The Effect of Denuded Epithelium on Responsiveness of Stress-Induced Guinea-Pig Isolated Trachea To Vip (Vasoactive Intestinal Peptide) and Salbutamol

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SUMMARY

Both neural and neuropeptide control mechanisms of airway might be affected by stress, and the responses of airway to stress might also be affected by epithelium. We, therefore, have investigated the effect of airway epithelium on responsiveness of stress-induced guinea-pig isolated trachea to VIP (Vasoactive Intestinal Peptide) and salbutamol. In addition, we histologically have examined the mast cells of the trachea. Contractile responses evoked by Acetylecholine (ACh) and histamine were more potent and rapid in stress-induced tracheal preparations with an intact epithelium than epithelium denuded. The most relaxing effect of VIP also occured in stress induced intact preparations precontracted by histamine. Although the relaxing responses of VIP absolutely depend on the presence of epithelium, salbutamol relatively depend. Histological results showed that degranulation and the number of mast cells may contribute to the etiology of stress-induced bronchial hyperreactivity or airway infections.

Keywords: VIP, Stress, Trachea epithelium, Mast cell, Salbutamol

ÖZET

Solunum yollanınn gerek sinirsel gerekse nöropeptid kontrol mekanizması stresden etkilenebilir. Solunum yollarının epitel yapısında stresse karşı oluşturulacak cevabı etkileyebilir. Bu nedenle, stres uygulanmış kobay izole trakea preparatının VİP ve salbutamole vermiş olduğu gevşetici cevaplara solunum yolları epilelinin etkisi incelenmiştir. Ayrıca histolojik olarak mast hücrelerine bakılmıştır. Stres uygulanmış, epiteli intakt trakea preparatlarında asetilkolin ve histamin ile oluşturulan kasılma cevapları daha kuvvetli ve hızlı gözlenmiştir. VİP'in kuvvetli gevşeme cevabı ise histamin ile bastırılmış, epiteli intakt stresli preparatlarda elde edilmiştir. VİP'in gevşetici cevaplarının gözlenebilmesi doğrudan epitelin intakt olmasına bağlı iken salbutamolun gevşetici cevaplarının ortaya çıkmasında epitele bağımlılık daha az olarak gözlenmiştir. Histolojik çalışmalar sonucunda stress uygulanmış preparatlarda mast hücre sayısının ve degranulasyonun arttığı saptanmıştır. Sonuç olarak solunum yollarının epitelindeki herhangi bir hasarın veya fonksiyon bozukluğunun, stresse bağlı aşırı duyarlılık ve solunum yolları enfeksiyonlarında rol oynayabileceği düşünülmekledir.

Anahtar Kelimeler: VİP, Stres, **Trakea** epiteli, Mast hücreleri, Salbutamol

Turk J Resc Med Sei. 1991, 9:71-76

T Klin Araştırma. **1991, 9:71-76**

Geliş Tarihi: 4.3.1989 **Kabul Tarihi: 23.1.1990**

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It is fairly established that acetylcholine (ACh)-induced relaxation of isolated artery preparations are dependent on the presence of an in-

tact endothelium (10,12). This relaxation seems to be mediated via the production of an endothelium-derived relaxing factor (EDrF). It was reported that the epithelium of isolated airway preparations also produce a relaxing factor (13). The epithelium-derived inhibitory factor (s), it is generaly believed, normally modulates the responsiveness of respiratory smooth muscle to contractile and relaxant agents. Removal of the epithelial cell layer alters the responiveness of in vitro preparations of the airway smooth muscle in several mammalian species. Therefore, the airway hyperreactivity, at least in part, results from the loss of an apethelium-dependent inhibitory influence on smooth muscle (14).

Vasoactive intestinal peptide (VIP), a 28-aminoacid peptide, have recently been indentified in airways and has potent effects on airway calibre in vitro, raising the possibility that it is a primary candidate for the neurotransmitter of non-cholinergic non-adrenergic (NANC) inhibitory nerves. Furthermore, in asthmatic patients a small bronchodilator response to VIP has been described (20,5,4).

Bronchial hyperresponsiveness, or excessive "twitchiness" of the airways was attributed to abnormal nervous mechanisms (5). Both neural and neuropeptide control mechanisms of airways might be affected by stress, and stressful events seem to promote respiratory infections (12,17). The response of airway to stress might also be affected by the epithelium.

On the other hand, mast cell degranulation may occur during stress due to an increased concentration of endogen opioid peptide (1). Casala et al (7), showed that human cutaneous mast cells respond to opiates and endogen opioid peptides by in vivo degranulation. In addition, it has been reported that opioid alkaloids and peptides increase the accumulation of calcium in mast cells and opiate receptors to be present in these cells (16).

