Effects of NZ-107 on Tracheal Responses to Adenosine in the Guinea Pig

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ABSTRACT—We have investigated the effect of NZ-107, an inhibitor of bronchoconstriction induced by slow reacting substance of anaphylaxis (SRS-A), on tracheal responses to adenosine in the guinea pig. In the presence of an adenosine uptake inhibitor, dipyridamole (1 μM), NZ-107 (0.3–1 μM) enhanced adenosine-induced relaxation in 30 nM leukotriene D4 (LTD4)-precontracted trachea, whereas aminophylline (AP, 10–30 μM), an adenosine receptor antagonist, markedly inhibited it. NZ-107 (1 μM) also enhanced the relaxation induced by forskolin, an adenylate cyclase activator, but not that by nitroprusside (NP), a guanylate cyclase activator. AP (30 μM) affected neither forskolin- nor NP-induced relaxation. NZ-107 (1 μM) and AP (30 μM) inhibited to about the same extent the contractile response to an adenosine A1 receptor agonist, the R(-)-enantiomer of N6-(2-phenylisopropyl)-adenosine (R-PIA). The R-PIA-induced contraction was completely blocked by 5 μM indomethacin. NZ-107 (1 μM) did not affect the contraction induced by PGD2, but significantly reduced that of PGF2α. AP (30 μM) had no effect on PGF2α- and PGD2-induced contractions. These results suggest that NZ-107 may have a unique profile for adenosine responses in bronchial asthma.

NZ-107 is an orally active and potent inhibitor of bronchoconstriction induced by slow reacting substance of anaphylaxis (SRS-A) in the guinea pig (1). In isolated trachea and lung parenchyma, NZ-107 more selectively inhibited antigen-induced contraction than that induced by the calcium ionophore A23187, and it also selectively inhibited antigen-induced SRS-A release from lung fragments (2). These profiles of NZ-107 suggested that it may be beneficial in the treatment of SRS-A predominant bronchial asthma.

Allergic mediators, leukotrienes (LT) (3), histamine and platelet activating factor (4), are thoroughly implicated in the pathogenesis of asthma. There is also increasing interest in the involvement of adenosine as a mediator in allergic asthma (5, 6). Adenosine is reported to be a potent bronchoconstrictor in allergic and non-allergic asthmatic subjects (6) and in allergic rabbits (7), and allergen-induced bronchospasm in asthmatics is associated with elevated plasma concentrations of adenosine (8).

Methylxanthine derivatives, such as theophylline and its diethylene salt aminophylline (AP), are widely used in the treatment of bronchial asthma. Theophylline at its therapeutic plasma concentration is only a weak inhibitor of phosphodiesterase (PDE) enzymes (9), but is an effective antagonist of adenosine
receptors (5). The other mechanism of action of theophylline has been reported to be antagonism against prostaglandins (PGs) (10).

We, therefore, investigated the effect of NZ-107 on adenosine receptor-mediated relaxation and contraction in the isolated guinea pig trachea, and the results were compared with those for AP.

MATERIALS AND METHODS

Materials

The following materials were used for these studies: adenosine, nitroprusside sodium (NP), histamine dihydrochloride (Wako Pure Chemicals, Japan); R(−)-enantiomer of N^6-2-phenylisopropyl-adenosine (R-PIA) (Research Biochemicals, MA); aminophylline, dipyridamole, indomethacin (Sigma Chemical Co., MO); leukotriene D₄ (LTD₄, Ultrafine Chemicals, U.K.); forskolin (Calbiochem, CA); prostaglandin F₂α (PGF₂α, Nacalai Tesque, Japan); and prostaglandin D₂ (PGD₂, Funakoshi, Japan). NZ-107 was synthesized by the Central Research Laboratory, Nissan Chemical Industries, Ltd. (Funabashi, Japan).

Methods

Generals: Male albino Hartley guinea pigs (300–500 g) were killed by a blow to the head. The trachea was removed and spirally cut and divided into two or three segments. Each was suspended under 1 g tension in an 8-ml organ bath containing modified Tyrode solution maintained at 37°C and aerated with 95% O₂ and 5% CO₂. The composition of the modified Tyrode solution was as follows: 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.3 mM NaHPO₄, 20 mM NaHCO₃ and 11 mM dextrose. Responses were recorded isotonically (Nihon Kohden, Type TD-112S).

