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Effect of an Anti-SRS-A Agent, NZ-107, on Airway Responses Induced by Ovalbumin and A23187 in the Guinea-pig

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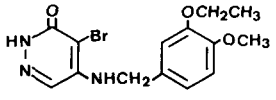
Abstract—The effects of the anti-SRS-A agent NZ-107 on antigen-(ovalbumin) and calcium ionophore A23187-induced airway responses in the guinea-pig have been investigated. In the presence of 5 μM indomethacin, NZ-107 (3 μM) did not affect the peak response in ovalbumin-induced contraction but did inhibit the prolonged response following the peak response in the tracheal strip. A higher concentration of NZ-107 (10 μM) completely blocked both peak and prolonged responses. Inhibitory effects of NZ-107 on ovalbumin responses were less in the lung parenchymal strip. The potency of NZ-107 in inhibiting ovalbumin-induced tracheal contraction was not changed in the absence of indomethacin but was reduced in the presence of 45 mM serine-borate, an inhibitor of the conversion of LTC_4 to LTD_4. NZ-107 inhibited A23187-induced contractions in both tracheal and parenchymal strips but in both cases the inhibitory potency was less than that on ovalbumin response. NZ-107 was a more potent inhibitor of ovalbumin-induced SRS-A release than histamine release in lung fragments but was ineffective in inhibiting A23187-induced SRS-A and histamine release. NZ-107 at a concentration of 10 μM more effectively inhibited LTC_4- and histamine-induced tracheal contractions than it did LTC_4 in the presence of 45 mM serine-borate. These results suggest that NZ-107 selectively inhibits antigen-induced SRS-A responses in airway tissues of the guinea-pig.

In allergic asthma, various bronchoactive substances, e.g. histamine, leukotrienes and other arachidonic acid metabolites, are released from mediator cells in airway tissues. Slow reacting substance of anaphylaxis (SRS-A), which consists of leukotrienes LTC_4, LTD_4 and LTE_4, may play a major role in allergic asthma. In human bronchial strips as well as in guinea-pig trachael strips SRS-A is a potent bronchoconstrictor (Dahlén et al 1980) and in addition leukotrienes may be involved in oedema formation and mucus hypersecretion in asthma (Woodward et al 1983). For these reasons, many leukotriene antagonists and inhibitors of leukotriene synthesis have been developed and evaluated for the therapy of bronchial asthma (I).



I. Structure of NZ-107.

We recently reported that NZ-107, a pyridazinone derivative, selectively inhibited LTD_4-induced airway smooth muscle contraction in-vitro and inhibited antigen- (ovalbumin) induced SRS-A-mediated bronchoconstriction when dosed orally to guinea-pig. On intravenous administration of NZ-107, the inhibition of antigen-induced SRS-A-mediated bronchoconstriction was similar to that of the SRS-A antagonist, FPL-55712, whereas in isolated trachea the inhibitory effect of NZ-107 against LTD_4-induced contraction was about 1/15 that of FPL-55712. The purpose of the present study was to investigate the inhibitory effects of NZ-107 on antigen-induced contraction in guinea-pig isolated airway tissues and to compare the effects with those on a calcium ionophore, A23187. We also investigated the inhibitory effect of NZ-107 on the release of SRS-A and histamine in lung fragment preparations.

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Materials and Methods

Materials

The following compounds were used: ovalbumin (grade II for sensitization and grade III for challenge), indomethacin, L-serine, boric acid, A23187 free acid (A23187), nordihydroguaiaretic acid (NDGA), pyrilamine maleate (Sigma Chemical Co. MO, USA), LTC_4 and LTD_4 monomethylesters (Paesel GmbH & Co. Germany), histamine dihydrochloride (Wako Pure Chemicals, Japan) and atropine sulphate (E. Merck, Germany). NZ-107 (4-bromo-5-(3-ethoxy-4-methoxy-benzylamino)-3(2H)-pyridazinone) and FPL-55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-chromene-2-carboxylate) were synthesized by the Central Research Laboratory, Nissan Chemical Industries (Funabashi, Japan). A23187, NZ-107, FPL-55712 and NDGA were dissolved in 100% dimethylsulphoxide (DMSO), indomethacin was dissolved in 100% ethanol, and other compounds were dissolved in distilled water. DMSO and ethanol in contact with tissue did not exceed 0·2% and 0·1% v/v, respectively.

