Novel Antiarrhythmic and Cardioprotective Effects of Brilliant Blue G

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Abstract: - In this study, we investigated the effects of the purinergic P2X7 receptor antagonist brilliant blue G (BBG) on cardiac arrhythmia and myocardial injury induced by intravenously (i.v.) administered epinephrine in anesthetized rats. We also examined the possible involvement of beta-adrenergic receptors or cholinergic mechanisms in the effects of BBG. Sprague-Dawley rats were treated with epinephrine (10 μg/kg, i.v.). Brilliant blue G (100 mg/kg) was intraperitoneally (i.p.) administered thirty minutes prior to i.v. epinephrine. The effects of pretreatment with propranolol (2 mg/kg, i.p.) or atropine (2 mg/kg, i.v.) given prior to BBG and epinephrine were examined. The control group received saline. Moreover, the effects of only BBG on electrocardiogram (ECG) parameters were investigated. Results showed that compared with the saline control, BBG caused significant bradycardia (from 405.8 ± 1.18 to 239.4 ± 6.69 beats/min), increased RR interval (from 0.149 ± 0.002 to 0.254± 0.007 sec) and PR interval (from 0.051 ± 0.0008 to 0.059 ± 0.0004 sec), increased R wave amplitude (from 0.238 ± 0.019 to 0.548 ± 0.009 mv), and shortened QTc interval (from 0.169 ± 0.006 to 0.141 ± 0.003 sec) over 15 minutes after of BBG administration. BBG did not cause cardiac arrhythmia. Meanwhile, epinephrine produced significant bradycardia (209.8 ± 28.78 *vs.* 405.8 ± 1.18 beats/min), increased PR interval, prolonged the QRS complex, shortened QTc interval, decreased R wave amplitude and induced ventricular tachycardia. Brilliant blue G given prior to epinephrine increased heart rate and completely suppressed the epinephrine-induced ventricular arrhythmia. The inhibitory effect of BBG on the arrhythmia caused by epinephrine was prevented by atropine. In contrast the epinephrine induced arrhythmia was completely suppressed with propranolol and BBG. The histopathological study showed that epinephrine caused necrosis and apoptosis of cardiac muscle cells, degeneration of cardiac muscle fibers, and interstitial haemorrhages. These changes were markedly prevented by BBG alone, propranolol/BBG and to a less extent by atropine/BBG pretreatment. The study provided the first evidence for a cardioprotective and anti-arrhythmogenic actions for BBG against epinephrine-induced arrhythmia and myocardial damage, and suggested that cholinergic mechanisms are involved in its anti-arrhythmogenic action.

Key-Words: - cardiac arrhythmia; epinephrine; brilliant blue G; epinephrine; myocardial injury; beta adrenergic receptors; atropine; cardioprotection

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1 Introduction

The vasopressor agent epinephrine is a mainstay in the treatment of life threatening conditions characterized by circulatory collapse such as cardiogenic shock, septic shock, and anaphylaxis [1], [2], [3]. Epinephrine has potent stimulatory action on β1- and β2- adrenoceptors in cardiac tissue with powerful inotropic and chronotropic effects on the heart, increasing myocardial contractility and heart rate, while its action on α1 adrenoceptors on arterial smooth muscle cells causes vasoconstriction [1]. The intravenous use of epinephrine also has the risk of provoking serious ventricular arrhythmia [4], [5]. A high dose of epinephrine can cause direct cardiac muscle damage, focal areas of necrosis]6], [7], and apoptosis of cardiomyocytes [6], [8], attributable to coronary vasoconstriction and to the oxidation products of catecholamines aminochromes and reactive oxygen species, leading to the formation of quinoproteins and depletion of intracellular reduced glutathione levels [9], [10], [11], [12]. Intravenously administered epinephrine is thus widely used as a model of ventricular arrhythmia for studying the pathogenetic mechanisms involved, and testing potential anti-arrhythmic drugs [13], [14].

Purinergic P2X7 receptors are members of the P2X family of ionotropic ATP-gated receptors, activated primarily by extracellular adenosine 5′-triphosphate (ATP). P2X7 receptors are expressed in immune cells such as macrophages, microglia, neurons, cardiac smooth muscle cells, epithelial cells, and endothelial cells. When stimulated by high concentrations of extracellular ATP, the P2X7 receptor acts as a nonselective cation channel, leading to K^+ efflux and the intracellular influx of Na^+ and Ca^{2+}. Excessive stimulation of P2X7 receptors can also open large transmembrane pores permeable to large molecular weight molecules and ions. The result is the activation of a number of downstream signaling events, including activation of inflammasome, release of proinflammatory cytokines such as interleukin-1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), increased generation of oxygen free radicals, and ultimately cell death [15], [16]. There is accumulating evidence for the involvement of purinergic P2X7 receptors in several cardiovascular disorders such as atherosclerosis, arrhythmia post-myocardial infarction, and cardiac fibrosis [17], [18], [19], [20]. Antagonists of P2X7 receptor are thus an intriguing approach to elucidate the role of these receptors in cardiovascular function under physiologic and pathophysiologic conditions.

