Reviews in Cardiovascular Medicine

IMR Press

ORIGINAL RESEARCH

Activation of β_1-adrenoceptors may not be involved in arrhythmogenesis in ischemic heart disease

Adriana D. Adameova[1], Sukhwinder K. Bhullar[2], Vijayan Elimban[2] and Naranjan S. Dhalla[2,*]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic, 83232
[2]Department of Physiology & Pathophysiology and Institute of Cardiovascular Sciences, Max Rady College of Medicine, St. Boniface Hospital Albrechtsen Research Centre, University of Manitoba, Winnipeg, R2H 2A6, Canada

*Correspondence to: Naranjan S. Dhalla, nsdhalla@sbrc.ca

DOI: 10.31083/j.rcm.2018.03.3181

Although ischemic heart disease is invariably associated with marked activation of sympathetic nervous system, elevated levels of circulating catecholamines and lethal ventricular arrhythmias, the mechanisms of arrhythmogenesis due to myocardial ischemia are not fully understood. Since catecholamines are known to produce stimulatory effects in the heart mainly by acting on β_1-adrenoceptors, this study was undertaken to test the involvement of these receptors in the development of arrhythmias due to myocardial infarction (MI) induced upon occluding the left coronary artery in rats for a period of 2 h. The animals were treated with or without atenolol (20 mg/kg; daily), a selective β_1-adrenoceptors blocker, for 14 days before inducing MI. No alterations in the number of MI-induced episodes and incidence or duration of different types of arrhythmias were observed. In fact, the incidence of trigemines and reversible ventricular fibrillation due to MI were significantly increased in the atenolol-treated animals. These observations support the view that the activation of β_1-adrenoceptors may not be exclusively involved in the development of arrhythmias during the occurrence of ischemic heart disease and other mechanisms can underlie the electric instability of such damaged heart.

Keywords
Arrhythmias; β_1-adrenoceptors; atenolol; myocardial ischemia

1. Introduction

It is now well known that the sympathetic nervous system (SNS) is activated under a wide variety of stressful conditions including ischemic heart disease (Dhalla et al., 1993; Randhawa et al., 2016). The activation of SNS for a prolonged period has been shown to be associated with lethal arrhythmias as a consequence of increased levels of circulatory catecholamines (Adameova et al., 2009; Dhalla et al., 2010; Dhalla, 2018). However, the role of high levels of circulatory catecholamines in inducing arrhythmias during the development of myocardial ischemia is not fully understood. Since catecholamines are known to produce positive inotropic and chronotropic actions on the heart by acting on β-adrenoceptors, resulting in increasing cyclic AMP and elevating the level of intracellular Ca^{2+} concentration (Adameova et al., 2009), it is generally considered that arrhythmias due to high levels of catecholamines in ischemic heart disease is a consequence of activation of β-adrenoceptors in the myocardium. This view is supported by clinical observations that long-term administration of certain β-adrenoceptors blockers improves survival of patients with myocardial infarction (MI), most probably by preventing ventricular fibrillation (Freemantle et al., 1999). Although some experimental studies have observed beneficial effects of different β-adrenoceptors blocking agents including propranolol, pindolol, atenolol, metoprolol and oxyprenol, in the ischemia-induced arrhythmias and atrial fibrillation (Abendroth et al., 1977; Fearon, 1967; Fitzgerald, 1982; Lepran et al., 1983), other investigations have failed to observe such actions in attenuating arrhythmogenesis due to ischemia as a consequence of β-adrenoceptors blockade in the myocardium (Ablad et al., 2007; Clements-Jewery et al., 2009; Daugherty et al., 1986; Rosati et al., 1966). In view of such controversial reports, the present study was undertaken to examine the effects of long term pretreatment with high doses of a selective β-adrenoceptors blocker, atenolol, on different types of arrhythmias induced upon occluding the coronary artery in anesthetized rats.

