Dexamethasone prevents virus-induced hyperresponsiveness via multiple mechanisms

Liliana Moreno,1 David B. Jacoby,1,2 and Allison D. Fryer1

1Department of Environmental Health Sciences, Bloomberg School of Public Health, and 2Division of Pulmonary and Critical Care Medicine, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205

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RESPIRATORY VIRAL INFECTIONS are an important cause of asthma attacks (13). Viral infections account for 80–85% of asthma exacerbations in children (17) and 50–55% in adults (4). Virus-induced hyperresponsiveness in animals and humans is blocked by atropine, indicating a role for the parasympathetic nerves (3, 6, 8). In the lungs, acetylcholine released from parasympathetic nerves onto postjunctional M3 muscarinic receptors causes smooth muscle contraction and bronchoconstriction. Acetylcholine also bind to inhibitory prejuncional M2 muscarinic receptors on parasympathetic nerves, suppressing acetylcholine release and decreasing vagally induced bronchoconstriction (5, 9). Blocking these neuronal M2 receptors with selective antagonists such as gallamine causes a 5- to 10-fold increase in vagally induced bronchoconstriction (1, 9, 10). Conversely, stimulating neuronal M2 muscarinic receptors with muscarinic agonists, such as pilocarpine, inhibits vagally induced bronchoconstriction (9).

During viral infection, neuronal M2 receptors are dysfunctional (2, 11). Both viral infection and interferon-γ (which is produced in response to viruses) decrease expression and function of the M2 receptors in cultured airway parasympathetic nerves from guinea pig trachea (14). The glucocorticoid dexamethasone increases expression of M2 receptors in these cells in culture and can prevent the interferon-induced decrease in M2 receptor expression in vitro (16).

These experiments were designed to test whether dexamethasone prevents virus-induced hyperresponsiveness and M2 receptor dysfunction in vivo.

METHODS

Animals. Pathogen-free Dunkin-Hartley guinea pigs (300–350 g; Hilltop Animal Farms, Scottsdale, PA) were kept in particulate-filtered-air cages. Guinea pigs were handled following the standards established by the U. S. Animal Welfare Acts set forth in National Institutes of Health guidelines and The Policy and Procedures Manual published by the Johns Hopkins School of Public Health Animal Care and Use Committee.

Viral infection and dexamethasone treatment. Pathogen-free guinea pigs were anesthetized with ketamine (30 mg/kg im) and xylazine (5 mg/kg im) and infected with parainfluenza virus type I (Sendai virus, ATCC-VR 105) intranasally on day 0 with 105 TCID50/ml. [TCID50 is defined as the amount of virus required to infect 50% of monolayers of cultured rhesus monkey kidney cells (15)]. Two and three days after infection, animals were treated with dexamethasone (6.5 or 65 μg/kg ip). Dexamethasone was also administered to noninfected controls. On day 4, 24 h after the last dose of dexamethasone, M2 receptor function, airway hyperresponsiveness, inflammation, and lung viral content were assessed.

Measurements of pulmonary inflation pressure. Guinea pigs were anesthetized with urethane (1.5 g/kg ip). Blood
pressure and heart rate were recorded from the carotid artery. The trachea was cannulated, and the guinea pig’s membranes were paralyzed with succinylcholine (10 μg/kg/min iv), treated with guanethidine (20 mg/kg iv) to deplete catecholamines, thereby eliminating sympathetic effects of vagal stimulation, and ventilated at a respiratory rate of 100 breaths/min and 10 ml/kg tidal volume. Pulmonary inflation pressure (Ppi) was measured from a side arm of the tracheal cannula. Bronchoconstriction (mmH2O) was measured as an increase in Ppi.

Studies of vagal hypersensitivity. Both vagus nerves were cut, and the distal ends were placed on platinum electrodes. Electrical stimulation of both vagus nerves (10 V, 0.2-ms pulse, 2–25 Hz, for 5 s at 120-s intervals) produced reversible bronchoconstriction and bradycardia that were blocked with atropine (1 mg/kg).

