

Research Article

Bradykinin-Induced Shock Increase Exhaled Nitric Oxide, Complement Activation and Cytokine Production in Pigs

Knut Fredrik Seip¹, Bjørg Evjenth², Anders Hovland³, Knut Dybvik⁴, Harald Thidemann Johansen⁵, Hilde Fure⁶, Tom Eirik Mollnes^{6,7} and Erik Waage Nielsen^{8*}

¹Department of Pharmaceutical Chemistry, University of Oslo, Norway

²Department of Pediatrics, Nordland Hospital, Norway

³Division of Internal Medicine, University of Tromsø, Norway

⁴Department of Anesthesiology, University of Nordland, Norway

⁵Department of Pharmaceutical Biosciences, Norway

⁶Research Laboratory, Nordland Hospital, Norway

⁷Department of Clinical Medicine, University of Tromsø, Norway

⁸Department of Anesthesiology, University of Nordland, Norway

*Corresponding author

Erik Waage Nielsen, Department of Anesthesiology, University of Nordland, Nordland Hospital, Prinsens gate 8095 Bodø, Norway, Tel: 47-90788035; Email: erikwn@me.com

Submitted: 25 January 2016

Accepted: 02 March 2016

Published: 04 March 2016

Copyright

© 2016 Nielsen et al.

OPEN ACCESS

Keywords

- Nitric oxide
- Bradykinin
- Macrophage
- Endothelial cell
- Plasma leakage
- Complement activation
- Coagulation
- Cytokines
- Porcine

Abstract

Bradykinin is an important mediator in blood pressure regulation, ischemic precondition and capillary leakage, allergy, anaphylaxis, inflammation, and nociception, at least partly via the generation of nitric oxide (NO). Macrophages are particularly abundant in the porcine lung circulation. Upon bradykinin binding macrophages release cytokines and endothelial cells increase plasma leakage. Both cells produce NO. The complement, hemostatic, fibrinolytic and kinin plasma cascade systems crosstalk and interacts with many inflammatory systems. In the present study we investigated the effect of the shock induced by intravenously infused bradykinin on the cascade systems, cytokines, plasma leakage and exhaled NO in pigs. The metabolite of bradykinin, BK1-5, was measured in plasma by a sensitive, specific and reliable liquid chromatography-mass spectrometry method to verify exposure and *in vivo* metabolism of bradykinin. We show for the first time *in vivo* how bradykinin exposure induced shock and increased exhaled NO, activated complement and hemostasis and induced cytokine production and capillary leakage. The results broaden our understanding of how bradykinin activates endothelial cells and macrophages to induce shock and inflammation. This should encourage further studies.

INTRODUCTION

Bradykinin is a potent nine amino acid short peptide generated from kininogen in plasma. Bradykinin is involved in blood pressure regulation, capillary leakage and ischemic preconditioning of the heart [1]. Bradykinin also mediates allergy, anaphylaxis, inflammation and nociception [2]. Bradykinin itself has an extremely short half-life (<30 s) due to breakdown by angiotensin converting enzyme (ACE) and other exopeptidases present in plasma. It is therefore difficult to detect *in vivo* [3]. The kinin system, the intrinsic pathway of coagulation, the complement system and the fibrinolytic system all cross-talk [4]. Macrophages and endothelial cells release nitric oxide (NO) and bradykinin markedly increase NO from endothelial cells [5]. Ovine and porcine lung parenchyma have large amounts

of macrophages in their circulation, and the macrophage densities are similar to that of Kupfer cells in the rat liver [6]. More than 25 years ago, bradykinin and its metabolite des Arg-bradykinin were shown to stimulate murine macrophages to release tumor necrosis factor (TNF) and interleukin-1 (IL-1) [7]. The quantitative relation between bradykinin and its effects on circulation and inflammation are largely unknown in all species. Therefore, we infused pigs with bradykinin to obtain shock and investigated the effects of bradykinin on exhaled NO, the plasma cascades and inflammation.

MATERIALS AND METHODS

Study design

In this porcine study we administered bradykinin in three

different ways. Animal A was given a continuing and steadily increasing infusion of bradykinin with bolus doses late in the experiment. Animal B was exposed to a high bolus dose of bradykinin early in the experiment. Animal C was pretreated with 1.5 mg captopril (ACE-inhibitor) prior to infusion of bradykinin. In all the pigs, the infusion of bradykinin was adjusted to maintain a critically low mean arterial pressure (MAP) of 40 mmHg.

