ผลของสารขยายหลอดเลือดต่อการหดตัวของหัวใจและการเกิดหัวใจเต้นผิดจังหวะในหนูแรทที่เหนี่ยวนำให้เกิด
ภาวะหัวใจขาดเลือด

นางสาวพรหมพร รักษาเสรี

EFFECTS OF VASODILATORS ON CARDIAC CONTRACTILITY AND ARRHYTHMOGENESIS IN

ISCHEMIC- INDUCED RAT HEART

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Thesis Title   Effects of vasodilators on cardiac contractility and
arrhythmogenesis in ischemic-induced rat heart

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พรหมพร   รักษาเสรี : ผลของสารขยายหลอดเลือดต่อการหดตัวและกลไกการเกิดหัวใจเต้นผิด จังหวะในหนูแรทที่เหนี่ยวนำให้เกิดภาวะหัวใจขาดเลือด (EFFECTS OF VASODILATORS ON CARDIAC CONTRACTILITY AND ARRHYTHMOGENESIS IN ISCHEMIC- INDUCED RAT HEART) อ.ที่ปรึกษา : อ.น.สพ.ดร.สุวรรณเกียรติ สว่างคุณ   อ.ที่ปรึกษาร่วม : ผศ.น.สพ.ดร. วุฒิชัย กลมเกลียว จำนวนหน้า 68 หน้า ISBN 974-17-6467-7

ทำการศึกษาผลของสารขยายหลอดเลือดต่อการหดตัวและการเต้นผิดจังหวะของหัวใจหนูแรทที่ เหนี่ยวนำให้ขาดเลือดจำนวน 60 ตัว แบ่งออกเป็น 4 กลุ่มทดลอง กลุ่มที่ 1   เป็นกลุ่มควบคุมได้รับ สารละลายเครบส์บัฟเฟอร์ กลุ่มที่ 2 ได้รับสารละลายเครบส์บัฟเฟอร์ร่วมกับยาอะทีโนลอลซึ่งเป็นยาใน กลุ่มเบตาวัน อะดรีเนอร์จิก แอนทาโกนิสต์ กลุ่มที่ 3 ได้รับสารละลายเครบส์บัฟเฟอร์ร่วมกับยาอะทีโนลอล และซาลบูทามอลซึ่งเป็นยาในกลุ่มเบตาทู อะดรีเนอร์จิก อะโกนิสต์ และกลุ่มที่ 4 ได้รับสารละลายเครบส์ บัฟเฟอร์ร่วมกับยาอะทีโนลอลและพราโซซินซึ่งเป็นยาในกลุ่มแอลฟาวัน อะดรีเนอร์จิก แอนทาโกนิสต์ ทำ การแยกหัวใจเพื่อนำมาแขวนในแลงเกนดอร์ฟ ทำการวัดความดันในหัวใจห้องล่างซ้าย อัตราการเต้นของ หัวใจ คลื่นไฟฟ้าหัวใจ ความเร็วในหดตัวและคลายตัวของหัวใจ อัตราการไหลของของเหลวที่ไหลผ่าน หลอดเลือดโคโรนารี และความแปรปรวนของจังหวะการเต้นของหัวใจ หลังจากให้สารละลายและยาไหล ผ่านหลอดเลือดหัวใจเป็นเวลา 10 นาทีจึงทำการผูกหลอดเลือดแดง เลฟ แอนทีเรีย ดีเซนดิงเพื่อเหนี่ยวนำ ให้เกิดภาวะหัวใจขาดเลือดเป็นเวลา 8 นาทีและทำการคลายหลอดเลือดเพื่อเหนี่ยวนำให้เกิดหัวใจเต้นผิด จังหวะ หลังจากให้ยาพบว่ากลุ่มที่ 3 เพิ่มอัตราการเต้นของหัวใจและอัตราการไหลของของเหลวที่ผ่าน หลอดเลือดแดงโคโรนารีในขณะที่กลุ่มที่ 4 ลดอัตราการเต้นของหัวใจและลดอัตราการไหลของของเหลว ผ่านหลอดเลือดแดงโคโรนารี ในภาวะหัวใจขาดเลือด พบการบีบตัวของหัวใจและอัตราการไหลของเหลว ผ่านหลอดเลือดแดงโคโรนารีลดลงในทุกกลุ่มและพารามิเตอร์เหล่านี้จะเพิ่มขึ้นเมื่อคลายหลอดเลือด ทุก กลุ่มทดลองเกิดหัวใจเต้นผิดจังหวะแบบ ซัซเตน เวนตริคูล่า ฟิบริเลชั่นทั้งหมด ในกลุ่มทดลองกลุ่มที่ 4 ซึ่ง ได้รับยาอะทีโนลอลและพราโซซินเพียงกลุ่มเดียวที่ไม่เกิดหัวใจเต้นผิดจังหวะแบบซัซเตน   เวนตริคูลา ฟิบริเลชั่น   นอกจากนี้การให้ยากลุ่มอะดรีเนอร์จิกนี้ไม่พบว่าทำให้เกิดอะปอปโตซีสในเซลล์กล้ามเนื้อ หัวใจเพิ่มขึ้นจากกลุ่มควบคุม จากการทดลองสรุปได้ว่าการให้ยากลุ่มเบตาวัน อะดรีเนอร์จิก แอนทาโก นิสต์ร่วมกับแอลฟาวัน อะดรีเนอร์จิก แอนทาโกนิสต์ให้ผลดีในการต้านการเกิดหัวใจเต้นผิดจังหวะจาก ภาวะหัวใจขาดเลือด ผลของสารขยายหลอดเลือดที่ให้ผลเด่นชัดในการขยายหลอดเลือดโคโรนารีและมีผล ต่อการหดตัวของหัวใจหนูแรทคือ เบตาทู อะดรีเนอร์จิก อะโกนิสต์

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PROMPORN RAKSASERI : EFFECTS OF VASODILATORS ON CARDIAC CONTRACTILITY AND ARRHYTHMOGENESIS IN ISCHEMIC-INDUCED RAT HEART THESIS ADVISOR: INSTRUCTOR SUWANAKIET SAWANGKOON, Ph.D., THESIS COADVISOR: ASST.PROF. WUTTICHAI KLOMKLEAW, Ph.D. pp.68 ISBN 974-17-6467-7

The objective of the present study was to investigate the effects of vasodilators (β_2-adrenergic agonist and α_1-adrenergic antagonist) on cardiac contractility and arrhythmogenesis in ischemic induced - rat hearts. Sixty male spraque-dawley rat hearts were randomized into 4 groups: the first group was perfused with Krebs- Henseleit bicarbonate (KHB) buffer and used as control group; the second group was perfused with KHB buffer and 10 µM atenolol (ATEN); the third group was perfused with KHB buffer 10 µM atenolol and 0.01µM salbutamol (ATEN/SALBU); the fourth group was perfused with KHB buffer, 10 µM atenolol and 5 µM prazosin (ATEN/PRAZ). Rat hearts were isolated and mounted in Langendorff apparatus. Left ventricular developed pressure, dP/dt$_{max}$ and dP/dt$_{min}$, Vmax, ECG, coronary flow, and RR-variability were recorded and determined. After drug perfusion for 10 minutes, the left anterior descending artery was occluded for 8 minutes and then reperfused to induce cardiac arrhythmias. Coronary flow and heart rates increased after ATEN/SALBU perfusion and decreased after ATEN/PRAZ. Ischemia deteriorated cardiac contractility and coronary flow in all treated groups. Recovery of contractile function and coronary flow were observed after reperfusion. Ischemic-reperfusion induced sustained ventricular fibrillation in all groups except ATEN/PRAZ treated group. Increases in cardiac myocyte apoptosis induced by adrenergic drugs were not observed in this study. In conclusion, the combination of β_1-antagonist and α_1-antagonist has antiarrhythmic action in ischemic-induced rat heart. However, β_2-agonist showed vasodilatory effect and increases in cardiac contractility.

Department	Physiology	Student's signature……………………...
Field of study	Animal Physiology	Advisor's signature………………………..
Academic year	2004	Co-advisor's signature……………………..

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

Heart Failure (HF), which affects many millions people around the world, is associated with high mortality as a result of contractile dysfunction (pump failure) or sudden death caused by ventricular arrhythmias (Packer, 1985). About 40% of sudden death victims have cardiac arrest at the first manifestation of coronary artery diseases (Zipes and Wellens, 2000). In patients with known coronary heart disease, sudden death is frequently caused by sudden rupture of an unstable plaque, leading to occlusion of the coronary artery previously having only a slight narrowing.

Disturbances of cardiac rhythm, including lethal ventricular arrhythmias and post-ischemic contractile dysfunction are a consequence of reperfusion following pathological and clinical instances of myocardial ischemia. One possible mechanism which might contribute to contractile dysfunction is oxidative stress (Bolli, 1998; Aiello et al., 1995). In addition, increasing in sympathetic activity during myocardial ischemia is arrhythmogenic actions (Du et al., 1999). The fall in intracoronary pressure downstream from an occlusion is itself a significant cause of the immediate decline in pump function (Katz, 2001).

Heart tissue contains α_1-adrenergic receptors, which can initiate inotropic responses and release of atrial natriuretic factor, but they do not appear to contribute substantially to sympathetic responses under physiological conditions. However, α_1-adrenergic receptors appear to play a more important role under pathological conditions, such as myocardial ischemia and reperfusion, where extensive norepinephrine release has been documented (Schomig, 1990). α_1-adrenergic receptor stimulation is capable of inducing ventricular arrhythmias during both ischemia and reperfusion (Sheridan, 1986), a response not observed in non-ischemia tissue. Myocardial ischemia has been reported to double α_1-adrenergic receptor density in the rat myocardium and increase sensitivity to norepinephrine stimulation (Butterfield and Chess-William, 1990), both of

which may contribute to arrhythmogenesis. Reperfusion of ischemically damaged myocardium is associated with large Ca^{2+} accumulations, which can be inhibited by α_1-adrenergic receptor blockade. Furthermore, α_1-adrenergic receptor has been shown to be antiarrhythmic during both early ischemia and postischemic reperfusion (Kurz et al., 1991). These studies suggest a link between α_1-adrenergic receptor stimulation and arrhythmias produced by myocardial ischemia and reperfusion.

Many studies both in human and animal models have produced somewhat inconsistent results with respect to the functional level of β-adrenergic mediating signaling in ischemic heart diseases. A general feature of the failing human heart is a decrease in the density of cardiac β-adrenergic receptor (β-AR) that in most cases are due to a selective decrease in the density of β_1-AR leading to a shift in the β_1 and β_2–ARs ratio towards β_2–ARs (Bristow,1993). Thus, in the setting of left ventricular dysfunction in heart failure, alterations in the expression and function of myocardial receptor-signal transduction pathways may play an important role in the onset and progression of the clinical syndrome in chronic heart failure (Lamba and Abraham, 2000). It has been reported that β_2–AR are not down regulated, but its sensitivity may be increased (Altschuld et al., 1995). In addition, recent evidence shows that apoptosis of cardiac myocytes is a feature in several myocardial disease states, including ischemia heart disease and congestive heart failure (Haunstetter and Izumo, 2000). The common inducers of apoptosis include oxygen free radicals, oxidative stress and Ca^{2+}, which are implicated in the pathogenesis of myocardial ischemic reperfusion injury (Maulik et al., 1997). Besides, pharmacologic studies of cardiac myocytes in vitro demonstrate that β-adrenergic receptor can stimulate apoptosis (Shizukuda et al., 1998; Singh et al., 2000; Zaugg et al., 2000). Nevertheless, it has been reported that apoptotic death signaling is selectively mediated by β_1-adrenergic receptors and inhibited by β_2–adrenergic receptors in cardiomyocytes (Communal et al., 1999; Zaugg et al., 2000). This finding is of great clinical interest, because selective pharmacological activation of β_2–adrenergic receptors–mediated inotropy or its overexpression through gene therapy might be used as a novel therapeutic approach in the failing heart.

β-blockers are commonly used for treatment of ischemic heart disease

(Erdmann,1998). Possible mechanisms contributing to its effect are including cardiac protection from toxic catecholamine effect inducing apoptosis, but also reduction in heart rate leading to lower myocardial expenditure (Reiter and Reiffel, 1998), prolong diastolic filling, and protection from cardiac arrhythmias (Kennedy et al., 1994). Nevertheless, acute administration of first-generation compounds, such as propanolol causes a decrease in contractile state (Haber et al., 1993), increases systemic vascular resistance (Armstrong et al., 1977; Bristow et al., 1998), and leads to decrease in cardiac output, which results in drug intolerance rate of more than 20% (Talwar et al., 1996). On the other hand, the second generation, β_1-selective blockers are less reflex vasoconstriction because unblocked peripheral vascular β_2-adrenergic receptors can mediate vasodilatation. The overall effect is that cardiac output and organ perfusion are reduced to a lesser degree than with the first-generation β-blocker (Bristow et al., 1998).

Nowadays, it seems that the third-generation carvedilol, a non-selective β-blocker with vasodilating activity, is the most effective β-blocker for the treatment of myocardial ischemia. It has the advantage of afterload reduction to counteract the negative inotropic properties of adrenergic withdrawal. The vasodilator properties of this third generation agents allow patients to be more tolerate (Bristow, 2000). There are many studies both in human and animal models that carvedilol provides potent cardioprotection for compromised ischemic myocardium (Australia-Newzealand Heart Failure Research Collaborative Group, 1995; Yaoita et al., 2002), attenuates cardiac dysfunction (Basu et al., 1997), and reduces mortality in acute infarction followed by reperfusion. Compared to β_1-selective blockers, carvedilol produces more beneficial effects on left ventricular function (Gilbert et al., 1996; Bristow et al., 1997). However, some data also demonstrate no difference between both of them (Kulkin et al., 1999).

It is interesting that vasodilating effect of carvedilol mediated by α_1-adrenergic antagonist maybe beneficial to patients with ischemic heart disease. Therefore, the β_2-adrenergic stimulation may increase cardiac contractility and provide vasodilating effect to patients with ischemic heart diseases. However, it has been reported by Billman et al. (1997) that the activation of β_2–adrenergic receptors in dog ventricular myocardium increasing intracellular Ca^{2+} transients may provoke malignant arrhythmias. In dog

model, myocardial infarction provoked an enhanced β_2–adrenergic receptors response in susceptible dogs (Houle et al., 2001). Therefore β_1-adrenergic antagonist combined with β_2-adrenergic agonist might inhibit sympathetic stimulation whereas, β_2-adrenergic stimulation might increase cardiac contractility and increase coronary blood flow in ischemic heart disease.

The objectives of this experiment are to study the effect of the combination of β_1-antagonist and β_2-adrenergic agonist and the combination of β_1- antagonist and α_1-adrenergic antagonist on cardiac contractility, arrhythmogenesis, apoptosis of cardiac myocytes in ischemic induced-rat heart.

CHAPTER 2
LITERATURE REVIEW

Adrenergic system controlling cardiac function

The sympathetic nervous system plays an important role on controlling cardiac function by activating adrenergic receptors. In the human heart there are α_1-, β_1- and β_2-adrenergic receptors, and possibly α_2-adrenergic receptors (Brodde et al., 2001). The coexistence of β_1- and β_2-adrenergic receptors has been demonstrated on isolated human ventricular cardiomyocytes (Del Monte et al., 1993). The β_1/ β_2 ratio in human heart is about 60 -70%:40-30% in the atria and about 70- 80%:30 - 20% in the ventricles (Brodde, 1999). Rodefeld et al. (1996) have shown that in human sinoatrial nodes, β-adrenergic receptors density is about 3-fold higher than that of the adjacent atrial myocardium. Although β_1-adrenergic receptors subtype predominates, the β_2 adrenergic receptors density is about 2.5-fold higher in the sinoatrial node than in the right atrial myocardium.

β-adrenergic receptors controlling cardiac function

Stimulations of both β_1- and β_2-adrenergic receptors cause increases in heart rate and force of contraction. Only β_1- adrenergic receptors stimulation causes maximal increase in force of contraction whereas β_2 adrenergic receptors stimulation causes only submaximal increase in force of contraction (Kaumann et al., 1989; Motomura et al., 1990). Hall et al.(1989) have shown that intracoronary injection of the β_2 adrenergic receptors agonist, salbutamol, causes increase in heart rate which is not blocked by the β_1-adrenergic receptors antagonist, practolol, but is blocked by the nonselective β-adrenergic receptors antagonist propanolol. These data support the view that positive chronotropic effect is also mediated via direct stimulation of cardiac β_2-adrenergic receptors.

Both β_1- and β_2-adrenergic receptors in the human heart couple to G_s to activate adenylyl cyclase, and stimulations of both receptors subtypes increase the intracellular level of cAMP. This subsequently leads to activation of PKA, which phosphorylates several sarcolemmal proteins, including L-type Ca^{2+} channel and phospholamban (Kaumann and Molenaar, 1997). Phosphorylation of L-type Ca^{2+} channels promotes Ca^{2+} influx, and thus enhances contraction. Phosphorylation of phospholamban may be involved in diastolic relaxation by increasing ca^{2+} uptake into the sarcoplasmic reticulum. Kaumann et al. (1999) has demonstrated that norepinephrine and epinephrine hasten human ventricular relaxation and promote phosphorylation of implicated proteins through both β_1- and β_2-adrenergic receptors, thereby potentially improving diastolic function. In addition, β_2 –adrenergic receptors also exist in smooth muscle of human coronary arterioles and small arteries. The passive diameter of arterioles was 168±8 µm. Norepinephrine (NE) elicited concentration-dependent dilations 47±4 µm. The administration of 10^{-5} mol/L of propanolol (β_1 and β_2 –blocker) or 10^{-6} mol/L of butoxamine (β_2 –blocker) to isolated coronary arteries from human with left ventricular hypertrophy completely eliminated the NE- induced vasodilation. These results suggested that human coronary arterioles and small arteries response to NE via β_2-receptors on smooth muscle (Sun et al., 2002).

α - adrenergic receptor controlling cardiac function

In the human heart, the presence of α_1-adrenergic receptors has been demonstrated at the mRNA level, the protein level, and in functional studies. Saito et al. (1994) have also demonstrated that sinoatrial node and atrioventricular node of rat hearts contains α_1-adrenergic receptors. These receptors are designated α_{1A}, α_{1B}, α_{1D}. On a protein level, there are α_1-adrenergic receptors; however, the density of α_1-adrenergic receptors is by far less than that of β-adrenergic receptors. The α_{1A}-adrenergic receptors is the most abundant α_1-adrenergic receptors subtype in the human heart (Brodde et al., 2001), but Luther et al. (2001) has clarified that α_{1B}-adrenergic receptors was preferentially expressed in the non-cardiomyocyte cell fraction. Early studies on the signal transduction mechanisms have shown that α_1-adrenergic receptors couple

primarily via a PTX-insensitive G-protein ($G_{q/11}$) to the phospholipase C/ inositol tris-phosphate/diacylglycerol (PLC/IP_3/DAG) system. It has been demonstrated in the human right atrial and ventricular tissues that noradrenaline increased formation of inositol phosphates via α_1-adrenergic receptors.