Test for neuronal M2 muscarinic receptor function with pilocarpine. We produced reproducible bronchoconstriction by stimulating both vagus nerves (2 Hz, 0.2-ms pulse, for 22 s at 1-min intervals). Voltage was chosen within the range of 10–30 V to give an increase in Ppi of 12–15 mmH2O. M2 receptor function was measured as the ability of the muscarinic agonist pilocarpine (0.1–100 μg/kg iv) to inhibit vagally induced bronchoconstriction. Data are expressed as the ratio of the bronchoconstriction in the presence of pilocarpine to the bronchoconstriction in the absence of pilocarpine.

Response of the airways to acetylcholine. We tested the function of M3 muscarinic receptors on airway smooth muscle in vagotomized guinea pigs by measuring bronchoconstriction induced by intravenous acetylcholine (1–10 μg/kg).

Bronchoalveolar lavage. At the end of each experiment, bronchoalveolar lavage was collected from each animal. In brief, lungs were lavaged with 10 ml of PBS via the tracheal cannula. The recovered fluid was centrifuged at 400 fl for 5 min. The cells were resuspended in 10 ml of PBS and counted in a hemocytometer (Hausser Scientific). Aliquots of this cell suspension were cytospun onto glass slides and stained with Diff-Quik (Baxter Healthcare) and counted to obtain differential cell counts.

Titration of virus in lungs. Infection was confirmed in all virus-infected animals as previously described (2). After physiological studies were completed, the guinea pig lungs were removed, weighed, and homogenized in 2 ml of PBS (Polytron, Brinkmann). We eluted virus from the tissue homogenate by incubating it at 37°C for 1 h. The suspension was centrifuged at 1,500 rpm for 30 min, and the supernatants were inoculated in serial 10-fold dilutions into rhesus monkey kidney cell monolayers. After incubation at 34°C for 1 wk, the monolayers were washed, and the medium was replaced with a 0.5% suspension of guinea pig red blood cells in Hanks’ buffered salt solution. After 1 h at 4°C, the red blood cells were washed off, and the monolayers examined under an inverted phase-contrast microscope for evidence of hemadsorption (adhesion of erythrocytes to monolayers because of the expression of viral hemagglutinin on infected cell membranes). Viral content was determined as the amount of lung homogenate required to produce infection in 50% of tissue titers were done with ANOVA (Statview, 4.51; Abacus Concepts).

Fig. 1. Virus-induced hyperresponsiveness is prevented by dexamethasone. Electrical stimulation (10 V, 0.2 ms, 5 s) of both vagi caused frequency-dependent bronchoconstriction, measured as an increase in pulmonary inflation pressure (Ppi) in controls (○). This is not affected by treatment with dexamethasone (Dex; 6.5 μg/kg ip, ■). Vagally induced bronchoconstriction was significantly potentiated by viral infection (●, P < 0.05). Dex completely prevented virus-induced hyperresponsiveness (■); n = 5–8.

### RESULTS

**Baselines.** Baseline heart rate (318.5 ± 27.7 beats/min) and blood pressure (systolic blood pressure 52.5 ± 9.4 mmHg, diastolic blood pressure 36.2 ± 8.5 mmHg) in controls were not changed either by viral infection or by dexamethasone. Ppi was significantly increased in virus-infected guinea pigs (control, 100 ± 5 mmH2O vs. virus, 150 ± 8 mmH2O). Dexamethasone (6.5 or 65 μg/kg ip) did not inhibit the virus-induced increase in resting Ppi.

**Effect of dexamethasone on vagally induced bronchoconstriction.** Electrical stimulation of both vagus nerves caused frequency-dependent bronchoconstriction that was significantly potentiated in virus-infected animals (Fig. 1). Dexamethasone (6.5 μg/kg ip) prevented virus-induced potentiation of vagally induced bronchoconstriction but did not change vagally induced bronchoconstriction in controls. (Fig. 1). Stimulation of the nerves also caused a frequency-dependent fall in heart rate. Neither viral infection nor dexamethasone altered vagally induced bradycardia (data not shown).