Effect of NZ-107 on the relaxants: Tissues were equilibrated for 50–60 min, and then the maximal response to histamine (100 μM) was obtained in each tissue. After the response reached a plateau, tissues were washed several times for 20 min until resting level tone was restored. Indomethacin (5 μM), dipyridamole (1 μM, in the experiment of adenosine), and NZ-107 or AP were added to the organ bath 30 min before LTD₄ was added. After the contractile response of LTD₄ reached a plateau, the relaxant was added cumulatively. The maximum response was obtained by 1 mM AP.

Effect of NZ-107 on constrictors: NZ-107 (0.3 or 1 μM) or AP (10 or 30 μM) and indomethacin (when PGs were used as constrictors) were administered to the organ bath 30 min before R-PIA or PGs were cumulatively added. The contractile responses were expressed as a percentage of the maximum response to histamine (100 μM). Other experimental conditions were similar to those of the relaxation experiment.

Statistics: Results are expressed as the mean ± S.E.M. pEC₅₀ was calculated from the negative logarithm of the concentration (molar) of compound required to induce 50% of the response. Statistical analysis was performed by Student's t-test or the paired t-test. P values of less than 0.05 were considered to be significant.

RESULTS

Effect of NZ-107 on adenosine-induced relaxation

In the presence of indomethacin and dipyridamole, adenosine (over 1 μM) caused concentration-dependent relaxation against 30 nM LTD₄-induced contraction, and the maximal response was obtained at 300 μM adenosine (90% relaxation as compared with 1 mM AP) (Fig. 1). NZ-107 (0.3 and 1 μM) caused a leftward shift of the concentration-relaxation curve to adenosine. In contrast, AP (10 and 30 μM) caused a rightward shift of the curve. NZ-107 (1 μM) partly inhibited 30 nM LTD₄-induced contraction, producing a level of contraction similar to the control response to 3 nM LTD₄. We, therefore, compared the pEC₅₀ value between similar levels of contractile response (11). The pEC₅₀ value in the presence of NZ-107 (1 μM) was significantly in-
increased as compared with 3 nM LTD₄ (Table 1). In contrast, the pEC₅₀ values in the presence of AP (10 and 30 μM) were markedly reduced as compared with the control.

Effect of NZ-107 on forskolin and NP-induced relaxation

Both an adenylate cyclase activator, forskolin (12), and a guanylate cyclase activator, NP (13), caused concentration-dependent relaxation against the contraction induced by 30 nM LTD₄ in the presence of indomethacin. The pEC₅₀ value for each relaxant is shown in Table 2. Preincubation of NZ-107 (1 μM) or AP (30 μM) attenuated 30 nM LTD₄-induced contraction to the same extent (less that about 15% inhibition, Table 2). NZ-107 appeared to enhance the relaxant response induced by forskolin but not that by NP. AP did not enhance the response of either relaxant.

Fig. 1. The effects of NZ-107 and aminophylline on adenosine-induced relaxation in isolated guinea pig trachea. (∗) Control, (∙) 0.3 μM NZ-107, (∆) 1 μM NZ-107, (□) 10 μM aminophylline, (△) 30 μM aminophylline. Contraction was induced by 30 nM LTD₄ in the presence of 1 μM dipyridamole and 5 μM indomethacin. Each point represents the mean ± S.E.M. of 5–12 experiments.

Effect of NZ-107 on forskolin and NP-induced relaxation

Both an adenylate cyclase activator, forskolin (12), and a guanylate cyclase activator, NP (13), caused concentration-dependent relaxation against the contraction induced by 30 nM LTD₄ in the presence of indomethacin. The pEC₅₀ value for each relaxant is shown in Table 2. Preincubation of NZ-107 (1 μM) or AP (30 μM) attenuated 30 nM LTD₄-induced contraction to the same extent (less that about 15% inhibition, Table 2). NZ-107 appeared to enhance the relaxant response induced by forskolin but not that by NP. AP did not enhance the response of either relaxant.

