TÜBİTAK

Original Article

Turk J Med Sci
2010; 40 (2): 279-286
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0812-11

# Morphine modulates microvascular leakage dose-dependently in the airway of ovalbumin-sensitized rats

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**Aim:** To investigate the effect of morphine on ovalbumin-evoked airway microvascular leakage in sensitized rats.

**Materials and methods:** Rats were sensitized on days 0, 14, and 21 with ovalbumin. Intravenous ovalbumin (2 mg/kg) or capsaicin (50 µg/kg) increased the extravasation of Evans blue dye in trachea, bronchi, and intra-pulmonary tissues of sensitized rats.

**Results:** Morphine (1-10 mg/kg) inhibited ovalbumin-evoked increase in microvascular plasma leakage in a dose-dependent manner; however, it had no significant effect at the doses of 0.1 or 30 mg/kg. In addition, morphine, at the doses of 1-30 mg/kg, abolished microvascular leakage increased by capsaicin. The inhibition caused by morphine was blocked by the peripheral opioid receptor antagonist, naloxone methiodide, in ovalbumin or capsaicin series. Morphine or naloxone methiodide has alone no effect on plasma leakage.

**Conclusion:** These results conclude that morphine inhibits microvascular leakage, maybe mediated by neurogenic inflammation in sensitized rats, via peripheral opioid receptors.

**Key words:** Microvascular leakage, airway, sensitization, ovalbumin, capsaicin, morphine, naloxone methiodide, rat

## Morfin, ovalbumin ile duyarlı kılınmış sıçanların solunum yolundaki dolaşım sıvısı çıkışını doza bağlı olarak değiştiriyor

**Amaç:** Bu araştırma, ovalbumin ile duyarlılaştırılmış sıçanlarda solunum yolundaki dolaşım sıvısı çıkışı üzerine morfinin etkisini görebilmek için düzenlendi.

**Yöntem ve gereç:** Sıçanlarda, 0, 14 ve 21. günlerde ovalbumin uygulanarak alerjik duyarlılık oluşturuldu. Ovalbumin (2 mg/kg) ve kapsaisin (50 µg/kg) sıçanlara damar içi verilerek, solunum yolundaki dolaşımda damar dışına Evans mavisi kaçışı ölçüldü.

**Bulgular:** Morfin (1-10 mg/kg aralığında) doza bağlı olarak ovalbuminin solunum yolunda yol açtığı plazma sızıntısını durdurdu (0,1 ve 30 mg/kg dozlarda ise önemli etkisi yoktu). Ayrıca morfin (1-30 mg/kg doz aralığında) kapsaisinin yaptığı plazma çıkışını engelledi. Morfinin çevre dokulardaki reseptör antagonisti olan nalokson metiyodid bu etkilerin tamamını tersine çevirdi. Morfin ve nalokson metiyodid tek başına uygulandıklarında plazma sızıntısına yol açmadılar.

**Sonuç:** Bu bulgular, morfinin solunum yolundaki alerjik duyarlı dokulardan plazma sızıntısını durdurduğunu ortaya koyuyor. Plazma sızıntısı nörojenik inflamasyonla ilişkili olabilir ve bu olayda çevresel opioid reseptörler rol alabilir.

**Anahtar sözcükler:** Plazma sızıntısı, solunum yolu, duyarlılık, ovalbumin, kapsaisin, morfin, nalokson metiyodid, sıçan

Received: 04.12.2008 – Accepted: 10.12.2009

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## Introduction

The leakage of plasma proteins from the microvasculature into airways tissue is an important factor in the pathogenesis of asthma inflammation in human (1). The microvascular leakage facilitates airway oedema, which may consequently produce epithelial cell damage, bronchospasm, and airway obstruction. This phenomenon has been modeled in rats that were sensitized and challenged with ovalbumin (2,3). The mechanism of microvascular protein leakage in the allergic asthma has recently been discussed; however, it has not been fully understood or explained as yet.