**Effect of dexamethasone on neuronal M2 muscarinic receptor function.** Electrical stimulation of the vagi was done with voltages (10–30 V) that were adjusted to yield similar bronchoconstrictions in all groups. Pilocarpine (0.1–100 μg/kg iv) inhibited vagally induced bronchoconstriction in control animals, in a dose-related manner, due to stimulation of the neuronal M2 receptors (Fig. 2). In contrast, pilocarpine did not decrease vagally mediated bronchoconstriction in virus-infected animals, indicating dysfunctional M2 receptors (Fig. 2). In virus-infected animals treated with 6.5 μg/kg dexamethasone, pilocarpine partially inhibited vagally mediated bronchoconstriction (Fig. 2A), whereas in those treated with 65 μg/kg iv dexamethasone, the inhibition of vagally induced bronchoconstriction by
pilocarpine was not different from uninfected controls (Fig. 2B). However, whereas 6.5 μg/kg iv dexamethasone had no effect on the ability of pilocarpine to inhibit vagally mediated bronchoconstriction in uninfected animals (Fig. 2A), 65 μg/kg iv dexamethasone increased the response to pilocarpine in uninfected guinea pigs (Fig. 2B), indicating a hyperfunctional M2 receptor.

Effect of dexamethasone on intravenous acetylcholine-induced bronchoconstriction. Intravenous acetylcholine caused a dose-related bronchoconstriction (1–10 μg/kg) that was not altered by viral infection or by dexamethasone (Fig. 3). In addition, acetylcholine also caused a fall in heart rate that was not affected by viral infection or dexamethasone (data not shown).

Effect of dexamethasone on inflammatory cells. Macrophages and neutrophils in bronchoalveolar lavage were significantly increased in lungs of guinea pigs infected with parainfluenza virus (Fig. 4). The lower dose of dexamethasone (6.5 μg/kg ip) did not inhibit virus-induced inflammation. In contrast, the higher dose of dexamethasone (65 μg/kg ip) inhibited virus-induced increase in inflammatory cells in bronchoalveolar lavage.

Effect of dexamethasone on viral infection of the lungs. Both 6.5 and 65 μg/kg ip dexamethasone significantly decreased the amount of virus recovered from infected guinea pig lungs (Fig. 5). In addition, a significant correlation between viral titer and maximal response to pilocarpine was found (Fig. 6).

DISCUSSION

We (2) and others (6) have previously shown that parainfluenza virus infection of guinea pigs causes...
hyperresponsiveness to vagal stimulation. This hyperresponsiveness is at the level of increased acetylcholine release from the nerves, as bronchoconstriction to exogenous acetylcholine is not increased by viral infection. Viral infection causes dysfunction of M2 muscarinic receptors, resulting in increased release of acetylcholine (2).

Dexamethasone (6.5 μg/kg) prevented virus-induced hyperresponsiveness (Fig. 1) and partially restored M2 receptor function (Fig. 2). This dose of dexamethasone did not affect either the airway response to vagal stimulation or M2 receptor function in uninfected guinea pigs. A higher dose of dexamethasone (65 μg/kg) completely prevented virus-induced M2 receptor dysfunction (Fig. 2). However, as we have previously shown, treatment with high-dose dexamethasone increased M2 receptor function in uninfected guinea pigs (16).

Virus infection induces inflammation in the lungs, consisting primarily of macrophages and neutrophils (Fig. 4). Although the higher dose of dexamethasone reduced virus-induced inflammation in the lungs, the lower dose of dexamethasone did not change virus-induced inflammation. Despite this lack of effect on inflammation, low-dose dexamethasone prevented virus-induced hyperresponsiveness and partially prevented virus-induced M2 receptor dysfunction. Thus the protective effects of low-dose dexamethasone on responsiveness and M2 receptor function are not dependent on suppression of inflammation.

Both doses of dexamethasone reduced viral content of the lungs. This antiviral effect was greater with the higher dose of dexamethasone than with the lower dose. Viral titers correlated inversely with M2 receptor function (Fig. 6), suggesting that suppression of viral replication may contribute to the protective effects of dexamethasone on M2 receptor function.