Sensitization

Male Hartley strain guinea-pigs, 200–250 g, were sensitized with ovalbumin (100 mg s.c. and 100 mg i.p.).

Contractile response induced by antigen and A23187

Tracheas and lungs were obtained 3–4 weeks after sensitization. Guinea-pigs that were not previously sensitized with

ovalbumin were used in the series of A23187 experiments. The trachea was spirally cut, divided into three or four equal segments each of which was suspended under 1 g tension in a 10 mL organ bath containing modified Tyrode solution maintained at 37°C and aerated with 95% O_2–5% CO_2. The composition of the modified tyrode solution was (mM): NaCl 137, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·0, $NaHPO_4$ 0·3, $NaHCO_3$ 20, and dextrose 11. Lung parenchymal strips were prepared by cutting from the distal edges of the lobes and were suspended as described above. One tissue preparation always served as a control. Contractions were measured using an isotonic transducer (Nihon Kohden, type TD-112s). Tissues were equilibrated for 50–60 min and then constant maximal responses to histamine (100 μM) were obtained. Tissues were washed several times for 20 min until resting level tone was restored. Indomethacin, serine-borate and NZ-107 were added to the organ bath, as appropriate, 30 min before challenge with ovalbumin (100 μg mL^{-1}) or A23187 (1 μg mL^{-1}). Contractile responses to ovalbumin and A23187 were measured as a percentage of response obtained with 100 μM histamine in each tissue, and the area under the curve for contraction (AUC) during the 60 min after challenge was calculated to give an indication of total contractile response.

Mediator release

Guinea-pigs were killed by a blow to the head. Lungs were perfused via the right ventricle with 20 mL modified Tyrode solution to remove blood. Lung parenchyma were cut into 2–3 mm segments. The fragments were washed several times with modified Tyrode solution and divided into portions weighing approximately 800 mg. Lung fragments were resuspended in 5 mL modified Tyrode solution and then incubated at 37°C for 5 min with 5 μM indomethacin and for an additional 10 min with NZ-107 or NDGA. Ovalbumin (10 μg mL^{-1}) or A23187 (10 μg mL^{-1}) was added to the test tubes and incubation was performed for 45 min. The incubation medium was recovered as supernatant following low speed centrifugation (2000 *g* for 5 min).

The release of SRS-A content was determined by a bioassay technique using guinea-pig isolated ileum. Ileal strips were suspended under 0·5 g tension at 30°C, and constant histamine (1 μM) responses were obtained. To obtain the standard concentration-response curve for LTD_4, synthetic LTD_4 was added to the organ bath in the presence of pyrilamine (1 μM) and atropine (1 μM). SRS-A content was expressed as the nanogram equivalent of LTD_4 released per gram lung (wet weight). Histamine was assayed using a fluorometric procedure as described by May et al (1970) and released histamine was expressed as the percentage of the total histamine content. Spontaneous histamine release was obtained in the absence of stimulant.

Histamine and LTC_4-induced tracheal contraction

Tracheae from normal guinea-pigs were used. The experiment was performed in a manner similar to that in ovalbumin- and A23187-induced contraction. LTC_4 or histamine was cumulatively added after 30 min incubation with NZ-107 and inhibitors.

Statistics

All data are presented as means ± s.e.m. A paired *t*-test was used to determine statistical significance ($P < 0·05$).

