4-Aminopyridine, A Blocker of Voltage-Dependent K^+ Channels, Restores Blood Pressure and Improves Survival in the Wistar Rat Model of Anaphylactic Shock

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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. Experiments were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee.

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Objectives: Anaphylactic shock is associated with severe hypotension. Potassium channel blockers, such as 4-aminopyridine, induce vasoconstriction. The objective of this study was to test the ability of 4-aminopyridine to restore blood pressure and increase survival in anaphylactic shock.

Design: Experimental study.

Setting: Physiology laboratory.

Subjects: Adult male Wistar rats.

Interventions: Rats were sensitized with ovalbumin (1 mg SC), and anaphylactic shock was induced by IV injection of ovalbumin (1 mg). Experimental groups included non-allergic rats (NA) ($n = 6$); allergic rats (Controls) ($n = 6$); allergic rats treated with 4-aminopyridine (4-aminopyridine) (1 mg/kg) ($n = 6$); and allergic rats treated with epinephrine (EPI) (10 µg/kg) ($n = 6$). Treatments were administered 1 minute after induction of anaphylactic shock.

Measurements and Main Results: Mean arterial blood pressure, heart rate, and survival were measured for 60 minutes. Plasma levels of histamine, leukotriene B_4, prostaglandin E_2, prostaglandin F_2, pH, and HCO_3 were measured. Mean arterial blood pressure was normal in the NA group; severe hypotension and high mortality were observed in controls; normalization of mean arterial blood pressure, heart rate, and increased survival were observed in 4-aminopyridine and EPI groups. All allergic 4-aminopyridine-treated rats survived after the induction of anaphylactic shock. Histamine level was higher in controls and the 4-aminopyridine group but reduced in the EPI group. Prostaglandin E_2 increased in controls and EPI group and decreased in 4-aminopyridine group;

prostaglandin F_2 increased in controls but decreased in 4-aminopyridine and EPI groups. Leukotriene B_4 decreased in 4-aminopyridine and EPI groups. Metabolic acidosis was prevented in the 4-aminopyridine group.

Conclusions: Our data suggest that voltage-dependent K+ channel inhibition with 4-aminopyridine treatment restores blood pressure and increases survival in the Wistar rat model of anaphylactic shock. 4-aminopyridine or related voltage-dependent K^+ channel blockers could be a useful additional therapeutic approach to treatment of refractory anaphylactic shock. (*Crit Care Med* 2016; XX:00–00)

Key Words: 4-aminopyridine; anaphylactic shock; histamine; leukotrienes; prostaglandins; voltage-dependent K^+ channel

Prevalence of allergy has increased dramatically in recent years (1, 2). It has been reported that 2–3% of the population will experience anaphylaxis during their lifetime (3–10). Anaphylaxis is frequently managed in prehospital settings or emergency departments (11–13).

Anaphylactic shock (AS) often results from an immunoglobulin E (IgE)-mediated systemic allergic reaction and is characterized by vasodilation and vascular leakage, causing a rapid, precipitous and sustained decrease in systemic arterial blood pressure (SABP) with a concomitant decrease of cardiac output (13–16). Anaphylaxis-induced decrease in SABP appears to be secondary to arterial vasodilation (14). Hypovolemia secondary to microcirculatory leak contributes to the onset of hypotension (17). The capillary leakage becomes evident within 5 minutes after AS induction (18).

Hypotension observed during AS is attributed to the action of released mast cell and basophile mediators. The principal allergic mediators released are histamine, tryptase, chymase, carboxy-peptides A, prostanoids, and eicosanoids, predominantly leukotriene B_4 (LTB_4), leukotriene C_4, prostaglandin E_2 (PGE_2), prostaglandin F_2 (PGF_2), and prostaglandin D_2 (19, 20). These mediators act through G protein-coupled receptors (20, 21). Suppression of the Gq/G11-mediated signaling pathway in the endothelium blocks nitric oxide (NO) production and damages the endothelial barrier (22). Activation of the sphingosine-1-phosphate receptor 2 (S1P2) inhibits Rac through G12/13 (23). S1rp2$^{-/-}$ mice exhibited exaggerated vascular leakage and hypotension (23). The protective effect of S1P2 is associated with reduced activation of NO synthase (NOS) type 2. Evidence of NO involvement in AS has been reported, mostly in mice (23–25). In contrast, the NOS inhibitor, L-NAME, is detrimental in rats (26, 27).

AS-associated vasodilation secondary to allergen-induced mediator release could also be mediated by vascular K^+ channels. We have previously shown that hypotension and survival in a rat model of AS improved significantly after postchallenge treatment with the K_{ATP} blocker, glyburide (28). K^+ channels identified in the vasculature include large conductance Ca^{2+}-activated (BKCa), intermediate conductance Ca^{2+}-activated, small conductance Ca^{2+}-activated, adenosine triphosphate (ATP)-sensitive (K_{ATP}), voltage-gated (Kv), and inward rectifier (see [29] for review). The two most abundant K^+ channels of vascular smooth muscle are the Kv and BKCa channels (29). The Kv channel blocker 4-aminopyridine has been approved in Europe and the United States under the names dalfampridine and fampridine for the treatment of multiple sclerosis.

Here, we report the first study exploring the effect of 4-aminopyridine administration in AS. The hypothesis tested in this study holds that the inhibition of voltage-dependent K^+ channels with 4-aminopyridine in the Wistar rat (Charles River Institute, Elphinstone, UK) model of AS can improve hypotension and survival.

MATERIAL AND METHODS

Animals and Immunization Protocol

Experiments were performed using 4-week-old male Wistar rats (body weight of 250 ± 15 g,) housed in groups of four in polypropylene cages with a 12-hour light-dark cycle at 24–26°C and ad libitum food and water. After 1-week acclimatization, rats were divided among four groups of six rats each. Ovalbumin (1 mg) was dissolved in a suspension of aluminum hydroxide (Al OH, 3.5 mg) in 1 mL of 0.9% sterile normal saline. The ovalbumin-Al OH suspension (1 mL) was injected subcutaneously at subscapular sites of rats on days 0, 5, and 14. Naive rats received only Al OH.

