

# THE ROLE OF PROSTAGLANDINS AND MAST CELLS IN THE MODULATION OF ACUTE ACID-INDUCED TRACHEAL CONTRACTION IN RAT

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**Abstract-** The exact role of the epithelial lining of the trachea in modulating the bronchial tone is controversial. The present study was an attempt to verify the role of both prostaglandins and mast cells in the acute phase of acid inspiration in rat. Four groups (n = 6) of N. Mari rats were employed. The first group was used as placebo control, and normal saline was injected. To the second group hydrochloric acid (25 µl) with pH of 1.3 was injected into the trachea through the criothyroid membrane. The third and fourth groups were pretreated for three consecutive days either with indomethacin (10 mg/Kg) or nebulized sodium cromoglycate (20 mg/Kg), 1 hour prior to installation of the acid. Three minutes after instillation of acid, the trachea was removed. The tracheal spirals were prepared and immediately suspended in an organ bath containing Tyrode's solution. Dose response curves to acetylcholine ( $10^{-9}$  to  $10^{-3}$ M) were constructed. The results showed that the responses to acetylcholine in the acid treated trachea were significantly ( $P < 0.01$ ) reduced compared with the control saline treated trachea due to acute acid-induced tracheal contraction. Incubation with atropine, induced reduction of baseline tension and reversed the responses to acetylcholine. Both indomethacin and sodium cromoglycate, reversed the responses to acetylcholine and were in similar range as the control trachea. In conclusion, it seems that both prostaglandins and mast cells are important mediators in the acute phase of airway smooth muscle contraction following instillation of acid.

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**Key words:** Acute acid-induced tracheal damage, acetylcholine, prostaglandin, mast cells, cromoglycate, indomethacin

## INTRODUCTION

One of the complications of post-operative anaesthesia is the aspiration of gastric contents with the possible serious consequences of aspiration pneumonia (1). Thus, the dictum of non-per-oral prior to anaesthetization is the norm in all elective surgical operations. However, in emergency situations such precautionary measures are not possible which carries an increased risk of aspiration of gastric contents. Inhalation of liquids or solids with a pH of less than 2.4 has been implicated to induce an immediate severe bronchoconstriction and, within hours, destruction of tracheal mucosa (2,3). The mechanisms by which the endogenous and exogenous substances implicated in the mediation of the sequela of the abnormalities

associated with tracheal dysfunction are varied and numerous. Furthermore, the exact role of the epithelial lining of the trachea in modulating the bronchial tone is controversial; It has been suggested to facilitate the release of noradrenalin (4). In addition, it causes the release of an inhibitory substance, which suppresses the release of inhibitory factors that inhibit the release of noradrenalin (4). It is proposed to be the source of the relaxatory prostaglandin intermediates (PGI and PGE<sub>2</sub>) (5), an epithelium relaxing factor (6) or nitric oxide (7).

On the other hand, contraction to acetylcholine in the rabbit trachea is not significantly increased in epithelium denuded relative to epithelium intact rings (5). In experimentally sensitized guinea-pig trachea, it has been shown that epithelium mediates the potentiation of anaphylactic contractions induced by ovalbumin (8). In addition, the epithelium has been reported to lack acetylcholinesterase (9). Perhaps these controversies may be due to species differences (10), since most studies have been conducted on different

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animal species. The presence of both sensory neurons in the bronchial epithelium has been shown to modulate the responses of the bronchial contractions. A variety of receptors including those responsive to nitric oxide (7), tachykinin (11), opioids (8), muscarinic M<sub>2</sub> (12), beta 2 adrenergic (4), prostaglandins (13), histamine (14), in addition to non-adrenergic non-cholinergic receptors (15), have been demonstrated. The degree of significance of each of these receptors in the control of the bronchial tonocity is still a matter of speculation. The aim of this study was an experimental attempt to elucidate the role of prostaglandins and mast cells in the early acute tracheal contraction following the instillation of hydrochloric acid in anaesthetized rat.

## MATERIALS AND METHODS

N-MRI rats weighing 140 to 180g were used in this study. The rats were purchased from Razi Institute in Tehran. The animals were housed in AM transparent PVC cages in groups of four, maintained in a 12 hourly light/dark cycle starting at 7 am to 7 PM, and had free access to food and tap water. The following two series of experiments were performed:

A. The rats were divided into four groups of six animals each. The animals were anaesthetized with sodium thiopental. An excision opening through the skin of the throat was made and the trachea was fully exposed. The first group was allocated as placebo control to which 25µl of normal saline was injected through the cricothyroid membrane using a Hamilton syringe. The second group was treated similarly but injected with 25 µl of hydrochloric acid at pH of 1.3 (11). The third and fourth groups were pretreated with indomethacin (10 mg/kg, i.p.), or nebulized with disodium cromoglycate (20 mg/kg) respectively, given daily for three consecutive days and one hour prior to acid treatment. After three minutes of exposure to the acid or normal saline, the trachea were removed, washed in aerated Tyrode's solution and freed from fat and connective tissues. Tracheal spiral were prepared and suspended, under 1g tension at 37 °C in an organ bath containing Tyrode s solution, and aerated with oxygen. Following stabilization after several washings at 15 minutes intervals, tissue responses to acetylcholine (10<sup>-8</sup> - 10<sup>-3</sup> M ) were recorded.

