Investigation of the Relaxant Effect Mechanism of Ketamine on Normal and Ovalbumin-Induced Trachea in Guinea Pigs

Ketaminin Gevşetici Etki Mekanizmasının Normal ve Ovalbumin Duyarlı Kobay Trakealarında İncelenmesi

Bülent SARAÇ, MD,[a]
Ahmet ALTUN, MD,[a]
Şahin YILDIRIM, MD,[a]
Mustafa Kemal YILDIRIM, MD,[a]
Tijen Kaya TEMİZ, MD,[a]
İhsan BAĞCİVAN, MD,[a]
Mehmet ŞENCAN, MD[b]

Departments of
[a]Pharmacology,
[b]Internal Medicine,
Cumhuriyet University
Faculty of Medicine, Sivas

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Yazışma Adresi/*Correspondence:*
Bülent SARAÇ, MD
Cumhuriyet University
Faculty of Medicine,
Department of Pharmacology,
Sivas,
TÜRKİYE/TURKEY
saracbulent@mynet.com

ABSTRACT Objective: The aim of this study was to investigate of effects and the possible mechanisms of effects of ketamine on the isolated trachea preparations from control and ovalbumin sensitized guinea pigs. **Material and Methods:** Adult male guinea pigs were randomly allocated to two experimental groups. We tested the relaxant effects of ketamine (10^{-8} M to 3 x 10^{-4} M) on the isolated trachea preparations precontracted with carbachol (10^{-6} M) in control and ovalbumin-sensitized guinea pigs. We also evaluated the effects of ketamine on the levels of cGMP in isolated tracheal smooth muscle strips with radioimmunoassay. **Results:** Although ketamine (10^{-8} to 3 x 10^{-4} M) produced concentration-dependent relaxation on isolated trachea preparations precontracted by carbachol (10^{-6} M) in both groups, relaxations in control group were significantly high when compared to ovalbumin-sensitized group ($p< 0.05$). Preincubation of trachea preparations by indomethacin (10^{-5} M) and propranolol (10^{-4} M) did not produce a significant alteration on ketamine-induced relaxation responses ($p> 0.05$), while preincubation by N(w)-nitro L-arginine methyl ester (3 x 10^{-5} M) significantly decreased the ketamine-induced relaxation responses in both groups, preincubation by aminoguanidine (3 x 10^{-5} M) decreased the ketamine-induced relaxation responses in ova-sensitized group ($p< 0.05$). **Conclusion:** Ketamine induced concentration-dependent relaxations in precontracted isolated trachea smooth muscle of guinea pigs in the both groups. These relaxations were independent from cyclooxygenase products released from respiratory epithelium and stimulation of beta adrenergic receptors. The relaxation caused by ketamine seems to be mainly related to the nitric oxide/cGMP pathway, especially eNOS pathway. Although ketamine caused much less relaxation in ova-sensitized group, ketamine can be used as a proper anesthetic agent in patients with airway hyperresponsiveness and bronchial asthma.

