The Effects of Nitric Oxide Synthase Inhibition on Epinephrine-Induced Arrhythmia and Myocardial Damage

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Abstract: - We have recently reported that methylene blue (MethyB) was able to inhibit epinephrine-induced arrhythmias and cardiac muscle injury. In this study, we investigated the effect of nitric oxide synthase inhibition by *NG*-nitro-L-arginine methyl ester (L-NAME) on cardiac arrhythmias, and myocardial damage induced by epinephrine in rats. Whether nitric oxide inhibition would affect the antiarrhythmic and cardiac protective actions of MethyB was also examined. L-NAME (40 mg/kg), L-arginine (200 mg/kg) + L-NAME, or MethyB (100 mg/kg) + L-NAME were given intraperitoneally (i.p.). Cardiac arrhythmia was then induced with intravenous (i.v.) injection of 10 μg/kg epinephrine. Results showed that epinephrine injection caused marked bradycardia (221.0 ± 1.37 *vs.* 409.4 ± 3.18 beats/min), shortened QTc interval (0.096 ± 0.0093 *vs.* 0.177 ± 0.0008 s), increased QRS duration (0.040 ± 0.0035 *vs.* 0.0185 ± 0.0002 s), decreased R wave amplitude (0.176 ± 0.0051 *vs.* 0.21 ± 0.0009 mv), ST segment height (-0.026 ± 0.007 *vs.* -0.002 ± 0.0005 mv), and induced ventricular extrasystoles. L-NAME given to untreated control rats resulted in a decrease in heart rate (288.2 ± 0.88 *vs.* 409.4 ± 3.18 beats/min), and increased R wave amplitude (0.436 ± 0.004 *vs.* 0.21 ± 0.0009 mv) compared to controls. L-NAME did not cause extrasystoles in untreated control rats but significantly increased the number of extrasystoles and duration of arrhythmia in the epinephrine-treated group. The administration of L-arginine (200 mg/kg, i.p.) to epinephrine plus L-NAME-treated rats resulted in increased heart rate and markedly decreased the number of extrasystoles and duration of arrhythmia. Methylene blue given at 100 mg/kg to rats treated with epinephrine and L-NAME caused a marked increase in heart rate. It also normalized QRS duration, prevented ST segment depression, markedly suppressed ventricular extrasystoles, and decreased the duration of arrhythmia compared with either epinephrine or L-NAME plus epinephrine-treated groups. Epinephrine injection caused disorganization, and necrosis of cardiac cells, interstitial hemorrhage, and cellular infiltrations. These changes were markedly improved by treatment with either L-NAME or L-NAME/MethyB. These results suggest that (i) inhibiting nitric oxide synthase by L-NAME increases epinephrine-induced arrhythmia which is inhibited by L-arginine or MethyB; (ii) either L-NAME alone or in combination with MethyB prevented cardiac muscle injury induced by epinephrine; (iii) L-NAME did not prevent the cardiac protective and antiarrhythmic actions of MethyB.

Key-Words: - cardiac arrhythmia; epinephrine; L-arginine; L-NAME; antiarrhythmic; cardioprotection; metylene blue; nitric oxide synthase

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1 Introduction

The development of cardiac arrhythmias is common during anesthesia and surgery. The intra-operative use of the vasopressor drug epinephrine to increase cardiac inotropy and maintain blood pressure can cause clinically relevant cardiac arrhythmias, [1], [2]. The administration of this catecholamine also carries the risk of causing direct cardiac muscle toxicity, apoptosis of cardiac myocytes, [3], [4], and focal necrosis of the myocardium, [3], [5]. These effects of catecholamines involve mainly ß-adrenergic receptors, [4], [5], [6], in addition to an α-adrenergic receptor-mediated vascular spasm and myocardial ischemia, [7], and are attributable to the oxidation products of catecholamines such as adrenochrome and other reactive oxygen species, [7], [8].

Nitric oxide plays an important role in cardiac physiology in the regulation of cardiac excitability and contractility and in the control of vascular tone and coronary blood flow, [9]. It is also involved in pathological disease states e.g., heart failure and coronary artery disease, [10]. Nitric oxide is produced from L-arginine by the action of nitric oxide synthases in endothelial cells, cardiac myocytes (endothelial nitric oxide synthase), and nerves (neuronal nitric oxide synthase). A third isoform (inducible nitric oxide synthase) is not constitutively expressed, but is induced by inflammatory signals, [11]. There is evidence that endogenous nitric oxide may have a cardiac protective role in suppressing cardiac arrhythmias caused by ischemia-reperfusion injury, by its ability to maintain synchronous beating and conductivity, coronary vasodilatation, a decrease in oxidative stress, [12], and suppression of sympathetic nerve activity, [9].

