Effects of neurokinin receptor antagonists in virus-infected airways

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Jacoby, David B., Bethany L. Yost, Thomas Elwood, and Allison D. Fryer. Effects of neurokinin receptor antagonists in virus-infected airways. Am J Physiol Lung Cell Mol Physiol 279: L59–L65, 2000.—We investigated the effects of a neurokinin-1 (NK1) receptor antagonist (SR-140333) and a NK2 receptor antagonist (SR-48968) on airway responsiveness and on the function of neuronal M2 muscarinic receptors, which normally inhibit vagal acetylcholine release, in guinea pigs infected with parainfluenza virus. Antagonists were given 1 h before infection and daily thereafter. Four days later, bronchoconstriction induced by either intravenous histamine (which is partly vagally mediated) or electrical stimulation of the vagus nerves was increased by viral infection compared with control. In addition, the ability of the muscarinic agonist pilocarpine to inhibit vagally induced bronchoconstriction was lost in virus-infected animals, demonstrating loss of neuronal M2 muscarinic receptor function. Macrophage influx into the lungs was inhibited by pretreatment with both antagonists. However, only the NK1 receptor antagonist prevented M2 receptor dysfunction and inhibited hyperresponsiveness (measured as an increase in either vagally induced or histamine-induced bronchoconstriction). Thus virus-induced M2 receptor dysfunction and hyperresponsiveness are prevented by a NK1 receptor antagonist, but not by a NK2 receptor antagonist, whereas both antagonists had similar anti-inflammatory effects.

M2 muscarinic receptors on the vagus nerves in the lung, which normally inhibit acetylcholine release (20), are no longer functional in virus-infected guinea pigs (18).

Multiple mechanisms are involved in virus-induced loss of neuronal M2 muscarinic receptor function. Dysfunction of inhibitory M2 muscarinic receptors may be a direct effect of the virus itself. Viral neuraminidase cleaves sialic acid residues from the M2 receptors, thereby decreasing agonist affinity for the receptors (17). Furthermore, parainfluenza virus decreases gene expression for the M2 receptors (26). Alternatively, inflammatory cells and their products may be important participants in loss of M2 receptor function in airway disease. Cyclophosphamide pretreatment of guinea pigs before viral infection depletes leukocytes, and in these animals, M2 receptor function is preserved except in animals with very severe infections in which the direct effects of the virus on M2 receptor function are seen (22).

In antigen-challenged guinea pigs, eosinophils are responsible for M2 receptor dysfunction (11, 16). Binding of eosinophil major basic protein to the M2 receptors blocks the negative feedback normally provided by binding of acetylcholine to the receptors (19, 24). We have shown that blockade of the neurokinin-1 (NK1) receptors prevents loss of M2 receptor function in antigen-challenged guinea pigs. However, the influx of eosinophils into the airways is not blocked by NK1 receptor antagonists. Because tachykinins can activate eosinophils and cause release of eosinophil granule proteins (30), it seems likely that the NK1 receptor antagonist prevents antigen-induced M2 receptor dysfunction by preventing eosinophil activation.

Eosinophils are not involved in virus-induced loss of M2 receptor function because depletion of eosinophils or of eosinophil major basic protein did not protect M2 function or prevent hyperresponsiveness (1). However, the effects of tachykinins in the airways are potentiated by viral infections. Virus-induced loss of neutral endopeptidase activity decreases the breakdown of

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tachykinins (25), potentiating their effects on airway smooth muscle (10) and increasing neurogenic inflammation (34). Because tachykinins may be involved in the activation of neutrophils and macrophages (4, 32) and these cells are increased with viral infection, we investigated the role of tachykinins in airway hyperresponsiveness and M₃ receptor dysfunction after viral infection.

METHODS

Virus stock and titration. Parainfluenza type 1 (Sendai virus, ATCC) was grown in rhesus monkey kidney cell monolayers in L-15 medium for 1 wk at 34°C. Cultures were frozen and thawed to disrupt cells, and the fluids were cleared by low-speed centrifugation and stored in aliquots at −70°C.