Key Words: Ketamine; trachea; asthma; guinea pigs

ÖZET Amaç: Bu çalışmada ketaminin kontrol ve ovalbumin duyarlı kobay izole trakea preperatlarındaki gevşetici etkisi ve olası etki mekanizmaları araştırıldı. **Gereç ve Yöntemler:** Yetişkin kobaylar rastgele 2 ayrı gruba ayrıldı. Karbakolle (10^{-6} M) kastırılmış, kontrol ve ovalbumin duyarlı kobay izole trakea preparatlarında, ketaminin (10^{-8}-3x10^{-4} M) gevşetici etkileri ve cGMP düzeyinde oluşturduğu değişiklikler organ banyosu deneyleri ve radyoimmünoassay ölçüm yöntemi ile araştırıldı. **Bulgular:** Karbakol (10-6M) ile kasılmış izole trakea preperatlarında ketamin (10^{-8}-3 x 10^{-4} M) her iki grupta da konsantrasyona bağımlı gevşeme oluşturdu. Kontrol grubundaki gevşemeler ovalbumin duyarlı gruba göre anlamlı düzeyde daha yüksekti ($p< 0.05$). Her iki grupta da indometazin (10^{-5} M) ve propranolol (10^{-4} M) varlığında ketamine bağlı gevşeme yanıtlarında anlamlı bir değişiklik yoktu ($p> 0.05$). N(w)-nitro L-arginine methyl ester (3 x 10^{-5} M) her iki grupta da ketamine bağlı gevşeme yanıtlarını anlamlı düzeyde azaltırken, aminoguanidin yalnızca ovalbumin duyarlı gruptaki gevşeme yanıtlarını azalttı ($p< 0.05$). **Sonuç:** İzole kobay trakea preperatlarında ketamine bağlı gevşemeler solunum epitelinden salınan siklooksijenaz ürünlerine ve β-adrenerjik reseptör uyarılmasına bağımlı değildir. Ketamine bağlı gevşemeler NO/cGMP yolağı ile özellikle de eNOS bağımlı NO ile ilişkili görünmektedir. Ketamin, ovalbuminle duyarlı grupta kontrol grubuna göre daha az gevşeme yapmış olsa da solunum yollarında aşırı duyarlılığı ve bronşiyal astımı olanlarda uygun bir anestezik ilaç olarak görünmektedir.

Anahtar Kelimeler: Ketamin; trakea; astım; kobaylar

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Asthma is a major public health issue with high and increasing prevalence rates[1] and a concomitant increase in morbidity and mortality.[2] Studies have shown that the lifetime prevalence of asthma among adults is 11%.[3]

The anesthetic management of patients with asthma still represents a challenge to anesthesiologists.[4] The occurrence of significant perioperative respiratory complications such as bronchospasm and pulmonary barotrauma are thought to be increased in persons with asthma, with reported frequencies as high as 30%. These reports suggest that these patients have a significant risk for development of perioperative respiratory complications that may lead to serious morbidity.[5,6]

Ketamine, a phencyclidine derivative, is a short-acting intravenous anesthetic, first used in humans in 1965 and is still applied in a variety of clinical settings nowadays.[7,8] Ketamine was very popular when it was get into clinic use at first, because it could produce a good anesthetic dept and did not cause respiratory or cardiovascular complications.[9] Ketamine has many pharmacological properties, including analgesic, anesthetic and sympathomimetic effects.[8] Owing to its ability to induce relaxation of bronchial smooth muscle, ketamine is recommended as an optimum anesthetic for asthmatic patients, and has been clinically used to treat bronchospasms, asthma exacerbation and status asthmaticus.[10-12]

Allergic asthma is characterized by airway hyperresponsiveness, allergen-specific immunoglobulin E in serum and infiltration of inflammatory cells in the airways.[13,14] Ovalbumin-induced asthma in guinea pigs is widely accepted as an experimental model of bronchial asthma and was applied in this study.[15] The aim of this study was to investigate the possible mechanism of these effects and effects of ketamine on the isolated trachea preparations from control and ovalbumin sensitized guinea pigs.

MATERIAL AND METHODS

EXPERIMENT AND SENSITIZATION OF GUINEA PIGS

Adult male guinea pigs, weighing 280-330 g, were randomly (by using random numbers table) allocated to two experimental groups each consisting of 10 animals. They were individually placed in metal cages and temperature-controlled room (22 ± 02 ºC) in which a 12-12 h light-dark cycle was maintained (08:00-20:00 h light). The animals were bred with feed and tap water ad libitum.

Guinea pigs (n= 10) in experimental group were sensitized by IM (intramuscular) injections of 0.30 ml of a 5% (w/v) ovalbumin/saline solution into each thigh (0.6 ml total) on days 1 and 4. Guinea pigs (n= 10) in control group received IM injections of 0.30 ml of saline solution into each thigh (0.6 ml total) on days 1 and 4 as placebo. The guinea pigs were ready for procedure after 25 days.

All protocols described in this study were approved by the local ethics committee for animal experimentation in Cumhuriyet University Faculty of Medicine.