The pre- or intra-operative use of the synthetic dye methylene blue (MethyB) is an effective rescue therapy in paraplegic shock, occurring during or after cardiac surgery requiring cardiopulmonary bypass and characterized by decreased systemic vascular resistance, and severe hypotension unresponsive to intravenous fluids and vasopressor drugs, [13], [14]. The vasoplegic syndrome is attributed to a systemic inflammatory response with excessive production of nitric oxide and inflammatory cytokines, [15]. Methylene blue, by virtue of its ability to inhibit nitric oxide synthases, [16] and inhibit soluble guanylate cyclase, thereby, preventing cyclic guanosine 3’5’-monophosphate–dependent vasorelaxant action of nitric oxide, [17], is thought to counteract the effect of the excessively released nitric oxide on vascular smooth muscle cells, and increase the response to vasopressors in vasoplegic shock, [15]. MethyB has been shown to exhibit cardioprotective effects, [18], [19], and to inhibit epinephrine-induced arrhythmias and cardiac muscle injury, [20].

The aims of this study were therefore to: (i) investigate the effects of inhibiting nitric oxide synthases by *NG*-nitro-L-arginine methyl ester (L-NAME) on the epinephrine-induced cardiac arrhythmias and muscle injury; (ii) examine the role of nitric oxide in the antiarrhythmic and cardiac protective actions of MethyB.

2 Materials and Methods

2.1 Animals

Male Sprague-Dawley rats weighing 170-180 g were used in the study. Rats were obtained from the Animal House Colony of the National Research Centre. Animals were kept under temperature- and light-controlled conditions (20–22 °C and a 12-hour light/dark cycle) and given free access to tap water and standard laboratory rodent chow. Animal procedures followed the guidelines of the Institute's ethics committee for the use of animals in experimental studies and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health.

2.2 Drugs and Chemicals

NG-nitro-L-arginine methyl ester (L-NAME), methylene blue (Sigma Chemical Co., St. Louis, MO, U.S.A), and epinephrine (Nile Co., Egypt) were used in the study and freshly dissolved in saline before the experiments to obtain the necessary doses.

2.3 Experimental Groups

Rats were randomly divided into six equal groups (n = 8 per group) and treated as follows:

Group 1 was given intraperitoneal (i.p.) saline (served as a negative control).

Group 2 was given i.p. saline before induction of cardiac arrhythmia by i.v. injection of 10 μg/kg of epinephrine (served as a positive control).

Group 3 was given L-NAME (40 mg/kg, i.p.) only

Group 4 was given L-NAME (40 mg/kg, i.p.), 30 min before the arrhythmia was induced by an i.v. injection of epinephrine.

Group 5 was given L-NAME (40 mg/kg, i.p.) 30 min before administering L-arginine (200 mg/kg), and followed 30 min later by an i.v. injection of epinephrine.

Group 6 was given L-NAME (40 mg/kg, i.p.) 30 min before administering MethyB (100 mg/kg), and followed 30 min later by an i.v. injection of epinephrine.

2.4 Electrocardiography

After 30 minutes of drug or saline administration, rats were anesthetized with an intraperitoneal injection of thiopental sodium (45 mg/kg). The ECG was then recorded with the ECG Powerlab module. The latter consisted of Powerlab/8sp and Animal Bio-Amplifier (Australia), in addition to Lab Chart 7 software with an ECG analyzer. After the establishment of a steady state, arrhythmia was induced by the i.v. injection of 10 μg/kg epinephrine. ECG recording was continued until the termination of the arrhythmia, [21]. The average heart rate, RR interval, PR interval, QRS interval, QTc (corrected QT interval), R wave amplitude, ST height, number of extrasystoles, and duration of arrhythmia after different treatments were determined over 15 minutes.

2.5 Cardiac Histopathology

Cardiac specimens were immediately fixed in 10% formalin at room temperature, treated with a conventional grade of alcohol and xylol, embedded in paraffin, and sectioned at 5 μm thicknesses. The sections were stained with haematoxylin and eosin (H&E) to study the histopathological changes using a light microscope (Olympus CX 41 with DP12 Olympus digital camera).

2.6 Statistical Analysis

Data are presented as mean ± SE for measurement variables over 15 minutes. Comparison between groups was performed with a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used, and differences were considered statistically significant when probability values were less than 0.05.

3 Results

3.1 Electrocardiographic Recordings

A representative electrocardiographic (ECG) trace of the saline control is shown in Figure 1. ECG recordings in the L-NAME-only group showing ST segment depression are presented in Figure 2. ECG recordings in the epinephrine control group showed the presence of bradycardia and ventricular extrasystoles (Figure 3). The group treated with L-NAME plus epinephrine showed ventricular extrasystoles and ventricular tachycardia (Figure 4 and Figure 5). ECG changes in the L-NAME plus epinephrine-treated group were prevented by prior treatment with MethyB (Figure 6).