Treatment Groups

The effect of 4-aminopyridine was studied in sensitized rats after induction of AS by IV bolus injection of ovalbumin. The four groups of rats were as follows: NA, non-sensitized rats receiving no treatment; controls, sensitized rats treated with normal saline, IV bolus, 1 minute after AS induction; 4-aminopyridine, sensitized rats treated with 4-aminopyridine (1 mg/kg, IV bolus) 1 minute after shock induction; EPI, sensitized rats treated with epinephrine (IV bolus, 10 µg/kg at 1 and 10 min after AS induction). All groups were perfused with normal saline at 2 mL/hr IV throughout the experimental period following surgery.

Hemodynamic and AS Protocol

One week after the last immunization, rats (7 wk old) were anesthetized with pentobarbital sodium solution (62.5 mg/kg) administered intraperitoneally. The tracheas were surgically cannulated for artificial ventilation through an endotracheal tube attached to a constant-volume ventilator (Harvard Apparatus, Edenbridge, United Kingdom). The ventilator was set for a respiratory rate of 60 breaths/min, a tidal volume of 6 mL/kg body weight, and an end-expiratory pressure of 5 cm H_2O, at 100% inspired oxygen. Body temperature was maintained at 37°C by temperature-controlled thermo-blanket (Harvard Apparatus, South Natick, MA). One catheter (PE10 tubing) was placed in the left jugular vein, connected to a slow injection/infusion pump (Harvard Apparatus) for IV treatment. Another catheter was placed in the left carotid

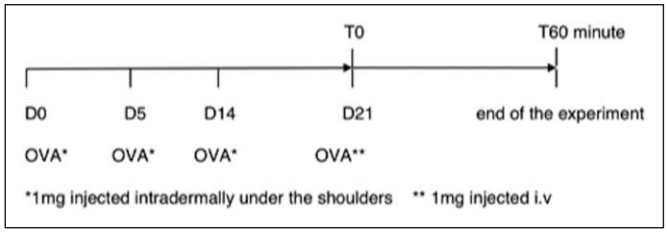
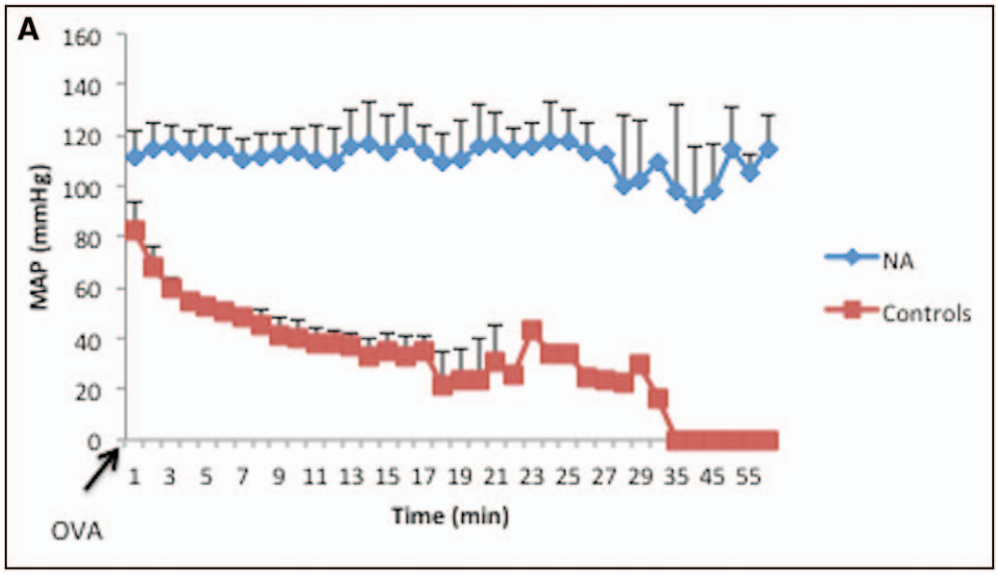


Figure 1. Design of the experiment. Rats were sensitized with ovalbumin (OVA) by three ID injections on day 0 (D0), day 5 (D5), and day 14 (D14). On day 21 (D21), rats were prepared for hemodynamic study by recording mean arterial blood pressure (MAP) and heart rate (HR) through a carotid artery catheter placed after anesthesia. Anaphylactic shock was induced by an IV bolus of OVA administered via jugular vein catheter. Hemodynamic parameters (MAP and HR) were recorded every minute for 30 min, and then every 5 min for 30 additional minutes. Total duration of the experiment post-4-aminopyridine administration was 60 min. NMDA= N-methyl-D-aspartate receptor.

artery, connected to the blood pressure module of the PowerLab (AD Instruments, Bella Vista, 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 widely used in the experiments in direct blood pressure recording has no detectable effect on cerebral blood flow. Normal saline (0.9%) was infused IV at 2 mL/hr by infusion pump (Harvard Apparatus) to compensate for estimated intra surgical fluid loss.

After the surgical procedures, a 30-minute stabilization period was allowed before measurement. The ovalbumin challenge consisted in 1 mL IV of ovalbumin followed by the constant infusion of normal saline. Treatment was administered IV as single bolus and the hemodynamic parameters (MAP and HR) were monitored. Measurement of hemodynamic parameters was performed every 5 minutes for a period of 30 minutes before the ovalbumin challenge. After the stabilization period, ovalbumin challenge was started, and IV treatments were administered 60 seconds later. The hemodynamic parameters were recorded for 60 minutes at 1 minute intervals.



