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# Glyburide, a $K^+_{ATP}$ channel blocker, improves hypotension and survival in anaphylactic shock induced in Wistar rats sensitized to ovalbumin

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ABSTRACT

Allergens can induce anaphylactic shock and death due to severe hypotension. Potassium channel blockers ($K^+_{ATP}$) such as glyburide (GLY) induce vasoconstriction. The effect of $K^+_{ATP}$ channel blockers on anaphylactic shock is poorly understood. Objective of the study was to test the hypothesis that GLY reduces hypotension induced in anaphylactic shock and increases survival. Rats were grouped into: G1-N=Naïve; G2-SC=Sensitized-Control; G3-SG=Sensitized-GLY (glyburide 40 mg/kg); G4-SE=Sensitized-EPI (epinephrine 10 μg/kg). G2 to G4 groups were sensitized with ovalbumin (OVA) and shock was induced by i.v. injection of OVA. Treatments were administered intravenously 5 min later. Mean arterial pressure (MAP), heart rate (HR), and mean survival time (MST) were measured for 60 min following OVA injection and treatments administration. At the end of the experiment, blood withdrawal was performed to measure plasma levels of histamine, leukotriene $B_4$ ($LTB_4$), prostaglandin $E_2$ ($PGE_2$) and prostaglandin $F_2$ ($PGF_2$). Additionally blood gas ($p_aO_2$, $p_aCO_2$, $S_aO_2$) and electrolytes ($Na^+$, $K^+$ and $Ca^{++}$) were measured. MAP was normal in G1-N; severe hypotension, negative inotropic and short MST were observed in G2-SC; normalization of MAP, with lesser negative inotropism and increased MST were observed in G3-SG; full recovery was observed in G4-SE. Histamine level was significantly higher in G2-SC; reduced in G3-SG and G4-SE. $PGE_2$ increased in G3-SG; $PGF_2$ increased in G2-SC and G3-SG. $Na^+$ and $Ca^{++}$ concentration decreased in sensitized rats but reversed in treated groups, without change in $K^+$ concentration. In conclusion, our data suggest that administration of GLY reduces hypotension and increases survival time in rat anaphylactic shock.



## 1. Introduction

Anaphylactic shock is a complex disease with multi-factorial causes. The prevalence of allergies has increased dramatically in recent years (Holt, 1998; de Bisschop and Bellou, 2012). Food allergy is growing significantly and can induce severe anaphylaxis, a life-threatening emergency (Neugut et al., 2001). According to recent epidemiological data, fatal anaphylaxis occurs in 2 to 20% of cases (Triggiani et al., 2008). Anaphylactic shock is characterized by complex cardiovascular disorders including severe hypotension and shock, cardiac arrhythmias, ventricular dysfunction and cardiac arrest (Enjeti et al., 1983, Pumphrey, 2004). Other clinical manifestations are observed such as bronchospasm, urticaria and angioedema (Triggiani et al., 2008). Arterial hypotension observed during anaphylactic shock is mainly attributed to the release of mediators via immune reactions involving IgE, mast cell and basophiles activation. Histamine, tryptase, chymase, carboxy-peptides A, prostanoids, and eicasanoids, predominantly leukotriene $B_4$ ($LTB_4$), leukotriene $C_4$ ($LTC_4$), Prostaglandin $E_2$, Prostaglandin $F_2$ and Prostaglandin $D_2$ are the principal mediators released after mast cells activation (Lewis and Austen, 1984; Triggiani et al., 2008).

The role of $K^+$ channels has been studied in different immune responses, as acute rejection reaction after organ transplantation and multiple sclerosis (Wulff et al., 2003, 2000). Maybauer et al. (2004) reported the effect of ATP-sensitive potassium ($K^+_{ATP}$) channel blockers like glibenclamide on cardiac manifestations. Glibenclamide (also known as glyburide (GLY) is an oral anti-diabetic drug and classified as sulfonylurea derivative. $K^+_{ATP}$ channel blockade with GLY has been previously reported to improve both mean arterial blood pressure

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(MAP) and cardiac tone, caused by hemorrhagic shock (Maybauer et al., 2004). Furthermore, the action of $K^+_{ATP}$ channel blockade results in cardiac protection, which is elicited by chronic hypoxia, and improves diastolic contraction (Milano et al., 2004). Largely, the mechanism of action of GLY was demonstrated by Gribble and Ashcroft (2000) who reported that GLY acts by targeting the sulfonylurea receptor subunits $SUR_1$ and $SUR_2$ of the $K^+_{ATP}$ channel. Despite the absence of randomized studies, epinephrine remains the first line treatment of anaphylactic shock. Because of ethical issues, animals such as the rat are used to explore new approach of anaphylactic shock treatments.

The aim of this study was to test the hypothesis that blockade of $K^+_{ATP}$ channel by GLY in Wistar rat anaphylactic shock induced by OVA will: (1) improve hypotension; and (2) improve survival.

## 2. Materials and methods

This project was reviewed and approved by the Institutional Review Board of the United Arab Emirates University, College of Medicine and Health Sciences, Al Ain, Abu Dhabi, United Arab Emirates, and experiments were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee.