B. In order to investigate the reasons of the reduced responsiveness to acetylcholine, separate complementary experiments comprising two groups

of six rats were employed. The first group was treated as control and the second group used as test group as above. Following the stabilization of the tracheal spirals, the responses to submaximal (10<sup>-4</sup>M) dose of acetylcholine were recorded under two conditions: a. as above and following stabilization of the tissues and b. after 90 minutes of atropine wash-out. The wash-out was performed at 15 minutes intervals after a 5-minute exposure to 10<sup>-6</sup> M of atropine. Samples from the trachea of all groups were taken for histological staining. Tissues were stained with either haematoxylin and eosin, or mast cell staining with toluidine blue.

ANOVA followed by Tukey method of analysis were employed for comparison between means of more than two groups. Furthermore, student *t*-test was used when comparison was made between means of two groups and *P* value less than 0.05 was considered significant.

## RESULTS

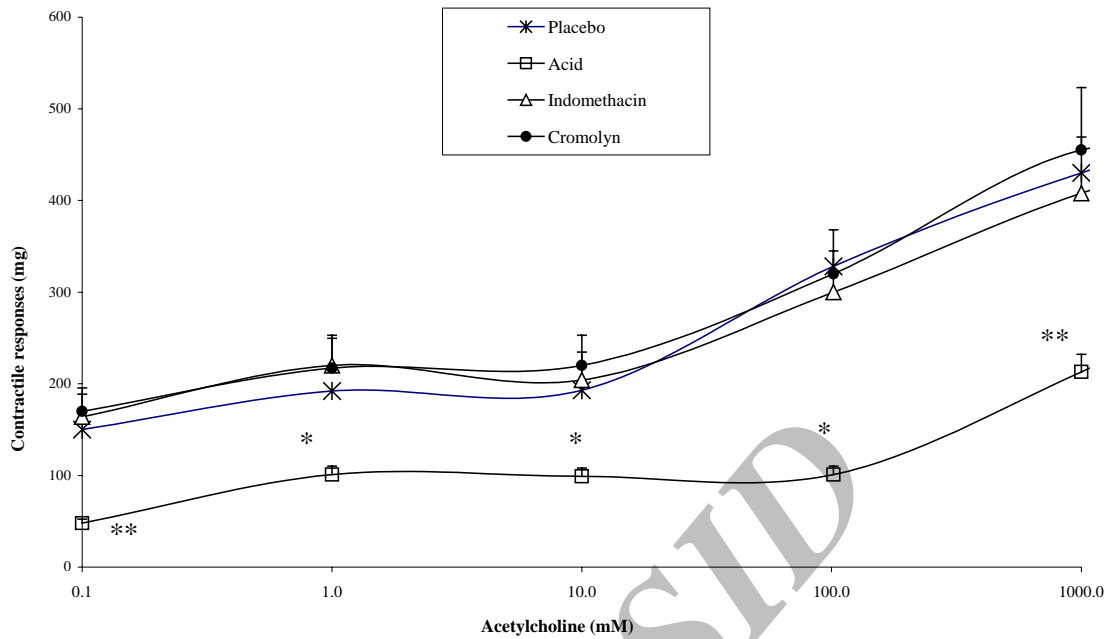
### Pharmacological investigations

The responses to acetylcholine: The dose response curves constructed for all the four groups of rats are illustrated in figure 1. The responses in the control normal saline-treated group attained its maximum level with 10<sup>-3</sup>M acetylcholine and produced 480 ± 22 mg of tension. The responses in both indomethacin and disodium cromoglycate-pretreated trachea are in similar range as those of the control group. However, the responses in the acid-treated trachea were significantly (*P*<0.01) reduced relative to control and attained maximum contraction with 10<sup>-3</sup> M acetylcholine producing 300 ± 15 mg tension.

Responses to acetylcholine following atropine wash-out: incubation with 10<sup>-6</sup> M atropine induced a gradual but sustained relaxation in the baseline tension of 80 ± 4 mg. This relaxation was maintained for more than 1 hour even after several washings. The control responses to 10<sup>-4</sup> M acetylcholine were 200 ± 14 mg, which significantly (*P*<0.01) increased to 430 ± 20 mg after atropine washout. This latter level of contraction was not significantly different from untreated trachea controls.

### Histological investigations

The instillation of hydrochloric acid induced an acute localized damage to the epithelial linings with loss of cells together with an increase in the number of infiltrated cells in the lamina propria.