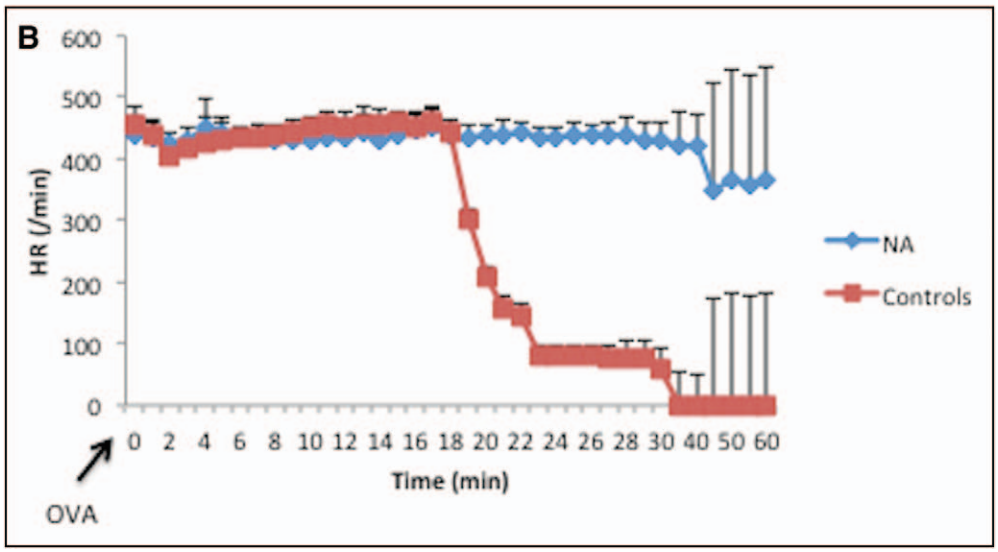


Figure 2. Time course of mean arterial blood pressure (MAP, mm Hg) (**A**) and heart rate (HR, beats/min) (**B**) after IV bolus of ovalbumin (OVA) in non-allergic rats (NA, ♦) and allergic rats (controls, ■). MAP and HR were recorded every minute until 30 min post-OVA injection, then every 5 min until the end of the experiment (at 60 min). Each point represents the mean (± SEM) of six rats. The difference between NA and control rats was significant ($p < 0.0005$, two-way analysis of variance).

Blood Collection

Whole blood samples were withdrawn from the carotid artery and immediately transferred to heparinized tubes when MAP fell to 25 mm Hg, defined as the end of the experiment, and corresponding to shock; or no later than 1 hour after ovalbumin injection. About 200 µL of the blood was used immediately for the determination of blood gas and electrolyte parameters. The remaining blood was centrifuged at 3,000*g* at 4°C for 15 minutes to separate the plasma. The separated plasma was stored in –80°C until used for further enzyme-linked immunosorbent assay (ELISA).

Determination of Plasma Concentration of Histamine, PGE_2, PGF_2, LTB_4

Plasma samples from treated or untreated animals either from naive or sensitized groups were used for ELISA measurements of concentrations of histamine, LTB_4, PGE_2, and PGF_2 (Cayman Chemical, Ann Arbor, MI).

Measurement of Blood Gas

Arterial blood collected anaerobically as described above (0.5 mL) was rapidly subjected to analysis of blood gases. $Paco_2$, Pao_2, bicarbonates (Hco_3^-), and pH were measured using the Osmetech OPTI Sensor Cassette, Type "E-Ca," BP7560, in the OPTI CC-TS device (Osmetech, Critical Care division, Roswell, GA), as suggested by the manufacturer, with recalibration using a standard reference cassette (level 1: BP7536 and level 3: BP7543) each day before assay of test samples, or after every on/off cycle.

Statistical Analysis

Results are expressed as the mean ± SEM. Statistical significance was measured using two-way analysis of variance (ANOVA) (e.g., naive × treatment; time as a repeated measure when needed) and Bonferroni comparisons of MAP and HR experiments. In other experiments, one-way ANOVA with Newman-Keuls comparisons was used to measure statistical significance. p value less than 0.05 was considered significant (SPSS 9.0 Advanced Models software, Chicago, IL). Group survival portrayed by Kaplan-Meier curves was compared by log rank test.

RESULTS

Rats were sensitized with ovalbumin administered at 0, 5, and 14 days prior to hemodynamic assessment. On day 21, animals were prepared for surgical intervention and induction of AS with one IV bolus of 1 mg of ovalbumin. **Figure 1** summarizes the design of the experimentation.

Evolution of MAP and HR After Induction of AS With Ovalbumin

MAP and HR were monitored in NA and controls (**Fig. 2**). All treatment groups' data were compared statistically with NA and controls at all time points until 60 minutes. In NA, MAP and HR were stable and unchanged throughout the 60 minutes duration of the experiment. In controls, MAP decreased by 66% within 5 minutes post ovalbumin challenge, and by 87% within 30 minutes (Fig. 2). This hypotension was significantly greater in controls when compared with NA at all time points from 5 minutes onward ($p < 0.0001$), as was the difference in the fall in MAP between controls and 4-aminopyridine group ($p < 0.0001$) (**Fig. 3**). In the 4-aminopyridine group, MAP post ovalbumin injection decreased by 29% at 5 minutes (vs 66% in controls), 24% at 30 minutes (vs 87% in controls), and 22% at 60 minutes (vs 100% in controls). After an initial decline in MAP in the 4-aminopyridine group, MAP stabilized, then slowly returned to match MAP values of NA. This rise of MAP in the 4-aminopyridine group was significant when compared with controls ($p<0.0001$).Epinephrine-treated