Several studies have demonstrated that opioids, during peripheral inflammation, have anti-inflammatory effects, including anti-oedema (4,5) and anti-exudation (6-8). In the periphery, opioid receptors are expressed on sensory nerves and on sympathetic postganglionic terminals, where they may participate in the modulation of vascular permeability under certain inflammatory conditions (9). Opioid receptors are also constitutively expressed in non-neuronal sites including vascular endothelial cells (10,11) and immune cells, such as macrophages and lymphocytes (12). They have been identified in rat airways (13). Previous reports have demonstrated that the activation of these receptors by opioids modulates the microvascular protein leakage in animal models. Opioids may reduce the cholinergic neural responses in airways via an inhibitory action on excitatory NANC nerves and by a direct effect on cholinergic neurotransmission (an in vitro study, 14). Morphine inhibits airway plasma leakage by presynaptic inhibition of release of neuropeptides from sensory nerves (an in vivo study, 15), and prevents peritonitis induced by zymosan via mast cell-derived factors (16). Opioid agonists increase, paradoxically, plasma leakage due to endogenous histamine release from mast cells (17), where histamine is an inflammatory mediator causing microvascular leakage in airway of rodents and asthmatic humans (18). There are also other controversial findings reported morphine effects on the airway microvascular leakage. Morphine does not alter microvascular leakage increased by airway hypocapnia in guinea pig trachea (19) and by N-formyl-methionyl-leucyl-phenylalanine (FMLP) inhalation in rabbit trachea (20). On the other hand, morphine has an inhibitory effect, through inhibition of neurotransmission, on airway plasma leakage induced by cigarette smoke (21). However, effect of morphine on microvascular plasma leakage induced by ovalbumin allergen in sensitized rats is unknown.

The aim of the present study was to investigate the effect of morphine on microvascular plasma leakage in the airways of sensitized rats and the role of peripheral opioid receptors in this effect. For this purpose, the studies of allergic asthma model were undertaken by capsaicin-, and ovalbumin-evoked microvascular plasma leakage in ovalbumin-sensitized rats. Microvascular plasma leakage was evaluated by measuring the leakage of Evans blue dye in Wistar-albino rat trachea, bronchi, and intrapulmonary airway.

## Materials and methods

### Animals

Pathogen-free female Wistar-albino rats (180-200 g in weight) were obtained from the Experimental Animal Unit of Zonguldak Karaelmas University and housed at 22 ± 1 °C under 12:12 h light-dark cycle. Animals were allowed free access to standard laboratory chow and water. All procedures complied with the standards for the care and use of animals as stated in *the Guide for the Care and Use of Laboratory Animals.*

### Experimental protocol

Rats were sensitized on days 0, 14, and 21 with ovalbumin (at 100 μg administered with aluminum hydroxide adjuvant at 100 mg, i.p.) and were ready to use from day 28.

Plasma leakage was measured as described by a previous study (3). Briefly, sensitized rats were anaesthetized with Na thiopental (50 mg/kg) and given Evans blue dye (25 mg/kg as 25 mg/mL in saline, i.v.). Two minute later, they were given ovalbumin (2 mg/kg, i.v.), and capsaicin (50 μg/kg, i.v.) or vehicle. Cervical jugular vein was used for intravenous injections. The dose of ovalbumin or capsaicin to alert the microvascular plasma leakage was chosen based on the literature (3,22). Animals were killed by Na thiopental overdose (100 mg/kg i.p.), 15 min after allergen administration.

The chest was opened and an incision was made in the left ventricle, then a cannula was inserted through the left ventricle and into the ascending aorta, and approximately 150 mL of sterile saline (0.9%) was perfused at a pressure of 100 mmHg. The heart and lungs were removed en bloc. The trachea, bronchi, and intra-pulmonary airways were each placed in 2 mL of formamide for 18 h at 40 °C to facilitate the extraction of Evans blue dye. The absorbencies of the resulting extracts were determined against standard concentrations of Evans blue at a 620 nm wavelength. The measurements were duplicated. The results are expressed as concentration of Evans blue dye (ng/mg of wet tissue).