2. Material and Methods

This study was carried out following the guidelines established by the Canadian Institute of Health Research and approved by the Animal Care Committee of the University of Manitoba. Sprague-Dawley male rats weighing 250 to 300 g were kept at 12-h day/night cycle and fed rat chow and water *ad libitum.*

2.1 Drug treatment and electrocardiographic (ECG) monitoring

Atenolol (20 mg/kg) was given to rats via a gastric tube daily for 15 days. The last dose of the drug was given 10 min before the induction of ischemia and the control rats received saline solution daily for 15 days. This protocol for atenolol treatment was similar to that used for this and other drugs (Adameova et al., 2019; Barta et al., 2008). Furthermore, the dose of atenolol used in this study is 5-fold higher than that used as a bolus injection for blocking the β-adrenoceptors in rats (Clements-Jewery et al., 2009), thereby considering also pharmacokinetics and bioavailability of this drug. The animals were anesthetized with 5% isoflurane and put on six-

lead ECG (leads I-III; augmented vector right, aVR; augmented vector left, aVL; augmented vector foot, aVF) monitoring system (Acknowledge 3.0.3 software). ECG was recorded continuously and was used for further analysis of ventricular arrhythmias.

2.2 Induction of myocardial ischemia

After a 10-min baseline ECG recording in rats, acute MI was induced by occlusion of the left coronary artery as described previously (Barta et al., 2008; Dixon et al., 1990). Briefly, the heart was exposed by thoracotomy and the left coronary artery was ligated at about 2 mm from its origin at the aorta. The heart was repositioned into the chest and the incision was closed with a purse string suture. Ischemia-induced ventricular arrhythmias were monitored for 2 h at 10 min intervals. The incidence, episodes, onset and duration of ventricular premature beats (VPB), bigemines (Big), trigemines (Trig), salvos as well as the most threatening arrhythmias such as ventricular tachycardia (VT) and ventricular fibrillation (VF) were analysed from the ECG recordings according to Lambeth Conventions (Curtis et al., 2013; Walker et al., 1988). In addition, each animal was evaluated for arrhythmias score by means of 6-point score system as described elsewhere (Adameova et al., 2007).

2.3 Statistical analysis

Data are presented as mean ± SEM. Student`s t-test was used to compare differences in normally distributed parametric variables between the groups. Differences in variables with non-parametric distribution, such as the number of episodes of ventricular tachycardia and ventricular fibrillation as well as the incidence of these arrhythmias (expressed as percentage) were compared using the Mann-Whitney test and 2 × 2 chi-square test, respectively. A P-value < 0.05 indicated a significant difference.

3. Results

Electrocardiographic examination of control and atenolol-treated animals revealed different types of ventricular arrhythmias during a period of 2 h after the induction of MI. The development of VPB was evident within 2 min whereas the occurrence of VT and tVT was apparent within 3 to 5 min after the induction of MI in both control and atenolol-treated animals. As shown in Figure 1 (A and B) and 2 (A and B), the incidence and episodes of different types of ventricular arrhythmias including VPB, Big, Salvos, VF and VT due to induction of MI were not affected by atenolol treatment. On the other hand, both the incidence and episodes of Trig, which were not seen in the control, became evident in atenolol treated animals (Fig. 1A and 2A). Furthermore, the incidence of reversible VF (rVF) was significantly increased and that of irreversible VF (iVF) was slightly decreased but the incidence of total VF (tVF) was not altered significantly in the atenolol-treated animals (Fig. 1B).

The data in Table 1 expressing the duration of different types of arrhythmias due to MI are in line with above-listed arrhythmias analysis indicating ineffectiveness of atenolol to mitigate MI-induced electrophysiological changes. Indeed, atenolol treatment was not able to alter the duration of MI-triggered ventricular arrhythmias. Likewsie, no significant differences in the onset of MI-induced arrhythmias including VPB, VT and tVF as well as arrhythmia score were observed in the control and atenolol treated animals (Fig. 3A and B).