In our study we investigated the role of airway epithelium in the response of stress-induced guinea-pig isolated trachea to contractile (ACh and histamine) and relaxant (VIP and salbutamol) agonists. In addition we examined histologically the mast cells of the stress-induced and non-stressed guinea-pig trachea.

MATERIAL AND METHODS

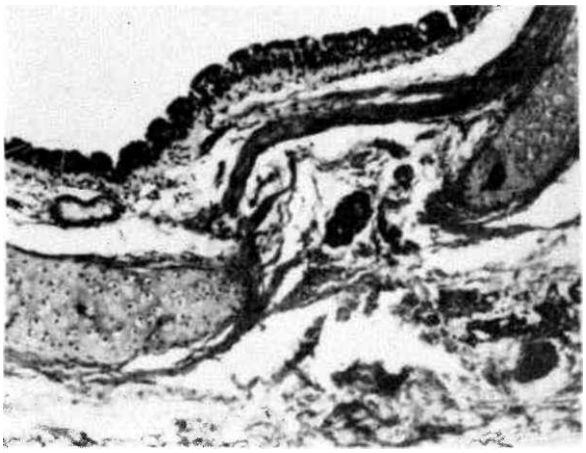
Adult guinea-pigs (250-300 g) of either six were used. The animals were divided into two groups; stress-induced and non-stressed as a control. In the stressed group, the animals were exposed to immobilization in cold for an hour and than to swimming. All animals were killed by cervical dislocation.

Trachea was divided into two segments, rostral and caudal. Each segment was cut spirally to attain an amount of muscle tissue equivalent to that of six tracheal segments. Each muscle strip was placed in a 20 ml organ bath (Hugo Sachs 4 container Schulcr organ bath) containing a modificd-Kreps-Hcnseleit solution aerated with 95% O_2 + 5% CO_2 and maintained at 37°C (2). They were mounted isometrically on forcd transducer (load, lg) (F-60 Narco Bio System). Responses were monitored on a pen recorder (MK IIIsS, Narco Bio System). Tissue baths and glass support rods were siliconized periodically to reduce the peptide binding to glass surfaces (3).

ACh (10^{-4}M)and histamine ($5x10^{-5}$M) were used to induce contraction, and VIP ($1.5x10^{-8}$" $1.5xlO^{-10}$M) and salbutamol (10^{-6}M) were added once the response reached a plateau. TheVIP and salbutamol-induccd relaxation was expressed as a percentage of the ACh and histamine-induced contractions. The results are given a means ±S.E.M. of the number (= 19) of experiments. Statistical analysis was conducted by the use of Student's t test for paired or unpaired.

The epithelium of the rostral and caudal tracheal strip were alternately removed with a smooth brush cleaner. The presence or absence of the epithelium was confirmed by histological examination. Sections were stained with hacmotoxylin and eosin and the integrity of the airway epithelium assessed at the light microscope (Figure 1).

A modified stainig tecnique used to demonstrate mast cells involves neutral formalin (%10) fixation of sections and toluidine blue staining. After staining, treatment of preparations immediately with absolute alcohol (Isopropanol or ethyl alcohol) protect the granules to lysis. In serial sections, the presence of mast cells were examined in light microscope histologically. Mast cells were considered to be "degranulated" if their cytoplasm showed some metachromasia or exocytosis (ink granules were observed outside the boundaries of mast cells).



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I . Lpithclium rubbed. ILL.