The experimental protocol for the effect of morphine or morphine plus naloxone methiodide (peripheral opioid receptor antagonist) was put on ovalbumin-, and capsaicin-induced microvascular leakage in the airways and plasma leakage was assessed as described above. Sensitized rats were received morphine (0.1, 1, 3, 10, and 30 mg/kg, s.c.) 20 min before ovalbumin or capsaicin injection. Naloxone methiodide (5 mg/kg, s.c.) was injected 5 min before morphine administration. The animals were killed 15 min after ovalbumin or capsaicin and tissues were removed for Evans blue dye extraction.

**Data analysis**

The plasma leakage results are expressed as mean ± s.e.m. of the concentration of Evans blue (ng/mg of tissue). Differences between the groups were analyzed by one way analysis of variance (ANOVA) and followed by the Bonferroni's multiple comparison test or Dunnet's multiple test, using GraphPad Prism, version 3.0. A value of $P < 0.05$ was considered as significant.

**Chemicals**

In this study, the following drugs and chemicals were used: Ovalbumin (grade V), naloxone methiodide, Tween 80, and formamide (Sigma, St. Lous, M.O., USA); capsaicin, Evans blue dye (Fluka Chemie GmbH, Buchs, Switzerland); morphine hydrochloride (Galen İlaç San. A.Ş., Türkiye); thiopental sodium (Pentothal sodium, Abbott Lab. Ltd. Şti, Türkiye); aluminum hydroxide. Capsaicin was prepared in 1:1:8 mixture of ethanol, Tween 80, and 0.9% sodium chloride and diluted further for use with 0.9% sodium chloride. Ovalbumin, morphine, and naloxone methiodide were dissolved in 0.9% sodium chloride.

## Results

**Effects of morphine or naloxone methiodide combination on ovalbumin-induced microvascular leakage in the airways of sensitized rats.**

Intravenous administration of ovalbumin (1 mg/kg) to ovalbumin-sensitized rats significantly increased microvascular leakage in airways (in trachea: 1.41 ± 0.47 vs 36.76 ± 3.63, $P < 0.001$; in bronchi: 2.16 ± 0.50 vs 41.76 ± 5.58, $P < 0.001$; and in intra-pulmonary airways: 4.16.21 ± 1.20 vs 51.76 ± 4.59, $P < 0.001$ ng/mg of tissue for vehicle and ovalbumin administration, respectively, Figure 1). Morphine (1-10 mg/kg, s.c.) given 20 min prior to ovalbumin induced dose-dependent inhibition of the microvascular leakage response in all airway tissues. Maximal inhibition was reached at the dose of 3 mg/kg, and microvascular leakages in trachea, bronchi, and in intra-pulmonary airways were obtained as 36.76 ± 3.63 vs 9.49 ± 1.38, $P < 0.001$; 11.99 ± 2.23, $P < 0.01$; 18.49±4.02, $P < 0.001$ compared to the ovalbumin group, ng/mg of tissue for that dose of morphine administration, respectively (Figure 1). However, doses of 0.1 and 30 mg/kg of morphine had no significant effect (Figure 1).

A dose of 3 mg/kg of morphine, which produced a maximal inhibitory response, was chosen for peripheral opioid antagonist, naloxone methiodide. Pretreatment with naloxone methiodide (5 mg/kg) 5 min before morphine application reversed the action of morphine and thus there were no significant between ovalbumin (alone) and morphine + naloxone methiodide combination for each one of 3 tissues (microvascular leakages in trachea, bronchi, and intra-pulmonary airways were obtained as 31.65 ± 5.00, 32.44 ± 5.30, and 40.26 ± 2.49, ng/mg of tissue for morphine plus naloxone methiodide administration, respectively, $P > 0.05$ compared to the ovalbumin group, Figure 1).