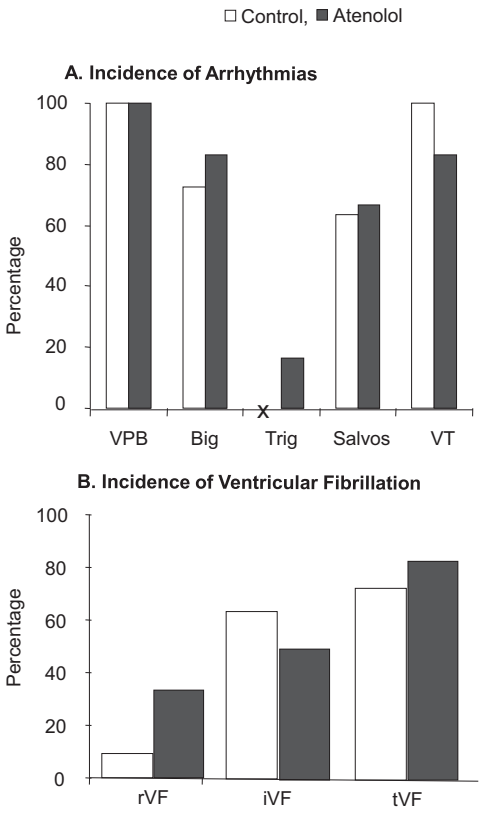

Figure 1. Effect of atenolol pretreatment (20 mg/kg daily for 14 days) on the incidence of different types of arrhythmias (A) and ventricular fibrillation (B) during 2 h of myocardial ischemia induced by occlusion of the left coronary artery in anesthetized rats. The values for number of episodes are mean ± SEM of 6-11 animals. VPB – ventricular premature beats; Big – bigemines; Trig – trigemines; VT – ventricular tachycardia; rVF – reversible ventricular fibrillation; iVF- irreversible ventricular fibrillation; tVF – total ventricular fibrillation; X – no evidence.

Table 1. Duration of different types of arrhythmias due to coronary occlusion in rats with or witout atenelol treatment.

Parameters	Duration of Arrhythmias (sec)	
	Control	Atenolol-treated
Ventricular premature beats	1.98 ± 0.45	3.95 ± 1.44
Bigemines	5.23 ± 2.38	7.21 ± 2.11
Trigemines	0.00 ± 0.00	0.19 ± 0.19
Salvos	0.54 ± 0.19	0.66 ± 0.25
Vetricular tachycardia	44.97 ± 17.70	32.34 ± 19.25
Ventricular fibrillation	274.59 ± 78.54	212.10 ± 95.24

Atenolol (20 mg/kg/day) was given orally by gavage for 14 days before the occlusion of left coronary artery in anesthetized animals. The values are mean ± SEM of 6-11 experiments.

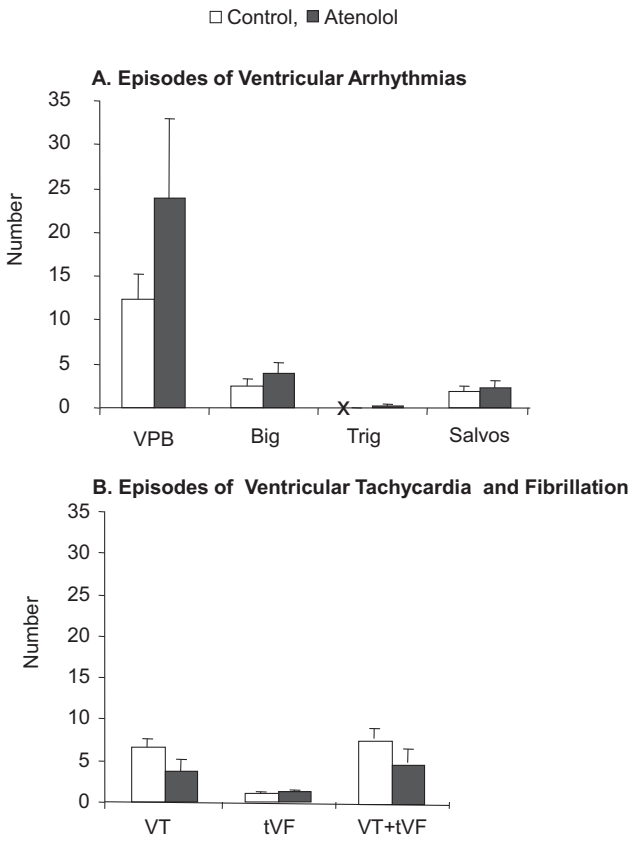

Figure 2. Effect of atenolol pretreatment (20 mg/kg daily for 14 days) on the number of episodes of different types of arrhythmias (A) and episodes of ventricular tachycardia and fibrillation (B) during 2 h of myocardial ischemia induced by occlusion of the left coronary artery in anesthetized rats. The values for number of episodes are mean ± SEM of 6-11 animals. VPB – ventricular premature beats; Big – bigemines; Trig – trigemines; VT – ventricular tachycardia; tVF – total ventricular fibrillation; X – no evidence.

