Study the Effects of Vernakalant on Ischemic-Reperfusion Dysrhythmias in Experimental Animals in Comparison with Amiodarone

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Abstract

Cardiac dysrhythmia is a term for any of large heterogeneous group of conditions in which there is abnormal electrical activity in the heart. Dysrhythmias may be life threatening medical emergencies that can result in cardiac arrest and sudden death; it may predispose the patient to potentially life threatening stroke and embolism.

The pathogenesis of cardiac dysrhythmia involves crossing of electrolytes through different ions channels on the cellular level that may be dysrhythmogenic.

In this study, ischemic-reperfusion dysrhythmia were performed in experimental animals, ischemia of the myocardium results in release of ischemic metabolites and slowing impulse propagation while reperfusion results in increase late inward Na current (INaL) which amplifies Na^+ influx and intracellular Na^+ concentration leading finally to calcium overload. The enhancement of Na^+ and $Ca2^+$ concentration causes electrophysiological instability, formation of free oxygen radicle and liberation of platelet activating factor.

Seeking for novel antidysrhythmogenic agents having ions channels inhibiting properties, this work aims to investigate the effects of vernakalant, antidysrhythmic drug with atrial selective and multi-channel blocking effect on ischemia-reperfusion dysrhythmia in experimental animals in comparison with amiodarone as a standard antidysrhythmic medication.

Keywords: Ischemic-Reperfusion Dysrhythmia, Vernakalnt & Amiodarone.

Introduction

Dysrhythmia is an abnormality of the rate, rhythm, site of origin of heart impulses or a hindrance in electrical conductive system of the heart that develop activation sequences of myocardium. The impulses of conductivity originate from the primary pacemaker, sinus node, spontaneously sending depolarization wave through the atrium, depolarizing the atrioventricular node then propagated to Purkinje fibres then depolarizing the ventricle in a systemic manner. Actually, there are more than hundred classes of cardiac dysrhythmias. The normal cardiac rhythm, sinus rhythm, could be disrupted due to failure of automaticity, such as sick sinus syndrome or due to over activity, such as inappropriate sinus tachycardia.

Ectopic foci cause premature excitation of the myocardium on single or continues basis leading to premature atrial contractions (PACs) and premature ventricular contractions (PVCs). Another classes of dysrhythmia, atrial fibrillation, paroxysmal atrial tachycardia (PAT) and supraventricular tachycardia (SVT), caused by micro or macro re-entry. In general, the seriousness of cardiac dysrhythmias depends on the presence or absence of structural heart diseases. [Fu, 2015]

The most common relatively benign dysrhythmia are atrial fibrillation, PACs and PVCs, being benign is in case of absence of structural heart lesion. In contrast, the presence of non-sustained ventricular tachycardia (VT) or syncope in coronary heart disease patient may be a harbinger of subsequent cardiac death and must not be ignored.

The most frequent complaints associated with cardiac dysrhythmia, dizziness, palpitations and syncope are noticed either by family physician or by the patient himself. On the contrary, these ubiquitous complaints, sudden cardiac death remains an important public health concern.

Centres for Disease Control and Prevention (CDC) have estimated sudden cardiac death in more than 600,000 per year. Up to 50% of patient have sudden death as the first manifestation of cardiac disease. [Grant, et al 2012]

Cardiac electrophysiology

The action potential of ventricular myocytes is the standard model of cardiac action potential and it is composed of 5 phases (numbered from 0-4). Figure 1 [Rudy, 2008] Phase 4 is the resting membrane potential, the cell is not being stimulated, when an electric current from the adjacent cell typically stimulates cardiac myocytes, a sequence of influx and efflux of various anions and cations that produce action potential of cardiac cells propagating the electric stimulation to different parts of the myocardium.
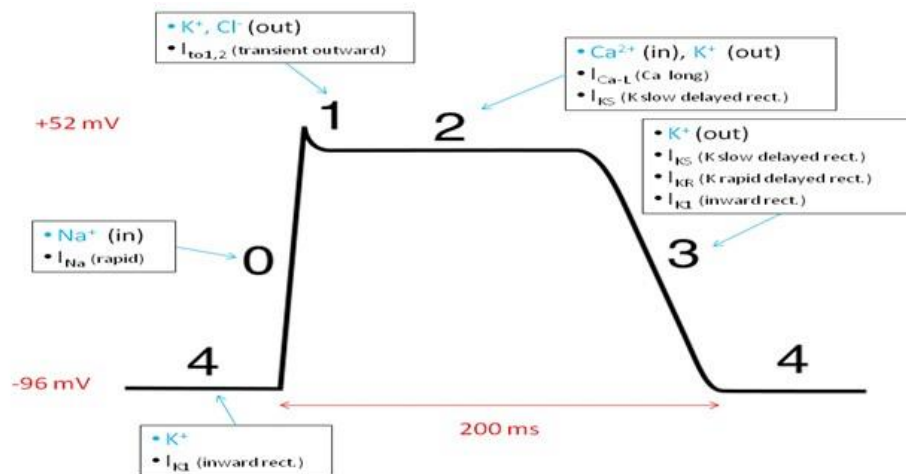

Figure 1. Five phases of cardiac action potential. [Rudy, 2008]

The resting membrane potential is caused by different ionic concentration and conductance through the cell membrane during phase 4 of the action potential. The normal resting membrane potential in ventricular myocardium is about -85 to -95 mV. This potential is determined by selective permeability of the cell membrane to different types of electrolytes. The cell membrane is most permeable to K^+ ions and relatively impermeable to the other ions. The resting membrane potential is there for dominated by the K^+ equilibrium potential according to K^+ gradient across the cell membrane. The membrane potential can be calculated using Goldman-Hodgkin-Katz voltage equation. [Grunnet, 2010] The stability of electrical gradient is maintained by different ions pump and exchange mechanism, involving Na^+/K^+ ion exchange pump, Na^+/Ca^+ exchanger current and Inwardly Rectifying K^+ current (Ikr). [Sipido, et al 2007].

Phase 0 is a rapid depolarization phase. The slope of phase 0 represents the maximum rate of depolarization of the cell and is known as dV/dt max. This phase is caused by fast opening of Na^+ channels leading to rapid Na^+ influx (I_{Na}) into the cell. The ability of the cardiac cells to open fast Na^+ channels during phase 0 is related to the membrane potential at the moment of excitation. If the membrane potential is at its baseline (about -85 mV), all the fast Na^+ channels are closed and the excitation will open them all causing large influx of Na^+ ions. If the membrane potential is less negative, some of the fast Na^+ channels will be inactivated and insensitive to opening, leading to lesser response to excitation of the cell membrane and a lower Vmax, thus the resting membrane potential become too positive, so that lead to delayed excitation and conduction, increasing risk for dysrhythmias. [Santana, et al 2010a].