### 2.1. Animals and immunization protocol

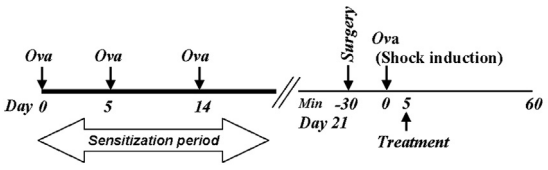
Experiments were performed using male Wistar rats (250 ± 15 g body weight, 4 weeks old). Rats were housed in groups of 4 in polypropylene cages with 12 h controlled light and dark cycle at 24–26 °C. Food and water were available ad libitum. All animals were acclimatized to these conditions for 1 week prior to the experiment. Six rats in each group were used and divided into four groups as described in the following section. OVA was dissolved in a suspension of aluminum hydroxide (Al OH) at a proportion of 1 mg of OVA+3.5 mg of Al OH in 1 ml 0.9% sterile normal saline. Al OH is used for increasing the antigenicity of OVA and optimizing the sensitization. The fine suspension of OVA in Al OH was injected subcutaneously, under the shoulder blades of rats treated by glibenclamide and controls on days 0, 5 and 14. Al OH is used for increasing the antigenicity of OVA and optimizing the sensitization. Naïve rats received only Al OH. Injections were made in a volume of 1 ml to each animal.

### 2.2. Treatment groups

The effect of Glyburide (a $K^+$—ATP blocker, GLY) on anaphylactic shock was studied in sensitized rats after injection i.v. bolus of OVA. Hence, the four groups of animals were divided, namely: G1-N=Naïve, non-sensitized rats which received no treatment; G2-SC= Sensitized-Controls, treated with normal saline, i.v. bolus, 5 min after shock induction; G3-SG=Sensitized-GLY treated with GLY (40 mg/kg, i.v. bolus) 5 min after shock induction; G4-SE= Sensitized-EPI treated with epinephrine (i.v. bolus, 10 μg/kg at 5 min and 10 min after the shock induction) (See illustration in Fig. 1). All the groups were perfused with normal saline 2 ml/h i.v. throughout the period of experiment following surgery.

### 2.3. Hemodynamic assessment and anaphylactic shock protocol

One week after the last immunization, rats (7 weeks old) were anesthetized with pentobarbital sodium solution (62.5 mg/kg) administered intraperitoneally (i.p.). The tracheas of the animals were surgically cannulated to permit artificially ventilated through an endotracheal tube attached to a constant-volume ventilator (Harvard Apparatus, Edenbridge, UK). The ventilator was set to deliver the following parameters: a respiratory rate of 60 breaths/min; a tidal volume of 6 ml/kg body weight; an end-expiratory pressure of 5 cm $H_2O$ and a concentration of 1.0 with the inspiratory oxygen. Body temperature was maintained at 37 °C by the temperature controlled thermo-blanket (Harvard Apparatus, MA, USA). One catheter (PE10 tubing) was placed in the left jugular left vein and connected to slow injection/infusion pump (Harvard Apparatus, MA, USA) for i.v. treatment. Another similar catheter was placed in the left carotid artery and connected to blood pressure module of PowerLab® (AD Instruments, NSW, Australia) system via a pressure transducer to measure systolic, diastolic and mean arterial blood pressure (MAP) and heart rate (HR). The ligature of one carotid artery is widely used in the experiments in direct blood pressure recording and has no effect on cerebral blood flow. Normal saline (0.9%) was given 2 ml/h., i.v. by infusion pump to compensate for the estimated loss of fluid during surgery.




| | Sensitization Period | Shock induction | Treatment |
|---|---|---|---|
| G1-N: Naive | Saline | Saline | Saline |
| G2-SC: Control | OVA | OVA | Saline |
| G3-SG: GLY | OVA | OVA | Glyburide (40 mg/kg, i.v.) |
| G4-SE: EPI | OVA | OVA | Epinephrine (10 μg/kg, i.v. at 1 min and 10 min after shock) |

**Fig. 1.** Experimental design showing sensitization period and shock induction.

After the surgical procedures, a thirty minutes stabilization period was allowed for all groups before any measurement. OVA challenge was made to all groups by injecting 1 ml of OVA fine suspension i.v. followed by the constant infusion of normal saline. In all groups, treatments were administered i.v. as single bolus and the hemodynamic parameters (MAP and HR) were monitored continuously and recorded. Measurement of hemodynamic parameters was performed every 5 min for a period of 30 min before the OVA challenge. After the stabilization period, OVA challenge was started and after 5 min treatments were given i.v. The hemodynamic parameters were continuously recorded at 1 min interval up to a period of 60 min.

### 2.4. Blood collection

The whole blood sample was withdrawn from the carotid artery (previously cannulated for direct blood pressure recording) and placed into heparinized tubes immediately when the MAP falls to 25 mmHg which correspond to shock and at the end of the experiment (1 h after OVA injection). 200 μl of the blood was used immediately for the determination of blood gas and electrolyte parameters. The remaining blood was centrifuged at 3000*g* at 4 °C for 15 min to separate the plasma. The separated plasma was stored in −80 °C until used for further ELISA assays.

### 2.5. Determination of plasma concentration of histamine, $LTB_4$, $PGE_2$ and $PGF_2$

Plasma samples from treated or untreated animals either from naïve or sensitized groups were used for histamine, $LTB_4$, $PGE_2$ and $PGF_2$ concentration measurements using ELISA commercial kits (Cayman Chemical, Ann Arbor, MI, USA).

