

Effects of a Beta Adrenergic Antagonist Combined with Vasodilators on Cardiac Arrhythmia in Ischemic-induced Rat Hearts

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Abstract

Sixty male Sprague-Dawley rats were used in this study. The hearts were isolated and placed on a constant-pressure Langendorff apparatus, and perfused with only Krebs-Henseleit buffer (control), atenolol, atenolol plus prazosin, and atenolol plus salbutamol. After that the left anterior descending artery was occluded for 8 minutes and reperfused for 30 minutes to induce cardiac arrhythmias. The left ventricular pressure and coronary flow rate were monitored, and ECGs were recorded to evaluate cardiac arrhythmias. The administration of atenolol (β_1 -adrenergic antagonist) combined with prazosin (α_1 -adrenergic antagonist) prolonged RR intervals whereas that of atenolol combined with salbutamol (β_2 -adrenergic antagonist) did not prolong RR intervals during the ischemic period. Moreover, reperfusion induced sustained ventricular fibrillation in all groups except the atenolol plus prazosin treated group. The decline of coronary flow rate after reperfusion in atenolol and atenolol plus salbutamol treated groups were lower than the atenolol plus prazosin group. Our results support the theory that α_1 -adrenergic receptors play an important role on reperfusion arrhythmia whereas coronary vasodilation is mediated through β_2 -adrenergic receptors. Furthermore, the combination of β_1 -adrenergic antagonist and α_1 -adrenergic antagonist could have a more beneficial effect for anti-arrhythmia in cardiac ischemia.

Keywords : Adrenergic, arrhythmias, beta blocker, ischemia, rat, vasodilator

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บทคัดย่อ

ผลของการใช้ยาเบต้าอะดรีโนร์จิกแอนทาโกนิสต์ร่วมกับยาขยายหลอดเลือดต่อการเกิดหัวใจเต้นผิดจังหวะในหัวใจหมูแรกที่เหนี่ยวนำให้เกิดภาวะขาดเลือด

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ทำการศึกษาหัวใจหมูแรกที่เหนี่ยวนำให้ขาดเลือดจำนวน 60 ตัว แบ่งออกเป็น 4 กลุ่มทดลอง กลุ่มที่ 1 กลุ่มควบคุมได้รับสารละลายบัฟเฟอร์ กลุ่มที่ 2 ได้รับสารละลายบัฟเฟอร์ร่วมกับยาอะทีโนโลลซึ่งเป็นยาในกลุ่มเบต้าวันอะดรีโนร์จิกแอนทาโกนิสต์ กลุ่มที่ 3 ได้รับสารละลายบัฟเฟอร์ร่วมกับยาอะทีโนโลล และชาลูนุ่มมาลซึ่งเป็นยาในกลุ่มเบต้าทูอะดรีโนร์จิกอะโกนิสต์ ส่วนกลุ่มที่ 4 ได้รับสารละลายบัฟเฟอร์ร่วมกับยาอะทีโนโลล และพรารोไซซินซึ่งเป็นยาในกลุ่มแอลฟ่าวันอะดรีโนร์จิกแอนทาโกนิสต์ ทำการแยกหัวใจเพื่อนำมาแขวนในแหล่งเก็บรักษาแล้วดักลิ่นไฟฟ้าหัวใจ ความดันในหัวใจห้องล่างซ้าย และอัตราการไฟของสารละลายผ่านหลอดเลือดโกรนารี หลังจากให้สารละลายและยาไฟของผ่านระยะหนึ่ง จึงทำการผูกหลอดเลือดแดงและที่เรียดเช่นดึงด้านซ้าย ซึ่งเป็นแขนงของหลอดเลือดแดงโกรนารีเพื่อเหนี่ยวนำให้เกิดภาวะหัวใจขาดเลือดเป็นเวลา 8 นาที แล้วคลายปมผูกหลอดเลือด ให้สารละลายไฟหล่นได้อีกรั้งเป็นระยะเวลา 30 นาที เพื่อเหนี่ยวนำให้เกิดหัวใจเต้นผิดจังหวะ จากผลการศึกษาพบว่า การให้ยาอะทีโนโลลร่วมกับพรารอไซซินส่งผลทำให้ระยะการร้ารื่งของคลื่นไฟฟ้าหัวใจยาวนานขึ้น ขณะที่การให้ยาอะทีโนโลลร่วมกับชาลูนุ่มมาลไม่มีผลทำให้ระยะการร้ารื่งเปลี่ยนแปลง นอกจานี้ทุกกลุ่มทดลองยังเกิดภาวะหัวใจเต้นผิดจังหวะแบบซ้ำๆ เต้นวนตอริคูลาไฟบริเลชัน ส่วนผลของยาต่ออัตราการไฟของสารละลายผ่านหลอดเลือดโกรนารีพบว่า กลุ่มที่ได้รับยาอะทีโนโลลย่างเดียวหรือได้รับยาอะทีโนโลลร่วมกับชาลูนุ่มมาลมีผลทำให้อัตราการไฟของสารละลายผ่านหลอดเลือดโกรนารีลดลงน้อยกว่ากลุ่มที่ได้รับยาอะทีโนโลลร่วมกับพรารอไซซินในช่วงระยะเวลาที่สารละลายไฟหลับเข้าใหม่อีกรั้งหนึ่ง จากการทดลองสรุปได้ว่ากลไกการเกิดหัวใจเต้นผิดจังหวะถูกควบคุมผ่านทางตัวรับแอลฟ่าวันอะดรีโนร์จิก และอัตราการไฟของสารละลายผ่านหลอดเลือดโกรนารีถูกควบคุมผ่านทางตัวรับเบต้าทูอะดรีโนร์จิก นอกจานี้ การให้ยาอะทีโนโลลร่วมกับยาอะทีโนโลลย่างเดียวหรือได้รับยาอะทีโนโลลร่วมกับชาลูนุ่มมาลมีผลทำให้อัตราการไฟหัวใจเต้นผิดจังหวะจากภาวะหัวใจขาดเลือด

