ABSTRACT The bronchodilator and cardiovascular effects of NKH477 (6-(3-dimethylaminopropionyl)forskolin hydrochloride) were evaluated. In anesthetized guinea pigs, i.v. bolus injections of NKH477 (1 - 100 μg/kg) inhibited the bronchoconstriction induced by inhaled leukotriene D4, increased the heart rate (HR) and decreased the diastolic arterial blood pressure (DBP) in a dose-dependent manner. The bronchodilator effect of NKH477 was 1500 times more potent than that of aminophylline and 17 times less potent than that of isoproterenol. The selectivity of NKH477 for bronchodilation vs an increase in HR was 15 times higher than that of isoproterenol and similar to that of aminophylline; and vs a decrease in DBP, the selectivity was 4 times higher than that of aminophylline and similar to that of isoproterenol. I.v. infusion of NKH477 (0.1 - 3 μg/kg/min) for 2 hr dose-dependently inhibited the bronchoconstriction induced by i.v. histamine. Isoproterenol (0.1 μg/kg/min, i.v.) enhanced the bronchoconstriction after termination of the infusion, whereas NKH477 did not. In conscious guinea pigs, inhalation of NKH477 (0.1 - 5 mg/ml) concentration-dependently inhibited the bronchoconstriction induced by inhaled histamine, and a high concentration of NKH477 (35.4 mg/ml) increased the HR. The bronchodilator effect of inhaled NKH477 was 15 times less potent than that of isoproterenol. The selectivity of inhaled NKH477 was similar to that of isoproterenol. These results indicate that NKH477 may be useful as a bronchodilator.

Keywords: NKH477, Bronchodilator, Heart rate, Blood pressure

β-Adrenergic-receptor agonists and methylxanthines, which can increase the intracellular levels of cyclic AMP, have found utility as bronchodilators in the treatment of bronchial asthma. Forskolin (Fig. 1), a diterpene isolated from the roots of Coleus forskohlii, can directly activate adenylate cyclase and raise cyclic AMP levels in a variety of tissues (1, 2). Therefore, forskolin would be expected to have potential as a bronchodilator. Indeed, the bronchodilator effects of forskolin have been demonstrated in guinea pigs (3, 4) and humans (5 - 7), although the poor water solubility of forskolin has limited its clinical use. NKH477, 6-(3-dimethylaminopropionyl)forskolin hydrochloride (Fig. 1), is a novel, water-soluble forskolin derivative (8). The cardiovascular profile of NKH477 in
anesthetized and conscious dogs was reported previously (9). NKH477, like forskolin, stimulated adenylate cyclase directly and did not inhibit phosphodiesterase activity in preparations from guinea pig hearts (9). In the present study, the bronchodilator and cardiovascular effects of NKH477 in anesthetized and conscious guinea pigs were investigated in comparison with those of other conventional bronchodilators. A preliminary report of these results has been presented (10).

MATERIALS AND METHODS

Anesthetized guinea pigs
Male Hartley guinea pigs weighing 390–770 g (Japan SLC, Inc., Hamamatsu) were anesthetized with pentobarbital (40 mg/kg, i.p.) and placed in a dorsal recumbent position on a small animal operating table. The trachea was cannulated, and the lungs were ventilated mechanically by a respirator (SN-480-7; Shinano Manufacturing Co., Ltd., Tokyo) at a rate of 60 breaths/min with an air volume of 10 ml/kg. Succinylcholine (5 mg/kg, s.c.) was given to the animals to prevent interference from spontaneous respiration. Pulmonary inflation pressure (PIP) was measured from a lateral port in the afferent limb of the ventilator circuit, with a pressure transducer (TP-200T; Nihon Kohden Co., Tokyo). A catheter connected to a pressure transducer (CP-01, Century Technology Co., Inglewood, CA, USA; or TP-602T, Nihon Kohden) was inserted into the left carotid artery to measure diastolic arterial blood pressure (DBP). Heart rate (HR) was counted with a cardiotachometer (AT-601G, Nihon Kohden) triggered by blood pressure pulses. The manus and pes veins were cannulated for i.v. administration of drugs. Rectal temperature was monitored and maintained at 37°C with a heat lamp.