### 2.6. Measurement of blood gas and electrolytes

The whole blood collected anaerobically as described in the earlier section were used for blood gas and electrolytes analysis. The equipment OPTI® CC-TS (Osmetech Inc., Critical Care division, GA, USA) was used for the determination of the following parameters; arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen ($PaO_2$), bicarbonates ($HCO_3$), total carbon dioxide ($tCO_2$), sodium ($Na^+$), potassium ($K^+$), ionic calcium ($Ca^{++}$), chloride (Cl), total hemoglobin (tv), saturated Oxygen ($SaO_2$) and hematocrit (HCT). The arterial blood was placed into the cassette (Osmetech OPTI Sensor Cassette, Type "E-Ca", BP7560) and the parameters were measured as described by the manufacturer. Arterial blood gas samples (0.5 ml) were rapidly analyzed after blood withdrawal. Equipment use was according to the method suggested by the manufacturer using a standard reference cassette ("SRC"—Level 1, BP7536 and Level 3, BP7543) every day before the test samples were applied or after every on/off cycle.

### 2.7. Statistical analysis

Results are expressed as the mean ± S.E.M. Statistical significance was measured using two-way analysis of variance (ANOVA) (e.g., naive x treatment; time as a repeated measure when needed) and Bonferonni comparisons of MAP and HR experiments. In other experiments, one-way ANOVA with Newman–Keuls comparisons was used to measure the statistical significance. $P<0.05$ was considered significant (Graphpad Software, San Diego, CA, USA). Survival was studied with Kaplan Meier curves.

## 3. Results

Rats were sensitized with OVA administered at 0, 5 and 14 days prior to the hemodynamic assessment as illustrated in Fig. 1. On day 21, animals were prepared for surgical intervention and for the induction of shock with one i.v. bolus of OVA. During the experiment, rats which served as a naïve group gained about 3.2% over their initial weight. Rats which received OVA for sensitization gained 2.7% of weight which was not significant as compared to their age-matched naïve group.

### 3.1. Hemodynamic assessment and anaphylactic shock protocol

Hemodynamic assessment was performed by measuring blood pressure and HR in naive and sensitized rat groups (Fig. 2). All treatment groups' data were compared statistically with naïve and control groups at their respective time points.

In naïve group (G1-N), MAP and HR were stable throughout the 60 min duration of the experiment. MAP was between 107 and 115 mmHg and HR between 380 and 439 per min. Changes in both MAP and HR were compared between 0 min to other time points and were not significant. In the control group (G2-SC), a fall in MAP occurred 5 min after OVA injection which continued to decrease. All rats died in 30 to 35 min. Hypotension was highly significant ($P<0.001$) as compared to naïve rats at all time points from 5 min to the end of the experiment. HR showed a marked fall within 5 min after OVA injection. Change in HR was highly significant ($P<0.001$) after 25 min of OVA injection. Glyburide treated rats (G3-SG: GLY), showed severe hypotension shortly after OVA injection within 5 min and continued to fall till 15 min but gradually increased to 95 mmHg in 50 min. Statistically, the severity of the fall was highly significant till 30 min ($P<0.001$), at 35 min ($P<0.1$), at 40 min ($P<0.05$) and after 45 min there was no significant difference with naïve rats until the end of the experiment. However, the HR decreased, but this decrease