Animals

Three Norwegian Landrace pigs of either sex (23 kg ±2) were treated in accordance with the Norwegian laboratory animal regulations and the study was approved by the Norwegian Animal Research Authority (FOTS #2843). Housekeeping, anesthesia, surgery and euthanasia were performed as previously described [8]. Ringer's acetate was given intravenously at 10 ml/kg/h.

Monitoring and examinations

Electrocardiography, end-tidal CO₂, pulse oximetry, regular interval blood gas, systemic intra-arterial blood pressure and pulmonary artery pressure were recorded as described previously [8]. Basic echocardiographic parameters were obtained by a Vivid S6 scanner (GE Vingmed, Horten, Norway) with a 1.5-3.6 MHz matrix array probe. All echocardiographic values are an average of five consecutive measurements with standard deviations.

Nitric oxide in exhaled gas

NO was measured online by the multiple breath method with a chemiluminescence analyzer, as described elsewhere [9,10].

Laboratory analyses

Cytokines, hemostatic markers and the terminal complement complex (TCC) were measured in blood samples at baseline and during bradykinin infusion by methods described previously [11].

Measurements of bradykinin and BK1-5

Measurement of the stable bradykinin metabolite BK1-5 was performed by a previously described liquid chromatography-mass spectrometry (LC-MS/MS) method [12]. Quantification of bradykinin was performed by a commercial ELISA-kit from Uscn Life Science Inc (Wuhan, China) that was validated by measuring the internal standard of bradykinin.

RESULTS

Bradykinin increased exhaled NO and induced shock and capillary leakage

Bradykinin dose dependently increased exhaled NO (Figure 1). Even though a large decrease in MAP was seen, mean pulmonary artery pressure (MPAP) remained constant (20-30 mmHg) throughout the infusion. Systemic vascular resistance index (SVRI) was reduced by 46% in animal A, 65% in animal B and 23% in animal C. A 37% and 30% fall in albumin was seen in animal B and C, respectively, but not in animal A. A complete echocardiographic assessment of left ventricular dimensions in animal A showed 2.4 cm (+/- 0.2 cm) end systolic parasternal long axis at baseline and 0.3 cm (+/- 0.1 cm) at peak bradykinin infusion.

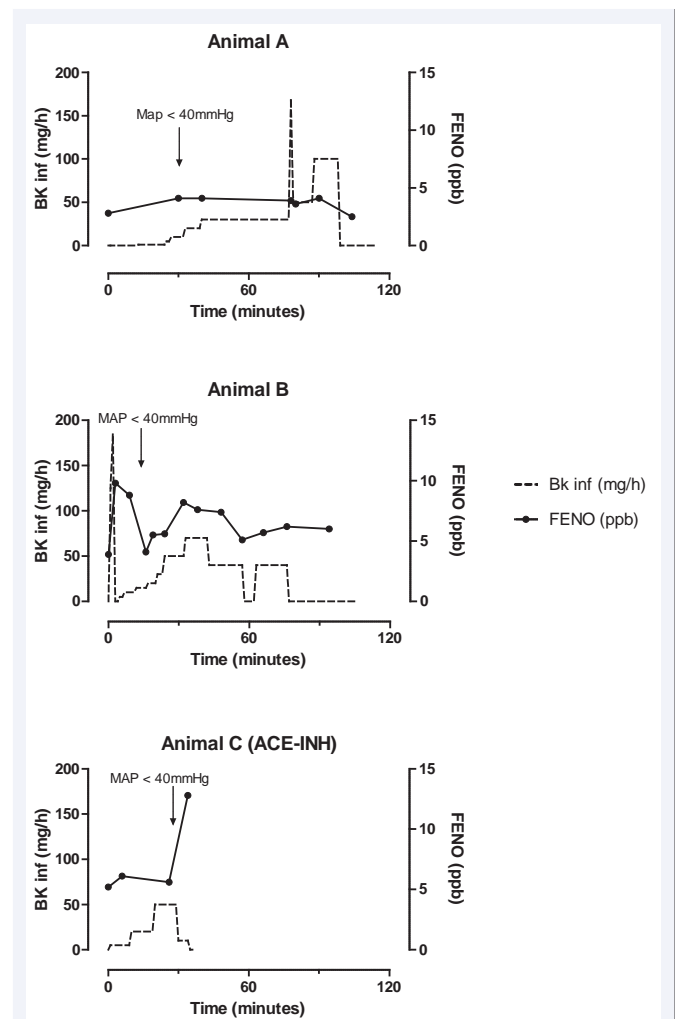


Figure 1 Effects of bradykinin infusion on exhaled NO.

