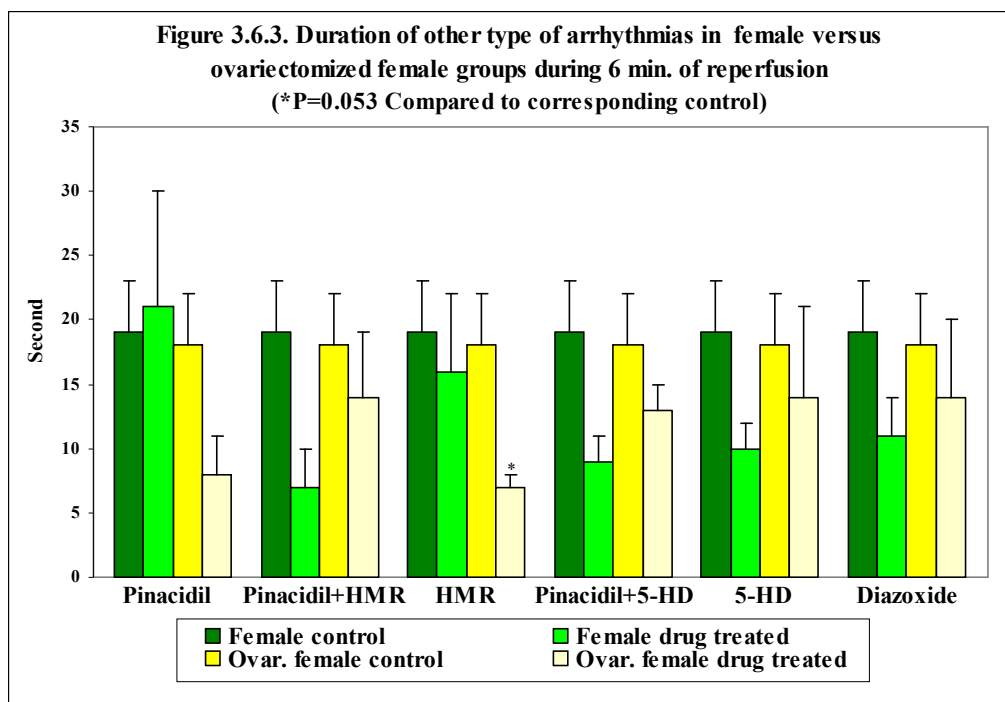
Both Pinacidil and HMR treatment decreased the length of arrhythmic period during 6 min. of reperfusion in ovariectomized female groups according to control ($P<0.05$). But they didn't decrease the arrhythmic period in control female (Figure 3.6.1), (Table 3.3.3), (Table 3.4.3).



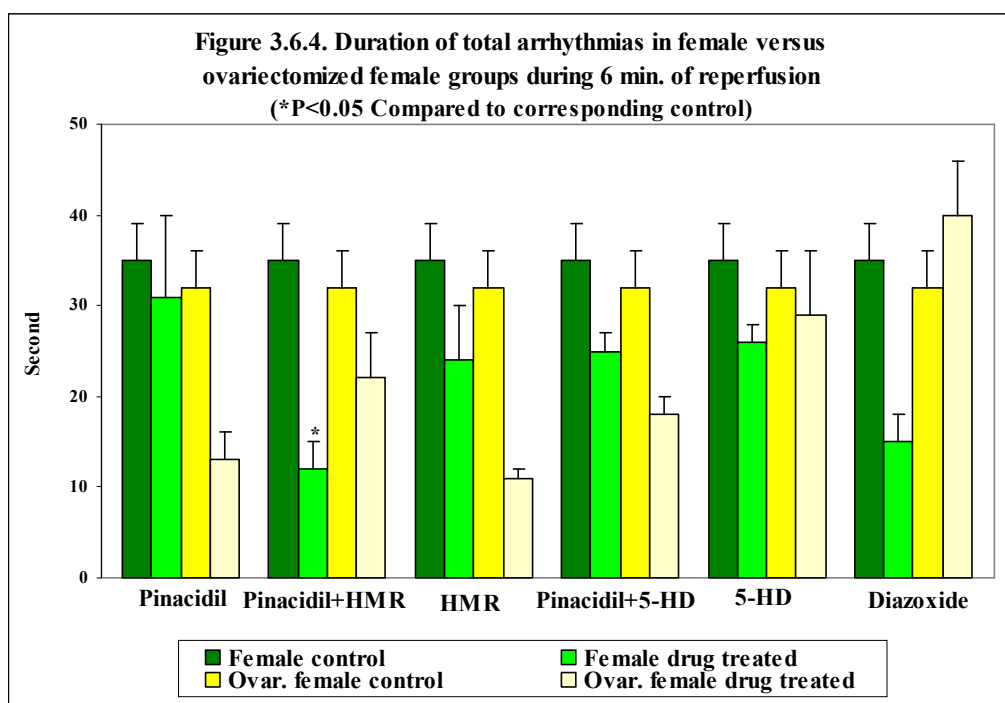
During 6 min. of reperfusion HMR treatment also decreased the score of arrhythmia in ovariectomized female ($P<0.05$). But it was not effective in unovariectomized female (Figure 3.6.2), (Table 3.3.4), (Table 3.4.4).