The stimulation of α_1-adrenergic receptors causes positive inotropic effects. By the use of phenylephrine in the presence of β-adrenergic receptor antagonists, positive inotropic effects could be demonstrated in human atria and ventricle preparations; the maximal inotropic effect was only 15-35% of that evoked by β-adrenergic receptors stimulation. Zhang et al. (1999) has demonstrated that α_{1A} and α_{1B} play a prominent role in the positive inotropic response to noradrenaline. Although α-adrenergic stimulation exerts the positive inotropic effect, it does not contribute to the maintenance of basal left ventricular contractile state in humans (Landzberg et al., 1991). The mechanism of positive inotropic effect induced by α_1-adrenergic receptor is still unclear. However, IP_3 formation released from the α_1-adrenergic receptor stimulation may cause the release of ca^{2+} from intracellular stores which could be involved in increase in force of contraction.

Ischemic-reperfusion heart

Ischemic heart diseases often occur as a result of an obstruction in the vascular bed, leading to compromised blood circulation. This results in ischemia (inability to provide adequate oxygen) to heart muscle and this can cause damage to the heart muscle. Complete occlusion of the blood vessel leads to heart attack and sudden death. When the ischemic myocardium is reperfused and oxygen reintroduced, there is a sudden burst of oxygen free radical production. This leads to the formation of other damaging reactive oxygen species such as hydroxyl radicals, hydrogen peroxide, and peroxynitrite. These reactive oxygen species damage cell membranes and impair cellular function. Studies have shown that scavenging oxygen free radicals can reduce reperfusion-associated ventricular dysfunction, arrhythmias, and infarct size (Bernier et al., 1986; Liu et al., 1997; Opitz et al., 1998).

Studies in animal models have produced somewhat inconsistence results with respect to the functional level of β-AR-mediated signaling in ischemia heart disease. Wistar rats with ischemic heart failure induced by coronary ligation show no changes in $G_s\alpha$ and $G_i\alpha$ concentrations. Myocardial NaF- and forskolin-stimulated adenylyl cyclase activities are significantly decreased, suggesting the presence of myocardial $G_s\alpha$ dysfunction that may contribute to the contractile abnormalities in ischemic heart failure (Yamamoto et al., 1994). β-AR down regulation has been demonstrated in animal models with heart diseases (Schmedtje et al., 1996) whereas human ischemic cardiomyopathy per se does not appear to significantly influence β-AR signaling. Not only β- adrenergic receptor but also α-adrenergic receptor was involved in ischemic-reperfusion. Hwang et al. (1996) has detected an increase in α_1-AR expression in ischemic hearts.

Ischemic-reperfusion and arrhythmogenesis

Disturbances of cardiac rhythm, including lethal ventricular arrhythmias and postischemic contractile dysfunction are consequences of reperfusion following myocardial ischemia (Manning and Hearse, 1984). Arrhythmias often occurred following reperfusion. It is postulated that arrhythmias result from alterations in membrane ionic currents or cell to cell coupling. These alterations may due to (1) sympathetic cathecolamine release and elevated intracellular cAMP (2) increase in oxidative stress and oxidative damage (Aiello et al.,1995), (3) alterations in ionic gradients, including nonhomogeneous changes in extra cellular K^+ content, increased intracellular levels of H^+, Ca^{2+} and/or Na^+, (4) depletion of high energy phosphates.

Several studies have suggested an important role for sympathetic nervous system activation on initiating arrhythmias following myocardial infarction, reperfusion or heart failure. High level of circulating cathecolamines as well as noradrenaline spillover (Esler et al., 1997) is associated with increased incidences of arrhythmias and mortality (Ferguson, 1997). Activation of sympathetic nerves, especially stimulation of left cervicothoracic ganglion, induces ventricular arrhythmias under conditions of ischemia,

infarction and heart failure (Schwatz et al., 1992; Du et al., 1999). Stimulation of β_2-adrenergic receptors in ischemic dog hearts increases intracellular calcium transient and provoke ventricular arrhythmia (Billman et al., 1997). Transgenic mice with cardiac over expression of $G_s\alpha$ have increase incidences of arrhythmias and mortality (Iwase et al., 1996). The importance of locally mediated release of noradrenaline, with subsequent activation of α_1-adrenergic receptors, in ischemia reperfusion, arrhythmias has been also investigated. Du et al. (1995) have shown that inositol triphosphate (Ins (1, 4, 5) P_3) release plays a pivotal role in mediating arrhythmias during early reperfusion. In addition, a good correlation between the quantity of noradrenaline release or the extent of α-adrenergic receptors activation and the severity of arrhythmias has been reported (Du et al., 1995; Schomig, 1990). In contrast, Chess-William et al. (2001) support hypothesis that myocardial α-adrenergic receptors did not have a primary role in arrhythmogenesis. It supports a role for these receptors in myocardial protection.

Additionally, oxygen radicals causing peroxidation of lipids and proteins may also play an important role in arrhythmogenesis and contractile dysfunction in ischemic-reperfusion. Direct effects of free radicals on Ca^{2+} regulating mechanisms of the cell as well as the contractile proteins and various ionic membrane currents have been described. Oxygen radicals also inhibit critical enzymes in anaerobic and aerobic metabolic pathways, which may limit the metabolic reserve of reperfused myocardium and contribute to intracellular Ca^{2+} overload (Heyndrickx, 2003). Inhibiting free radical accumulation during myocardial ischemia/reperfusion by free radical scavengers has been demonstrated to reduce the severity of myocardial stunning, irreversible injury, and reperfusion arrhythmias in many, but not all, studies (Bernier et al., 1986; Goldhaber and Weiss, 1992). There was an investigation in guinea pig right ventricular wall by Aiello et al. (1995). Pretreatment with scavenger cocktail affected neither electrical nor contractile activities before or during no-flow ischemia, but it accelerated recovery of resting membrane potential and action potential duration during reperfusion. Moreover, it reduced the incidence of tachyarrhythmia and improved contractile function. Some β-blockers have free radical scavenging effect such as carvedilol, a vasodilating β-blocker

with potent antioxidant activity has been to prevent myocardial ischemia-reperfusion–induced apoptosis without restoration of β- adrenergic adrenergic receptor density (Flesch, 1999). In addition, prazosin which is an α_1- antagonist was also shown to have hydroxyl scavenging effect and attenuate lipid peroxidation in the heart (Akahira et al., 1998).

There are some evidences indicated that involvement of alteration in ionic gradients produces cardiac arrhythmias.   During subsequent ischemia-reperfusion, enhanced and maintained glycolysis would occur leading to more pronounced intracellular acidosis, Na^+ and Ca^{2+} overload via Na^+/H^+ and Na^+/Ca^{2+} exchangers, and increased susceptibility to arrhythmias (Tani and Neely, 1989; Scholz et al., 1995).   One of the best established pathways is that protons produced during ischemia leave the myocytes on the Na^+/H^+ exchanger during reperfusion, and cause Na^+ loading. Subsequently, Ca^{2+} loading occurs as Na^+ leaves the cell on the Na^+/Ca^{2+} exchangers. The resulting rise in $[Ca^{2+}]i$ is believed to trigger Ca^{2+} - activated proteases and phospholipases that cause the cellular damage (Xiao and Allen,1999). Influx of Ca^{2+} into myocytes via Na^+/Ca^{2+} exchanges may be stimulated by the high levels of intracellular Na^+ and changes in membrane potential known to occur during ischemia-reperfusion. Pogwizd et al. (2001) has shown that Na^+/Ca^{2+} exchange upregulation appears to be critical link between contractile dysfunction and arrhythmogenesis.

β-blockers in treatment of ischemic heart disease

Beta blockers are one of current used drugs for angina pectoris and antiarrhythmias which are characterized as class II in the Vaughan Williams classification of antiarrhythmic agents. However, they also have therapeutic effects in many other clinical disorders including systemic hypertension, hypertrophic cardiomyopathy, mitral valve prolapse, and silent myocardial ischemia. Beta blockers have been effective drug for reducing the cardiovascular mortality from myocardial infarction (Sleight, 1986).

The chronically increased cardiac sympathetic drive in the failing human heart causes deleterious adverse effects on the cardiac myocyte via stimulation of β-

adrenergic pathway. Long term treatment with β-blocker in patients with chronic heart failure has beneficial effects (Bristow et al., 1997; Lechat et al, 1998). One possible mechanism of beneficial effects of β-blockers could be that they up regulate the cardiac β-adrenergic receptors. Because the human heart contains only a few spare β-adrenergic receptors (Brodde et al., 1995), such an up-regulation would be helpful in restoring maximal contractile response to β-adrenergic receptors stimulation.

Long-term treatment of patients with coronary disease with β_1-adrenergic receptor antagonists such as atenolol, metoprolol, or bisoprolol sensitizes cardiac β_2-adrenergic receptors function in vitro (Hall et al.1990; Motomura et al., 1990) and in vivo (Hall et al, 1991). Another possible mechanism could be that β-adrenergic receptor antagonists decrease heart rates. This might shift the force frequency relationship toward lower rates of beating, and may improve contractility in patients with chronic heart failure (Brodde et al., 1999). It seems that the effect of β-blockade on ischemia cardiac function depends on the different properties of β-blockers and the doses used. The oral administrations of carvedilol, a non-selective β-blocking and vasodilating effect, metoprolol (β_1- selective blocker), propanolol (a non-selective β-blocker) were performed in rats with coronary stenosis and coronary occlusion *in vivo*. Carvedilol at relatively high doses (10 and 30 mg/kg BW) attenuated the increase in left ventricular end- diastolic diameter (LVEDD) and left end- systolic diameter(LVESD) including increases in myocardial blood flow. At 30 mg/kg BW, Carvedilol attenuated the decrease in left ventricular ejection fraction in rats with coronary stenosis though it did not attenuate them at any doses in rats with permanent coronary occlusion. Propanolol and metoprolol at the same doses have shown similar effects as carvedilol on lowering heart rate and systolic blood pressure in the resting state and tended to attenuate the increase in LVEDD. Carvedilol at doses 30 mg/kg BW, modulation of left ventricular function and morphology is likely to be associated intimately with an increase in myocardial blood flow and coronary flow rate. Increases in MBF and CFR seem to be attributable to improvement of coronary microvascular dilating function. In addition, carvedilol at 30 mg/kg BW reduced the level of ascorbyl free radical which reflected the antioxidant

activity. The antioxidant activity may have contributed to the improvement of coronary microcirculation in coronary stenosis models. These results suggested that carvedilol provided potent cardioprotection for compromised ischemic myocardium compared to metoprolol and propanolol (Yaoita et al., 2002).

Atenolol (β_1-selective antagonists)
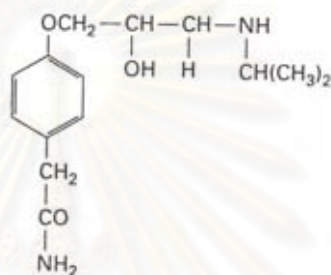
Figure 2.1 Structure of atenolol (β_1-selective antagonist)

Atenolol, a water soluble agent, has strong β_1- selective blockade about 30 fold (Abrahamsson et al., 1988). It has no intrinsic sympathomimetic activity and membrane stabilizing activity. It has been reported that atenolol was used for patients with hypertension, angina pectoris. Atenolol also reduces the risk of cardiovascular mortality in survivors of myocardial infarction (Sleight, 1986). Atenolol slows heart rate, and decreases cardiac contractility leading to decline of cardiac output. In isolated rat heart model, 10 µM atenolol had no effect on cardiac performance after drug perfusion. It also significantly protected against ischemic-reperfusion-induced change in cardiac performance and sarcoplasmic reticulum function (Temsah et al., 2000). The protective effect against free radical produced by atenolol has been reported. Pretreatment of canine cardiomyocytes membranes with 20 and 200 µM resulted in decreases of malondialdehyde formation (84.3 and 73.0% respectively) (Tong Mak and Weglicki, 1988). Because free radicals are mediated in cardiac arrhythmias, atenolol also has antiarrhythmic actions. It appeared to decrease the incidence of supraventricular tachycardia, ventricular tachycardia and left ventricular mass index in patients with left ventricular hypertrophy when treated for 6 months (Novo et al., 2001).

Effects of vasodilators on the cardiac function

Prazosin (α_1-selective antagonist)
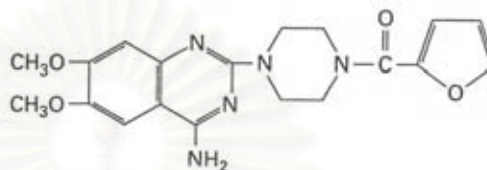
Figure 2.2 Molecular structure of prazosin (α_1-selective antagonist)

Prazosin exerts a vasodilating effect by reversible blockade of post-synaptic α_1-adrenergic receptors. The blockade of α_1-adrenergic receptors results in relaxation of vascular smooth muscle. This drug was used as first-step antihypertensive agent accompanying with little or no increase in heart rate, plasma renin activity, or circulating catecholamines. It has been attributed by some investigators that prazosin produces negative chronotropic effect independent of its peripheral vascular effect (Ribner et al., 1982). In addition, α-adrenergic blocking drugs appear particularly attractive for use in the treatment of heart failure because they hold possibility of reproducing balanced reductions in resistance and capacitance beds. Studies evaluating the acute hemodynamic effects of prazosin in patients with congestive heart failure consistently find significant reduction in systemic and pulmonary vascular resistances and left ventricular filling pressures associated with increases in stroke volume. In most studies, there was no change in or decrease in heart rate. Because α-adrenergic receptors mediate coronary vasoconstriction, a pathologic alteration of this system may be the mechanism of coronary spasm in some patients with angina pectoris (Orlick et al., 1978). According to arrhythmias, it has been postulated that enhanced α-adrenergic responsiveness occurs during myocardial ischemia and that is the primary mediator of the electrophysiologic derangements. Myocardial ischemia has been reported to doubled α_1-adrenergic receptor density in rat myocardium (Butterfield and Chess-

Williams, 1990). The enhancement of α_1-adrenergic receptor results in malignant arrhythmias induced by catecholamines during myocardial ischemia and reperfusion (Sheridan, 1986). There have been favorable reports of the use of α-blocker in the treatment of ventricular arrhythmias (Hanaki et al., 1988; Dabrowska et al., 1995).

Salbutamol (β_2-adrenergic agonist)
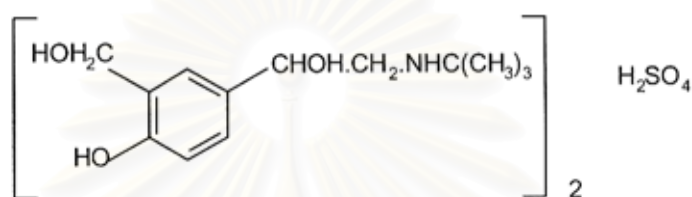
Figure 2.3 Molecular structure of salbutamol (β_2-adrenergic agonist)

Salbutamol is a selective β_2-adrenergic receptor agonist. At the therapeutic dose it acts on the β_2-adrenergic receptors of pulmonary bronchial muscle with little or no action on the β_1-adrenergic receptors of the cardiac muscle. Salbutamol stimulates the production of cyclic AMP, enhances the binding of intracellular calcium to cell membrane and sarcoplasmic reticulum (Hool et al., 1997).

The cardiovascular effects of salbutamol in humans have been described. The study was conducted in 5 healthy human volunteers to compare the effect on heart rate and blood pressure. Single dose of salbutamol at 4 mg was orally administered after 4 mg propanolol or 50 mg metoprolol. Salbutamol alone increased heart rate significantly. The maximum change was +18 beats/ minute. After metoprolol the maximum change was +7 beats/ minute. Propanolol completely blocked salbutamol-induced increase in heart rate. According to this experiment, metoprolol had partially blocked whereas propanolol completely blocked the effects of salbutamol on heart rate. These data suggested that salbutamol increases heart rate through β_1 and β_2-adrenergic receptors. Moreover, salbutamol also increases maximal change in systolic

blood pressure +12 mmHg and decreases maximal change in diastolic blood pressure 6 mmHg. It appeared that salbutamol which acted via β_2-adrenergic receptors was involved more in chronotropic than inotropic action (Shrivastava et al., 1985). In agreement with Hall et al.(1989) who showed that intracoronary injection of the β_2 – adrenergic receptor agonist, (salbutamol) cause increase in heart rate which is not affected by the β_1-adrenergic receptor antagonist (practolol) but is blocked by the nonselective β-adrenergic receptor antagonist (propanolol). These data support the view that positive chronotropic effects are mediated via direct stimulation of cardiac β_2-adrenergic receptors.

The role of sympathoexcitatory cardiac β_2-adrenergic receptor was described by Newton et al. (1999). Salbutamol was infused into the left coronary artery in 3 groups of patients: group1 with no β-blockade therapy, group2 with atenolol therapy and, group 3 with nadolol (nonselective β-blockade therapy). Left ventricular +dP/dt in response to increasing concentrations of salbutamol were measured. Salbutamol resulted in a 44±6% significantly increase in +dP/dt in group1, a 25±6% increase in group2, and no increase in group3. Salbutamol also resulted in a 124±37% increase in cardiac norepinephrine spillover in group1.Evidence that salbutamol increased NE release from cardiac sympathetic nerves was provided by the observation that atenolol suppressed inotropic response, demonstrating that this response was mediated in part by β_1-adrenergic receptors. This provided in vivo evidence in humans for the role of sympathiexcitatory cardiac β_2 –adrenergic receptors.

As we known, β_2 adrenergic receptors are arrhythmogenic. Long term efficacy and potential side effects of oral salbutamol in the treatment of congestive heart failure has been studied in human. Oral administration of 6 mg. salbutamol increased cardiac index (1.9 to 2.3 L/min/M2) and heart rate (92 to 97 bpm). Salbutamol increased the number of patients having ventricular tachycardia from two to six. Salbutamol also increased the number of episodes of ventricular tachycardia from 2 to 27 (Mettauer et al., 1985). In addition, there was a study in humans which defined the effects of β_2-

adrenergic receptor stimulation on ventricular repolarization *in vivo*. Intravenous and intracoronary salbutamol 10-30 µg/min and 1-10 µg/min were infused during fixed atrial pacing. Salbutamol decreased QT $_{onset}$ and QT $_{peak}$ but increased QT $_{end}$ duration, resulting in T wave prolongation (201msec to 233 msec ;p<0.01). The increase in dispersion of repolarization provided a mechanism whereby catecholamines acting through this receptor subtype may trigger ventricular arrhythmias (Lowe et al., 2001).