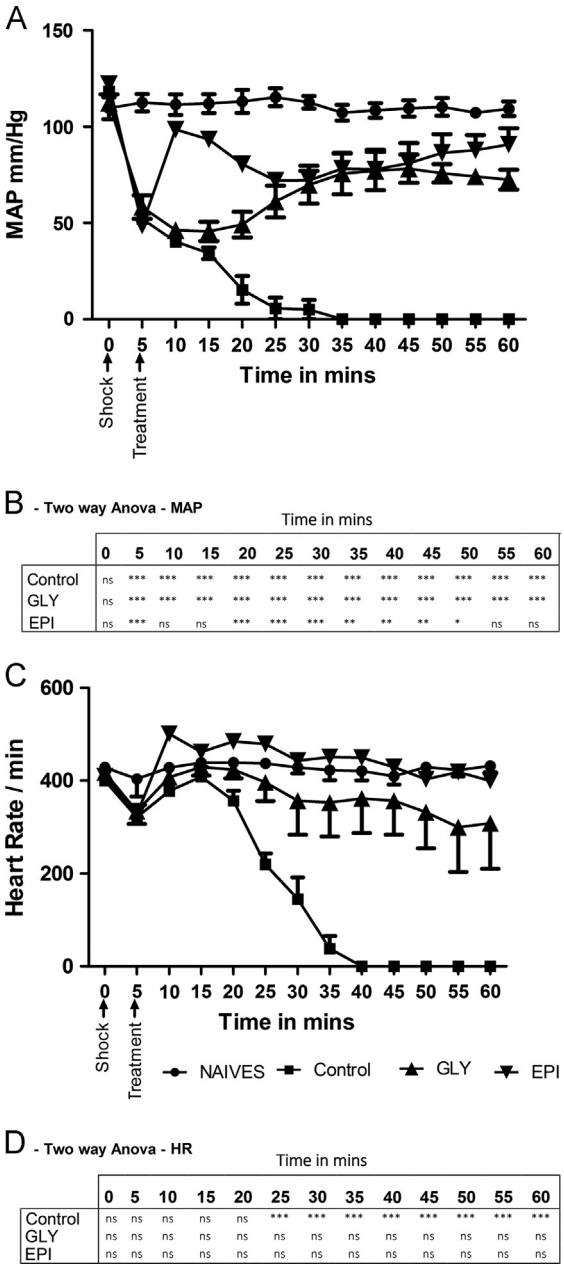



**Fig. 2.** Evolution of mean arterial blood pressure (MAP, mmHg) (A) and heart rate (per min) (C) after i.v. bolus of OVA in rat groups (—●— NAIVE —■—Control —▲— GLY. —▼— EPI). Each point represents the mean (± S.E.M.) of six rats in panel A and C. All results were analyzed by two way ANOVA test and are tabulated in the panel (B) for MAP and panel (D) for Heart rate. ***$P<0.001$, **$P<0.01$, *$P<0.05$, ns = not significant.

was not statistically significant, and did not show a significant change throughout the experiment as compared to naïve rats. The difference in MAP and HR between controls and GLY group was significant ($P<0.0001$). The rise of MAP in GLY group is significant as compared to controls ($P<0.001$). Epinephrine treated group (G4-SE: EPI) showed similar hypotension as other groups ($P<0.001$) which occurred immediately after OVA injection but a transient rise in the MAP was observed after EPI injection and was statistically not significant as compared to naïve rats but significant when compared to control rats ($P<0.001$). The transient rise in MAP did not sustain with the time and started falling till 40 min. However, after about 40 to 45 min, the MAP slowly recovered to 90 mmHg from 55 to 60 min. There is a significant difference between rats treated with EPI and GLY from 10 to 20 min after OVA injection ($P<0.001$) but after the 20th min the

difference disappeared. HR, showed a remarkable fall quickly after the OVA injection up to 5 min till EPI administration. Acute tachycardia was recorded immediately after EPI injection and contained the HR throughout the experiment above the naive group.

Kaplan–Meier survival curves (Fig. 3) show the survival rate for rats in each group. Naïve and the EPI groups survived till the end of the experiment. The control group showed a severe mortality which reached 100% at 40 min. Subsequently to the control group, GLY group showed 80% of survival till the end of experiment.

### 3.2. Effect of OVA induced anaphylactic shock on histamine release

The levels of histamine in naïve and treated rats were recorded and shown in Fig. 4(A). The control group rats (G2-SC), showed a significant ($P < 0.001$) rise in the histamine level when compared to naïve group (G1-N) which represents the effect of OVA on mast cells. However, the group which received glyburide (G3-SG) and the group with epinephrine (G4-SE) showed no significant changes in histamine levels when compared to naïve group. Among sensitized groups (G2-SC, G3-SG and G4-SE), glyburide treated (G3-SG) and epinephrine treated (G4-SE) groups showed a relatively lesser rise in histamine level as compared to the control group (G2-SC).

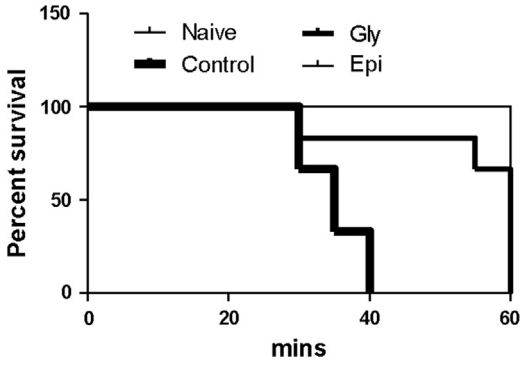



**Fig. 3.** Effect of treatments on survival after i.v. bolus of OVA. Survival of rat groups (naïve, control, glyburide, GLY and epinephrine, EPI) is represented in Kaplan–Meier survival curve.

### 3.3. Effect of OVA induced anaphylactic shock on LTB4 release

$LTB_4$ plasma levels were shown in Fig. 4(B). $LTB_4$ levels in GLY treated (G3-SG) and epinephrine treated (G4-SE) groups showed a lesser concentration as compared to the control group (G2-SC). However, statistical analysis revealed no significant difference. There was no difference in sensitized rats compared to the unsensitized naïve rats.

### 3.4. Effect of OVA induced anaphylactic shock on $PGE_2$ release

The plasma concentrations of $PGE_2$ were measured and reported in Fig. 4(C). Our results showed a significant rise in the levels of $PGE_2$ in all sensitized groups (G2-SC, G3-SG and G4-SE) when compared to the unsensitized naïve group. But glyburide treated (G3-SG) group showed a highly significant ($P < 0.01$) increase in the concentration of $PGE_2$.

### 3.5. Effect of OVA induced anaphylactic shock on $PGF_2$ release

Measurements of $PGF_2$ are presented in Fig. 4(D). All sensitized rats (G2-SC, G3-SG and G4-SE groups) showed an increase in the levels of $PGF_2$ without statistical difference within the sensitized groups. Nevertheless, levels of $PGF_2$ in naïve rats were relatively lower when compared to the sensitized group. The variations in $PGF_2$ concentration displayed a significant increase ($P < 0.05$) in G2-SC and G3-SG group of rats when compared to naïve group with the exception of epinephrine treated group (G4-SE).