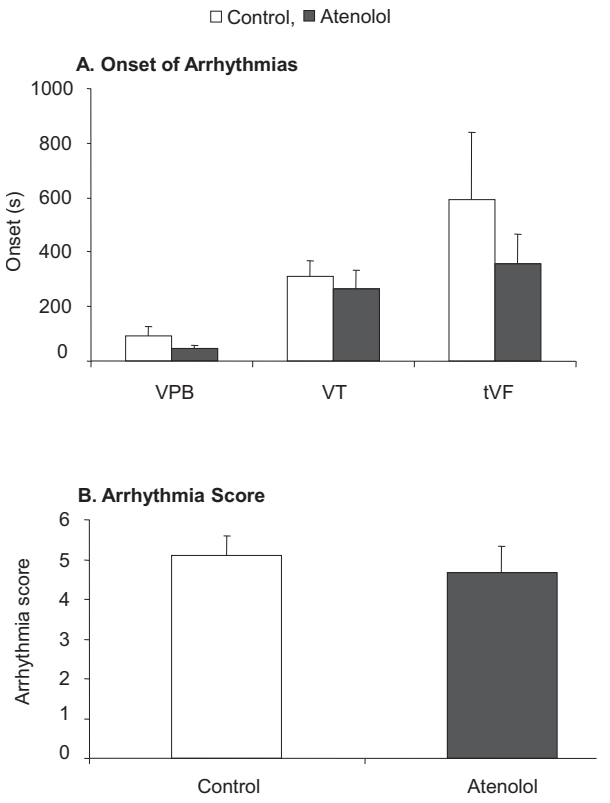

Figure 3. Effect of atenolol pretreatment (20 mg/kg daily for 14 days) on onset (A) and score (B) of arrhythmias due to 2-h myocardial ischemia induced by occlusion of the left coronary artery in anesthetized rats. The values for the onset and score of arrhythmias are mean ± SEM of 6-11 animals. VPB – ventricular premature beats, VT – vetricular tachycardia, tVF – total ventricular fibrillation.

4. Discussion

In agreement with previous reports employing both conscious and anesthetized animals (Barta et al., 2008; Clark et al., 1980; Opitz et al., 1995; Rosati et al., 1966), occlusion of the left coronary artery for 2 h in anesthetized rats was observed to induce a wide variety of ventricular arrhythmias including VPB, Big, Salvos, VT and VF. These MI-induced arrhythmias are considered to be a consequence of myocardial ischemia as the appearance of arrhythmias and the extent of ischemic area during early periods of the coronary occlusion were found to show excellent correlation (Lepran et al., 1983). In view of the occurrence of the premature beats within 2 min of occluding the coronary artery, it is unlikely that ischemia-reperfusion plays any role in the development of arrhythmias at this early stage. Since the coronary occlusion has been observed to be associated with marked increase in the plasma levels of both catecholamines and angiotensin II as well as various oxidants in the heart (Babick et al., 2013; Dhalla et al., 2000a; Shao et al., 1996), it has been suggested that the MI-induced cardiac effects, hemodynamic changes and arrhythmias are elicitepd by neurohormonal activation and oxidative stress (Dhalla et al., 2000b).

In this study, we have shown that the treatment of rats with a high dose of atenolol (20 mg/kg, daily) for 14 days did not affect the incidence, number of episodes or duration of the MI-induced VPB, Big, Salvos and VT. Likewise, the onset of VPB, VT or VF was not modified by the drug. Although the incidence of MI-induced rVF had tendency to be increased and that of iVF was slightly decreased, the incidence and episodes for tVF were not altered by atenolol treatment significantly. In addition, in spite of the fact that Trig, which was not detected in control animals, became evident in the atenolol-treated animals but the overall arrhythmia assessment score did not differ between the groups. These observations suggest that the selective blockade of β_1-adrenoceptors in the myocardium by atenolol treatment does not exert any influence on the MI-induced arrhythmias. A bolus injection or acute infusion of a low dose (4 mg/kg) of atenolol in animals has also been reported to show no effect on the MI-induced arrhythmias (Ablad et al., 2007; Clements-Jewery et al., 2009). In contrast, different investigators have shown the beneficial antiarrhythmic effects of some β-adrenoceptors antagonists, such as metoprolol, propranolol and oxyprolol, in animals subjected to MI. However, it should be pointed out that this cardioprotective action of the non-selective beta-blockers has been shown to be due to their quinidine-like,

nonspecific or central actions rather than due to the blockade of β-adrenoceptors in the myocardium (Ablad et al., 2007; Clements-Jewery et al., 2009; Daugherty et al., 1986; Rosati et al., 1966). Likewise, genetic polymorphism in the β_1-adrenoreceptors and race has been discussed to affect the anti-arrhythmic efficacy of beta-blockers (Taylor et al., 2014).

