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Anti-allergic Effect of *N*-Acetylneuraminic Acid in Guinea-pigs

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Abstract—The in-vivo anti-allergic effect of *N*-acetylneuraminic acid (NANA) in guinea-pigs passively sensitized with anti-ovalbumin rabbit serum has been studied. NANA (20 mg kg^{-1} i.v.) inhibited bronchial anaphylaxis and the release of histamine into bronchoalveolar lavage fluid. NANA dose-dependently inhibited heterologous passive cutaneous anaphylaxis and haemorrhaging in the passive Arthus reaction. However, it did not inhibit the release of histamine from sensitized minced lung tissue.

Sialic acids, *N*- and *O*-acyl derivatives of neuraminic acid, constitute a group of sugars that occur mainly as the terminal components of glycoconjugates, and are thought to be involved in cell recognition, receptor functions and immunological reactions (Schauer 1982).

Gorog & Kovacs (1978) reported that an intravenously administered sialic acid exhibited an anti-inflammatory property as determined in carrageenan-induced rat paw oedema and pleurisy tests. We have shown that *N*-acetylneuraminic acid (NANA) exhibited an anti-inflammatory effect in the respiratory tract (Miyata et al 1988, 1990). In an in-vitro study, an anti-allergic effect of a sialic acid was demonstrated in the human basophil (Jensen et al 1986); the present study was carried out to examine the anti-allergic action of NANA in-vivo in guinea-pigs.

Materials and Methods

Chemicals

Sodium *N*-acetylneuraminate was a gift from Mect Co. Ltd, Tokyo (Japan). L-Ascorbic acid sodium salt, histamine dihydrochloride, (±)-isoprenaline hydrochloride and (±)-propranolol hydrochloride were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Atropine sulphate (monohydrate) was obtained from Wako Pure Chemical Ind., Ltd (Osaka, Japan). Ovalbumin (grade VI) and pyrilamine maleate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Evans Blue (Merck, Darmstadt) and Freunds' complete adjuvant (Difco Lab., Detroit, MI, USA) were also used.

Animals

Male Hartley guinea-pigs, 300–400 g, were used.

Antiserum

Male New Zealand White rabbits, 2·0–2·2 kg, were immunized by subcutaneous injection with 1 mL of an emulsion of 20 mg of ovalbumin and Freund's complete adjuvant, five times, at intervals of one week, and bled one week after the last antigenic stimulation. Anti-ovalbumin serum was separated and stored at −20°C.

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Effect on bronchial anaphylaxis

Guinea-pigs were passively sensitized by intravenous injection of the antiserum (5 mL kg^{-1}) diluted 1 in 10 with 0·9% NaCl (saline). After 18 h, a conscious guinea-pig was placed in a body plethysmograph with head and body compartments, which comprised two cylindrical boxes, separated by a neck restrainer. Bronchial anaphylaxis was provoked by inhalation of an aerosol of an ovalbumin solution (2·2 mL solution min^{-1}). The aerosol was generated with an ultrasonic nebulizer (TUR-3200; Nihon Kohden) connected to the body box and change of the tidal volume was recorded with a pen recorder (PMP-3004; Nihon Kohden). To evaluate the degree of bronchial anaphylaxis, the time of onset of dyspnoea was determined from the respiration pattern (Fig. 1) and mortality. If dyspnoea did not occur, the time of the onset was taken as the time of termination of the observation. Each experiment was performed as follows: NANA was administered at 30 min before the antigen challenge (1 mg mL^{-1} for 10 min or 5 mg mL^{-1} for 3 min). Ovalbumin inhalation (5 mg mL^{-1} for 3 min) led to dyspnoea but not to death. Arterial blood was obtained from the abdominal aorta with a heparinized syringe and then bronchoalveolar lavage was carried out immediately after the ovalbumin inhalation. The partial pressures of oxygen (Po_2) and carbon dioxide (Pco_2), and pH were determined using an Auto Blood Gas Analyzer System at 37°C (IL1304; Instrumentation Laboratory, Milano, Italy). The lavage was performed with 7 mL of saline. Firstly, 3 mL of lavage fluid was recovered and then re-instilled into the lungs. Secondly, the same volume of lavage fluid was recovered. The bronchoalveolar lavage fluid (BALF) was centrifuged at 1400 *g* for 10 min. The histamine content of the supernatant was determined by the method of Pruzansky & Patterson (1966).