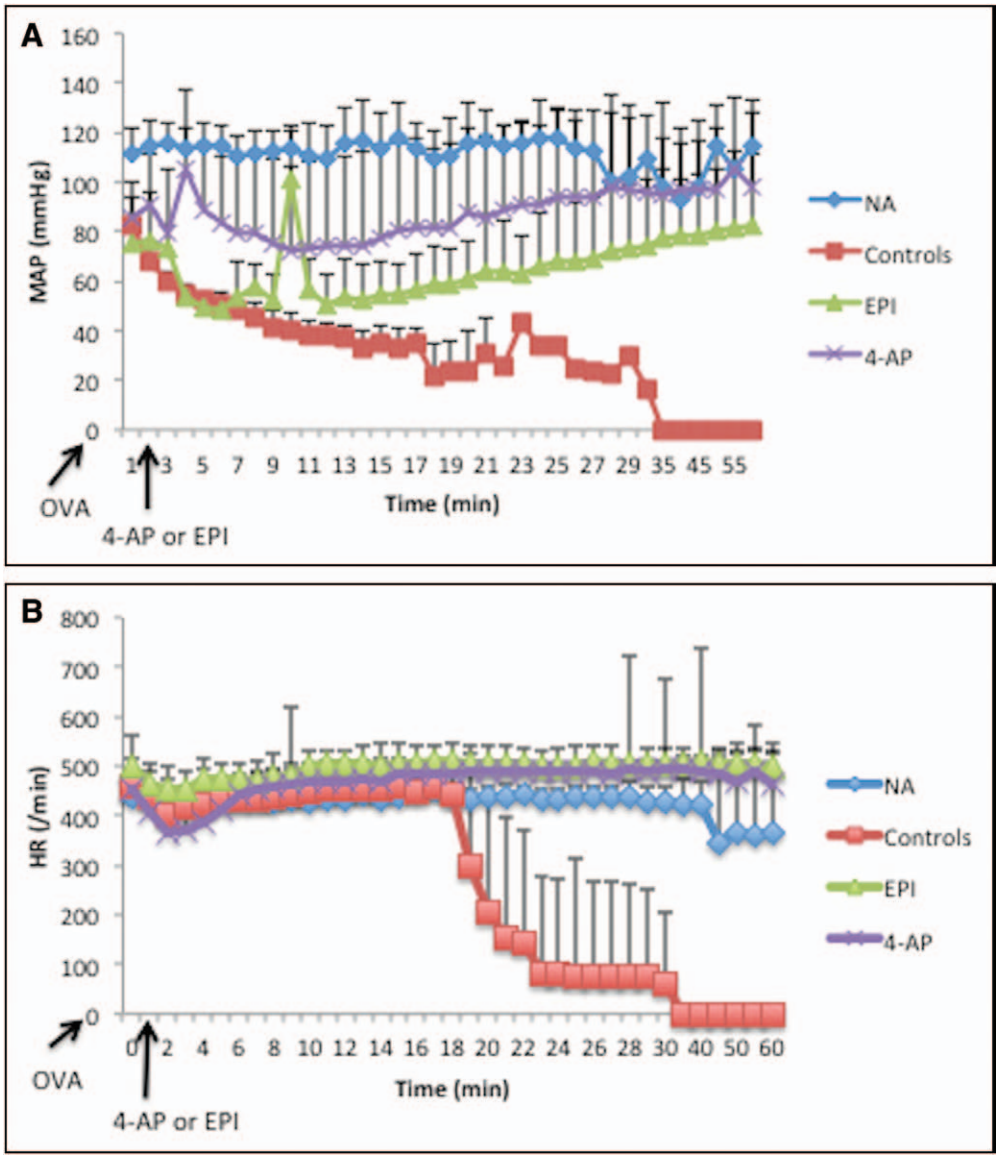


Figure 3. Effect on mean arterial blood pressure (MAP, mm Hg) (**A**) and heart rate (HR, beats/min) (**B**) of 4-aminopyridine (AP, ×) or of epinephrine (EPI, ▲) administered 1 min after induction of anaphylactic shock with IV ovalbumin (OVA). MAP and HR were recorded every minute until 30 min post-OVA injection, then every 5 min until the end of the experiment at 60 min. 4-AP and EPI groups were compared to non-allergic rats (NA, ♦) and allergic rats (control groups, ■). Each point represents the mean (± SEM) of six rats. The difference between 4-AP and EPI and NA and control rats was significant ($p < 0.0005$, two-way analysis of variance).

group followed the same pattern as the 4-aminopyridine group but with MAP values consistently lower than those of the 4-aminopyridine group. Thus, at 5 minutes post ovalbumin challenge, EPI group MAP decreased by 62% versus 29% in the 4-aminopyridine group. In controls, HR showed a marked drop from 457 ± 37.3/min to 207.3 ± 231.4/min within 20 minutes after ovalbumin injection, and differed significantly from the NA at 25 minutes post ovalbumin injection. After an initial post ovalbumin fall in HR in all groups, HR began to rise within 1 minute postinjection of 4-aminopyridine, and normalized by 6 minutes post-4-aminopyridine injection, remaining stable thereafter. HR did not differ between EPI and 4-aminopyridine groups.

Five of six non-allergic rats were alive at 60 minutes, with an average survival time of 45 minutes. In contrast, no controls remained alive at 60 minutes, and average survival time was 23 minutes. All 4-aminopyridine and EPI rats remained alive at 60 minutes. The Kaplan-Meier curves confirmed that 4-aminopyridine treatment of allergic rats improved survival when compared with that of controls ($p = 0.0004$) (**Fig. 4**).

Effect of Ovalbumin on Histamine Release in AS

The control group rats showed a significant rise in histamine level when compared with the NA, representing the effect of ovalbumin on mast cells (**Fig. 5*A***). Among sensitized treated rats, the EPI group showed significant reduced elevation in histamine levels compared to the controls. Histamine levels were not decreased in the 4-aminopyridine group.

Effect of Ovalbumin on PGE_2 and PGF_2 Release in AS

The plasma levels of PGE_2 in the NA and controls were indistinguishable ($p = 0.68$), but PGE_2 levels in the 4-aminopyridine group differed compared with the control group ($p = 0.0005$) (**Fig. 5*B***). PGE_2 levels were unchanged in the EPI group ($p = 0.233$). Plasma levels of PGF_2 significantly decreased in 4-aminopyridine and EPI groups when compared with controls ($p = 0.0005$) (**Fig. 5*C***).

Effect of Ovalbumin on LTB4 Release in AS

LTB_4 plasma levels in the 4-aminopyridine and EPI groups showed a significant reduced elevation compared with controls (**Fig. 5*D***).

Blood Gas Changes in Ovalbumin-Induced AS

The metabolic acid–base status was assessed by measurement of arterial blood pH and plasma concentration of HCO_3^-. Controls exhibited severe metabolic acidosis after AS induction (HCO_3^-, 8.48 ± 4.7; pH, 7.2 ± 0.19), reflecting AS-associated tissue hypoperfusion. In contrast, metabolic acidosis was significantly improved in 4-aminopyridine-treated rats (HCO_3^-, 17 ± 4.9; pH, 7.44 ± 0.07) ($p = 0.016$ vs controls) (**Fig. 5**, ***E*** and ***F***).