Animal A: The infusion of bradykinin was gradually increased with a corresponding increase in exhaled NO. MAP fell below 40 mmHg after 30 minutes and was maintained at a low level as the infusion rate was kept constant.

Animal B: An initial bolus dose of bradykinin was given (initial peak) which induced a substantial increase in exhaled NO. The infusion rate was quickly escalated after the initial bolus and the levels of exhaled NO closely followed the infusion rate and remained at a relatively high level throughout the experiment. In this case, MAP dropped below 40 mmHg after 15 minutes.

Animal C: Captopril was given before bradykinin and exhaled NO quickly increased to more than twice the initial concentration after bradykinin infusion was started. The animal died after only 34 minutes after a large fall in MAP. BK inf. Bradykinin infusion, FENO: fractional exhaled nitric oxide, ppb: parts per billion.

Bradykinin activated both complement and hemostasis and induced cytokine production

Inflammatory and hemostatic markers, including TCC, thrombin-antithrombin complex (TAT), plasminogen activator inhibitor-1 (PAI-1), IL-1 β , TNF, interleukin-6 (IL-6) and interleukin-8 (IL-8), were measured during the experiments. After bradykinin infusion, TCC, TNF and IL-6 increased markedly in Animal A, while all markers rose in Animal B (Figure 2). Animal

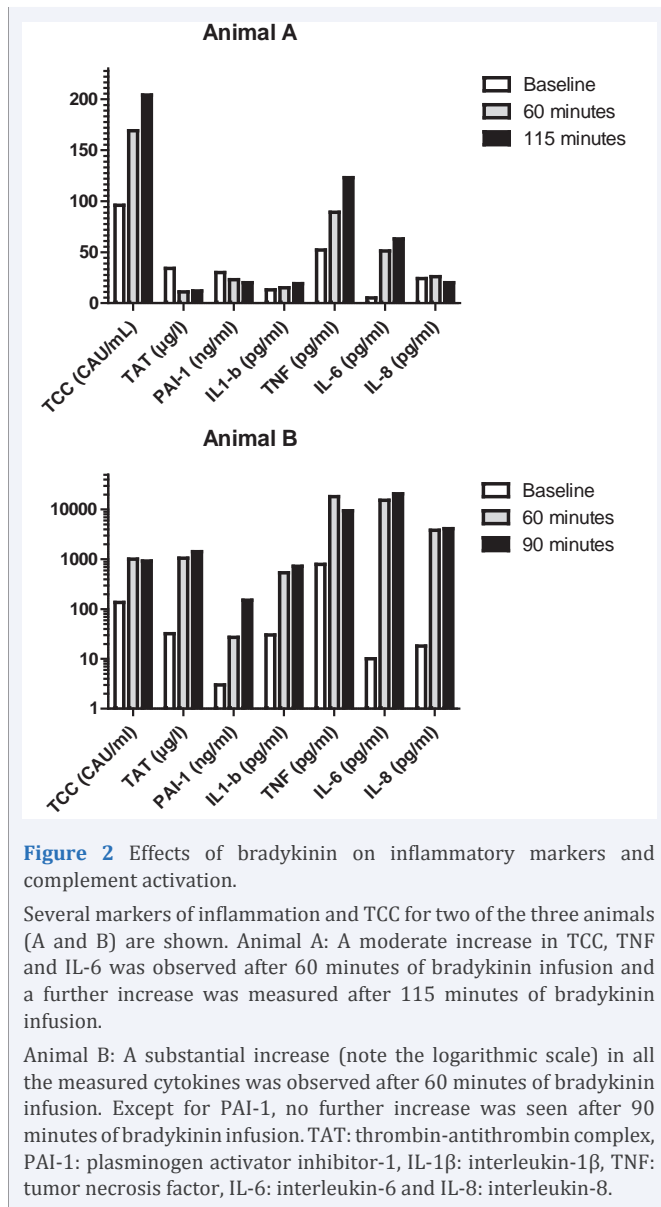


Figure 2 Effects of bradykinin on inflammatory markers and complement activation.