Cardiac myocytes apoptosis

Apoptosis is a process of transcriptionally regulated, programmed cell death. It has been noted that apoptosis contributes to pathophysiology of myocardial failure. It is observed in experimental heart failure due to multiple intracoronary embolization, tachycardia, postmyocardial infarction, or left ventricular hypertrophy, are seen in human hearts with myocardial infarction, ischemic cardiomyopathy, and idiopathic dilated cardiomyopathy (Shizukuda et al.,1998). Assessment of apoptosis was usually performed using a combination of terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) by light microscopy and genomic deoxyribonucleic acid (DNA) ladder detection (Chesley et al., 2000: Scarabelli et al., 2001).

Apoptosis contrasts to necrosis in the modes of cell death. In apoptosis, plasma membrane is intact until late in process whereas it is destroyed early in necrosis. Chromatin condensation (pyknosis), nuclear fragmentation (karyorhexis) and cell shrinkage give the appearance of apoptotic body. In necrotic cell, mitochondrial swelling occurs but, there was no swelling of mitochondria until late process of apoptosis. Following the definition of apoptosis, its biochemical characteristics were elucidated that adenosine triphosphate (ATP) requires step-wise DNA fragmentation culminating in the formation of mono- and/or oligomers of 180-200 base pairs by endonucleases. In contrast, necrosis shows nonspecific degradation of DNA. Specific proteases are required for protein degradation in apoptosis whereas nonspecific proteases can be involved in case of necrosis. Finally, apoptotic cells are eliminated with little inflammation

while necrotic cells will have leakage of contents and secondary inflammation will be occurred (Yaoita et al., 2000).

Ischemic –reperfusion and cardiac myocyte apoptosis

Ischemic/reperfusion induced cardiac myocyte apoptosis has shown both in animal models (Maulik et al., 1997: Inserte et al., 2000: Palojoki et al., 2001) and in human (Olivetti et al., 1997). Induction of apoptosis in ischemic/reperfused hearts has been suggested to be mediated by a variety of pathways which may or may not be interrelated, including: (a) activation of Fas or tumor necrosis factor-α (TNFα) receptors, (b) activation of p53 and c-Jun kinase pathways, (c) downregulation of antiapoptotic Bax protein and upregulation of proapoptotic Bax protein, (d) infiltration and activation of neutrophils and or macrophages. These pathways are more likely to be involved during reperfusion whereas ischemic itself may initiate apoptosis via a mitochondrial pathway.

Mitochondria play several roles in apoptosis: (a) they supply ATP that is necessary for execution of apoptosis; (b) they release cytochrome c and apoptosis-inducing factor proteins that they are involved in caspase activation and nuclear fragmentation; (c) they release proteins (second mitochondria-derived activator of caspases that neutralize endogenous inhibitors of apoptosis. There is a report demonstrated whether ischemic alone induce apoptosis. Scarabelli et al. (2001) have shown that ischemia alone is not sufficient to complete the apoptotic death of myocyte and nonmyocyte cells accessed by TUNEL and electron microscopy. In Langendorff mode, 35-minutes occlusion of the left coronary artery was performed followed by 5-60 minutes of reperfusion. The endothelial cells of large coronary vessels become TUNEL positive. Although TUNEL-positive cells were seen after ischemia alone, some endothelial cells were stained with an antibody that recognized the cleaved from caspase-3. This suggests that ischemia without reperfusion can initiate the molecular pathway of apoptosis, although reperfusion is required to complete DNA fragmentation and morphological changes characteristic of an end-stage apoptotic cell.

The common inducers of apoptotic include oxygen free radicals, oxidative stress and Ca^{2+} which are implicated in the pathogenesis of myocardial ischemic reperfusion injury. Maulik et al. (1997) have shown that the presence of apoptotic cells and DNA fragmentation in the myocardium are abolished by preperfusing the hearts in the presence of ebselen, which also removed the oxidative stress developed in the heart. These results may indicate that oxidative stress can induce apoptosis in cardiac myocytes. The possible mechanism involved in apoptosis is caspase activation. The major intermediate regulator of caspase activation is a mitochondrial pathway. At present two apoptogenic factors are known; ∼ 50 kDA apoptosis-inducing factor (AIF) and cytochrome *c*. AIF which acts by activating caspases does not require cytochrome *c* to exert its apoptotic action. Cytochrome *c* itself is not apoptogenic but holocytochrome *c*, which lacks a heme group, is released reversibly from mitochondria injured by numerous factors including reactive oxygen species and Ca overload. Cytochrome *c* binds to apoptotic protease-activating factor-1 (Apaf-1). Since Apaf-1 has an ATP binding site, this activates the caspase cascade in an ATP-dependent manner. Caspase activation via release of cytochrome *c* from mitochondria is induced by oxidative stress to the cytoplasm or mitochondria and possibly by unidentified signals from activated death factor receptors at the plasma membrane, such as Fas and tumor necrosis factor (TNF)-receptors. In the presence of deoxy-ATP, Apaf-1 activates caspase-9, which is an upstream enzyme in the caspase cascade, and then caspase-3 is activated. Caspase-3 induces Caspase activated DNase (CAD) activation, which leads to DNA fragmentation, and it also cleaves cytoskeletal proteins such as actin, fodrin and lamin, leading to alteration of cytoskeleton (Yaoita et al., 2000).

It has been postulated that exposure to high level of catecholamine might be toxic to cardiac myocytes. Norepinephrine stimulates apoptosis in adult rat cardiac myocytes in vitro. This effect was completely blocked by propanolol (non-selective β-adrenergic antagonist) but was not affected by prazosin (α_1 adrenergic antagonist). This result revealed that apoptosis acts via β-adrenergic pathway (Communal et al., 1998). In transgenic mice overexpression of β_1-adrenergic receptor or $G_\alpha s$ is

associated with myocyte apoptosis and the development of dilated cardiomyopathy. β_1-AR stimulate apoptosis *in vitro* and *in vivo* whereas β_2-AR may either stimulate or inhibit apoptosis and myocardial failure depending on the level of expression (Singh et al., 2000). It has been elucidated by Zaugg et al. (2000) that atenolol, a β_1-adrenergic antagonist, abolished the norepinephrine-induced increase in nick end-labeling (TUNEL)-positive cardiomyocytes but, ICI118551, a highly selective β_2-AR antagonist, did not decrease the percentages of norepinephrine, isoproterenol, and albuterol (β_2-AR agonist)–induced apoptosis. These observations provide evidence that β-AR-mediated apoptotic death signaling is largely dissociated from β_2-ARs and selectively mediated by β_1-ARs in adult rat ventricular myocytes. In agreement with Chesley et al. (2000) who has demonstrated that β_2–ARs activate phosphatidylinositol-3'-kinase-dependent, pertussis toxin-sensitive signaling pathway in cardiac myocytes that is required for protection from apoptosis-inducing stimuli often associated with ischemia stress. Because β-adrenergic is associated with apoptosis, there were many reports which demonstrated the antiapoptotic properties of β-blocker. Chronic therapy with metoprolol (β_1-selective blocker) attenuates apoptosis in dogs with heart failure. Metoprolol not only improved left ventricular ejection fraction but also attenuate progressive left ventricular remodeling in heart failure. In heart failure, apoptosis also has been shown to occur with decrease in left ventricular function. DNA fragmentation, marker of apoptosis, was reduced after three months therapy with metoprolol in dogs with heart failure induced by intracoronary microembolizations (Subbah et al., 2000). In addition, carvedilol, a non-selective β-blocker with α_1-blocker, also had antiapoptotic properties and reduced infarct size (Yue et al., 1998).

Nowadays, several lines of evidence suggest that antiapoptotic treatment might become a new therapeutic option. Cardioprotective agents must act at the pre-mitochondrial stage of apoptosis. Interventions acting only at a post-mitochondrial stage may reduce apoptosis but may not reduce infarct size (Yaoita, 2000).

CHAPTER 3

MATERIALS AND METHODS

Experimental animals

60 male Spraque-dawley rats weighing about 250-350 grams were used in these experiments (46 male Spraque-dawley rats were used to determine cardiac contractility, heart rate, R-R variability, coronary flow, and ECG and 60 male Spraque-dawley rats were used to determine incidences of arrhythmias and arrhythmias score). They were randomly divided into 4 groups.

1. The first group (control) was perfused with Krebs-Henseleit bicarbonate (KHB) buffer in the absence of any drugs.

2. The second group (ATEN) was perfused with KHB buffer in the presence of 10 μM atenolol (Sigma-Aldrich[®], MO, USA), β_1-selective antagonist.

3. The third group (ATEN/SALBU) was perfused with KHB buffer in the presence of 10 μM atenolol and 0.01μM salbutamol (Ventolin nebules[®], OH ,USA), β_2-selective agonist

4. The fourth group (ATEN/PRAZ) was perfused with KHB buffer in the presence of 10 μM atenolol and 5 μM prazosin (Sigma-Aldrich[®], MO, USA), α_1- selective antagonist).

The 10 μM concentration of atenolol for use in this study was based on the work of other investigator (Temsah et al., 2000). The 5 μM concentration of prazosin was based on Akahira et al. (1998). The 0.01 μM concentration of salbutamol was based on ED$_{50}$ (MDS Pharma Services).

Isolated rat heart preparation (Langendorff apparatus)

Spraque-dawley rats were anesthetized with pentobarbital (60 mg/kg) intraperitonially. After intravenous administration of heparin (500 IU/kg), the chest was opened, the hearts were rapidly excised and canulated on a non-recirculating Langendorff perfusion apparatus (Maulik et al., 1997). Retrograde perfusion was established at a pressure of 80 mmHg with an oxygenated normothermic (37°C) Kreb-Henseleit bicarbonate (KHB) buffer with the following ion concentration (in mM): 118 NaCl, 24.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.7 $CaCl_2$, and 10.0 glucose. The KHB buffer had been previously equilibrated with 95% O_2 / 5%CO_2, pH 7.4 at 37°C. The oxygen saturation of the buffer measured by blood gas analyzer was 99%.

Experimental protocol

To monitor isometric tension developed by the left ventricle, a latex balloon tipped catheter connected with pressure transducer was inserted into the left ventricle via small incision made in the left atrium.

The balloon was filled with saline until left ventricular diastolic pressure was 10 mmHg. Two electrodes were placed on the cardiac epicardium to monitored electrograms. The positive pole was placed on the surface of the right atrium and the negative pole was placed on the apex of the heart. To obtain stable cardiac function, all hearts were placed for a period of 10 minutes before baseline recording. The pressure transducer was connected to a physiograph (Grass Model 79, Grass Instruments Co., Mass., USA) and then data were transferred to a computer by an A/D converter (Powerlab ADInstruments, CO, USA). The left ventricular pressure was recorded at a sample rate of 400 samples / sec for 30 seconds and electrogram was recorded at a sample rate of 100 samples / sec for 5 minutes. Hearts were then perfused with Krebs - Henseleit buffer (KHB) in group 1, 10μM atenolol in group2, 10μM atenolol + 0.01μM salbutamol in group3, and 10μM atenolol + 5 μM prazosin in group 4 for 10 minutes and the data were recorded the same as the baseline period.

After that, the left anterior descending artery was ligated for 8 minutes to induce ischemia and then reperfused for 60 minutes by unligating the coronary artery. At first 30 minutes of reperfusion, left ventricular pressure (LVDP) and heart rate were monitored via the water-filled latex balloon inserted to left ventricle.

Determination of Left ventricular developed pressure

Left ventricular developed pressure was defined as the difference between systolic pressure and diastolic pressure (Tosaki et al., 1998).

LVDP=SP-DP (mmHg)

Determination of rate of rise and fall (dP/dt$_{max}$, dP/dt$_{min}$)

The maximum first derivative of left ventricular developed pressure (LV$_{max}$dP/dt) was made at each wave of LVDP at the period of isovolumic contraction and relaxation (Tosaki et al., 1998).

dP/dt$_{max}$ is commonly used as an index of cardiac contractility while dP/dt$_{min}$ is used as an index of cardiac relaxation.

Determination of heart rate

The heart rate was calculated by the apparent QRS complex in a minute.

Determination of coronary flow rate

Coronary flow rate was measured by a time collection of the coronary effluent that dripped from the heart (Tosaki et al., 1998).

Determination of V_{max}

V max was determined by the method of Mason et al. (1971). Extrapolation of the pressure-velocity descending limb to zero load allows estimation of the maximal intrinsic velocity of the contractile elements or V_{max}.

V_{max} is related directly to the contractile state of heart muscles, and this measure is not altered by variations of the end-diastolic fiber length.

V_{CE} = dP/dt/ (Kx IP)

IP = isovolumic pressure   (Left ventricular developed pressure)

K is series elastic constant of 32 per muscle length at body temperature

V_{max} = V_{CE} at zero load
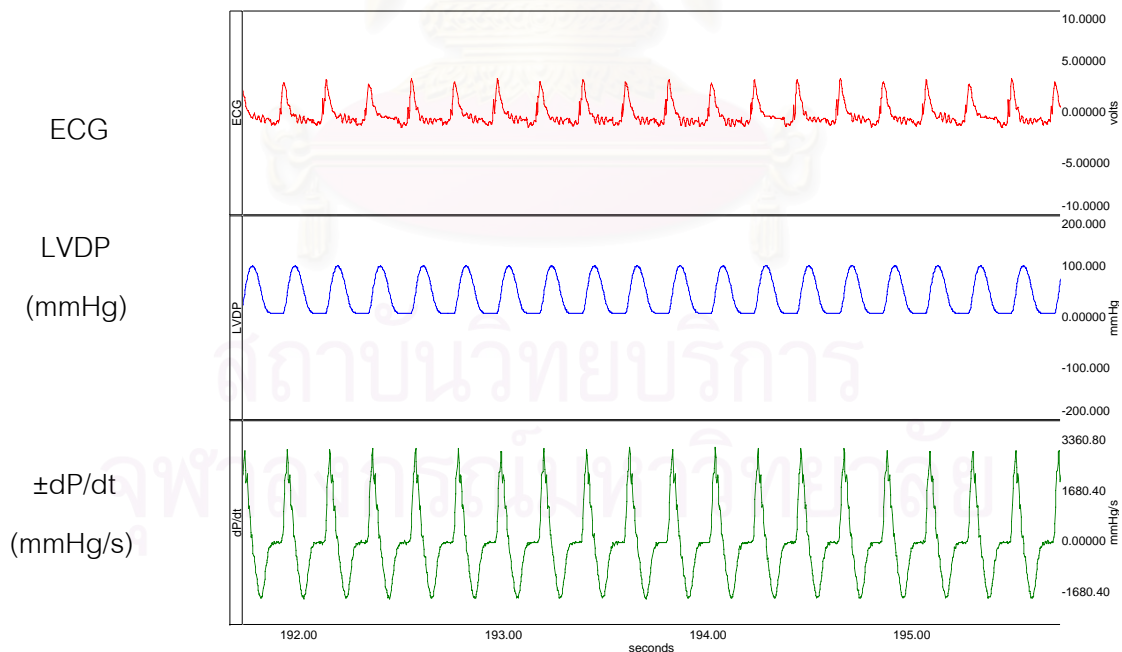
Figure 3.1 Tracing shows ECG, LVDP, dP/dt$_{max}$, and dP/dt$_{min}$ of an isolated rat heart.
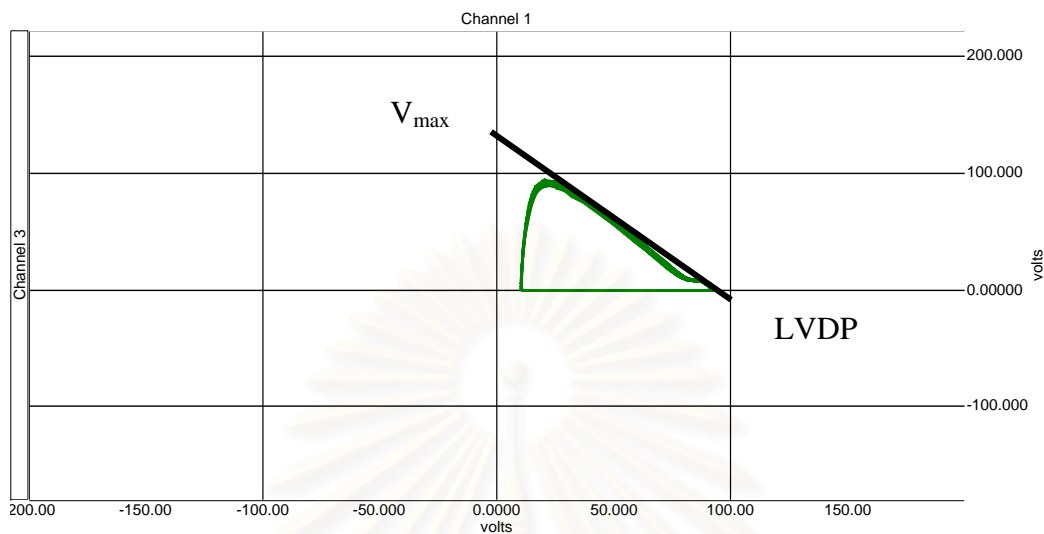
Figure 3.2 The graph shows maximal velocity of shortening (V_{max}) of an isolated rat
heart.

Determination of RR variability in time domain

RR variability represents one of most promising such markers which describe variations of both instantaneous heart rates and RR intervals. It permits insight into the autonomic control to the heart (Aubert et al., 1999).

In a continuous ECG record, time between each QRS complex was detected, and so called normal to normal (NN) intervals which were all intervals between adjacent QRS complexes resulting from sinus node depolarization. In NN interval series obtained from short term recordings, the formula of standard deviation can be applied either duration of individual intervals or to the differences between the neighboring intervals. Before applying any statistical method to the data of RR interval durations of consecutive heart rates, visual checks and manual corrections of the automatic ECG analysis have to ensure that all coupling intervals and compensatory pauses of premature cycles have been excluded (Malliani et al., 1997).

For each rat, 3 periods which are baseline and drug perfusion periods of were recorded for 5 minutes to determine the RR interval and heart rate variability.

1. SDNN

$$SDNN = \sqrt{1/n \sum_{i=1}(NN_i - m)^2}$$

SDNN is standard deviation of the NN intervals. This parameter reflects all the cyclic components responsible for variability in the period of recording.

NN_i is the duration of i-th NN interval in the analysed ECG, n is the number of all NN intervals, and m is their mean duration.

2. rMSSD

$$rMSSD = \sqrt{1/n\text{-}1 \sum_{i=1}(NN_{i+1} - NN_{i-1})^2}$$

rMSSD is the square root of the mean squared difference of successive NN intervals.

NN is the duration of the i-th NN interval in analysed ECGs and n is the number of all NN intervals. This simple form of the formula assumes that there are not any ectopic beats in the ECG that would lead to the omission of some RR intervals.

3. *pNN10*

pNN10 was determined as described by Aubert et al. (1999).