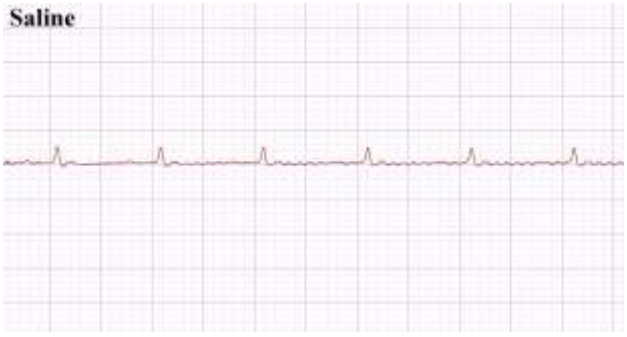

Fig. 1: Representative ECG tracing in the saline control group.

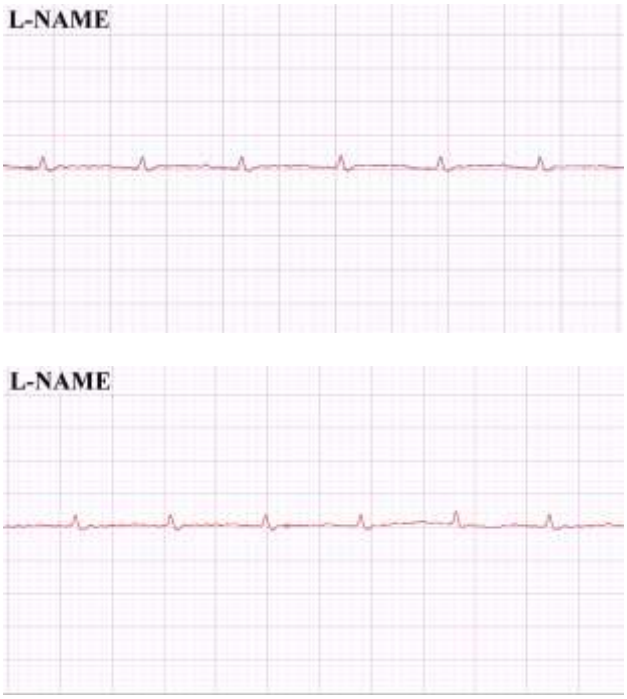

Fig. 2: Representative ECG changes in L-NAME-only-treated group.

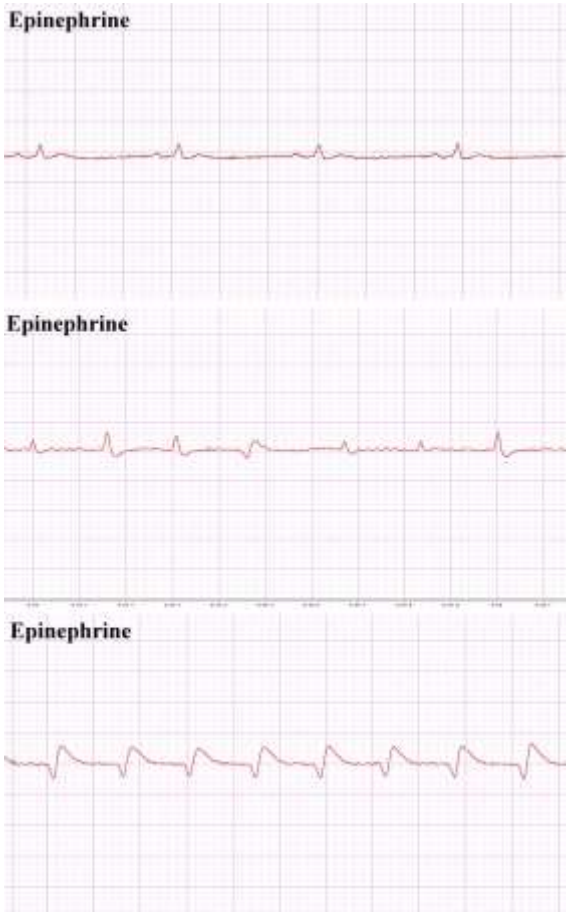

Fig. 3: Representative ECG tracings of the changes induced by intravenous epinephrine injection. Bradycardia, ventricular premature beats, and ventricular tachycardia.