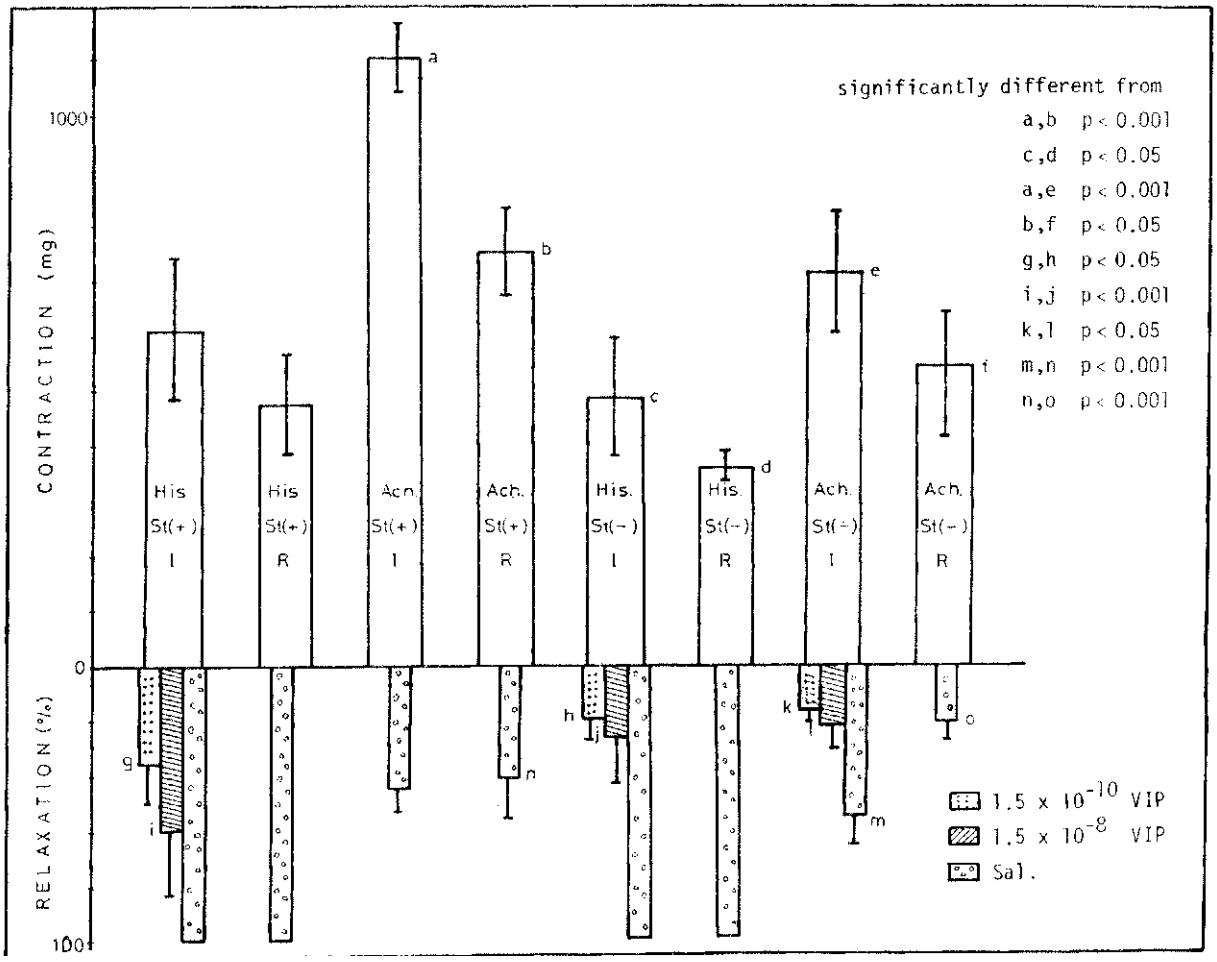

Figure **2.** Contraction (mg) and relaxation (%) responses ol trachea smooth muscle to ACh. His, **VIP,** Sal. (FLpithclium intact, R:lipithelium rebbed, St(+): Stress-induced, St(-): Non-stressed, His: Histamine, Sal: Sulbulamol).

RESULTS

Contractile responses evoked by ACh and histaminc were more potent and rapid in all the stress-induced tracheal muscle strips (with or without epithelium) than control groups. But statistical significance was observed only in the ACh-induccd