Leukotriene D₄ challenge was achieved by exposure for 30 sec to an aerosol of a leukotriene D₄ solution (300 ng/ml). The aerosol was generated by an ultrasonic

![Fig. 2.](image-url)
nebulizer (TUR-3000; Nihon Kohden, modified for small animals) permanently connected in series with the afferent limb of the ventilator circuit. To prevent interference from indirect bronchoconstriction mediated by the release of thromboxanes, indomethacin (10 mg/kg, i.v.) was given to the animals 10 min before the leukotriene D₄ challenge. Test drugs were administered i.v. 1 min before the challenge. Doses that produced a 50% inhibition of the increase in PIP induced by leukotriene D₄ (ED₅₀) at 1.5 min after the start of the inhalation were determined from the dose-response curves fitted by non-linear regressions with a personal computer. Doses that produced a 15% increase in HR (ED₁₅) and a 20% decrease in DBP (ED₂₀) were determined from the individual values by linear regressions.

Histamine challenge (10 μg/kg, i.v.) was repeated 20 times at 15-min intervals. To keep a constant anesthesia, animals were supplemented with infusions of pentobarbital (8 mg/kg/hr, i.p.) and succinylcholine (1 mg/kg/hr, s.c.) during the experiments. I.v. infusions of test drugs were continued for 2 hr from 5 min after the third histamine challenge.

**Conscious guinea pigs**

Male Hartley guinea pigs weighing 420–690 g (Japan SLC) were used in a constant-temperature room (25°C).

Each animal was exposed two times to aerosols of 0.9% saline for 30 sec in 15-min intervals. Specific airway conductance (SGaw) was measured 2 min after the start of the inhalation, and the mean value of two measurements was defined as the baseline SGaw value. Fifteen minutes later, each animal was exposed for 30 sec to aerosols of histamine solutions (0.05, 0.071, 0.1, 0.14 and 0.2%) at 15-min intervals. The concentration that produced a 40% decrease in the SGaw was determined by linear interpolation. After 90 min, each animal was exposed five times to aerosols of that concentration of histamine solution for 30 sec at 90-min intervals, and the SGaw was measured 2 min after the start of the inhalation. Animals were divided into three groups, to which NKH477, isoproterenol, or 0.9% saline was administered. In the first (initial) and fifth histamine challenges, all animals were pre-exposed to aerosols of 0.9% saline for 2 min from 3 min before the challenge. In the second, third and fourth histamine challenges, each animal of the groups was pre-exposed to aerosols of NKH477 solutions (0.1, 0.71 and 5 mg/ml), isoproterenol solutions (0.002, 0.014 and 0.1 mg/ml) or 0.9% saline for 2 min from 3 min before histamine inhalation.

SGaw were measured in intact, conscious, spontaneously breathing guinea pigs placed in a constant-volume body plethysmograph. The technique used was similar to that described previously (11). Briefly, each animal was positioned in a two-compartment chamber designed to keep the animal’s head fixed and isolated from its body and plethysmograph. Flow at the snout was measured using a pneumotachograph (TV-142T, Nihon Kohden) connected to a differential pressure transducer (TP-602T, Nihon Kohden). To prevent rebreathing of carbon dioxide from the dead space around the nares and within the

![Fig. 2. Time course of effects of i.v. bolus injections of NKH477 (a), aminophylline (b) and isoproterenol (c) on the increase in pulmonary inflation pressure (PIP) induced by inhaled leukotriene D₄ (LTD₄), heart rate (HR) and diastolic blood pressure (DBP) in anesthetized guinea pigs. Guinea pigs were pretreated with succinylcholine and indomethacin. Each animal was exposed to an aerosol of a LTD₄ solution (300 μg/ml) for 30 sec (time 0 to 0.5 min). Test drugs were administered i.v. (time −1 to −0.67 min). Values are expressed as changes from the values just before the drug injections. Basal values (mean ±S.D., n=82): PIP, 15.3±2.7 cmH₂O; HR, 235±23 beats/min; and DBP, 100±18 mmHg. Each point and vertical bar represent a mean value and S.E. *P<0.05: Significantly different from the control (Bonferroni method).](image-url)
pneumotachograph, a constant flow of air (1 l/min) was provided at the nares except during the time the SGaw was measured. The animal in the chamber was placed in the plethysmograph, which was equipped with another transducer (MP45; Validyne Engineering Co., Norridge, CA, USA) for measuring changes in box pressure. Air flow and box pressure signals were displayed simultaneously on an X-Y oscilloscope (DS-6411; Ishatsu Electric Co., Ltd., Tokyo). The angle described during the rapid inspiratory phase of the animal's breathing was measured, and the SGaw was calculated from it (12).