Viral content was determined by exposing fresh rhesus monkey kidney cell monolayers to serial 10-fold dilutions of the stock solution. After 1 wk of incubation at 34°C, the monolayers were washed and the medium was replaced with a 0.5% suspension of guinea pig erythrocytes. After 1 h, the erythrocytes were washed off and the monolayers were examined under an inverted phase-contrast microscope (Olympus) for evidence of hemadsorption (sticking of erythrocytes to the surface of cells expressing the viral hemagglutinin on their surfaces (38)). Viral content was determined as the multiple of the amount of stock solution required to produce infection in 50% of rhesus monkey kidney monolayers [the 50% tissue culture infection dose (TCID₅₀)].

Virus infection. Dunkin-Hartley guinea pigs (female, 250–350 g, specific pathogen free; Hilltop Animal Farms; Scottsdale, PA) were anesthetized with ketamine (45 mg/kg im) and xylazine (8 mg/kg im). Animals in the infected group were inoculated intranasally with 1 ml of virus solution that contained 10⁸ TCID₅₀/ml obtained by diluting the viral stock in Dulbecco’s phosphate-buffered saline. Animals in the uninfected (control) group were inoculated intranasally with fluids obtained from virus-free rhesus monkey kidney cells that were prepared and diluted in phosphate-buffered saline in the same way as the viral solutions. Control and infected animals were housed in separate laminar flow hoods.

Virus isolation and titration. After physiological studies were completed, the guinea pig lungs were removed, weighed, and homogenized in 2 ml of phosphate-buffered saline (Polytron, Brinkman; Lucerne, Switzerland). Virus was eluted from the tissue homogenate by incubation at 34°C for 1 h. The suspensions were centrifuged at 400 g for 30 min, and the viral centrifugate was determined by inoculating rhesus monkey kidney cell monolayers as above.

Treatment with neurokinin receptor antagonists. Guinea pigs were treated with either a NK₁ receptor antagonist [SR-140333, 1 mg/kg sc (2)] or a NK₂ receptor antagonist [SR-48968, 0.2 mg/kg sc (12)]. In three guinea pigs, the effect of a higher dose of the NK₂ antagonist (1 mg/kg) was also tested. Antagonists were given 1 h before infection and daily thereafter for a total of four doses. The final dose of antagonist was given 24 h before physiological studies were done. To exclude the possibility that the effects of the NK₁ antagonist on M₃ receptor function were the result of residual NK₁ antagonist, we treated two virus-infected guinea pigs with the NK₁ receptor antagonist (1 mg/kg iv) at the time of the physiological experiments and tested the effects on M₃ receptor function as described below.

Anesthesia and measurement of pulmonary inflation pressure. Four days after inoculation with virus or with control medium, guinea pigs were anesthetized with urethan (1.5 g/kg) injected intraperitoneally. This dose of urethan produces a deep anesthesia lasting 8–10 h. None of the experiments described lasted longer than 3 h, and the depth of anesthesia was monitored by observing for fluctuations in heart rate and blood pressure. Guinea pigs were handled in accordance with the standards established by the Animal Welfare Acts set forth in National Institutes of Health guidelines and the Policy and Procedures Manual published by the Johns Hopkins University School of Hygiene and Public Health Animal Care and Use Committee.

Once the guinea pigs were anesthetized, a carotid artery was cannulated for measurement of blood pressure and heart rate. Cannulas were placed into both jugular veins for the administration of drugs. Both vagi were cut, and the distal portions were placed on shielded platinum electrodes immersed in a pool of liquid paraffin. The body temperature of the animal was maintained at 37°C using a heating blanket.

The animals were paralyzed with succinylcholine (infused at 10 μg · kg⁻¹ · min⁻¹), tracheostomized, and ventilated using a positive-pressure, constant-volume animal ventilator (Harvard Apparatus; South Natick, MA; tidal volume 2.5–3.5 ml, 100 breaths/min). Pulmonary inflation pressure (Pₚᵢ) was measured with a Spectromed pressure transducer (DTX). All signals were displayed on a Grass polygraph.

A positive pressure of 85–100 mmH₂O was needed for adequate ventilation of the animals. Given constant flow and volume, bronchoconstriction was measured as the increase in Pₚᵢ over the baseline inflation pressure (9). The Pₚᵢ signal from the driver was fed into the input of the preamplifier of a second channel on the polygraph, and the baseline Pₚᵢ was subtracted electrically. Thus Pₚᵢ was recorded on one channel and increases in Pₚᵢ were recorded on a separate channel at a higher sensitivity. With this method, it was possible to accurately measure increases in Pₚᵢ as small as 2 mmH₂O above baseline.