In addition, HMR treatment decreased the other type of arrhythmias in ovariectomized female during 6 min. of reperfusion ($P=0.053$). But it was not effective in unovariectomized females (Figure 3.6.3), (Table 3.3.3), (Table 3.4.3).



The combination of Pinacidil and HMR treatment decreased the total length of arrhythmias in unovariectomized female ($P < 0.05$). But it was not effective in ovariectomized female during 6 min. of reperfusion (Figure 3.6.4), (Table 3.3.3), (Table 3.4.3).



4. DISCUSSION

4.1. THE EFFECT OF THE ESTROGEN AND SEX ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS

In the present study, the score and duration of ischemia reperfusion arrhythmias were lower in female control groups than a comparable male group. This finding is consistent with our previous results (23). Numerous studies reported the conflicting observations about the susceptibility of males and females to the ischemia and I/R arrhythmias. Some of them reported the gender specific effect on ischemia and reperfusion arrhythmias (24, 260) but the others did not found this kind of effect (257, 258, 261, 262, 273). The reason why some outhors have not observed sex related differences in I/R induced arrhythmias could be different evaluations of arrhythmia (258). In some of these studies, only incidence of arrhythmias was calculated (273). Conversely, in our study, arrhythmias were evaluated according to Lambeth conventions.

The arrhythmic activity is directly proportional to body weight so that overweighing animals exhibit more pronounced arrhythmic activity (285). In the present study, the average body weight and the heart size of control females was smaller than that of males. The low arrhythmic activity in female may depend on the smaller body weight and heart size in our study. However in the studies by Humphreys RA. and colleagues (24), it was shown when male and female rats were matched by weight, still the lower arrhythmic activity was seen in females.

It has been also demonstrated that sex hormones decreased the severity of ventricular arrhythmias induced by regional myocardial ischemia and reperfusion in an *in vivo* ovariectomized rat model of coronary artery occlusion (263). Physiological supplementation of 17- β estradiol after ovariectomy resulted in preservation of

contractile function (265) and plays cardioprotective role in global ischemia and reperfusion (264, 274). Similarly the exogenously given estrogen significantly decreased ischemia and reperfusion injury and arrhythmias when it was given to ovariectomized females (25, 242, 259, 262, 276-278). It was suggested that this effect may be related to an increase in NO synthesis and/or prevention of oxidative stress, then improving endothelial function and coronary blood circulation (266, 267). In a comparable study, Humphreys RA. and his colleagues (24) documented that attenuation in arrhythmic activity during 30 min. of coronary artery occlusion was most pronounced during the estrus state which estrogen reach to highest level in respect to nonestrus females.