EXPERIMENTAL PROCEDURES

Guinea pigs were stunned and killed by decapitation. The trachea was removed rapidly and transverse rings (3 mm long) were cut and then mounted in thermostatically controlled (37 ºC) organ baths. The organ baths contained 10 ml Krebs–Henseleit solution (KHS) of the following composition (mmol/L): NaCl, 120; KCl, 4.6; $MgSO_4$, 1.2; $NaHCO_3$, 22; NaH_2PO_4, 1.2; $CaCl_2$, 2.5; glucose, 11.5. The solution was adjusted to pH 7.4 by aerating with a 95% O_2 and 5% CO_2 gas mixture. Isometric tension was continuously measured with a force transducer (Grass FT 03, Quincy, MA, USA). The tissues were stretched for 60 min under a resting tension of 1 gram. The preparations were washed with KHS every 15 min during the equilibration period.

ISOMETRIC MEASUREMENTS ON ISOLATED TRACHEAL SMOOTH MUSCLE (TSM) STRIPS

After the equilibration period, the tissues were contracted with a submaximal concentration of carbachol (10^{-6} M). This concentration of carbachol was determined from preliminary experiments to elicit 70% of its maximum contraction. We tested the effects of ketamine (10^{-8} M to 3 x 10^{-4} M) on the resting tension and precontracted with car-

bachol on the isolated trachea preparations from control and ovalbumin-sensitized guinea pigs. After the addition of each dose, we waited until a plateau response was obtained before adding the next one. Ketamine was added in a cumulative manner in to the organ bath. At the end of the experiment, papaverine (10^{-4} M) was added to the organ bath to obtain the maximal relaxation and to control the relaxing ability of the trachea preparations. The preparations were then washed three times before antagonists were applied and the tissue was allowed to return to baseline tension. N (w)-nitro L-arginine methyl ester (L-NAME) (3×10^{-5} M), a non-specific inhibitor of nitric oxide synthase (NOS); aminoguanidine, selective inhibitor of inducible nitric oxide synthase (iNOS) (3×10^{-5} M); indomethacin (10^{-5} M); an inhibitor of cyclooxigenase, propranolol (10^{-4} M), a beta adrenergic receptors blocker were added to the organ bath that the trachea preparations were placed in. Twenty minutes later, the trachea preparations were contracted with carbachol separately, and relaxation responses to ketamine were obtained. The doses of antagonists were chosen based on previous studies. Four antagonists were tested in each preparation. The effects of antagonists on ketamine-induced relaxation were evaluated by comparing the response before and after the addition of antagonists in the same preparation.

MEASUREMENT OF cGMP LEVELS IN GUINEA PIG TSM STRIPS

The isolated trachea preparations were mounted in organ bath, and equilibrated in KHS (composition as above), continuously gassed with a mixture of 95% O_2 and 5% CO_2 at 37° C (pH 7.40) for 60 min. Four sets of experimental studies were performed except to control group. Tissues were exposed to 10^{-6} M carbachol for 15 min in the first set. In the second set, tissues were exposed to 10^{-5} M ketamine after 15 min exposure of 10^{-6} M carbachol. In the third set, 3×10^{-5} M L-NAME was added to the organ bath. Then 10^{-6} M carbachol added to the organ bath. Finally ketamine (10^{-5} M) added to the organ bath after 15 minutes exposure of carbachol. In the fourth set, 3×10^{-5} M aminoguanidine was added to it. Then 10^{-6} M carbachol, finally ketamine (10^{-5} M) were added to the organ bath after 15 minutes exposure of carbachol. At the end of a total incubation period, the samples were immediately transferred into liquid nitrogen, homogenized with ethanol and incubated for 30 min at 12000 x g. The supernatants were poured into a clean test tube and dried in a vacuum at 50 ºC to remove ethanol. Aliquots of the supernatant were tested for cGMP by radioimmunoassay (RE 110 21 and RE 290 71 respectively; IBL Hamburg, Germany). The samples were then processed according to instructions provided with the kits for determination of cGMP levels. Sampling data were divided according to the weight of isolated trachea preparations and the results were expressed in fmol/mg TSM strips.

DRUGS

The following drugs were all obtained from Sigma Chemical Co. (St. Louis, MO, USA): carbachol chloride, ketamine, papaverine hydrochloride, L-NAME, aminoguanidine, indomethacin, propranolol. All drugs were dissolved in distilled water except for indomethacin, which was dissolved in dimethyl sulfoxide (DMSO) and then diluted with distilled water to prepare decreasing concentrations of these drugs. All drugs were freshly prepared on the day of the experiments. No effect with the DMSO was observed on the isolated trachea preparations.