Airway response to vagal stimulation. All animals were pretreated with guanethidine (10 mg/kg iv) to deplete noradrenaline. The vagus nerves were cut bilaterally, and the distal portions were placed on pairs of platinum electrodes. The vagi were stimulated bilaterally (1–25 Hz, 0.2-ms pulse duration, 10 V, 44 pulses per train), and the increase in Pₚᵢ was recorded.

M₃ receptor function. Airway neuronal M₃ muscarinic receptor function was assessed as the ability of the muscarinic agonist pilocarpine to inhibit the airway response to vagal stimulation. The nerves were stimulated regularly at 1-min intervals (2 Hz, 0.2-ms pulse duration, 4–10 V, 44 pulses per train). Once a stable baseline response to vagal stimulation had been established, pilocarpine (0.1–100 μg/kg) was administered intravenously and the effect on vagally induced bronchoconstriction was determined. Doses of pilocarpine greater than 30 μg/kg produced a transient bronchoconstriction. Therefore, the effect of these doses of pilocarpine on vagally induced bronchoconstriction was measured after the Pₚᵢ had returned to baseline. Doses of pilocarpine greater than 100 μg/kg were not used because they caused sustained bronchoconstriction via stimulation of muscarinic receptors on the airway smooth muscle. At the end of each experiment, vagally induced bronchoconstriction and bradycardia were abolished by atropine (1 mg/kg iv), indicating that both of these responses were mediated via release of acetylcholine onto muscarinic receptors.

Responsiveness to histamine. Histamine (1–20 nmol/kg) was injected intravenously, and the increase in Pₚᵢ was recorded. Histamine responsiveness was measured in animals before and after vagotomy. In the absence of vagotomy,
RESULTS

Viral infection. All animals exposed to parainfluenza virus became infected as shown by recovery of infectious virus from lung homogenates into rhesus monkey kidney cells. Viral content of lungs from non-neurokinin antagonist-treated animals was $3.9 \times 10^6 \pm 2.4 \times 10^6$ TCID$_{50}$/g lung tissue ($n = 13$), whereas that from NK$_1$ antagonist-treated animals was $4.8 \times 10^6 \pm 2.7 \times 10^6$ TCID$_{50}$/g lung tissue ($n = 13$) and that from NK$_2$ antagonist-treated animals was $4.4 \times 10^6 \pm 3.3 \times 10^6$ TCID$_{50}$/g lung tissue ($n = 7$). There were no significant differences among the viral titers in the three groups, and no virus was recovered from control (uninfected) animals.

Physiological measurements and $M_2$ receptor function. Baseline $P_{pi}$ was increased by viral infection [87 ± 5.7 mmHg in control and 155 ± 5.9 mmHg in infected animals ($P < 0.0001$)]. This virus-induced increase was attenuated but not abolished by pretreatment with either the NK$_1$ antagonist (115 ± 9.5 mmHg; $P = 0.0013$ vs. virus alone, $P = 0.036$ vs. control) or the NK$_2$ antagonist (126 ± 7.4 mmHg; $P = 0.0084$ vs. virus alone, $P = 0.002$ vs. control). Baseline heart rate was not affected by any of the treatments.

Electrical stimulation of the vagus nerves caused bronchoconstriction (measured as an increase in $P_{pi}$) and bradycardia. Both responses were frequency dependent. Vagally induced bronchoconstriction was potentiated in virus-infected guinea pigs (Figs. 1 and 2). This hyperresponsiveness to vagal stimulation was prevented by pretreatment with the NK$_1$ receptor antagonist (Fig. 1) but not by the NK$_2$ receptor antagonist (Fig. 2).

Fig. 1. Pretreatment with a neurokinin-1 (NK$_1$) receptor antagonist (Ant) prevents hyperresponsiveness to vagal stimulation in virus-infected guinea pigs. Stimulation of the vagi (1–25 Hz, 0.2-ms pulse duration, 10 V, 44 pulses per train) causes a frequency-dependent bronchoconstriction, reflected as an increase in pulmonary inflation pressure. This response is potentiated in virus-infected animals ($n = 5$) compared with uninfected controls ($n = 6$). In animals that were pretreated with the NK$_1$ receptor antagonist SR-140333, this hyperresponsiveness to vagal stimulation was prevented ($n = 5$; means ± SE).