In the present study, females showed low arrhythmic activity than males and we expected that ovariectomy would increase the susceptibility of animals to reperfusion induced arrhythmias, however, this was not observed. The duration of arrhythmias did not increase in ovariectomized females compared to unovariectomized females although the plasma estradiol hormone levels on the day of study were lower in the ovariectomized females ($P<0.05$; 47 ± 6 pg/ml in female, 22 ± 4 pg/ml in ovariectomized female versus 59 ± 12 pg/ml in sham operated control). In a comparable study, the measured plasma estradiol levels were similar to the values in the present study (27 ± 1 pg/ml versus 60 ± 6 pg/ml) (264). There was no significant different in the severity of arrhythmias between sham operated female and unovariectomized females. This shows the ovariectomize operation should not be considered as a factor that affect the occurrence of arrhythmias. Since less arrhythmia was observed in isolated female rat hearts where the influence of circulating hormone is removed, Humphreys RA. and his colleagues (24) suggested that an attenuated arrhythmic activity in female rats does not depend on circulating estrogen. Our results

are consistent with the results reported by Humphreys RA. and his colleagues (24). According to these results the preservation of protection against arrhythmias may depend on sustained effect of estrogen even after removal of ovaries.

The presence of estrogen may regulate the expression of the some myocardial ion channels and structures. It has been demonstrated that sex-specific resistance to myocardial ischemia reperfusion injury is dependent on sarcK_{ATP} channel activity during ischemia (286). Female guinea pig ventricular myocytes express more sarcolemmal K_{ATP} channels than males (22). It has been proposed that lower arrhythmic activity seen in females may depend on the higher density of K_{ATP} channels (22).

In the present study, the lower arrhythmic activity seen in females may be also due to the higher expression of K_{ATP} channel in female subjects. Ovariectomy did not eliminate the lower susceptibility to I/R arrhythmias. The reason may be still higher K_{ATP} channel density in ovariectomized females three weeks after the surgery.

It has been suggested that both antiarrhythmic and infarct size limiting effect of exogenous estrogen may be also mediated by myocardial mitoK_{ATP} channels (25, 259, 277). Endogen estrogen may also act on these effectors; mitoK_{ATP} channels. However the effect of the endogen estrogen on the expression of mitoK_{ATP} channels is unknown. The lower arrhythmic activity in female and ovariectomized female may involve in different expression of these channels.

Estrogen has been shown to potentiate parasympathetic activity on the heart (252). This result is supported by another study that found differences in heart rate between male and female following coronary ligation (253). Heart rate was found higher in males than females during ligation and after the vagotomy this differences was abolished. In contrast to these finding, in the present study, heart rate and blood

pressure are not different between males and females during ligation and reperfusion. This result was consistent with the studies by Humphreys RA. et al. (24). In the present study, ovariectomy did not generate any effect on heart rate in female rats as it was reported in a previous study (273). Differences in results related with heart rate may be originated from different anesthetic and their dose used during ligation period.

4.2. THE EFFECT OF K_{ATP} MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN MALES

The present results demonstrate that Pinacidil, a nonselective activator of K_{ATP} channels and HMR 1098, a selective sarcolemmal K_{ATP} channels blocker decreased ventricular arrhythmias during reperfusion. These results are coincident with previous *in vivo* (13, 17-19) and *in vitro* studies (192, 193) that Pinacidil was shown to decrease the arrhythmias. In those studies which Pinacidil used in 0.1 mg/kg decreased the arrhythmias in anaesthetized and conscious rats during ischemia and reperfusion (13, 18). In our previous study, Pinacidil was found antiarrhythmic in a dose of 1 mg/kg (19). In the present study we used Pinacidil in a dose of 0.1 mg/kg to avoid its hemodynamic effect. Pinacidil was also found to decrease arrhythmias at low dose. In the above studies it was suggested that the reduction in action potential duration by Pinacidil was thought to prevent intracellular Ca⁺⁺ overload which may be responsible for triggered activity and afterdepolarization (17-19, 194, 195). It has been also suggested that Pinacidil could further shorten the action potential duration of the slightly injured fibers in the heart during ischemia and reperfusion. This makes the injured myocardium more homogeneous and diminishes the development of life threatening ventricular arrhythmia (13, 18, 171).