The fast Na^+ channels are being controlled by numbers of gates, each gate can attain a value between 1 (fully open) and 0 (fully closed). The product of all gates denotes the percentage of channels available to conduct Na^+. According to Hodgkin and Huxley model, the Na^+ channels

contains 3 gates: m, h and j. In the resting state, m gate is closed (zero) and h and j gates are open (one). Upon electric stimulation of cardiac myocytes, m gates opens quickly while simultaneously h and j gates close more slowly. For a brief period of time, all gates are open (non-zero) and Na^+ enter the cell following electrochemical gradient, thus, if the resting membrane potential is too positive, the h or j gates may be considerably less than one, such that the product of m, h and j becomes too small upon depolarization. [Grunnet, 2010].

Phase 1 action potential is due to inactivation of Na^+ channels. The transient net outward current causing small downward deflection of the action potential is due to movement of K^+ and Cl^- ions, carried by I_{to1} and I_{to2} currents respectively. Particularly the I_{to1} contributes to the notch of ventricular myocytes action potentials. [Santana, et al 2010b].

The plateau phase of cardiac action potential is maintained by balance between inward movement of Ca^+ (ICa^+) through L-type calcium channel and outward movement of K^+ ions through the slow delayed rectifier K^+ channels (IKs). [Grunnet, 2010] During phase 3 (rapid repolarization), the L-type calcium channels are closed while the slow delayed rectifier K^+ channel are still open. This ensure a net outward current, corresponding to negative change in membrane potential, thus allowing more types of K^+ channels to open. These are primarily the rapid delayed rectifier K^+ channels (IKr) and the inwardly rectifier K+ current (IK_1). This net outward, positive current (equal to loss of positive charge from the cell) cause the cell to depolarize. The delayed rectifier K^+ channels close when the membrane potential is restored to about -80 to -85 mV, while IK_1 remains conducting throughout phase 4, contributing to set the resting membrane potential. [Kubo, et al 2005].

Pathophysiology of dysrhythmia

The pathogenesis of dysrhythmia is involving three main mechanisms: enhance or supressed automaticity, triggered activity or re-entry. **Automaticity** is a natural property of cardiac myocytes, suppression of automaticity of sinoatrial node (SAN) can lead to sinus node dysfunction and sick sinus syndrome (SSS) which is the most common indication for permanent pacemaker implantation. In contrast, enhance automaticity can result in multiple dysrhythmias, both atrial and ventricular. **Triggered activity** occurs in case of early afterdepolarization and delayed afterdepolarization initiate spontaneous multiple depolarization precipitating ventricular dysrhythmia such as Torsades de pointes and digitalis induced ventricular dysrhythmia. Probably, the most common mechanism of arrhythmogenesis results from **re-entry** that include bidirectional conduction and unidirectional block. "Micro" level re-entry results in ventricular tachycardia from conduction around the scar of myocardial infarction and "Macro" level of re-entry results in conduction through Wolff-Parkinson-White [WPW] syndrome concealed accessory pathway. [Nakagawa, et al 2001]

Atrial fibrillation is the most common type of supra-ventricular dysrhythmia (SVD) associated with significant morbidity, mortality and affecting quality of life. [Camm, et al 2012] Despite significant progress in catheter and surgical ablation techniques, antidysrhythmic drugs remain first line therapy for rhythm control. [Fragakis & Katritsis, 2012] Currently available and commonly used antidysrhytmic drugs are limited by incomplete efficacy for achieving and maintaining sinus rhythm and/or by adverse effects such as life threatening ventricular pro-dysrhythmia or severe extra-cardiac toxicities. [Lafuente, et al 2006] [Camm, 2012] Amiodarone is the most effective medication for rhythm control but it is often discontinued due to numerous systemic side effects such as thyroid and lung dysfunction. [Lafuente, et al 2006] [Camm, 2012] Therefor, there is a clear need for safer and more effective pharmacological strategies for rhythm control. [Dobrev & Nattel, 2010] In light of these unmet needs, the following research has been focused to design novel pharmacological target aiming to treat most common type of SVD (atrial fibrillation) with higher efficacy and less risk. An attractive prospect for AF therapy has been considered the introduction of agents with selective affinity to ions channels specifically or predominately involved in atrium. [Dobrev, et al 2010]

Indeed, this research is currently focused on the development of agent targeting to modification of those pathway and molecular mediators which are involved in propagation and maintenance of supraventricular dysrhythmias. [Fedida, et al 2005]

Vernakalant

Vernakalant has highly selective atrial ion-channel blocking properties that has recently involved in management of acute atrial fibrillation (AF). [Dobrev & Nattel, 2010] [Dobrev, et al 2010] Intravenous vernakalant has been approved for the alteration of recent-onset AF in Europe and other parts of the world, but not in the USA. Vernakalant inhibits atrial-selective K^+ currents, including the ultra-rapidly activating delayed rectifier K^+ current (I_{Kur}) and acetylcholine-activated inward rectifier K^+ current ($I_{K,ACh}$), and causes rate-dependent atrial-preferential Na^+ channel block, with only a small inhibitory effect on the rapidly activating delayed rectifier K^+ current (I_{Kr}) in the ventricle.[Fedida, et al 2005] Due to its atrial-selective properties, vernakalant prolongs the effective refractory period (ERP) of the atria with moderate effects on the ventricles [Dorian, et al 2007] which explains the low pro-dysrhythmic risk for torsades de pointes (TdP) dysrhythmias. [Dobrev & Nattel, 2010] [Dobrev, et al 2010].