Morphine or naloxone methiodide has alone no effect on plasma leakage (data not shown).

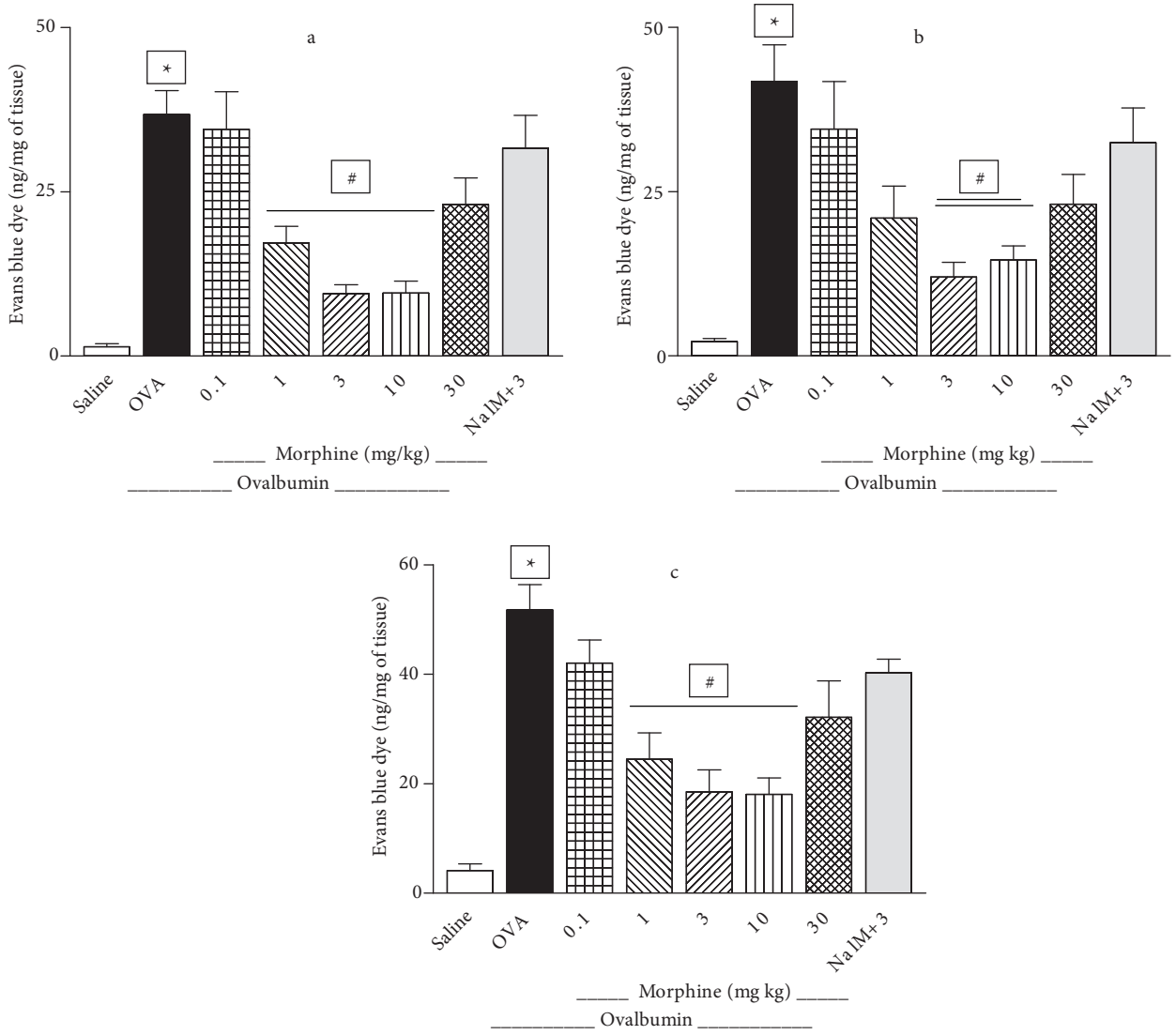


Figure 1. Effect of morphine on ovalbumin-evoked microvascular plasma leakage into trachea (**a**), bronchi (**b**), or intra-pulmonary (**c**) of antigen-sensitized rat, in vivo.