Results

Antigen-induced contraction

In the presence of 5 μM indomethacin, tracheal contractions induced by ovalbumin (100 μg mL^{-1}) reached peak responses within 5-8 min of ovalbumin challenge. The percent contraction at peak response was 97 ± 4% and that at 60 min was 63 ± 6% (n = 11). NZ-107 (1 μM) had no significant effect on the ovalbumin response, whereas 3 μM of NZ-107 significantly inhibited the later phase of the response (contraction at 60 min, 32 ± 13%, $P < 0·05$ vs control, Fig. 1a) without affecting the peak response (82 ± 11%, $P > 0·05$ vs control). A high concentration of NZ-107 (10 μM) blocked both peak and prolonged responses. The area under the curve for contraction (AUC) was significantly reduced by 3 and 10 μM of NZ-107 (Table 1). A leukotriene antagonist FPL-55712 (1 μM) significantly reduced the AUC.

In lung parenchymal strips, ovalbumin-induced contractions developed slowly and the pattern of the time-course curve was different from that in the trachea (Fig. 1b). The peak response was 131 ± 8% and the response at 60 min was 121 ± 7%. NZ-107 (10 μM) significantly inhibited both peak and prolonged responses at 60 min (Fig. 1b, Table 1). However, the potency of inhibition was less than that observed in trachea.

The inhibitory effect of NZ-107 on ovalbumin responses was investigated in tracheal strips in the absence of indomethacin. The control response was significantly less than that in the presence of indomethacin (peak response: 72 ± 3%; response at 60 min: 33 ± 6%; $P < 0·05$ vs responses with indomethacin). In this condition, the potency of NZ-107 in inhibiting the ovalbumin response was about equivalent to that in the presence of indomethacin (Table 1). A similar inhibition was also observed with FPL-55712.

We further investigated the effect of NZ-107 on ovalbumin responses in the presence of L-serine-borate (45 mM), an inhibitor of γ-glutamyltranspeptidase (Tate & Meister 1978). The control tracheal response was not changed by L-serine-borate (peak response: 89 ± 5%; response at 60 min: 80 ± 4%), and NZ-107 (1 μM) had no significant effect on the response. However, a high concentration of NZ-107 (10 μM)

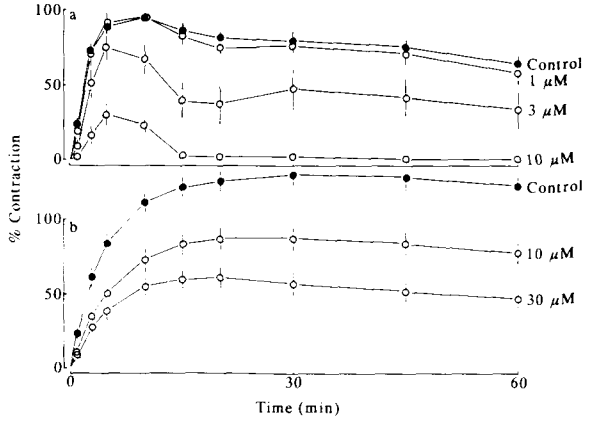

FIG. 1. Effects of NZ-107 on contraction induced by ovalbumin (100 μg mL^{-1}) in guinea-pig (a) trachea and (b) lung parenchyma in the presence of indomethacin (5 μM). Each point represents the mean ± s.e.m. of 6–11 experiments.

Table 1. Effects of NZ-107 and FPL-55712 on airway contraction induced by ovalbumin (100 $\mu g\ mL^{-1}$) or A23187 (1 $\mu g\ mL^{-1}$).

	Compound	Concn (μM)	% Control AUC Trachea Mean ± s.e.m. (n)	% Control AUC Lung Mean ± s.e.m. (n)
Ovalbumin				
Indomethacin (5 μM)	NZ-107	1	95 ± 5 (11)	—
		3	49 ± 13* (6)	—
		10	6 ± 2* (11)	66 ± 4* (6)
		30	—	44 ± 3* (6)
	FPL-55712	1	44 ± 7* (5)	—
		10	20 ± 5* (5)	—
No indomethacin	NZ-107	1	81 ± 24 (4)	—
		10	1 ± 1 (4)	—
	FPL-55712	1	57 ± 12* (5)	—
		10	18 ± 5* (5)	—
Indomethacin (5 μM) + serine-borate (45 mM)	NZ-107	1	105 ± 16 (5)	—
		10	30 ± 4* (5)	—
A23187				
Indomethacin (5 μM)	NZ-107	1	87 ± 6* (6)	—
		10	43 ± 2* (6)	103 ± 10 (6)
		30	—	55 ± 9* (6)

* $P < 0.05$ by paired t-test.

significantly inhibited the ovalbumin response, but the degree of inhibition was less than that in the absence of serine-borate (Table 1).