To examine the effect of NANA on bronchial anaphylaxis mainly provoked by slow reacting substances of anaphylaxis (SRS-A), three antagonists (pyrilamine 2·5, propranolol 1 and atropine 1 mg kg^{-1}) were injected intravenously 5 min before the ovalbumin inhalation (10 mg mL^{-1} for 1·5 min)

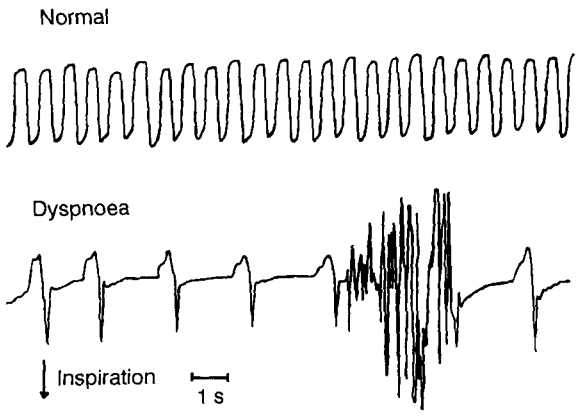

FIG. 1. The pattern of respiration in dyspnoea induced by inhalation of antigen in a passively sensitized guinea-pig. The tidal volume was measured by body plethysmography. The time of onset of dyspnoea was determined from the tidal volume.

(Terasawa et al 1983). Observation was continued for 20 min after the end of the challenge.

In another schedule, NANA was administered with the antiserum simultaneously. Eighteen hours later, the ovalbumin challenge (1 or 10 mg mL^{-1} for 5 min) was made. In all these experiments, NANA (20 mg kg^{-1}) or saline (1 mL kg^{-1}), as control, was injected intravenously according to the schedule described above.

Effect of heterologous passive cutaneous anaphylaxis (PCA)
Guinea-pigs received intradermal injections of 0·1 mL of the antiserum, 1 in 16000 dilution, and saline, as a blank, at two points on the shaved back. After 4 h, PCA was provoked by intravenous injection with 10 mg kg^{-1} of ovalbumin and 20 mg kg^{-1} of Evans Blue. The animals were killed 30 min later and skin was removed for determination of extravasated dye at each reaction site. NANA (1, 5, 20 or 100 mg kg^{-1}) or saline (1 mL kg^{-1}) was intravenously administered simultaneously with or 30 min before the ovalbumin challenge. For determination of the reaction strength, dye extraction and measurement by spectrometry were according to Katayama et al (1978). The results were corrected for the blank and expressed as percentages of the saline control.

Effect on the passive Arthus reaction
The passive Arthus reaction was essentially as described by Katayama et al (1981). Briefly, guinea-pigs were injected intravenously with 1 mL of the antiserum. Thirty minutes after sensitization each animal was injected intradermally at two points on the abdomen with a 0·05 mL solution containing 0·025 mg and 0·05 mg of ovalbumin. The diameter of the Arthus reaction site was macroscopically determined as the diameter of the haemoerrhaging at 2 h and 24 h after the antigenic challenge. NANA (1, 5 or 20 mg kg^{-1}) or saline (1 mL kg^{-1}) was administered i.v. 30 min before the antigenic challenge.

Effect on histamine release from minced lung tissue
Guinea-pigs were passively sensitized as for bronchial anaphylaxis. Eighteen hours after the antiserum injection, the animals were exsanguinated via the abdominal aorta under ether anaesthesia and then the lungs were perfused with ice-cold Tyrode solution adjusted to pH 7·4 before use. The lungs were chopped into small fragments of ca 1 mm^3 and then were washed with approximately 200 mL of Tyrode. A 1 g portion of the fragments was suspended in 1·5 mL of Tyrode and then mixed with 50 μL of a test compound solution. The mixture was preincubated at 37°C for 30 min in a plastic test tube. To induce histamine release, 50 μL of an ovalbumin solution was added to the mixture, to give a final concentration of 1 μg mL^{-1} and the whole mixture was further incubated at 37°C for 30 min. After exposure to ovalbumin, the reaction was terminated by chilling the tube in an ice-water bath and by adding 0·5 mL of ice-cold Tyrode to the mixture. The lung fragments were then separated by centrifugation (1400 *g* for 10 min). The fragments were boiled in 0·4 M perchloric acid for 10 min to release residual histamine. Individual supernatants were assayed for histamine according to the method described above. Control samples, i.e. containing no test compound, were prepared in the same manner. Histamine release was expressed as a percentage of the total histamine content. All values were corrected for the spontaneous secretion occurring in the absence of ovalbumin. The results were expressed as percentage of the control release.