DATA ANALYSIS

Carbachol-induced (10^{-6} M) contractions were considered as reference response. Relaxation responses were expressed as percentage of the carbachol-induced contractions. The effects of cumulative concentrations of ketamine on carbachol-induced contractions in the absence or presence of antagonists or inhibitors were measured, and values for-Log_{10} half maximal effective concentration (pD_2) and mean maximal inhibition (E_{max}) were compared. Maximal inhibitor effects were calculated for each concentration-response curve. The EC_{50} value represents 50% of the maximal inhibitor effect. EC_{50} values were calculated by linear regression of the probit of response vs. log_{10} molar concentration for ketamine. Experi-

mental values were presented as means ± S.E.M. and analyzed by repeated measures of analysis of variance (ANOVA) with the Tukey HSD as post-hoc test. A p value of <0.05 was considered significant. All statistical analyses were performed using Statistica for Windows (Statsoft, Inc., Tusla, USA).

RESULTS

ISOMETRIC STUDIES

Ketamine (10^{-8} to 3 x 10^{-4} M) did not produce any response on basal tension of isolated trachea preparations from control and ovalbumin sensitized guinea pigs. Although ketamine (10^{-8} to 3 x 10^{-4} M) caused concentration-dependent relaxation on isolated trachea preparations precontracted by carbachol (10^{-6} M) in both control and ovalbumin-sensitized groups, relaxations in ovalbumin- sensitized group were significantly low when compared control group (Figure 1). In trachea preparations from ovalbumin-sensitized guinea pigs, E_{max} value of ketamine was significantly different as compared to control group ($p< 0.05$) (n= 10, for each group).

None of the antagonists investigated had a significant influence on basal tonus of isolated trachea preparations from control and ovalbumin-sensitized guinea pigs. Preincubation of trachea preparations by indomethacin (10^{-5} M) and propranolol (10^{-4} M) did not produce a significant alteration on ketamine-induced relaxation responses in both group (Figure 2), while preincubation by L-NAME (3 x 10^{-5} M) significantly decreased the ketamine-induced relaxation responses in both groups, preincubation by aminoguanidine (3 x 10^{-5} M) decreased the ketamine-induced relaxation responses in ova-sensitized group (Figure 3) (n= 10, for each antagonist or inhibitor). There was no significant difference between pD_2 values in all groups in the presence and absence of antagonists. In addition, there was no significant difference between ketamine-induced E_{max} in the presence of antagonists ($p> 0.05$), except aminoguanidine and L-NAME in both groups ($p< 0.05$).

MEASUREMENT OF cGMP IN TSM STRIPS

We examined the effect of ketamine on cGMP levels in the presence and absence of the non-selective NOS inhibitor L-NAME and selective iNOS inhibitor aminoguanidine in guinea pig TSM strips. The basal release of cGMP was 0.43 ± 0.08 pmol/mg protein, in control group (n= 6), whereas basal re-

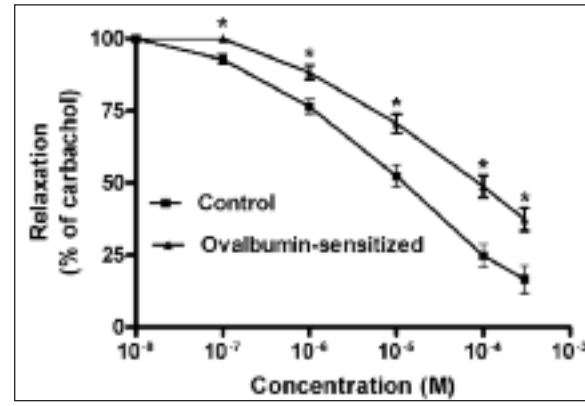

FIGURE 1: The effect of ketamine (10^{-8} to 3 x 10^{-4} M) in the isolated trachea preparations precontracted with carbachol (10^{-6} M) from control and ovalbumin-sensitized guinea pigs. Relaxation responses were expressed as a percentage of carbachol-induced contraction and shown as means ± SEM (n= 10, each experiments).