Brilliant blue G (BBG), also known as Coomassie brilliant blue, is used to stain proteins in biomedical applications [21] and as an ophthalmic solution in vitreoretinal surgery [22]. The dye is a noncompetitive antagonist of P2X7 receptors with nanomolar affinity [23]. Blockade of P2X7 receptors with BBG has been shown to attenuate systemic inflammation [24], [25]. Whether P2X7 antagonism by BBG would be protective against catecholamine-induced cardiac arrhythmia is not known.

The aims of this study were therefore to: (i) investigate the effects of P2X7 receptor antagonist BBG in the epinephrine model of cardiac arrhythmia and myocardial injury; (ii) examine the role of beta-adrenoreceptors or cholinergic mechanisms in the effects of BBG.

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 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 (Publication No. 85-23, revised 1996).

2.2 Drugs and Chemicals

Brilliant blue G (Sigma Chemical Co., St. Louis, MO, U.S.A), epinephrine (Nile Co., Egypt), and propranolol (AstraZeneca-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 equal treatment groups (6 rats per group). The following groups were studied:

Group 1 received i.p. saline and served as normal control.

Group 2 received i.p. saline thirty minutes before i.v. epinephrine (10 μg/kg), and served as epinephrine control.

Group 3 received i.p. brilliant blue G (100 mg/kg).

Group 4 received i.p. brilliant blue G (100 mg/kg) thirty minutes before i.v. epinephrine injection.

Group 5 received i.v. atropine (2 mg/kg) followed thirty minutes later by i.p. brilliant blue G (100 mg/kg), and thirty minutes thereafter by i.v. epinephrine.

Group 6 received i.p. propranolol (2 mg/kg) followed thirty min later by i.p. brilliant blue G (100 mg/kg), and thirty minutes thereafter by i.v. epinephrine.

2.4 Electrocardiography

After 30 minutes of drug or saline administration, rats were anesthetized with 10% chloral hydrate (300 mg/kg, i.p.). 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 was recorded thereafter for 15 min in each group. The average heart rate, RR interval, PR interval, QRS interval, QTc interval (corrected QT interval), R wave amplitude, ST segment height, number of ventricular premature beats, ventricular arrhythmia, and duration of ventricular arrhythmia after different treatments were determined over a period of 15 minutes [26], [27]. The mean value of three successive 5 min of ECG recordings for each group obtained in the first 15 min after saline, BBG or in the first 15 min after the i.v. injection of epinephrine was used for the calculations. Arrhythmia was assessed by counting the number of premature ventricular beats, missed beats, and runs of ventricular tachycardia during the first 15 min after different agents or after i.v. epinephrine. Arrhythmias were defined according to the Lambeth conventions [28]. Ventricular extrasystole is defined as a single premature ventricular complex, and ventricular tachycardia is defined as 4 or more consecutive ventricular premature beats.

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) in order to study the histopathological changes using a light microscope (Olympus CX 41 with DP12 Olympous digital camera).

2.6 Statistical analysis

Data are presented as mean ± SE for measurement variables over a period of 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 Changes in electrocardiographic parameters

The representative ECG changes in the different treated groups are shown in Figs. 1-7. Results of the ECG parameters are presented in tables 1 & 2 plus Figs. 8 & 9.

3.1.1 Effects of brilliant blue G

Compared with the saline control, BBG administration resulted in significant bradycardia, increased RR and PR intervals, increased R wave amplitude, shortened QTc interval but did not cause arrhythmia. Heart rate decreased significantly by 41% (from 405.8 ± 1.18 to 239.4 ± 6.69 beats/min) over 15 min after BBG administration. This reduction in heart rate by BBG was consistent during the successive 5 min of recordings that followed its i.p. administration. Values were 218.5 ± 6.72, 227.2 ± 8.36, 272.4 ± 2.70 compared with the corresponding saline control values of 410.1 ± 2.95, 404.0 ± 0.18, 403.4 ± 0.05 beats/min, respectively.

Meanwhile, the RR interval significantly increased by 69.4% from 0.149 ± 0.002 to 0.254 ± 0.007 sec, and the PR interval increased by 16.6% from 0.051 ± 0.0008 to 0.059 ± 0.0004 sec). The QRS complex was not changed compared with the saline control but the R wave amplitude increased by 130.2% from 0.238 ± 0.019 to 0.548 ± 0.009 mv. The QTc interval fell from 0.169 ± 0.006 to 0.141 ± 0.003 sec over 15 min of ECG recording. BBG significantly increased the ST amplitude relative to the saline control (Table 1, Figs. 8 & 9). The representative

ECG tracings of the saline and BBG only groups are shown in Figs. 1 & 2.

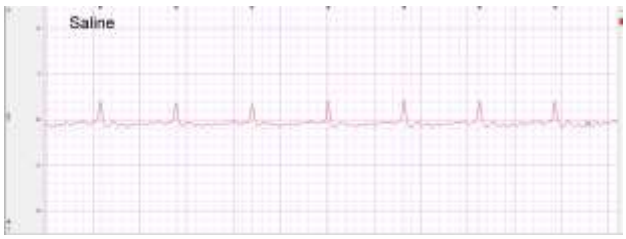

Fig. 1 Representative ECG tracing in saline control group.

