Electrophysiological changes in response to L-arginine infusion in isolated mammalian heart

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Arrhythmia is one of the major detrimental risk factors for cardiac arrest and death especially those associated with prolonged Q-T interval. Several antiarrhythmic and cardiac agents prolong the Q-T interval as class I-a and class III anti-arrhythmic agents. The cGMP is an important second messenger formed by the NO induced-guanylyl cyclase in response to L-arginine infusion. The aim of the present work is to investigate the relation between L-arginine infusion and different electrocardiograph (ECG) intervals. Isolated hearts from 6 male rabbits were perfused using Langendorff’s apparatus in which the perfusion fluid was ringer-Locke solution, applied at constant flow rate and was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Each heart served as its own control before infusion of adrenaline and then L-arginine at concentration of 3 mmol/L. With the help of Power Lab data acquisition and analysis system and Chart 7 program (ADInstruments Australia), the force of contraction, heart rate, and ECG were recorded for 5 min. NO generation and cGMP generation produces negative chronotropic effect with significant decrease in the heart rate from (125.2 ± 8.320) to (93.67 ± 7.04) /min. and significant prolongation of the Q–T interval 34% from (199.5 ± 22.35) to (268.4 ± 9.948) m.sec. and the Q-Tc by 24% from (291.0 ±35.98) to (361.2 ± 13.23) m.sec. L-arginine infusion with NO generation in isolated mammalian produces negative inotropic effects as well as prolongs Q-T and Q-Tc intervals.

Key words: L-arginine, Q-T interval, arrhythmias, isolated heart.

INTRODUCTION

Arrhythmias is one of the major cardiovascular causes of mortality caused by abnormality in the generation or propagation of the cardiac electricity. Some of these arrhythmias are paroxysmal with life threats, others have tremendous effects ending with death as torsades de pointes (TdP) which is a polymorphic ventricular tachycardia characterized by a distinctive pattern of undulating QRS complexes that twist around the isoelectric line. TdP is usually self-terminating or can subsequently degenerate into ventricular fibrillation, syncope, and sudden death (Blancett et al., 2005). The electro cardio graph (ECG) intervals includes R-R, P-R and QT intervals. QT interval (also termed electrical systole), the period between the beginning of the QRS complex and the end of the T wave of the electrocardiogram, reflects the ventricular action potential duration (APD) and represents the required period for ventricular depolarization and repolarization. This duration is determined by the balance of inward and outward currents occurring during depolarization and repolarization phases of ventricular action potential (AP). TdP has been associated with QT interval prolongation of the electrocardiogram; therefore,
the QT interval has come to be recognized as a surrogate marker for the risk of TdP (Van et al., 2004).

Nitric oxide (NO), essential for the proper functioning of the cardiovascular system, is derived from L-arginine by NO synthase (NOS) in endothelial cells as shown in Figure 1. NO through cGMP generation produces negative inotropic and chronotropic effects on isolated mammalian heart (Sakr et al., 2010). NO donors or the precursor for NO synthesis, L-arginine, can ameliorate reperfusion-induced arrhythmias and reduce ischemic/reperfusion injury in rabbits. Several previous studies investigated the effects of l-arginine on the Q-T interval and Q-Tc in the presence of other variables such as exercise (Bednarz et al., 2000) and hypercholesterolemia (Kumar et al., 2009). So the aim of the present work is to clarify the ECG intervals changes in response to L-arginine infusion on isolated mammalian heart in the absence of other variables.

MATERIALS AND METHODS

Animals

Six adult white adult newzealand male rabbits weighing between 2 and 3 kg were used for the experiments with the approval of Ethical Committee of the Medical School, King Khalid University, Abha, Saudi Arabia. The animals were obtained from the animal house of the College of Medicine of King Khalid University where they were fed with standard rabbit pellets and allowed free access to water. They were housed at a controlled ambient temperature of 25 ± 2°C and 50 ± 10% relative humidity, with 12-h light/12-h dark cycles. All studies were conducted in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals (NIH, 1996).