**Effects of morphine or naloxone methiodide combination on capsaicin-induced microvascular leakage in the airways of sensitized rats**

Intravenous administration of capsaicin (50 μg/kg) caused significant increases in microvascular leakage of airways of ovalbumin-sensitized rats (in trachea: 9.67 ± 1.66 vs 79.26 ± 5.3, $P < 0.001$; in bronchi: 12.17 ± 1.51 vs 71.76 ± 8.40, $P < 0.001$; and in intra-pulmonary airways: 7.17 ± 1.71 vs 66.76 ± 9.01, $P < 0.001$ ng/mg of tissue for vehicle and capsaicin administration, respectively, Figure 2). Morphine (1-30 mg/kg, s.c.) given 20 min prior to ovalbumin induced the inhibition of the microvascular leakage response in all airway tissues. Microvascular leakages in trachea, bronchi, and in intra-pulmonary airways were obtained as 15.97 ± 2.65, 22.72 ± 4.43, and 28.47 ± 6.18, $P < 0.001$ compared to the capsaicin group, ng/mg of tissue for morphine (1 mg/kg) administration, respectively (Figure 2). On the other hand, at a dose of 0.1 mg/kg, it had no significant effect in all tissues ($P > 0.05$ compared to the capsaicin group, Figure 2).