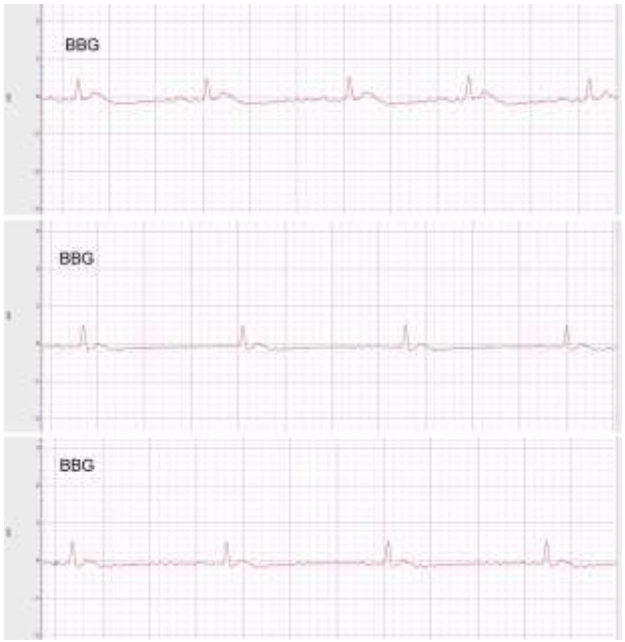

Fig. 2 Representative ECG tracings in brilliant blue G only group showing sinus bradycardia. These are ECG recordings from 5, 10 and 15 min time periods after i.p. BBG.

3.1.2 Effects of epinephrine

Compared with the saline control, i.v. epinephrine caused significant bradycardia, increased RR and PR intervals, decreased R wave amplitude, increased QRS duration, and shortened QTc interval. The heart rate significantly decreased by 88.1% from 410.1 ± 2.95 to 48.69 ± 1.78 beats/min in the first 5 minutes that followed epinephrine injection. It decreased by 48.3% from 405.8 ± 1.18 to 209.8 ± 28.78 beats/min in the 15 minutes after i.v. epinephrine injection.

The RR and PR intervals significantly increased from 0.149 ± 0.002 to 0.554 ± 0.115 sec and from 0.051 ± 0.0008 to 0.056 ± 0.002 sec. In contrast, QTc interval significantly decreased from 0.169 ± 0.006 to 0.099 ± 0.015 sec over 15 min of epinephrine injection. Epinephrine significantly decreased R wave amplitude from 0.238 ± 0.019 to 0.183 ± 0.013 mv, and increased QRS duration from 0.0187 ± 0.0007 to 0.0418 ± 0.005 sec. Epinephrine injection resulted in significantly depressed ST segment compared to the saline control (Table 1, Figs. 8 & 9). Multiple premature ventricular contractions and runs of ventricular tachycardia occurred after epinephrine administration (Table 2, Figs. 3).

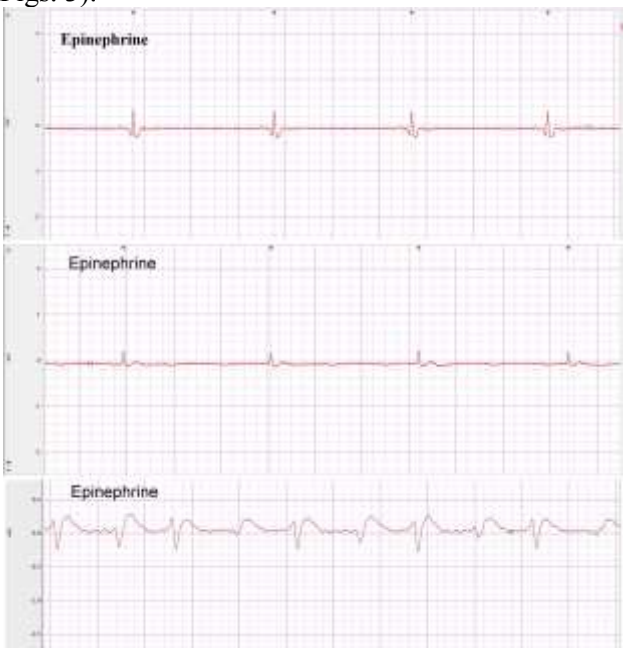

Fig. 3 Representative ECG tracings of the changes induced by intravenous epinephrine (10 μg/kg) showing bardycardia (upper two tracings) and polymorphic ventricular tachycardia (lowest tracing). These are ECG recordings from 5, 10 and 15 min time periods after epinephrine injection.

3.1.3 Effects of brilliant blue G and epinephrine

Brilliant blue G given prior to i.v. epinephrine resulted in increased heart rate, decreased RR interval, prolonged PR interval, increased R wave amplitude and normalized QRS duration and QTc interval compared to epinephrine-treated control rats. The reflex bradycardic effect observed early after i.v. epinephrine was largely prevented by BBG. The heart rate significantly increased from 48.69 ± 1.78 to 196.4 ± 10.54 beats/min in the first 5 minutes and from 209.8 ± 28.78 to 267.4 ± 12.75 beats/min in the 15 minutes that followed epinephrine injection. The RR interval significantly decreased from 0.554± 0.115 to 0.236± 0.014 sec. The PR interval did not change significantly (0.061 ± 0.0004 *vs*. 0.056 ± 0.002 sec). Brilliant blue G in addition, increased R wave amplitude from 0.183 ±

0.013 to 0.612 ± 0.012 mv and caused significantly raised ST segment compared to epinephrine control group (Table 1, Figs. 8 & 9). Brilliant blue G completely inhibited the development of epinephrine-induced arrhythmia (Table 2, Fig. 4).