Thus there may be multiple mechanisms by which dexamethasone prevents virus-induced airway hyperresponsiveness. First, dexamethasone may have a direct effect on the M2 receptor, increasing expression and function (16) and thereby compensating for virus-induced M2 receptor dysfunction. Consistent with this possibility is our observation that, although interferon-γ decreases M2 receptor expression and function in nerve cell cultures, these effects can be reversed by dexamethasone (16). Second, dexamethasone has an anti-inflammatory effect, and high doses of dexamethasone can attenuate virus-induced inflammation. Third, dexamethasone suppresses viral replication in the lungs. The more complete reversal of virus-induced M2 receptor dysfunction with the higher dose of dexamethasone may suggest that all these mechanisms contribute to the beneficial effects of dexamethasone.

We have previously shown that blocking the inflammatory response to viral infection (using cyclophosphamide) prevents M2 receptor dysfunction in some, but not all, virus-infected animals (12). It is likely to be significant that, in those animals that were treated with cyclophosphamide, animals with high viral content in the lungs were the ones that lost M2 receptor function, whereas those with lower viral content had normal M2 receptor function. This supports our hypothesis that the antiviral effect of dexamethasone contributes to inhibiting virus-induced hyperresponsiveness in the present study, as viral content correlated inversely with M2 receptor function.

There have been few other reports of antiviral effects of glucocorticoids. Kimsey and colleagues (18) reported that pretreating hamsters with steroids prevents subsequent infection with parainfluenza virus. Although the mechanism of this effect is not known, they speculate that decreased viral receptors on epithelial cells might be responsible. Decreased viral receptors ac-

![Fig. 5. Virus titers from guinea pig lungs. Treatment with Dex (both 6.5 and 65 μg/kg) significantly reduced virus titers (*P < 0.05). Results represent the multiple of the amount of virus required to produce infection in 50% of rhesus monkey kidney cells (TCID50) normalized to lung wet weight (n = 5–7; means ± SE).](image1)

![Fig. 6. M2 receptor function is significantly correlated with viral titer. (r = 0.69, P < 0.05). Results are graphed as the viral titers (TCID50/g lung) vs. the ratio of bronchoconstriction before and after pilocarpine (100 μg/kg iv). ○, Animals treated with Dex; ●, animals not treated with Dex.](image2)
counts for the antiviral effects of steroids on infectability of cultured epithelial cells with rhinovirus (19). In this case, expression of ICAM-1, the receptor for most rhinoviruses, was suppressed by steroid pretreatment. In contrast, Domachowsk and colleagues (7) showed that treating mice with steroids increases titers of pneumonia virus of mice (a virus related to human respiratory syncytial virus). They speculate that this might be due to an antiviral effect of eosinophils in this infection and the suppression of eosinophilia by the steroid treatment. Pretreatment of rhesus monkey kidney cells with dexamethasone inhibits the ability of parainfluenza virus to grow in these cells (Moreno L, Jacoby DB, and Fryer AD, unpublished observations), supporting our in vivo observation that dexamethasone is antiviral.

Thus dexamethasone reverses virus-induced airway hyperresponsiveness and M2 muscarinic receptor dysfunction via multiple mechanisms. High-dose dexamethasone suppresses virus-induced inflammation and decreases viral replication. At high doses, dexamethasone also increases M2 receptor function in uninfected animals, possibly by increasing M2 receptor gene expression (16). All these effects may contribute to the protective effects of high-dose dexamethasone in virus-infected airways. In contrast, low-dose dexamethasone also prevents virus-induced hyperresponsiveness. However, it does so without inhibiting virus-induced inflammation or increasing expression of M2 receptors in uninfected animals. It partially restores M2 receptor function, possibly by decreasing viral titers, and this is sufficient to prevent virus-induced hyperresponsiveness.

Glucocorticoids are part of the standard treatment for acute asthma attacks. In view of multiple studies showing that viral infections are a major cause of asthma attacks, it is important to consider the effects of this treatment on both virus-induced hyperresponsiveness and viral replication in the lungs. The results of this study show that glucocorticoids ameliorate virus-induced hyperresponsiveness via multiple mechanisms and that they restore the function of M2 receptors in virus-infected airways. Furthermore, at least for parainfluenza virus, treatment with glucocorticoids not only does not hamper the body’s ability to contain the infection but actually reduces viral replication in the lungs. This may have implication not only for the management of asthma attacks but also for the overall strategies of managing viral infections of the lungs.

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DISCLOSURES
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REFERENCES