Vernakalant is an antidysrhythmic agent that has predominant properties on supraventricular electrophysiology. A human electrophysiological study demonstrated that vernakalant infusion dose-dependently prolongs atrial ERP. [Dorian, et al 2007] Atrial selectivity, thereby avoiding ventricular pro-dysrhythmia, can be achieved by aiming towards atrial-selective channels, such as I_{Kur} and $I_{K,ACh}$, by atrial-preferential inhibition of excitability through exploiting state-selective Na^+ channel blocking properties or by high selectivity for rapid rhythms like AF. [Dobrev & Nattel, 2010]

Vernakalant blocks several K^+ channels. It inhibits I_{Kur} in the open state, with preserved efficacy at high stimulation frequencies. [Fedida, et al 2005] The atrial-selective $I_{K,ACh}$ current is potently blocked by vernakalant. [Fedida, 2007] [Wettwer, et al 2013] Vernakalant also targets Kv4.3 and human ERG (hERG) channels that correspond to the transient outward current (I_{to}) and I_{Kr}, respectively, although the contribution of I_{to} to repolarization is lower in ventricles than in atria. [Fedida, et al 2005] In contrast, I_{Kr} is an important repolarizing current in ventricular cells. Its blockade causes QT interval and action potential duration prolongation, predisposing to TdP arrhythmias through the development of dysrhythmia-triggering early afterdepolarization and/or an increased dispersion of repolarization. [Dobrev & Nattel, 2010] However, the potency of vernakalant in blocking hERG channels is up to 100-fold lower than that of class IC antiarrhythmic drugs (flecainide or propafenone). [Fedida, et al 2005] Late Na^+ current ($I_{Na, late}$) inhibition by vernakalant is protective against the proarrhythmia from I_{Kr} blockade. [Orth, et al 2006]

Vernakalant causes an open-channel block of Na^+ channel Nav1.5 α-subunits that underlie the atrial I_{Na}. [16] [18] At physiological heart rates, the block of Nav1.5 channels by vernakalant is weak because of its rapid unbinding kinetics from the channel, [Fedida, et al 2005] [Fedida, 2007] which is consistent with the small increase in QRS interval (a marker of ventricular conduction velocity) observed in clinical trials. [Roy, et al 2008] [Pratt, et al 2010] [Carmeliet & Mubagwa, 1998] In addition, the effects of vernakalant on Na^+ channels are voltage and rate dependent, resulting in an enhanced inhibitory potency at depolarized potentials and rapid rates, like in fibrillating atria. [Fedida, et al 2005] The resting membrane potential of normal atrial myocytes is 10 mV more depolarized than that of normal ventricular myocytes. When atrial myocytes fail to repolarize fully, as can happen during AF, the atrioventricular difference in resting membrane potential is further accentuated and a large fraction of atrial Na^+ channels is inactivated. This reduces the Na^+ channel reserve predominantly in the atria and allows vernakalant to inhibit preferentially atrial Na^+ channels. [Fedida, et al 2005] Although such voltage and rate dependency is also typical for flecainide and propafenone, they do not show atrial selectively, [Fedida, et al 2006] [Carmaliet & Mubagwa, 1998] and the important difference is that vernakalant exhibits rapid unbinding kinetics from Na^+ channels. [Trop-Pedersen, et al 2001] Therefore, Na^+ channel block with rapid unbinding kinetics has recently been identified as a promising option for atrial-selective drug treatment of AF. [Comtois, et al 2008] [Antzelevitch, et al 2010]

Amiodarone

Amiodarone is a broad spectrum anti-dysrhythmic drug against numerous types of irregular heartbeats including ventricular tachycardia, ventricular fibrillation, atrial fibrillation & paroxysmal supraventricular tachycardia. [Porid, 1995]

The antiarrhythmic effect of amiodarone is due to non-competitive alpha and beta adrenergic inhibition, class II activity, in addition, amiodarone is a very effective blocker of sodium channels, class I activity, moreover, it has a week calcium channel blocking effect, class IV activity. [Du, et al 1995]

Amiodarone increases the cardiac refractory period without influencing resting membrane potential, except in automatic cells where the slope of pre-potential is reduced, generally reducing automaticity [Varro & Robloczky, 1986]. Amiodarone relaxes vascular smooth muscle, reduces peripheral vascular resistance (after load) and slightly increases cardiac index. [Singh, 1970] After oral dosing, however, amiodarone produces no significant changes in left ventricular ejection fraction (LVEF), even in patients with depressed LVEF. [Twidale, et al 1993] After acute intravenous dosing in man, amiodarone may have a mild negative inotropic effect. [Gangol, et al 1985]

Amiodarone does not alter vagal reflexes or the responsiveness of cardiac cholinergic receptors but it causes some non-competitive alpha and beta adrenergic blockade. [Biggera & Hoffman, 1992] Amiodarone has also a selective inhibition of the effect of T3 on myocardium that may contribute to prolongation of the action potential duration and refractoriness. [Melmed, et al 1981]

The Pharmacokinetic of numerous drugs, including many that are commonly administered to individuals with heart disease, is affected by amiodarone. Particularly, doses of digoxin should be halved in individuals taking amiodarone since amiodarone decreases renal and non-renal clearance of the digitalis glycosides and increases its bioavailability. These effects appear related to the dose of amiodarone, with higher doses of amiodarone being associated with the greatest increase in digoxin concentration. [Achilli & Serra, 1981]

Amiodarone potentiates the action of warfarin. Individuals taking both of these medications should have their warfarin dose halved and their anticoagulation status, measured as prothrombin time & international normalized ratio, measured more frequently. Amiodarone decreased the total body clearance of warfarin in normal subjects but did not change volumes of distribution. Amiodarone is a general inhibitor of the cytochrome P450 catalyzed oxidation of warfarin. [Larry, et al 1991]

The FDA revised the labels of amiodarone and simvastatin in 2002 to warn of increased risk of rhabdomyolysis, the most severe form of myopathy, when the two drugs are taken concomitantly in doses greater than 20 mg per day of simvastatin. [Karimi, et al 2010] There are many other drugs should not be taken with amiodarone: cimetidine, clopidogrel, cyclosporine, dextromethorphan, diclofenac, loratedine, a beta-blocker, potentiation, and Ca2+ channel blockers. [Singh, et al 1989]

Amiodarone has numerous side effects. Most individuals administered amiodarone on a chronic basis will experience at least one side effect [Vanerven & Schalij, 2010], Decrease heart rate and increase incidence of heart block, interstitial lung disease, Some individuals developed pulmonary fibrosis after a week of treatment, Amiodarone is structurally similar to thyroxin, which contributes to the effects of amiodarone on thyroid function, both under and over activity of the thyroid may occur on amiodarone treatment [Batcher, et al 1989], Corneal micro-deposits, Corneal verticillata, Abnormal liver enzyme results are common in patients on amiodarone. [Flaharty, et al 1989]

According to the numerous drug interactions and adverse effects caused by amiodarone, this research investigates the effect of a novel antidysrhythmic drug, vernakalant, on reperfusion dysthymia in rats in comparison with amiodarone, standard broad spectrum antidysrhythmic drug.