DISCUSSION

Severe AS was induced in our rat model of AS. Acute IV challenge with ovalbumin induced severe hypotension and bradycardia, leading to the death of all allergic untreated rats. This model of a severe type of AS, observed in humans, allows exploration of clinically relevant pathophysiologic mechanisms in AS. It is well known that mediators released after allergen-activation of mast cells or basophils are key factors in the clinical manifestations of AS. However, the mechanism of action of these mediators remains incompletely understood. The role of NO in AS is still debated. NO is produced in endothelial cells after the binding of anaphylactic mediators on mast or basophil cells and seems to be involved in the vasodilation of vascular smooth muscle cells (VSMC) (24). NO causes vasodilation by activating guanylyl cyclase, increasing the concentration of cyclic guanosine monophosphate in smooth muscle, and inducing relaxation of VSMC.

Our data suggest that K^+ channel activation also contributes to the induction of AS. In our model, administration of the Kv inhibitor 4-aminopyridine restored blood pressure, HR, and improved survival. 4-aminopyridine blocked the AS-associated fall in blood pressure and accelerated blood pressure recovery to pre ovalbumin challenge values. These findings suggest that 4-aminopyridine protects against the cardiovascular effects of AS.

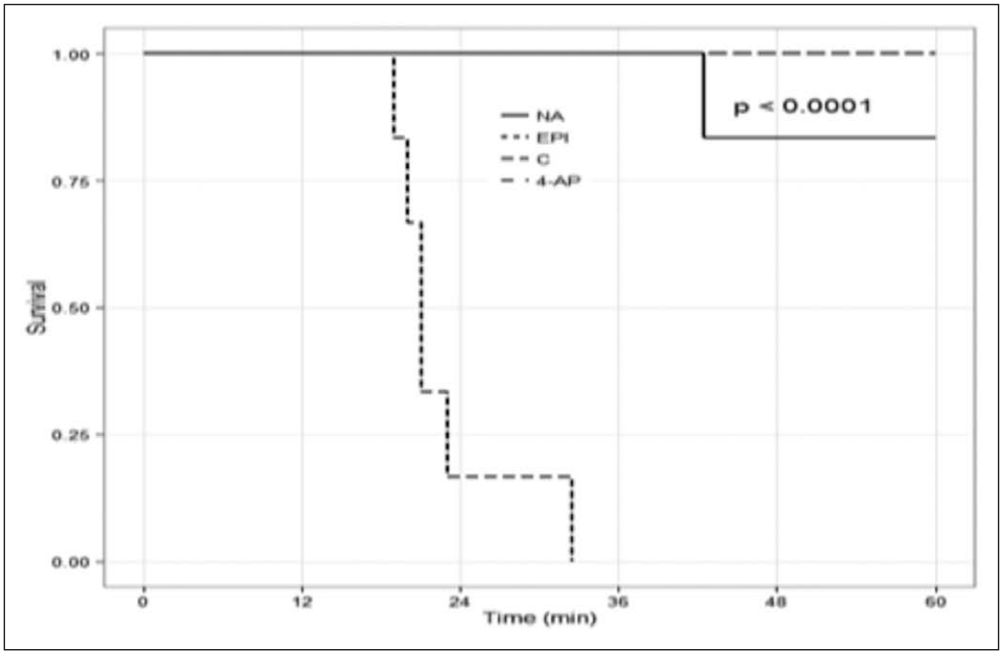


Figure 4. Kaplan-Meier curve. Analysis of survival time after IV administration of ovalbumin (OVA) to non-allergic (NA), allergic rats (control group [C]), 4-aminopyridine (AP)-, and epinephrine (EPI)-treated groups. Survival time was measured during 60 min after IV bolus of OVA. Groups were compared with Log Rank (Mantle-Cox) test. The difference was significant when *p* value less than 0.05.