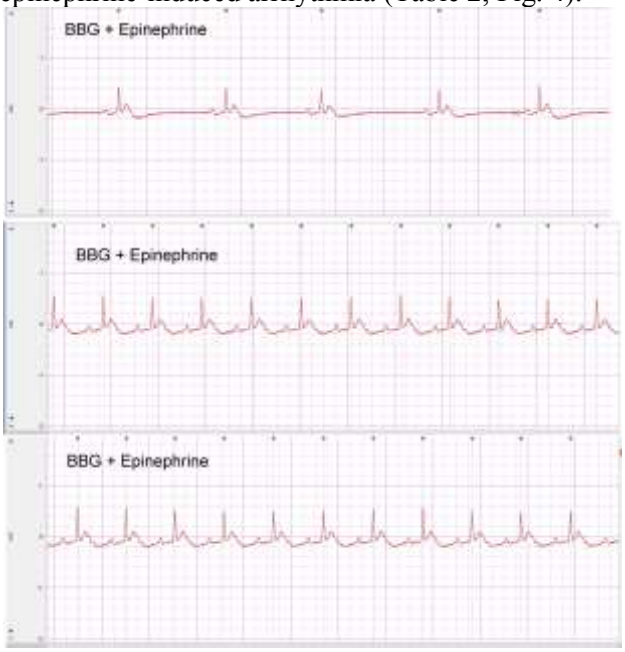

Fig. 4 Representative ECG tracings of the changes induced by i.p. BBG administration prior to i.v. epinephrine (10 μg/kg). These are ECG recordings from 5, 10 and 15 min time periods after epinephrine injection.

3.1.4 Effects of atropine, BBG and epinephrine

No significant difference in heart rate was observed in the 15 minutes that followed epinephrine injection between rats that received atropine/BBG/epinephrine and the BBG/epinephrine group. Values were 275.0 ± 16.18 and 267.4 ± 12.76 beats/min, respectively.

The PR interval normalized, but the QRS duration increased from 0.0181 ± 0.0006 to 0.0251 ± 0.001 sec, the QTc interval decreased from 0.152 ± 0.006 to 0.093 ± 0.008 sec, the R wave decreased in amplitude from 0.612 ± 0.012 to 0.288 ± 0.076 mv, and the ST segment was significantly depressed compared with the BBG/epinephrine group (Table 1, Figs. 8 & 9). The inhibitory effect of BBG on arrhythmia caused by epinephrine was almost prevented by atropine. The duration of arrhythmia increased in atropine/BBG/epinephrine group with respect to the BBG/epinephrine group (Table 2). The ECG showed marked bradycardia, ventricular premature beats, and sinus arrest (Fig. 5 & 6).

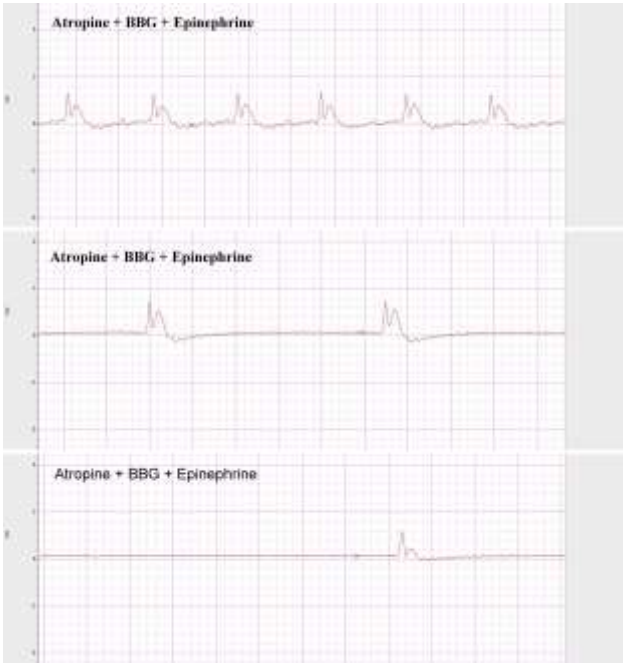

Fig. 5 Representative ECG tracings of the changes induced by i.p. atropine and BBG given prior to i.v. epinephrine (10 μg/kg) showing bradycardia and ST segment elevation (upper and middle tracings), and sinus pause (lowest tracing). These are ECG recordings from 5, 10 and 15 min time periods after epinephrine injection.