Several markers of inflammation and TCC for two of the three animals (A and B) are shown. Animal A: A moderate increase in TCC, TNF and IL-6 was observed after 60 minutes of bradykinin infusion and a further increase was measured after 115 minutes of bradykinin infusion.

Animal B: A substantial increase (note the logarithmic scale) in all the measured cytokines was observed after 60 minutes of bradykinin infusion. Except for PAI-1, no further increase was seen after 90 minutes of bradykinin infusion. TAT: thrombin-antithrombin complex, PAI-1: plasminogen activator inhibitor-1, IL-1β: interleukin-1β, TNF: tumor necrosis factor, IL-6: interleukin-6 and IL-8: interleukin-8.

C developed an uncontrollable hypotension and died too early to deliver data on these markers.

Massively increased BK1-5 measured by LC-MS/MS

After 60 minutes, animal A and B showed substantially elevated levels of BK1-5. The signals were about two orders of magnitude above the detection method's validated maximum (17.7 nmol/mL). Animal C, which was given an ACE-inhibitor before bradykinin, had lower (15.8 nmol/mL), but elevated levels of BK1-5 after 30 minutes of bradykinin infusion. The baseline levels were below the method's detection limit (35.4 pmol/mL) for all the animals. ELISA measurements of bradykinin were done in animal A, but failed to show the extensive exposure to bradykinin as they never reached the lowest standard of 24 pg/ml, which is consistent with its short half-life.

DISCUSSION

In this study, effects of intravenously infused bradykinin in

the absence or presence of an ACE-inhibitor was investigated in pigs. We found an increase in exhaled NO, activation of complement and hemostasis, increased cytokine production and capillary leakage.

In all the animals, there was a clear link between the infused amount of bradykinin and a severe lowering of MAP, whereas MPAP remained unaffected. The sizeable reduction in end systolic diameter of the left ventricle during peak infusion also indicates that bradykinin reduces afterload and increases contractility. This is consistent with earlier findings on the hemodynamic effects of bradykinin infusion [13], and bradykinin's known activity as a vasodilator [14]. Our results also fit well with an early study of bradykinin infusion to humans [15], and suggest a different effect of bradykinin in the pulmonary circulation than in the systemic circulation. It is tempting to speculate that the high concentration of ACE on pulmonary endothelium protects the lungs from bradykinin induced edema. This could also explain why pulmonary edema is rare in patients with hereditary angioedema who excessively produce bradykinin [16].

By measuring the NO concentration in expiration gas, the effects of bradykinin could be indirectly monitored. A close and rapid relation between exhaled NO and rate of bradykinin infusion was seen (Figure 1). Whether bradykinin released NO directly or indirectly via preformed cytokine cell stores, remains to be investigated. However, the slow and moderate increase of cytokines we measured suggest bradykinin as dominant directly. Elevated exhaled NO after infused bradykinin is, to our knowledge, for the first time observed and documented in the present study. The increased exhaled NO was related to a quantifiable shock. Stimulation of the B2-receptor by bradykinin is known to release plasminogen-activator and produce NO and prostaglandin I₂ (PGI₂) from the endothelial cells [14,17,18]. Upon stimulation by lipopolysaccharides macrophages per cell release ten times more NO than do endothelial cells upon exogenously added bradykinin [5]. The enzyme endothelial NO synthase (eNOS) is released within seconds upon receptor stimulation and could explain the rapid NO response [19]. A difference in exhaled NO was seen between animal A and B (Figure 1). This should be expected as animal A and B received bradykinin infusions at varying rates. The difference may suggest a desensitizing effect by prolonged and gradually increased exposure to bradykinin. A previous publication also found desensitization after internalization of the bradykinin-B2 receptor complex [20].