For a selected threshold t of RR interval prolongation or shortening, the number of cases may be counted in which a NN interval is prolonged or shortened by more than t within one cardiac cycle, that is the number of NN intervals that are longer than $NN' + t$, where NN' is the duration of the immediately preceding NN interval. Originally, the method has been proposed with the threshold t of 10 milliseconds (value of 10 msec were selected due to the high mean heart rate in rats which is300-350 beat per minutes). Therefore pNN_{10} is the proportion derived by deviding NN_{10} by the total number of all NN intervals.

$$pNN_{10} = NN_{10}/ n$$

NN$_{10}$ is the number of interval differences of successive NN intervals greater than 10 msec, n is total numbers of NN interval.

ECG Analysis

The ECG signal was amplified on a Power lab instrument. The sampling rate was 400 Hz per channel. ECG was recorded continuously for 5 minutes in baseline conditions followed by 5 minutes of each period of drugs perfusion, ischemic conditions and reperfusion conditions.

1. The RR-interval

The RR-interval (msec) was measured from the R wave to the consecutive R wave.

2. The QT and QT$_c$ interval

Measurement of this interval can be used to evaluate the effects of drugs and diseases on the time-dependent properties of the ion channels responsible for ventricular repolarization. QT prolongation is clinically important because delayed repolarization is the substrate for arrhythmias and sudden death (Katz, 2001).

The QT-interval was measured from the beginning of the Q wave to the end of T wave, defined as the point at which the voltage returns to the isoelectric baseline (Tosaki et al., 1998).

The corrected QT intervals (QT$_c$) were calculated using Bazett's formula.

$$QT_c = QT/RR^2$$
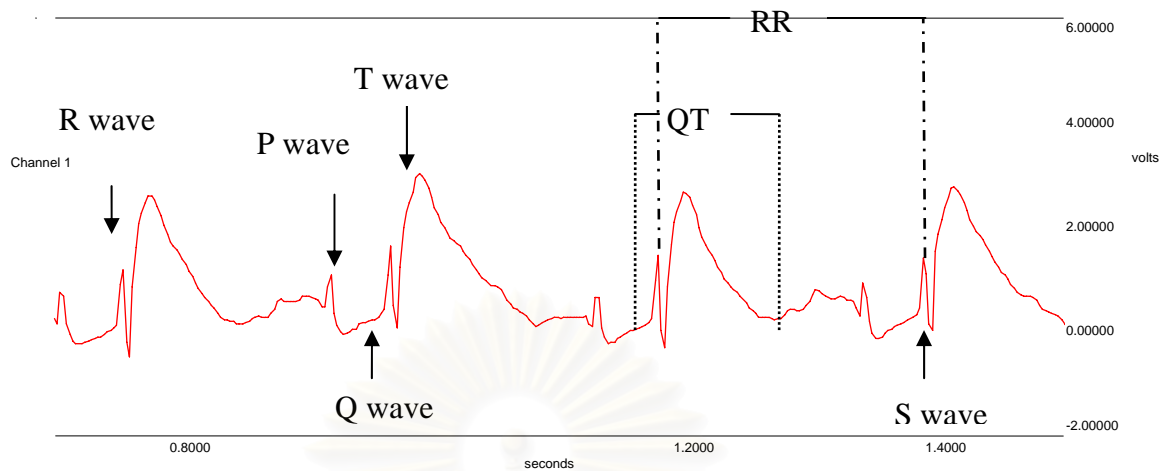
Figure 3.3 Tracing shows ECG recorded from an isolated rat heart.

Arrhythmia analysis

The acquired single-lead ECG tracings were displayed and analysed.

After coronary reperfusion, ECG and left ventricular pressure were continuing recorded for 30 minutes to evaluate cardiac arrhythmia. Ventricular premature beat (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF) were defined according to the Lambeth convention criteria (Walker et al., 1988) with more stringent modifications. Specifically, VPB was defined as ventricular contraction without atrial depolarization.

A ventricular premature beat was classified as singlet.

Two ventricular premature beats in a row was classified as couplets.

Three ventricular premature beats were classified as triplets or salvo

Ventricular tachycardia (VT) was defined as 4 or more consecutive ventricular premature beats (Walker et al., 1988).

Ventricular fibrillation was defined as a signal that changed from beat to beat in rate and morphology or a signal in which individual QRS deflections could not easily be distinguished from one another.

Arrhythmia was classified into sustained ventricular tachycardia when ventricular premature systole lasts longer than 30 seconds.

Arrhythmia was classified into non-sustained ventricular tachycardia when the arrhythmia is converted to normal sinus rhythm within 30 seconds.

As expected in this model, a high incidence of spontaneously reversible and nonfatal VF was found (Opitz et al., 1995).

The ranking scores are arbitrary numerical grades of different sorts of ventricular arrhythmias. The scaling applied was as follows: 0=no arrhythmias, 1=single VPB, 2=couple or salvos of VPB, 3=ventricular tachycardias, 4=sustained ventricular tachycardias, 5=ventricular fibrillation. When multiple forms of arrhythmias occurred in one heart, only the highest single score was used (Di Napoli et al., 1998). The incidence of ventricular arrhythmias was also present according to the experiment of Du et al. (1999).

Evaluation of apoptosis

Apoptotic cell death was evaluated by Terminal deoxynucleotidyltransferase-mediated nick end-labeling (TUNEL) method using APOP TAG$^{®}$ Peroxidase In Situ Apoptosis Detection Kit for immunoperoxidase staining (Intergen Company, NY, USA). After finishing Langendorff preparation, all hearts were immediately fixed in 10% (v/v) neutral buffered formalin for 24 hours at $4^{°}$C. Three areas of the heart were selected. The first area was selected from tissue just right below the ligation site of coronary artery as ischemic area. The second was cut from area beside that artery and the last area was selected from the right ventricle which was non-ischemic area as control. Beside that, thymus from the same rat was sectioned to use as positive control. After that, these tissues were processed through an automatic tissue fixing machine. The tissues were

carefully embedded in the molten paraffin in metallic blocks and kept in refrigerator to allow the paraffin to solidify. The metallic containers were removed and tissues became embedded in paraffin on the plastic molds. Three of 6 µm thick sections were cut. Prior to analyzing tissues for apoptosis, tissue sections were deparaffinized with xylene and washed in succession with different concentrations of ethanol (absolute, 95%, 70%). Tissues were then treated with proteinase k (Sigma-Aldrich$^{®}$, MO, USA) for 15 minutes at room temperature and quenched in 3.0% hydrogen peroxide in PBS for 5 minutes at room temperature. Excess liquid was gently tapped off from the sections. Equilibration buffer was applied directly onto the specimens and incubated for 5 minutes at room temperature. Specimens were treated with terminal deoxynucleotidyl transferase (Tdt) and covered with plastic cover slips at 37°C for 1 hour in a humidified chamber. After 1 hour, cover slips were removed, and specimens were placed in a coplin jar containing stop/wash buffer for 10 minutes at room temperature. Working strength anti-digoxigenin peroxidase conjugate was added to the slides and incubated for 30 minutes at room temperature, washed in PBS, and working strength peroxidase substrate (DAB dilution buffer plus DAB substrate) was applied to completely cover the specimens, stained for 6 minutes at room temperature. Wash the specimen with de-ionized water in coplin jar, then incubated the slides for 5 minutes at room temperature.   The tissues were counterstained in 0.5 % methyl green for 10 minutes at room temperature, washed in water and N-butanol. Tissues were then mounted in a mounting medium and covered by a cover slip. Apoptotic cells were visualized under a light microscope. This method was based on the new 3'-OH DNA ends generated by DNA fragmentation and typically localized in morphologically identifiable nuclei and apoptic bodies. In contrast, non-apoptotic nuclei, which had relatively insignificant numbers of DNA 3'-OH ends, was not stained with this reagent.

For each section, the number of TUNEL-positive myocyte nuclei was manually counted for 1000 cells per section. Only nuclei that were clearly located in cardiac myocytes were counted.

Data analyses

All data are presented as the mean $\pm$SD. Statistical significant of difference among groups was determined by one-way ANOVA followed by Student- Newman Keuls. The cardiac functions among periods were done by ANOVA with repeated measures design (i.e. LVDP, dP/dt$_{max}$, dP/dt$_{min}$, Vmax, HR, coronary flow). Non-parametric method was used in some data which normality failed, one way ANOVA with repeated measures on ranks was used. Paired t-test was used to compare the data before and after drug perfusion (i.e. SDNN, rMSSD, *pNN10*). A level of $P<0.05$ was considered the threshold for statistical significance between the control and experimental groups.

Arrhythmia scores were compared using the unpaired t-test when results passed the normal distribution test, or otherwise, the Mann-whitney rank sum test when the normality failed. The Chi-square test was used for comparison of the incidences of arrhythmias (Du et al., 1999).

Apoptotic quantitative results were calculated using as means $\pm$SD. The differences in the amounts of apoptotic cardiomyocytes among each group were compared using Chi-square or Fisher's exact test (Du et al., 2000).

Sigma-stat software (Jandel Scientific) was used for statistical analysis.

CHAPTER 4

RESULTS

Effects of adrenergic drugs on cardiac contractility

1. Effects of adrenergic drugs on left ventricular developed pressure (LVDP)

LVDP changes in each period are presented in figure 4.1. At baseline period, LVDP in control, atenolol (ATEN), atenolol combined with salbutamol (ATEN/SALBU), and atenolol combined with prazosin (ATEN/PRAZ) treated groups were 102.2±10.9, 104.5±9.2, 101.4±11.0, and 103.6±18.2 mmHg, respectively. The significant decreases in LVDP following drug perfusion were observed in ATEN/PRAZ, ATEN/SALBU, and ATEN/PRAZ treated groups. Although LVDP in control group was also decreased during drug perfusion period but there was not a significant difference versus the baseline. After drug perfusion, LVDP in control, ATEN, ATEN/SALBU, and ATEN/PRAZ treated group were significantly decreased to 97.1±13.8, 96.2±11.3, 95.1±10.1, and 95.0±19.1 mmHg (P<0.05), and coronary occlusion caused marked decreases in LVDP to 55.9±13.3, 52.7±13.1, 53.2 ±13.8, and 54.2 ±9.4 mmHg, respectively (P<0.05). Significant increases in LVDP during reperfusion period compared with ischemia periods were seen in ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups. LVDP in reperfusion periods were 63.8 ±15.7, 60.7±9.9, 70.1±11.5, and 81.3 ±21.9 in control, ATEN, ATEN/SALBU, and ATEN/PRAZ treated group, respectively. At the same period, the comparison of LVDP among groups was not significant difference. Interestingly, LVDP in ATEN/PRAZ group was the highest compared to other experimental groups.
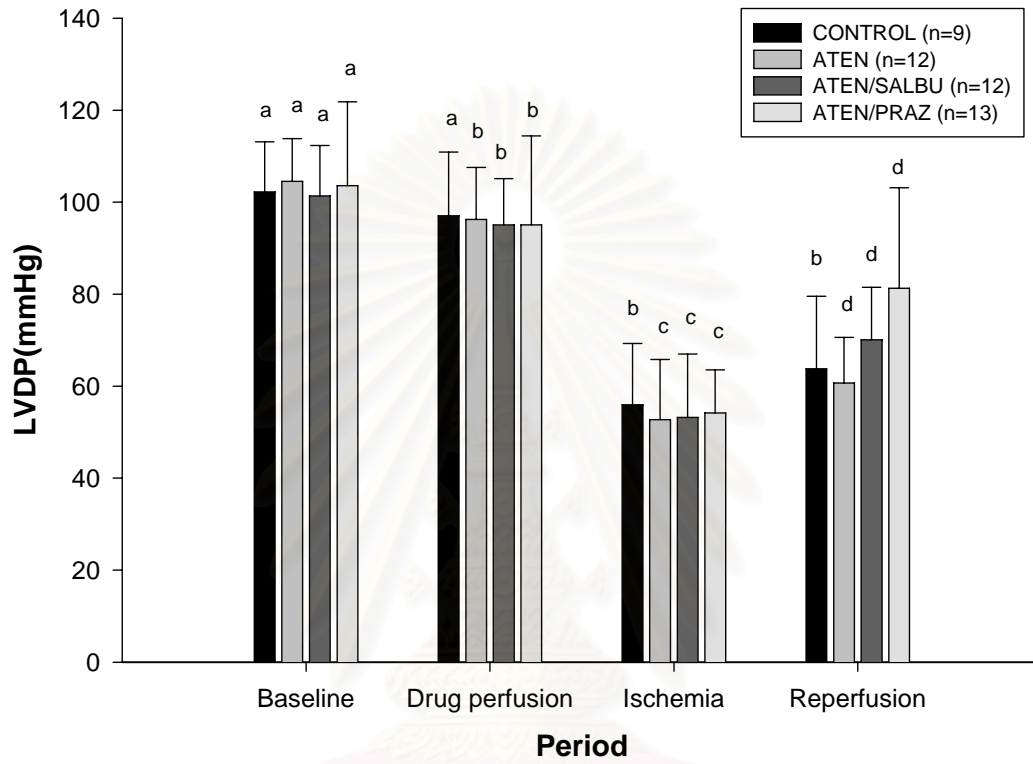
Figure 4.1 Effects of adrenergic drugs on left ventricular developed pressure

(mean ± SD)

[a, b, c] and [d] superscripts represent significant differences (P<0.05) among periods

in the same treated group (followed periods of time).

2. Effects of adrenergic drugs on dP/dt_{max}

The dP/dt_{max} changes are presented in figure 4.2. At the baseline, dP/dt_{max} in control, ATEN, ATEN/SALBU, and ATEN/SALBU groups were 3221±268, 3235±490, 3311±432, and 3350±470 respectively and dP/dt_{max} slightly decreased after drug perfusion in all groups. During cardiac ischemia, the dP/dt_{max} in all groups decreased to 2077±351, 1933±635, 2041±636, and 1887±414, respectively (P<0.05). After coronary reperfusion, the increases in dP/dt_{max} were observed in ATEN/SALBU and ATEN/SALBU treated groups to 2543±709 and 2554±658 mmHg/s (P<0.05). However, there was no significant difference in dP/dt_{max} between ATEN/SALBU and ATEN/PRAZ groups.

Figure 4.2 Effects of adrenergic drugs on dP/dt_{max} (mean±SD)

[a, b] and [c] superscripts represent significant differences (P<0.05) among

periods in the same treated group (followed periods of time).

3. Effects of adrenergic drugs on dP/dt$_{min}$

As shown in figure 4.3, dP/dt$_{min}$ at baseline periods in control, ATEN, ATEN/SALBU and ATEN/PRAZ group were 2601± 705, 2235±294, 2434±410, and 2394±655 – mmHg/s, respectively. dP/dt $_{min}$ slightly declined in all groups nevertheless the statistical significances were detected only in control and ATEN/PRAZ treated groups during drug perfusion. The marked decreases in dP/dt$_{min}$ were observed in all groups after coronary ligation. The dP/dt$_{min}$ in control, ATEN, ATEN/SALBU and ATEN/PRAZ groups in ischemic periods were 1179±403, 1080±405, 1251±432, and 1111±435, respectively. These data have demonstrated that ischemia caused the decreases in cardiac relaxation. During reperfusion, the dP/dt$_{min}$ in ATEN/SALBU and ATEN/PRAZ were 1522±472 and 1393±390 –mmHg/s, respectively (P<0.05).

Figure 4.3 Effects of adrenergic drugs on dP/dt$_{min}$ (mean±SD)

[a, b, c] and [d] superscripts represent significant differences (P<0.05) among

periods in the same treated group (followed periods of time).