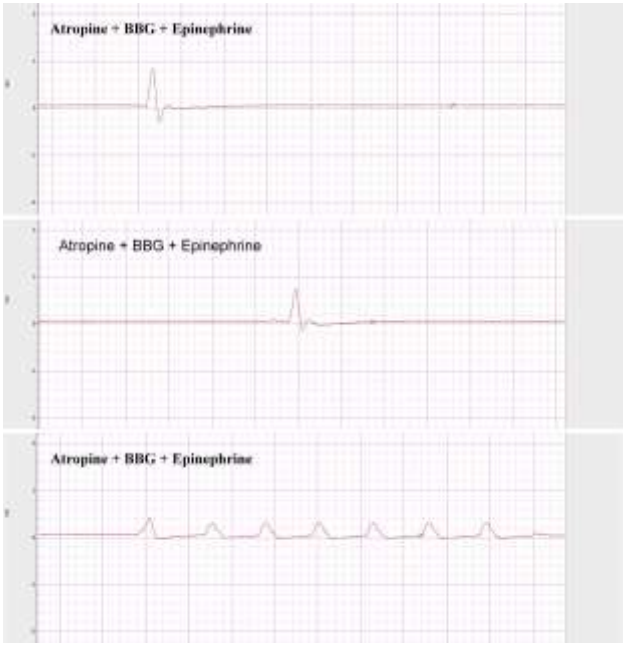

Fig. 6 Representative ECG tracings of the changes induced by i.p. atropine and BBG given prior to i.v. epinephrine (10 μg/kg) showing ventricular premature beat and sinus pause (upper and middle tracings), monomorphic ventricular tachycardia (lower tracing). These are ECG recordings from 5,

10 and 15 min time periods after epinephrine injection.

3.1.5 Effects of propranolol, BBG and epinephrine

Compared with the BBG/epinephrine group, rats receiving propranolol/BBG prior to i.v. epinephrine showed significantly increased heart rate by 30.3% in the 5 minutes that followed i.v. epinephrine administration (255.9 ± 1.46 *vs.* 196.4 ± 10.54 beats/min). However, no significant difference in heart rate was observed between the two groups over the 15 min following epinephrine injection (287.9 ± 8.79 *vs.* 267.4 ± 12.76 beats/min). The PR interval significantly decreased from 0.061 ± 0.0004 to 0.047 ± 0.001 sec, the QRS complex duration increased from 0.0181 ± 0.0006 to 0.0256 ± 0.0008 sec, and the QTc interval decreased from 0.152 ± 0.006 to 0.099 ± 0.002 sec. The R wave amplitude showed significant increase from 0.612 ± 0.012 to 0.839 ± 0.009 mv (Table 1, Figs. 8 & 9). The arrhythmia induced by epinephrine was completely suppressed by the administration of the beta adrenoceptor blocker and BBG (Table 2, Fig. 7).

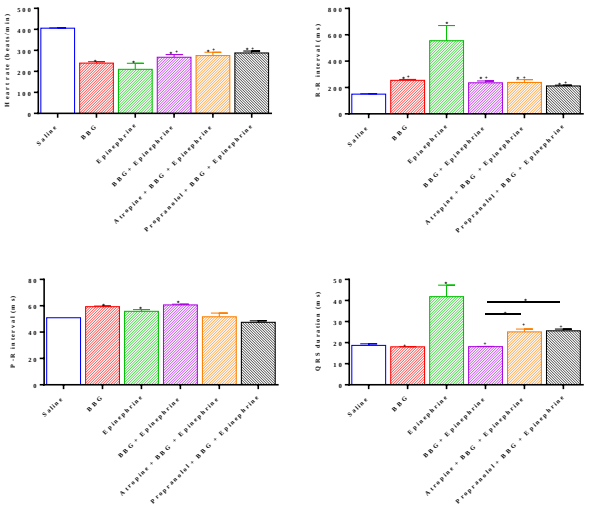

Fig. 8 Changes in heart rate, RR interval, PR interval, and QRS duration in different treatment groups. *: p<0.05: significantly different from normal control group and between different groups as shown in the figure. +: p<0.05: significantly different from epinephrine control group.

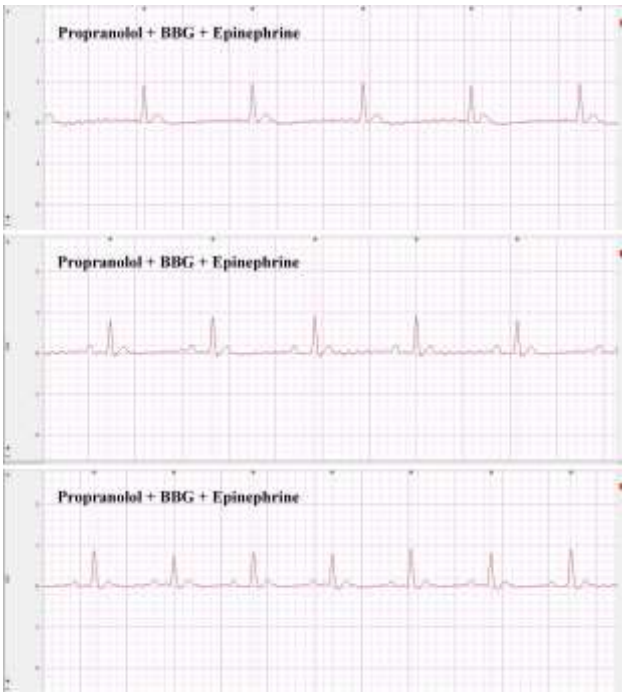

Fig. 7 Representative ECG tracings of the changes in rats treated with i.p. propranolol and BBG prior to i.v. epinephrine (10 µg/kg) showing increased heart rate and increased R wave amplitude compared with the epinephrine control. These are ECG recordings from 5, 10 and 15 min time periods after epinephrine injection.