HMR 1098, HMR 1883, a sulfonylurea plasma membrane K_{ATP} channel blocker was developed as a cardioselective sarcK_{ATP} channel blocker with significantly higher affinity to cardiac myocardial cells (214). HMR 1883 has been shown to reduce the incidence of myocardial ischemia-induced ventricular fibrillation in conscious dogs (226), arrhythmias induced by programmed electrical stimulation in anaesthetized dogs (287), arrhythmias induced by pretreatment with isoproterenol in anesthetized rats (215) and arrhythmias induced by ischemia and reperfusion in isolated rabbit hearts (288). In another recent study, HMR 1098 reduced the number of ventricular premature beats, but did not decrease the incidence of reperfusion induced ventricular fibrillation in isolated rat hearts (223). In more comparable studies with our result, HMR 1883 in a dose dependent manner 1, 3 and 10 mg/kg reduces the incidence of irreversible ventricular fibrillation during acute myocardial ischemia in conscious and anesthetized rats (229). HMR 1098 of a 3mg/kg dose decreased the ventricular arrhythmias during 30 min. of coronary ligation in rats (230). In the present study, similar dose of HMR 1098 was used and found to decrease duration of ventricular arrhythmia in reperfusion period.

In these studies the antiarrhythmic effect of the K_{ATP} channel blockers was attributed to the inhibition of the K^+ loss from jeopardized cells and decreased nonuniform shortening of the action potential duration during the progressive 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, antifibrillatory action. Apart from the inhibition of reentrant mechanisms based on the shortening of the action potential, K_{ATP} channel blockade may prevent extracellular potassium accumulation and the depolarizing effect of high extracellular potassium (9, 11-13,

18, 23, 214, 215). These mechanisms may also involve the antiarrhythmic effect of the HMR 1098.

The present results demonstrated that the inhibition of the mitoK_{ATP} channels by 5-HD, selective inhibitor of mitoK_{ATP} channels also decreased ventricular arrhythmias during reperfusion in male rats. There is relatively few data on the effect of 5-hydroxydecanoate on arrhythmias in the setting of myocardial infarction or ischemia/reperfusion. Niho T. et al. reported an antifibrillatory effect of 5-HD following coronary artery ligation in rats (289). However other studies showed no antiarrhythmic effect of 5-HD in dogs (290), in rabbits (143) and rats (229, 230, 291). In a recent more comparable study, 5-HD in a dose of 5 mg/kg did not decrease the ischemia and reperfusion arrhythmias in pentobarbitone anesthetized rats (229).

In the present study, the opening of the mitoK_{ATP} channels by Diazoxide, selective activator of mitoK_{ATP} channels decreased ventricular arrhythmias during reperfusion. This result is in line with the results of previous studies on isolated rat heart model (292). However, it has been also reported that that Diazoxide increases arrhythmia in isolated rat heart model (293). Vegh A. and Parratt JR. et al. have showed that the opening of mitochondrial K_{ATP} channel by Diazoxide suppressed the arrhythmias during coronary ligation in chloralose-urethane anaesthetized dogs (145). We haven't found any work investigated the effect of Diazoxide on ischemia reperfusion arrhythmia model.

In the present study, although Pinacidil decrease the score of arrhythmia and the duration of VF during reperfusion, the combination of 5-HD with Pinacidil abolished antiarrhythmic effect in reperfusion period. The combination of HMR 1098 with the Pinacidil did not affect antiarrhythmia produced by pinacidil. Similar results were observed in ischemia induced arrhythmia. Pinacidil treatment

significantly decreased the life threatening arrhythmias. However, pretreatment with 5-HD but not HMR 1098 abolished the antiarrhythmic effect of Pinacidil on ischemia reperfusion induced arrhythmias. This indicate that an antiarrhythmic effect of Pinacidil may be produced via the selective activation of mitochondrial K_{ATP} channels rather than sarcolemmal K_{ATP} channels. These results are supported by the studies performed by Das B. and Sarkar C. et al. in rabbits (143, 144, 294). In those studies the antiarrhythmic effects of 3-Pyridyl pinacidil, nicorandil, cromakalim and minoxidil were abolished when it is combined with 5-HD. In recent year Fischbach PS. and his collageus (207, 208) demonstrated that the proarrhythmic effect of Pinacidil was found to depend on the activation of the sarc K_{ATP} channels in *in vitro* studies in rabbits.