*, $p< 0.05$, statistically different from ketamine relaxation response (from control group).

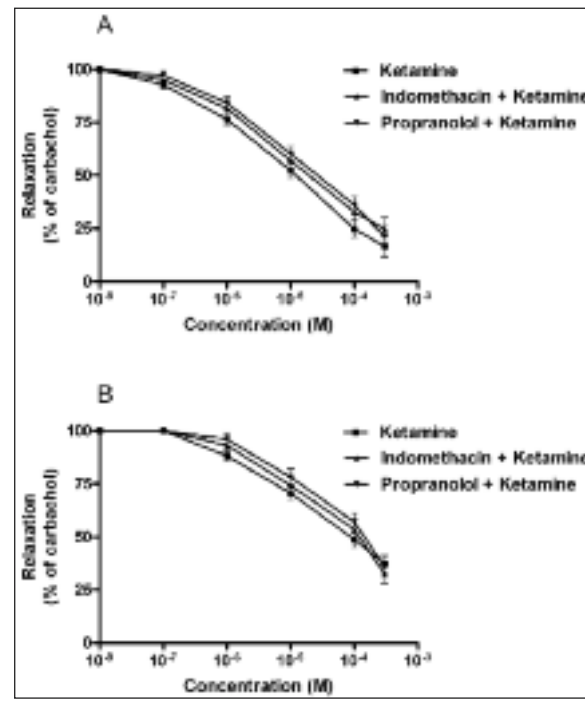

FIGURE 2: The effect of ketamine (10^{-8} to 3 x 10^{-4} M) in the absence or presence of indomethacin, and propranolol in the isolated trachea preparations precontracted with carbachol (10^{-6} M) from control **(A)** and ovalbumin-sensitized **(B)** guinea pigs. Relaxation responses were expressed as a percentage of carbachol-induced contraction and shown as means ± SEM (n= 10, in each drug).

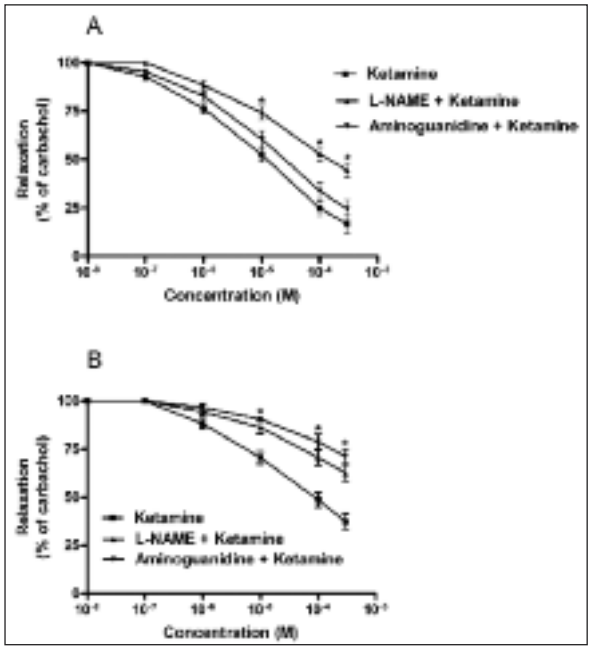

FIGURE 3: The effect of ketamine (10^{-8} to 10^{-4} M) in the absence or presence of L-NAME (3 x 10^{-5} M) and aminoguanidine (3 x 10^{-5} M) in the isolated trachea preparations precontracted with carbachol (10^{-6} M) from control **(A)** and ovalbumin-sensitized **(B)** guinea pigs. Relaxation responses were expressed as a percentage of carbachol-induced contraction and shown as means ± SEM (n= 10, in each drug).