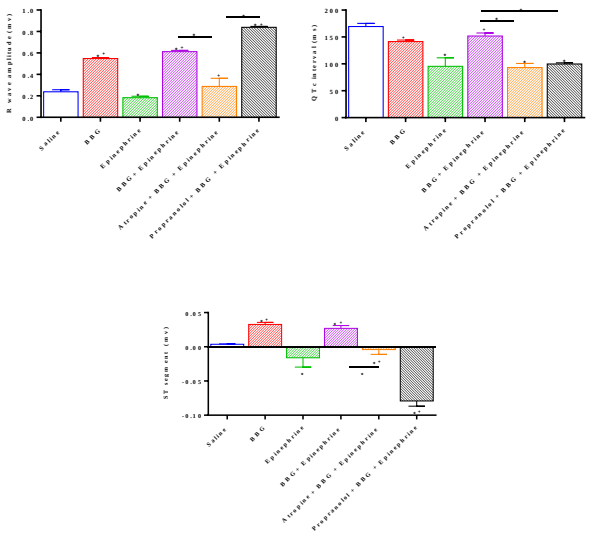

Fig. 9 Changes in R wave amplitude, QTc interval, and ST segment height in different treatment groups. *: p<0.05: significantly different from normal control group and between different groups as shown in the figure. +: p<0.05: significantly different from epinephrine control group.

Table 1. The changes in ECG parameters in different treatment groups.

Group/ Parameter	Normal control	BBG	Epinephrine	BBG + Epinephrine	Atropine + BBG + Epinephrine	Propranolol + BBG + Epinephrine
Heart rate/min	405.8±1.18	239.4±6.69*	209.8±28.78*	267.4±12.76^{+}	275.0±16.18^{*+}	287.9±8.79^{*+}
RR interval (sec)	0.149±0.002	0.254±0.007^{*+}	0.554± 0.115*	0.236±0.014^{*+}	0.238±0.021^{*+}	0.212±0.006^{*+}
PR interval (sec)	0.051±0.0008	0.059±0.0004*	0.056±0.002*	0.061±0.0004*	0.052±0.003	0.047±0.001
QRS duration (sec)	0.0187±0.0007	0.018±0.0001*	0.0418±0.005*	0.0181±0.0006^{+}	0.0251±0.001$^{+\#}$	0.0256±0.0008^{+}
QTc interval (sec)	0.169±0.006	0.141±0.003^{+}	0.099±0.015*	0.152±0.006^{+}	0.093±0.008$^{*\#}$	0.099±0.002*
R wave amplitude (mv)	0.238±0.019	0.548±0.009^{*+}	0.183 ±0.013*	0.612±0.012^{*+}	0.288±0.076$^{+\#}$	0.839±0.008^{*+}
ST segment height (mv)	0.00373±0.0004	0.0327±0.003^{*+}	-0.016±0.0137*	0.027±0.004^{*+}	-0.004±0.007^{*+}	-0.0792±0.008^{*+}

Data were expressed as mean ± SE (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *: $p<0.05$: significantly different from normal control group. +: $p<0.05$: significantly different from epinephrine control group. #: $p<0.05$: significantly different from the BBG + Epinephrine group.

Table 2. Effect of brilliant blue G (BBG) on epinephrine-induced ventricular arrhythmia/min.

Group/ Parameter	Normal control	BBG	Epinephrine	BBG + Epinephrine	Atropine + BBG + Epinephrine	Propranolol + BBG + Epinephrine
Number of ventricular premature beats	0.0±0.0	0.0±0.0	54.56±1.25*	0.0±0.0^{+}	41.28±1.09$^{*+\#}$	0.0±0.0^{+}
Duration of arrhythmia (sec)	0.0± 0.0	0.0±0.0	51.21±1.02*	0.0±0.0^{+}	57.11±0.61$^{*+\#}$	0.0±0.0^{+}

Data were expressed as mean ± SE (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *: $p<0.05$: significantly different from normal control group. +: $p<0.05$: significantly different from epinephrine control group. #: $p<0.05$: significantly different from the BBG + Epinephrine group.

3.2 Cardiac histopathology

Examination of sections of the cardiac muscle of saline control rats showed that the myocardium is striated and arranged in a linear array that branches and anatomizes in a specific pattern giving the appearance of a sheet. The cardiac muscle fibers are joined together by intercalated discs, and cardiac muscle fibers are separated by a delicate layer of connective tissue, with well evidenced myocardial blood capillaries (Fig. 10A). In the epinephrine treated rats there was necrosis of cardiac muscle cells that had lost their normal structure. Some myocardial cells appeared apoptotic and there was severe inflammatory cell infiltration. Degeneration of cardiac muscle fibers, interstitial haemorrhages and oedema were present (Figs. 10B & C). Sections from the group that received BBG prior to i.v. epinephrine showed improvement in the histological picture (Fig. 10D). Cardiac sections of rats treated with propranolol and BBG prior to i.v. epinephrine showed the normal histological structure of cardiac muscle fibers; although focal hyalinization of cardiac muscle fibers was still present (Fig. 10E). Sections from the group treated with atropine and BBG prior to i.v. epinephrine revealed a decrease in myocardial lesions in the form normal striation of cardiac muscle, although some areas showed splitting of muscle fibers, interstitial haemorrhage, some nuclei appeared flattened and there was swelling of other nuclei (Fig .10F).