Material and method

The animals used in the experiments were 40 adult male albino rats weighting 170-200 g. The animals were handled according to the guide lines of local ethical committee which comply with the international laws for use and care of laboratory animals.

The animals were divided into four groups, each group contained 10 rats.
- **(Control Group I)** normal (did not receive any medications)
- **(Control Group II)** diseased group (reperfusion dysrhythmia, adult male rats were anaesthetized by intramuscular injection of 25% solution of urethane in a dose of 0.7ml/100g body weight. The trachea was exposed and tracheotomy was done through which a Y-shaped glass tube was cannulated. The animal was artificially ventilated in a respiratory rate of 40/minute and tidal

volume of 6 ml/kg [Harkness & Wagner, 1989] throughout the experiment to avoid any respiratory disturbances during the experiment.

The left jugular vein was cannulated by pediatric cannula (size 24G). The chest was opened by midline thoracotomy at the xiphesternal junction. After opening the pericardium, the heart is exteriorized by gentle pressure on the chest wall then a snap was taken by a proline 5-0 thread around left anterior descending branch of the left main coronary artery and the two ends of the thread were put into a plastic tube closed by a clamp for 15 min & subsequent reperfusions for 30 min. [Abraham, et al 1989]

- (Group III) received vernakalant after induction of dysrhythmia (after 30 minutes reperfusion) by a 10-minute infusion of 3 mg/kg followed by a 15-minute observation period then a second 10-minute infusion of 2 mg/kg if still in AF. [Tian & Frishman, 2011]
- (Group IV) received amiodarone 100 mg IV [loading dose] approximately 30 min prior to induction of dysrhythmia. [Nadkarni, et al 2010]

In all groups, limb electrodes of the electrocardiogram recorder of the power lab device were connected to three limbs of the animal with the following order:

- The negative electrode (black) is connected to the right upper limb.
- The positive electrode (red) is connected to the left lower limb.
- The indifferent electrode (green) is connected to the left upper limb.

The standard lead II was adjusted by the power lab and the heart rate was recorded by the power lab device (Model no. 866. MLA1215 Animal Bio AMP lead wires set of three 2 mm pins to micro hook lead wires).

Animals were anesthetized and prepared as the previous groups. After recording of normal ECG, reperfusion dysrhythmia was induced in the same previous manner with ECG recording every 5 minutes From T0 to T23.

For each animal, the heart rate, time of appearance of cardiac dysrhythmias and disturbances ECG were recorded.

Statistical methods

Data were statistically described in terms of range, mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Kruskal Wallis analysis of variance (ANOVA) test. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, and USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Control group I

Heart rate

The normal heart rate ranged between 226 and 312 beats/minute, with a mean ± SD of 286.20 ±26.377 ranged from T0-T23.

S-T segment

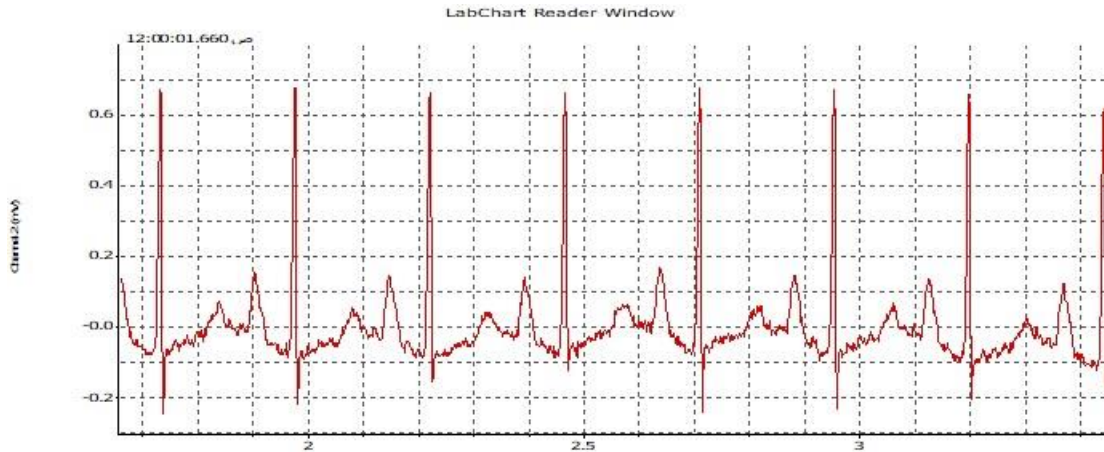There were no significant changes in the S-T segment with normal ECG. (Figure 2)

Figure 2. Regular ECG & Regular heart rate in control group I

Control group II

Heart rate

By induction of dysrhythmia, there were no statistically significant changes in heart rate up to the T4 (Closure of coronary arteries). From T5 (Reperfusion), there was statistically significant reduction in heart rate down to the T 23,177.5±14.849 with a ratio of reduction of 38%. (Figure 3)
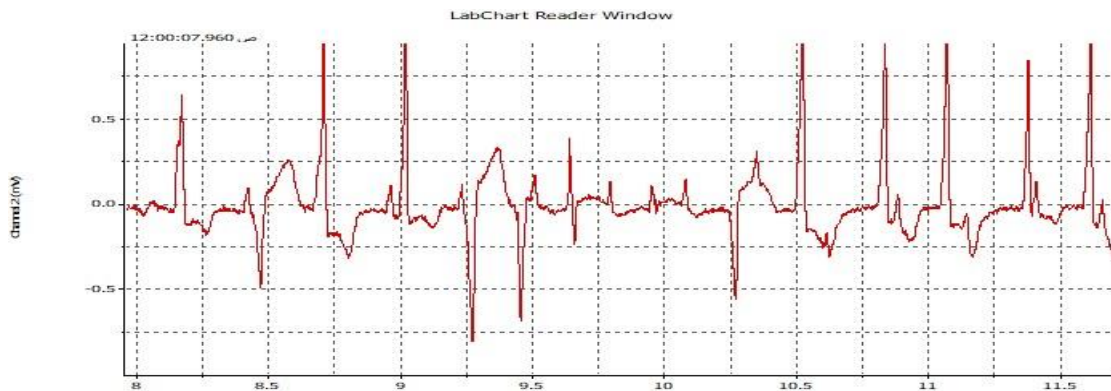