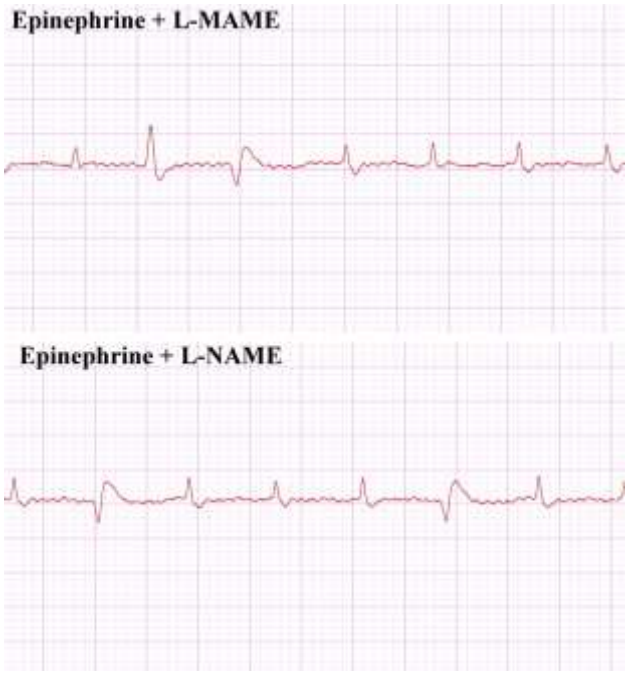

Fig. 4: Representative ECG tracings of the changes in the epinephrine and L-NAME-treated group. Ventricular premature beats.

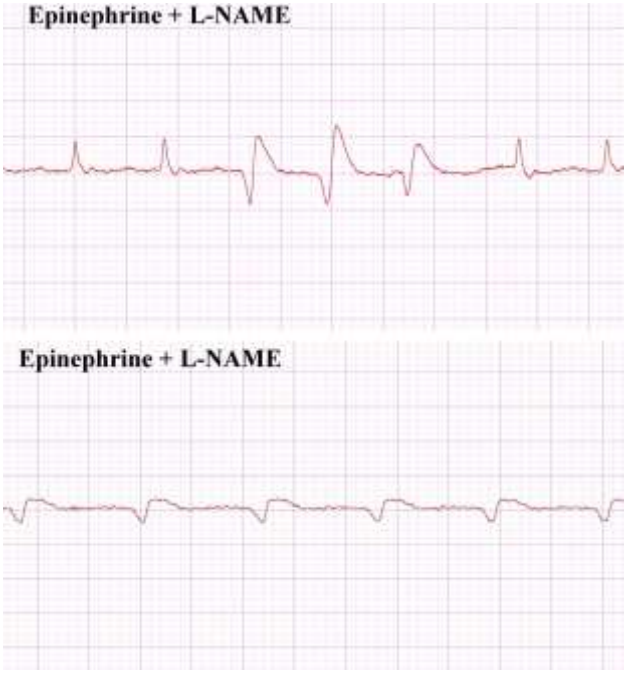

Fig. 5: Representative ECG tracings of the changes in the epinephrine and L-NAME-treated group Ventricular premature beats and ventricular tachycardia.

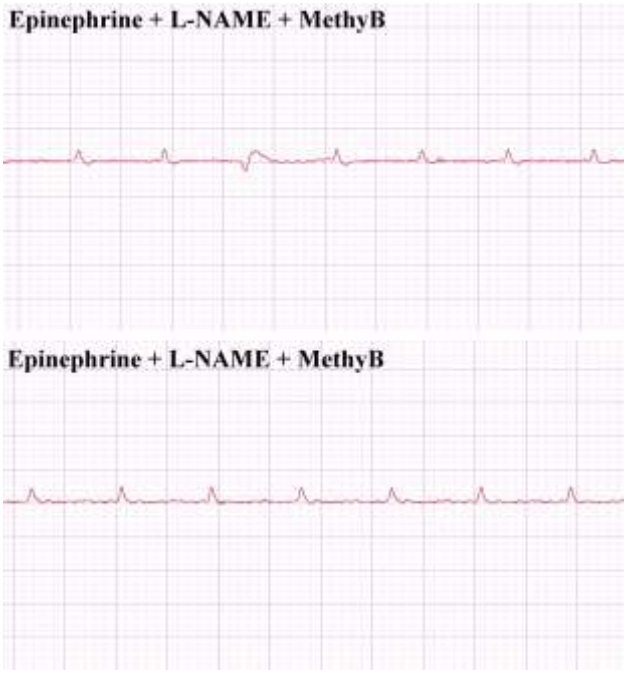

Fig. 6: Representative ECG tracings of the epinephrine, L-NAME, and MethyB-treated group.

3.2 Electrocardiographic Parameters

3.2.1 Effects of Epinephrine

The heart rate in the saline control group was 409.4 ± 3.18 beats/min. The i.v. administration of epinephrine caused marked bradycardia (221.0 ± 1.37 *vs.* 409.4 ± 3.18 beats/min), increased PR

interval (0.057 ± 0.001 *vs.* 0.043 ± 0.0002 s), RR interval (0.548 ± 0.016 *vs.* 0.148 ± 0.001 s), shortened QTc interval (0.096 ± 0.0093 *vs.* 0.177 ± 0.0008 s), increased QRS duration (0.040 ±0.0035 *vs.* 0.0185 ± 0.0002 s), decreased R wave amplitude (0.176 ± 0.0051 *vs.* 0.21 ± 0.0009 mv), ST segment height (-0.075 ± 0.007 *vs.* 0.002 ± 0.0005 mv), and induced ventricular extrasystoles (Table 1, Figure 7 and Figure 8).