Aerosols were generated by ultrasonic nebulizers (NE-U11B; Omron Co., Ltd., Kyoto) containing 0.9% saline or solutions of various concentrations of histamine, NKH477 or isoproterenol. The nebulizers were attached to a large-bore, 8-way stopcock, which was connected to the aerosol inlet of the anterior chamber of the restrainer by tubing. To deliver aerosol, 1 l/ml of air was passed through the nebulizer. The concentrations that produced a 50% inhibition of the decrease in the SGaw induced by histamine (EC50) were determined from the concentration-response curves fitted by non-linear regressions with a personal computer.

For HR measurement, other guinea pigs were used. Under ether anesthesia, bipolar needle electrodes connected to a telemeter for transmission (ZB-141G, Nihon Kohden) were inserted in the skin for reading the electrocardiogram. After waking from anesthesia, each animal was placed in a constant-volume body plethysmograph, and HR was counted with a cardiotachometer (AT-601G, Nihon Kohden) triggered by R waves of the electrocardiogram from a telemeter for reception (ZR-601G, Nihon Kohden) were inserted in the skin for reading the electrocardiogram. After waking from anesthesia, each animal was placed in a constant-volume body plethysmograph, and HR was counted with a cardiotachometer (AT-601G, Nihon Kohden) triggered by R waves of the electrocardiogram from a telemeter for reception (ZR-601G, Nihon Kohden). Animals were treated with aerosols of NKH477 solutions (0, 0.1, 0.71, 5 and 35.4 mg/ml) or isoproterenol solutions (0, 0.002, 0.014, 0.1, 0.71 and 5 mg/ml) in the manner described above for 2 min at 45-min intervals. The concentrations that produced a 15% increase in HR (EC15) were determined from the individual values by linear regressions.

**Drugs**

NKH477 was prepared by Nippon Kayaku Co., Ltd., Tokyo. Other drugs and chemicals used were obtained from the following sources: aminophylline, L-ascorbic acid sodium, histamine dihydrochloride, indomethacin, (−)-isoproterenol hydrochloride and procaterol hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); pentobarbital sodium and succinylcholine chloride (Tokyo Kasei Kogyo Co., Ltd., Tokyo); leukotriene D4 (Wako Pure Chemical Co., Ltd., Osaka).

NKH477 was dissolved in 0.9% saline. Indomethacin was dissolved in 1 M Tris-HCl buffer (pH 8.5). Isoproterenol was dissolved in 0.9% saline for i.v. injections and inhalations or 0.9% saline including a molar equivalent of ascorbic acid for i.v. infusions. Leukotriene D4 methanol solution was diluted with 0.9% saline. Other drugs and chemicals were dissolved in 0.9% saline.

**Statistical analyses**

The results are expressed as the mean±S.E., unless otherwise noted. Significant differences (P<0.05) between groups were determined with one-way analysis of variance followed by the Bonferroni or Dunnett method.