คำสำคัญ : อะดรีโนร์จิก หัวใจเต้นผิดจังหวะ เบต้าลีอคเกอร์ ภาวะหัวใจขาดเลือด หมู ยาขยายหลอดเลือด

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Introduction

Cardiac disease, which affects many millions people around the world, is a leading cause of death as a result of contractile dysfunction, or sudden cardiac death caused by ventricular arrhythmia (Packer, 1985). About 40% of sudden death victims experience cardiac arrest at the first manifestation of coronary artery diseases (Zipes and Wellens, 2000).

Disturbances of cardiac rhythm, including lethal ventricular arrhythmia and post-ischemic contractile dysfunction are a consequence of reperfusion following myocardial ischemia. Many possible mechanisms contribute to contractile dysfunctions. The first one is an increase in sympathetic activity during myocardial

ischemia leading to cardiac arrhythmia (Du et al., 1999). Oxidative stress is also a cause of contractile dysfunction (Aiello et al., 1995; Bolli, 1998). In addition, the fall in intracoronary pressure downstream from an occlusion is itself a significant cause of an immediate decline in pump function (Katz, 2001).

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 disease. A general feature of the failing human heart is a decrease in the density of β_1 -adrenergic receptor (AR) leading to shifts in β_1 and β_2 -ARs ratio towards β_2 -ARs (Bristow, 1993). It has been reported that β_2 -ARs are not down-regulated but

their sensitivity may be increased (Altschuld et al., 1995).

The α_1 -adrenergic receptors in the heart can initiate an inotropic response under physiological conditions while they also play an important role in reperfusion arrhythmia, where extensive norepinephrine release has been documented (Sheridan, 1986; Schomig, 1990). In addition, reperfusion of ischemically damaged myocardium is associated with large Ca^{2+} accumulations, which can be inhibited by α_1 -adrenergic receptor blockade. These studies suggest a link between α_1 -adrenergic receptor stimulation and arrhythmia produced by myocardial ischemia and reperfusion.

β -adrenergic antagonists are commonly used for the treatment of ischemic heart disease (Erdmann, 1998). Possible mechanisms contributing to their effects include cardiac protection from the toxic catecholamine effect inducing apoptosis but also a reduction in heart rate leading to lower myocardial energy expenditure (Reiter and Reiffel, 1998), prolong diastolic filling, and protection from cardiac arrhythmia (Kennedy et al., 1994). Nevertheless, acute administration of first generation adrenergic antagonists, such as propanolol, causes a decrease in the contractile state (Haber et al., 1993), increased systemic vascular resistance (Armstrong et al., 1977; Bristow et al., 1998), and leads to a decrease in cardiac output, which results in a drug intolerance rate of more than 20% (Talwar et al., 1996). On the other hand, the second generation, β_1 -selective blockers have less reflex vasoconstriction because unblocked peripheral vascular β_2 -adrenergic receptors may respond and cause vasodilation. The overall effect is that cardiac output and organ perfusion are reduced to a lesser degree than the first-generation β -blockers (Bristow et al., 1998). Nowadays, it seems that a third-generation β -blocker, carvedilol, a non-selective β -blocker with vasodilating properties, is an 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 (Bristow, 2000). Compared to β_1 -selective blocker, carvedilol produces more beneficial effects on the left ventricular function (Gilbert et al., 1996; Bristow et al., 1997). However, some data also demonstrated no difference between them (Kulkin et al., 1999). Furthermore, the high price of newly developed drugs may limit drug availability especially in developing

countries.

It is interesting that the combination of vasodilating drugs mediated through α_1 -adrenergic antagonist or β_2 -adrenergic stimulation with a beta blocker can have beneficial effects on patients with ischemic heart diseases. However, the effects of these combined drugs on cardiac arrhythmia in ischemic hearts are still unclear. Therefore, the present study was designed to observe the effects of drug combinations on cardiac arrhythmia in ischemic rat hearts.

Materials and Methods

Isolated heart preparations (Langendorff apparatus)

Sixty male Sprague-dawley rats weighing between 250-350 grams were used in this study. The animals were anesthetized with pentobarbital sodium (60 mg/kg) intraperitoneally. After the intravenous administration of heparin (500 IU/kg), the chest was opened and the heart was rapidly excised before cannulated on a non-recirculating Langendorff apparatus (Maulik et al., 1997). Retrograde perfusion was established at a constant pressure of 80 mmHg with an oxygenated (95% O_2 /5% CO_2) normothermic (37°C) Krebs-Henseleit bicarbonate (KHB) buffer at pH 7.4.