### 3.6. Blood gas changes in OVA induced anaphylactic shock

$PaO_2$, $SaO_2$ and $PaCO_2$ levels in naïve and sensitized groups were measured (Fig. 5; Panels A–C respectively). Both $PaO_2$ and $SaO_2$ measurements showed no significant difference between each treatment within sensitized group; and between sensitized

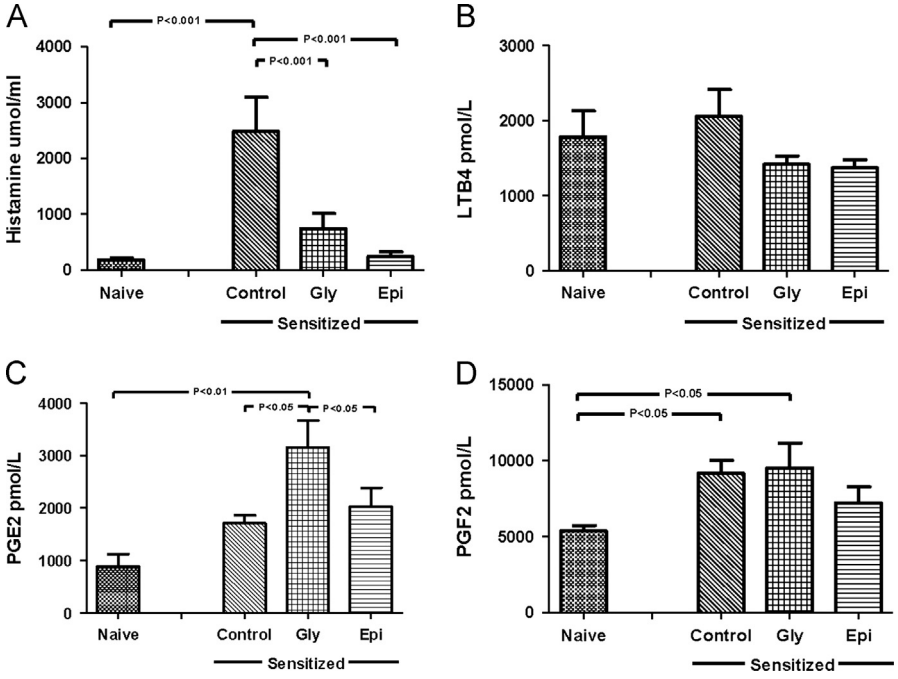



**Fig. 4.** Plasma levels of histamine (A), $LTB_4$ (B), $PGE_2$ (C) and $PGF_2$ (D) after induction of anaphylactic shock in sensitized treated rats and sensitized untreated rats. Values are expressed as means ( ± S.E.M.) for 6 rats in each group.

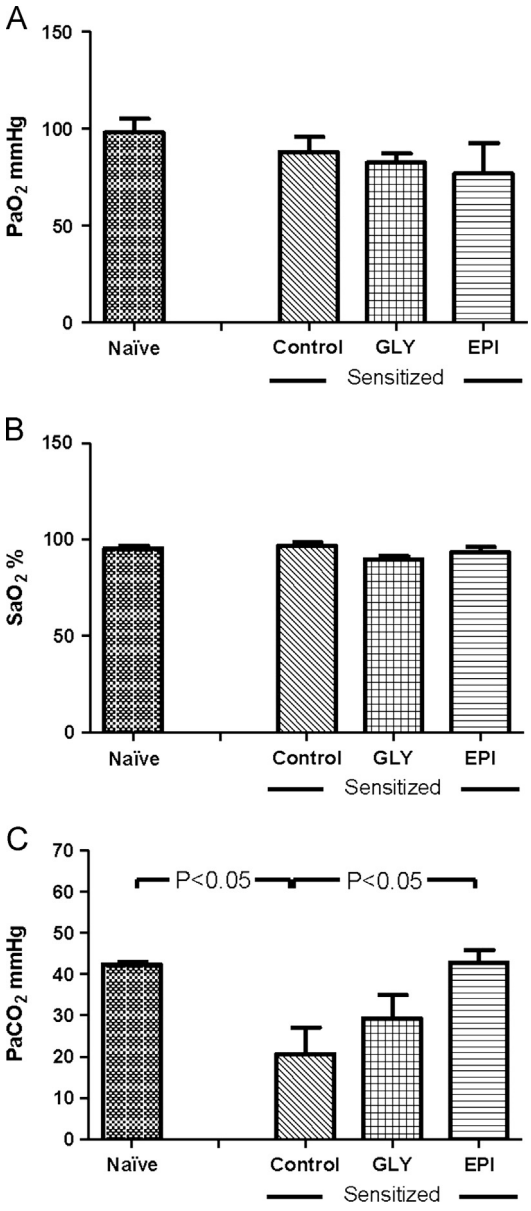



**Fig. 5.** Blood levels of $PaO_2$ (A), $SaO_2$ (B) and $PaCO_2$ (C) in sensitized untreated rats (Control) and sensitized treated rats (GLY, EPI). Values are expressed as means ( ± S.E.M.) for 6 rats in each group.