**RESULTS**

**Effects of i.v. bolus injections of NKH477, aminophylline and isoproterenol on the bronchoconstriction induced by inhaled leukotriene D4 in anesthetized guinea pigs**

As shown in Fig. 2a, administration of NKH477 (1−100 μg/kg) 1 min before leukotriene D4 inhalation produced a dose-dependent inhibition of bronchoconstriction. At 10 μg/kg of NKH477, the inhibition was 76, 73, 68 and 66% at 1.5, 2, 3 and 4 min after the inhalation, respectively. NKH477 also increased HR and decreased DBP dose-dependently, and it produced peak effects within 8 and 2 min after administration, respectively. Aminophylline (1000−100000 μg/kg), like NKH477, caused an inhibition of bronchoconstriction, an increase in HR and a decrease in DBP (Fig. 2b). Isoproterenol (0.01−1 μg/kg) inhibited bronchoconstriction and increased HR dose-dependently, but exerted biphasic effects on DBP (Fig. 2c). At 0.3 μg/kg of isoproterenol, the inhibition of bronchoconstriction was 81, 68, 40 and 18% at 1.5, 2, 3 and 4 min, respectively, after leukotriene D4 inhalation. Thus, the duration of bronchodilator effects of NKH477 was longer than that of isoproterenol. The dose-response curves to NKH477, aminophylline and isoproterenol for inhibition of bronchoconstriction at 1.5 min after leukotriene D4 inhalation were 12 nmol/kg (6.5 μg/kg), 18000 nmol/kg (8100 μg/kg) and 0.70 nmol/kg (0.17 μg/kg), respectively. Thus, the bronchodilator effect of NKH477 was 1500 times more potent than that of aminophylline and 17 times less potent than that of isoproterenol. The selectivity of NKH477 for bronchodilation vs an increase in HR (ED15/ED50=3.3) was 15 times higher than that of isoproterenol (ED15/ED50=0.22) and similar to that of aminophylline (ED15/ED50=4.3). The selectivity of NKH477 vs a decrease in DBP (ED20/ED50=0.60) was 4.0 times higher than that of aminophylline (ED20/ED50=0.15) and similar to that of isoproterenol (ED20/ED50=0.95).
Effects of i.v. infusion of NKH477, aminophylline, isoproterenol and procaterol on the bronchoconstriction induced by i.v. histamine in anesthetized guinea pigs

NKH477 (0.1–3 μg/kg/min, Fig. 4a) and aminophylline (300 and 1000 μg/kg/min, Fig. 4b) infused for 2 hr inhibited the bronchoconstriction induced by histamine and increased HR in a dose-dependent manner. The DBP was reduced at 3 μg/kg/min of NKH477 and 1000 μg/kg/min of aminophylline. The onset of the effects of NKH477 was more immediate than that of aminophylline. The effects of NKH477 recovered gradually after termination of the infusion, but aminophylline-induced hypotension did not recover within 2 hr after termination of the infusion. Isoproterenol (0.01–0.1 μg/kg/min, Fig. 4c) and procaterol (0.01 and 0.03 μg/kg/min, Fig. 4d) inhibited the bronchoconstriction and increased HR dose-dependently, but had no effects on DBP. Isoproterenol at a high dose (0.1 μg/kg/min) enhanced the histamine-induced bronchoconstriction 40 min after termination of the infusion, whereas NKH477, aminophylline and procaterol at any doses examined did not. The selectivity of NKH477 for bronchodilation vs an increase in HR was about 4 times higher than that of isoproterenol, about 2 times higher than that of aminophylline and about 2 times lower than that of procaterol. The selectivity of NKH477 for bronchodilation vs a decrease in DBP was about 2 times higher than that of aminophylline.

Effects of inhalation of NKH477 and isoproterenol on the bronchoconstriction induced by inhaled histamine in conscious guinea pigs