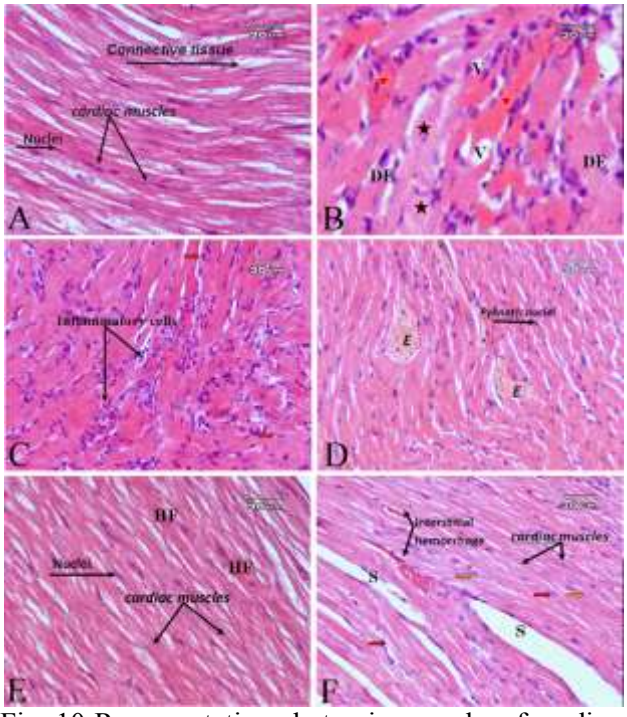

Fig. 10 Representative photomicrographs of cardiac muscles of rats after treatment with **(A)** Saline showing longitudinally arranged cardiac muscle fibers with regular striation, which have acidophilic cytoplasm, and central vesicular oval nuclei (black arrow); **(B)** Epinephrine showing degeneration of the cardiac muscle (DE), interstitial haemorrhage (red star), focal necrosis of muscle fibers (black star) and vacuolation (V); **(C)** Epinephrine (another field) showing severe inflammatory cell infiltration (black arrow). Some cardiac muscle cells appeared apoptotic (red arrow); **(D)** BBG and epinephrine showing improvement of the histological changes, but oedema (E) and apoptotic cells were seen; **(E)** Propranolol, BBG and epinephrine showing normal histological changes of cardiac muscle fibers, although focal hyalinization of muscle fibers was still present (HF); **(F)** Atropine, BBG and epinephrine showing normal striation of cardiac muscle, although some areas showed splitting of cardiac muscle fibers (S), and interstitial haemorrhage was seen. Some nuclei appeared flattened (red arrow) while swelling of other nuclei was noted (orange arrow).

4 Discussion

The purpose of this study, was to examine the effects of the purinergic P2X7 receptor antagonist brilliant blue G (BBG) on the epinephrine-induced arrhythmia and cardiac muscle injury. In addition, we investigated the role of beta adrenoceptors and cholinergic mechanisms in the effects of BBG. The main findings in the study can be summarized as follows: (i) the i.p. administration of BBG resulted in significant bradycardia, increased PR interval and R wave amplitude, shortened QTc interval, but did not change QRS duration. BBG itself caused no ventricular arrhythmia; (ii) epinephrine given i.v. produced significant bradycardia, increased PR interval, caused QRS widening, shortened QTc interval, and decreased R wave amplitude. First degree heart block, and polymorphic ventricular tachycardia occurred following i.v., epinephrine injection; (iii) when given prior to i.v. epinephrine, BBG, however, counteracted the bardycardiac response, normalized QRS duration and QTc interval, increased R wave amplitude and completely suppressed the development of arrhythmia; (ii) the inhibitory effect of BBG on ventricular arrhythmia was prevented with prior atropine administration; (iv) BBG provided histologic protection against myocardial damage caused by epinephrine.

In rats, i.v. administered epinephrine causes cardiac arrhythmias including premature ventricular beats, runs of ventricular tachycardia and missed beats [27[, [29], [30], [31], as well as severe myocardial

damage [27], [31], [32]. The catecholamines-induced arrhythmia and cardiac muscle injury are considered to result from stimulation of α-adrenoceptors in coronary arteries, producing coronary vasospasm and subsequent cardiac muscle ischaemia, as well as stimulation of β-adrenoceptors in cardiomyocytes, increasing cyclic adenosine monophosphate (cAMP) concentrations, and increasing intracellular Ca^{2+} levels. Aminochromes and reactive oxygen species produced during the oxidation of catecholamines also contribute to coronary vasospasm, myocardial cellular injury, and the development of ventricular arrhythmia [10].