Statistical analysis
The results are presented as means ± s.e. for at least 6 experiments. For statistical analysis of data, the Mann Whitney U-test for onset time of dyspnoea and heterologous passive cutaneous anaphylaxis and Student's *t*-test for other comparisons were used, $P < 0.05$ was considered significant.

Results

Effect on bronchial anaphylaxis
The results of NANA administration at 30 min before antigen exposure (1 mg mL^{-1} for 10 min or 5 mg mL^{-1} for 3 min) are shown in Fig. 2. At neither antigen concentration did death occur and NANA (20 mg kg^{-1} i.v.) delayed the time of onset of dyspnoea. Furthermore, NANA inhibited the increase in the histamine content of BALF (control: 1·1 ± 0·2; NANA: 0·6 ± 0·1 μg mL^{-1}, $P < 0.05$) and the decrease in Po_2 (control: 48 ± 9; NANA: 77 ± 9, $P < 0.05$) occurring in bronchial anaphylaxis provoked by antigen inhalation (5 mg mL^{-1} 3 min). The base levels without antigen inhalation were: Po_2, 83 ± 5; Pco_2, 38 ± 3 mm Hg.

Administration of NANA simultaneously with intravenous antiserum injection before the antigen challenge had inhibitory effects as judged by onset time and death rate (Fig. 3). On treatment with three antagonists, NANA administration delayed the time of onset of dyspnoea and reduced the death rate (Fig. 2).

Effect on heterologous PCA
Table 1 shows that administration of NANA (1 or 20 mg kg^{-1}) simultaneously with antigenic challenge had an inhibitory effect. Furthermore, the administration of NANA 30 min before the challenge had an inhibitory effect at doses of 5 and 20 mg kg^{-1}.

Effect on the passive Arthus reaction
Fig. 4 shows that the administration of NANA dose-

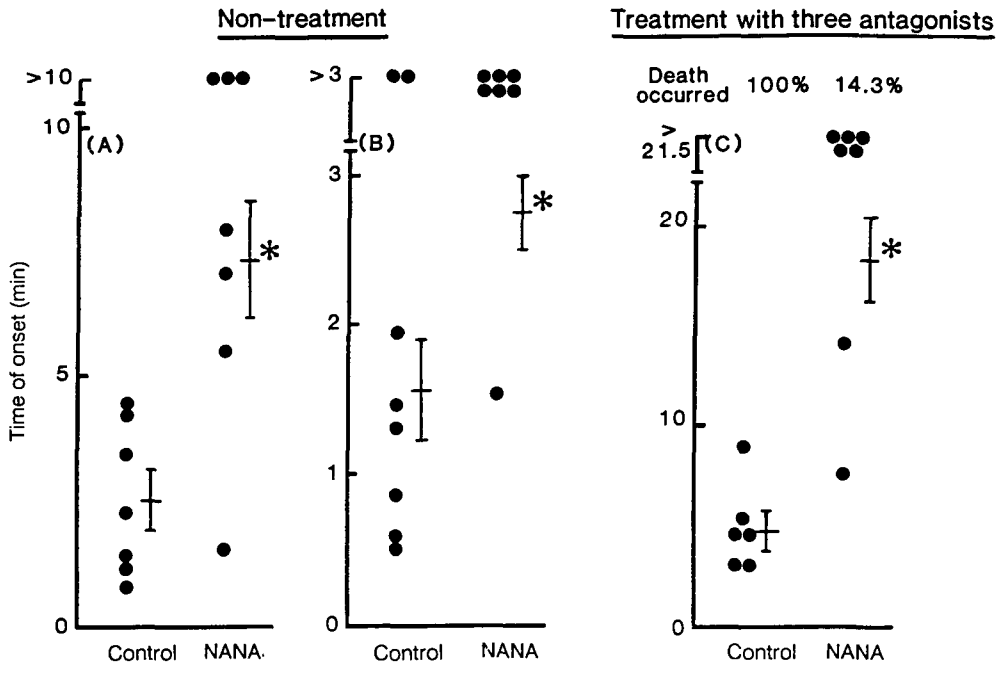

FIG. 2. Effect of NANA on bronchial anaphylaxis provoked by inhalation of ovalbumin: 1 mg mL^{-1} for 10 min (A) or 5 mg mL^{-1} for 3 min (B) or 10 mg mL^{-1} for 1·5 min and treatment with three antagonists (C) in passively sensitized guinea-pigs. The three antagonists were: 2·5 mg kg^{-1} pyrilamine, 1 mg kg^{-1} propranolol and 1 mg kg^{-1} atropine, i.v. NANA (20 mg kg^{-1}) or saline (1 mL kg^{-1}) was administered i.v. 30 min before antigen exposure. The time of onset of dyspnoea and mortality were determined with the tidal volume measured by body plethysmography. If a guinea-pig did not die within the observation period (10 or 3 min without treatment or 21·5 min with treatment), the onset time was taken as the time of termination of the observation. * $P < 0.05$ compared with control.