Effect of NZ-107 on contraction induced by R-PIA

A stable adenosine analogue, R-PIA (14), which is relatively selective for adenosine A₁ receptors, contracted guinea pig trachea at concentrations ranging from 0.01–1 μM. The maximum response at 1 μM was about 25% of the response to 100 μM histamine (Fig. 2). Both NZ-107 and AP reduced R-PIA-induced contraction in a concentration-dependent manner, and 1 μM NZ-107 and 30 μM AP significantly reduced it. A cyclooxygenase inhibitor, indomethacin (5 μM), completely blocked R-PIA-induced contraction (data not shown).

Effect of NZ-107 on contraction induced by PGD₂ and PGF₂α

In a separate experiment, PGF₂α produced concentration-dependent contraction in the presence of 5 μM indomethacin (Fig. 3, a and b). NZ-107 (1 μM) caused a significantly rightward shift of the PGF₂α curve (Table 3).

Table 1. Effect of NZ-107 and aminophylline (AP) on adenosine-induced relaxation in the isolated guinea pig trachea

<table>
<thead>
<tr>
<th>Condition</th>
<th>LTD₄ (nM)ᵃ</th>
<th>% His maxᵇ</th>
<th>pEC₅₀ᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>65 ± 3</td>
<td>5.01 ± 0.07</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>45 ± 3</td>
<td>5.02 ± 0.16</td>
</tr>
<tr>
<td>NZ-107, 1 μM</td>
<td>30</td>
<td>45 ± 4ᵇ</td>
<td>5.39 ± 0.12ᵇ</td>
</tr>
<tr>
<td>AP, 10 μM</td>
<td>30</td>
<td>73 ± 9ᵇ</td>
<td>4.49 ± 0.09ᵇ</td>
</tr>
<tr>
<td>30 μM</td>
<td>30</td>
<td>72 ± 7ᵇ</td>
<td>4.30 ± 0.13ᵇ</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. of 5–12 observations. ᵃTrachea was precontracted by the indicated concentration of LTD₄. ᵇPercent maximum contractile response to histamine. ᶜNot significantly different from the value of the 3 nM LTD₄ control. ᵈNot significantly different from the value of the 30 nM LTD₄ control. ᵉSignificantly different from the value of the 3 nM LTD₄ control. ᶠSignificantly different from the value of the 30 nM LTD₄ control.
Table 2. Effects of NZ-107 and aminophylline (AP) on forskolin- and nitroprusside-induced relaxation in the isolated guinea pig trachea

<table>
<thead>
<tr>
<th>Condition</th>
<th>LTD₄ (nM)ᵃ</th>
<th>% Hist maxᵇ</th>
<th>pEC₅₀</th>
<th>% Hist maxᵇ</th>
<th>pEC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>79 ± 4</td>
<td>6.23 ± 0.06</td>
<td>80 ± 4</td>
<td>6.50 ± 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>63 ± 2</td>
<td>6.17 ± 0.03</td>
<td>63 ± 3</td>
<td>6.66 ± 0.08</td>
</tr>
<tr>
<td>NZ-107, 1 µM</td>
<td>30</td>
<td>65 ± 6</td>
<td>6.50 ± 0.06ᵇ</td>
<td>66 ± 5ᵇ</td>
<td>6.64 ± 0.07ᵇ</td>
</tr>
<tr>
<td>AP, 30 µM</td>
<td>30</td>
<td>68 ± 4ᵇ</td>
<td>6.25 ± 0.11ᵇ</td>
<td>77 ± 9ᵇ</td>
<td>6.38 ± 0.07ᵇ</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. of 5–6 observations. ᵃTrachea was precontracted by the indicated concentration of LTD₄. ᵇPercent maximum contractile response to histamine. ᵇNot significantly different from the value of the 10 nM LTD₄ control. ᵇNot significantly different from the value of the 30 nM LTD₄ control. *Significantly different from the value of the 10 nM LTD₄ control.