Figure 3. Irregular heart rate at T16 in control group II

S-T segment

There were no significant changes in the S-T segment before induction of dysrhythmia, T0-T4. Reperfusion produced an equal percentage of S-T segment depression (Figure 4) and elevation (Figure 5) about 10% for each at T5 and then there was statistically significant increase in the percentage of S-T segment depression to reaches 60% at T9 then decreased gradually from T14, 50%, to reach 0% at T23. The percentage of elevated S-T segment increased gradually to reach 66% at T22.
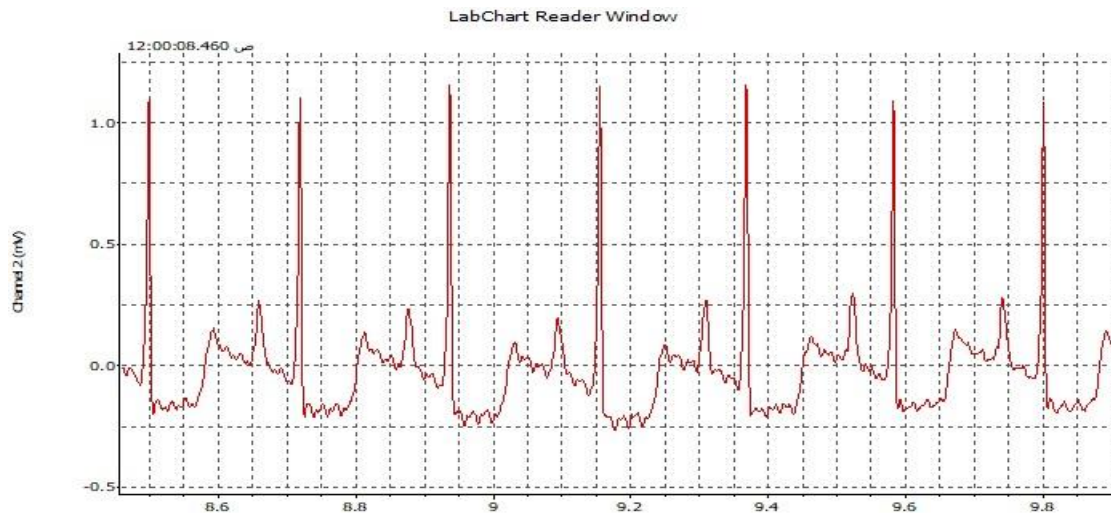
Figure 4. Depressed S-T segment occurred at T 9 at control group II.

Figure 5. Elevated S-T segment occurred at T 12 at control group II

Dysrhythmia

Coronaries reperfusion resulted in induction of dysrhythmia starting with the T5 in 2 animals, 20%, in the form of SVEs and SVT in one animal and VEs in the second animal. The incidence of dysrhythmias increased gradually to affect all animals (100%) by T9. Death of the animals started to occur after T12 in one animal, 10%, and increased gradually to reach 40% after T20. As regards the types of reperfusion dysrhythmia to the non-treated rats, control group I, the following four types of cardiac dysrhythmias developed, ventricular tachycardia (V.T.) (Figure 6), multiple ventricular extra systoles (V.Es) (Figure 7), multiple supraventricular extra systoles (S.V.Es) (Figure 8) and supraventricular tachycardia (S.V.T) (Figure 9).
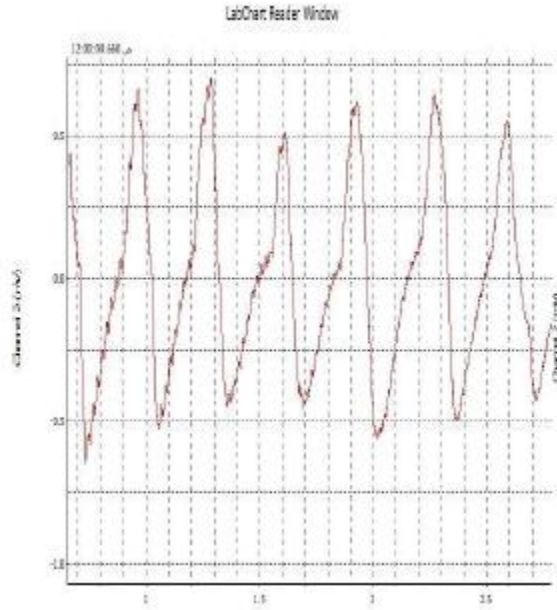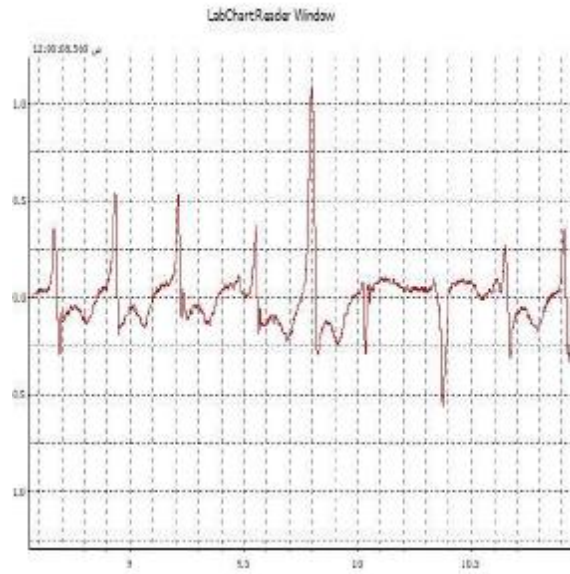
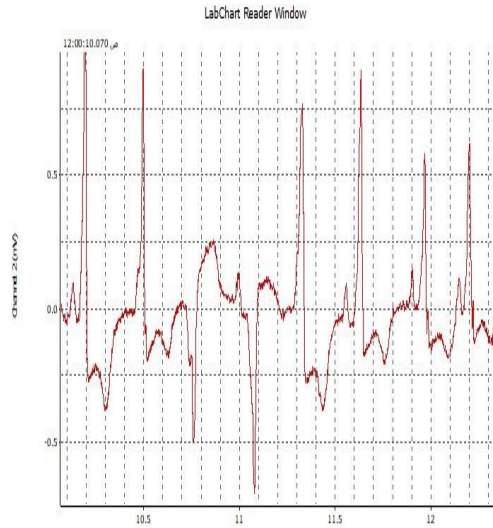**Figure 6.** Ventricular tachycardia