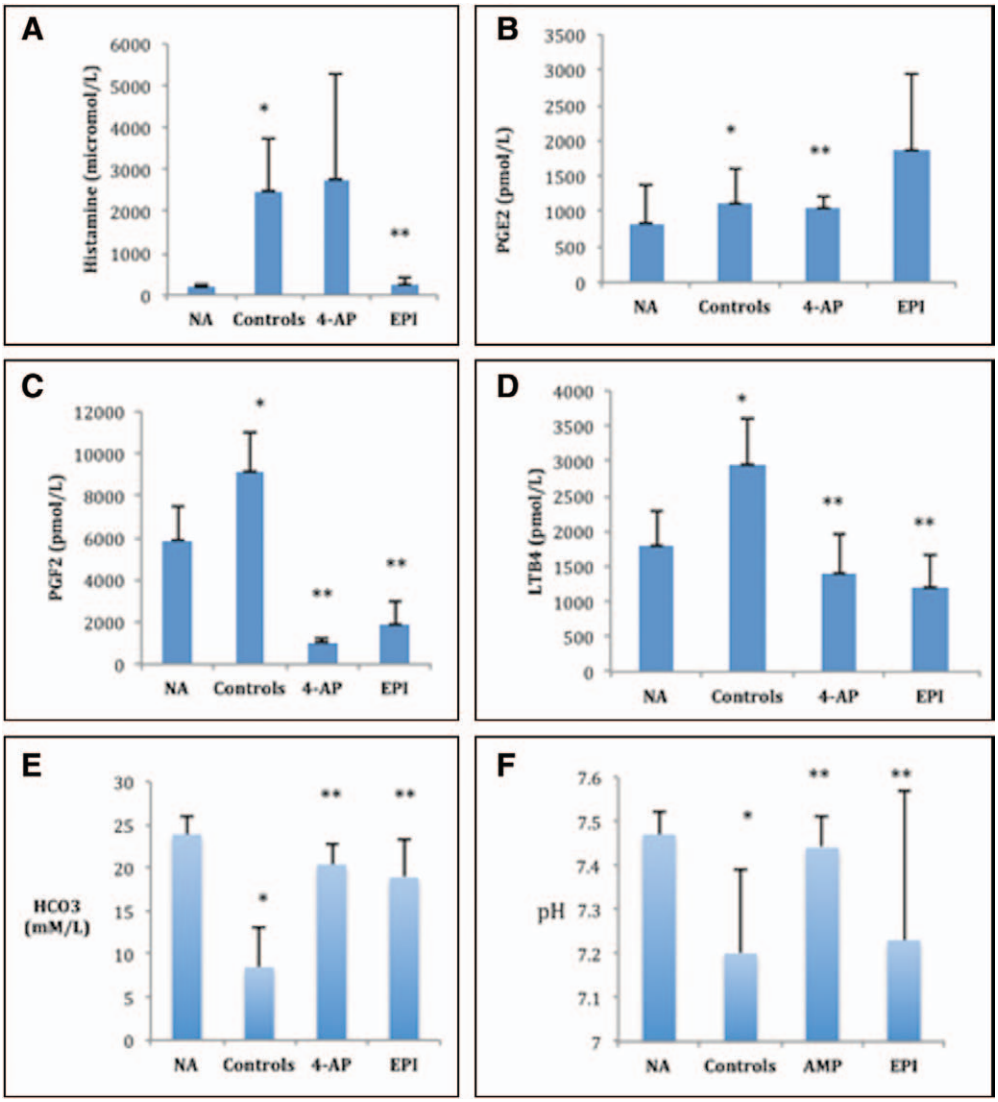


Figure 5. Plasma concentrations of histamine (**A**), prostaglandin E2 (PGE_2) (**B**), prostaglandin F2 (PGF_2) (**C**), leukotriene B4 (LTB_4) (**D**) and arterial blood levels of Hco_3^- (**E**) and pH (**F**) after injection of ovalbumin in non-allergic rats (NA), allergic rats (controls), and rats treated with 4-aminopyridine (4-AP or AMP) or epinephrine (EPI). Values are expressed as means (± SEM) for six rats in each group. $^{**}p < 0.05$, 4-AP or EPI versus controls.

One explanation of 4-aminopyridine's salutary effect on hypotension could be the blockade of hyperpolarization induced by anaphylactic mediators in endothelial cells. Increasing evidence shows involvement of K^+ channels in the development of sepsis-associated systemic vasodilatation and hyporesponsiveness to vasoconstrictors (30–32). Endothelial K^+ channels have been implicated in endothelium-dependent vasodilation (33). Endothelial cell hyperpolarization secondary to K^+ channel activation facilitates Ca^{2+} influx by increasing the inward driving force for Ca^{2+} (33), and enhances the Ca^{2+}-dependent increase in endothelium-dependent vasorelaxants NO and prostacyclin (33). Hyperpolarization propagates to VSMC or may induce endothelial release of factors that activate hyperpolarizing K^+ channels of VSMC, leading to VSMC relaxation and vasodilation.

4-aminopyridine has been used in many VSMC studies for pharmacologic isolation of Kv current from BKCa current, also activated by membrane depolarization (34–36). Kv channel blockade can depolarize VSMC promoting Ca^{2+} influx and activating actin-myosin contraction and vasoconstriction. Kv channels are a tetramer of Kv α-subunits forming an ion conductive pore, associated with KV β accessory subunits (37, 38). More than 30 genes encoding several subfamilies of Kv α-subunits have been described (39), and VSMC express members of the Kv 1.5 and 1.6 families (37–39). Kv channel blockers such as 4-aminopyridine (33, 37, 38) potentiate vasoconstrictor-induced depolarization of arteriolar smooth muscle. Kv blockade can also directly depolarize VSMC, increasing intracellular Ca^{2+} entry via L-type Ca^{2+} voltage-dependent channels to promote vasoconstriction and prevent AS-induced hypotension. Among several types of K^+ channel inhibitors tested, only 4-aminopyridine evoked a marked, concentration-dependent vasoconstriction, consistent with the dominant role of Kv channels in isolated perfused rat mesenteric artery (40). Inhibition of Kv channel-mediated endothelial cell hyperpolarization might reduce the driving force for endothelial cell Ca^{2+} entry through store-operated and ligand-operated Ca^{2+}-permeable cation channels, thus decreasing intracellular [Ca^{2+}] and blunting NOS activation and NO production. However, in our model, we observed no beneficial effect of L-NAME, a blocker of NOS (results not shown).

The effect of 4-aminopyridine on mediator release was unexpected. However, elevation of plasma histamine was not altered by 4-aminopyridine, consistent with the effectively instantaneous release of preformed histamine from mast cells in response to allergen activation of the IgE-Fcε receptor I complex. Thus, our delayed administration of 4-aminopyridine in the rat-ovalbumin model of AS could not block ovalbumin-triggered mast cell release of preformed mediators such as histamine. In contrast, the effect of 4-aminopyridine on PGE_2, PGF_2, and LTB_4 was remarkable. Both 4-aminopyridine and EPI decreased release of these mediators generated

de novo from membrane phospholipids. The release of these mediators involved in AS-associated vasodilation and vascular leakage is delayed when compared with histamine release. Nevertheless, we can postulate that the effects of second-wave anaphylactic mediators released during the development of AS in our model might themselves be regulated by Kv channel block. Prostaglandins and leukotrienes may also influence cardiac contractility by reducing myocardial perfusion (15), contributing to myocardial depression and impaired ventricular function, and so potentiating hemodynamic failure in AS. In our model, the severe bradycardia observed in controls disappeared after 4-aminopyridine treatment. Finally, AS-associated hypotension was corrected and AS-associated metabolic acidosis was prevented by 4-aminopyridine posttreatment.

Our model has limitations. In the absence of measured plasma levels of angiotensin II and vasopressin, we cannot exclude the compensatory roles of hypotension-induced vasoconstrictor release. Indeed, angiotensin II and vasopressin levels are increased in both early and recovery stages of AS in a rat model (41), in which initiation of blood pressure recovery and vasoconstrictor release were both detected within 30 minutes postallergen challenge, and progressed in parallel during the 120 minutes duration of that experiment. In contrast, recovery from AS in our model occurred only in 4-aminopyridine-treated rats; all control rats died quickly without evidence of recovery. Thus, compensatory increases in vasoconstrictor levels do not suffice to explain 4-aminopyridine-induced blood pressure recovery in our model. We therefore postulate that the main mechanism for blood pressure recovery is Kv channel inhibition by 4-aminopyridine.