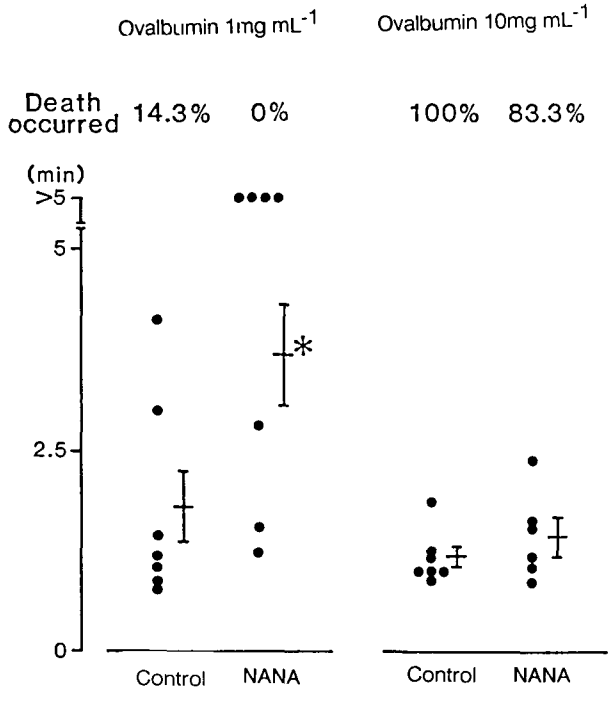

FIG. 3. Effect of NANA on bronchial anaphylaxis provoked by inhalation of ovalbumin (1 or 10 mg mL^{-1} for 5 min). NANA (20 mg kg^{-1}) or saline (1 mL kg^{-1}) was simultaneously administered i.v. with i.v. injection of antiserum. The time of onset of dyspnoea and mortality were determined. The results are expressed as in Fig. 2. * $P < 0.05$ compared with control.

Table 1. Effect of NANA on heterologous PCA.

		NANA (mg kg^{-1} i.v.)		
	Control	1	5	20
Simultaneous administration	100	27 ± 15*	—	36 ± 10*
30 min before	100	128 ± 35	52 ± 13*	41 ± 5*

The results are expressed as percentage of the control, after subtracting dye extravasation induced by the solvent (blank). Data are means ± s.e. for six to eight experiments. * $P < 0.05$ compared with control.

dependently inhibited the haemorrhaging in the skin. This inhibitory effect continued for 24 h after the antigenic challenge.

Effect on histamine release from minced lung tissue

Pretreatment with NANA (10^{-4} to 10^{-2} M) did not lead to significant inhibition of the anaphylactic histamine release from minced lung tissue (Table 2). Moreover, the histamine release was markedly inhibited by pretreatment with (±)-isoprenaline (at a final concentration of 10^{-6} M), used as a positive control (Assem & Schild 1971).

Discussion

In the present study, NANA was found to inhibit anaphylactic bronchoconstriction as well as heterologous PCA.