Experimental procedure

This experiment was carried out in accordance with the Langendorff (1985) procedure. Each rabbit was injected with 1000 IU of heparin intravenously through the marginal ear vein. Five minutes later, a blow on the neck of the rabbit made them unconscious. The chest was opened and the heart was dissected out with about 1 cm of aorta attached, and washed quickly as possible with oxygenated Ringer-Locke solution (NaCl; 45.0 g, NaHCO₃; 1.0 g, D-glucose; 5.0 g, KCl; 2.1 g, CaCl₂·2H₂O; 1.6 g, in 5 L of distilled water). The isolated heart was gently squeezed several times to remove as much residual blood as possible. The heart was then transferred to the perfusion apparatus (Radnoti isolated heart system, AD instrument, Australia) and tied to a stainless steel canula through the aorta. The perfusion fluid was warm Ringer-Locke solution which was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide and was applied at a constant perfusion pressure of 70 mm Hg (Langendorff, 1985). Temperature was continuously monitored by a thermo-probe inserted into the perfusion fluid tank and maintained between 36.5 and 37.5°C. The hearts were allowed to stabilize for 30 min before any drug interventions. 1 ml of Ringer-Locke solution containing 3 mmol/L of L-arginine was injected over 30 s with the aid of 1 ml syringe through the perfusion line above the aortic line, and the changes in the cardiac parameters were recorded (Figures 2 and 3). Parameters measured are heart rate (beats/min) and ECG for rhythm monitoring. During the experiments each heart served as its own control before infusion of each solution.

Statistical analysis

Results were expressed as the mean value ±SD. Statistical differences between groups were assessed using the Graph pad5 software by t -test. Values of P<0.05 were considered significantly different (95% confidence interval).

RESULTS AND DISCUSSION

Table 1 shows that the infusion of 3 mmol/L L-arginine reduces the heart rate significantly by 25% from (125.2 ± 8.320) to (93.67 ± 7.04) /min. Table 1 shows that the infusion of L-arginine increases the R-R interval significantly by 35% from (496.1 ± 25.83) to (670.2 ± 18.79) m.sec and decreases the P-R interval significantly by 40% from (52.00 ± 6.106) to (32.09 ± 2.401) m.sec. Also, NO generation in response to L-arginine infusion significantly prolongs the Q-T interval by 34% from (199.5 ± 22.35) to (268.4 ± 9.948) m.sec. and the Q-Tc by 24% from (291.0 ±35.98) to (361.2 ± 13.23) m.sec.

Nitric oxide (NO) synthetized by essentially all cardiac cell types exerts a key role in regulating cardiac function (Kelly et al., 1996). NO is a highly diffusible gas that spreads greatly from its site of synthesis and a free radical highly reactive with other species, notably oxygen, superoxide and iron-containing haeme groups which act as NO scavengers (Massion et al., 2003). For this reason, the half-life of NO is limited to seconds and its effects are localized close to where it is synthetized. NO generated within the cardiomyocytes can exert intracrine effects or modify the functional properties of adjacent cardiomyocytes (Schulz et al., 2005). NO generated from non-cardiomyocyte sources (coronary, endocardial, and endothelial cells, autonomic nerves and ganglia, and blood-formed elements) can exert direct effects on cardiomyocytes and indirect effects by modulating coronary blood flow and/or autonomic transmission (Ziolo et al., 2004; Seddon et al., 2007). The heart produces NO on a beat-to-beat basis in response to changes in coronary flow and myocardial loading. In rabbit hearts, NO levels reach peak values during diastole and lowest during systole. NO concentrations were 15% lower in rat hearts (Pinsky et al., 2007).

The ventricular AP Figure (5) can be divided into 5 phases. When a wave of depolarization reaches ventricular myocytes, a rapid opening of voltage-gated sodium channels (INa), allows for the influx of Na⁺ into the ventricular myocytes; this produces phase 0 of ventricular AP, and produces depolarization, which is represented by the QRS complex on the surface ECG. Immediately after maximal depolarization of Phase 0, INa is in the inactivated stage, and repolarization begins with activation of the transient outward potassium current (Yan and Antzelevitch, 1996). This process causes a brief rapid repolarization and yields a notch on the ventricular action potential known as Phase 1. This phase is followed by a slower phase of repolarization called Phase 2 (the plateau). Phase 2 of the AP is generated mainly by
Figure 1. Mechanism of NO generation in the endothelial cells and its activation on guanylyl cyclase, in the presence of endothelial nitric oxide synthase (eNOS), L-arg arginine is converted into NO. NO diffuses to the ventricular muscle fiber forming cGMP from GTP by the action of guanylyl cyclase.

Figure 2. Baseline recording of ECG from the rabbit’s heart.

the inward L-type calcium current (ICaL) and outward K⁺ currents. The delayed rectifier potassium currents also begin to activate at this phase. The activation is slow and the currents have a reduced conductance at positive transmembrane potentials causing the prolonged AP (Sanguinetti and Tristani-Firouzi, 2006).