Despite its negative chronotropic effect (295-297), in the present study, heart rate in Pinacidil and Pinacidil+HMR groups was found higher than in the controls both before and during ligation. This may depend on the different response of animals to anesthesia, since heart rate was higher in Pinacidil and Pinacidil and HMR group already before drug administration.

4.3. THE EFFECT OF K_{ATP} MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN FEMALES

Pinacidil, HMR 1098, Diazoxide and 5-HD did not significantly affect the duration of arrhythmias in females. No comparable studies were found in the up to day literature. Although in female, Pinacidil itself was not found antiarrhythmic, the combination of HMR 1098 (selective sarc K_{ATP} channel blocker) with pinacidil significantly decreased the total duration of arrhythmias, and nonsignificantly decreased duration of VPC. These results suggest that the sarc K_{ATP} channel opening may be arrhythmogenic. This is supported by studies performed by Fischbach PS. et

al. (207, 208). In that work, proarrhythmic effect of Pinacidil was abolished by selective inhibition of the sarcK_{ATP} channels with HMR 1098 in both sexes of rabbits in isolated heart model (207).

The activation of the sarcK_{ATP} channels and the inhibition of the mitoK_{ATP} channels induced by the combination of the Pinacidil with 5-HD did not significantly affect the duration of arrhythmias during ischemia and reperfusion. Similarly the selective inhibition of the mitoK_{ATP} channels by 5-HD itself also did not affect the duration of arrhythmias in present study. This results show that the inhibition of the mitoK_{ATP} channels in addition to sarcK_{ATP} channel activation does not result in antiarrhythmic effect in females.

The sarcK_{ATP} channel blockage with HMR 1098 alone was also not found effective to decrease the arrhythmias in females. Similarly, the nonselective blockage of the K_{ATP} channels with glibenclamide was not found antiarrhythmic in previous study performed in female rats (23). This result suggest that blockage of mitoK_{ATP} channels by glibenclamide may involve in the absence of antiarrhythmic effect in reperfusion period. But in present study, although the sarcK_{ATP} channel is selectively blocked by HMR 1098 with no effect on mitoK_{ATP} channels, the arrhythmias induced by reperfusion did not decrease.

5-HD and Diazoxide were not found effective to decrease arrhythmia, but Diazoxide nonsignificantly decreased the score and total length of arrhythmias during reperfusion ($P>0.05$) similar to the combination of Pinacidil and HMR group. This result may indicate that the decrement in total length of arrhythmias provided by the combination of Pinacidil and HMR may have resulted from the opening of the mitoK_{ATP} channels .

In present study, Pinacidil was found antiarrhythmic in males but not in females. Recent years, Kocic I. et al. reported that estrogen specifically modulate Pinacidil action in guinea pig right atria (296). They suggest that sex hormones change the modulation of sarcK_{ATP} channel binding site for Pinacidil (297). Similarly, the estrogen hormone in female rats may prevent the modulation of sarcK_{ATP} channels with Pinacidil if it can change the modulation of channels in rat ventricular myocytes. The antiarrhythmic effect of Pinacidil was not abolished by the blockage of the sarcK_{ATP} channels with HMR 1098 in males. But in females, Pinacidil was only found antiarrhythmic when the activation of the sarcK_{ATP} channels produced by Pinacidil was blocked with HMR 1098. This results indicate that sarcK_{ATP} channel activation is arrhythmogenic stimuli in females. Since the numbers of sarcK_{ATP} channels are lower in males, opening of the channels may not be effective on arrhythmias. But since Pinacidil may be more effective on mitoK_{ATP} channel, it may decrease arrhythmia in males but not in females. The sarcK_{ATP} channel blockage induced by the HMR 1098 is also antiarrhythmic in males but not in females. These results may depend on the effect of estrogen other than on K_{ATP} channels in ischemia reperfusion induced arrhythmia (298).

NOS activation and NO generation is more in female (238, 239). Sato T. et al. (217) has revealed that NO directly activates mitoK_{ATP} channels and potentiates the ability of Diazoxide to open these channels. In the present study, the mitoK_{ATP} channel blockage by the 5-HD is an antiarrhythmic in males but not in females. 5-HD also did not increase the arrhythmias in females. These results show that the less frequent arrhythmias in females may result from opening of the mitoK_{ATP} channels induced by endogenous NO or due to the ineffectiveness of 5-HD to block mitoK_{ATP} channel in the presence of excess NO.

