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Review

The Role of Mast Cells in Citric Acid-Induced Airway Constriction and Cough

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Abstract

Inhalation of citric acid (CA) causes airway constriction and coughing. To investigate the role of mast cells in CA-induced airway constriction and cough, three experiments using guinea pigs were carried out. In the first experiment, we used compound 48/80 to deplete mast cells, cromolyn sodium to stabilize mast cells, MK-886 to inhibit synthesis of leukotrienes, pyrilamine to antagonize histamine H_1 receptor, methysergide to antagonize serotonin receptor, and indomethacin to inhibit cyclooxygenase. In the second experiment, compound 48/80-pretreated animals were divided into 2 parts; the first one was used to test the role of exogenous leukotriene (LT) C_4, while the second one to test the role of exogenous histamine. Decreases in respiratory compliance (Crs) and forced expiratory volume in 0.1 sec ($FEV_{0.1}$) were used as indicators for airway constriction in anesthetized guinea pigs. CA-induced cough was recorded for 12 min using a barometric body plethysmograph in conscious animals. In the third experiment, the activation of mast cells upon CA inhalation was investigated by determining lung tissue or arterial plasma histamine concentration in animals. Exposure to CA induced marked airway constriction and increase in cough number. Compound 48/80, cromolyn sodium, MK-886 and pyrilamine, but not indomethacin or methysergide, significantly attenuated CA-induced airway constriction and cough. Injection of LTC_4 or histamine caused a significant increase in CA-induced airway constriction and cough in compound 48/80-pretreated animals. In addition, CA inhalation caused significant increase in lung tissue and plasma histamine concentrations, which were blocked by compound 48/80 pretreatment. These results suggest that mast cells play an important role in CA aerosol inhalation-induced airway constriction and cough *via* perhaps mediators including LTs and histamine.

Key Words: histamine, leukotrienes, noncholinergic airway constriction, Tussive effect

Introduction

Inhalation of citric acid (CA) aerosol causes coughing (17, 33), airway hyperresponsiveness (18), bronchoconstriction (30, 34, 38, 51, 63), and plasma extravasation in the lungs (51). These effects of CA-induced pulmonary alterations are attenuated or prevented by capsaicin pretreatment to deplete tachykinins (18, 51), the selective capsaicin antagonist capsazepine (51), and the NK_2 receptor antagonist SR 48968 (18, 51). Capsazepine interferes with proton-sensitive ion channels (3) *via* occupation of the proposed capsaicin receptor site (57). Therefore, the above CA-induced changes are related closely with the activation of afferent C-fibers in the lungs *via* capsaicin receptors.

We postulated that mast cells have a close relationship with CA-induced airway constriction and cough. Our reason is based on the morphological fact that there is a close anatomical association between mast cells and nerve fibers, especially fibers containing substance P (a tachykinin) (4). Also, mast cells have

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been demonstrated to be involved with noncholingergic airway constriction (25, 32). In addition, priming of mast cells to subsequent stimulus by picomolar concentrations of substance P had been previously demonstrated by patch-clamp studies (24). Therefore, the purpose of this article was to review our studies in exploring the role of mast cells in CA-induced airway constriction and cough.

Citric Acid-Induced Airway Constriction and Cough

Micro-aspiration of acid into the lower airways may contribute to the pathophysiology of asthma, but the specific mechanism of acid-induced airway constriction is still not clear (23). Acid-induced airway responses are blocked by pretreatment with high does of capsaicin (16) and capsazepine (selective capsaicin antagonist) (35), suggesting the involvement of tachykinin-containing sensory nerves. Both capsazepine and SR48968 (selective neurokinin$_2$, NK_2, receptor antagonist) markedly inhibited CA-induced airway constriction in guinea pigs, indicating that tachykinins, such as neurokinin A (NKA), are locally released from sensory nerves and cause bronchoconstriction, mainly by NK_2 receptor mechanism (51). The available evidence indicates that protons (H^+) and capsaicin share a common mechanism of neuronal activation. A proton should be viewed as a mediator that elicits a protective response with reflex respiratory response (8, 48). Vanilloid receptor-1 is a thermosensitive, nonselective cation channel (2) that is expressed by capsaicin-sensitive afferent sensory nerves and is activated by noxious heat, acidic pH (proton) and the irritant capsaicin (52). On the other hand, bronchoconstriction provoked by CA inhalation in guinea pigs was not significantly blocked by atropine, excluding involvement of the cholinergic pathway (48).

