Influence of influenza viral infection on airway smooth muscle activity

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The contractility of airway smooth muscle (ASM) plays an important role in pathophysiology of several bronchial disorders. Increased contraction of ASM during asthma and respiratory viral infection has been attributed to the release of mediators acting through different receptors. In the present study, influence of influenza type A virus (H1N1) infection has been examined on ASM responsiveness to various bronchoactive agents e.g. adenosine, histamine, 5-hydroxytryptamine (5-HT) and isoproterenol in an organ bath set up for isolated tissue preparation. The contractile effect of adenosine, histamine and 5-HT was enhanced, however, relaxant response of isoproterenol was attenuated with the duration following viral exposure. The most prominent response was observed 48 to 72 hr after infection and tissues from multiple exposure to virus infected animals showed the maximum contractile response. Results demonstrated the deleterious effect of viral infection on ASM function and the findings will be helpful in understanding the mechanism of influenza virus induced bronchoconstriction.

The contractility of airway smooth muscle (ASM) plays an important role in pathophysiology of several bronchial disorders. Hyper-responsiveness leading to contraction of ASM is recognized as the main cause of acute respiratory disorders¹. Acute changes in airway function during asthma leads to contraction of ASM by disturbance in signalling pathways. The infection due to certain respiratory viruses causes bronchoconstriction and alters the sensitivity of ASM to various drugs. Respiratory viruses increase inflammation and worsen the injury in diseased airway by increasing cytokines production and modulating epithelial cells, thereby causing bronchospasm². Influenza type A virus is the most important of respiratory viruses with respect to morbidity and mortality in civilian population, national as well as globally.

The receptors commonly implicated, as the precipitating factors for bronchoconstriction in asthmatic cases with respiratory tract infection, are histaminic, β-adrenergic, purinergic, serotonergic and cholinergic¹. The most extensively spread signalling cascade, G-proteins are coupled with ASM receptors. It has been suggested that respiratory viral infection may impair airway function through attenuation of receptor sensitivity and post-receptor activation of adenylate cyclase activity². Studies have documented that these receptors are also coupled to a variety of effector systems e.g. K⁺ channels, Ca²⁺ channels, Na⁺-Ca⁺ exchange, guanylate cyclase etc.²

However, even after better understanding of the mechanisms of action of bronchoactive drugs on ASM and their clinical importance, little is known about the duration of response during influenza virus infection. Therefore, the present study has been undertaken to examine the role of some bronchoactive drugs e.g. adenosine, histamine, isoproterenol and 5-hydroxytryptamine (5-HT) on isolated tissue preparations obtained from influenza virus infected guinea pigs. Tissues were taken from guinea pigs at 24, 48 and 72 hr after influenza virus (H1N1) infection. Tissues from control animals and animals subjected to multiple exposure to virus were also tested. Concentration response curves of various bronchoactive agents were examined in different group of animals.

Materials and Methods

Animals—Experiments were performed on healthy adult guinea pigs of either sex, weighing 500-600 g. The animals were provided standard feed and water ad libitum.

Infection of guinea pigs—Animals were inoculated with 0.1 ml of virus divided into half in each nostril.
Results

In the present study airway smooth muscle became more sensitive towards different vasoactive drugs with the duration of inoculation with influenza virus and there was an increase in the amount of antibodies present in blood of the animals.

Adenosine showed the typical biphasic concentration dependent contractile response in rings obtained from control animals. The response remained same in tissues obtained 24 hr after viral infection. However, in tissues after 48 and 72 hr of viral infection there was a significant increase in contractile response to adenosine and 82.3 ± 11.4% contraction was recorded at 10⁻⁸ M concentration (Fig. 1a). The contraction started declining after 72 hr of infection. Preparations from animals subjected to multiple exposure showed a strong contractile response even at lower concentrations (Fig. 1a) and tissue exhibited contractile response (30.35 ± 7.9%) also at higher (10⁻⁴ M) concentrations.

The sensitivity of virus infected tissues for histamine was enhanced in the present study and the contractile response increased with duration of viral infection. A maximum of 280 ± 31.2% contraction was observed after 72 hr, which decreased with time following viral infection. The animals with multiple virus exposure (Fig. 1b) became more sensitive to even lower concentrations and a significant (415.8 ± 36.4%) contraction recorded at 10⁻⁷ M concentration remained same and did not change on adding higher concentration of histamine.

There was a reduction in relaxant response of isoproterenol with the duration of viral infection. Rings from control animals showed up to 100% relaxation. However, there was a significant reduction in relaxant response in animals with multiple exposure of virus as only 64.2 ± 13.3% relaxation was recorded at 10⁻⁷ M concentration (Fig. 1c). A weak relaxant response was observed in rings of 48 and 72 hr post infected animals as up to 10⁻⁹ M concentration no significant relaxation was observed. Response to isoproterenol was very slow in animals with multiple exposure to virus.