Our results showed that L-arginine infusion produced a significant negative chronotropic effect with decreasing the heart rate by about 25% and significant prolongation of the R-R interval. These data were previously concluded by other studies that proved that effect. NO generated under the influence of NO synthase stimulated the guanylyl cyclase yielding the highly important second messenger cGMP. cGMP decreased the rhythmicity by the activation of the acetyl choline dependent K channels in the sino-atrial node facilitating excess K efflux with hyperpolarization generation. Our data was in accordance with Kiziltepe et al. (2004) who discovered
Figure 3. Recording of ECG from the rabbit’s heart in response to L-arginine.

Table 1. The effect of L-arginine infusion 3 mmol/L on isolated mammalian heart on heart rate, R-R interval, P-R interval, Q-T interval and Q-Tc intervals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>L-arginine infusion</th>
<th>Percent of change</th>
</tr>
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<tbody>
<tr>
<td>Heart rate /min</td>
<td>125.2 ± 8.320</td>
<td>93.67 ± 7.04</td>
<td>-25 %</td>
</tr>
<tr>
<td>R-R interval (m.sec)</td>
<td>496.1 ± 25.83</td>
<td>670.2 ± 18.79</td>
<td>+35 %</td>
</tr>
<tr>
<td>P-R interval (m.sec)</td>
<td>52.00 ± 6.106</td>
<td>32.09 ± 2.401</td>
<td>+40 %</td>
</tr>
<tr>
<td>Q-T interval (m.sec)</td>
<td>199.5 ± 22.35</td>
<td>268.4 ± 9.948</td>
<td>+34 %</td>
</tr>
<tr>
<td>Q-Tc interval (m.sec)</td>
<td>291.0 ±35.98</td>
<td>361.2 ± 13.23</td>
<td>+24 %</td>
</tr>
</tbody>
</table>

Figure 4. Effect of L-arginine (3 mmol/L) on isolated mammalian heart at horizontal scaling, showing the ECG recording, heart rate. (on scaling 500:1).

that L-arginine may be a natural anti-arrhythmic agent upon consideration of its effect in restarting normal sinus rhythm at the completion of heart surgery. The P-R interval introduces an idea about the conduction of the electrical impulse in the atrial wall as well as the atrioventricular node. Naturally the atrioventricular nodal
Figure 5. Action potential of ventricular muscle fiber phase 0: rapid depolarization, phase 1 slow partial repolarization, phase 2: plateau, phase 3: rapid repolarization and phase 5: complete repolarization.

(AVN) is characterized by the slowest velocity of conduction in the myocardium which offers sufficient time for atrial contraction before ventricular contraction and protects the ventricles from high atrial rhythm. In disagreement with our results, conduction through the AVN was previously studied by Khor et al. (2011) who concluded that NO generation in response to L-arginine had stimulatory effect on AV nodal properties through decreasing the refractory period. The mechanism of impulse conduction facilitation could be attributed due to the activation of protein kinase G in response to cGMP. Previous research suggested that the NO-cGMP-PKG pathway contributes to phosphorylation of K(ATP) channels in rabbit ventricular myocytes producing depolarization of the myocytes in the AVN and enhanced conduction (Tamargo et al., 2010).

Our results showed that NO generation produces prolongation of the Q-T and Q-Tc intervals significantly. The QT interval is measured from the beginning of the QRS complex to the end of the T wave, therefore it represents the duration of depolarization and repolarization of ventricular muscle fibers which is roughly parallel to the ventricular absolute and relative refractory period. The QT interval consists of 2 components: the QRS complex represents ventricular depolarization and the JT interval, a measure of the duration of ventricular repolarization.

Since QT duration changes inversely with heart rate; the slower the heart rate the longer the QT interval. Hence, a QT correction formula is needed to substitute for each measured QT interval. The corrected QT (QTC) value corresponds to one that would have been measure-
unless the QTc exceeds 500 ms (Bednar et al., 2001).

The mechanism of Q-T prolongation in response to L-arginine seems to be unclear. Several previous works by Horimoto et al. (2000) and Stavrou et al. (2001) established that NO generation increases the ventricular muscle action potential duration and the absolute refractory period independent to the ATP sensitive K+ channels, meaning that Q-T prolongation in response to L-arginine is not related to the change in the ventricular repolarization.