Figure 7. Ventricular extrasystole

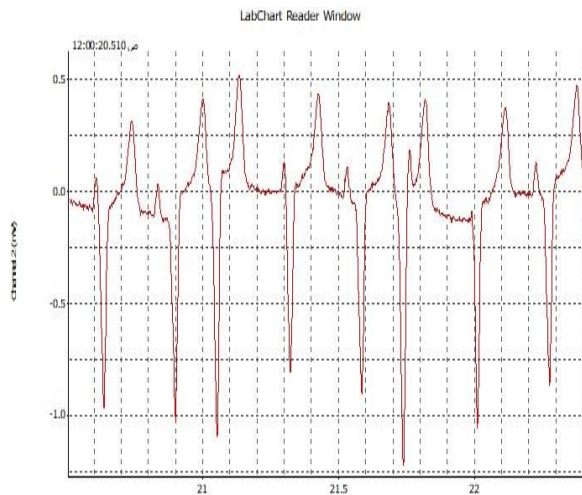**Figure 8.** Supraventricular extrasystole

Figure 9. Supraventricular Tachycardia

Table 1. Summary of types of coronaries reperfusion dysrhythmias

	Animal's number									
Dose	1	2	3	4	5	6	7	8	9	10
T1										
T2										
T3										
T4										
T5	SVE+SVT				VE					
T6	SVE+SVT				SVE			SVE		
T7	SVE+SVT	VE			SVE	SVT		VT	VE	
T8	SVE+SVT	VE	VE	SVE	SVE	VT	SVE	VT	VE	
T9	SVE+SVT	VE	VE	SVE	SVE	VT	SVE	VT	VE+VT	SVE
T10	SVE+SVT	VE +VT	VE	SVE	SVE	VT	SVE+SVT	VT	VE+VT	SVE
T11	SVE+SVT	VE +VT	VE	SVE	SVE	VT	VT	VT	VE+VT	SVE

T12	SVE+SVT	VE +VT	VE	VE +VT	SVE	VT	VT	VT	VT	VE+ VT
T13	SVE+SVT	VE +VT	VT	VE+ VT		VT	VT	VT	VT	VE+ VT
T14	SVT	VT	VT	VE+ VT			VT	VT	VT	VE+ VT
T15	SVT	VT	VT	VE+ VT			VT	VT	VT	VE+ VT
T16	SVT	VT	VT	VT			VT	VT	VT	
T17	SVT	VT	VT	VT			VT	VT	VT	
T18	SVT	VT	VT	VT			VT	VT	VT	
T19	SVT	VT	VT	VT			VT	VT	VT	
T20	SVT	VT	VT	VT					VT	
T21	SVT	VT	VT						VT	
T22	SVT	VT							VT	
T23	SVT	VT								

Comparison between group III (Vernakalant treated group) & group IV (Amiodarone treated group) against group II (reperfusion dysrhythmias)

Heart rate

There were no statistically significant differences in the resting heart rate, T0, between control group II, both group III and IV. By induction of dysrhythmia in group III, vernakalant reduced bradycardia gradually from 38% to 18.5% (Figure 10), while in group IV, amiodarone decreased the bradycardia from 38% to 17.5% (Figure 11).
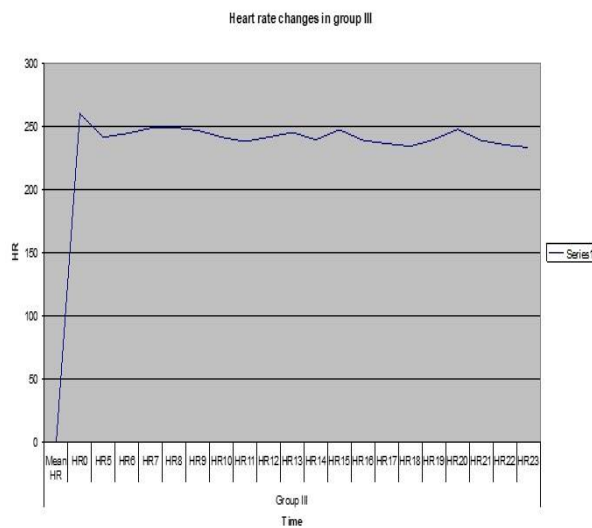

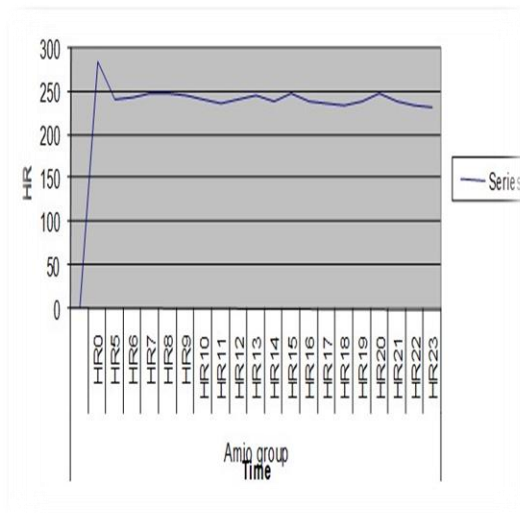**Figure 10.** Different heart rate changes in group III

Figure 11. Different heart rate changes in group IV

S-T segment

There are no significant changes in the S-T segment before induction of dysrhythmia, T0 –T4 in the control group II and both group III and group IV. There was slight increase in the percentage of elevated S-T segment starting from T5 to T10 on comparison between the control group II and group III, then this percentage gone within normal range with the control group with no statistically significant changes up to T 23. In group IV, the percentage of elevated S-T segment markedly decrease to reach 0% during the whole experiment and the percentage of normal S-T segment stays at a high level ranged from 100% at T5 to 80% at T23 with statistically significant changes starting from T7 to T21 (Table 2).