4.4. THE EFFECT OF K_{ATP} MODULATORS ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN OVARECTOMIZED FEMALES

In the present study, the blockage of sarcK_{ATP} channels with HMR 1098 treatment decreased the arrhythmias during reperfusion in ovariectomized females. Since there were no studies found related with the effect of ovariectomy on reperfusion induced arrhythmias, these results are not comparable. The effectiveness of HMR 1098 in decreased arrhythmia might be related with the effect of estrogen which increases the expression of the sarcK_{ATP} channels (26). It was reported that the production of NO is greater in female than male and ovariectomy decrease NO production in female so this abolishes the difference in NO level between female and male (234). In a previous study it was suggested that the less effect of NOS inhibition by L-NAME (L-nitro arginine methyl ester) on I/R arrhythmias in females was depended on more NO release in females than males (299). The intracellular level of NO may change the effect of HMR 1098 on K_{ATP} channel (300). In ovariectomized female, HMR 1098 can easily bind to K_{ATP} channel probably due to the decreased NO production and so HMR 1098 decrease reperfusion induced arrhythmia in female. Similarly the reason of nonsignificant effect of diazoxide to decrease arrhythmia may depend on the changes in the modulation of mitoK_{ATP} channel in the absence of NO. There was proarrhythmic effect of 5-HD in ovariectomized female during ligation period in respect to control. In the present study, this proarrhythmic effect was not observed in unovariectomized female. Kawahara K. et al (293) pointed out that there is a relation between mitoK_{ATP} channel activation and NO level. So the proarrhythmia produced by 5-HD during ligation period in ovariectomized female may depend on the changes in the modulation of mitoK_{ATP} channels in the lower level of NO.

5. CONCLUSION

As a conclusion, females have lower arrhythmic activity than males following ischemia and reperfusion in rats. Ovariectomy does not abolish this effect although plasma estrogen level decrease. This may indicate that there is no direct correlation between the plasma estradiol level and the cardioprotection in females against ischemia reperfusion arrhythmias.

Pinacidil was found antiarrhythmic in males but not in females. This may result from the changes in the modulation of the sarcK_{ATP} channels by estrogen (297). Pinacidil in female decreases the arrhythmias when it is combined with the sarcK_{ATP} channel blocker, HMR 1098. This result is thought that sarcK_{ATP} channel opening is proarrhythmic stimulus at least in females. Mitochondrial K_{ATP} channel activation produce antiarrhythmic stimulus in both sexes.

In male rats, the antiarrhythmic effect of Pinacidil was abolished by the blockage of the mitoK_{ATP} channels with 5-HD but not the blockage of the sarcK_{ATP} channels with HMR 1098. This indicates that the antiarrhythmic effect of nonselective activation of the K_{ATP} channels with Pinacidil is result from the activation of the mitoK_{ATP} channel.

Only in ovariectomized females, the blockage of the sarcK_{ATP} with HMR 1098 decreased the arrhythmias. This may indicate that estrogen may change either the expression of the K_{ATP} channel subtype or specific binding site for drugs.

6. FUTURE PROSPECTIVES

Studies to clarify whether the level of estrogen is important to change the expression of channels or binding sites to drugs, the effect of exogenous estrogen in ovariectomized female and the effect of ovariectomy on the expression of the sarcK_{ATP} channel proteins should be researched.

In the present study we have not used selective K_{ATP} channel opener. Selective sarcK_{ATP} channel opener, P-1075 should be studied in this model to clarify only the effect of the opening of the sarcK_{ATP} channels on ischemia reperfusion arrhythmias.

The activation of the mitoK_{ATP} channel and the inhibition of the sarcK_{ATP} channel may have strong antiarrhythmic effect. To correct this hypothesis, the combination of the Diazoxide (mitoK_{ATP} activator) and HMR 1098 (selective sarcK_{ATP} channel blocker) should be used in this model. The effect of bepridil which is selective mitoK_{ATP} channel opener and sarcK_{ATP} channel blocker on ischemia reperfusion arrhythmias can be studied.

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