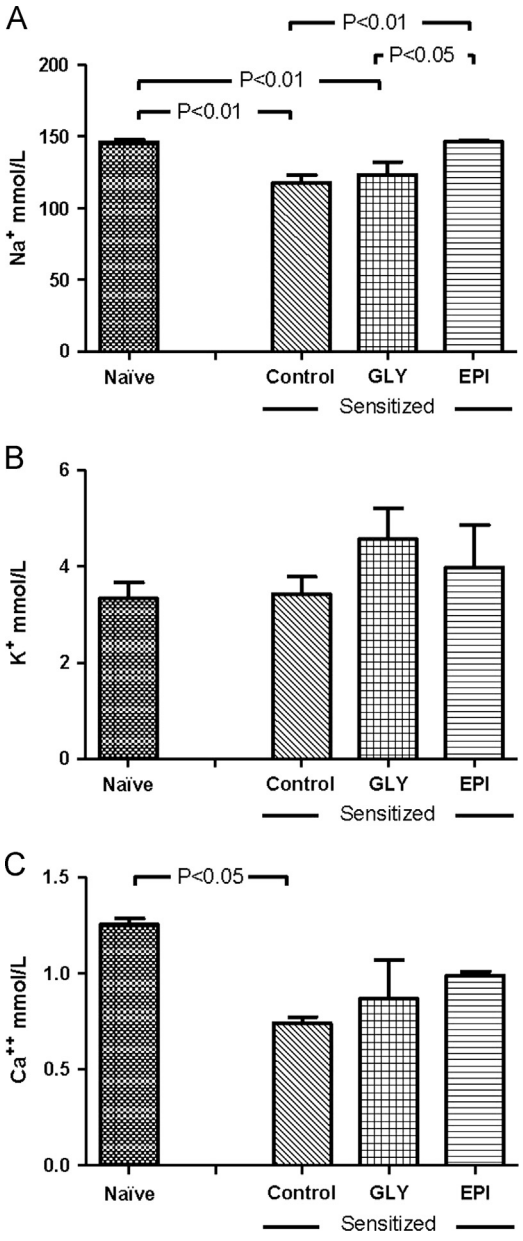



**Fig. 6.** Blood levels of $Na^+$ (A), $K^+$ (B) and $Ca^{++}$ (C) in sensitized untreated rats (Control) and sensitized treated rats (GLY, EPI). Values are expressed as means ( ± S.E.M.) for 6 rats in each group.

and unsensitized groups. $PaO_2$ in naïve group was 98 ± 7.6 mmHg; 88 ± 7.8 in G2-SC group; and 82.5 ± 4.7 in G3-SG group; 76.8 ± 15.8 mmHg in G4-SE group. Likewise, $SaO_2$ in naïve group was 95.3 ± 1.4; 96.6 ± 1.8 in G2-SC group; 89.7 ± 1.7 in G3-SG group; and 92.3 ± 2.9 mg/l in G4-SE group. $PaCO_2$ was 42.3 ± 0.6 in naïve group; 20.6 ± 6.5 mmHg in G2-SC group; 29.3 ± 5.7 in G3-SG; and 42.8 ± 2.9 mmHg in G4-SE. $PaCO_2$ was significantly reduced in control sensitized rats group (G2-SC), as compared to naïve group ($P < 0.05$). However, $PaCO_2$ in G3-SG treated group reduced but did not show statistical significance. Furthermore, epinephrine treated group displayed a significant difference ($P < 0.05$) when compared to control group. The level of epinephrine group showed similarity to the level of naïve group indicating a protective action induced on sympathetic tone on the respiratory system.

### 3.7. Blood electrolyte changes in OVA induced anaphylactic shock

$Na^+$, $K^+$ and $Ca^{++}$ ion levels in naïve and sensitized rats were illustrated in Fig. 6, Panel A–C, respectively. The levels of $Na^+$ reduced significantly in G2-SC and G3-SG group when compared to naïve group. Na+ levels were 146 ± 1.6 in G1-N group; 117.5 ± 5.4 in G2-SC ($P < 0.01$); and 123 ± 9.3 in G3-SG ($P < 0.01$); 146 ± 0.8 mmol/ in G4-SE (P: ns). No noteworthy difference was observed in the levels of $K^+$ in all sensitized groups compared to naïve rats. $Ca^{++}$ ions, decreased significantly when compared to naive and control groups ($P < 0.05$) but exhibited no significant difference among other groups of animals (Fig. 7).

## 4. Discussion

We demonstrated that GLY improves hypotension and increases survival in a model of anaphylactic shock in Wistar rats sensitized to ovalbumin. This model mimics severe anaphylactic shock observed in humans. All sensitized untreated rats died within 30 min as compared to GLY treated rats where 80% survived. Our results