In the ischaemic injury to the myocardium and subsequent tissue damage, there is the release of large amounts of adenosine 5′-triphosphate (ATP) resulting in excessive stimulation of the P2X7 receptor, triggering the release of proinflammatory cytokines e.g., IL-1β, IL-8, reactive oxygen species and recruitment of leucocytes into the ischaemic region. The resultant inflammatory response will lead to the enhancement of the myocardial tissue damage, and increases the susceptibility for the development of arrhythmias [17], [18]. There is also evidence that inflammation and proinflammatory cytokines e.g., interleukin-1beta (IL-1β) have direct arrhythmogenic effects on cardiac cells, and are important contributors to arrhythmogenesis in the post-myocardial infarction heart [33], [34].

Brilliant blue G by virtue of its ability to act as a non-competitive antagonist of P2X7 receptor [23] is likely to interfere with the inflammatory cascade, thereby, preventing excessive cardiac tissue damage and arrhythmia. The findings in the present study are in support of this notion, where BBG given prior to a toxic dose of epinephrine prevented the cardiac histologic damage, and completely suppressed the ventricular arrhythmia.

Brilliant blue G was shown to inhibit the systemic inflammatory response, oxidative stress, and tissue damage caused by i.p. lipopolysaccharide in the rat [25]. Other studies showed that blockade of P2X7 receptors with BBG attenuated brain inflammation *via* a number of mechanisms such as reduced activation of microglia cells [35], decreased infiltration of neutrophils into the area of damage [36], and decreased levels of inflammatory cytokines IL-1β, IL-10 [37], IL-6 [38]. The dye also exhibited an inhibitory effect on lipid peroxidation, nitric oxide production and nuclear factor-kappaB activation [38], [39], and exerted antiapoptotic action by decreasing caspase-3 activation [39]. It is obvious therefore, that the above mentioned effects of BBG will act to inhibit the inflammatory response during myocardial ischaemia, and thus limit or minimize the extent of cardiac tissue damage and the potential for arrhythmogensis.

The arrhythmogenicity of epinephrine involves shortening of sinus cycle length, an increase in atrial and ventricular automaticity, enhancement of atrioventricular nodal conduction, and a decrease in ventricular effective refractory period [30]. These electrophysiologic effects of epinephrine are the result of stimulating beta adrenoceptors [29]. When administered acutely, beta-adrenoceptor blockers prevent the increase in Purkinje fiber automaticity, and the decrease in ventricular fibrillation threshold induced by catecholamines, and are effective antiarrhythmic drugs in patients with ventricular arrhythmias [40]. In the present study, we found that pretreatment with i.p. BBG counteracted the reflex bradycardia observed early following i.v. injection of epinephrine. It also normalized the QRS duration, and QTc interval, and counteracted the decrease in R wave amplitude by epinephrine. The i.v. administration of epinephrine produces bradycardia and heart block. This is a reflex response to an abrupt increase in mean arterial blood pressure by epinephrine [41]. On the other hand, the bradycardia observed in the present study when administering BBG suggests a cardiac depressant effect, which could be vagally mediated. It has been suggested that ATP acting *via* P2X-purinoceptors has the effect of stimulating vagal afferent nerve terminals in the left ventricle myocardium, and eliciting cardiocardiac vagal depressor reflex [42]. Brilliant blue G may therefore, prevent arrhythmia by restoring the sympatho-parasympathetic balance in the cardiac conduction system.

The present study also examined the effects of beta-adrenergic blockade or cholinergic mechanisms in the antiarrhythmic action of BBG. We found that propranolol given prior to BBG had no effect on the antiarrhythmic action of BBG in that the arrhythmia induced by epinephrine was completely suppressed by the administration of propranolol and BBG. Moreover, the cardiac tissue from this group showed normal histological appearance of cardiac muscle fibers. Indeed, the protective effects of beta adrenoceptor blockade against myocardial cell injury and arrhythmia induced by epinephrine or cardiac ischaemia have been demonstrated *in vitro* [8], [9] and *in vivo* [32], [40[, [43]. In contrast, the inhibitory effect of BBG on the arrhythmia induced by epinephrine was prevented by prior

administration of atropine, which may suggest a cholinergic component in the antiarrhythmic action of BBG.

5 Conclusion

The present results provided the first evidence that the purinergic P2X7 receptor antagonist brilliant blue G protects against epinephrine-induced dysrhythmia and cardiac muscle damage and suggested the involvement of a cholinergic mechanism for the anti-arrhythmogenic action of brilliant blue G. The study also indicated a role for purinergic P2X7 receptor in the pathogenesis of epinephrine -induced arrhythmias and myocardial injury, and suggested that P2X7 receptor antagonism could be valuable in patients who develop arrhythmia during epinephrine infusion. These results are of considerable clinical relevance in elucidating the potential future of P2X7 antagonists in treatment of cardiac arrhythmias. The pharmacological inhibition of P2X7 receptors may offer a distinct therapeutic approach for the treatment of cardiac rhythm abnormalities. Obviously, there is a need for future research in this field in order to explore the different mechanisms by which brilliant blue G exerts its antiarrhythmogenic and cardioprotective effects.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

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

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