A23187-induced contraction

In preliminary experiments, A23187 (1 $\mu g\ mL^{-1}$) caused contractions of a magnitude similar to those elicited by ovalbumin in both trachea and lung parenchyma preparations. Since the contractions induced by A23187 developed slowly with no apparent peak response, the effect of NZ-107 on the A23187 response was examined after 60 min and the AUC calculated. In the trachea, NZ-107 (1–10 μM) dose-dependently inhibited A23187 responses (Fig. 2a). The potency of inhibition at 10 μM of NZ-107 was weaker than that observed in response to ovalbumin (Table 1).

In lung parenchymal strips, NZ-107 tended to produce a lesser inhibitory activity compared with that in the trachea

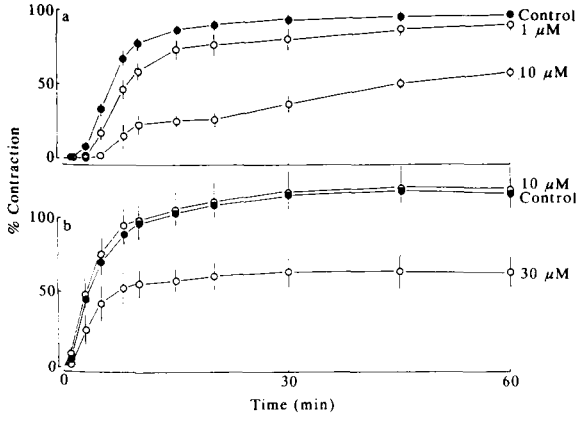

FIG. 2. Effects of NZ-107 on contraction induced by A23187 (1 $\mu g\ mL^{-1}$) in guinea-pig (a) trachea and (b) lung parenchyma in the presence of indomethacin (5 μM). Each point represents the mean ± s.e.m. of 6 experiments.

(Fig. 2b). NZ-107 (10 μM) did not decrease the AUC (103 ± 10% of control) but at 30 μM, NZ-107 inhibited the A23187 response to the same extent as the ovalbumin response (Table 1).

SRS-A release

Ovalbumin (10 $\mu g\ mL^{-1}$) and A23187 (10 $\mu g\ mL^{-1}$) released SRS-A from lung fragment preparations; the amount of released SRS-A was 68 ± 15 ng g^{-1} wet tissue and 67 ± 3 ng g^{-1} wet tissue, respectively. NZ-107 (1–100 μM) caused dose-dependent and significant inhibition of SRS-A release stimulated by ovalbumin (Table 2). In contrast, although NZ-107 10 μM significantly inhibited A23187-induced SRS-A release, no such inhibition occurred with 100 μM (Table 2). A lipoxygenase inhibitor NDGA, significantly inhibited both ovalbumin- and A23187-induced SRS-A release (Table 2).

Histamine release

The amount of histamine (percent of total histamine content) released by ovalbumin (10 $\mu g\ mL^{-1}$) and A23187 (10 $\mu g\ mL^{-1}$) was 6·2 ± 0·8 and 20 ± 1·0%, respectively. NZ-107 (1–100 μM) inhibited ovalbumin-induced histamine release in

Table 2. Effects of NZ-107 and NDGA on SRS-A release induced by ovalbumin (10 $\mu g\ mL^{-1}$) or A23187 (10 $\mu g\ mL^{-1}$).

Compound	Concn (μM)	% Inhibition Ovalbumin Mean ± s.e.m. (n)	% Inhibition A23187 Mean ± s.e.m. (n)
NZ-107	1	19 ± 5* (9)	—
	10	75 ± 5* (9)	22 ± 9* (6)
	100	100* (9)	15 + 9 (6)
NDGA	1	−14 ± 13 (9)	16 ± 7 (4)
	10	45 ± 7* (9)	51 ± 7* (4)
	100	94 ± 2* (9)	61 ± 4* (4)

* $P < 0.05$ by paired t-test.