Both NO and PGI₂ relax smooth muscles in the arterial wall and systemic vascular resistance drops accordingly. The shock we observed mimicked severe sepsis, and bradykinin mediates inflammation [8]. A fall in albumin, as we observe during bradykinin infusion, is also seen during sepsis [8]. It supports the role of bradykinin as a mediator of capillary leakage in these conditions. In a recent study exogenously infused bradykinin to rats reduced the tight junction proteins claudin-5 and occludin and increased plasma leakage. The mechanism in this brain tumor model was up regulation of the eNOS and neuronal NOS and the transcriptional repressor ZO-1 associated nucleic acid binding protein ZONAB [21]. In macrophages the inducible NOS (iNOS) is the key inflammatory enzyme and produce such high levels of NO that in one study setting upon LPS administration

to mice genetically defect of iNOS, the fall in blood pressure was markedly attenuated and early death averted [22]. Consequently, the relative contribution by macrophages and endothelial cells to the capillary leakage and NO-production we observed after bradykinin infusion is difficult to ascertain. The presence of approximately 4000 bradykinin receptors per guinea pig macrophage [23], and the reciprocal activation by NO and cytokines on macrophages and endothelial cells gives the impression that both cell types contribute to the results in our study.

The inflammatory markers TCC, TNF and IL-6 were markedly increased after bradykinin infusion (Figure 2). Interestingly, in animal B (Figure 2), where a large bolus dose of bradykinin was instantly followed by a large increase in exhaled NO and a marked drop in MAP, these increases were several orders of magnitude higher than in animal A. A marked increase in coagulation, represented by TAT and PAI-1 was also present. It is possible that this massive generation was a result of the very early bradykinin bolus. It also suggests that bradykinin might be better tolerated when its infusion rate is gradually increased. This also fits well with the previously mentioned desensitizing theory from prolonged bradykinin exposure. Even more interesting is the possibility of a direct cross-talk between the kinin system on one side and the complement and coagulation pathways on the other. To our knowledge, we present here the first *in vivo* evidence that bradykinin alone activates these cascade systems directly.

The BK1-5 measurements showed a massive signal after infusion of bradykinin (possibly more than 100 000 times increase compared to baseline levels), whereas measurements of bradykinin by ELISA did not reveal any changes in concentration. This illustrates the very rapid metabolism of bradykinin [3]. The significance was further exemplified in animal C. Here the increase in BK1-5 was modest, as the conversion of bradykinin into BK1-5 is hampered by inhibition of ACE [24]. Bönner et al. found that an ACE-inhibitor potentiated a bradykinin infusion 20-fold [15]. This is in line with our observations in animal C. Although the bradykinin infusion in animal C was reduced compared to animal A and B, the presence of an ACE-inhibitor caused a massive bradykinin buildup and a rapid circulatory collapse. It also explains why the exhaled NO in animal C reached the highest value in our experiment.

CONCLUSION

This *in vivo* study shows for the first time how infused bradykinin induced shock and markedly increased exhaled NO, activated the coagulation and complements system and induced several pro-inflammatory cytokines. The rise in these markers was related to the stable bradykinin metabolite BK1-5 with a sensitive LC-MS/MS method. These results suggest that bradykinin directly activates macrophages and endothelial cells and contributes to cytokine response, plasma leakage and increased exhaled NO. Our study expands the understanding of bradykinin's many roles *in vivo*.

ACKNOWLEDGMENTS

This study was financially supported by The Norwegian Council on Cardiovascular Disease and the European

Community's Seventh Framework Programme under grant agreement n°602699 (DIREKT).