3.2.2 Effects of L-NAME

L-NAME given to untreated control rats resulted in sinus bradycardia (288.2 ± 0.88 *vs.* 409.4 ± 3.18 beats/min), increased RR interval (0.20 ± 0.001 *vs.* 0.148 ± 0.001 s), increased PR interval (0.057 ± 0.001 *vs.* 0.043 ± 0.0002 s), increased QRS duration (0.030± 0.0009 *vs.* 0.0185 ± 0.0002 s), shortened Qtc interval (0.092 ± 0.0024 *vs.* 0.177 ± 0.0008 s) and increased R wave amplitude (0.436 ± 0.004 *vs.* 0.21 ± 0.0009 mv) and decreased ST segment height (-0.075 ± 0.0009 *vs.* 0.0023 ± 0.0005 mv) (Table 1, Figure 7 and Figure 8).

3.2.3 Effects of Epinephrine and L-NAME

In epinephrine-treated rats, L-NAME decreased heart rate (155.9 ± 1.42 *vs.* 221.0 ± 1.37 beats/min), increased R wave amplitude (0.36 ± 0.0018 ± 0.0018 *vs.* 0.176 ± 0.0051 mv) and decreased ST segment height (-0.054 ± 0.0011 *vs.* -0.026 ± 0.007 mv) compared to epinephrine control. On the other hand, the number of extrasystoles and duration of arrhythmia induced by epinephrine injection was significantly increased by administering L-NAME (Table 1, Figure 7, and Figure 8).

3.2.4 Effects of epinephrine, L-NAME and L-arginine

The administration of L-arginine to epinephrine and L-NAME-treated rats resulted in increased heart rate (307.3 ± 2.10 *vs.* 155.9 ± 1.42 beats/min), increased R wave amplitude (0.480 ± 0.0015 *vs.* 0.36 ± 0.0018 mv) and markedly decreased the number of extrasystoles and duration of arrhythmia compared to epinephrine + L-NAME group (Table 1, Figure 7 and Figure 8).

3.2.5 Effects of epinephrine, L-NAME, and MethyB

The ECG changes induced by epinephrine and L-NAME in heart rate, QRS duration, and ST segment height were markedly ameliorated by prior treatment with MethyB which also caused marked inhibition of extrasystoles and markedly shortened the duration of arrhythmia compared to rats treated with epinephrine or L-NAME + epinephrine (Table 1, Figure 7 and Figure 8).

Table 1. Effect of L-NAME or L-NAME + MethyB on epinephrine-induced electrocardiogram changes and arrhythmia.

Parameter/ Group	Normal control	L-NAME	Epinephrine	Epinephrine + L-NAME	Epinephrine + L-NAME + L-arginine	Epinephrine + L-NAME+ MethyB
Heart rate/min	409.4 ± 3.18	288.2 ± 0.88^{*+}	221.0 ± 1.37*	155.9 ± 1.42^{*+}	307.3 ± 2.10$^{*+\#}$	320.3 ± 1.72$^{*+\#}$
RR interval (s)	0.148 ± 0.001	0.20 ± 0.001^{*+}	0.549 ± 0.016*	0.446 ± 0.01^{*+}	0.198 ± 0.004$^{*+\#}$	0.178 ± 0.003$^{*+\#}$
PR interval (s)	0.043 ± 0.0002	0.057± 0.0018*	0.057 ± 0.001*	0.056 ± 0.0001*	0.054 ± 0.007*	0.052 ±0.0011*
QTc interval (s)	0.177 ± 0.0008	0.092 ± 0.0024*	0.096 ± 0.0093*	0.102 ± 0.009*	0.106 ± 0.003*	0.115± 0.010^{*+}
QRS duration (s)	0.0185 ± 0.0002	0.030± 0.0009^{*+}	0.040 ±0.0035*	0.031± 0.001^{*+}	0.024 ±0.002$^{*+\#}$	0.017± 0.002$^{*+\#}$
R wave amplitude (mv)	0.21 ± 0.0009	0.436 ± 0.004^{*+}	0.176 ± 0.0051$^{*\#}$	0.36 ± 0.0018^{*+}	0.480 ± 0.0015$^{*+\#}$	0.32 ± 0.0063$^{*+\#}$
ST segment height (mv)	-0.0023 ± 0.0005	-0.075 ± 0.0009^{+}	-0.026 ± 0.007	-0.054 ± 0.0011^{*+}	-0.035 ± 0.0051$^{*\#}$	-0.003 ± 0.0012$^{+\#}$
Duration of arrhythmia (s)	0.0 ± 0.0	0.0 ± 0.0	831.7 ± 16.98*	3191 ± 75.46^{*+}	249 ± 10.3$^{*+\#}$	100.1 ± 12.39$^{*+\#}$
Number of extrasystoles/15 min	0.0 ± 0.0	0.0 ± 0.0	995.8 ± 19.22*	1101 ± 40.14^{*+}	252.5 ± 8.1$^{*+\#}$	130.1 ± 10.01$^{*+\#}$