DISCUSSION

Previous studies showed that two surface receptors for adenosine are recognized by substitute analogues of adenosine. A₁ receptors are inhibitory to adenylate cyclase and associated with a fall in intracellular cyclic AMP (cAMP), while A₂ receptors are stimulatory to adenylate cyclase and associated with a rise in cAMP (15). A₁ receptors have a high affinity for adenosine (Ka: 5 nM to 50 nM), while A₂ receptors show low affinity (Ka: 5 µM to 20 µM) (15). In guinea pig trachea, adenosine-induced relaxation is reported to be mediated by
A2 receptors (16, 17). The stable adenosine analogue R-PIA, which is relatively selective for adenosine A1 receptors (14), elicits a contractile response in guinea pig trachea at concentrations lower than about 1 μM (18, 19). In the present study, adenosine caused only relaxation at concentrations higher than 3 μM, and R-PIA caused contraction at concentrations less than 1 μM, suggesting that adenosine and R-PIA act on A2 and A1 receptors, respectively, in the guinea pig trachea.

It is interesting that NZ-107 enhanced the relaxation of adenosine and inhibited contraction of R-PIA, while AP inhibited both the relaxant response and contractile response. These dual inhibitory effects of AP may be due to non-selective antagonism at adenosine A1 and A2 receptors. NZ-107, also potentiated the relaxation induced by forskolin but not that by NP. This result suggests that the effect of NZ-107 on adenosine A2 receptor-mediated relaxation is partly mediated by the elevation of cAMP levels. The ineffectiveness of AP on forskolin-induced relaxation may be related to the lack of the increase in cAMP at 30 μM. Farmer (18) reported that a xanthine derivative, enprofylline, which is not an adenosine antagonist but a potent PDE inhibitor (20), had little effect on R-PIA-induced contraction in the guinea pig trachea. Thus it is unlikely that the inhibitory effect of NZ-107 on A1-mediated contraction results from PDE inhibition.

Table 3. Effect of NZ-107 and aminophylline (AP) on PGF2α- and PGD2-induced contraction in the guinea pig trachea

<table>
<thead>
<tr>
<th>Condition</th>
<th>PGF&lt;sub&gt;2α&lt;/sub&gt;</th>
<th>PGD&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.10 ± 0.06</td>
<td>6.03 ± 0.13</td>
</tr>
<tr>
<td>NZ-107, 1 μM</td>
<td>5.77 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.09 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>6.29 ± 0.08</td>
<td>6.37 ± 0.04</td>
</tr>
<tr>
<td>AP, 30 μM</td>
<td>6.23 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.28 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. of 5–6 observations. <sup>a</sup>Not significantly different from the paired control. *Significantly different from the paired control.

Since the cyclooxygenase inhibitor indomethacin completely inhibited R-PIA-induced contraction, it is suggested that the contractile response elicited by R-PIA is mediated by cyclooxygenase metabolites, such as constrictor PGs. Similar results have been reported by Farmer et al. (18) and Caparrotta et al. (19). Since NZ-107 (1 μM) significantly inhibited the contraction induced by PGF2α, it is suggested that NZ-107 reduces R-PIA-induced contraction by inhibiting the contraction induced by endogenously released PGF2α. NZ-107, however, did not affect the contractile response of PGD2, another constrictor PG. The reason for the inability of NZ-107 to inhibit the PGD2 response is not clear, but it may be related to the different contractile mechanism between PGD2 and PGF2α, because we have previously found that PGF2α-induced contraction is partly conducted by LT-like substances in the guinea pig trachea (21) and NZ-107 is an inhibitor of LTD4 contraction of the guinea pig trachea (1). Therefore, it is a possible interpretation that NZ-107 predominantly blocks LTD4 contraction through the involvement of PGF2α rather than PGD2. We, therefore, suggest that NZ-107 enhances adenosine A2-mediated relaxation and inhibits adenosine A1-mediated contraction of the guinea pig trachea by elevating the cAMP level and blocking the LTD4 response, respectively. Since AP did not affect PGF2α- and PGD2-induced contraction, the inhibitory effect of AP on R-PIA response is not due to the inhibition of PG but due to adenosine antagonism.

Adenosine is a potent bronchoconstricotor in asthmatic subjects but not in normal subjects (6). It is possible that adenosine acts preferentially on the A1 receptor and induces bronchoconstriction in asthmatics. In light of this hypothesis, the inhibitory effect of NZ-107 on R-PIA-induced contraction may be a useful profile for an anti-asthma drug.

In conclusion, NZ-107 enhanced adenosine A2 receptor-mediated relaxation and reduced adenosine A1 receptor-mediated contractions at the same concentration in the guinea pig trachea.
REFERENCES