Although our data do not allow unique attribution of the effects of 4-aminopyridine to inhibition of Kv channels, previous in-vitro studies show that 4-aminopyridine selectively inhibits Kv channels in VSMC (42), exhibiting different affinities for Kv1.X subunits and for Kv2.X channel family. Further experiments will be needed to identify the specific Kv channel subtype mediating the effect of 4-aminopyridine in our model. We chose to study 4-aminopyridine at 1 mg/kg, guided by Food and Drug Administration dosage recommendations for multiple sclerosis. Laci et al (43) showed that 0.5 and 2 mg/kg improved sensorimotor responses, but optimal 4-aminopyridine dosage and dosing schedule for potential treatment of AS with minimal side effects remain to be determined. Finally, 4-aminopyridine can be proconvulsant, as shown by case reports in the setting of 4-aminopyridine overdoses (44). Seizures have been associated with high systemic doses or high plasma 4-aminopyridine concentrations. However, 4-aminopyridine-induced seizure activity in experimental animals has been successfully treated with leptin, with NMDA receptor inhibitors, and by isovaline (45). Moreover, 4-aminopyridine derivatives with reduced blood-brain barrier permeability are under development (46). Although we observed no apparent arrhythmias during the 60-minute period postinjection of 4-aminopyridine, cardiac toxicity has been reported in the setting of 4-aminopyridine overdose (47).

CONCLUSION

We have previously demonstrated that administration of a K^+_{ATP} channel blocker improves hypotension and survival in the rat-ovalbumin AS model (28). The present study demonstrates for the first time that the Kv channel blocker 4-aminopyridine dramatically ameliorates hypotension induced by ovalbumin in the rat model of AS and improved survival. Although epinephrine remains the gold standard of AS treatment (48), our current data support the proposal of Kv channels as a potential therapeutic target in the treatment of AS. Severe vasodilation resistant to epinephrine (adrenaline) has been described (49). We propose that 4-aminopyridine treatment might be considered as an alternative treatment in severe AS unresponsive to epinephrine.

REFERENCES

1. Holt PG. Atopic allergy and other hypersensitivities. The aetiology and pathogenesis of allergic disease: New insights and new challenges. *Cur Opin Immunol* 1998; 10:605–606
2. De Bisschop MB, Bellou A: Anaphylaxis. *Curr Opin Crit Care* 2012; 18:308–317
3. Neugut AI, Ghatak AT, Miller RL: Anaphylaxis in the United States: An investigation into its epidemiology. *Arch Intern Med* 2001; 161:15–21
4. Decker WW, Campbell RL, Manivannan V, et al: The etiology and incidence of anaphylaxis in Rochester, Minnesota: A report from the Rochester Epidemiology Project. *J Allergy Clin Immunol* 2008; 122:1161–1165
5. Poulos LM, Waters AM, Correll PK, et al: Trends in hospitalizations for anaphylaxis, angioedema, and urticaria in Australia, 1993-1994 to 2004-2005. *J Allergy Clin Immunol* 2007; 120:878–884
6. González-Pérez A, Aponte Z, Vidaurre CF, et al: Anaphylaxis epidemiology in patients with and patients without asthma: A United Kingdom database review. *J Allergy Clin Immunol* 2010; 125:1098–1104.e1
7. Lin RY, Anderson AS, Shah SN, et al: Increasing anaphylaxis hospitalizations in the first 2 decades of life: New York State, 1990-2006. *Ann Allergy Asthma Immunol* 2008; 101:387–393
8. Liew WK, Williamson E, Tang ML: Anaphylaxis fatalities and admissions in Australia. *J Allergy Clin Immunol* 2009; 123:434–442
9. Gupta RS, Springston EE, Warrier MR, et al: The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics* 2011; 128:e9–e17
10. Liu AH, Jaramillo R, Sicherer SH, et al: National prevalence and risk factors for food allergy and relationship to asthma: Results from the National Health and Nutrition Examination Survey 2005-2006. *J Allergy Clin Immunol* 2010; 126:798–806.e13
11. Bellou A, Manel J, Samman-Kaakaji H, et al: Spectrum of acute allergic diseases in an emergency department: An evaluation of one years' experience. *Emerg Med (Fremantle)* 2003; 15:341–347
12. Gaeta TJ, Clark S, Pelletier AJ, et al: National study of US emergency department visits for acute allergic reactions, 1993 to 2004. *Ann Allergy Asthma Immunol* 2007; 98:360–365
13. Nolan JP, Soar J, Zideman DA, et al; ERC Guidelines Writing Group: European Resuscitation Council Guidelines for Resuscitation 2010 Section 1. Executive summary. *Resuscitation* 2010; 81:1219–1276
14. Brown SG: Cardiovascular aspects of anaphylaxis: Implications for treatment and diagnosis. *Curr Opin Allergy Clin Immunol* 2005; 5:359–364
15. Triggiani M, Patella V, Staiano RI, et al: Allergy and the cardiovascular system. *Clin Exp Immunol* 2008; 153(Suppl 1):7–11
16. Helbling A, Hurni T, Mueller UR, et al: Incidence of anaphylaxis with circulatory symptoms: A study over a 3-year period comprising 940,000 inhabitants of the Swiss Canton Bern. *Clin Exp Allergy* 2004; 34:285–290