*MethyB: methylene blue. Data were expressed as mean ± SE (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *: p<0.05: significantly different from the normal control group. +: p<0.05: significantly different from the epinephrine control group. #: p<0.05: significantly different from the epinephrine + L-NAME group.*

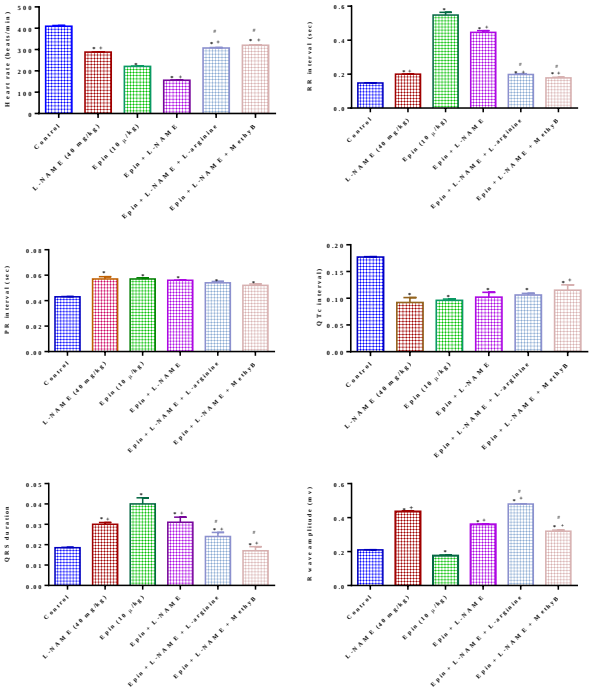

Fig. 7: Effects of treatment with L-NAME, L-NAME + L-arginine, or L-NAME plus methylene blue (MethyB) on the epinephrine-induced changes in heart rate, RR interval, PR interval, QTc, QRS duration, and R wave amplitude. *: p<0.05: significantly different from the normal control group. +: p<0.05: significantly different from the epinephrine control group. #: p<0.05: significantly different from the epinephrine + L-NAME group.

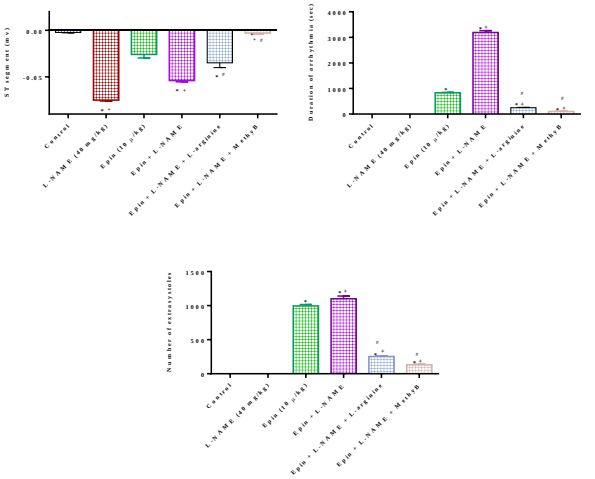

Fig. 8: Effects of treatment with L-NAME, L-NAME plus L-arginine or L-NAME and methylene blue (MethyB) on the epinephrine-induced changes in ST wave height, duration of arrhythmia, and number of ventricular extrasystoles. *: p<0.05: significantly different from the normal control group. +: p<0.05: significantly different from the epinephrine control group. #: p<0.05: significantly different from the epinephrine + L-NAME group.

3.2 Cardiac Histopathology

Sections of the saline control group showed normal myocardial architecture. The myofibers were intact, branching, and cylindrical with acidophilic cytoplasm and exhibited vesicular nuclei, transverse striations, and obvious intercalated discs. They were separated by scanty connective tissue containing fibroblasts that were identified by their flat nuclei (Figure 9A). Cardiac tissues of rats treated with epinephrine showed many histopathological changes, especially in the cardiac cells. There were disorganized cardiac cells, necrotic cardiac cells with focal areas of degeneration, widening of the intercellular spaces, and deeply stained (pyknotic) nuclei. Congestion and dilatation of blood vessels with interstitial haemorrhage were seen. Marked cellular infiltrations were common in many sections (Figure 9B).

The group treated with epinephrine and L-NAME showed nearly normal myocardial architecture, with mild interstitial haemorrhage and congestion of blood vessels. Most myofibers were intact, while others had mild widening of the intercellular spaces and few pyknotic nuclei (Figure 9C). Meanwhile, rats treated with epinephrine and L-NAME with MethyB showed almost normal myocardium and minimal interstitial haemorrhage. Most myofibers were intact, while others had mild widening of the intercellular spaces and few pyknotic nuclei (Figure 9D).

