Original Article

Role of in-vitro ketamine in protecting the airways from bradykinin induced contraction

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Objective: To study the dilatory effects of ketamine on the tracheal muscle against bradykinin induced bronchoconstriction**.**

Methodology: Guinea pig tracheal chain was first dissected and cut into 2 – 3 mm wide rings and was placed in Krebs Henseleit solution. The muscle activity was recorded by Research Grade Isometric Force Transducer (DT – 475, USA) on power lab data acquisition unit. Effect of increasing concentration of acetylcholine (control group) and bradykinin were recorded and semi-log dose response curves were plotted. Response with acetylcholine was considered to be 100 percent. Ketamine was then added in a fixed dose and its effect with increasing doses of bradykinin was recorded and plotted on a cumulative response curve.

Results: Acetylcholine produced maximum tissue contraction with mean ± SEM of 0.015 ± 0.0006. Bradykinin produced reversible contraction at maximum mean ± SEM of 0.014 ± 0.0007 mV. Ketamine reduced the contraction of the guinea pig tracheal muscle at 300 µM having a mean ± SEM value of 0.010 ± 0.0003 mV. A maximum response of 38% was seen with the dose response curve shifting downwards and to the right.

Conclusion: Bradykinin stimulated tracheal muscle was significantly ameliorated by ketamine, indicating that this intravenous general anesthetic can be given in asthmatic individuals or those having airway hyperresponsiveness undergoing intubation or surgery. The underlying mechanism of action of ketamine in these individuals can be linked to the inhibition of bradykinin, which is an important mediator in asthma.

Keywords: Acetylcholine, bradykinin, ketamine, tracheal muscle, bronchodilatation.

INTRODUCTION

Asthma is a heterogenous syndrome that substantially effects the quality of life.[1] It has affected almost 300 million people worldwide and this number continues to rise as it may even reach 400 million by 2025.[2] Apart from the well-defined role of inflammatory markers in asthma, the proinflammatory mediators like histamine, bradykinin and leukotrienes take part in stimulating the bronchial tone.

Out of these mediators, bradykinin is mainly involved in producing airway hyperresponsiveness, airway inflammation and remodeling, subsequently increasing the airway resistance.[3] Bradykinin not only plays a role in acute asthma but it is also involved in its chronicity and severity.[4] Mechanism of bradykinin induced contraction is thought to be linked to either direct activation of bradykinin receptors on the smooth muscles, through the neural cholinergic pathway or by stimulation of capsaicin-sensitive sensory fibers.[5]

These asthmatic individuals are at high risk of having bronchospasm, especially while they are undergoing surgery or during intubation.[6] Many intravenous general anesthetics have a key role in abolishing this bronchospasm because of their bronchodilatory properties.[7] Among these, ketamine is a commonly used agent, as its pharmacological effects vary from sedation and analgesia to induction as well as maintenance of anesthesia. It has a unique dissociative profile that makes it the agent of choice for different surgeries in a variety of patients.[8] Also, its known bronchodilatory property makes it preferable to be used in asthmatic individuals.

Underlying mechanism for this relaxation of airways which can either be through the inhibition of the vagal system, stimulation of adrenergic system, blocking NMDA receptor induced bronchospasm, reducing the release of inflammatory mediators or by inhibiting the histamine induced contraction.[9] Ketamine has also inhibited the increase in the intracellular Ca^{++} induced by stimulants.[10] No study has yet shown the bronchorelaxant effects of ketamine against bradykinin induced contraction of tracheal muscles. The present

study aimed to find the cardinal mechanism of action of ketamine for bronchodilatation particularly in asthmatic individuals.

METHODOLOGY

This laboratory based randomized control trial was conducted on 18 adult healthy guinea pigs in The Department of Pharmacology in collaboration with the Department of Physiology, Army Medical College, Rawalpindi, for duration of one year. Both male and female guinea pigs aged 6 to 9 weeks of Dunkin Hartley variety were used. They were first selected through non-probability convenience method and then were randomly assigned 3 groups each containing six animals. The study protocol was approved from Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi.

The guinea pigs were first killed by cervical dislocation. The trachea chain was dissected out and was cut into rings 2 – 3 mm width and its epithelium was gently removed. These rings were then opened by a vertical cut that formed the tracheal smooth muscle in the center and cartilaginous part at the periphery. These tracheal rings were then transferred to the organ bath containing Krebs Henseleit solution at room temperature of 37°C, with continuous supply of oxygen.