Table 2. Comparison between groups II, group III, group IV the percentage of normal, elevated and depressed S-T segment

S-T segment									
	Group II			Group III			Group IV		
Time	Normal	Elevated	Depressed	Normal	Elevated	Depressed	Normal	Elevated	Depressed
T5	80%	10%	10%	60%	40%	0%	100%	0%	0%
T6	70%	10%	20%	60%	40%	0%	100%	0%	0%
T7	30%	10%	60%	50%	40%	10%	100%	0%	0%
T8	30%	20%	50%	50%	40%	10%	100%	0%	0%
T9	20%	20%	60%	50%	40%	10%	100%	0%	0%
T10	20%	40%	40%	50%	40%	10%	100%	0%	0%
T11	20%	40%	40%	50%	40%	10%	90%	0%	10%
T12	20%	50%	30%	50%	40%	10%	80%	0%	20%
T13	22.2%	44.4%	33.3%	50%	40%	10%	80%	0%	20%
T14	12.5%	37.5%	50%	50%	40%	10%	80%	0%	20%
T15	12.5%	37.5%	50%	40%	50%	10%	80%	0%	20%
T16	12.5%	37.5%	50%	40%	50%	10%	80%	0%	20%
T17	12.5%	50%	37.5%	40%	50%	10%	80%	0%	20%
T18	12.5%	62.5%	25%	40%	50%	10%	80%	0%	20%
T19	25%	50%	25%	50%	40%	10%	80%	0%	20%
T20	16.7%	33.3%	50%	50%	40%	10%	80%	0%	20%
T21	25%	50%	25%	50%	40%	10%	80%	0%	20%
T22	33.3%	66.7%	0%	50%	40%	10%	80%	0%	20%
T23	50%	50%	0%	50%	40%	10%	80%	0%	20%

Dysrhythmias

There were regularly paced complexes with normal shape without any statistically significant differences between group II, group III and group IV up to T4.

In group III and IV, the heart rate stayed regular all up to T23. There were statistically significant changes in the regularity of the heart beats between the control group II and both group III and IV starting from T7 until T23.

VT appeared only in one animal at T22 in group III, while in group IV, there were not any types of dysrhythmia occurred all through adrenaline doses. (Figure 12)
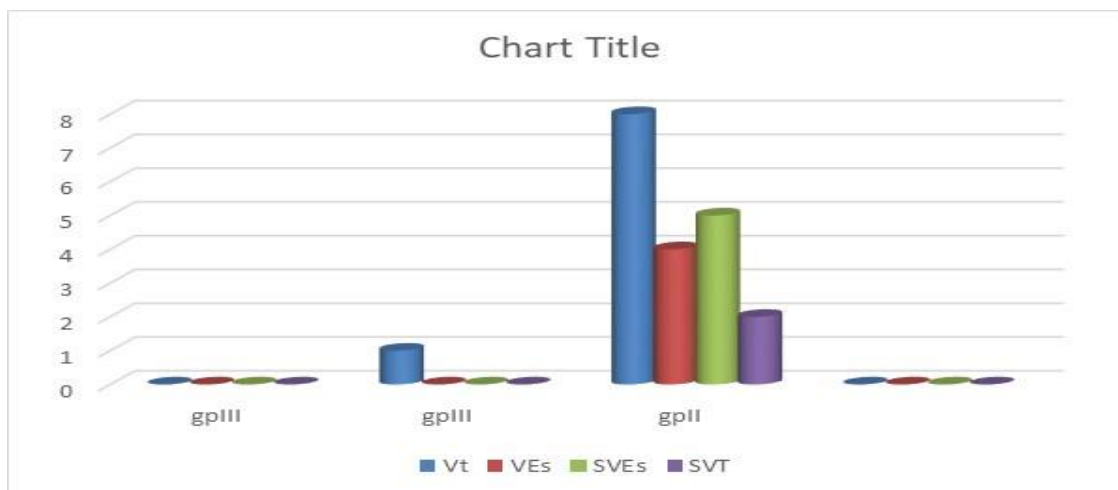

Figure 12. Chart showing percentage of different types of cardiac dysrhythmias in group II, III and IV.

Discussion

Cardiac dysrhythmias occur when the electrical signals to the heartbeats are not working probably. For instance, some people experience irregular heartbeats, which may feel like a racing heart or fluttering. Many types of cardiac dysrhythmias are harmless, however, if they are particular abnormalities resulting from weak or damage heart, dysrhythmia can cause serious and even potentially fatal symptoms. Dysrhythmias are life threatening medical emergencies that may cause cardiac arrest and sudden death. Up to 65% of patients had sudden cardiac death as first manifestation of cardiac dysrhythmia. In the United States, more than 850,000 people are hospitalized for a dysrhythmia each year. [John, et al 2010]

Supraventricular dysrhythmia is a complicated type of dysrhythmia that is hard to treat with habitual antidysrhythmic medications. Novel pharmacological methodologies are in advance concentrated on the advancement of specialists with selective affinity to ion channels predominately engaged with the atrium. In parallel, inquire about endeavors have been focused on the development of agents focusing to adjustment of those pathways which are associated with the proliferation and maintenance of atrial fibrillation (AF). [Ferrari, et al 2015]

Novel ion channels inhibition agents developed to treat AF are broadly separated into two categories such as "atrial-selective" compounds and "multi-channel blockers". Vernakalant is predominantly "atrial-selective" blocker while amiodarone is considered as a "multi-channel blocker".

Vernakalant is an antiarrhythmic atrial-selective compound acting by blockade of IKur, which is exclusively expressed in the atria. Furthermore, it is a multichannel blocker that affects the sodium channel and IK-Ach both expressed predominantly in the atria. [Burashnihov, et al 2010]

This study aimed to investigate the possible antidysrhythmic effect of vernakalant on coronary reperfusion cardiac dysrhythmias, in comparison with amiodarone. In this work, adult male albino rats were used. Their heart structure is relatively close to that of the humans and their size made it easy to induce ischemia-induced dysrhythmias. Amiodarome was chosen as a comparator, because it is a standard antidysrhythmic drug with broad spectrum properties against different types of cardiac dysrhythmia with different mechanisms.

In this work, adult male albino rats weighting 170-200 g were kept in normal environment without any procedures or medications given, as a standard (control) group I with normal heart rate and normal ECG recording.

In group II (diseased group), It was noticed that there is no changes in heart rate during closure of coronary arteries (up to T4), after reperfusion (T5) there was statistically significant reduction in the heart rate with irregular cardiac rhythm, it was explained by [Jurkovicova and Cagan, 1998] that abnormal cardiac rhythm originate as a consequence of the complex of cellular and humeral reactions accompanying the opening of coronary artery leading to release of chemical substances such as calcium, thrombin, platelet activating factor, inositol triphosphate & angiotensin II which operate as modulators of cellular electrophysiology causing complex changes at the level of ions channels.