4. Effects of adrenergic drugs on maximal velocity of fiber shortening (V_{max})

As shown in figure 4.4, V_{max} between baselines and drug perfusion periods were not significant differences in all groups. Decreases in V_{max} were detected during ischemic periods in control, ATEN, ATEN/SALBU treated groups (from 4.0±0.3 to 3.8±0.3, 4.0±0.4 to 3.7±0.3, 4.1±0.6 to 3.7±0.7, respectively) whereas V_{max} in ATEN/PRAZ treated group did not decline. During reperfusion, V_{max} in all groups were not change from ischemia period.
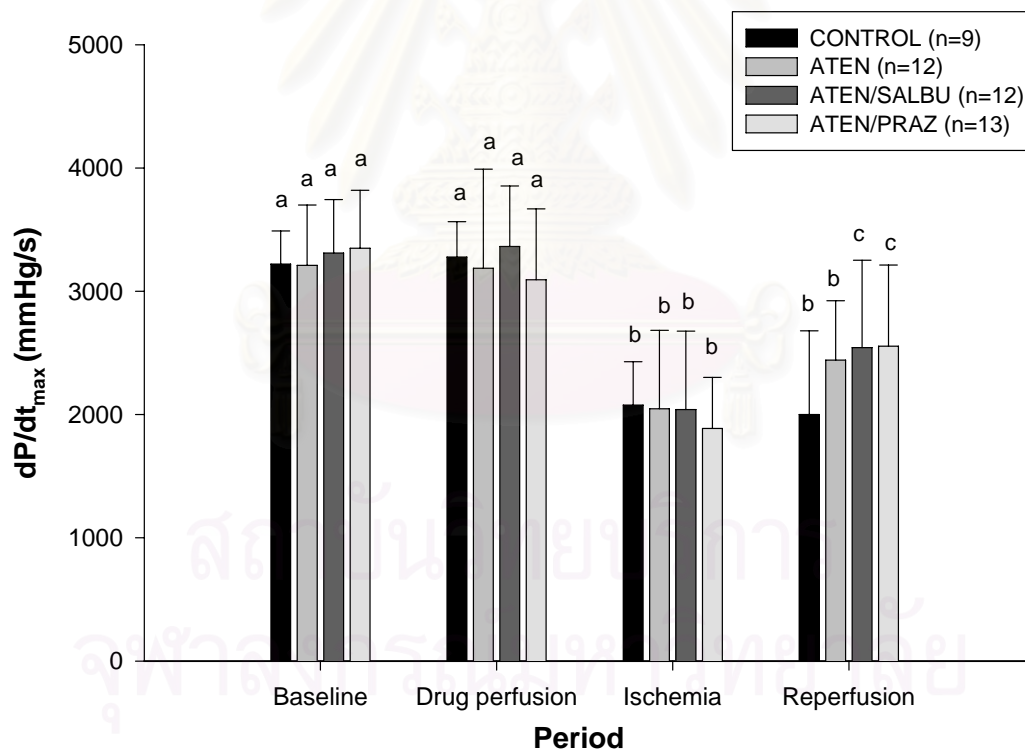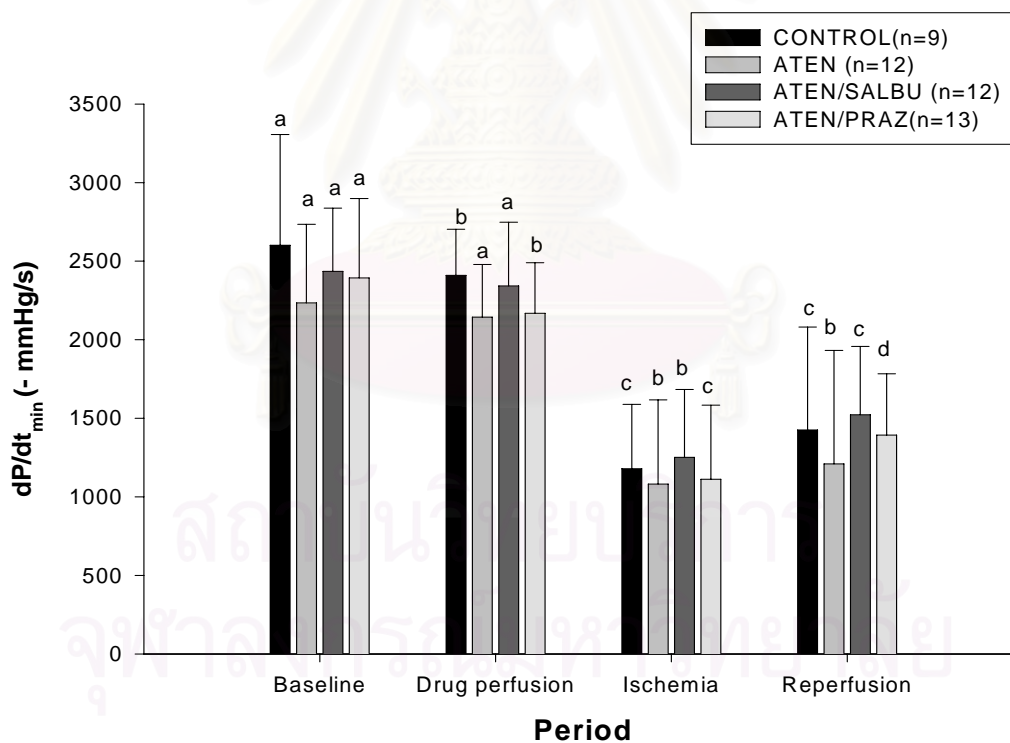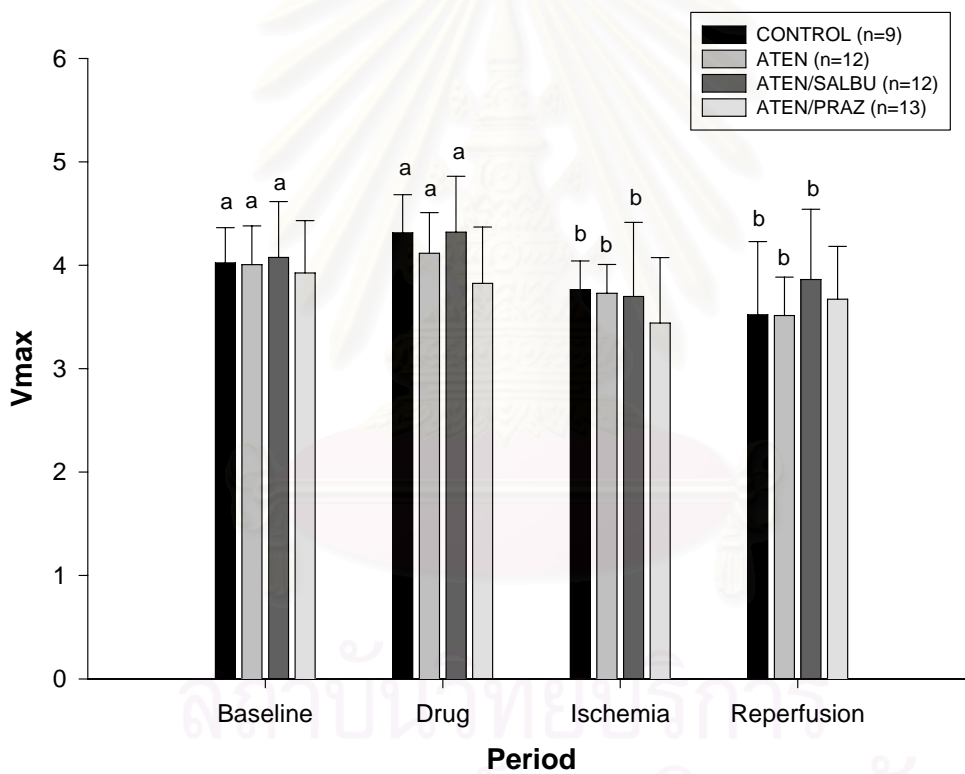

Figure 4.4 Effects of adrenergic drugs on V_{max} (mean±SD)

[a] and [b] superscripts represent significant differences (P<0.05) among periods in the same treated group (followed periods of time).

Effects of adrenergic drugs on coronary flow

Table 4.1 Effects of adrenergic drugs on coronary flow rate (ml/min)

Group	Baseline	Drug perfusion	Ischemia	Reperfusion
Control (n=9)	21.0 ± 3.6^{a}	19.7 ± 3.5^{b}	15.0 ± 3.9^{c}	18.2 ± 3.3^{c}
ATEN (n=12)	20.4 ± 3.0^{a}	19.8 ± 3.9^{a}	14.7 ± 3.4^{b}	17.3 ± 4.0^{c}
ATEN/SALBU(n=12)	21.5 ± 3.4^{a}	21.4 ± 3.0^{a}	15.5 ± 5.6^{b}	17.8 ± 5.5^{c}
ATEN/PRAZ (n=13)	22.9 ± 4.7^{a}	22.0 ± 5.8^{b}	12.1 ± 4.5^{c}	15.6 ± 4.6^{d}

The data were present as mean ± SD.

[a, b, c] and [d] superscripts represent significant differences (P<0.05) among periods in the same treated group.

Coronary flow in each groups were shown in table 4.1. At the baseline, coronary flow in control, ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups were not different. After drug perfusion, declines in coronary flow were detected in control and ATEN/PRAZ treated groups compared to the baselines (P<0.05). Whereas coronary flow in ATEN and ATEN/SALBU treated groups were slightly decreased compared to the baselines. Coronary flow dropped in all groups during ischemic period and increased after reperfusion (P<0.05). Only control group that coronary flow during reperfusion period was not different from drug perfusion period. There was no significant difference in coronary flow among groups at the same period.

Effects of adrenergic drugs on heart rates

Effects of adrenergic drugs on heart rate are shown in figure 4.5. In control group, heart rates during drug perfusion and ischemic periods were not different from the baseline whereas heart rate during reperfusion period was lower than the baseline.

In ATEN treated group, ischemic heart remarkably decreased in heart rate. During reperfusion period, heart rate was higher than that of ischemic period. Interestingly, the perfusion of atenolol combined with prazosin showed significant decrease in heart rate ($P<0.05$). Moreover, ischemia-reperfusion did not alter heart rate in this group. ATEN/SALBU did not alter heart rate at all periods.

In conclusion, there were no differences in heart rate among 4 groups at the baseline. During drug perfusion, ischemic- and reperfusion period, only ATEN/SALBU and ATEN/PRAZ treated groups were shown a statistically significant difference in heart rate ($P<0.05$). Heart rate in ATEN/SALBU treated group was remarkably higher than ATEN/PRAZ treated group.

Effects of adrenergic drugs on electrocardiogram

Effects of adrenergic drugs on ECGs in terms of R-R, Q-T and Q-Tc intervals are shown in table 4.2. There were no significant differences in R-R and Q-T intervals among experimental groups at the baselines. During drug perfusion period, only ATEN/PRAZ caused marked widening R-R intervals and shortening Q-T intervals ($P<0.05$). R-R intervals of ATEN/PRAZ treated group was much wider than that of ATEN/SALBU treated group during reperfusion ($P<0.05$). Prolonged R-R intervals after ischemia was observed in control, ATEN, ATEN/SALBU and ATEN/PRAZ treated group nevertheless there was a significant difference only in ATEN/PRAZ treated group ($P<0.05$). Although Q-T intervals in periods of ischemia and reperfusion in ATEN/PRAZ treated group much wider than the baseline ($P<0.05$), Q-T intervals during ischemia and reperfusion periods did not differ from the drug perfusion period. Even though R-R intervals in ATEN/PRAZ treated group

did not alter during ischemia, they were increased after reperfusion. It should be noted that Q-T intervals in ATEN/PRAZ treated group were wider than other groups (P<0.05). Interestingly, ATEN/PRAZ prolonged R-R intervals at all periods.

Q-T_c intervals in all groups were not different at all periods. These data demonstrated that all drugs using in this experiment were not alter Q-T_c intervals. ATEN/SALBU caused shortening of R-R intervals whereas ATEN/PRAZ caused widening of R-R intervals during drug perfusion.
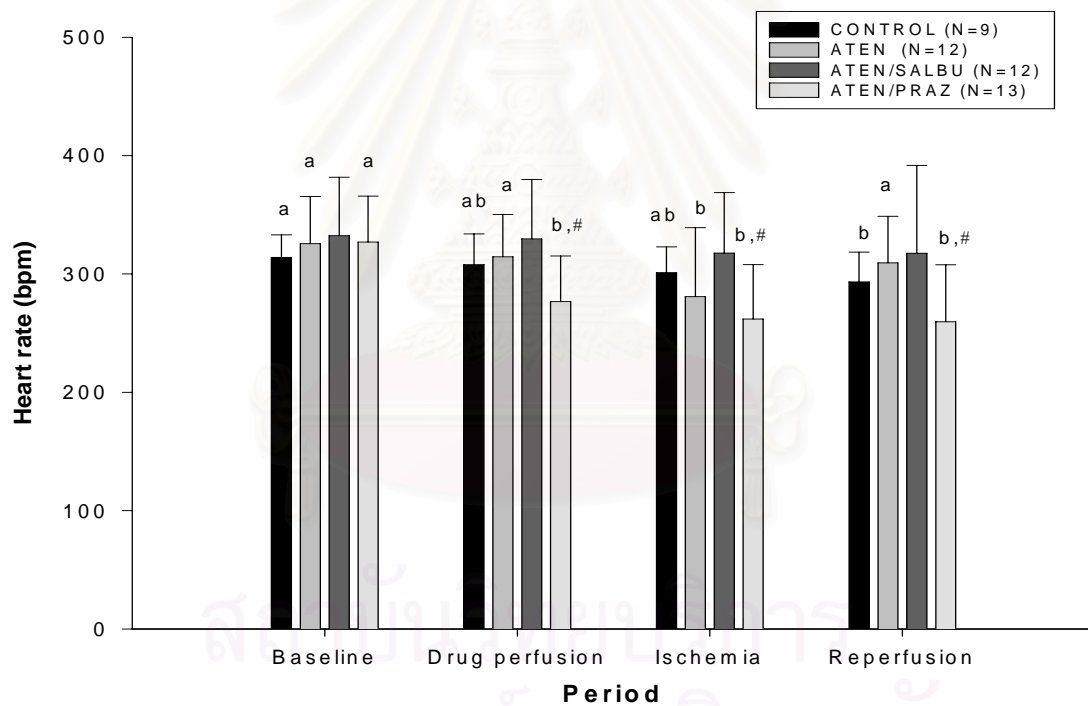
Figure 4.5 Effects of adrenergic drugs on heart rates

[a] and [b] superscripts represent significant differences (P<0.05) among periods in the same treated group (followed periods of time).

[#] represents significant difference from ATEN/SALBU treated group (P<0.05).

Table 4.2 Effects of adrenergic drugs on R-R, Q-T, and Q-Tc intervals

Group/Period		Baseline	Drug	Ischemia	Reperfusion
Control (n=9)	RR (msec)	194±11	195±15	201±12	206±21
	QT (msec)	83±12	83±10	85±14	82±12 [a]
	QT$_c$ (msec)	190±27	188±24	188±30	181±32
ATEN (n=12)	RR (msec)	188±19	193±22	204±23	196±19
	QT (msec)	86±7	83±4.7	85±5	80±8.7 [a]
	QT$_c$ (msec)	198±18	190±14	190±16	182±24
ATEN/SALBU (n=12)	RR (msec)	188±28	187±33	194±40	201±67
	QT (msec)	84±3	84±6	86±8	85±3 [a]
	QT$_c$ (msec)	195±15	196±17	195±14	194±16
ATEN/PRAZ (n=13)	RR (msec)	187±26 [a]	219±33 [b,#]	230±39 [b]	240±47 [c]
	QT (msec)	85±3 [a]	84±24 [b]	94±13 [b]	93±10 [b,*]
	QT$_c$ (msec)	200±13	196±22	198±31	181±32

The data were present as mean ± SD.

[a, b] and [c] superscripts represent significant differences (P<0.05) among periods in the same treated group.

*superscript represents significant difference from control group (P<0.05)

#superscript represents significant difference from ATEN/SALBU treated group (P<0.05)

Effects of vasodilators on RR variability

<u>Table 4.3</u> Effects of adrenergic drugs on RR variability

Group	Period					
	Baseline			Drug perfusion		
	SDNN (msec)	rMSSD (msec)	pNN10 (%)	SDNN (msec)	rMSSD (msec)	pNN10 (%)
Control (n=9)	9±2	11±3	14.2±11.5	12±5	15±8	16.0±18.9
ATEN (n=12)	12±9	15±14	11.0±12.6	14±5	21±9	27.8±23.2
ATEN/SALBU (n=12)	7±3	11±4	8.43±13.5	7±4	11±4	11.8±14.8
ATEN/PRAZ (n=13)	8±4	19±27	10.2±15.5	15±13	21±19	6.3±9.9

The data were present as mean ± SD.

Time domain of RR variability data are shown in table 4.3. The SDNN at baseline in control, ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups are 9±2, 11 ±9, 7±3, and 8±4 msec, respectively. There were no significant differences in SDNN among treatment groups. After drug perfusion, SDNN tended to be increased in all groups except ATEN/SALBU treated group nevertheless there were no significant differences between baseline and drug perfusion periods in all groups.

The rMSSD at baseline in control, ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups were 12±5, 13±5, 7±4, and 15±13 msec, respectively. The increases in rMSSD were found in all treatment groups even though there were no significant differences. pNN10 at baseline and drug perfusion periods were no significant differences in all groups.

Effects of adrenergic drugs on cardiac arrhythmias

<u>Table 4.4</u> Incidence of arrhythmias following reperfusion in controal and drug treated

groups

Group	%VPB (singlet)	%VPB (couplet)	%VPB (triplet)	%non-sustained VT	%sustained VT	%VF	Arrhythmia score
Control (n=12)	83.3 (10/12)	66.7 (8/12)	33.3 (4/12)	58.3 (7/12)	8.3 [a] (1/12)	66.7 (8/12)	4.2±1.4
ATEN (n=14)	92.8 (13/14)	78.6 (11/14)	57.1 (8/14)	64.3 (9/14)	0 [a,#] (0/14)	50.0 (7/14)	3.6±1.5
ATEN/SALBU (n=19)	94.7[a] (18/19)	73.7[a] (14/19)	31.6[b] (6/19)	63.2[a] (12/19)	5.3[b] (1/19)	68.4[a] (13/19)	4.1±1.4
ATEN/PRAZ (n=15)	100 [a] (15/15)	40.0[b] (6/15)	40.0 [b] (6/15)	33.3 [b] (5/15)	0 [c,#] (0/15)	0 [c,#] (0/15)	1.8±0.9[*]

VPB= ventricular premature beat, VT=ventricular tachycardia, VF= ventricular fibrillation

The incidences of ventricular arrhythmias were present as percent of the incidence and

its proportion. The arrhythmia scores were present as mean ± SD.

[#]superscript represents significant difference in the incidence of ventricular

arrhythmias among treated groups ($P<0.05$)

[a, b,] and [c] superscripts represent significant differences in types of arrhythmias within

the same treated group.

[*] superscript represents significant different among groups in arrhythmia score

($P<0.05$)

Figure 4.6 Tracings show ECG, LVDP, dP/dt$_{max}$ and dP/dt$_{min}$ during baseline,