This inhibitory effect on bronchial anaphylaxis lasted for

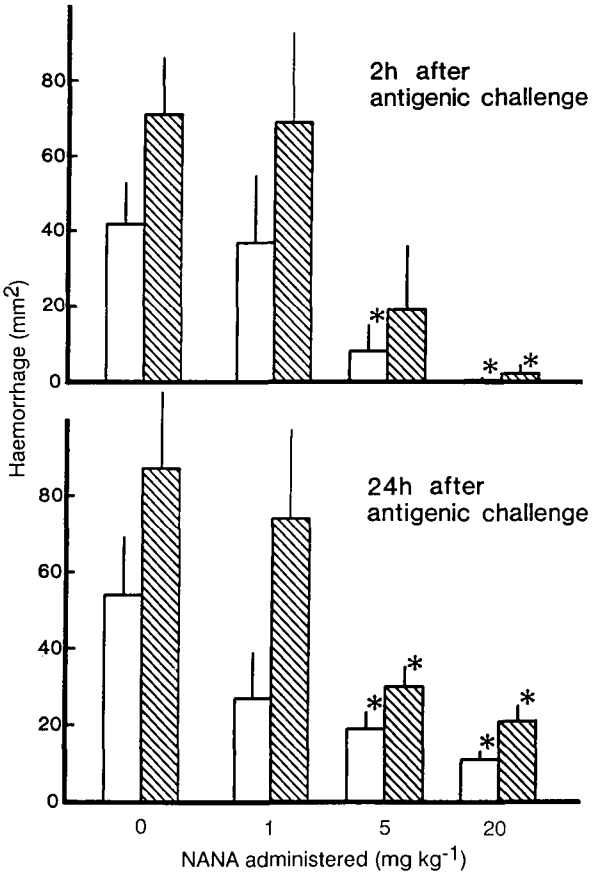

FIG. 4. Effect of NANA on the passive Arthus reaction. NANA or saline was administered i.v. 30 min before the antigenic challenge. The doses of the antigen were 0·025 (open columns) and 0·05 mg/site (hatched columns). Data are means ± s.e. for six experiments. * $P < 0.05$.

Table 2. Effect of NANA on histamine release from minced lung tissue.

M	% of control
Control	100
NANA	
10^{-4}	89·6 ± 4·1
10^{-3}	93·0 ± 8·1
10^{-2}	95·9 ± 15·2
(±)-Isoprenaline	
10^{-6}	45·5 ± 7·5*

Data are means ± s.e. for six experiments.
* $P < 0.01$ compared with control.

18 h. Furthermore, the inhibitory effect of NANA on heterologous PCA was observed with a lower dose on simultaneous administration with the antigenic challenge than that at 30 min before the antigenic challenge. This suggests that NANA immediately inhibits the anaphylactic reaction and that its action is long-lasting.

NANA inhibited the increase in the histamine content of BALF occurring on bronchial anaphylaxis. This suggests that NANA inhibits the release of chemical mediators from mast cells.

NANA also inhibited the bronchoconstriction provoked by anaphylaxis on treatment with three antagonists (pyrilamine, propranolol and atropine, i.v.). This suggests that NANA can inhibit the anaphylactic response to not only histamine but also to SRS-A.

Although homologous PCA is thought to be simply due to mediator release from mast cells following the antigen-antibody reaction, heterologous PCA might partially be mediated by neutrophil lysosomes, i.e. caused by Arthus-like mechanisms (Taichman & Movat 1968; Taichman et al 1971). NANA dose-dependently inhibited the passive Arthus reaction, which is an allergic inflammatory reaction induced by immune complex deposition in and around the vessel walls. According to Gorog & Kovacs (1978) and Atherton & Born (1973), sialic acids in-vivo decreased the inflammatory response to carrageenan in rats, and this inhibitory effect was based on the suppression of neutrophil accumulation and adherence. Thus the inhibitory effect on the Arthus reaction and the partial effect on PCA of NANA, as shown here, may be due, at least partly, to the suppression of neutrophils in-vivo, as neutrophils play roles in the Arthus reaction (Mielens et al 1984).

In our in-vitro study, NANA was found not to affect anaphylactic histamine release from lung tissue. This result was inconsistent with those of Jensen et al (1986), possibly due to different experimental conditions. Failure of NANA to inhibit mediator release from rat mast cells induced by non-immunological agents was reported by Coleman (1982). Consequently, our findings suggest that the in-vivo anti-allergic action of NANA is indirectly rather than directly affecting mast cells.

Two mechanisms may be proposed for these actions of NANA. Firstly, the actions may be due to the anti-recognition (Goldstein & Chapman 1981; Rinsum et al 1986) or calcium-binding activity (Jaques et al 1977) of NANA itself; NANA may thus block enzymes involved in the allergic reactions. Secondly, the actions may be due to a modification of the glycoconjugate structure or function caused by non-enzymatic sialylation (McKinney et al 1982). Such modification may lead to masking of the recognition sites on the cell surface. It has been reported that the level of tracheal gangliosides, namely sialosphingolipids, especially NANA, is associated with bronchial hyperreactivity in guinea-pigs (Banerjee 1982), and activation of an alternative pathway is inhibited on insertion of NANA, as a ganglioside component, into an artificial membrane (Michalek et al 1988).

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