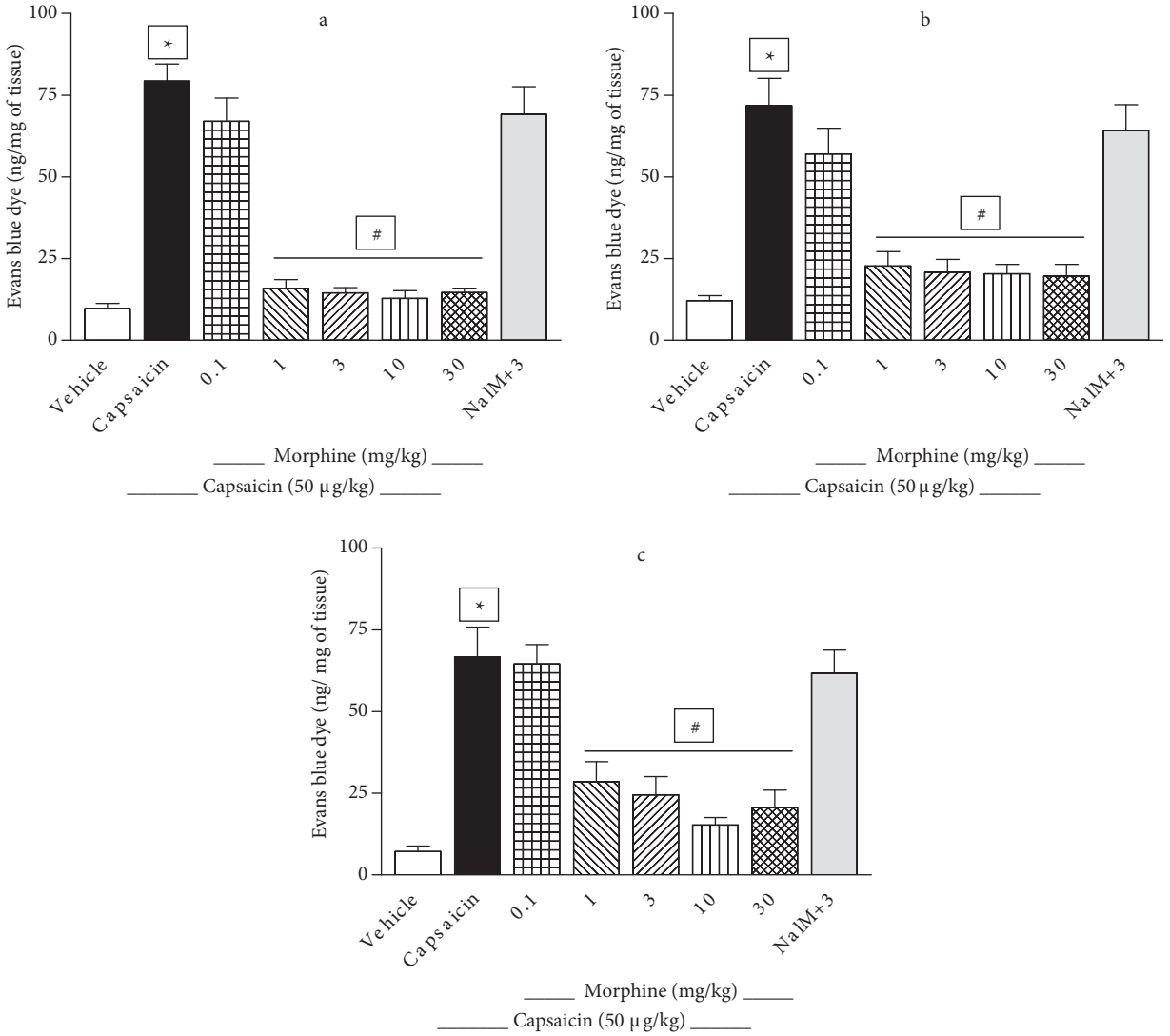


Figure 2. Effect of morphine on capsaicin-evoked microvascular plasma leakage into trachea (**a**), bronchi (**b**), or intra-pulmonary (**c**) of antigen-sensitized rat, in vivo.

Pretreatment with naloxone methiodide (5 mg/kg) reversed morphine (1 mg/kg) action and thus there were no significant difference between ovalbumin (alone) and morphine + naloxone methiodide combination for each one of the 3 tissues (microvascular leakages in trachea, bronchi, and intra-pulmonary airways were obtained as 69.15 ± 8.44, 64.15 ± 7.97, and 61.65 ± 7.13, ng/mg of tissue for morphine plus naloxone methiodide administration, respectively, $P > 0.05$ compared to the ovalbumin group, Figure 2).

## Discussion

The leakage of plasma proteins from the microvasculature into airway tissue is an important consequence of asthmatic airway inflammation. The leakage of plasma proteins was evaluated by measuring the tissue accumulation of Evans blue dye, which binds to proteins. The present experimental model was undertaken on ovalbumin-sensitized rats that were evoked with intravenously administration of ovalbumin and capsaicin. Our results show that either of ovalbumin and capsaicin can cause microvascular leakage into the airway tissue. The

microvascular leakage was observed to be distributed throughout the trachea, bronchi, and intra-pulmonary airways. The increase in airway microvascular leakage induced by ovalbumin could be inhibited by dose-dependent pretreatment with morphine (1-10 mg/kg), while the increased leakage could be decreased by the high dose of morphine (30 mg/kg) pretreatment. Furthermore, morphine significantly inhibited microvascular leakage induced by capsaicin in the airway. Previous studies showed that microvascular airway leakage is increased in sensitized rats, mice, and guinea pigs by ovalbumin (3,23-25) and capsaicin (22,23).

The increased airway microvascular leakage induced by i.v. application of ovalbumin to ovalbumin-sensitized rats could be a result of several factors. Ovalbumin antigen may elicit the release of inflammatory mediators from inflammatory and structural cells in airways (3). These mediators can lead to the typical pathophysiological changes of asthma, including microvascular plasma leakage. Many mediators are released in asthma, and it is clear that these mediators interact with each other in some ways. Mediators may act synergistically to enhance each other's effects, or one mediator may modify the release or action of another mediator (2,18).