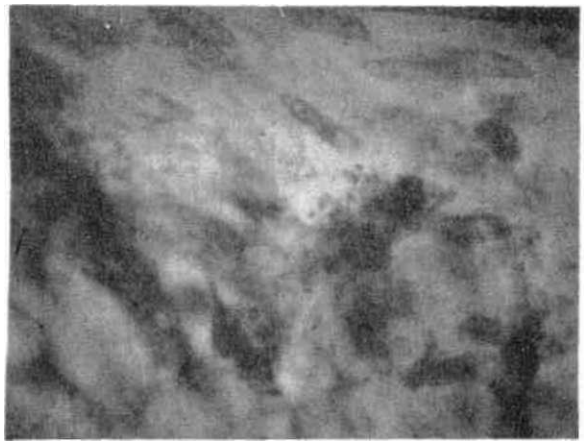

Figure 3*. Slrcss-inducd cxoeylosis. Touidmc blue X.120

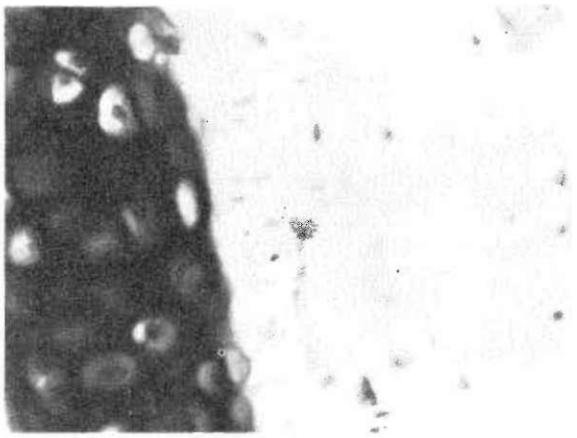

Figure 3 ' Slrcss-inducd cxocxtosis. Toluidinc blue X128

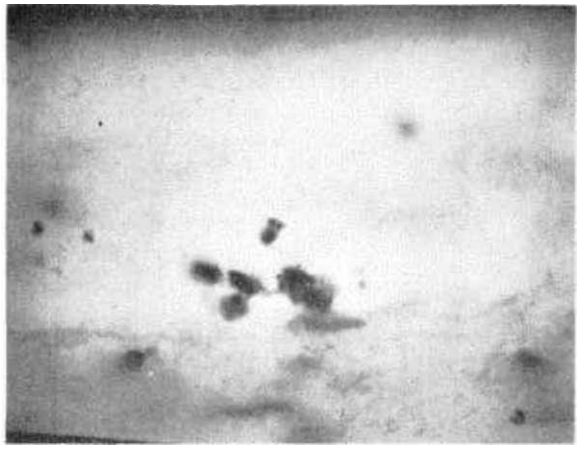

Figure 4 .Stress-induced nichac'hromasi and cxoeylosis. I oliidinc blue. **X320**

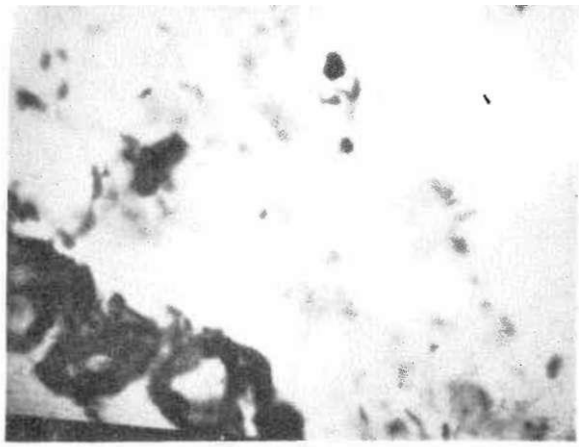

Figure 4[b]. Stress-induced methachromasi. Toluidine blue-X128.

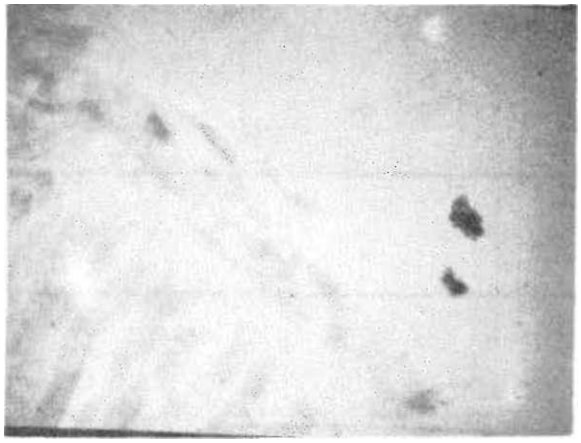

Figure 5*. Non-stressed strong methachromasi. Toluidinc blue X320.

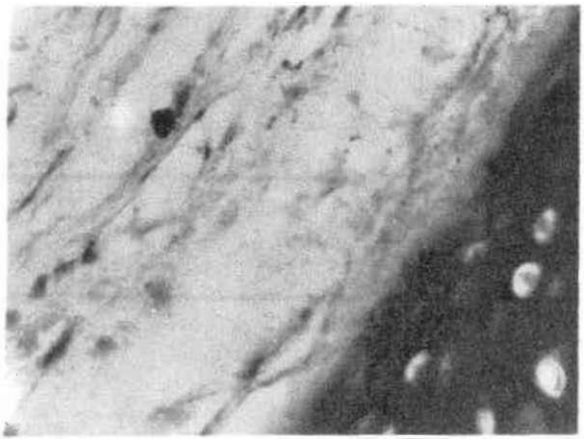

Figure S'. Non-stressed strong methachromasi. Toluidine blue. **XI**28.

contractions. The removal of the epithelium slightly reduced the sensitivity of isolated tracheal strips to A C h had histamine in both the stressed and non-stressed groups (Figure 2). Also, the most relaxing effect of VIP occurcd in stress-induced intact preparations precontracted by histamine. VIP

failed to bring about relaxation in stress-induced precontracted by ACh preparations; furthermore, it potentiated the responses of ACh when the epithelium was rubbed. VIP was more effective in relaxing the tracheal smooth muscle precontracted by histamine than those contracted with ACh in the non-stressed group with intact epithelium. Neither the stressed nor non-stressed group with intact epithelium. Neither the stressed nor non-stressed groups with rubbed epithelium precontracted by both ACh and histamine gave any relaxing response to VIP (Figure 2).