Activation of afferent C-fibers also causes coughing. Aerosol inhalation with low concentration of capsaicin or CA caused cough and airway constriction in unanesthetized guinea pigs (61). Capsaicin and CA activated afferent C-fibers in airways and induced directly airway constriction. Following afferent C-fibers were activated their impulses were sent to the cough center for generating cough. In addition, tachykinins released from activated C-fibers can stimulate rapidly adapting receptors and the information can also be conducted to the cough center to generate cough. These capsaicin- and CA-induced responses could be antagonized by capsaicin pretreatment to deplete afferent C-fibers or by ruthenium red (61). Furthermore, CA-induced cough was also blocked by NK_2 receptor antagonist SR 48968 (17) and by NK_1 receptor antagonist FK 888 (64) in conscious guinea pigs.

Reactive Oxygen Species (ROS) and Endogenous Elastase in CA-Induced Airway Constriction

We demonstrated previously that ROS are involved in the activation of afferent C-fibers which release tachykinins. The involvement of ROS has been found in several types of noncholinergic airway constriction such as that caused by capsaicin (29), hyperventilation (14), exsanguinations (65), and CA aerosol inhalation (30, 34, 63). It is not clear how ROS are generated during the process of afferent C-fiber activation. Goldman *et al*. (19) found that acid aspiration caused increases in both ROS and lung permeability. Similarly, we (34) observed that CA inhalation induced an increase in chemiluminescene counts of bronchoalveolar fluid and airway constriction. Both lucigenin-initiated and t-butyl hydroperoxide (TBHP)-initiated chemiluminescene counts were increased by CA. Lucigenin-initiated chemiluminescene is an effective monitor of mitochondrial superoxide generation (47). On the other hand, TBHP-initiated chemiluminescene is an effective monitor of lipid peroxide generation (7) and has been used to detect decreased levels of endogenous antioxidants in liver and cardiac tissues (46). Thus, our data suggest that the decreased antioxidant activity following CA inhalation is partially caused by the superoxide pathway, while remote pathophysiological events are mediated by defective scavenging defences. The generation of free radicals in the presence of defective scavenging defences might be the cause of the stimulation of afferent C-fibers, resulting in noncholinergic airway constriction. Due to the interaction between the production of ROS and tachykinin release, thus CA aerosol inhalation can induce a sustained airway constriction (30).

In addition, using an elastase inhibitor eglin-c, we found that endogenous elastase plays an important role in hyperpnea- (32) and CA-induced airway constriction (34). It is possible that endogenous elastase could cause airway constriction directly (56) or indirectly *via* its enhancement of the release of bronchoconstrictors and/or ROS. For example, serine proteinases augment the release of histamine in sheep (41), rats (13) and humans (22).

Mast Cells in CA-Induced Airway Constriction

In order to explore the role of mast cells in CA-induced airway constriction, our study was divided into three experiments (38). We assumed that mast cells have several types of mediators: serotonin, leukotrienes, histamine, and cyclooxygenase products. Each guinea pig was anesthetized, cannulated, paralyzed, artificially ventilated and pretreated with

atropine (1 mg/kg, i.v.). The protocol included the baseline, CA inhalation and recovery periods. Except the third experiment, we measured dynamic respiratory compliance (Crs) and forced expiratory volume in 0.1 sec ($FEV_{0.1}$) during either baseline or recovery period. Decreases in Crs and $FEV_{0.1}$ were used as indicators for airway constriction.