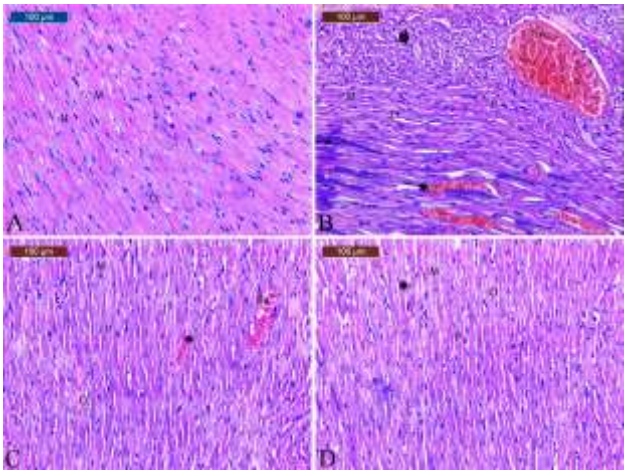

Fig. 9: Representative photomicrographs of Hx & E stained heart tissue sections. **(A)** Saline control shows the normal histological architecture of cardiac myocytes (M). Most appear longitudinally with rounded vesicular centrally located nuclei (N). In between the cardiac myocytes, there was a delicate layer of connective tissue. (**B**) Epinephrine

showing disorganized cardiac cells, necrotic cardiac cells with focal areas of degeneration (arrowhead), widening of the intercellular spaces (Ct) and deeply stained (pyknotic) nuclei (P), congestion and dilatation of blood vessels (Bv) with interstitial haemorrhage (star) and marked cellular infiltrations (arrow). (**C)** Epinephrine and L-NAME showing nearly normal myocardial architecture, with mild interstitial haemorrhage (star) congestion blood vessels (Bv). Most myofibers were intact while others had mild widening of the intercellular spaces (Ct) and few pyknotic nuclei (P). (**D)** Epinephrine + L-NAME + MethyB showing almost normal myocardium and minimal interstitial haemorrhage (star). Most myofibers were intact while others had mild widening of the intercellular spaces (Ct) and few pyknotic nuclei (P).

4 Discussion

In this study, we investigated whether nitric oxide synthase blockade with L-NAME has a modulating effect on the development of arrhythmias and cardiac muscle injury after epinephrine infusion in rats. Because MethyB, an inhibitor of nitric oxide synthase exerts anti-arrhythmic and cardiac protective effects, we also investigated whether these actions of MethyB could be affected by NOS inhibition with L-NAME. The results of this study showed that L-NAME itself induced ECG changes in the form of sinus bradycardia, shortened Qtc interval, and an increase in QRS duration and R wave amplitude. On the other hand, in epinephrine-treated rats, L-NAME caused an increase in the bradycardiac response but counteracted the increase in QRS duration and R wave amplitude induced by epinephrine. However, L-NAME increased the number of ventricular extrasystles and the duration of the arrhythmia induced by epinephrine. L-arginine, the precursor of nitric oxide was found to counteract the bradycardiac response and suppress the number of ventricular extrasystles and duration of arrhythmia compared with either epinephrine alone or epinephrine plus L-NAME. These findings may suggest that endogenous nitric oxide is endued with an anti-arrhythmic function. Other researchers reported an increase in blood pressure and a bradycardiac response to i.v. L-NAME (7.5 mg/kg) in intact anesthetized rats, [22]. Our results are also supported by the study of Pabla and Curtis, [23], in the rat-isolated heart where L-NAME caused significant bradycardia. In their study, L-NAME exacerbated ventricular fibrillation in hearts subjected to 60 minutes of ischaemia. Because these L-NAME effects were counteracted by L-arginine, the substrate for nitric oxide synthase, they were attributed to a decrease in nitric oxide bioavailability.

Nitric oxide produced from the cardiac endothelial cells, cardiac myocytes, and nerves participates in the regulation of cardiac excitability and contractility, [9]. Studies have suggested that the provision of nitric oxide protection, whereas inhibition of nitric oxide synthesis increased ischemia-reperfusion injury, [12]. Moreover, a study by [21], has suggested that the release of nitric oxide and prostaglandins may account for the protection against epinephrine-induced arrhythmia by bradykinin. Several mechanisms have been postulated to account for the anti-arrhythmogenic action of nitric oxide, including effects on ion channels and gap junctions, increase in intracellular cyclic GMP *via* guanylyl cyclase, and a decrease in oxidative stress-mediated arrhythmogenesis, [12]. Moreover, the basal tone of nitric oxide was found to suppress the stimulatory action of sympathetic nerve activity in the heart, [9]. Accordingly, blockade of nitric oxide synthesis by L-NAME would be expected to enhance epinephrine-mediated stimulation of the myocardium. Nitric oxide is also important in the maintenance of vascular tone and thus coronary blood flow, [11], and it is possible that blockade of nitric oxide synthesis by L-NAME with a consequent decrease in cardiac muscle perfusion accounted for the exacerbation of ventricular arrhythmias following epinephrine infusion in the present study.