Experimental protocol

To monitor the isometric tension developed by the left ventricle, a latex balloon tipped catheter connected to a pressure transducer was inserted into the left ventricle via a small incision made in the left atrium. The pressure transducer was connected to a physiograph (Grass Model 79, Grass instruments Co., MA, USA), and the data was transferred to a computer by an A/D converter (Powerlab ADInstruments, CO, USA). Two electrodes were placed on the cardiac epicardium to monitor electrograms (epicardial ECG). To obtain a stable cardiac function, all hearts perfused with KHB were placed for a period of 10 minutes before baseline recording. After that, the isolated hearts were then perfused with Krebs-Henseleit buffer (KHB) in group 1 (control), 10 μM atenolol (a β_1 -selective antagonist, Sigma-Aldrich, MO, USA) in group 2 (ATEN), 10 μM atenolol + 0.01 μM salbutamol (a β_2 -selective agonist, Ventolin, OH, USA) in group 3 (ATEN/SALBU), and 10 μM atenolol + 5 μM prazosin (an α_1 -selective

antagonist, Sigma-Aldrich, MO, USA) in group 4 (ATEN/PRAZ) for 10 minutes, and the data was recorded as a perfusion period. After that, the left anterior descending artery was ligated for 8 minutes and then reperfused for 30 minutes.

Data analysis

Left ventricular developed pressure was defined as the pressure difference between systolic pressure and diastolic pressure (Tosaki et al., 1998), and the maximal first derivative of left ventricular developed pressure (LV dP/dt_{max}) was made at each wave of LVDP at the period of isovolumic contraction and relaxation (Tosaki et al., 1998). Coronary flow rate was measured by a time collection of the coronary effluent that dripped from the heart (Tosaki et al., 1998).

The ECG signals were recorded continuously for 5 minutes in the baseline condition followed by 5 minutes at each period of drug perfusion, ischemic conditions and reperfusion conditions. The RR, QT, and QT_c calculated by Bazett's method were determined. The heart rate was calculated by the apparent QRS complex in a minute.

For arrhythmia analysis, the acquired single-lead ECG tracings were displayed and analyzed. After coronary reperfusion, ECG was recorded for 30 minutes to evaluate cardiac arrhythmia. Ventricular premature beats (VPB) classified as singlet, couplet, triplet, non-sustained ventricular tachycardia (VT), sustained VT, and ventricular fibrillation (VF) were determined according to the Lembeth convention criteria (Walker et al., 1988) with more stringent modifications. The ranking scores are arbitrary numerical grades of different sorts of ventricular arrhythmias. The scaling was applied as follows: 0 = no arrhythmias, 1 = single VPB, 2 = couple or salvos of VPB, 3 = VT, 4 = sustained VT, 5 = VF. 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 arrhythmia was also present according to the experiment of Du et al. (1999).

All data is presented as mean \pm SD. Changes in indices of left ventricular function were compared using repeating-measure analysis of variance with appropriate statistical contrasts employed to make all pair-wise between-group and within-group followed by Student-Newman Keuls. The non-parametric method

was used if the data had failed normality, and a one way analysis of variance with repeated measures on ranks was used instead. 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 a comparison of the incidences of arrhythmia. $p<0.05$ was considered for statistical significance.

Results

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

At baselines, LVDP in the 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 (Fig.1). Although slight decreases in LVDP following drug perfusion period were observed in ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups, a marked decrease in LVDP could be seen in all groups during coronary occlusion which were 55.9 ± 13.3 , 52.7 ± 13.1 , 53.2 ± 13.8 , and 54.2 ± 9.4 mmHg respectively ($p<0.05$). However, LVDP quickly increased upon reperfusion to 60.7 ± 9.9 , 70.1 ± 11.5 , and 81.3 ± 21.9 mmHg in ATEN, ATEN/SALBU, and ATEN/PRAZ, respectively, whereas LVDP in the control group did not recover from the occlusion period. Interestingly, LVDP in the ATEN/PRAZ treated group was the highest compared to other groups during reperfusion.

Effects of adrenergic drugs on dP/dt_{max}

At baselines, dP/dt_{max} in the control, ATEN, ATEN/SALBU, and ATEN/PRAZ groups were 3221 ± 268 , 3235 ± 490 , 3311 ± 432 , and 3350 ± 470 mmHg/s respectively and slightly decreased after drug perfusion (Fig.2). During cardiac ischemia, the dP/dt_{max} in all groups dropped to 2077 ± 351 , 1933 ± 635 , 2041 ± 636 , and 1887 ± 414 mmHg/s, respectively ($p<0.05$). After coronary reperfusion, increases in dP/dt_{max} were observed only in the ATEN/SALBU and ATEN/PRAZ treated groups which were 2543 ± 709 and 2554 ± 658 mmHg/s, respectively ($p<0.05$). However, there was no significant difference in dP/dt_{max} between these two groups.

Effects of adrenergic drugs on dP/dt_{min}

As shown in Fig. 3, the dP/dt_{min} at baselines in the control, ATEN, ATEN/SALBU and ATEN/PRAZ