The first experiment was carried out to examine effects caused by blocking agents of mast cell mediators. 67 young Hartley guinea pigs were divided into 7 groups: saline + CA; methysergide + CA; MK-886 + CA; mepyramine + CA; indomethacin + CA; cromolyn sodium + CA; and compound 48/80 + CA. In the control group, saline was intravenously injected 30 min before CA challenge to induce airway constriction. Methysergide is a serotonin receptor antagonist and was intravenously injected (0.5 mg/kg) 30 min before CA challenge. MK-886 is an inhibitor for lipoxygenase and was intravenously infused (2 mg/kg) for 30 min just prior to CA. Mepyramine is a histamine H_1 receptor antagonist and was intravenously injected (30 mg/kg) 30 min before CA challenge. Indomethacin is an inhibitor for cyclooxygenase and was intravenously injected (5 mg/kg) 10 min before CA challenge. Cromolyn sodium is a stabilizer for mast cells and was intravenously injected (20 mg/kg) 30 min before CA challenge. Compound 48/80, a mast cell degranulation agent, was employed to deplete mast cells *via* chronic treatment. Compound 48/80 was given to each animal by subcutaneous injection for 3 days before the study. Three consecutive daily doses were 6, 9, 10 mg/kg (32, 49). CA aerosol inhalation caused decreases in Crs and $FEV_{0.1}$, indicating CA-induced airway constriction in the control group. This airway constriction was significantly attenuated by MK-886, mepyramine, cromolyn sodium and compound 48/80, but not by either methysergide or indomethacin.

In the second experiment, we tested whether exogenous leukotriene C_4 (LTC_4) or histamine enhances CA-induced airway constriction in compound 48/80-pretreated guinea pigs. Forty compound 48/80-pretreated guinea pigs were divided into 5 groups: saline infusion + CA; LTC_4 infusion; LTC_4 infusion + CA; histamine infusion; and histamine infusion + CA. Saline, LTC_4 (1 μg/kg), or histamine (10 μg/kg) was infused intravenously and continuously for 5 min prior to the CA inhalation period. Both LTC_4 and histamine infusions significantly increased the magnitude of CA-induced airway constriction in compound 48/80-pretreated guinea pigs.

Finally, in the third experiment, we detected histamine level, an index of pulmonary mast cell degranulation, in the bronchoalveolar lavage (BAL) and lung tissue samples. Animals were divided into three groups: saline + saline; saline + CA; and compound 48/80 + CA. Immediately after CA or saline inhalation, BAL and lung tissue samples were collected for determination of histamine using histamine enzyme immunoassay kit. In animals with no compound 48/80 pretreatment, CA inhalation caused significant increases in histamine levels in both BAL and lung tissue. These increases in histamine levels were significantly suppressed by compound 48/80.

Many mediators, cytokines, chemokines, and ROS released from mast cells may augment CA-induced airway constriction. In this study, we examined only the roles of leukotrienes, histamine, products of cyclooxygenase, and serotonin. Both leukotriene synthesis inhibitor and histamine H_1 receptor antagonist prevented CA-induced airway constriction. Also, exogenous LTC_4 and histamine augmented CA-induced airway constriction in animals pretreated with compound 48/80 to deplete endogenous mast cell constituents. Accordingly, these results suggest that the involvement of mast cells in CA-induced airway constriction may be mediated *via* leukotrienes and/or histamine. These results are compatible with the fact that leukotriene receptor antagonist FPL 55712 and leukotriene synthesis inhibitor MK-886 significantly attenuated hyperpnea-induced noncholinergic airway constriction in guinea pigs (31, 43). Also in rats, FPL 55712 markedly, whereas atropine only weakly, inhibited CA-induced airway constriction (20). Our results agree also with the fact that cromolyn sodium and mepyramine inhibited substance P-induced neurogenic edema (36). However, both indomethacin and methysergide did not alter CA-induced airway constriction. Our result of indomethacin was consistent with the fact that indomethacin had little or no effect on substance P- (54) or calcium ionophore (an inflammatory agent) A23187-induced (53) airway constriction in guinea pigs. This inefficacy of indomethacin could be due to the fact that indomethacin is a general inhibitor for cyclooxygenase but not a very specific inhibitor for the production of a special cyclooxygenase product. The failure of methysergide in preventing airway constriction of guinea pigs might be related to the fact that serotonin is the main mast cell mediator in rats (21) but not in guinea pigs. Also, serotonin has been found to induce both constriction and relaxation of the guinea pig airway (1).