Table 3. Effects of NZ-107 on histamine release induced by ovalbumin (10 $\mu g\ mL^{-1}$) or A23187 (10 $\mu g\ mL^{-1}$).

		% Inhibition	
NZ-107	Concn (μM)	Ovalbumin Mean ± s.e.m. (n)	A23187 Mean ± s.e.m. (n)
	1	19 ± 13 (5)	12 ± 5 (5)
	10	37 ± 11* (5)	5 ± 5 (5)
	100	72 ± 1* (5)	4 ± 6 (5)

* $P < 0.05$ by paired t-test.

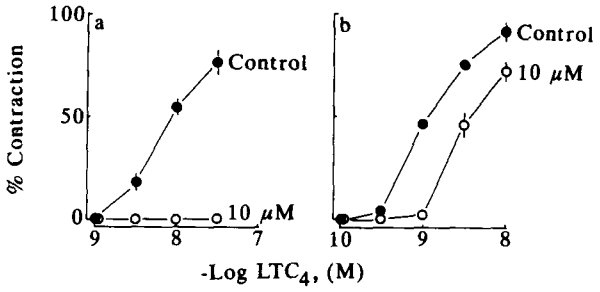

FIG. 3. Effects of NZ-107 (10 μM) on LTC_4-induced contraction in guinea-pig trachea in (a) absence and (b) presence of serine-borate (45 mM). Each point represents the mean ± s.e.m. of 4 experiments.

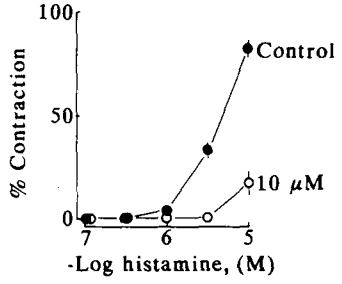

FIG. 4. Effects of NZ-107 (10 μM) on histamine-induced contraction in guinea-pig trachea. Each point represents the mean ± s.e.m. of 4 experiments.

a concentration-dependent manner and significant inhibition was observed at 10 and 100 μM of NZ-107 (Table 3). NZ-107, however, showed no significant inhibition of A23187-induced histamine release (Table 3).

LTC_4- and histamine-induced tracheal contractions

NZ-107 (1 and 10 μM) inhibited LTC_4-induced tracheal contractions; 10 μM of NZ-107 caused complete inhibition (Fig. 3a). In the presence of 45 mM serine-borate, the inhibitory effect of 10 μM of NZ-107 was markedly reduced (Fig. 3b). NZ-107 (10 μM) markedly inhibited histamine-induced contractions (Fig. 4).

Discussion

Antigen-induced contractions of airway tissues isolated from the guinea-pig have been used as a model for evaluating anti-asthma drugs and investigating their mode of action. Such contractions are caused by many bronchoactive substances released from mediator cells (Burka 1986; Chand et al 1986; Jones et al 1988). Adams & Lichtenstein (1979) reported that histamine was involved in the early phase of contraction whereas SRS-A was involved in the prolonged phase of airway contraction stimulated by antigen. We recently reported (Hibi et al 1989) that NZ-107 inhibited antigen-induced SRS-A mediated bronchoconstriction and selectively inhibited LTD_4-induced tracheal contraction in the guinea-pig. In this study, NZ-107 selectively inhibited the prolonged phase of antigen-induced tracheal contraction and SRS-A release. Moreover, a high concentration of NZ-107 potently inhibited histamine-induced contraction and histamine release. The data suggest that NZ-107 is a selective SRS-A agent.