drug perfusion, ischemic, and reperfusion periods in ATEN/SALBU treated

group. Tracing shows VT and VF during reperfusion.   (VT= ventricular

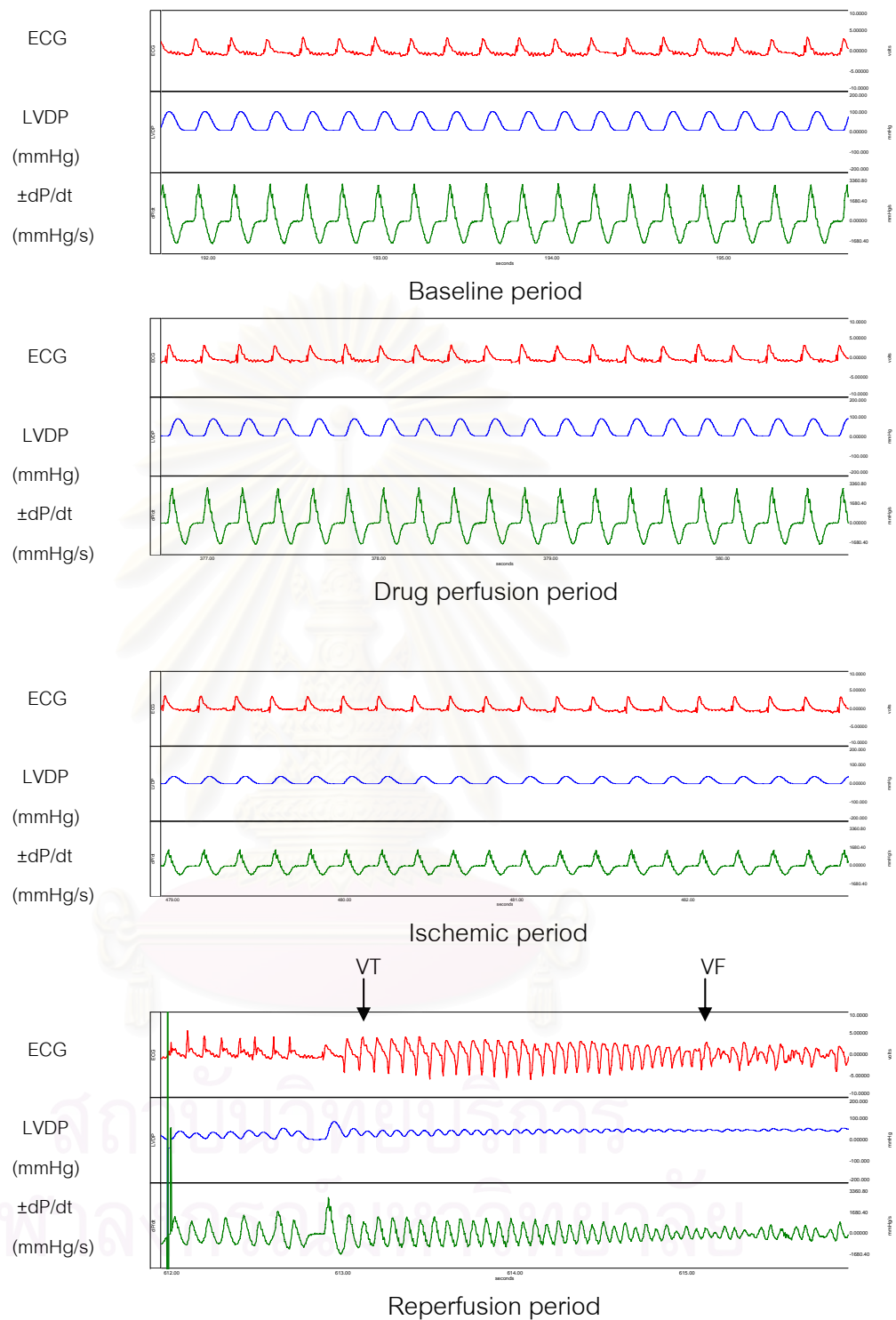tachycardia, VF = ventricular fibrillation)
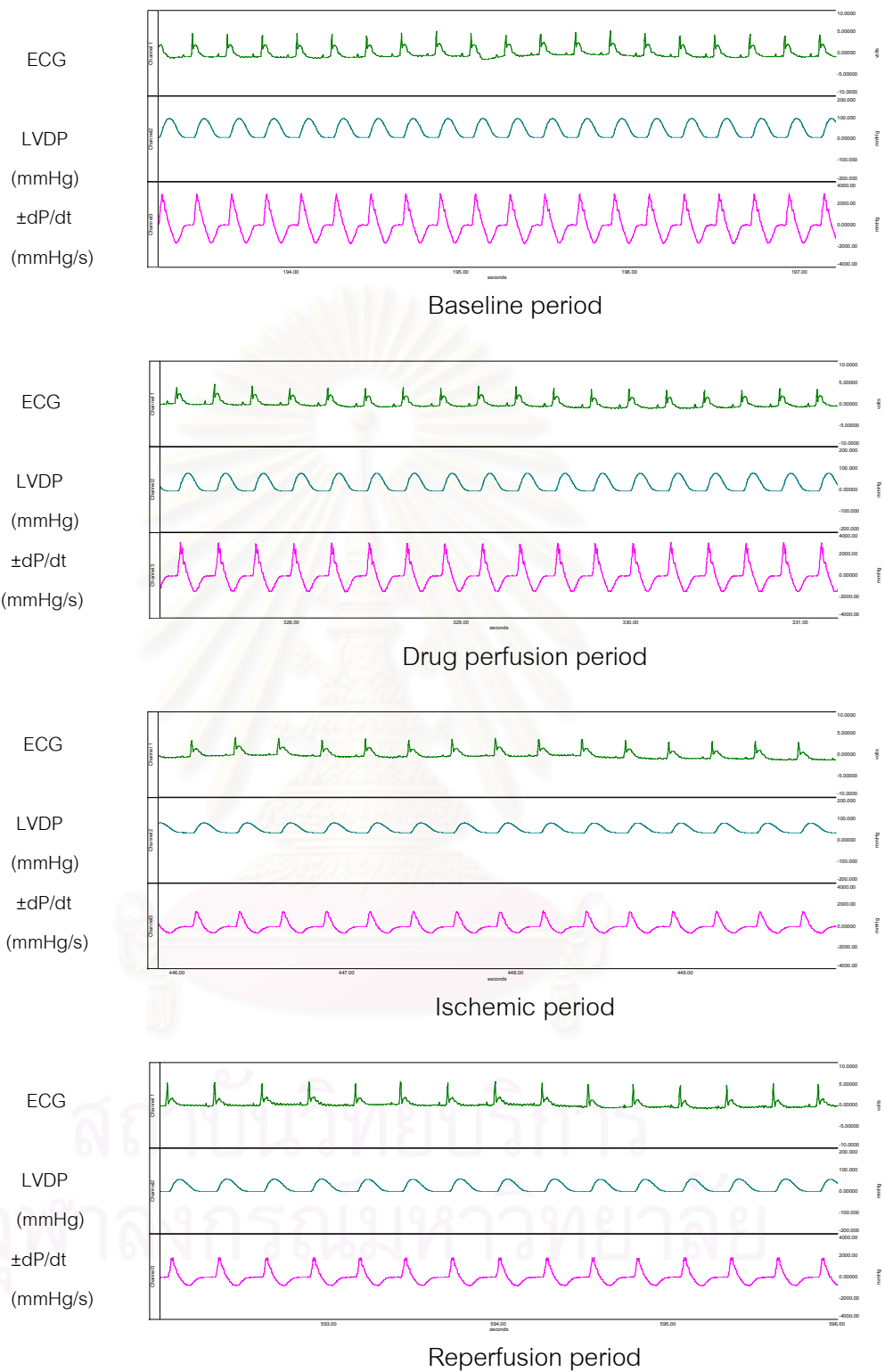
Figure 4.7 Tracings show ECG, LVDP, dP/dt$_{max}$ and dP/dt$_{min}$ during baseline, drug perfusion, ischemic, and reperfusion periods in ATEN/PRAZ treate group. The tracing during reperfusion shows no evidence of ventricular arrhythmias

As shown in table 4.4, analysis of 30 minute reperfusion following coronary ischemia demonstrated ventricular arrhythmias in all experimental groups. Singlet, couplet, and triplet ventricular premature beat (VPB) appeared in all groups during coronary reperfusion. Non-sustained ventricular tachycardia were present in all groups nevertheless sustained ventricular tachycardia were present only in control and ATEN/SALBU treated group. However, there was not a significant difference in the incidence of singlet, couplet, triplet, non-sustained VT and sustained VT among groups. The incidence of ventricular fibrillation was highest in ATEN/SALBU treated group although there was no significant difference when compared to the control group. ATEN slightly reduced the occurrence of ventricular fibrillation when compared to the control group. Notably, there was no incidence of ventricular fibrillation in ATEN/PRAZ treated group after coronary reperfusion. There was a significant decrease in incidences of ventricular fibrillation in ATEN/PRAZ treated group compared to the others (P<0.001). The arrhythmia score was also lowest in ATEN/PRAZ treated group which was a significant difference when compared to control, ATEN, and ATEN/SALBU treated groups (P<0.001). The arrhythmia scores of control, ATEN, and ATEN/SALBU treated groups were not a significant difference. From the results, ATEN did not decrease the incidences of ventricular arrhythmias compared to control group whereas ATEN/SALBU did not enhance the incidence of ventricular arrhythmias in reperfusion hearts. ATEN/PRAZ appears to decrease incidences of sustained ventricular tachycardia and ventricular fibrillation. However, it should be noted that atenolol combined with prasozin had potent antiarrhythmic effect.

Effects of adrenergic drugs on cardiac myocytes apoptosis

Table 4.5 Number of apoptotic nuclei in different areas of rat hearts treated by different
adrenergic drugs.

Group	Control area (nuclei/1000 nuclei)	Peri-ischemic area (nuclei/1000 nuclei)	Ischemic area (nuclei/1000 nuclei)
Control (n=5)	0.2±0.4	0.4±0.5	1.8±0.8
ATEN (n=5)	0.6±0.9	2.2±2.8	1.6±2.6
ATEN/SALBU (n=5)	0.2±0.4	2.2±3.3	1.8±2.5
ATEN/PRAZ (n=5)	0.4±0.5	0.6±0.5	1.2±1.3

Data were present as mean ± SD.

From the results, of all treated group, apoptotic cells were rarely found in the non-ischemia area (control area) with the use of the TUNEL assay. In contrast, the apoptotic cells were more numerous in peri–ischemic and ischemic area in all groups. However, there was no significant difference in apoptotic positive cells either among treated groups at the same area or among areas in the same group.

Figure 4.8 Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling
(TUNEL)-positive cardiomyocytes in the infarcted zone of ischemic-induced rat
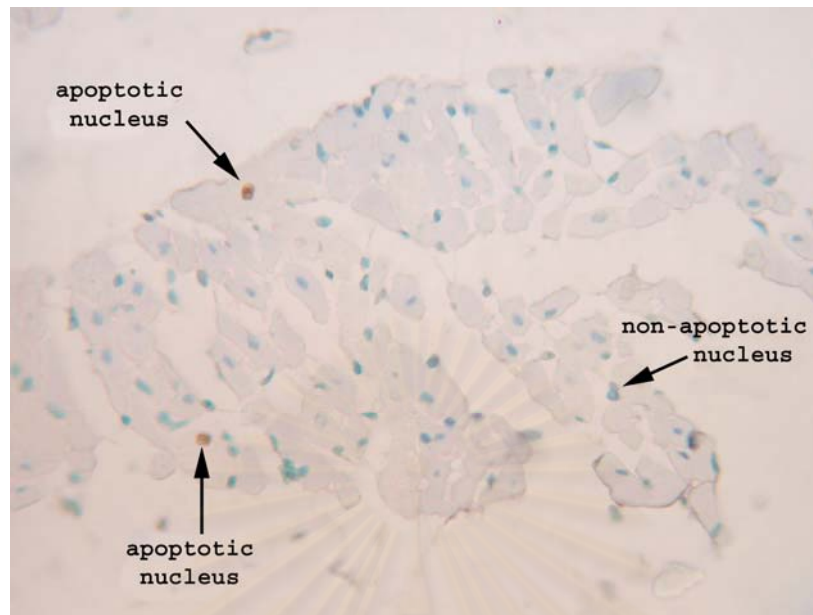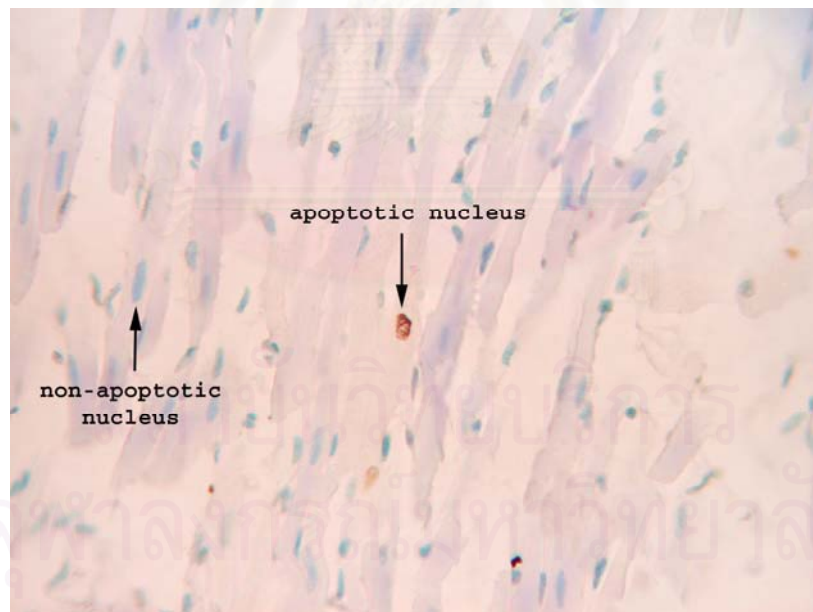myocardium (cross section, 400 X magnification)

Figure 4.9   Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling
(TUNEL)-positive cardiomyocytes in the infarcted zone of ischemic-induced rat
myocardium (longitudinal section, 400 X magnification)

CHAPTER 5

DISCUSSION

All hearts in this model showed decrease in cardiac contractility and coronary flow during ischemia and reperfusion. Ca^{2+}-overload and increased production of free radicals are the two most important mechanisms responsible for reperfusion injury. Ca^{2+} overload is caused by several mechanisms. During ischemia, intracellular acidosis stimulates Na^+/H^+ exchanger activity. Due to inhibition of energy-dependent Na^+/K^+ -ATPase, Na^+ tends to accumulate. Intracellular Ca^{2+} increases through activation of the Na^+/Ca^{2+} exchanger. The increase in the intracellular Ca^{2+} content activates phospholipases, which may alter phospholipids. Cytosolic Ca^{2+} overload can result also from influx across the sarcolemmal cation channels or release from sarcoplasmic reticulum (Peuhkurinen, 2000). Intracellular acidosis, Na^+ and Ca^{2+} overload via Na^+/H^+ and Na^+/Ca^+ exchanges may increased susceptibility to arrhythmias. (Pogwizd et al., 2001; Tani et al., 1989)

Potential electrophysiologic and antiarrhythmic effects of α_1-adrenergic blocker

In the present study, atenolol, a selective β_1-adrenergic blocker combined with a selective α_1-adernergic blocker, prazosin, showed the highest potential antiarrhythmic effects whereas atenolol alone does not demonstrate antiarrhythmic effects. In concurrence with Daugherty et al. (1986) who suggested that arrhythmia production was not a consequence of β- adrenergic stimulation. These results indicate that α_1-ARs play an important role on reperfusion arrhythmias. Many reports demonstrated the cardiac arrhythmias induced by α_1-adrenergic stimulation. Alpha-adrenergic stimulation which induced delayed afterdepolarization was studied in Purkinje fibers isolated from cat hearts in the presence of an elevated Ca^{2+} concentration (Kimura et al., 1984). Kurz et al. (1991) have supported the hypothesis that the α_1-adrenergic stimulation of canine hypoxic cardiac myocytes with norepinephrine resulted in the appearance of delayed

after depolarizations. The delayed afterdepolarization may response to an increase in intracellular Ca^{2+}. Thandroyen et al. (1987) studied the arrhythmogenic action of α_1- and α_2-adrenergic receptor stimulation in isolated perfused hearts. The α_1-adrenergic stimulation in the presence of β_1-selective antagonist decreased the ventricular fibrillation threshold in the normoxic rat myocardium. Prazosin prevented the methoxamine, α_1- adrenergic agonist-induced fall in ventricular fibrillation threshold. The enhance vulnerability ventricular fibrillation induced by α_1-adrenergic agonist could be demonstrated only at supraphysiological extracellular calcium concentrations but not at physiological calcium concentrations. The arrhythmogenic and inotropic effects of α_1-adrenergic agonists were prevented by inhibition of sarcolemmal Ca^{2+} ion influx by inhibition of Ca^{2+} release from sarcoplasmic reticulum. Likewise, in the present study, ischemic-reperfusion causes the Ca^{2+} ion influx through the sarcolemma and induces ventricular arrhythmias (Lu, 1999). Reperfusion arrhythmias could be prevented by the blockade of α_1-adrenergic blocker, prazosin. Prazosin not only has α_1-adrenergic blocking effect but also free radical scavenging effect which may reduce incidences of cardiac arrhythmias. The effects of prazosin on the hydrogen peroxide-induced mechanical and metabolic derangements were studied in the isolated rat heart. The H_2O_2 -induced mechanical and metabolic derangement were attenuated by 2.5, 5,or 10 μM prazosin, and an increase in a level of malondialdehyde (lipid peroxidation) was attenuated by 5 or 10 μM prazosin (Akahira et al., 1998). According to our studies, 5μM prazosin was used and their hydroxyl radical scavenging effect might be responsible for antiarrhythmic effect. Another possible mechanism which is responsible for antiarrhythmic effect of prazosin is Na^+ channel blocking effect. Prazosin has a Na^+ channel blocking action as well as the α_1-blocking action. Su et al. (1995) have found that prazosin (1-10 μm) inhibits inward Na^+ current in the rat, guinea pig, and human myocardium. This action of prazosin is unrelated to blockade of α_1-AR. In addition, the pharmacological properties of prazosin had demonstable local anesthetic properties and there was no relationship between potency of α_1- adrenergic blockade and antiarrhythmic efficacy (Daugherty et al., 1986). Therefore, it is possible that the

antiarrhythmic action of prazosin may not due to the α_1-adrenergic blockade but, it due to the local anesthetic properties from the inhibition of inward Na^+ current.

In the heart, as in other tissues, α_1-adrenergic receptors are coupled to the phosphatidylinositol (PtdIns) turn over pathway. In the model of myocardial ischemia using isolated perfused rat hearts, the 2-minute reperfusion following 20-minute ischemia produced a rapid and transient release of inositol phosphates which were inhibited by perfusion of 10 µM prazosin. These results reveal that ischemia-reperfusion produced inositol phosphates mediated by α_1-adrenergic receptors (Anderson et al, 1995). Because the rapid release of Ins (1, 4, 5) P_3 caused Ca^{2+} oscillations, the accumulation of inositol phosphates between 1-2 minutes after initiation of reperfusion might be related to reperfusion arrhythmias.

Although our experiment indicates that the combination of atenolol and prazosin show antiarrhythmic effect, there was an experiment which demonstrated that prazosin (5.2 µM) alone could not prevent reperfusion arrhythmias in rat isolated heart. The left main coronary artery of rat hearts were ligated for 10 minutes and followed by reperfusion. The same as our experiment, ventricular arrhythmias did not occur during the occlusion period. Reperfusion was followed by ventricular fibrillation and ventricular tachycardia in all hearts in the control group. These data demonstrated that 5.2 µm prazosin alone was not antiarrhythmic (Bralet et al., 1985). Therefore, the combination of β_1-blocker and α_1-blocker produce antiarrhythmic effect stronger than α_1-blocker or β_1-blocker alone.

From the results, prazosin prolongs R-R intervals and decreases heart rate at each period. These data indicate that α_1-adrenergic receptors play an important role in negative chronotropy of rat hearts. In agreement with other investigations, phenylephrine (α_1- agonist) induced a positive chronotropic effects in isolated rat right atria (Williamson et al., 1994). In addition, Saito *et al.* (1994) have demonstrated that sinoatrial node and atrioventricular node of rat hearts contain α_1-adreoceptors which play an important role in the conduction system. In contrast, the negative chronotropic effect induced by

phenylephrine was observed in right vagally innervated Langendorff-perfused guinea pig hearts (Chevalier et al., 1998). These studies probably reflect species differences in effects of α_1-adrenergic stimulation.