Both FPL 55712 (a LT receptor antagonist) and MK-886 significantly attenuated hyperpnea-induced noncholinergic bronchoconstriction in guinea pigs (31). Besides, mepyramine significantly inhibited neurogenic and SP-induced edema (36). However, methysergide (1 mg/kg) and indomethacin (1 mg/kg) were completely ineffective (20). LTD_4 potentiated histamine-induced bronchoconstriction was pre-

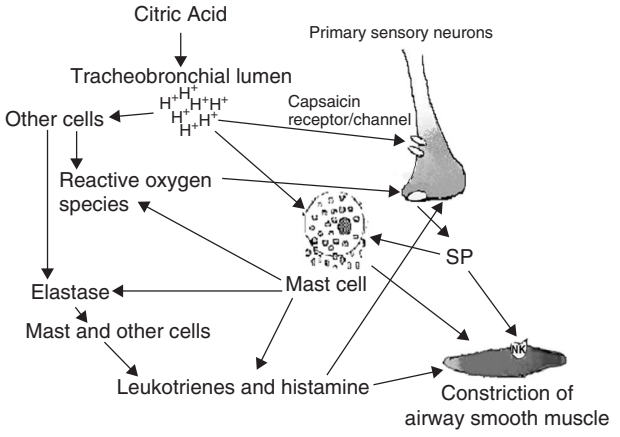

Fig. 1. A diagram to illustrate possible routes for the involvement of mast cell in citric acid-induced airway constriction in this study.

vented by atropine in guinea pigs (55). In this study, we used not only mepyramine to block endogenous histamine but also atropine to inhibit cholinergic effect. Thus, leukotrienes potentiated histamine effect may be prevented in our experimental model. We found the similar results in this study that either MK-886 or mepyramine attenuated CA-induced airway constriction in guinea pigs. But contractions induced by NKA were largely reduced by the methysergide, 5-HT antagonist (27). We postulated the variation from the different species and different experimental procedures. Thus, we further tested whether LTC_4 or histamine enhances CA-induced airway constriction in compound 48/80-pretreated guinea pigs. Intravenous infusion of LTC_4 (1 μg/kg) or histamine (10 μg/kg) caused airway constriction before the CA inhalation. This is because LTC_4 and histamine are the classic mediators from mast cells and cause contraction of airway smooth muscle *via* Cys-LT receptor and H_1-receptor, respectively (44). Additionally, we found that LTC_4 or histamine replacement augmented CA inhalation-induced airway constriction in guinea pigs with depleted mast cells *via* compound 48/80 pretreatment. Accordingly, we concluded that LTC_4 and/or histamine from mast cells were involved in CA-induced airway constriction.

It is not clear how leukotrienes and histamine can augment CA-induced airway constriction. We postulated that both leukotrienes and histamine augment CA-induced airway constriction *via* their action on afferent C-fibers (Fig. 1). Bloomquist and Kream (5) as well as Martins *et al*. (40) have demonstrated that leukotrienes activate afferent C-fibers while Martins *et al*. (40), Saria *et al*. (50) and Undem and Carr (60) have shown that histamine stimulates C-fibers. In addition, CA with high proton level may also activate afferent C-fibers (2). Subsequently, the activation of afferent C-fibers may enhance CA-induced airway constriction *via* the release of tachykinins (Fig. 1). Furthermore, the released tachykinins may act on mast cells because substance P is known to activate mast cells (15, 37, 58). It is also possible that released tachykinins may again stimulate afferent C-fibers (40), and thus augment CA-induced airway constriction.

In addition to depleting mast cells, mentioned above, compound 48/80 also stimulates sensory fibers (39, 49), and thus compound 48/80 pretreatment could produce partial depletion of sensory fibers. Cromolyn sodium and nedocromil sodium are very effective inhibitors for agent-induced airway response (11). Cromolyn sodium inhibits unmyelinated afferent vagal fibers in dogs (12) and in some other species. Thus, the inhibitory effect of cromolyn sodium on CA-induced airway constriction could be explained by the inhibition of afferent fibers. Furthermore, Cheung *et al*. (9) and Joos *et al*. (26) found that cromolyn sodium suppressed airway constriction induced by NKA in human subjects. Our results with compound 48/80 pretreatment and cromolyn administration may thus be mediated partially at least *via* altered release of tachykinins from afferent C-fibers.