*, $p < 0.05$, statistically different from ketamine relaxation response (from control group).

lease of cGMP was 0.26 ± 0.03 pmol/mg protein in Ova-sensitized group. The difference was significant as compared to control group (p= 0.008). Carbachol (10^{-6} M) significantly augmented cGMP levels in both control and Ova-sensitized groups (p< 0.05). This increase was more prominent in control group when compared to Ova-sensitized group (p= 0.032). In addition, we observed the effects of ketamine alone and in the presence of L-NAME and aminoguanidine. Administration of ketamine (10^{-5} M) significantly augmented cGMP levels in both control and Ova-sensitized groups (p< 0.05). This increase was significantly higher in control group as compared to Ova-sensitized group (p= 0.027). The effects of ketamine in the presence of L-NAME on cGMP levels were significantly different from those in the absence of L-NAME. In the presence of L-NAME, cGMP levels were significantly lower as compared to carbachol + ketamine group (p= 0.06). Also the cGMP levels in the presence of L-NAME was significantly higher in control group when compared to Ova-sensitized group (p= 0.010). The effects of ketamine on cGMP levels in the presence of aminoguanidine were not significantly different from those in the absence of aminoguanidine (p= 0.830) (Figure 4).

DISCUSSION

Asthma is a major public health issue with high and increasing prevalence rates and a concomitant increase in morbidity and mortality.[1,2] The pathophysiological hallmarks of asthma are narrowing in airway diameter due to the contraction of smooth muscle, vascular congestion, oedema of the bronchial wall, and tenacious secretions.[16] Bronchial hyperreactivity associated with asthma is an important risk factor for perioperative bronchospasm. The occurrence of this potentially life-threatening condition in anesthesia practice varies from 0.17% to 4.2%.[17]

Ketamine is an IV (intravenous) general anaesthetic that is considered as an attractive choice because of its effectiveness at preventing and reversing wheezing in patients with asthma who require anesthesia and intubation.[18] Ketamine relaxes the bronchiolar musculature and prevents the bronchoconstriction induced by histamine, decreasing the risk of bronchospasm during the induction of anesthesia. It has been speculated that these effects

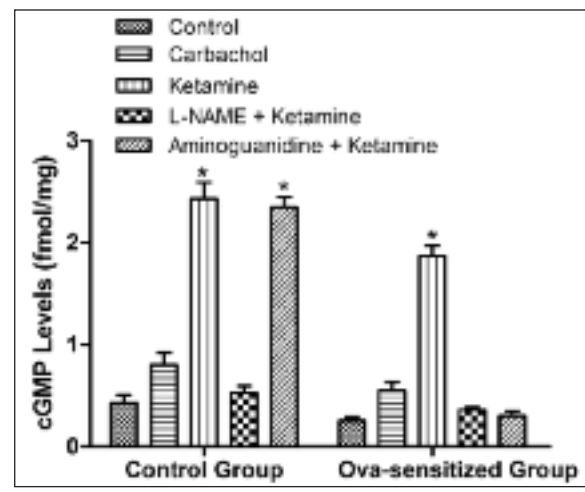

FIGURE 4: Effect on cGMP levels of the ketamine in absence or the presence of L-NAME and aminoguanidine in isolated trachea preparations precontracted with carbachol.

Data were expressed as the means ± SEM of six experiments.

*, $p < 0.05$, significantly different from all groups.

derive from a direct action on bronchial muscle as well as a potentiation of cathecolamines.[19]

Ketamine is also known to produce bronchodilatation in asthmatic patients who do not respond to conventional therapy.[12] Although the mechanism of this spasmolytic effect of ketamine is still unclear, previous studies suggested that Ca^{2+} channel blocking effects,[20-22] NO/cGMP pathway,[23] catecholamine release and/or inhibition of catecholamine uptake[24,25] may contribute.

The main opinion of this study seems that relaxative effect of ketamine on both groups of control and ovalbumin sensitized guinea-pigs is independent from products of cyclooxygenase and stimulation of beta adrenergic receptor, besides, this relaxant effect may be related to NO/cGMP pathway.

The products of cyclooxigenase pathway, prostanoids, are centrally involved in pulmonary physiology and have been implicated in asthma, especially as a potential regulator of the bronchial tone.[26] Some studies showed the potential for the bronchial epithelial cells both in vivo and in vitro to produce substantial amounts of prostanoids and suggested that airway epithelium actively regulates airway function by generating PGE_2.[27] β_2-receptors are richly expressed in airway smooth muscle.[28] β_2-selective adrenoceptor agonists are widely used for treatment of asthma, strongly relax this smooth muscle.[29]

In the light of this evidence, we used both inhibitors of beta adrenergic receptors and cyclooxygenase pathways. In the present study, both propranolol and indomethacin did not change relaxations of ketamine. It suggests that relaxations of ketamine are independent from stimulation of beta adrenergic receptor and cyclooxygenase products released from airway epithelium.