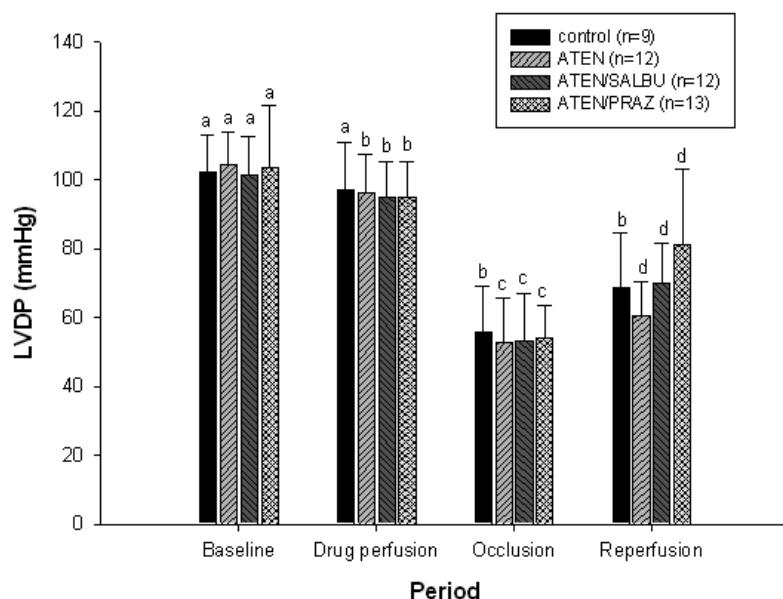


Figure 1 Comparison of the effect of perfusion with a Krebs-Henseleit buffer (control; n=9), 10 μ M atenolol (ATEN; n=12), 10 μ M atenolol combined with 0.01 μ M salbutamol (ATEN/SALBU; n=12), or 10 μ M atenolol combined with 5 μ M prazosin (ATEN/PRAZ; n=13) on left ventricular developed pressure (LVDP) during the baseline, drug perfusion, occlusion, and reperfusion periods of isolated rat hearts. LVDP was decreased significantly after drug perfusion in ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups. Marked decreases of LVDP during the coronary occlusion period were observed in all treated groups. After reperfusion, LVDP increased in all groups compared to the occlusion period. Interestingly, LVDP in ATEN/PRAZ group was the highest. The data is presented as mean \pm SD. ^{a,b,c} and ^d represent significant differences ($p<0.05$) among periods in the same treated group.

treated group were 2601 ± 705 , 2235 ± 294 , 2434 ± 410 , and 2394 ± 655 mmHg/s, respectively. The dP/dt_{min} slightly declined in all groups nevertheless statistical significances were detected only in the control and the ATEN/PRAZ treated groups during drug perfusion. The dP/dt_{min} markedly decreased to 1179 ± 403 , 1080 ± 405 , 1251 ± 432 , and 1111 ± 435 mmHg/s following coronary occlusion. This data had demonstrated that coronary occlusion caused the decrease in cardiac relaxation. During reperfusion, the dP/dt_{min} in ATEN/SALBU and ATEN/PRAZ increased to 1522 ± 472 and 1393 ± 390 mmHg/s, respectively ($p<0.05$).

Effects of adrenergic drugs on heart rates

In this model, heart rates did not significantly change during drug perfusion and coronary occlusion whereas a decrease in heart rate was observed in the control group during reperfusion. The ATEN/PRAZ treated group showed a significant decrease in heart rate during drug perfusion ($p<0.05$), but not in the coronary occlusion and reperfusion periods. In contrast, the ATEN/SALBU treated group did not have alterations of heart rates at any period. Interestingly, heart rates in the ATEN/SALBU treated group were higher than the

ATEN/PRAZ treated group as shown in Fig. 4.

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

The coronary flow rates of all groups are shown in Table 1. At baselines, coronary flow rates in the control, ATEN, ATEN/SALBU, and ATEN/PRAZ treated groups were not different. After drug perfusion, declines in coronary flow were detected in the control and ATEN/PRAZ treated groups compared to their baselines ($p<0.05$), whereas coronary flows in ATEN and ATEN/SALBU treated groups were slightly decreased compared to the baselines. Coronary flow rates dropped in all groups during the coronary occlusion period and increased after perfusion ($p<0.05$). Only the control group showed that coronary flow rate during the reperfusion period was not different from the drug perfusion period. There was no significant difference of coronary flow rate among groups at the same period.

Effects of adrenergic drugs on electrograms

As shown in Table 2, there were no significant differences in RR and QT intervals among experimental groups at the baselines. During the drug perfusion period, only ATEN/PRAZ prolonged RR intervals and shortened

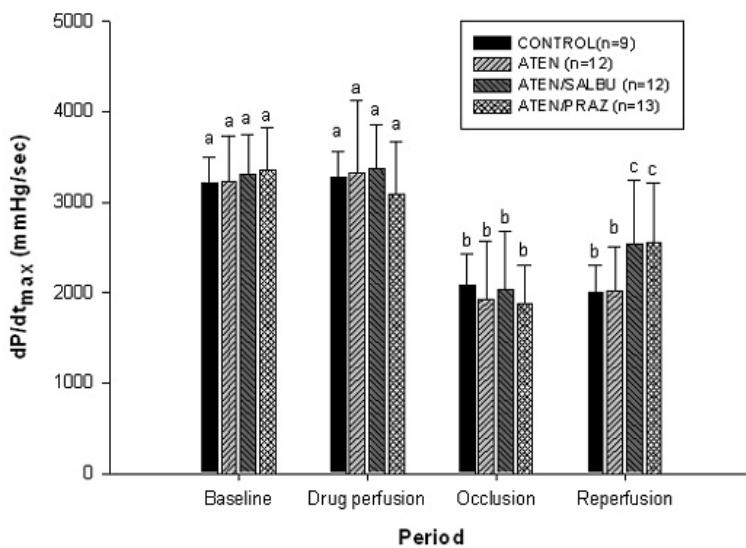


Figure 2 Comparison of the effect of perfusion with a Krebs-Henseleit buffer (control; n=9), 10 μ M atenolol (ATEN; n=12), 10 μ M atenolol combined with 0.01 μ M salbutamol (ATEN/SALBU; n=12), or 10 μ M atenolol combined with 5 μ M prazosin (ATEN/PRAZ; n=13) on the rate of rise (dP/dt_{max}) during the baseline, drug perfusion, occlusion, and reperfusion of isolated rat hearts. The dP/dt_{max} decreased significantly after drug perfusion, and then abruptly decreased during occlusion period in all groups ($p<0.05$). After coronary reperfusion, increases in dP/dt_{max} were observed in the ATEN/SALBU and ATEN/PRAZ treated groups although there was no significant difference between these two groups. The data is presented as mean \pm SD. ^{a,b} and ^c represent significant differences ($p<0.05$) among periods in the same treated group.