The inability of the blockade of β_1-adrenoceptors by atenolol treatment to modify the MI-induced arrhythmias should not be taken to suggest that the activation of sympathetic nervous system and elevated levels of plasma catecholamines do not participate in the genesis of arrhythmias during the development of ischemic heart disease. Indeed, recently we have shown that the epinephrine-induced arrhythmias in rats were also not altered by atenolol treatment (Adameova et al., 2019), which observation has been considered to provide further evidence that the ventricular arrhythmias induced by an excessive amount of catecholamines due to sustained activation of the sympathetic nervous system may be induced by the oxidation products of catecholamines such as aminochrome and oxyradicals, rather than by catecholamine *per se* (Dhalla, 2018). It is also pointed out that MI-induced arrhythmias is a complex problem involving several pathomechanisms such as the involvement of elevated levels of angiotensin II as a consequence of the activation of renin-angiotensin system (de Boer et al., 2002; Fleetwood et al., 1991; Matsuo et al., 1997; Shao et al., 1996), increased concentrations of plasma 5-HT due to platelet activation (Barta et al., 2008; Brasil et al., 2002), occurrence of intracellular Ca^{2+}-overload due to increased Ca^{2+}-influx (Curtis et al., 1984), development of oxidative stress due to ischemia (Dhalla et al., 2000a,b) as well as inflammation due to the activation of neutrophils (Clements-Jewery et al., 2009). Although we did not measure the infarct size in control and atenolol groups, it is unlikely that changes in the infarct size can be considered responsible for the observation reported here. Nonetheless, the results of this study indicating the ineffectiveness of atenolol to alter the MI-induced arrhythmias seem to indicate that the activation of β_1-adrenoceptors in the myocardium may not be involved in arrhythmogenesis in the ischemic heart disease. It can also be suggested that selective blockade of β_1-adrenoceptors may not be sufficiently effective in the prevention and/or treatment of the MI-triggered arrhythmias and a combination therapy targeting multiple signaling in the cardiovascular system is likely to exert more beneficial action.

Acknowledgments

The financial support for this work as well as the infrastructure for this project were provided by the St. Boniface General Hospital Research Foundation.

Conflict of Interest

The authors declare no conflict of interest associated with this work.

References

Abendroth RR, Meesmann W, Stephan K, et al. Effects of the beta-blocking agent atenolol on arrhythmias, especially ventricular fibrillation and ventricular fibrillation threshold after acute experimental coronary occlusion. Z Kardiol. 1977;66:341-350.

Ablad B, Bjuro T, Bjorkman J-A, et al. Prevention of ventricular fibrillation requires central β-adrenoceptor blockade in rabbits. Scand Cardiovasc J. 2007;41:221-229.

Adameova A, Abdellatif Y, Dhalla NS. Role of the excessive amounts of circulating catecholamines and glucocorticoids in stress-induced heart disease. Can J Physiol Pharmacol. 2009;87:493-414.

Adameova A, Elimban V, Ganguly PK, et al. Both β-1 adrenoceptors and AT1 receptors may not be involved in the catecholamine-induced lethal arrhythmias. Can J Physiol Pharmacol. 2019.

Adameova A, Kuzelova M, Andelova E, et al. Hypercholesterolemia abrogates an increased resistance of diabetic rat hearts to ischemia-reperfusion injury. Mol Cell Biochem. 2007;295:129-136.

Babick A, Elimban V, Zieroth S et al. Reversal of cardiac dysfunction and subcellular alterations by metoprolol in heart failure due to myocardial infarction. J Cell Physiol. 2013;228:2063-2070.

Barta J, Sanganalmath SK, Kumamoto H, et al. Antiplatelet agents sarpogrelate and cilostazol affect experimentally-induced ventricular arrhythmias and mortality. Cardiovasc Toxicol. 2008;8:127-135.

Brasil D, Temsah RM, Kumar K, et al. Blockade of 5-HT_2A receptors by sarpogrelate protects the heart against myocardial infarction in rat. J Cardiovasc Pharmacol Therapeut. 2002;7:53-59.