Previous work investigated the significant correlation between the activation recovery intervals and the action potential duration (Haws and Lux, 1990; Millar et al., 1985). Wang (2003) investigated the activation-recovery intervals from epicardial ECGs leads and recorded that intravenous administration of N\(^{G}\)-nitro-L-arginine, a NO synthase inhibitor, increased left ventricular systolic pressure from 101±7 to 118±10 mmHg (P=0.02), and left ventricular end diastolic pressure from 6.3±1.5 to 8.8±1.8 mmHg (P<0.01) without changing the heart rate (96±4 beats/min versus 94±3 beats/min, P=0.06). Wang (2003) concluded that NO synthase inhibition with N\(^{G}\)-nitro-L-arginine did not change the configuration of epicardial ECGs or influence the activation-recovery intervals. These data indicate that basal NO inhibition has no significant effect on ventricular repolarization.

Evidence also suggests that NO is involved in certain drug induced reduction of action potential duration. In guinea pig ventricular papillary muscles, inhibition of NO synthase with NG-monomethyl-L-arginine (L-NAME) attenuates lipopolysaccharide-induced shortening in action potential duration (Chen et al., 2000). In normoxic rabbit Purkinje fibres, NO donors, S-nitrosoglutathione and spermine NONOate shorten the action potential duration to the level seen in hypoxic preparations (Baker, 2001). The shortening of action potential duration can be abolished by an NO remover such as carboxy-PTIO (Baker, 2001). Another NO donor, sodium nitroprusside, decreases the duration of repolarization and increases the pacemaker activity of the isolated guinea pig sinus node. However, sodium nitroprusside has no significant effect on the action potential duration of ventricular papillary muscles (Joa et al., 2000).

Prolongation of the ventricular action potential and consequently the Q-T interval could be attributed to Na+ and Ca\(^{2+}\) permeability. NO inhibits Na influx in isolated mouse and guinea pig ventricular myocytes without changing channel kinetics (Ahmmed et al., 2001). This inhibition is due to a decrease in open probability (Po) without changes in single-channel conductance and involves the activation of both protein kinase G (PKG) and protein kinase A (PKA). However, in rat ventricular myocytes, NO donors induce a late Na+ current (INaL) because Na+ channels fail to inactivate completely or close and then reopen at depolarized potentials, that is, during the plateau phase of the AP(Ahern et al., 2000). Cardiac depolarization opens L-type Ca\(^{2+}\) channels (LTCC) generating an ICa that is responsible for the AP plateau and triggers a larger release of Ca\(^{2+}\) through the opening of RyR. The ICa is also responsible for phase 0 depolarization and the slow diastolic depolarization in sinoatrial (SAN) and AVN cells.

NO produces contradictory effects on ICa, increasing, (Wang, 2000) decreasing, (Abi-Gerges et al., 2002) or producing a biphasic effect (Campbell et al., 1996). In human atrial myocytes, the NO donor SIN-1 stimulates ICa, an effect that decreases at concentrations of 1 mM (Stavrou et al. 2001). The increase in ICa is produced via cGMP-inhibited PDE3, which increases intracellular cAMP levels (Kirstein et al., 1995); however, it has also been attributed to a cAMP-independent activation of PKG (Wang, 2000).

In accordance to our data, the prolongation of the Q-T and Q-Tc may be related to the cGMP effect on the L-type Ca\(^{2+}\) channels which was confirmed by Tohse et al. (1995) who studied the effect of the cGMP generated in response to the atrial natriuretic peptide on the rabbit ventricular muscle action potential and reviewed that cGMP inhibits the Ca\(^{2+}\) current through blockade of the L-type Ca\(^{2+}\) channels. These data was also confirmed by another study performed on guinea pig myocytes and evidenced that cGMP regulated the Ca\(^{2+}\) current (Levi et al., 1989).

Conclusion

From these data we can conclude that in spite of its cardioprotective effects; NO generation in response to L-arginine infusion prolongs the Q-T interval and consequently the corrected Q-T. Further studies are needed to investigate the effects of NO generating drugs as Na nitroprisde and hydralazine on the Q-T interval and their impact in patients with cardiac arrhythmia.

Conflict of interest

The authors have no conflict of interest to declare.

ABBREVIATIONS

PAAET, Public Authority for Applied Education and Training; Tdp, Torsades de pointes; ECG, electrocardiograph; APD, action potential duration; AP, action potential; NO, nitric oxide; NOS, nitric oxide synthase; Po, open probability; PKG, protein kinase G; PKA, protein kinase A; LTCC, L-type Ca2+ channels; SAN, sinoatrial; AVN, atrioventricular nodal; ICa, inward current; CGMP, Cyclic guanosine monophosphate, RyRC, bryanodine receptor calcium release channel.

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