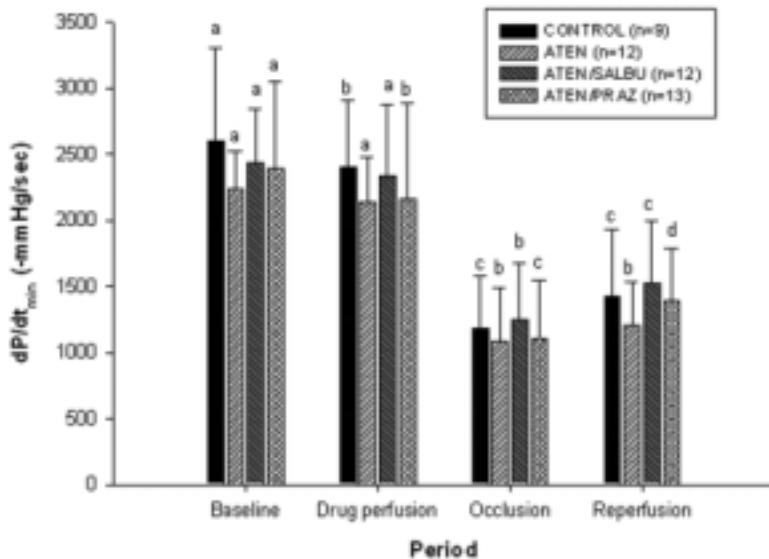


Figure 3 Comparison of the effects of perfusion with a Krebs-Henseleit buffer (control; n=9), 10 μ M atenolol (ATEN; n=12), 10 μ M atenolol combined with 0.01 μ M salbutamol (ATEN/SALBU; n=12), and 10 μ M atenolol combined with 5 μ M prazosin (ATEN/PRAZ; n=13) on the rate of fall (dP/dt_{min}) during the baseline, drug perfusion, ischemia, and reperfusion of isolated rat hearts. The dP/dt_{min} decreased significantly after drug perfusion, and then abruptly decreased during coronary occlusion period in all groups ($p<0.05$). After coronary reperfusion, increases in dP/dt_{min} were observed in the ATEN/SALBU and ATEN/PRAZ treated groups. The data is presented as mean \pm SD. ^{a,b,c}, and ^d represent significant differences ($p<0.05$) among periods in the same treated group.

QT intervals ($p<0.05$). RR intervals of ATEN/PRAZ treated group was much wider than that of ATEN/SALBU treated group during reperfusion ($p<0.05$). Prolonged RR intervals during coronary occlusion were observed in all groups nevertheless there was a significant difference

only in the ATEN/PRAZ treated group ($p<0.05$). Interestingly, ATEN/PRAZ increased RR intervals at all periods.

QT intervals were prolonged in all groups during reperfusion, and it should be noted that QT intervals in

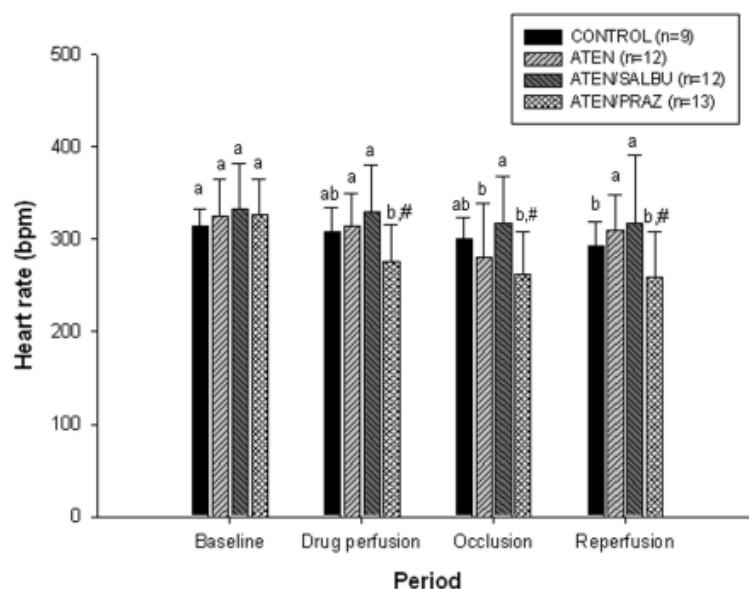


Figure 4 Comparison of the effect of perfusion with a Krebs-Henseleit buffer (control; n=9), 10 μ M atenolol (ATEN; n=12), 10 μ M atenolol combined with 0.01 μ M salbutamol (ATEN/SALBU; n=12), or 10 μ M atenolol combined with 5 μ M prazosin (ATEN/PRAZ; n=13) on heart rates (HR) during the baseline, drug perfusion, occlusion, and reperfusion of isolated rat hearts. After drug perfusion, ATEN/PRAZ caused decreases in HR ($p<0.05$) in all following periods. On the other hand, there was no change in HR in ATEN/SALBU at any period. During the drug perfusion, occlusion and reperfusion periods, HR in the ATEN/SALBU treated group was much higher than that of the ATEN/PRAZ treated group ($p<0.05$). The data is presented as mean \pm SD. ^a and ^b represent significant differences ($p<0.05$) among periods in the same treated group. [#]represents significant difference of the ATEN/PRAZ treated group from the ATEN/SALBU treated group ($p<0.05$).

the ATEN/PRAZ treated group were wider than other groups ($p<0.05$). However, QTc intervals in all groups were not different at any period. This data demonstrated that no drugs used in this experiment altered QTc intervals.