Effects of β_2-adrenergic stimulation on cardiac contractility, electrophysiology and coronary vasodilatation in ischemic-reperfusion heart

ATEN/SALBU produced recovery of cardiac contractility via the stimulation of β_2-ARS in I/R heart. The β_2 activation exerted positive inotropic and chronotropic effects (Kaumann et al., 1989; Motomura et al., 1990). During reperfusion, ATEN/SALBU treated heart showed an increase in diastolic function accessed by dP/dt_{min}. The cardiac relaxation via β_2-ARs has been explained by Kaumann et al. (1999). This receptor subtype hastens cardiac relaxation and associated with phosphorylation of phospholamban, troponin I, and C-protein. Although salbutamol has an arrhythmogenic effect mediating by increases in cytosolic Ca^{2+} transient (Billman et al., 1997), it was not found the arrhythmogenic effect in our experiment. The 10 μM atenolol (β_1-adrenergic receptor antagonist) combined with 0.01μM salbutamol (β_2-adrenergic receptor agonist) did not show arrhythmogenic effect compared to the control group. This may due to the low dose of salbutamol which produced only positive inotropic and chronotropic effects via β_2-ARs at postganglionic cardiac sympathetic nerve terminals. The arrhythmogenic effect of salbutamol may also suppressed by atenolol via β_1- AR in the role of sympathoexcitatory cardiac β_2-ARs in human hearts which demonstrated by Newton et al. (1999). It has been found that the inotropic response of intracoronary injection of salbutamol was abolished by atenolol. It has been postulated that myocardial β_2-ARs play a role in the induction of malignant arrhythmias during myocardial infarction. The β_2-AR stimulation resulted in cytosolic Ca^{2+} transient increases. These may lead to afterpotentials that ultimately trigger VF in dog cardiomyocytes (Billman et al., 1997). Additionally, there has been reported in patients that salbutamol increased number of episodes of ventricular tachycardia and may cause serious arrhythmias in patients predisposed to develop arrhythmias. ATEN/SALBU increased in heart rate and tended to decrease in RR-interval during drug perfusion. In agreement with other studies that the

stimulation of β_2-adrenergic receptors exerts positive chronotropic effects. The electrophysiologic effects of salbutamol were studied in human in vivo. Salbutamol infusion produced electrophysiologic properties in both myocardial and nodal tissues, with significantly greater effects on nodal properties. An interesting result was a significant increase in QS interval which in the presence of unchanged His-Purkinje conduction represents slower depolarization of the ventricle. Furthermore, QT dispersion also increased (Insulander et al., 2003). An another experiment, intravenous and intracoronary salbutamol 10-30 μg/min and 1-10 μg/min were infused during fixed atrial pacing. Salbutamol decreased QT $_{onset}$ and QT $_{peak}$ but increased QT $_{end}$ duration, resulting in T wave prolongation (201msec to 233 msec; p<0.01) (Lowe et al., 2001). The increase in dispersion of repolarization provided a mechanism whereby cathecolamines acting through the β_2-adrenergic receptor subtype may trigger ventricular arrhythmias. In contrast, ATEN/SALBU did not alter QT$_c$ in our experiment. Atenolol which is β_1-blocker may prevent the prolongation of QT$_c$ intervals affected by salbutamol. As an experiment in healthy human in vivo, the QT interval was significantly longer (from 9 to 16 msec) after acute atenolol administration at heart rates between 80 and 120 beats per minute (Viitasalo and Karjalainene, 1992).

According to our studies, the coronary flow in control and ATEN/PRAZ treated group declines by time after drug perfusion whereas ATEN and ATEN/SALBU did not. Our results indicated that the β_2-AR stimulation produce vasodilation of coronary artery greater than the α_1-AR blockade. β_2- adrenergic receptors may play an important role in vasodilation of the coronary artery. In agreement with the study in isolated human coronary arterioles from the left ventricle, administration of 10^{-5} mol/L of propanolol (a nonspecific β-blocker)or 10^{-6} mol/L of butoxamine (β_2-blocker) completely eliminated the norepinephrine (NE)-induced dilation whereas the constriction to NE were inhibited by 10^{-6} mol/L of prazosin only 2 of 39 vessels. These data indicated that isolated coronary arterioles and small artery from the heart of patients with dilated cardiomyopathy dilated to NE via β_2- ARs on smooth muscle (Sun et al., 2002). As we known, coronary perfusion occurred in the period of cardiac relaxation. The cardiac β_2-AR stimulation increased

cardiac relaxation (Kaumann et al., 1999). This reason may cause the increase in coronary flow in ATEN/SALBU treated group.

Effects of adrenergic drug on RR-variability

Although there was no significant difference in measure of RR-variability between baseline and drug perfusion, the trend of RR–variability could be observed. Both SDNN and rMSSD are increased after drug perfusion in all groups except ATEN/SALBU treated group. SDNN and rMSSD of ATEN/PRAZ treated group are mostly increased. These data reveal that a decrease in RR-variability due to the effects of salbutamol and the increase in RR-variability due to the effects of prazosin. In accordance with the experiment in human in vivo, a decrease in heart rate variability using frequency domain of the acute effects of salbutamol was observed in adult asthmatic patients. HRV analysis was performed for each 5-minutes segment, 5 minutes before inhalation of the study drug and 5, 10, 15, 20, 25, and, 30 minute after inhalation. Total power (TP) (<0.40Hz), high frequency power (0.15-0.40 Hz), low frequency power (0.04-0.15 Hz) and LF/HF ratio were calculated. The LF, LF/HF ratio increased and TP decreased at 5, 10, 15, and 20 minute after salbutamol inhalation, HF did not change significantly. These finding showed the inhalation of β_2-adrenergic agonists caused an acute and statistically significant augmentation of LF and HR and decrease in TP, which is considered to reflected sympathetic activity (Eryonucu et al., 2001). Previous study, scatterplot has also shown the same results that salbutamol reduced HRV fraction significantly (Silke et al., 2000). These results reveal that salbutamol alter RR-variability by influence the sinus node. However, the adrenergic drug at the doses used in this experiment did not influence the sinus discharge rate.

Effects of adrenergic drug on cardiac myocyte apoptosis

As other investigations, ischemia-reperfusion in this study has shown cardiac myocyte apoptotic cells (Fliss and Gattinger, 1996). In our investigations, apoptotic cardiomyocytes were rarely found in non-ischemic area and more numerous in peri - infarction and infarction areas nevertheless there was no significant difference. It is

possible that the time of coronary ligation is short and not enough to induce numerous apoptotic cells.   Previous study had shown that continuous ischemia or ischemia followed by reperfusion could cause apoptosis in the rat model of coronary artery occlusion. The myocardium subjected to 45 minutes of ischemia followed by 1 hour of reperfusion appears to undergo accelerated apoptosis. The cardiomyocytes apoptosis was determined by in situ end labeling and DNA ladder (Fliss and Gattinger, 1996). In addition, even 30 minutes of ischemia in langendorff model could not induce apoptosis as evidenced under propidium iodide filter exposure. There was no sign of tunnel of fragmented nuclear DNA in rat myocardium biopsies (Maulik et al., 1997). Although the time of coronary occlusion just only 8-minute followed by 1 hour of reperfusion can caused very little cardiac myocyte apoptosis, the numbers of apoptotic cells in our experiment were near to the patients with heart failure. Recently, myocyte apoptosis has been noted in failing human heart. There has been reported that approximately 80-250 heart muscle cells per 10^{5} cardiac nuclei commit suicide at any given time in patients with late-stage dilated cardiomyopathy (Olevetti et al., 1997; Guerra et al., 1999).

It could be concluded from this experiment that 8 minutes ischemia followed by 1 hour reperfusion caused physiological function changed independent from cardiac myocyte apoptosis.   There were many reports demonstrated that reactive oxygen species promotes myocyte apoptosis observed in many pathological situations such as ischemic heart (Palojoki et al., 2001). Although reactive oxygen species formation was not detected in this study, the deteriorated left ventricular function following reperfusion was improved by antioxidant property of prazosin. It is important to consider that apoptosis may not be the whole mechanism responsible for left ventricular dysfunction in I/R heart.

In conclusion, the combination of atenolol (β_1-adrenergic antagonist) and prazosin (α_1-adrenergic antagonist) has the most potential antiarrhythmic effects whereas the combination of atenolol (β_1-adrenergic blocker) and salbutamol (β_2-adrenergic agonist) provide recovery in contractile function and coronary flow after ischemic-reperfusion. Possible mechanisms which prazosin can reduce ventricular arrhythmias are Ca^{2+} overload reduction, free radical scavenging and Na^+ channel blocking effect. However, the combination of atenolol and prazosin causes marked decrease in heart rates compared to the use of atenolol alone. Although atenolol slightly reduced incidences of ventricular arrhythmias, it produces slightly drop in heart rate. Therefore, the use of atenolol plus prazosin should be caution with marked decrease in heart rate. In vivo study should be confirmed the effect of the combination of β_1- and α_1-adrenergic antagonist. According to the combination of β_1- adrenergic antagonist and β_2- agonist, salbutamol produces recovery of contractile function by the activation of β_2-adrenergic receptors which exert positive inotropic and lusitropic effects. In addition, the administration of β_2-adrenergic agonist tends to increase coronary flow in I/R heart via the stimulation of β_2- adrenergic receptors which are main receptors responsible for coronary vasodilation. However, the stimulation of these receptors may increase incidence of ventricular arrhythmias which depends on the dosages used.

REFERENCES

Abrahamsson, T., Ek, B. and Nerme, V. 1988. The beta 1- and beta 2- adrenergic receptors affinity of atenolol and metoprolol. A receptor-binding study performed with different radioligands in tissues from the rat, the guinea pig and man. *Biochem. Pharmacol.* 37(2): 203-208. (abstract)

Aiello, E.A., Jabr, R.I. and Cole, W.C. 1995. Arrhythmia and delayed recovery of cardiac action potential during reperfusion after ischemia; Role of oxygen radical induced no-reflow phenomenon. *Circ. Res.* 77: 153-162.

Akahira, M., Hara, A., Hashizume, H., Anderson, K.E., Dart, A.M. and Woodcock, E.A. 1995. Inositol phosphate release and metabolism during myocardial ischemia and reperfusion in rat heart. *Circ. Res.* 76: 261-268.

Akahira, M., Hara, A., Hashizume, H., Makamura, M. and Abiko, Y. 1998. Protective effect of prazosin on the hydrogen peroxide-induced derangements in the isolated perfused rat heart . *Life Sci.* 62(19): 1755-1766.

Allen, D.G. and Orchard, C.H. 1987. Myocardial contractile function during ischemia and hypoxia. *Circ. Res.* 60: 153-168.

Altschuld, R.A., Staring, R.C., Hamlin, R.L., Billman, G.E., Hensley, J., Castillo, L., Fertel, R.H., Hohl, C.M., Robitaille, P-M.L., Jones, L.R., Xiao, R-P and Lakatta, E.G. 1995. Response of failing canine and human heart cells to β_2-adrenergic stimulation. *Circulation.* 92: 1612-1618.

Anderson K.E., Dart A.M. and Woodcock E.A. 1995. Inositol phosphate release and metabolism during myocardial ischemia and reperfusion in rat heart. *Circ. Res.* 76: 261-268.

Armstrong, P.W., Chiong, M.A. and Packer, J.O. 1977. Effect of propanolol on the hemodynamic, coronary sinus blood flow and myocardial metabolic response to atrial pacing. *Am. J. Cardiol.* 40: 83-89.

Aubert, A.E., Ramaekers, D., Beckers, F., Breem, R., Denef, C., Van de Werf, F. and Ector, H. 1999. The analysis of heart rate variability in unrestrained rats. *Comput Methods Programs Biomed.* 60: 197-213.

Australia-New zealand Heart Failure Research Collaborative Group. 1995. Effects of Carvedilol, a vasodilator-β-blocker, in patients with congestive heart failure due to ischemic heart disease. *Circulation*. 92: 212-218.

Basu, S.,Senior, R.,Raval, U.,Lahiri, A. 1997. Beneficial effects of intravenous and oral carvedilol treatment in acute myocardial infarction: a placebo-controlled, randomized trial. *Circulation*. 96: 183-191.

Bernier, M., Hearse, D.J. and Manning, A.S. 1986. Reperfusion-induced arrhythmias and oxygen derived free radicals: Studies with anti-free radical interventions and a free radical-generating system in the isolated perfused rat heart. *Circ. Res*. 58: 331-340. (abstract)

Billman, G.E., Castillo, L.C., Hensley, J., Holh, C.H. and Altschuld, R.A. 1997. β_2-adrenergic receptor antagonists protect against ventricular fibrillation: In vivo and in vitro evidence for enhanced sensitivity to β_2- adrenergic stimulation in animals susceptibilible to sudden death. *Circulation*. 96: 1914-1922.

Bolli, R. 1998. Why myocardial stunning is clinically important. *Basic Res. Cardiol*. 93: 169-172.

Bralet, J., Didier, J., Moreau, D., Opie, L.H. and Rochette, L. 1985 . Effect of alpha-adrenergic receptors antagonists (phentolamine, nicergoline and prazosin) on reperfusion arrhythmias and noradrenaline release in perfused rat heart. *Br. J. Pharmacol*. 84: 9-18. (abstract)

Bristow, M.R. 1993. Changes in myocardial and vascular receptors in heart failure. *J. Am. Cardiol*. 22(suppl A): 61A-71A.

Bristow, M.R., Abraham, W.T., Yoshigawa, T., White, M., Hattler, B., Crisman, T., Lowes, B., Larrabee, P. and Gilbert, E.M. 1997. Second and Third beta blocking agents in the treatment of chronic heart failure. *Cardiovasc Drugs Ther*. 11: 291-296.

Bristow, M.R., Roden, R.L., Rowes, B.D., Gilbert, E.M. and Eichhorn, E.J. 1998. The role of third generation β_b-blocking agents in chronic heart failure. *Clin Cardiol*. 21: I3-I13.

Bristow, M.R. 2000. β-adrenergic blockade in chronic heart failure. *Circulation*. 101: 558-569.

Brodde, O.E., Michel, M.C. and Zerkowski, H.R. 1995. Signal transduction mechanisms controlling cardiac contractility and their alterations in Chronic heart failure. *Cardiovasc. Res* . 30: 570-584.

Brodde, O.E. 1999. Adrenergic and muscarinic receptors in the human heart. *Pharmacol. Rev*. 51(2): 651-668.

Brodde, O.E., Bruck, H., Leineweber, K. and Seyfarth, T. 2001. Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. *Basic Res. Cardiol.* 96: 528-538.

Butterfield, M.C. and Chess-Williams, R. 1990. Enhanced alpha-adrenergic receptors responsiveness and receptor number during global ischemia in the Langendorff perfused rat heart. *Br. J. Pharmacol*. 100: 641-645.

Chesley, A., Lundberg, M.S., Asai, T., Xioa, R.P., Ohtsni, S., Lakatta, E.G. and Crow, M.T. 2000. The β_2-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G_i-dependent coupling to phosphatidylinositol 3'kinase. *Circ Res*. 87: 1172-1179.

Chess-Williams, R. and Milton, H.L. 2001. Arrhythmogenesis in isolated rat heart with enhanced alpha-adrenergic receptors-mediated responsiveness. *J Auton Pharmacol*. 21(1): 39-35. (abstract)

Chevalier, P., Ruffy, F., Dalino, P. and Rosen, M.R. 1998. Interaction between Alpha-1 adrenergic and vagal effects on cardiac rate and repolarization. *J. Pharmacol. Exp Ther*. 284: 832-837.

Communal, C., Singh, K., Pimental, D.R. and Colucci, W.S. 1998. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the β-adrenergic pathway. *Circulation*. 98: 1329-1334.

Communal, C., Singh, K., Sawyer, D.B. and Colucci, W.S. 1999. Opposing effects of β_1- and β_2-adrenergic receptors on cardiac myocyte apoptosis: Role of pertussis toxin-sensitive G protein. *Circulation*. 100: 2210-2212.

Dabrowska, B., Pruszezyk, P., Dabrowski, A., Feltynowski, T., Wocial, B. and Januszewicz, W. 1995. Influence of alpha-adrenergic blockade on ventricular

arrhythmias, QT_c interval and heart rate variability in phaeochromocytoma. *J Hum Hypertens.* 9(11): 925-929.

Daugherty, A., Frayn, K.N., Redfern, W.S. and Woodward, B. 1986. The role of catecholamines in the production of ischaemia-induced ventricular arrhythmias in the rat in vivo and in vitro. *Br. J. Pharmacol.* 87: 265-277.

Del Monte, F., Kaumann, A.J., Poole-Wilson, P.A.,Wynne, D.G.,Pepper, J. and Harding, S.E. 1993. Coexistence of functioning β_1-and β_2- adrenergic receptors in single myocytes from human ventricle. *Circulation.* 88: 854-863.

Di Napoli, P., Contegiacomo, G., Di Crecchio, A., Di Muzio, M., Tiloca, P., Taccardi, A.A., Maggi, A. and Barsotti, A. 1998. Ischaemic preconditioning rat myocardium: effects on postischaemic coronary endothelium hyperpermeability and microcirculatory damage. *J Clin Bas Cardiol.* 1: 37-42.

Du, X.J, Anderson, K.E., Jacobsen, A., Woodcock, E.A. and Dart, A.M. 1995. Suppression of ventricular arrhythmias during ischemia-reperfusion by agents inhibiting Ins (1, 4, 5) P_3 release. *Circulation.* 91: 2712-2716.

Du, X.J, Cox, H.S., Dart, A.M. and Esler, M.D. 1999. Sympathetic activation triggers ventricular arrhythmias in rat heart with chronic infarction and failure. *Cardiovasc. Res.* 43: 919- 929.

Erdmann, E. 1998. Chronic cardiac insufficiency: therapy with beta-adrenergic blockers. *J Clin Bas Cardiol.* 1: 10-13.

Eryonucu, B., Uzun, K., Guler, N. and Bilge, M. 2001. Comparison of the acute effects of salbutamol and terbutaline on heart rate variability in adult asthmatic patients. *Eur. Respir. J.* 17: 863-867.

Esler, M., Kaye, D., Lambert, G., Esler, D. and Jennings, G. 1997. Adrenergic nervous system in heart failure. *Am. J. Cardiol.* 80(11A): 7L-14L.

Ferguson, D.W. 1997. Sympathetic mechanisms in heart failure. Pathophysiological implications. *Circulation.* 87(Suppl VII): VII68-VII75.

Flesch, M., Maack, C., Cremers, B., Bäumer, A.T., Südkamp and Böhm M. 1999. Effects of β-blockers on free radical-induced cardiac contractile dysfunction. *Circulation.* 100: 346-353.

Fliss, H. and Gattinger, D. 1996. Apoptosis in ischemic and reperfusion rat myocardium. *Circ. Res.* 79: 949-956.

Gilbert, E.M., Abraham, W.T., Olsen, S., Hattler, B., White, M., Mealy, P., Larrabee, P. and Bristow, M.R. 1996. Comparative hemodynamic, left ventricular functional, and antiadrenergic effects of chronic treatment with metoprolol versus carvedilol in the failing heart. *Circulation*. 94: 2817-2825.

Goldhaber, J.I. and Weiss, J.N. 1992. Oxygen free radicals and cardiac reperfusion abnormalities*. Hypertension*. 20(1): 118-27.

Guerra, S., Leri, A., Wang, X., Finato, N., Di Loreto, C., Beltrami, C.A., Kajstura, J. and Anversa, P. 1999. Myocyte death in the failing human heart is gender dependent. *Circ. Res.* 85: 856-866.

Haber, H.L., Christopher, L.S., Gimple, L.W., Bergin, J.D., Subbiah, M.E., Jayaweera, A.R., Powers, E.R. and Feldman, M.D. 1993. Why do patients with congestive heart failure tolerate the initiation of β-blocker therapy? *Circulation*. 88: 1610-1619.

Hall, J.A., Petch, M.C. and Brown, M.J. 1989. Intracoronary injection of salbutamol demonstrate the presence of functional β_2-adrenergic receptors in the human heart. *Circ .Res*. 65: 546-553.

Hall, J.A., Petch, M.C. and Brown, M.J. 1991. In vivo demonstration of cardiac β_2-adrenoceptor sensitization by β_1-antagonist treatment. *Circ. Res.* 69: 959-964.