**Fig. 1.** Effect of instillation of hydrochloric acid (25 $\mu$ l, pH 1.3) on the contractile responses of rat trachea to acetylcholine ( $10^{-7}$  to  $10^{-3}$   $\mu$ M) and after disodium cromoglycate (nebulised 20 mg/Kg) and indomethacin (10 mg/Kg, i.p.) pre-treatment. \* $P$ <0.01 & \*\* $P$ <0.001 relative to acid treatment, ANOVA followed by Tukey method of analysis,  $n=6$ .

In addition, there was localized hemorrhage, with some damage to the cartilaginous structures. No damage was seen to occur in the muscular areas of the bronchus. There was an extensive mast cell degranulation both in the lamina propria and in the remaining of the still attached epithelial layer in the control acid-treated group, but no degranulation of mast cells was observed in both the disodium cromoglycate, and indomethacin treated groups.

## DISCUSSION

In the present study, we have demonstrated that both prostaglandins and intermediate agents released from the mast cells were involved in the acute phase of tracheal contraction following instillation of hydrochloric acid in this experimental model. Both indomethacin, a potent prostaglandin inhibitor, and disodium cromoglycate, a mast cell stabilizing agent, were equally effective in maintaining the functional responsiveness to acetylcholine and inhibiting the acute tracheal contraction induced by acid instillation in the rat trachea. There are three possibilities for the reduced contraction to the *in vitro* addition of acetylcholine following the *in vivo* instillation of the acid in the trachea: The first is that the acid has

induced damage to the neuronal and smooth muscles underlying the submucosal layer. This is unlikely, because our histological investigations revealed no damage to this layer, and is supported by previous studies (11). The second is the shift of balance in favor of bronchocontractile prostaglandins (5). This suggestion seems to be less likely, at least by direct action, since incubation of the trachea with atropine, a selective muscarinic blocker, induced reduction to the baseline tension. The third, that our results suggest, is the presence of a pre-existing sustained contraction due to activation of the post synaptic muscarinic receptors. We were able to show that atropine induced a relaxation in the baseline tension and a reversal of the contractions to normal range. This observation suggests that, perhaps an excessive and a persistent irritation in the trachea induced a continuous release of acetylcholine at tracheal parasympathetic nerve endings and to be responsible for such reduction in responses, since the trachea was already in a sustained contraction.

Following instillation of the acid, indomethacin was found to prevent the degranulation of mast cells and produced a similar degree of protection as cromoglycate upon the acute tracheal contraction. These findings suggest that prostaglandins play a central role in this process. Namely they have a direct

action on mast cells and induce its degranulation, in addition to their involvement in tracheal contraction (13). Furthermore, prostaglandins and other intermediates released from mast cells (16) seem to have direct tracheal parasympathetic nerve stimulation. Cromoglycate stabilization action on mast cells not only nullified the effects of the released prostaglandins, but also abolished the release of various intermediates known to be released from these cells.

The overall conclusion from these observations suggests that both the prostaglandins and mediators released from the mast cells act via a common pathway resulting in the stimulation of the parasympathetic neuronal pathway in the tracheal tissues. The reason for arriving at this conclusion comes from several observations: Since both disodium cromoglycate and indomethacin produced similar degree of reversal of responses to acetylcholine, it seems that the prostaglandins act via the mast cells (16). The histological results also agree with these conclusions in that there was reduction in the inflammatory changes and absence of mast cell degranulation in both disodium cromoglycate and indomethacin-treated groups. In addition, *in vitro* studies have demonstrated that acetylcholine induced prolonged contractile responses in rat trachea (5), perhaps similar sustained contractions do occur under *in vivo* conditions as a result of excessive acetylcholine release.

What is the relevance of these findings to clinical practice? Although extrapolation of data from animal studies to human beings needs to be very guarded, the findings of this study dictate further studies on human subjects. Cromoglycate, a safe and nebulizable drug, can be given prophylactically, in order to prevent possible acid-aspiration damage, to patients on emergency operations and in whom prior non-per-oral regimen is not possible. This study is underway in our centre. One interesting finding from this study is that atropine was able not only to induce reduction in the baseline tension, but also in reversing the functional contractions to acetylcholine to normal control level. Perhaps drugs with similar mechanisms of action (like ipratropium bromide) can be used to increase the functional responsiveness in asthmatic patients to bronchodilator drugs that act via activation of adrenoceptor as well. However, further studies are required in order to support this hypothesis. The complex nature and involvement of many intermediates in the process of inflammation in the bronchial responses makes this type of research a challenging one. In this study, we have attempted to make an inroad towards solving one of many aspects

of acute bronchial damage. The prostaglandins and mast cells have been demonstrated to play an important role in the acute bronchial responses following acid instillation in this experimental model and deserve further investigation.

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