Effects of adrenergic drugs on cardiac arrhythmias

The 30 minutes reperfusion following coronary occlusion produced ventricular arrhythmia in all experimental groups. As shown in Table 3, singlet, couplet, and triplet ventricular premature beats (VPB) appeared in all groups during coronary reperfusion.

Non-sustained VT was present in all groups nevertheless sustained ventricular tachycardia was present only in the control and the ATEN/SALBU treated group. However, there was no significant difference in the incidence of singlet, couplet, and triplet, non-sustained, and sustained VT among groups. The incidence of VF was highest in the ATEN/SALBU treated group as shown in Table 3 and Fig. 5 although there was no statistically significant difference compared to other groups. ATEN slightly reduced the occurrence of VF compared to the control. Notably, there was no incidence of VF in the ATEN/PRAZ treated group after reperfusion as shown in Table 3

Table 1 Effects of adrenergic drugs on coronary flow rate

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

The data is presented as mean \pm SD. ATEN, atenolol; ATEN/SALBU, atenolol combined with salbutamol; ATEN/PRAZ, atenolol combined with prazosin.

^{a,b,c} and ^d represent significant differences ($p<0.05$) among periods in the same treated group.

Table 2 Effects of adrenergic drugs on R-R, Q-T, and Q-T_c intervals

Group/Period	ECG interval	Baseline	Drug	Occlusion	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 is presented as mean ± SD. ATEN, atenolol; ATEN/SALBU, atenolol combined with salbutamol; ATEN/PRAZ, atenolol combined with prazosin.

^{a,b}, and ^c represent significant differences ($p<0.05$) among periods in the same treated group.

*represents the significant difference of the ATEN/PRAZ treated group from the control group ($p<0.05$) in the same period.

[#]represents significant difference of the ATEN/PRAZ treated group from the ATEN/SALBU treated group ($p<0.05$) in the same period.

Table 3 Incidences of ventricular arrhythmia after reperfusion in control and drug treated groups

Group	%VPB (singlet)	%VPB (couplet)	%VPB (triplet)	% Non- sustained VT	% Sustained VT	% VF	Arrhythmia score
Control (n=12)	83.8 (10/12)	66.7 (7/12)	33.3 (8/12)	58.3 (4/12)	8.3 ^a (1/12)	66.7 (1/12)	4.2±1.4
	92.8 (13/14)	78.6 (11/14)	57.1 (8/14)	64.3 (9/14)	0 ^{a, #} (0/14)	50.0 (1/14)	3.6±1.5
ATEN (n=14)	94.7 ^a (18/19)	73.7 ^a (14/19)	31.631.6 ^b (6/19)	63.2 ^a (12/19)	5.3 ^b (1/19)	68.4 ^a (13/19)	4.1±1.4
	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 [*]

Incidence of ventricular arrhythmia were present as percentage and its proportion, and arrhythmia scores were present as mean ± SD. VPB, ventricular premature beat; VT, ventricular tachycardia; VF, ventricular fibrillation; ATEN, atenolol; ATEN/SALBU, atenolol combined with salbutamol; ATEN/PRAZ, atenolol combined with prazosin.

^{a,b}, and ^c represent significant differences in the types of arrhythmia within the same treated group.

*represents significant differences of arrhythmia score in the ATEN/PRAZ treated group from other groups ($p<0.05$)

[#]represents significant differences of arrhythmia incidences among treated groups ($p<0.05$).