Hanaki, Y., Saito, H., Sugiyama, S. and Ozawa, T. 1988. Effect of the alpha1-blocker bunazosin on reperfusion-induced mitochondrial dysfunction in canine hearts. *Arzneimittelforschung*. 38(1): 11-13. (abstract)

Haunstetter, A. and Izumo, S. 2000. Toward antiapoptosis as a new treatment modality. *Circ. Res.* 86: 371-376.

Heyndrickx, G.R. 2003. Myocardial stunning: an experimental act with a large clinical audience. *Arch Mal Coeur*. 96: 665-670.

Hool, L.C. and Harvey, R.D. 1997. Role of β_1- and β_2- adrenergic receptors in regulation of Cl^- and Ca^{2+} channels in guinea pig ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 273: H1669-H1676.

Houle, M.S., Altschuld, R.A. and Billman, G.E. 2001. Enhanced in vivo and in vitro contractile responses to β_2- adrenergic receptor stimulation in dogs susceptible to lethal arrhythmias. *J Appl Physiol*. 91: 1627-1637.

Hwang, K.C, Gray, C.D., Sweet, W.E., Moravec, C.S. and Im, M.J. 1996. α_1-Adrenergic receptor coupling with G_h in the failing human heart. *Circulation*. 94: 718-726.

Inserte, J.,Taimor, G., Hofstaetter, B., Garcia-Dorado, D. and Piper, H.M. 2000. Influence of simulated ischemia on apoptosis induction by oxidative stress in adult cardiomyocytes of rats. *Am. J. Physiol. Heart Circ. Physiol.* 278: H94-H99.

Insulander, P., Juhlin-Dannfelt, A., Freyschuss, U. and Vallin, H. 2003. Electrophysiologic effects of salbutamol, a β_2-selective agonist. *J. Cardiovasc. Electrophysiol*. 15(3): 316. (abstract)

Iwase, M., Bishop, S.P., Uechi, M., Vatner, D.E. et al. 1996. Adverse effects of chronic endogenous sympathetic drive induced by cardiac Gsα overexpression. *Circ. Res*. 78: 517-524.

Katz, .AM. 2001. Physiology of the heart. 3rd ed. Philadelphia: Lippincott Williams & Wilkins.

Kaumann, A.H., Hall, J.A., Murray K.J., Wells F.C., Brown M.J. 1989. A comparison of the effects of adrenaline and noradrenaline on human heart: the role of β_1- and β_2-adrenergic receptors in the stimulation of adenylate cyclase and contractile force. *Eur. Heart. J*. 10: 29-37

Kaumann, A.J. and Molenaar, P. 1997. Modulation of human cardiac function through 4 β-adrenergic receptors population. *Naunyn-Schmiedeberg's Arch Pharmacol*. 355: 667-681.

Kaumann, A., Bartel, S., Molenaar, P., Sanders, L., Burrell, K., Vetter, D., Hempel, P., Karczewski, P. and Krause, E.G. 1999. Activation of β_2-adrenergic receptors hastens relaxation and mediates phosphorylation of phospholamban, troponin I, and C-protein in ventricular myocardium from patients with terminal heart failure. *Circulation*. 99: 65-72.

Kennedy, H.L., Brooks, M.M., Barker, A.H., Bergstrand, R., Huther, M.L., Beanlands, D.S., Bigger, J.T. and Goldstein, S. 1994. Beta-blocker therapy in the cardiac arrhythmia suppression trial.Cast investigstors. *Am. J. Cardiol.* 74(7): 674-680.

Kimura, S., Cameron, J.S., Kozlovskis, P.L., Bassett, A.L. and Myerburg, R.J. 1984. Delayed afterdepolarizations and triggered activity induced in feline purkinje fibers by alpha-adrenergic stimulation in the presence of elevated calcium levels. *Circulation.* 70: 1074-1082.

Kulkin, M.L., Charney, R.H., Levy, D.K., Buchholz-Varley, C., Ocampo, O.N. and Eng, C. 1999. Prospective, randomized comparison of effect of long-term treatment with metoprolol or carvedilol on symptoms, exercise, ejection fraction, and oxidative stress in heart failure. *Circulation.* 99: 2645-2651.

Kurz, T.,Yamada, K.A., Datorre, S.D. and Corr, P.B. 1991. Alpha1-adrenergic system and arrhythmias in ischaemic heart disease. *Eur Heart J.* 12: 88-98.

Lamba, S. and Abraham, W.T. 2000. Alterations in Adrenergic Receptor Signaling in Heart Failure. *Heart Fail Rev.* 5: 7-16.

Landzberg, J.S., Parker, J.D., Guathier, D.F. and Colucci, W.S. 1991. Effects of myocardial alpha-1adrenergic receptor stimulation and blockade on contractility in humans. *Circulation.* 84: 1608-1614.

Lechart, P., Packer, M., Chalon, S., Cucherat, M., Arab, T. and Boissel, J.P. Clinical effects of β-adrenergic blockade in chronic heart failure: A meta-analysis of double blind, placebo-controlled, randomized trials. *Circulation.* 98: 1184-1191.

Liu, P., Hock, C.E., Nagele, R. and Wong, P.Y. 1997. Formation of nitric oxide, superoxide, and peroxynitrite in myocardial ischemia-reperfusion injury in rats. *Am. J. Physiol. Heart Circ. Physiol.* 272: H2327-H2336.

Lowe, M.D., Rowland, E., Brown, M.J. and Grace, A.A. 2001. β_2 Adrenergic receptors mediate important electrophysiological effects in human ventricular myocardium. *Heart.* 86: 45-51.

Lu, H.R., Yang, P., Remeysen, P., Saels, A., Dal, D.Z. and De Clerck, F. 1999. Ischemic/reperfusion-induced arrhythmias in anesthetized rat: a role of Na$^+$ and Ca^{2+} influx. *Eur .J. Pharmacol.* 22; 365(2-3): 233-239.

Luther, H.P., Podlowski, S., Schulze, W., Morwinski, R., Buchwalow, I., Baumann, G. and Wallukat, G. 2001. Expression of alpha1-adrenergic receptor subtypes in heart cell culture. *Mol. Cell. Biochem.* 224(1-2): 69-79. (abstract)

Malliani, A., Montano, N. and Pagani, M. 1997. Physiological background of heart rate variability. *Card Electrophysiol Rev.* 1(3): 343-346.

Mason, D., Spann, J. and Zelis, R. 1970. Quantification of the contractile state of the intact human heart. *Am. J. Cardiol.* 26: 248-257.

Manning, A.S. and Hearse, D.J. 1984. Reperfusion-induced arrhythmias: mechanisms and prevention. *J. Mol. Cell. Cardiol.* 16: 497-518.

Maulik, N.,Yoshida, T. and Das, D.K. 1997. Oxidative stress developed during the reperfusion of ischemic myocardium induces apoptosis. *Free. Radic. Biol. Med*. 24(5): 869-875.

MDS Pharma Services. Pharmacology profiling, Tissue assays of adrenergic drug [Online].Available from: http://www.discovery.mdsps.com/Catalog/Assays/Assay Details.aspx?id=407500

Mettauer, B., Rouleau, J.L. and Burgess, J.H. 1985. Detrimental arrhythmogenic and sustained beneficial hemodynamic effects of oral salbutamol in patients with chronic congestive heart failure*.   Am. Heart J*. 109(4): 840-847.

Motomura, S., Zerkoski, H.R., Daul, A. and Brodde, O.E. 1990. On the physiological role of beta-2 adrenergic receptors in the human heart: In vitro and in vivo studies. *Am. Heart J.* 119: 608-619.

Newton, G.E., Azevedo, E.R. and Parker, J.D. 1999. Inotropic and sympathetic responses to the intracoronary infusion of a β_2-receptor agonist: A human in vivo study. *Circulation.* 99: 2402-2407.

Novo, S., Abrignani, M.G., Novo, G., Nardi, E., Dominguez, L.J, Strano, A. and Barbagalo, M. 2001. Effects of drug therapy on cardiac arrhythmias and

ischemia in hypertensives with left ventricular hypertrophy. *Am. J. Hypertens.* 14(7 pt 1): 637-643.

Olivetti G., Abbi, R., Quaini, F., Kajstura, J., Cheng, W., Nitahara, J.A., Quaini, E., Di Loreto, C., Beltrami, C.A., Krajewski, S., Reed, J.C. and Anversa, P. 1997. Apoptosis in the failing human heart. *N. Eng. J. Med.* 336: 1131-1141.

Opitz, C.F., Mitchell, G.F., Pfeffer, M.A. and Pfeffer, J.M. 1995. Arrhythmias and death after coronary artery occlusion in the rat: Continuous telemetric ECG monitoring in conscious, untethered rats. *Circulation.* 92: 253-261.

Opitz, C.F., Finn, P.V., Pfeffer, M.A., Mitchell, G.F. and Pfeffer, J.M. 1998. Effects of reperfusion on arrhythmias and death after coronary artery occlusion in the rat: Increased electrical stability independent of myocardial salvage. *J. Am. Coll. Cardiol.* 32: 261-267.

Orlick, A.E., Ricci, D.R. and Cipriano, P.R. 1978. The contribution of alpha-adrenergic tone to resting coronary vascular resistance in man. *J. Clin. Invest.* 62: 459.

Palojoki, E., Saraste, Antti, Erikkson, A., Pulkii, K., Pulkki, K., Kallajoki, M., Voipio-Pulkki, L.M. and Tikkanen, I. 2001. Cardiomyocyte apoptosis and ventricular remodeling after myocardial infarction in rats. *Am. J. Physiol. Heart Circ. Physiol.* 280: H2726-H2731.

Packer, M. 1985. Sudden unexpected death in patients with congestive heart failure: a second frontier. *Circulation.* 2: 681-685.

Peuhkurinen KJ. 2000. Ischemic heart disease at the cellular level. *Int J. Bioelectromagn.* 2: 1-8

Pogwizd, S.M., Schlotthauer, K., Li, L., Yuan, W. and Bers, D.M. 2001. Arrhythmogenesis and contractile dysfunction in heart failure roles of sodium-calcium exchange, inward rectifier potassium current, and residual β-adrenergic responsiveness. *Circ. Res.* 88: 1159-1167.

Reiter, M.J. and Reiffel, J.A. 1998. Importance of beta blockade in the therapy of serious ventricular arrhythmia. *Am. J. Cardiol.* 82: 9I-19I.

Ribner, H.S., Bresnahan, D. and Hsieh, A.M. 1982. Acute hemodynamic responses to vasodilation therapy in congestive heart failure. *Prog Cardiovasc Dis.* 25:1.

Rodefeld, M.D., Beau, S.L., Schuessler, R.B., Boineau, J.P. and Saffitz, J.E. 1996. Beta-adrenergic and muscarinic cholinergic receptor densities in the human sinoatrial node: identification of a high beta2-adrenergic receptor density. *J. Cardiovasc. Electrophysiol*. 7(11): 1039-1049.

Sabbah, H.N., Sharov VG, Gupta RC, Todor A, Singh, V and Goldstein S. 2000. Chronic therapy with metoprolol attenuates cardiomyocyte apoptosis in dogs with heart failure. *J. Am. Coll. Cardiol*. 36(5): 1698-1705. (abstract)

Saito, K., Suetsugu, T., Kuroda, A. and Tanaka, H. 1994. Alpha1- adrenergic receptors in the conduction system of rat hearts. *Br. J. Pharmacol*. 111: 465-468.

Scarabelli, T., Atephanou, A., Rayment, N., Pasini, E., Comini, L., Curello, S., Ferrari, R., Night, R. and Latchman, D. 2001. Apoptosis of endothelial cells precedes myocyte cell apoptosis in ischemia/reperfusion injury. *Circulation*. 104: 253-256.

Schmedtje, J.F., Liu, W.L. and Chen, Y.M. 1996. pH is critical to the regulation of expression of the β_2-adrenergic receptor gene in hypoxia. *Biochim. Biophys. Acta*. 1314: 25-33.

Scholz W, Albus U, Counillon L et al. 1995. Protective effects of HOE642, a selective sodium-hydrogen exchange subtype I inhibitor, on cardiac ischemia and reperfusion. *Cardiovasc. Res*.29:160-268

Schomig, A. 1990. Cathecolamines in myocardial ischemia: Systemic and cardiac release. *Circulation*. 82(suppl II): II13-II22.

Schwartz, P.J., La Rovere, M.T. and Vanoli, E. 1992. Autonomic nervous system and sudden cardiac death. Experimental basis and clinical observation for post-myocardial infarction risk stratification. *Circulation*. 85: I77-I91.

Sheridan, D.J. 1986. Alpha adrenergic receptors and arrhythmias. *J Mol Cell Cardiol*. 18: 59-68.

Shizukuda, Y., Buttrick, P.M., Geenen, D.L., Borczuk, A.C., Kitsis, R.N., and Sonnenblick, E.H. 1998. β-adrenergic stimulation causes cardiocyte apoptosis: influence of tachycardia and hypertropht. *Am. J. Physiol. Heart Circ. Physiol*. 275(44): H961- H968.

Shrivastava, M.P., Dhrmadhikari, S.D., Gharoure, K.J. and Dashputra, P.G. 1985. Cardiovascular effects of β_2 stimulants and their mechanisms. *Indian J Pharmacol*. 18: 168-170.

Silke, B., Hanratty, C.G., Veres, S.M. and Riddel, J.G. 2000. β-adrenergic receptors modulation and heart rate variability-The value of scatter plot measures of compactness. *Cardiovasc Drugs Ther*. 14(4): 433-440.

Singh, K., Communal, C., Sawyer, D. and Colucci, W.S. 2000. Review adrenergic regulation of   myocardial apoptosis. *Cardiovasc. Res*. 45: 713-719.

Singh, K., Xiao, L., Remondilo, A., Sawyer, D.B. and Colucci, W.S. 2001. Review article Adrenergic regulation of cardiac myocyte apoptosis. *J. Cell. Physiol.* 189(3): 257-265. (abstract)

Sleight, P. 1986. Use of beta adrenergic receptors blockade during and after acute myocardial infarction. *Annu. Rev. Med*. 37: 415-425.

Sun, D., Huang, A., Mital, S., Kichuk, M.R., Marboe, C.C., Addonizio, L.J., Michler, R.E., Koller, A., Hintze, T.H. and Kaley, G. 2002. Norepinephrine elicits $\beta_2$receptor-mediated dilation of isolated human coronary arterioles. *Circulation*. 106: 550-555.

Talwar, K.K., Bhargava, B., Upasani, P.T., Verma, S., Kamlaka, T. and Chopra, P. 1996. Hemodynamic predictors of early intolerance and long-term effects of propanolol in dilated cardiomyopathy. *J. Card. Fail*. 2: 273-277.

Tani, M., and Neely, J.R. 1989. Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemia rat hearts. *Circ. Res*. 65: 1045-1056.

Thandroyen, F.T., Flint, N.S., Worthington, M.G. and Opie, L.H. 1987. Arrhythmogenic of alpha1-adrenergic receptors stimulation in normoxic rat ventricular myocardium : influence of nisodipine reduced extracellular Ca^{2+} and ryanodine. *J. Mol. Cell. Cardiol*. 19(9): 841-851

Temsah, R.M., Dyck, C., Netticadan, T., Chapman, D., Elimban, V. and Dhalla, N.S. 2000. Effect of β-adrenergic receptors blockers on sarcoplasmic reticular

function and gene expression in the ischemic-reperfused heart. *JPET*. 293: 15-23.

Tong Mak, I. and Weglicki, W.B. 1988. Protection by β-blocking agents against free radical-mediated sarcolemmal lipid peroxidation. *Circ. Res*. 63: 262-266.

Tosaki, A., Maulik, N., Elliott, G.T., Blasig, I.E., Engelman, R.M. and Das, D.K. 1998 Preconditioning of rat heart with monophosphoryl lipid A: A role for nitric oxide. *J. Pharmacol. Exp. Ther.* 285:1274-1279.

Viitasalo, M. and Karjalainene, J. 1992. QT intervals at heart rates from 50 to 120 beats per minute during 24-hour electrocardiographic recordings in 100 healthy men: Effect of atenolol. *Circulation*. 86: 1439-1442. (abstract)

Walker, J.A., Curtis, M.L.,Hearse, D.J.,Campbell, W.F., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, W.G., Higgins, A.J., Julian, D.G., Lab, M.J, Manning, A.S, Northover, B.J., Parratt, J.R., Riemersms, R.A., Riva, E.,

Russel, D.C., Sheridan, D.J., Winslow, E. and Woodward, B. 1988. The Lembeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovasc. Res*. 22: 447-455.

Williamson, A.P., Seifen, E., Lindemann, J.P. and Kennedy, R.H. 1994. Alpha_{1A}-adrenergic receptor mediated positive chronotropic effect in right atria isolated from rats. *Can. J. Physiol. Pharmacol.* 72: 1574-1579.

Xiao, X.H. and Allen, D.G. 1999. Role of Na^+/H^+ exchanger during ischemia and preconditioning in isolated rat heart. *Circ. Res*. 85: 723-730.

Yamamoto, J., Ohyanagi, M., Morita, M. and Iwasaki, T. 1994. β-Adrenergic receptors-G-protein-adenylate cyclase complex in rat heart with ischemia heart failure produced by coronary artery ligation. *J. Mol. Cell. Cardiol*. 26: 617-626.

Yaoita ,H., Ogawa, K., Maehara, K. and Maruyama, Y. 2000. Apoptosis in relevant clinical situations: contribution of apoptosis in myocardial infarction. *Cardiovasc. Res*. 45: 630-641.

Yaoita, H., Sakabe, A., Maehara, K. and Maruyama, Y. 2002. Different effects of Carvedilol, Metoprolol, and Propanolol on left ventricular remodeling after

coronary stenosis or after permanent coronary occlusion in rats. *Circulation.* 105: 975-980.

Yue, T.L., Ma, X.L, Wang, X., Romanic, A.M., Liu, G.L., Louden, C., Gu, J.L., Kumar, S., Poste, G., Ruffolo, R.R., et al. 1998. Possible involvement of stress activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia/reperfusion-induced cardiomyocyte apoptosis by carvedilol. *Circ. Res.* 82:166-172.

Zaugg, M., Xu, W., Lucchinetti, E., Shafiq, S.A., Jamali, N.Z. and Siddiqui, M.A.Q. 2000. β-adrenergic receptor subtypes differentially affect apoptosis in adult rat ventricular myocytes. *Circulation.* 102: 344-350.

Zhang, Y.Y., Xu, K.M. and Han, C. 1999. α_1-Adrenergic receptors subtypes mediating inotropic responses in rat heart. *J. Pharmacol. Exp. Ther.* 291: 829-836.

Zipes,D.P. and Wellens, H.J. 2000. What have we learned about cardiac arrhythmias? *Circulation.* 102: IV-52-IV-57.

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