One end of the tracheal tissue was attached to the oxygen tube and was placed in the organ bath and the other end to a research grade Isometric Force Transducer DT – 475 (USA). An equilibrium period of 15 minutes was given to the tissue for rest, after which the displacement transducer was used to record the tracheal muscle activity. With the help of Power Lab data acquisition unit (AHK/214 iworx) dose response curves were plotted.

Group I: A dose response curve was plotted using acetylcholine (Ach) in a concentration range of 10^{-3} to 10^{-6} M. When Subsequent doses of 3, 6, 9, 12, 15 and 18 µg were added cumulatively, maximum effect was produced. After achieving the maximum effect, the tracheal tissue was washed and allowed to relax. The response documented was taken to be 100 percent as this group was a control group.

Group II: In this group, a cumulative semi-log dose response curve was produced by taking bradykinin with increasing doses of 11, 22, 33, 44, 55 and 66 µg at maximum concentration of 10^{-4} M.

Group III: In group III, fixed dose 300 µM of ketamine was then used to plot a semi-log dose response curve against increasing concentrations of bradykinin ranging from 11 to 66 µg.

Statistical Analysis: Data were analysed using SPSS version 22. Difference between two groups was attained by applying student's t-test. $p < 0.05$ was considered significant.

RESULTS

There were dose dependent contractions in the tracheal muscle chains with Ach and bradykinin. The amplitude of contraction was recorded in milli volts. Maximum amplitude of contraction produced by Ach was at 0.015 ± 0.0006 mV (Fig. 1) and that seen by bradykinin was 0.014 ± 0.0007 mV (Fig. 2). Maximum percent responses for both of these groups were found to be 100% and 96% (Table 1), respectively. The group pretreated with ketamine showed an amplitude of contraction ranging from 0.002 0.0003 mV to 0.010±0.0003 mV (Fig. 3) showing a maximum percent response ranging from 11 to 63%. A comparison of percent responses between group II (bradykinin alone) and group III (bradykinin pretreated with ketamine) was done and it was found to be statistically significant ($p = 0.0031$).

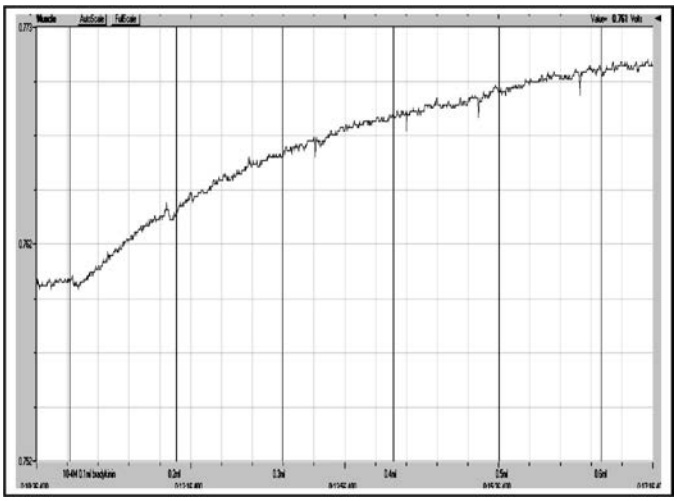


Fig. 1: Cumulative dose response curve of bradykinin (n = 6) showing increase in response with increasing doses.

The percent inhibition for group II and III was also calculated ranging from 47 to 34%. A cumulative response curve was finally plotted to compare group II and III, which showed a right shift of the curve demonstrating an ameliorating effect against bradykinin induced contractions in the tracheal tissue.

DISCUSSION

In addition to its dissociative anesthetic and analgesic effects, ketamine has sympathomimetic and bronchodilatory effects as well.[11] In our first group, Ach