Salbutamol relaxed all tracheal strips precontracted by histamine. The relaxation was more rapidly induced in both the stressed and non-stressed epithelium intact groups than the rubbed ones. On the other hand, salbutamol moderately relaxed the ACh precontracted tracheal smooth muscle, and the relaxation of stress-induced epithelium intact groups was slower and weaker than non-stressed epithelium intact groups. But the results were statistically non-significant. On the contrary, the response of the epithelium-rubbed stress-induced tracheal strips to salbutamol was significantly higher than the non-stressed, epithelium-rubbed preparations (Figure 2).

Histological results showed an increase in the degranulation and number of mast cells in the stress-induced tracheal strips (Figs: 3a-b, 4a-b, 5a-b).

DISCUSSION

Our findings showed that the relaxation by VIP definitely depends on the presence of the epithelium, while the response to salbutamol was less dependent on its presence. There was no difference in the ability of salbutamol to relax responses produced by histamine with or without epithelium. Salbutamol was much less potent at reversing contraction produced by ACh than those elicited by histamine, and it is slightly more potent when the epithelium is present.

Autoradiographic identifications clearly show that a greater density of Bcta-adrenoceptors exist in epithelial cells than in smooth muscle (9,11). Although the presence of the epithelium docs not appear to be necessary for Beta-adrenoceptor mediated relaxation, it acts to augment the direct effect of Beta-adrcnoccptor stimulation. Thus, the reduced responsiveness of epithelium-denuded tracheal rings to salbutamol apparently results from the lack of a modulating role of the epithelial cells on Beta adrenergic responsiveness of airway smooth muscle. During stress this sympathomimetic drug is less effective due to the more intense bronochoconstriction elicited by ACh in the epithelium intact preparations. Several investigators have demonstrated that increasing the degree of muscarinic activation in airway smooth muscle decresacs the sensitivity to Beta-adrenergic agonists (15). In the stress-induced preparations ACh contractions was possibly potentiated by SRS (LTC4: LeukotrieneC4+LTD4: LeukotriencD4) which is released by mast cells. This compound causes bronchoconstriclions, and salbutamol is much less potent at reversing contraction produced by LTC4 hay ct al (1988). Besides, it was reported that the mucosal mast cell LTC4 content is higher than that of connective tissue mast cell (18).

As the most relaxing effect of VIP occured only in the histamine-prccontracted stress-induced epithelium intact preparations, the epithelium may play an important role in the responds of airways to stress. A relaxant factor generated by the tracheal epithelium might have a permissive effect for VIP to exert its relaxation effect (9,11,13). Culz et al. (8). have reported that VIP or closely related peptide, occurs in lung, small intestine and peritoneal rat mast cells, from which it can be released by histamine releasers, and that VIP could have an important modulating influence on the release of histamine and other mediators from mast cells. Furthermore, when discharged from pulmonary mast cells, VIP may exert its relaxant action on bronchial smooth muscle. Stress may have a role as a histamine releaser due to the increased opiatcrgic system activity (1,7,16). This suggestion is supported by our findings. However, the effect of VIP under such conditon is closely related to the presence of the intact epithelium.

Studies on the effect of epithelium on the responsiveness of airway smooth muscle have been reported by other groups (4,9,11,19). They showed that removal of the epithelium from the bronchial rings enhanced responses to ACh, histamine and

5-HT but attenuated the responses to isoprenalin and tulobuterol. These reports are not in complete accord with the present study, particularly with regard to contractile agonists. We have observed that the removal of epithelium dose not enhance the sensitivity of guinea-pig isolated tracheal strips to ACh and histamine. However, the effect of relaxant agonists have been reduced and inhibited by epithelium denuation.

In the light of the peresent study damage to or disfunction of respiratory epithelial cells may contribute to the etiology of stress induced bronchial hyperreactivity or airway infections. Stress-induced responsiveness of tracheal smooth muscle to contractile agonists, can be relaxed more easily by relaxant agonists in the presence of intact epithelium. Otherwise, the airway hyperreactivity would continue and bronchoconstriction would not be reversed.

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