Fig. 2. Pretreatment with a NK$_2$ receptor antagonist does not prevent hyperresponsiveness to vagal stimulation in virus-infected guinea pigs. The response to vagal stimulation remains potentiated in virus-infected animals that were pretreated with the NK$_2$ receptor antagonist SR-48968 ($n = 4$) compared with uninfected controls ($n = 6$; means ± SE). Control and virus-alone animals are the same as in Fig. 1.

Measurement of inflammatory cells. At the end of each experiment, after the physiological measurements were completed, the lungs were lavaged five times with 10-ml aliquots of warm phosphate-buffered saline. Lavage fluids were centrifuged (400 $g$, 10 min) and the pellets were resuspended in normal saline. Cell counts were done using a hemocytometer. Slides made of lavaged cells using a cytospin were stained with Diff-Quik (American Scientific Products) and used to determine cell differentials.

Drugs. Pilocarpine, succinylcholine, atropine, histamine, guanethidine, urethan, and L-15 medium were purchased from Sigma (St. Louis, MO). Ketamine was purchased from Aveco (Fort Dodge, IA). Xylazine was purchased from Mobay (Shawnee, KS). SR-140333 and SR-48968 were generous gifts from Dr. X. Emonds-Alt (Sanofi Recherche; Montpellier, France). All drugs were dissolved and diluted in 0.9% NaCl.

Statistics. Results are expressed as the means ± SE. The effects of viral infection and of pretreatment with neurokinin receptor antagonists on dose-response curves to pilocarpine were compared using two-way analysis of variance (ANOVA) for repeated measures. Similar analysis was used to determine the effects of viral infection, vagotomy, and pretreatment with neurokinin receptor antagonists on histamine-induced bronchoconstriction as well as the effects of viral infection and neurokinin receptor antagonists on vagally induced bronchoconstriction. Viral content of the lungs and cell counts and differentials in various groups of animals were compared using one-way ANOVA. $P < 0.05$ was considered significant.
In uninfected animals, vagally induced bronchoconstriction was inhibited by pilocarpine in a dose-dependent fashion, demonstrating M\(_2\) receptor function. This response was lost in virus-infected animals (Figs. 3 and 4), demonstrating loss of M\(_2\) receptor function. In animals that were pretreated with the NK\(_1\) receptor antagonist SR-140333, loss of M\(_2\) receptor function was prevented (n = 6; means ± SE).

In uninfected animals, vagally induced bronchoconstriction was inhibited by pilocarpine in a dose-dependent fashion, demonstrating M\(_2\) receptor function. This response was lost in virus-infected animals (Figs. 3 and 4), demonstrating loss of M\(_2\) receptor function. M\(_2\) receptor dysfunction was prevented by pretreatment with the NK\(_1\) receptor antagonist (Fig. 3) but not by the NK\(_2\) receptor antagonist (Fig. 4). A higher dose of the NK\(_2\) receptor antagonist (1 mg/kg) also failed to prevent virus-induced M\(_2\) receptor dysfunction (data not shown). In virus-infected animals not previously treated with the NK\(_1\) antagonist, acute administration of the NK\(_2\) antagonist (1 mg/kg iv) at the time of the physiological experiments did not restore M\(_2\) receptor function (data not shown).

Intravenous injection of histamine caused dose-dependent bronchoconstriction. Histamine-induced bronchoconstriction was potentiated in virus-infected guinea pigs (Fig. 5). This hyperresponsiveness to histamine was largely, but not completely, reversed by vagotomy. Likewise, pretreatment with the NK\(_1\) receptor antagonist largely, but not completely, prevented virus-induced hyperresponsiveness to histamine (Fig. 5). In virus-infected animals that had been pretreated with the NK\(_1\) antagonist, vagotomy returned the responsiveness to histamine to control levels (Fig. 5).