In vernakalant treated group III the resting heart rate did not differ significantly from that of the control group I, vernakalant reduced the coronary-reperfusion induced bradycardia from 38% to 18.5%. It was stated by [Bechard et al, 2011] that vernakalant is an antiarrhythmic atrial-selective compound acting by blockade of IKur, which is exclusively expressed in the atria. Furthermore, it is a multichannel blocker that affects the sodium channel and IK-Ach both expressed predominantly in the atria. Inhibiting potassium currents, vernakalant causes prolongation of atrial refractoriness which contributes to the efficacy of the drug. Besides, it exerts frequency- and voltage-dependent sodium channels block, including the INaL, causing significant effect on the intra-atrial conduction particularly at fast rates.

In the amiodarone pretreated group IV, the resting heart rate did not differ significantly from those of the control group I and vernakalant treated group III. The effects of amiodarone on resting heart are evaluated in different studies. [Mason, 1987] stated that amiodarone decreases sinus rate about 15 to 20% and attributed this effect to its ability to inhibit intracellular conversion of thyroxin T4 to T3. [Djandjighian et al, 2000] also observed that amiodarone significantly and dose-dependently lowered the resting heart rate in animals and reduced the exercise-induced tachycardia which is probably due to its calcium channel and β-adrenoceptor blocking effects. These changes should not require discontinuation of amiodarone as they are evidence of its pharmacological action. [Arrendono, et al 1986]

In group IV, the mean heart rate decreased from T4 up to T23 with a percentage of reduction 17.5%, on comparison with that of the control group I, 38%. The difference in heart rate reduction in both groups, I and IV, was statistically significant. The antagonistic effect of amiodarone to bradycardia induced by reperfusion may be explained by its vasodilator effect [Zipese, et al 1984] and it was explained by [Patel et al, 2009] that amiodarone acts as a multichannel blocker by inhibiting a wide range of ion channels including IKs, IKr, IKur, IK-Ach, ICaL, INa+.

The antagonistic effects of both vernakalant and amiodarone on reperfusion induced bradycardia were comparable with no statistically significant difference.

Coronary reperfusion (group II) resulted in changes in ST segment and T-wave inversion. There was an elevation of S-T segment in one animal, 10%, and a depression in another animal, 10%, after T5 then the percentages of animals showing elevation and those showing depression increased significantly to reach 40% for each after T10. From T12 and up to T22 there were also comparable ST segment elevation and depression with more tendencies to elevation.

It is contrary to that stated by [Heper et al, 2008] that successful reperfusion causes normalization or more than 50% regression of S-T segment elevation, T-wave inversion or any other dysrhythmias observed by electrocardiograph, S-T segment return is explained by rapid normalization of myocardial cell membrane potentials in the ischemic area as myocardial cells are capable of normalizing their membrane potential immediately as oxygen become available.

Treatment of the animals in group III with vernakalant increased insignificantly the percentages of animals showing elevated ST-segment compared with that in control group I. Vernakalnt decreased significantly the percentages of animals showing ST-segment depression. In accordance with the observed results in this work, vernakalant could produce a dose-dependent reduction in ST-segment depression induced by exercise in experimental animals, suggesting that vernakalnt's beneficial mechanism of action is due to an improvement in regional coronary blood flow in areas of myocardial ischemia mainly for non-transmural, subendocardial ischemia, it was stated by [Roy et al, 2004] that

in vivo human electrophysiology study and the CRAFT trial did not find a significant change in QRS or heart rate-corrected QT interval (QTc) by the infusion of vernakalant. In contrast, pivotal trials (ACT I, II, and III) showed that vernakalant increases the QRS and QTc intervals between 5 minutes and 2 hours after the start of infusion [Pratt, et al 2010]

Treatment of the experimental animals in group IV with amiodarone, abolished S-T segment elevation completely with a minor percentage of depressed S-T segment, remaining in only 10%-20%. Similar results were observed in the experimental animals by [Lindenmeyer et al, 1984]

Based on aforementioned results, it could be concluded that amiodarone is slightly more effective than vernakalnt in correction of both elevated, transmural ischemia, and depressed S-T segment and non-transmural ischemia.

There were no significant changes in the regularity of the heart rate in the control group I up to T4. The dysrhythmias began from the T5 in 20% of animals and increased gradually, 30%, after T6 to 90% after T8 and at T9, 100%. These results were compatible to that stated by [Murdock et a, 1980] that the incidence of reperfusion-induced ventricular fibrillation increased when occlusion periods were lengthened from 5 minutes to 20 or 30 minutes and decreased when reperfusion was delayed beyond 30 to 60 minutes. Also, reperfusion-induced fibrillation tended to occur more often when severe arrhythmias developed during occlusion. It was also stated by [Casio et al, 2001] that changes in extracellular potassium (K) has been shown to fluctuate with coronary occlusion and reperfusion and that is also related to alterations in conduction that cause arrhythmias.

In the vernakalnt treated group, III, the regularity of the heart beats was maintained up to T 23. Cardiac dysrhythmia did not develop in 90% of the rats and only 10% developed ventricular tachycardia with high doses, at T22 and T23 for 10 minutes. The anti-dysrhythmic action of vernakalant could be attributed to its atrial-selective ion-channel blocking properties that has recently been introduced for the acute management of cardiac dysrhythmias. [Dobrev & Nattel, 2010] Vernakalant inhibits atrial-selective K currents, including the ultra-rapidly activating delayed rectifier K current (I_{Kr}) and acetylcholine-activated inward rectifier K current ($I_{K, Ach}$), and causes rate-dependent atrial-preferential Na channel block, with only a small inhibitory effect on the rapidly activating delayed rectifier K current (I_{Kr}) in the ventricle. [Fedida, et al 2005]

Ttreatment of animals with amiodarone, Group IV, resulted in prevention of development of all type of dysrhythmias (100%) up to T23. The antidysrhythmic effect of amiodarone could be attributed to its due non-competitive alpha and beta adrenergic inhibition, class II activity, in addition, amiodarone blocks sodium channels, class I activity, moreover, it has a weak calcium channel blocking effect, class IV activity. [Gill, et al 1992]

Conclusion

Vernakalant showed a powerful antidysrhythmic action against ischemic-reperfusion cardiac dysrhythmias in experimental animals, comparable to that exerted by amiodarone with less recorded adverse drug effects causing beneficial properties of vernakalant against different types of cardiac dysrhythmias.

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