In summary, this study found that mast cells play an important role in CA-induced airway constriction. We further demonstrated that leukotrienes and histamine originating from mast cells contribute to this type of airway constriction. According to our reasoning mentioned above, possible ways of mast cell in CA-induced airway constriction are summarized in Fig. 1.

Mast Cells in Citric Acid-Induced Cough

To investigate the role of mast cells in CA-induced cough, three experiments were carried out in conscious, unanesthetized guinea pigs (33).

In the first experiment, 59 guinea pigs were divided into seven groups: vehicle (saline) + CA (n = 8), compound 48/80 (for depletion of mast cell content) + CA (n = 10), cromolyn sodium (for stabilization of mast cells) + CA (n = 8), MK-886 (for inhibition of leukotriene syntheses) + CA (n = 8), pyrilamine (antagonist of histamine H_1 receptor) + CA (n = 8), methysergide (antagonist of serotonin receptor) + CA (n = 8), and indomethacin (for inhibition of cyclooxygenase) + CA (n = 9). Each animal in the vehicle (saline) + CA group received intraperitoneal injection of vehicle (saline solution) 30 min before the cough procedure. Compound 48/80, a mast cell degranulating agent, was given to the animal by subcutaneous injection for 3 days before the study. Three consecutive daily doses were 6, 9, 10 mg/kg, respectively.

Usually the daily dose was divided into two injections for each animal each day (49). Cromolyn sodium (20 mg/kg) was intraperitoneally injected 15 min prior to the cough procedure. MK-886 (1 mg/kg, i.p.) was given to animals 60 min prior to the cough study. Pyrilamine (30 mg/kg, i.p.), methysergide (10 mg/kg, i.p.) and indomethacin (10 mg/kg, i.p.) were each administered 60 min before the cough study. Exposure to CA induced a marked increase in cough number. Compound 48/80, cromolyn sodium, MK-886 and pyrilamine, but not indomethacin or methysergide, significantly attenuated CA-induced cough.

In the second experiment, 56 compound 48/80-pretreated animals were divided into 2 parts; the first one was used to test the role of exogenous leukotriene (LT) C_4, while the second one to test the role of exogenous histamine, in CA-induced cough. After CA aerosol inhalation, there were about 2 coughs produced in the vehicle group. Both injection of LTC_4 (2 μg/kg, i.p.) and histamine infusion (10 μg/kg, i.v. infusion) caused a significant increase in CA-induced cough in compound 48/80-pretreated animals.

For the first and second experiments, each animal was placed inside a barometric plethysmograph and was exposed sequentially to saline (baseline) and CA (0.6 M) aerosols, each exposure for 3 min. After each exposure, the cough response was recorded for 12 min. Cough was denoted by a large (2 × normal) inspiration followed by immediately a rapid, forceful expiration (2 × normal excursion).

In the third experiment, the activation of mast cells upon CA inhalation was investigated by determining arterial plasma histamine concentration in 17 animals. These animals were divided into three groups: vehicle + normal saline; vehicle + CA; and compound 48/80 + CA. CA inhalation caused significant increase in plasma histamine concentration, which was blocked by compound 48/80 pretreatment.

We investigated the role of mast cells in CA-induced cough by using compound 48/80 pretreatment and cromolyn sodium. Compound 48/80 is widely used as a mast cell degranulating agent leading to acute release of histamine and other mast cell constituents (45), and thus chronic treatment of compound 48/80 depletes mast cells. This study confirmed this point based on the finding that the pretreatment of compound 48/80 significantly reduced citric acid-induced increase in arterial plasma histamine level. In addition to deplete mast cells, compound 48/80 stimulates sensory fibers (39, 49), and thus compound 48/80 pretreatment could produce partial depletion of sensory fibers. As mentioned above, the inhibitory effect of cromolyn sodium on CA-induced cough could be explained by the inhibition of afferent fibers. Similarly, Cheung *et al*. (9) and Joos *et al*. (26) found that cromolyn sodium suppressed airway constriction induced by NKA in human subjects. Our results with compound 48/80 pretreatment and cromolyn administration suggest that mast cells play an important role in CA-induced cough.