REFERENCES

1. Sharma R, Randhawa PK, Singh N, Jaggi AS. Bradykinin in ischemic conditioning-induced tissue protection: Evidences and Possible mechanisms. *Eur J Pharmacol.* 2015; 768: 58-70.
2. Maurer M, Bader M, Bas M, Bossi F, Cicardi M, Cugno M, et al. New topics in bradykinin research. *Allergy.* 2011; 66: 1397-1406.
3. Cyr M, Lepage Y, Blais C, Gervais N, Cugno M, Rouleau J-L, et al. Bradykinin and des-Arg9-bradykinin metabolic pathways and kinetics of activation of human plasma. *Am J Physiol - Heart Circ Physiol.* 2001; 281: 275-283.
4. Nickel KF, Renné T. Crosstalk of the plasma contact system with bacteria. *Thromb Res.* 2012; 130: 78-83.
5. Woldman YY, Eubank TD, Mock AJ, Stevens NC, Varadharaj S, Turco J, et al. Detection of nitric oxide production in cell cultures by luciferin-luciferase chemiluminescence. *Biochem Biophys Res Commun.* 2015; 465: 232-238.
6. Winkler GC. Pulmonary intravascular macrophages in domestic animal species: review of structural and functional properties. *AmJAnat.* 1988; 181: 217-234.
7. Tiffany CW, Burch RM. Bradykinin stimulates tumor necrosis factor and interleukin-1 release from macrophages. *FEBS Lett.* 1989; 247: 189-292.
8. Nielsen EW, Hellerud BC, Thorgersen EB, Castellheim A, Pharo A, Lindstad J, et al. A new dynamic porcine model of meningococcal shock. *Shock.* 2009; 32: 302-309.
9. Archer S. Measurement of nitric oxide in biological models. *FASEB J.* 1993; 7: 349-360.
10. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide. *Am J Respir Crit Care Med.* 2005; 171: 912-930.
11. Barratt-Due A, Thorgersen EB, Egge K, Pischke S, Sokolov A, Hellerud BC, et al. Combined Inhibition of Complement (C5) and CD14 Markedly Attenuates Inflammation, Thrombogenicity, and Hemodynamic Changes in Porcine Sepsis. *J Immunol.* 2013; 191: 819-827.
12. Seip KF, Bjercknes KC, Johansen HT, Nielsen EW, Landrø L, Reubsæet L. Bradykinin analysis revived – A validated method for determination of its stable metabolite in whole blood by LC-MS/MS. *J Chromatogr B.* 2014; 947-948: 139-144.
13. Barratt-Due A, Johansen HT, Sokolov A, Thorgersen EB, Hellerud BC, Reubsæet JL, et al. The Role of Bradykinin and the Effect of the Bradykinin Receptor Antagonist Icatibant in Porcine Sepsis. *Shock.* 2011; 36: 517-523.
14. Schmaier AH. Physiologic activities of the Contact Activation System. *Thromb Res.* 2014; 133: 41-44.
15. Bonner G, Preis S, Schunk U, Toussaint C, Kaufmann W. Hemodynamic effects of bradykinin on systemic and pulmonary circulation in healthy and hypertensive humans. *J Cardiovasc Pharmacol.* 1990; 15: 46-56.
16. Agostoni A, ygoren-Pursun E, Binkley KE, Blanch A, Bork K, Bouillet L, et al. Hereditary and acquired angioedema: Problems and progress: Proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. *J Allergy Clin Immunol.* 2004; 114: 51-131.
17. Leeb-Lundberg LMF, Marceau F, Müller-Esterl W, Pettibone DJ, Zuraw BL. International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to

- pathophysiological consequences. *Pharmacol Rev.* 2005; 57: 27–77.
18. Sainz IM, Pixley RA, Colman RW. Fifty years of research on the plasma kallikrein-kinin system: from protein structure and function to cell biology and in-vivo pathophysiology. *Thromb Haemost.* 2007; 98: 77–83.
19. Ricciardolo FLM, Sterk PJ, Gaston B, Folkerts G. Nitric Oxide in Health and Disease of the Respiratory System. *Physiol Rev.* 2004; 84: 731–65.
20. Windischhofer W, Leis HJ. [3H] Bradykinin Receptor-Binding, Receptor-Recycling, and Receptor-Internalization of the B2 Bradykinin Receptor in the Murine Osteoblast-like Cell Line MC3T3-E1. *J Bone Miner Res.* 1997; 12: 1615–1625.
21. Liu L-B, Liu X-B, Ma J, Liu Y-H, Li Z-Q, Ma T, et al. Bradykinin increased the permeability of BTB via NOS/NO/ZONAB-mediated down-regulation of claudin-5 and occludin. *Biochem Biophys Res Commun.* 2015; 464: 118-125.
22. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, Trumbauer M, et al. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell.* 1995; 81: 641–650.
23. Böckmann S, Paegelow I. Bradykinin receptors in signal transduction pathways in peritoneal guinea pigs macrophages. *Eur J Pharmacol.* 1995; 291: 159–165.
24. Sheikh IA, Kaplan AP. Mechanism of digestion of bradykinin and lysylbradykinin (kallidin) in human serum. Role of carboxypeptidase, angiotensin-converting enzyme and determination of final degradation products. *Biochem Pharmacol.* 1989; 38: 993–1000.

Cite this article

Seip KF, Evjenth B, Hovland A, Dybwik K, Johansen HT, et al. (2016) Bradykinin-Induced Shock Increase Exhaled Nitric Oxide, Complement Activation and Cytokine Production in Pigs. *J Cardiol Clin Res* 4(2): 1057.