Excessive amounts of epinephrine can cause direct cardiac toxicity, band necrosis, and stimulation of myocyte apoptosis, [3], [4], [5]. Myocardial cellular damage caused by epinephrine is due to the direct stimulation of adrenergic beta 1 receptors in cardiac myocytes, which involves increases in cAMP and Ca^{2+} influx, [4], [6]. In addition, there is the effect of stimulation of α-adrenergic receptors in coronary arteries, which results in a decrease in coronary perfusion, and thus myocardial ischemia, [7]. There is also evidence for the involvement of adrenochrome, an oxidation metabolite of epinephrine, [8], and reactive oxygen metabolites, [7], in causing myocardial cell damage. In the present study, we found that inhibition of nitric oxide synthesis with L-NAME conferred protection against the severe cardiac muscle injury caused by epinephrine. The mechanism underlying this effect of L-NAME is not clear but may be related to decreased formation of oxyradicals such as peroxynitrite and other reactive species that are generated during myocardial ischemia and/or oxidation of catecholamines, [7], [8]. In support of

this notion are studies that have reported an antiarrhythmic effect for antioxidants in epinephrine-induced arrhythmia in rats, which could be caused by the antioxidant's ability to decrease the circulating levels of aminochromes produced by the oxidative metabolism of the catecholamine, [24], [25], [26].

MethyB protects against epinephrine-induced arrhythmia and cardiac muscle damage, [20]. Because MethyB inhibits nitric oxide synthesis, [16], [17], we thought that the cardiac protective effects of MethyB would be affected by decreasing nitric oxide bioavailability using L-NAME. Interestingly, we found that after inhibiting nitrite oxide synthases by L-NAME, MethyB was capable of suppressing ventricular extrasystoles and almost restoring the normal cardiac rhythm. Moreover, it afforded almost complete protection of the myocardium in rats treated with epinephrine or L-NAME plus epinephrine. These findings may suggest that mechanisms other than lowering nitric oxide levels underlie the beneficial effects of the dye in the epinephrine model of arrhythmia and cardiac damage. It is also suggested that while cardiac nitric oxide dyshomeostasis after L-NAME is involved in the exacerbation of arrhythmia, the development of myocardial injury after epinephrine infusion is likely to be largely mediated by other pathways, including the adrenergic stimulation of the myocardium ($\beta 1$), coronary vasospasm and decreased myocardial perfusion ($\alpha 1$) and the release of the oxidation products of epinephrine. Hence, blocking nitric oxide synthesis with L-NAME did not affect the cardioprotective action of MethyB. MethyB may exert its cardioprotective action *via* an antioxidant mechanism. In biological systems, MethyB cycles between its oxidized and reduced forms. It is reduced by the enzyme NADPH or thioredoxin to the colorless leucoMethyB, to be re-oxidized by reacting with O_2, [27]. The redox cycling property of MethyB may help block the production of reactive oxygen metabolites by the mitochondria, [28]. The antioxidant action of MethyB has been shown both *in vitro*, [29], and *in vivo*, [30], [31]. MethyB has been shown to inhibit the production of superoxide radicals ($O_2^{-\bullet}$) by xanthine oxidase, and thus prevent free radical-mediated tissue injury, [29]. The dye was also shown to improve mitochondrial respiratory function in cardiac mitochondria, [18], restore the depleted energy stores in cardiomyocytes following hydrogen sulfide toxicity, [19], and preserve intracellular Ca^{++} homeostasis and excitation-contraction coupling in mouse myocytes treated with sodium cyanide, [32].

5 Conclusion

The present results indicate that pretreatment with the nitric oxide synthase inhibitor L-NAME caused a significant increase in epinephrine arrhythmias, which could be ameliorated with L-arginine or MethyB. L-NAME, however, like MethyB, afforded histological protection against the deleterious cardiac muscle damage evoked by the catecholamine. Our results also indicate that L-NAME did not inhibit the cardiac protective and antiarrhythmic actions of MethyB. This finding is of particular clinical relevance as it suggests that these cardiac beneficial effects of MethyB may not be mediated by inhibition of nitric oxide release. Further research is, therefore, required to delineate the mechanism (s) underlying the cardiac protective properties of MethyB.

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Omar Abdel-Salam, Marawan Abd El Baset, and Amany Sleem designed the study. Marwan Abd El Baset conducted the experiments. Enayat Omara performed the histological studies and their interpretation. Omar Abdel-Salam prepared the manuscript. Omar Abdel-Salam, Marawan Abd El Baset, Amany Sleem, and Enayat Omara approved the final version of the manuscript.

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Conflict of Interest

The authors have no conflict of interest to declare.