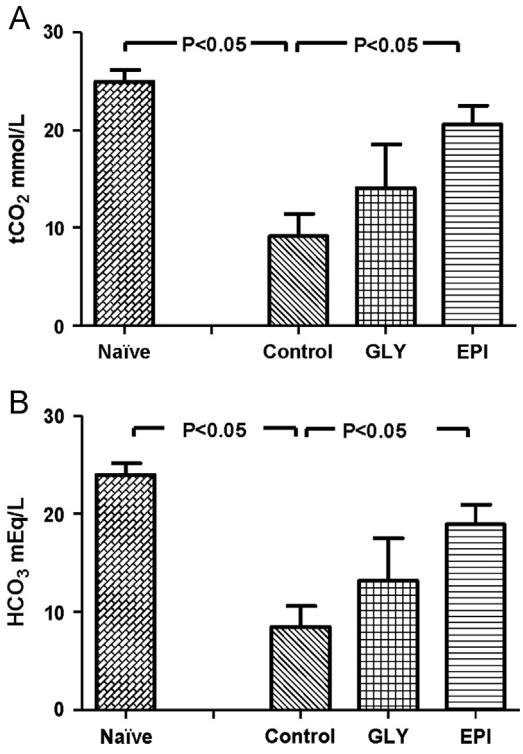



**Fig. 7.** Blood levels of $tCO_2$ (A) and $HCO_3$ (B) in sensitized untreated rats (Control) and sensitized treated rats (GLY, EPI). Values are expressed as means ( ± S.E.M.) for 6 rats in each group.

demonstrate that $K^+$ ATP channels are involved in vasodilation induced by OVA in allergic rats. We surmise that the blockade of $K^+$ ATP channels induces vasoconstriction when it is administered after the induction of anaphylactic shock. But the effect starts 15 min and 10 min after the occurrence of shock and administration of GLY, respectively. Surprisingly, the effects of epinephrine were not immediate (5 min after administration) and GLY and epinephrine followed the same curve after 25 min post treatment. It's important to note that rats treated with epinephrine received 2 boluses i.v. Effect of epinephrine was studied by Dewachter et al. (2007) in a Brown Norway anaphylactic rat model. In the epinephrine group, blood pressure recovered after initial decrease with 84% survival. As in our model, epinephrine did not block the occurrence of anaphylactic shock.

OVA, a potent food allergen, induced anaphylactic shock in human (Leonard et al., 2012; Shek et al., 2004) and in small animals (Demirturk et al., 2007; Ligeiro de Oliveira et al., 2012; Lu et al., 2010). In the present study, sensitization of rats was induced by OVA by three injections on days 0, 5 and 14. Aluminum hydroxide was mixed with OVA to increase the allergenicity of OVA enhancing sensitization. After the sensitization phase, a single dose (i.v., bolus) of OVA was administered one week after the last sensitization on day 14 to induce anaphylactic shock with severe hypotension and bradycardia followed by cardiac arrest and death. Previously, the anaphylactic effects of OVA were demonstrated in Wistar rats (Demirturk et al., 2007; Knippels et al., 1999; Lu et al., 2010) either with a longer sensitization period or with different way of sensitization such as the intra-nasal route, and in different strains (Brown Norway rats) (Bellou et al., 2003b). In our study design, Wistar strain, possessed relatively high sensitivity, towards IgE antigens (Knippels et al., 1999) and the hypotensive effect was similar to previous reports (Bellou et al., 2003b). It was shown that Wistar rats demonstrated more serious clinical manifestations and histopathologic changes that may have important implications for any OVA-induced anaphylaxis (Sun et al., 2013).

In our study, we observed a significant improvement in the cardiovascular manifestations in Wistar rats treated with GLY. A 40 mg/kg (i.v., bolus) reversed the fall in MAP and HR, slowly and gradually. Maybauer et al. (2004) used a hemorrhagic shock model, by bleeding the animal to hypovolemic state in sheep treated by GLY, and showed a transient rise in blood pressure.

Potassium and calcium are involved in the vasodilation induced by $K^+$ channels activation (Kefaloyianni et al., 2012; Ko et al., 2008). GLY blocks the $K^+$ ATP channel by binding $SUR_1$ and $SUR_2$, the sulfonylurea receptor subunits (Gribble and Ashcroft, 2000). Experimental studies in small animal models suggested that this blockade results in reversing hypotension induced by endotoxin (Landry and Oliver, 1992; Vanelli et al., 1995) or hemorrhage (Salzman et al., 1997). In Wistar rats, Szabó and Salzman (1996) demonstrated that GLY improves hemodynamics during hemorrhagic shock. These previous findings in vasodilatory shock (e.g., septic and hemorrhagic shock) support our results in anaphylactic shock.

The implication of histamine and other mediators released after mast cell and basophil activation were studied by analyzing the plasma level in our model to observe the effect of GLY. Histamine is one of the most important mediators acting immediately after its release by mast cells and basophils. Histamine induces a severe vasodilation through the activation of NO synthase which produce NO—one of the most potent vasodilators (Bellou et al., 2003a). It has been previously demonstrated (Middleton, 1984; Rodger, 1985) that the allergic histamine release and vasculitis are $Ca^{++}$ dependent (Hiroichi et al., 1991). Histamine and other mediators, $LTB_4$, $PGE_2$ and $PGF_2$ were analyzed to cover the spectrum of mediators that are involved in anaphylaxis. Our experiments showed 5 fold increase in the levels of histamine when compared with the naïve group which clearly indicates that OVA activates mast cells and basophils in sensitized rats and induces release of histamine. This effect was reported by other authors and was considered to be a marker of the magnitude of anaphylactic shock (Basheer et al., 2010; Triggiani et al., 2008; Vadas et al., 2012). GLY treated group showed a significant fall in the levels of histamine when compared to the sensitized control group. The levels of histamine in the EPI group, which served as a positive control, were also reduced significantly. These observations suggest a protection of GLY and EPI treated rats, probably by reducing the release of histamine from mast cells and basophils. However, despite this effect on production of histamine, hypotension continued 15 min after GLY administration suggesting that other mediators are involved in the acute phase of anaphylactic shock.