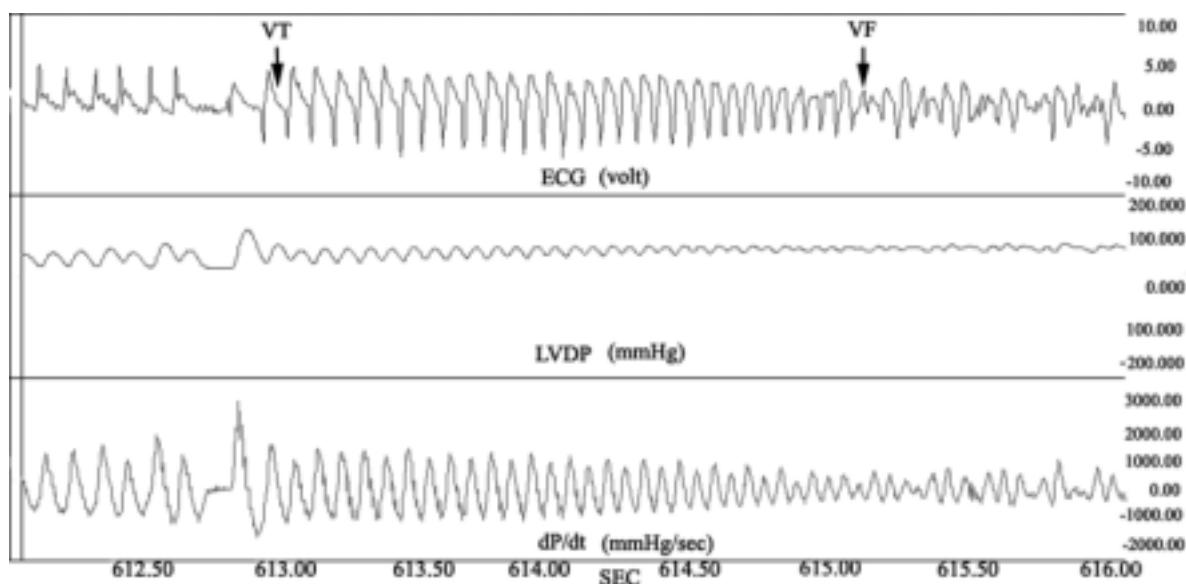


Figure 5 Tracing shows electrocardiogram (ECG), left ventricular developed pressure (LVDP), and the rate of rise and fall (dP/dt) during reperfusion in isolated rat hearts perfused with 10 μ M atenolol combined with 0.01 μ M salbutamol (ATEN/SALBU). A few seconds after reperfusion, the normal ECG changed to ventricular tachycardia (VT) and finally ventricular fibrillation (VF). LVDP and dP/dt also decreased suddenly during reperfusion.

and Fig. 6, and the decreased incidence of VF in ATEN/PRAZ treated group was significantly lower compared to other groups ($p<0.001$). Moreover, ATEN/PRAZ also appeared to decrease incidences of sustained VT. In concurrence with arrhythmia incidences, the arrhythmia score was the lowest in the ATEN/PRAZ treated group ($p<0.001$) while there were no significant difference of the scores among control, ATEN, and ATEN/SALBU treated groups. However, ATEN did not decrease the incidences of ventricular arrhythmia compared to the control whereas ATEN/SALBU did not enhance the incidence of ventricular arrhythmia in reperfusion hearts.

Discussion

In the present study, atenolol, a selective β_1 -adrenergic antagonist combined with a selective α_1 -adrenergic antagonist, prazosin, showed the highest potential antiarrhythmic effects whereas atenolol alone does not have significant antiarrhythmic effects. These results indicate that α_1 -AR plays an important role on reperfusion arrhythmias. Firstly, it is possible that α_1 -AR stimulation may induce delayed afterdepolarization in reperfused myocardium, the same as an investigation in Purkinje fibers isolated from cat hearts in the presence of an elevated Ca^{2+} concentration (Kimura et al., 1984).

Moreover, the α_1 -adrenergic stimulation of canine hypoxic cardiac myocytes with norepinephrine resulted in the appearance of delayed afterdepolarizations which may be a response to an increase in intracellular Ca^{2+} (Kurz et al., 1991). Secondly, α_1 -AR antagonist may prevent a fall in the ventricular fibrillation threshold. As previously described, prazosin prevented an α_1 -adrenergic agonist, metoxamine, induced a fall in the ventricular fibrillation threshold in normoxic rat myocardium (Thandroyen et al., 1987), and the enhanced vulnerability ventricular fibrillation induced by α_1 -adrenergic agonist could be demonstrated only at supraphysiological extracellular calcium concentrations, but not at physiological calcium concentrations (Thandroyen et al., 1987). Prazosin also blocked the metoxamine-induced increases in I_{Ca} (L) in chick and human single cardiac cells (Bkaily et al., 2003). Furthermore, the arrhythmogenic effects of α_1 -adrenergic agonists may be prevented by blocking L-type calcium channel. Likewise, in the present study, ischemic-reperfusion which causes Ca^{2+} ion influx through the sarcolemma and induces ventricular arrhythmia has been described (Lu et al., 1999), and reperfusion arrhythmias could be prevented by the blockade of α_1 -adrenergic receptors with prazosin. Moreover, prazosin not only has α_1 -adrenergic blocking