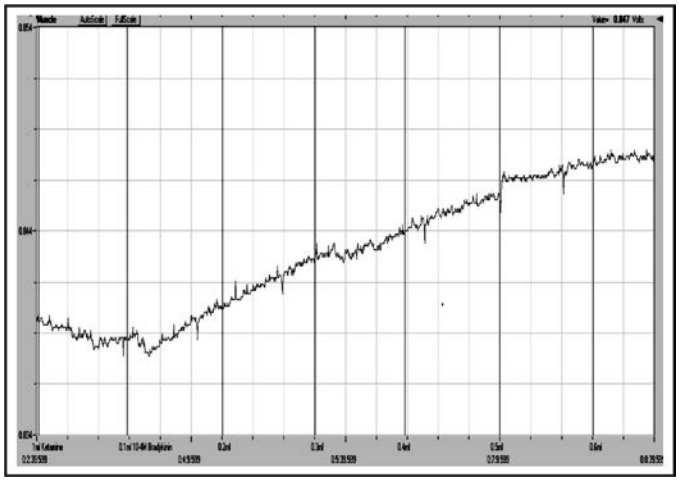
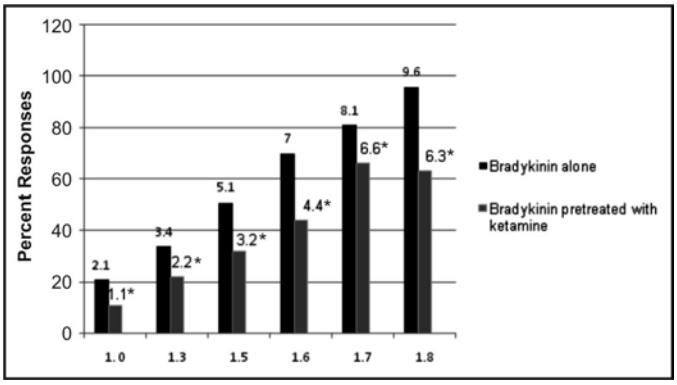


Fig. 2: Cumulative dose response curve of bradykinin in the presence of fixed dose (300 µM) of ketamine (n = 6) showing a decreased response with increasing dose.



*(mean p = 0.0031) Significant ($p < 0.05$)

Fig. 3: Comparison between group 2 illustrating bradykinin induced contraction and group 3 illustrating the contraction after pretreatment with ketamine.

Table 1: Comparison of group 2 (bradykinin) with group 3 (bradykinin after pretreatment with fixed dose 300 µM of ketamine).

Dose of Bradykinin (µg)	Response of Bradykinin Mean ± SEM (mV)	Response (%)	Response of Ketamine Mean ± SEM (mV)	Response (%)	Percent Inhibition (%)
11	0.003 ± 0.0003	21	0.002 ± 0.0003	11	47
22	0.005 ± 0.0003	34	0.003 ± 0.0004	22	35
33	0.008 ± 0.0005	51	0.005 ± 0.0005	32	37
44	0.011 ± 0.0006	70	0.007 ± 0.0003	44	37
55	0.012 ± 0.0007	81	0.008 ± 0.0003	56	32
66	0.014 ± 0.0007	96	0.010 ± 0.0003	63	34

showed concentration dependent contraction of the tracheal smooth muscles at a dose of 15 µg. This role of Ach is as the main neurotransmitter of the airways producing M_3 receptor induced contraction. The findings of our control group are consistent with those of Kieffer and Abel, who also demonstrated Ach induced contraction in tracheal muscles of mouse.[12] Another study by Malik et al showed that maximum effect of Ach was seen at in a concentration range of 10^{-6} to 10^{-3} M.[13] Our findings are similar.

Bradykinin, in a manner similar to Ach also produced dose dependent contraction but to a lesser extent than that of Ach, as shown in Fig. 1 and 2. In asthmatic individuals, bradykinin has been implicated as an important mediator, mediating its effects mainly through the stimulation of B_2 receptors.[3] Noor et al showed a maximum response with bradykinin at the dose of 77 µg in guinea pig trachea. This was in conjunction to our study where we used the dose of bradykinin ranging from 11 µg to 66 µg.[14] Keir et al, also demonstrated that bradykinin causes contraction of epithelium denuded trachea in guinea pigs.[15]

In the next set of experiment, effect of ketamine was seen on bradykinin induced contractions. Ketamine produced a reduced response with a maximum of 0.010 ± 0.0003 mV. This significant change was produced in a dose of 300 µM, which was similar to the study conducted by Yamaguchi et al, who performed on isolated rat trachea in the same dose.[16] In another study, ketamine significantly suppressed the ovalbumin (OVA) induced airway hyperresponsiveness in mice.[17]

The comparison of bradykinin alone and bradykinin with ketamine clearly showed attenuation in the contractile response produced by bradykinin on the guinea pig airway. The percent responses of both the groups were found to be statistically significant ($p < 0.05$). The concentration response curve shifted downwards and towards right and the mean percent

inhibition was calculated to be 37%, showing a significant reduction in response. The protective effect of ketamine in tracheal tissue has been shown in many studies against Ach, histamine or calcium ions induced contractions but none have shown its effects against bradykinin.