Our results indicate that morphine has a biphasic response to the airway microvascular leakage increased by ovalbumin antigen, in such that it significantly inhibits microvascular leakage at lower doses (1-10 mg/kg) and the degree of inhibition decreases at a higher dose (30 mg/kg). This effect of morphine on airway microvascular leakage in sensitized rats might be explained by the reason of unexpected action on histamine. A pharmacology textbook states that morphine is contraindicated in asthmatic humans, because it can cause severe airway bronchospasm via histamine release action (26). It is known that morphine induces histamine release via mast cell degranulation. This effect likely emerges at high doses (over 20 mg/kg) (16). Therefore, it appears that further studies are warranted to understand the interaction of opioid and histamine with regard to microvascular leakage in the airway of sensitized animals.

Neurogenic inflammation may be stimulated by ovalbumin antigen as another mechanism (24,27). Capsaicin causes microvascular leakage. This effect may be relevant by release of tachykinins from sensory nerves. Among the tachykinins, SP is the most potent to cause leakage. It can induce microvascular leakage in guinea pig airways when it is administered alone (24,28). The increased airway microvascular leakage induced by i.v. application of capsaicin to ovalbumin-sensitized rats could be a result of several factors: 1) This effect of capsaicin might be a result of an increased release of tachykinins and neuropeptides from sensory nerves, and 2) It could be the consequence of an increased sensitivity of the sensory nerves for various stimuli. It is known that cytokines as a tumor necrosis factor (TNF)-alfa can alter the sensitivity of the sensory nerves for neurogenic stimulants. It enhances the capsaicin-induced release of calcitonin gene-related peptide from sensory nerves (29). Our findings show that morphine decreases significantly microvascular leakage induced by capsaicin in airway in sensitized rats. Opioids can reduce the neurogenic inflammation mediated by capsaicin sensitive sensory nerves in the airways; this effect is probably associated with the inhibition of tachykinin and neuropeptide release. This hypothesis is supported by previous studies showing that morphine inhibits neurogenic inflammation induced by capsaicin in non-sensitized animals (15), by cigarette smoke (17,21), and by HCl intra-oesophageal instillation (28), where this action is shown with the inhibition of plasma leakage. Interestingly, a recent study reports that morphine inhibits nocifensive responses of ovalbumin-sensitized mice (30).

Opioid receptors have been localized in the airways of human and animals (13,31,32). It is clear from the data presented here that the treatment of sensitized rats with naloxone methiodide, a peripheral opioid receptor antagonist, causes a significant reversal of morphine effect, which inhibits airway microvascular leakage increased by ovalbumin and capsaicin. It is possible to think about the peripheric opioid receptors are involved in the inflammation of the airways.

Our data demonstrate that the doses of 1-10 mg/kg of morphine have an inhibitory dose-dependent effect on airway microvascular leakage induced by ovalbumin, while a dose of 30 mg/kg has no inhibitory effect, and the doses of 1-30 mg/kg of morphine have an inhibitory effect on airway

microvascular leakage induced by capsaicin in sensitized rats. These inhibitory effects are reversed by naloxone methiodide, a peripheral opioid receptor antagonist. These findings support that the modulated effect of lower doses of morphine in airway plasma leakage might be due to the inhibition of the release of neuropeptides and tachykinins from sensory nerve endings, which is stimulated by capsaicin, and that peripheral opioid receptors have a role in the microvascular leakage responses to ovalbumin and capsaicin in sensitized-rat airways.

**Acknowledgment**

The author wish to thank Dr F. Bayıroğlu for many helpful discussions and for the critical review of the manuscript. This work was supported by intramural funding of the Office of Research and Sponsored Programs, Zonguldak Karaelmas University. Preliminary results of the present study were presented at the 18th National Pharmacology Congress of Turkish Pharmacological Society, 28 September-1 October 2005, İzmir, Türkiye.

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