Clark C, Foreman MI, Kane KA, et al. Coronary artery ligation in anesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. J Pharmacol Meth. 1980;3:357-368.

Clements-Jewery H, Andrag E, Hearse DJ, et al. Complex adrenergic and inflammatory mechanisms contribute to phase 2 ventricular arrhythmias in anaesthetized rats. Br J Pharmac. 2009;156:444-453.

Curtis MJ, Hancox JC, Farkas A, et al. The Lambeth Conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. Pharmacol Ther. 2013;139:213-248.

Curtis MJ, MacLeod BA, Walker MJ. Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. Br J Pharmacol. 1984;83:373-385.

Daugherty A, Frayn KN, Redfern WS, et al. The role of catecholamines in the production of ischaemia-induced ventricular arrhythmias in the rat in vivo and in vitro. Br J Pharmac. 1986;87:265-277.

de Boer RA, van Geel PP, Pinto YM, et al. Efficacy of angiotensin II type 1 receptor blockade on reperfusion-induced arrhythmias and mortality early after myocardial infarction is increased in transgenic rats with cardiac angiotensin II type 1 overexpression. J Cardiovasc Pharmacol. 2002;39:610-619.

Dhalla NS, Adameova A, Kaur M. Role of catecholamine oxidation in sudden cardiac death. Fund Clin Pharmacol. 2010;24:539-546.

Dhalla NS, Afzal N, Beamish RE, et al. Pathophysiology of cardiac dysfunction in congestive heart failure. Can J Cardiol. 1993;9:873-887.

Dhalla NS. Formation of aminochrome leads to cardiac dysfunction and sudden cardiac death. Circ Res. 2018;123:409-411.

Dhalla NS, Elmoselhi AB, Hata T et al. Status of myocardial antioxidants in ischemiareperfusion injury. Cardiovasc Res. 2000a;47:446-456.

Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. J Hypertension 2000b;18:655-673.

Dixon IM, Lee SL, Dhalla NS. Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ Res. 1990;66:782-788.

Fearon RE. Propranolol in the prevention of ventricular fibrillation due to experimental coronary artery occlusion. Am J Cardiol. 1967;20:222-228.

Fitzgerald JD. The effects of β-adrenoceptor blocking drugs on early

arrhythmias in experimental and clinical myocardial ischemia. In: J. R. Parratt, editors. Early Arrhythmias Resulting from Myocardial Ischemia. London: Macmillan;1982;295-316.
Fleetwood G, Boutinet S, Meier M, et al. Involvement of the renin-angiotensin system in ischemic damage and reperfusion arrhythmias in the isolated perfused rat heart. J Cardiovasc Pharmacol. 1991;17:351-356.
Freemantle N, Cleland J, Young P, et al. β-Blockade after myocardial infarction: systematic review and meta regression analysis. Br Med J. 1999;318:1730-1737.
Lepran I, Koltai M, Siegmund W, et al. Coronary artery ligation, early arrhythmias, and determination of the ischemic area in conscious rats. J Pharmacol Meth. 1983;9:219-30.
Matsuo K, Kumagai K, Annoura M, et al. Effects of an angiotensin II antagonist on reperfusion arrhythmias in dogs. Pacing Clin Electrophysiol. 1997;20:938-945.
Opitz CF, Mitchell GF, Pfeffer MA, et al. Arrhythmias and death after coronary artery occlusion in the rat. Continuous telemetric ECG monitoring in conscious, untethered rats. Circulation. 1995;92:253-261.
Randhawa AS, Dhadial RS, Adameova A, et al. The role of the sympathetic nervous system in sudden cardiac death. Curr Res Cardiol. 2016;3:83-88.
Rosati RA, Alexndra JA, Wallace AG, et al. Failure of beta-adrenergic blockade to alter ventricular fibrillation threshold in the dog. Evidence for extra-adrenergic effects of pronethalol. Circ Res. 1966;19:721-725.
Shao Q, Takeda N, Temsah R, et al. Prevention of hemodynamic changes due to myocardial infarction by early treatment of rats with imidapril. Cardiovasc Pathol. 1996;1:180-186.
Taylor B, Moffett BS, Krenek M, et al. Race contributes to beta-blocker efficacy in pediatric patients with arrhythmias. Pediatr Cardiol. 2014;35:641-644.
Walker MJ, Curtis MJ, Hearse DJ, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. Cardiovasc Res. 1988;22:447-455.