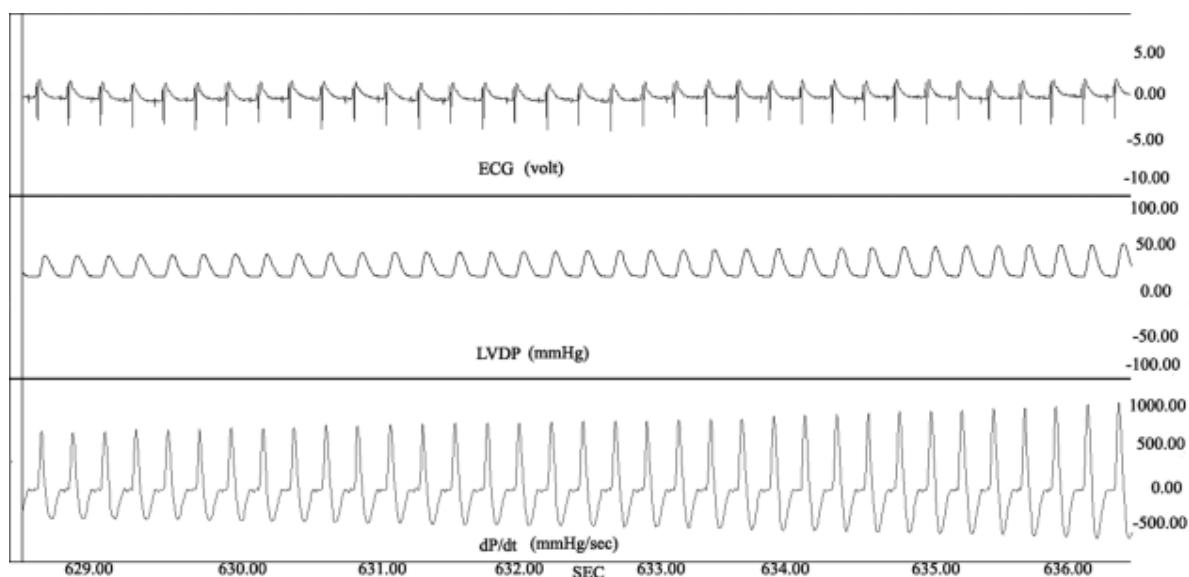


Figure 6 Tracing shows electrocardiogram (ECG), left ventricular developed pressure (LVDP), and rate of rise and fall (dP/dt) during reperfusion in isolated rat hearts perfused with 10 μ M atenolol combined with 5 μ M prazosin (ATEN/PRAZ). No evidence of sustained ventricular tachycardia (VT) or ventricular fibrillation (VF) was seen during reperfusion in this group.

effect but also has free radical scavenging activity, which may reduce incidences of cardiac arrhythmias. 5 and 10 μ M of prazosin attenuated H_2O_2 induced mechanical and metabolic derangements that have been demonstrated in isolated rat hearts (Anderson et al., 1995). In addition, Prazosin (1-10 μ M) has been shown to inhibit inward Na^+ current in the rat, guinea pig, and human myocardium (Su et al., 1995). This action of prazosin demonstrates local anesthetic properties and unrelated to blockade of α_1 -AR. Therefore, antiarrhythmic action of prazosin may not only due to the α_1 -AR blockade but also the local anesthetic properties from the inhibition of inward Na^+ current.

In the heart, α_1 -ARs are coupled to the phosphatidylinositol (PtdIns) turnover 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 μ M prazosin (Anderson et al., 1995). These results reveal that ischemia-reperfusion produces inositol phosphates mediated through α_1 -ARs, and the rapid release of inositol phosphates causes Ca^{2+} oscillations. Because of these, accumulation of inositol phosphate between 1-2 minutes after initiation of reperfusion may be related to reperfusion arrhythmia.

However, there was an experiment demonstrating that prazosin (5.2 μ M) alone could not prevent reperfusion arrhythmias in the isolated rat heart (Bralet et al., 1985). In this study, the left main coronary artery was ligated for 10 minutes and followed by reperfusion. This data demonstrates that 5.2 μ M prazosin alone was not antiarrhythmic. Therefore, the combination of a β_1 -adrenergic antagonist and an α_1 -adrenergic antagonist in our study may produce stronger antiarrhythmic effects compared to α_1 - or β_1 -adrenergic antagonist alone.

In our study, the coronary flow rate in the ATEN/SALBU treated group tended to be the highest during ischemia. This implies that β_2 -adrenergic receptors may play an important role on coronary vasodilation. In agreement with our study, a study shows that administration of 10^{-5} mol/L of propanolol or 10^{-6} mol/L of butoxamine in isolated human coronary arterioles completely eliminated norepinephrine (NE)-induced dilation whereas 10^{-6} mol/L of prazosin inhibited NE induced constriction only 2 of 39 vessels. This data indicated that isolated coronary arterioles from patients with dilated cardiomyopathy may mediate vasodilation via β_2 -ARs (Sun et al., 2002).

In conclusion, the combination of β_1 -adrenergic antagonist and β_1 -adrenergic antagonist has the most potential antiarrhythmic effects compared to the

β_1 -adrenergic antagonist alone or β_1 -adrenergic antagonist plus β_2 -adrenergic agonist. Our results support that α_1 -ARs play an important role on reperfusion arrhythmia whereas coronary vasodilation mediates through β_2 -ARs in ischemia/reperfusion isolated rat hearts.

Acknowledgement

We would like to thank the Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University for the laboratory room and equipment. This study was supported by the Ministry of University Affairs (MUA) - CU Thesis Grant.

References

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.

Altschuld, R.A., Staring, R.C., Hamlin, R.L., Billman, G.E., Hensley, J., Castillo, L., Fertel, R.H., Hohl, C.M., Robitaille, 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.

Bkaily, G., El-Bizri, N., Bui, M., Sukarieh, R., Jacques D., and Fu, M. 2003. Modulation of intracellular Ca^{2+} via L-type calcium channels in heart cells by autoantibody directed against the second extracellular loop of the α_1 -adrenoceptors. *Can. J. Physiol. Pharmacol.* 81: 234-246.

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 -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.

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., 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.

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.

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.

Katz, A.M. 2001. The working heart. In: Physiology of the Heart. 3rd ed. Katz, A.M. (ed.). Philadelphia: Lippincott Williams & Wilkins. 418-450.

Kennedy, H.L., Brooks, M.M., Barker, A.H., Bergstrand, R., Huther, M.L., Beanlands, D.S., Bigger, J.T., Goldstein, S. 1994. Beta-blocker therapy in the cardiac arrhythmia suppression trial cast investigators. *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., Corr, P.B. 1991. Alpha1-adrenergic system and arrhythmias in ischaemic heart disease. *Eur. Heart J.* 12: 88-98.

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 Ca²⁺ influx. *Eur. J. Pharmacol.* 365(2-3): 233-239.

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

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

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.

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

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

Su, M.J., Chu, J.F., and Chu, S.H. 1995. Effects of prazosin on rat, guinea pig and human cardiac tissues. *Chin. J. Physiol.* 38(3):159-169.

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 β_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.

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 Ca²⁺ and ryanodine. *J. Mol. Cell Cardiol.* 19(9): 841-851

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.

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.

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