Many mediators, cytokines, chemokines, and ROS released from mast cells may augment CA-induced cough. In this study, we examined only the roles of leukotrienes, histamine, products of cyclooxygenase, and serotonin. Both leukotriene synthesis inhibitor and histamine H_1 receptor antagonist prevented CA-induced cough. Also, exogenous LTC_4 and histamine augmented this cough response in animals pretreated with compound 48/80 to deplete endogenous mast cell constituents. Accordingly, these results suggest that the involvement of mast cells in CA-induced cough may be mediated *via* leukotrienes and/or histamine. These results are compatible with the fact that leukotriene receptor antagonist zafirlukast (10) and H_1 antihistamine loratadine (59) are effective antitussive agents in human subjects. However, both indomethacin and methysergide did not alter CA-induced cough. Our result of indomethacin was consistent with the fact that indomethacin did not affect allergic cough but inhibited only slightly capsaicin-induced cough in guinea pigs (6). The inefficacy of indomethacin and methysergide may be explained by the reasons mentioned above for CA-induced airway constriction.

It is not clear how leukotrienes and histamine can augment CA-induced cough. We postulated that both leukotrienes and histamine augment cough *via* their action on afferent C-fibers. Bloomquist and Kream (5) as well as Martins *et al*. (40) have demonstrated that leukotrienes activate afferent C-fibers while Saria *et al*. (50) and Undem and Carr (60) have shown that histamine stimulates Aδ and C-fibers. In addition, CA with high proton level may also activate afferent C-fibers (2). Subsequently, the activation of afferent C-fibers may initiate cough *via* the release of tachykinins. Released tachykinins can induce neurogenic inflammation and activate rapidly adapting receptors (and thus Aδ fibers) to cause cough (62). In addition, tachykinins such as substance P can directly induce cough response (28). Furthermore, the released tachykinins may act on mast cells because substance P is known to activate mast cells (15, 37, 58). It is also possible that released tachykinins may again stimulate afferent C-fibers (40) and then Aδ fibers, and thus augment cough. Tachykinins may also act on the central nervous system and initiate cough because central administration of substance P augments a central reflex related to cough (42).

In summary, exposure to CA aerosol induced a marked increase in cough number, which was mast cell-dependent. It is possible that mast cell con-

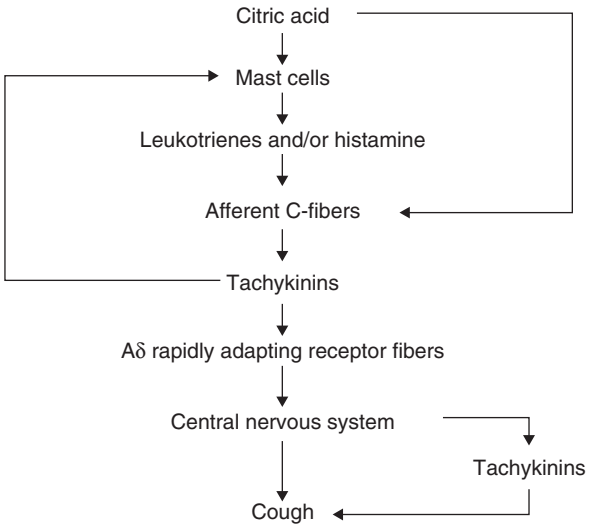

Fig. 2. A flow-chart diagram to illustrate possible routes of mast cells involved in citric acid aerosol-induced cough.

stituents leukotrienes and histamine are important components to initiate cough. According to our reasoning mentioned above, the possible routes of mast cells involved in CA-induced cough are illustrated in Fig. 2 using a flow-chart diagram.

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