CONCLUSION

This invitro study clearly showed protective response of ketamine against bradykinin induced tracheal muscle contractions. It can be concluded that ketamine can be safely used in asthmatic individuals or hyperresponsive patients undergoing surgery due to its broncho dilatory effect. Our study also indicates that the underlying mechanism of action of ketamine in asthmatic patients is mediated bradykinin inhibition. Further studies can be done to evaluate the mechanism of broncho dilatory effect in ketamine.

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REFERENCES

1. Eyerich S, Metz M, Bossios A, Eyerich K. New biological treatments for asthma and skin allergies. Allergy 2020;75:546-60.
2. Loftus PA, Wise SK. Epidemiology of asthma. Curr Opin Otolaryngol Head Neck Surg 2016;24:245-9.
3. Ricciardolo FL, Petecchia L, Sorbello V, Di Stefano A, Usai C, Massaglia GM et al. Bradykinin B2 receptor expression in the bronchial mucosa of allergic asthmatics: the role of NF-kB. Clin Exp Allergy 2016;46:428-38.
4. Hewitt MM, Adams G, Mazzone SB, Mori N, Yu L, Canning BJ. Pharmacology of bradykinin-evoked coughing in guinea pigs. J Pharmacol Exp Ther 2016;357:620-8.
5. Ricciardolo FL, Folkerts G, Folino A, Mognetti B. Bradykinin in asthma: Modulation of airway inflammation and remodelling. Eur J Pharmacol 2018;827:181-8.
6. Li HY, Meng JX, Liu Z, Liu XW, Huang YG, Zhao J. Propofol attenuates airway inflammation in a mast cell-dependent mouse model of allergic asthma by inhibiting the toll-like receptor 4/reactive oxygen species/nuclear factor κB signaling pathway. Inflammation 2018;41:914-23.
7. Khan KS, Hayes I, Buggy DJ. Pharmacology of anesthetics agents I: intravenous anaesthetic agents. Continuing Educ Anesthesia Crit Care Pain 2014;14:100-5.
8. Natoli S. The multiple faces of ketamine in anaesthesia and analgesia. Drugs Context 2021;10:2020-12-8.
9. Radvansky BM, Puri S, Sifonios AN, Eloy JD, Le V. Ketamine-a narrative review of its uses in medicine. Am J Ther 2016;23:e1414-26.
10. Trimmel H, Helbok R, Staudinger T, Jaksch W, Messerer B, Schöchl H, et al. S (+)-ketamine. Wien Klin Wochenschr 2018;130:356-66.
11. Barrett W, Buxhoeveden M, Dhillon S. Ketamine: a versatile tool for anesthesia and analgesia. Curr Opin Anaesthesiol 2020;33:633-8.
12. Kieffer CM, Abel PW. Pharmacokinetic Modeling of Acetylcholine-Induced Contraction of Mouse Trachea. Faseb J 2018;32:834-1.
13. Malik FQ, Khan BT, Sharif M. protective effect of lignocaine against acetylcholine and bradykinin induced tracheal tissue contraction of guinea pigs in vitro. Pak Armed Forces Med J 2019;69:1221-6.
14. Noor A, Najmi MH, Bukhtiar S. Effect of Montelukast on bradykinin-induced contraction of isolated tracheal smooth muscle of guinea pig. Indian J. Pharmacol 2011;43:445.
15. Keir SD, Spina D, Page CP. Bradykinin and capsaicin induced airways obstruction in the guinea pig are platelet dependent. Pulm Pharmacol Ther 2015;33:25-31.
16. Yamaguchi M, Shibata O, Saito M, Yoshimura M, Nishioka K, Makita T, et al. Effects of propofol and ketamine on ATP-induced contraction of the rat trachea. J Anesth 2007;21:37-41.
17. Song L, Sen S, Sun Y, Zhou J, Mo L, He Y. Ketamine inhalation ameliorates ovalbumin-induced murine asthma by suppressing the epithelial-mesenchymal transition. Med Sci Monit 2016;22:2471.