17. Fisher MM: Clinical observations on the pathophysiology and treatment of anaphylactic cardiovascular collapse. *Anaesth Intensive Care* 1986; 14:17–21
18. Faye N, Fournier L, Balvay D, et al: Macromolecular capillary leakage is involved in the onset of anaphylactic hypotension. *Anesthesiology* 2012; 117:1072–1079
19. Lewis RA, Austen KF: The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. *J Clin Invest* 1984; 73:889–897
20. Sendo T, Sumimura T, Itoh Y, et al: Involvement of proteinase-activated receptor-2 in mast cell tryptase-induced barrier dysfunction in bovine aortic endothelial cells. *Cell Signal* 2003; 15:773–781
21. Brown SL, Jala VR, Raghuwanshi SK, et al: Activation and regulation of platelet-activating factor receptor: Role of G(i) and G(q) in receptor-mediated chemotactic, cytotoxic, and cross-regulatory signals. *J Immunol* 2006; 177:3242–3249
22. Korhonen H, Fisslthaler B, Moers A, et al: Anaphylactic shock depends on endothelial Gq/G11. *J Exp Med* 2009; 206:411–420
23. Cui H, Okamoto Y, Yoshioka K, et al: Sphingosine-1-phosphate receptor 2 protects against anaphylactic shock through suppression of endothelial nitric oxide synthase in mice. *J Allergy Clin Immunol* 2013; 132:1205–1214.e9
24. Cauwels A, Janssen B, Buys E, et al: Anaphylactic shock depends on PI3K and eNOS-derived NO. *J Clin Invest* 2006; 116:2244–2251
25. Olivera A, Eisner C, Kitamura Y, et al: Sphingosine kinase 1 and sphingosine-1-phosphate receptor 2 are vital to recovery from anaphylactic shock in mice. *J Clin Invest* 2010; 120:1429–1440
26. Bellou A, Lambert H, Gillois P, et al: Constitutive nitric oxide synthase inhibition combined with histamine and serotonin receptor blockade improves the initial ovalbumin-induced arterial hypotension but decreases the survival time in brown Norway rats anaphylactic shock. *Shock* 2003; 19:71–78
27. Mitsuhata H, Saitoh J, Hasome N, et al: Nitric oxide synthase inhibition is detrimental to cardiac function and promotes bronchospasm in anaphylaxis in rabbits. *Shock* 1995; 4:143–148
28. Dhanasekaran S, Nemmar A, Aburawi EH, et al: Glyburide, a K(+)(ATP)channel blocker, improves hypotension and survival in anaphylactic shock induced in Wistar rats sensitized to ovalbumin. *Eur J Pharmacol* 2013; 720:166–173
29. Dora KA: Coordination of vasomotor responses by the endothelium. *Circ J* 2010; 74:226–232
30. Vanelli G, Hussain SN, Aguggini G: Glibenclamide, a blocker of ATP-sensitive potassium channels, reverses endotoxin-induced hypotension in pig. *Exp Physiol* 1995; 80:167–170
31. Gardiner SM, Kemp PA, March JE, et al: Regional haemodynamic responses to infusion of lipopolysaccharide in conscious rats: Effects of pre- or post-treatment with glibenclamide. *Br J Pharmacol* 1999; 128:1772–1778
32. Lange M, Szabo C, Van Aken H, et al: Short-term effects of glipizide (an adenosine triphosphate-sensitive potassium channel inhibitor) on cardiopulmonary hemodynamics and global oxygen transport in healthy and endotoxemic sheep. *Shock* 2006; 26:516–521
33. Yamanaka A, Ishikawa T, Goto K: Characterization of endothelium-dependent relaxation independent of NO and prostaglandins in guinea pig coronary artery. *J Pharmacol Exp Ther* 1998; 285:480–489
34. Okabe K, Kitamura K, Kuriyama H: Features of 4-aminopyridine sensitive outward current observed in single smooth muscle cells from the rabbit pulmonary artery. *Pflugers Arch* 1987; 409:561–568
35. Smirnov SV, Aaronson PI: Ca(2+)-activated and voltage-gated K+ currents in smooth muscle cells isolated from human mesenteric arteries. *J Physiol* 1992; 457:431–454
36. Nelson MT, Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 1995; 268:C799–C822
37. Cox RH, Petrou S: Ca(2+) influx inhibits voltage-dependent and augments Ca(2+)-dependent K(+) currents in arterial myocytes. *Am J Physiol* 1999; 277:C51–C63
38. Cheong A, Dedman AM, Beech DJ: Expression and function of native potassium channel [K(V)alpha1] subunits in terminal arterioles of rabbit. *J Physiol* 2001; 534:691–700
39. Kerr PM, Clément-Chomienne O, Thorneloe KS, et al: Heteromultimeric Kv1.2-Kv1.5 channels underlie 4-aminopyridine-sensitive delayed rectifier K(+) current of rabbit vascular myocytes. *Circ Res* 2001; 89:1038–1044
40. Sung DJ, Noh HJ, Kim JG, et al: Serotonin contracts the rat mesenteric artery by inhibiting 4-aminopyridine-sensitive Kv channels via the 5-HT2A receptor and Src tyrosine kinase. *Exp Mol Med* 2013; 45:e67
41. Wang M, Shibamoto T, Kuda Y, et al: Angiotensin II and vasopressin are involved in the defense system against anaphylactic hypotension in anesthetized rats. *Eur J Pharmacol* 2014; 731:38–43
42. Baeyens N, Bouryi V, Morel N: Extracellular calcium modulates the inhibitory effect of 4-aminopyridine on Kv current in vascular smooth muscle cells. *Eur J Pharmacol* 2014; 723:116–123
43. Iaci JF, Parry TJ, Huang Z, et al: Dalfampridine improves sensorimotor function in rats with chronic deficits after middle cerebral artery occlusion. *Stroke* 2013; 44:1942–1950
44. Pickett TA, Enns R: Atypical presentation of 4-aminopyridine overdose. *Ann Emerg Med* 1996; 27:382–385
45. Yu W, Smith AB, Pilitsis J, et al: Isovaline attenuates epileptiform activity and seizure behavior in 4-aminopyridine treated rats. *Epilepsy Res* 2014; 108:331–335
46. Mayorov AV, Willis B, Di Mola A, et al: Symptomatic relief of botulinum neurotoxin/a intoxication with aminopyridines: A new twist on an old molecule. *ACS Chem Biol* 2010; 5:1183–1191
47. King AM, Menke NB, Katz KD, et al: 4-aminopyridine toxicity: A case report and review of the literature. *J Med Toxicol* 2012; 8:314–321
48. Muraro A, Roberts G, Worm M, et al; EAACI Food Allergy and Anaphylaxis Guidelines Group: Anaphylaxis: Guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy* 2014; 69:1026–1045
49. Schummer W, Schummer C, Wippermann J, et al: Anaphylactic shock: Is vasopressin the drug of choice? *Anesthesiology* 2004; 101:1025–1027