Leukocytes in lung lavage fluid. In lung lavage fluid, total leukocyte counts were increased by viral infection, and this increase was prevented by treatment with either the NK\(_1\) or the NK\(_2\) antagonist (Fig. 6). The increase in total cells was made up primarily of neutrophils. A small but significant attenuation of the neutrophil influx was seen after pretreatment with the NK\(_2\) antagonist. Macrophages were not increased significantly by viral infection. However, macrophages were decreased in virus-infected animals by pretreatment with either the NK\(_1\) or the NK\(_2\) antagonist, although the effect of the NK\(_2\) antagonist did not achieve statistical significance (P = 0.06). Thus the effects of the antagonists on total cell numbers are the result of combined effects on neutrophils and macrophages.
Although the effect of the NK 2 antagonist did not achieve statistical significance, either M2 receptor dysfunction (Fig. 4) or the resulting hyperresponsiveness to vagal stimulation (Fig. 1) and largely attenuated the hyperresponsiveness to histamine (Fig. 5). Treatment with the NK2 antagonist did not prevent the loss of M2 muscarinic receptors via mechanisms that are largely dependent on stimulation of the NK1 receptors. In control guinea pigs, the muscarinic agonist pilocarpine inhibited vagally induced bronchoconstriction in a dose-dependent manner, confirming that neuronal M2 muscarinic receptors were functioning to inhibit release of acetylcholine (20). In contrast, viral infection caused loss of M2 muscarinic receptor function because pilocarpine no longer inhibited vagally induced bronchoconstriction (Figs. 3 and 4). Viral infection also induced hyperresponsiveness to both histamine and vagal nerve stimulation compared with noninfected controls (Figs. 1, 2, and 5).

In virus-infected animals that were treated with the NK1 receptor antagonist, M2 receptor function was preserved (Fig. 3). This prevented hyperresponsiveness to vagal stimulation (Fig. 1) and largely attenuated the hyperresponsiveness to histamine (Fig. 5). Treatment with the NK2 antagonist did not prevent either M2 receptor dysfunction (Fig. 4) or the resulting vagal hyperresponsiveness (Fig. 2).

Blocking the NK1 receptors resulted in significant suppression of the inflammatory response to viral infections, primarily by inhibiting the influx of macrophages (Fig. 6). However, blocking the NK2 receptor had a similar effect (Fig. 6) but did not prevent M2 receptor dysfunction or the airway hyperresponsiveness. Thus preventing the influx of inflammatory cells into the airways does not appear to be the mechanism for the effects of the NK1 receptor on the M2 receptor and on vagal responsiveness.

The lack of concordance between the effect on the inflammatory response and the effect on M2 receptor function is similar to our previous description of the effects of neurokinin receptor antagonists on antigen-induced hyperresponsiveness and M2 receptor dysfunction (7). In this case, neither NK1 antagonists nor NK2 antagonists blocked antigen-induced airway inflammation (which consisted largely of eosinophils). However, NK1 antagonists (but not NK2 antagonists) prevented M2 receptor dysfunction and airway hyperresponsiveness. We subsequently demonstrated the importance of NK1 receptors stimulating release of major basic protein from eosinophils, causing M2 receptor dysfunction and airway hyperresponsiveness (14).

Although tachykinins have important direct effects on cholinergic neurotransmission, these are not likely to be responsible for the current findings. Physiological studies were done at least 24 h after the last dose of neurokinin receptor antagonist when no antagonist would be expected to remain. Furthermore, we have shown that the NK1 antagonist, given at the time of the physiological studies, did not acutely reverse the M2 receptor dysfunction (data not shown).

We have previously demonstrated multiple mechanisms by which viral infections can cause M2 receptor dysfunction and airway hyperresponsiveness (22). Depleting inflammatory cells by pretreating with cyclophosphamide prevents M2 receptor dysfunction in the majority of virus-infected guinea pigs. In some animals, however, M2 receptor function is lost despite the prevention of lung inflammation by cyclophosphamide. The viral content of the lungs is substantially higher in guinea pigs that lose M2 receptor function despite cyclophosphamide treatment than in those in which the M2 receptor function is preserved. This suggests the possibility that in animals with severe viral infections, there may be a direct effect of the virus on M2 receptor function.

We have suggested two mechanisms by which the virus may directly affect M2 receptor function. Parainfluenza virus-induced pulmonary M2 receptor dysfunction may be the result of cleavage of sialic acid residues from M2 receptors by viral neuraminidase, which decreases agonist affinity for the receptors by an order of magnitude (17). Alternatively, virus-induced airway M2 receptor dysfunction may be a direct effect of viral infection on M2 receptor gene expression in the airway nerves. Using primary cultures of airway parasym pathetic nerves, we have shown that viral infection of these cells decreases M2 receptor gene expression by...
two orders of magnitude (26). This loss of M₂ receptor gene expression is accompanied by increased release of acetylcholine and by functional evidence of loss of inhibitory M₂ receptor effect.