5-HT showed an enhanced contractile response, there was no noticeable change after 24 hr of viral infection, however, a gradual increase in contraction with duration of viral exposure (48 and 72 hr) (Fig. 1d) was observed. In multiple exposure animals, a significant 145 ± 33.1 % contraction was recorded at 10⁻⁶ M (Fig. 1d).
Discussion
Influenza type A virus was isolated over half a century ago. Extensive work has been done in vaccine development and effective control of the respiratory disorder caused by influenza type A virus. Histopathological examinations showed acute necrotizing tracheobronchitis with loss of ciliated epithelial cells, interstitial edema and intrabronchial hemorrhage, leading to metaplastic epithelial regeneration and extensive fibrosis by influenza virus infection.

There was an increase in contraction of ASM to acetylcholine \((10^{-7} M)\) with the duration of viral infection (unpublished data). It may have resulted in selective loss of M2-receptors due to the action of viral neuraminidase on sialic acid residue that is necessary for the action of cholinergic receptors.

In the present study the contractile response of histamine increased after 48, 72 hr and multiple exposure, suggesting that the tissues become more sensitive to histamine. Histamine receptors \((H_1, H_2, H_3)\) mediate bronchoconstriction, airway microvascular leakage, modulation of mucous secretion. The present results are in agreement with earlier findings that bronchial responsiveness to inhaled histamine aerosol increases 4-5 times when hyper-responsiveness was related to infection, due to a greater degree of inflammation and airway epithelial damage by influenza type A virus infection. The tissues from animal with multiple exposure to virus remained constricted even at higher concentration of histamine, suggestive of changes in smooth muscle function. Antihistamines cause bronchodilation in asthmatic patients however, indication of certain degree of histamine-induced “tone” was presumably due to release of histamine from activated mast cell even during normal condition.

Adenosine has a biphasic bronchoconstrictor effect in asthmatic patients and releases histamine from primed mast cell in A2-receptors. The findings demonstrated that contractile response was maximal in tissues with multiple exposure to virus, indicating the upregulation of adenosine receptors due to viral infection. The response of adenosine results from the balance between A2-mediated relaxation and A1-mediated contraction. The contractile effect of adenosine in guinea pig isolated trachea potentiated by epithelial abrasion, related to the removal of sites

![Fig. 1](image-url)

*Fig. 1—Concentration-response curves of adenosine (a), histamine (b), isoproterenol (c), 5-hydroxytryptamine (d), in isolated tracheal rings obtained from guinea pigs 24 (-Y-), 48 (-A-), 72 (-Δ-) hr after single exposure to influenza virus, after multiple viral exposure (- ■ -) and from control animals (- ○ -) not exposed to virus. [Rings were precontracted with M acetylcholine and percentage contractions are expressed as percent increase in contraction produced by \((10^{-5} M)\) acetylcholine. All values are mean and vertical bars represent ± SEM. *Represents the statistically significant difference from respective control value \((P<0.05;\ n = 10)\).
of metabolism and reduction in diffusion barrier and attenuation of receptor and post receptor activation of adenylate cyclase activity.

Isoproterenol, a β-receptor agonist showed a bronchodilatory effect, which was significantly attenuated after exposure to virus. Bronchodilator effect of isoproterenol is mediated through β2-adrenergic receptors. β2-adrenergic receptors are important regulators of smooth muscle tone and are highly susceptible to rapid desensitization by inflammatory agents11. Mainly cytokines12 and PKC activation enhances the airway relaxant response to β2-adrenoceptor stimulation and the later effect is dependent on potentiated stimulation of airway electrogenic Na+-K+ pump13. Cytokines increased during exacerbation of asthmatic symptoms directly impair β-adrenoceptor function in ASM cells and is suggested to be one of the mechanisms by which inflammation impairs β-adrenoceptor signal transduction12.

There was a significant increase in contractile response of ASM to 5-HT with the increase in infection periods. Animals with multiple exposure to virus showed prolonged contractile effects even at maximum concentration. One of the principal locations of 5-HT is epithelium, it plays an important role in the uptake and release of 5-HT acting on ASM14. The exacerbation of 5-HT response in virus exposed preparations may be correlated with the damage to the epithelium with duration of infection.

Respiratory tract viral infections are associated with a wide array of pro-inflammatory cytokines, endothelins and reactive oxygen intermediates15. Infection impairs airway function through abrasion of epithelial cells and attenuation of receptors and post receptor activation of adenylate cyclase activity. The symptoms of influenza-A virus infection may last for 2 to 7 days16. However, plasma derived proteins in nasal lavage fluid show an early peak and then decrease, which was the main reason for studying the response 24, 48 and 72 hr after exposure to influenza virus.

The results of the present study demonstrated a significant change in the ASM response to bronchoactive agents during influenza-A viral infection. A prominent contractile response of ASM was observed between 48 to 72 hr of infection. Tissue from animals with multiple exposure to virus became more sensitive to even lower concentrations. The bronchoconstriction due to adenosine, histamine and 5-HT was enhanced and relaxant response to isoproterenol was attenuated with the duration of influenza virus infection, infection exacerbates breathlessness, especially in asthmatic patients possibly by altering the sensitivity of ASM to receptor mediated actions on the airways.

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References