Since nitric oxide (NO) was first described as endothelium derived relaxing factor (EDRF), there has been compelling evidence that NO is involved in very many biological processes.[30,31] There is increasing evidence that endogenous NO plays an important role in the physiologic regulation of human airways, and is implicated in the pathophysiology of airway disease.[32] NO increases in various inflammatory diseases of the airways such as bronchial asthma. Indeed, higher amounts of NO in exhaled air have been documented in asthmatic patients compared to normal controls.[33] NO is synthesized by three isoforms of NOS.[34] The constitutive isoforms (cNOS) are expressed in neurones (nNOS, type I) and endothelial cells (eNOS, type III) of the airway.[35] The third isoform is induced following exposure to proinflammatory cytokines (iNOS, type II) and is expressed in epithelial cells and inflammatory cells of the airway.[36] It is well known that high concentrations of iNOS-derived NO are also produced in asthmatic airway inflammation. Thus, NO is detectable in the exhaled air from humans and various experimental animals, and its concentration increases in exhaled air of patients with chronic asthma.[37,38] The increase in exhaled NO can be normalized after the administration of oral glucocorticosteroids or inhalation of the selective iNOS inhibitor aminoguanidine, indicating that high exhaled NO concentrations in these patients may reflect inflammation-induced enhanced expression of iNOS.[39,40] Consistently in the present study we showed that administration of all ketamin, L-NAME + ketamine and aminoguanidine + ketamine produced a relaxation in TSM strips precontracted with charbachol. In the control group, while administration of L-NAME reduced relaxation caused by ketamine, aminoguanidine had no effect. This may show that inducing eNOS and enhancing production of NOS are the main mechanisms of action of ketamine. In addition, cGMP results confirm this finding. In this study, we observed that administration of ketamine caused a high increase in cGMP level of TSM strips. In the control group, while this increase was reversed by pretreatment with L-NAME, aminoguanidine had no effect. In ova-sensitized group both L-NAME and aminoguanidine pretreatment decreased cGMP levels increased by ketamine. These different responses of isometric studies and cGMP measurements in the control and ova-sensitized groups may show that the action mechanism of ketamine, probably eNOS activation, may be affected in ova-sensitized group. This would be possible if there is a shift from eNOS to iNOS in

asthmatic conditions. Additionally, we observed that pretreatment with aminoguanidine caused a non-significant decrease in ketamine relaxation and cGMP levels increased by ketamine in the control group. This may suggest that there is no enhancement iNOS expression in tracheal smooth muscles in normal condition. It is well known that there is an important increase in production of iNOS in bronchial asthma.[37] Therefore it is sensible that aminoguanidine has significantly decreased ketamine induced relaxation in ova-sensitized group. Enhanced iNOS activity in asthma may cause desensitization of guanylate cyclase and partly inhibit cGMP production.[41] This may explain lower relaxation responses and cGMP levels in ova-sensitized group when compared to the control group.

In conclusion, ketamine induced concentration-dependent relaxations in precontracted isolated trachea smooth muscle of guinea-pigs in both control and ovalbumin-sensitized groups, but these relaxations in ovalbumin-sensitized group were significantly less when compared control group. These relaxations are independent from cyclooxygenase products released from airway epithelium and stimulation of beta adrenergic receptors. The effects of NOS inhibitors on ketamine-induced relaxation in tracheal smooth muscles and changed levels of cGMP in antagonist studies may suggest that NO/cGMP pathway has an important role in the action of ketamine. The decreased relaxation response to ketamine in ova-sensitized guinea pigs' trachea may be due to enhanced iNOS activity. All these findings may show that, although ketamine caused much less relaxation in ova-sensitized group, ketamine can be used as an appropriate anesthetic agent in patients with airway hyperresponsiveness and/or bronchial asthma.

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