Loss of M₂ receptor function may also be the result of airway inflammation, as is the case after inhalation of antigen or ozone (11, 16, 23). In both ozone- (40) and antigen-exposed (15, 19) guinea pigs, release of eosinophil major basic protein, which is an M₂ receptor antagonist (24), is responsible for loss of M₂ receptor function.

In contrast, eosinophils are not responsible for virus-induced M₂ receptor dysfunction and hyperresponsiveness. Depleting eosinophils using antibody to interleukin-5 does not prevent these effects of viral infections, and virus-induced M₂ receptor dysfunction cannot be reversed by neutralizing major basic protein (22).

Although the precise inflammatory cells responsible for virus-induced M₂ receptor dysfunction have not yet been identified, we have demonstrated that interferon-γ, which is produced in response to viral infections, may be involved. Treating primary cultures of airway parasympathetic neurons with interferon-γ (300 U/ml) for 24 h caused substantial loss of M₂ receptor function and increased acetylcholine release. This was accompanied by a marked decrease in M₂ receptor gene expression (26).

Tachykinins, signaling via the NK₁ receptor, may be involved in production of interferon-γ. Substance P has been shown to stimulate production of interferon-γ by clones of T helper cells (31) and to potentiate the stimulated release of interferon-γ by peripheral blood mononuclear cells (33). Furthermore, treatment of mice with the NK₁ receptor antagonist Spantide II decreases interferon-γ production in mice infected with Salmonella (29), whereas the production of interferon-γ is markedly decreased in NK₁ receptor knockout mice infected with Schistosoma mansoni (5).

We have demonstrated the role of M₂ receptor dysfunction in increasing vagally mediated bronchoconstriction in several models of airway disease (18, 21, 36). Furthermore, in antigen-challenged guinea pigs, the increased vagal acetylcholine release that results from M₂ receptor dysfunction is responsible for the increased response to histamine (8). Thus it is the reflex component of the response to histamine that is potentiated.

Studies in humans with naturally occurring viral infections likewise suggest that reflex bronchoconstriction is potentiated. Empey et al. (13) demonstrated that the response to inhaled histamine was increased in subjects with viral infections. The response to histamine was returned to the level of controls if subjects were treated with atropine, demonstrating that it was the vagal reflex component of the response that was increased. Similar effects were found by Aquilina et al. (3) using cold air as the stimulus.

Hyperresponsiveness to histamine in virus-infected guinea pigs is also largely vagally mediated. Buckner et al. (6) demonstrated that atropine reverses hyperresponsiveness to histamine in parainfluenza virus-infected guinea pigs. In the current studies, we showed that the vagally mediated component of the response to histamine is increased by M₂ receptor dysfunction in virus-infected animals. Vagotomy substantially decreases the hyperresponsiveness to histamine. However, it does not abolish it, suggesting that another, vagally independent mechanism exists in addition. Likewise, pretreatment with the NK₁ receptor antagonist largely, but not completely, blocked the increase in responsiveness to histamine. In these animals, subsequent vagotomy eliminated the residual histamine hyperresponsiveness.

One possibility to explain the vagus-independent hyperresponsiveness to histamine was proposed by Sekizawa et al. (37). In these studies, parainfluenza infection of guinea pigs decreased the activity of the histamine-degrading enzyme histamine N-methyltransferase and in this way increased the effects of histamine on the airway smooth muscle. The role of stimulation of NK₁ receptors in mediating this effect of viral infections has not been investigated.

In conclusion, viral infection causes M₂ muscarinic receptor dysfunction, leading to increased vagal acetylcholine release. Both effects appear to require stimulation of NK₁ receptors and are blocked by a NK₁ receptor antagonist but not by a NK₂ receptor antagonist. The hyperresponsiveness to histamine is also largely mediated through increased vagal reflex resulting from M₂ receptor dysfunction. This is again attenuated by the NK₁ receptor antagonist. The NK₁ receptor antagonist suppresses the inflammatory response to viral infections, but in this respect it does not appear to be substantially different from the NK₂ receptor antagonist. This suggests that the effects of the NK₁ receptor antagonist are not the result of an interruption of the recruitment of inflammatory cells, although they may be the result of interference with inflammatory cell activation.

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