Triggiani et al. (2008) reported that mast cell activation during anaphylactic shock induces release of pro-inflammatory mediators and strongly influence ventricular function, cardiac rhythm and coronary artery tone. Histamine, as well as other factors released during the response, exert a negative inotropic effect and induce myocardial depression (Triggiani et al., 2008). Endou and Levi (1995) reported that a large amount of histamine is released from the heart during anaphylaxis which induces major cardiovascular effects. These effects are due mainly to the activation of $H_1$ receptors expressed in vascular smooth muscle (Endou and Levi, 1995). Consistent with these reports, our results showed a significant fall in heart rate in sensitized control rats leading to severe and complete cardiac arrest within 35 to 40 min of induction of anaphylactic shock.

In this study, levels of $LTB_4$ were only slightly affected. A mild increase in the control group and a slight fall in the GLY and EPI groups were observed but without significant difference. $LTB_4$ was previously shown to be a potent vasoconstrictor (Bäck et al., 2004; Sakata et al., 2004) and has cardio depressor effects. Prostaglandins $PGE_2$ and $PGF_2$ alpha have shown significant differences, suggesting that OVA induced shock influences prostaglandin release. PGE2 has a vasodepressor action under certain conditions and increases after OVA injection (Bellou et al., 2003a).

$PGE_2$ signals through four subtypes of G-protein coupled receptors designated as $EP_1$ to $EP_4$ and acts on vessels (Swan and Breyer, 2011). $PGE_2$ when injected intravenously, decreased MAP in a dose dependent manner; but, when injected intra-cerebral ventricular route, it increases the MAP addressing the fact that the $PGE_2$ actions depend on the site of receptors activation (Kondo et al., 1979). Supporting these findings, $PGE_2$ level increased in sensitized rat groups in which high levels was observed in GLY treated rats. We don't have explanation on this unpredictable result we observed in our experiment. Despite the improvement of hypotension, plasma concentration of PGE2 was still high. Further studies are needed to understand the role of $PGE_2$ in anaphylactic shock.

Among the dissolved blood gas, $PaCO_2$ and $tCO_2$ levels were significantly reduced in all sensitized rats. This reduction may be related to the development of respiratory alkalosis. The low $PaCO_2$ levels in sensitized groups could also be attributed to other factors such as increased acidity from uncontrolled diabetes, kidney disease, metabolic disorders, or may be due to chronic mechanical hyperventilation. However, in our experiments none of the animals demonstrated any signs of illness during the experiment. The possible effect of metabolic disorders in these experimental animals could not be excluded as the sensitized animals did show respiratory alkalosis with reduced dissolved carbonate and bicarbonate level. However, levels of blood gas tension were within the range of observed values published previously (Goundasheva, 2000; Nemmar et al., 2010) and hence reduce the likelihood that mechanical hyperventilation influenced the values of our treated groups. The evaluation of oxygen metabolism during anaphylactic shock is not sufficient by arterial blood gas analysis. Dewachter et al. (2005) showed that anaerobic metabolism in skeletal muscle was associated with profoundly decrease of Ptio2 (tissue oxygen pressure) values. The severe decrease in PtiO2 during anaphylactic shock was associated with anaerobic metabolism without inhibition of the respiratory chain (Dewachter et al., 2005).

Finally, ions $Na^+$, $K^+$ and $Ca^{++}$ had significant changes depending on the treatment administered. Sodium was decreased significantly in sensitized groups. Among the sensitized groups, control and GLY groups showed no statistical difference but showed a significant difference in the EPI treatment. Although, the levels of extracellular $K^+$ measured in blood were higher in the sensitized group than the naïve group, we did not observe any statistical differences. The levels of $Ca^{++}$ were reduced in all sensitized rats as compared to un-sensitized naïve rats. However, the statistical difference was observed only between control and naïve group with mild increase in GLY and EPI groups suggesting that treatments could have indefinite influence.

## 5. Conclusion

In summary, the $K^+_{ATP}$-channel inhibitor GLY improved hemodynamics and had an effect on mediators release in response to murine anaphylactic shock. GLY exhibited a protective effect against hypotension and significantly increased survival. Thus, treatment with GLY may be a new approach in the treatment of patients with anaphylactic shock. Further studies are needed to confirm the effect of $K^+_{ATP}$ blocker such as GLY in human anaphylactic shock. The present study also provides evidence that Wistar rat is a suitable model for studying mechanisms of anaphylaxis and for testing new strategies of treatment.

## Author's contribution

Abdelouahab Bellou is the principal investigator of the study, obtained the grant, set the model and participated in the creation and written of the paper. Subramanian Dhanasekaran realized the experiments, collected data, analyzed the results and wrote the paper. Abderrahim Nemmar supervised blood analysis, interpreted the results, statistical studies and participated in the elaboration of the paper. Elhadi Aburawi did a review of the hemodynamics results of this study and participated in the elaboration of the paper. Elsadig E. Kazzam participated in the planning, budgeting and elaborating the discussions. Abdishakur Abdulle did the measurement of mediators and participated in the elaboration of the model. Moufida Bellou did all preliminary experiments before the current experiments started. She contributed in the elaboration of the paper and read carefully all sections.

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