The inhibitory effect of NZ-107 on antigen-induced tracheal contraction was not modulated by indomethacin. Burka (1985) reported that indomethacin enhanced antigen-induced tracheal contraction, inhibiting bronchodilator prostaglandins such as in the PGE series, and this was not a result of conversion of arachidonic acid to lipoxygenase products. However, the inhibitory effect of the leukotriene antagonist FPL-55712 on antigen-induced contraction has been reported to be reduced by treatment with indomethacin (Burka 1986). In the present study, we observed that FPL-55712 did not change inhibitory activity in the absence or presence of indomethacin. The reason for the discrepancy is not clear but may be because we used a high concentration of ovalbumin (100 $\mu g\ mL^{-1}$) for the challenge and the total amount of mediators and their involvement in contraction was therefore different from those in Burka's study.

NZ-107 inhibited antigen-induced contraction even in the presence of serine-borate. We have observed that the effect of FPL-55712 on the antigen-induced response was greatly reduced in the presence of serine-borate (Yamamoto et al 1991). NZ-107 moderately inhibited the contraction induced by synthetic LTC_4 in the presence of serine-borate (Fig. 3b). Therefore, it is suggested that NZ-107 also inhibits the response produced by endogenously-released LTC_4. The contribution of LTC_4 to the asthmatic response in man is not clear. However, if the conversion of LTC_4 to LTD_4 is insufficient, the resulting accumulation of LTC_4 may be implicated in the pathogenesis of asthma and NZ-107 could be a beneficial anti-asthmatic drug.

NZ-107 preferentially inhibited the tracheal contraction rather than that of the lung parenchymal strip stimulated by both antigen and A23187. This result is consistent with that seen with the LTD_4 antagonist FPL-55712 (Burka 1986). The reason for the weak inhibitory effect of NZ-107 and FPL-55712 in lung parenchyma is not clear but may be the result of contraction of many small pulmonary vessels in lung tissue. It is conceivable that NZ-107 and FPL-55712 failed to inhibit LTD_4-induced contraction of pulmonary vessels. The contractile effect of leukotrienes on the pulmonary artery has been reported by Berkowitz et al (1984).

NZ-107 is a more potent inhibitor of SRS-A release than of histamine release stimulated by antigen in lung fragment preparations. In contrast, NZ-107 did not inhibit A23187-induced SRS-A release nor histamine release. The reason for the inability of NZ-107 to inhibit A23187-induced SRS-A (or histamine) release is not clear but may be related to the fact that antigen- and A23187-induced SRS-A release occurs at different sites by different mechanisms in guinea-pig lung

tissue. Antigen-induced SRS-A release is usually dependent upon mast cell activation (Krell & Kusner 1984), whereas that of A23187 occurs not only in broncho-alveolar macrophages (Sirois 1980) but also in the pulmonary arteries (Fleisch & Haisch 1982). However, a lipoxygenase inhibitor, NDGA, inhibited both antigen- and A23187-induced SRS-A release. From these results, it is possible that NZ-107 blocked the release of arachidonic acid after antigen-antibody binding or that NZ-107 selectively inhibited the lipoxygenase pathway activated by antigen.

NZ-107 inhibited A23187-induced contraction in trachea and lung parenchyma, but the inhibitory potency tended to be less than that against antigen-induced contraction. Since NZ-107 failed to inhibit A23187-induced SRS-A or histamine release, it is suggested that inhibition of A23187-induced contraction by NZ-107 is the result of direct inhibition of mediator-induced contractions. It is not clear what kind of mediator is involved in A23187-induced contractile response. Burka (1986) suggested that mediators other than peptide-leukotrienes are responsible for A23187-induced contraction, because FPL-55712 had no effect on contractile response. It is important to clarify which mediators are responsible for the contraction stimulated by A23187.

In conclusion it is suggested that low concentrations of NZ-107 ($\leqq 3$ μM) inhibit antigen-induced tracheal contraction by inhibiting SRS-A release and its response, whereas at higher concentrations NZ-107 inhibits histamine release and its response. This inhibitory profile of NZ-107 suggests that it may have beneficial therapeutic effects for allergic bronchial asthma. Although NZ-107 failed to inhibit A23187-induced SRS-A and histamine release, the ability of NZ-107 to inhibit A23187-induced contractions suggests that NZ-107 may also be beneficial in the treatment of bronchial asthma induced by non-allergic stimuli.

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