As shown in Table 1, inhalation of NKH477 (0.1–5 mg/ml solutions) and isoproterenol (0.002–0.1 mg/ml solutions) for 2 min from 3 min before histamine inhalation caused a concentration-dependent inhibition of bronchoconstriction. Inhalation of 0.9% saline had no influence on the histamine-induced bronchoconstriction. The time courses of the inhalation effects of NKH477 (0.1–35.4 mg/ml solutions) and isoproterenol (0.002–5 mg/ml solutions) on heart rate are shown in Table 2. Inhalation of NKH477 at a high concentration (35.4 mg/ml) and isoproterenol at high concentrations (0.71 and 5 mg/ml) increased the HR, and peak effects occurred within 20 min. The dose-response curves to NKH477 and isoproterenol for inhibition of the bronchoconstriction and maximal increase in HR are shown in Fig. 5. The
Fig. 4. Effects of i.v. infusion of NKH477 (a), aminophylline (b), isoproterenol (c) and procaterol (d) on the increase in pulmonary inflation pressure (PIP) induced by i.v. histamine, heart rate (HR) and diastolic blood pressure (DBP) in anesthetized guinea pigs. Guinea pigs were pretreated with succinylcholine. Histamine challenge (10 μg/kg) was repeated 20 times at 15-min intervals. Infusions of test drugs were continued for 2 hr from 5 min after the third challenge. Responses of PIP were evaluated from histamine-induced increases in PIP from the baseline just before the third challenge, and they are expressed as percent changes of increased PIP before drug infusion. HR and DBP values are expressed as percent changes from the values just before the third histamine challenge. Basal values (mean ± S.D., n = 60): increased PIP, 28.4 ± 6.6 cmH₂O; HR, 238 ± 17 beats/min; and DBP, 33 ± 4 mmHg. Each point and vertical bar represent the mean value and S.E. of five animals. *P < 0.05: Significantly different from the control (Dunnett method).

Table 1. Effects of inhalation of NKH477 and isoproterenol on the decrease in specific airway conductance (SGaw) induced by inhaled histamine in conscious guinea pigs

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Baseline SGaw (1/sec · 1/cmH₂O)</th>
<th>Used-histamine concentration (log%)</th>
<th>Initial bronchoconstriction (decrease% in SGaw)</th>
<th>Inhibition of histamine-induced bronchoconstriction (% vs initial reaction) 2nd 3rd 4th 5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>6</td>
<td>535 ± 19</td>
<td>0.52 ± 0.03</td>
<td>-1.04 ± 0.08</td>
<td>81.7 ± 4.9</td>
<td>2.1 ± 12.1 9.9 ± 8.6 5.6 ± 11.1 2.4 ± 14.5</td>
</tr>
<tr>
<td>NKH477 (Used dose)</td>
<td>8</td>
<td>543 ± 19</td>
<td>0.48 ± 0.03</td>
<td>-1.04 ± 0.07</td>
<td>89.3 ± 2.2</td>
<td>8.5 ± 5.2 57.5 ± 16.0* 77.0 ± 3.8* 12.7 ± 9.0 (0.1 mg/ml) (0.71 mg/ml) (5 mg/ml) (0 mg/ml)</td>
</tr>
<tr>
<td>Isoproterenol (Used dose)</td>
<td>8</td>
<td>533 ± 21</td>
<td>0.46 ± 0.02</td>
<td>-0.99 ± 0.06</td>
<td>81.2 ± 6.4</td>
<td>3.3 ± 12.7 46.9 ± 11.9 73.9 ± 14.5* 0.4 ± 5.0 (0.002 mg/ml) (0.014 mg/ml) (0.1 mg/ml) (0 mg/ml)</td>
</tr>
</tbody>
</table>

Each guinea pig was exposed five times to aerosols of the same histamine solution for 30 sec at 90-min intervals. In the second, third and fourth histamine challenges, each animal was pre-exposed to aerosols of NKH477 solutions (0.1, 0.71 and 5 mg/ml), isoproterenol solutions (0.002, 0.014 and 0.1 mg/ml) or 0.9% saline for 2 min from 3 min before histamine inhalation. Values represent the mean ± S.E. *P < 0.05: Significantly different from the control (Bonferroni method).
Table 2. Time course of effects of inhalation of NKH477 and isoproterenol on the heart rate in conscious guinea pigs

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Heart rate before saline inhalation (beats/min)</th>
<th>Used dose (mg/ml)</th>
<th>Increase in heart rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKH477</td>
<td>8</td>
<td>568 ± 39</td>
<td>303 ± 3</td>
<td>0 (Saline)</td>
<td>3 min: 1.5 ± 1.3</td>
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<td>0.1</td>
<td>6 min: 0.1 ± 1.4</td>
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<td>0.01</td>
<td>10 min: 0.2 ± 1.4</td>
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<td>0.001</td>
<td>20 min: 0.3 ± 1.4</td>
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<td></td>
<td>0.002</td>
<td>40 min: 0.4 ± 1.5</td>
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<tr>
<td>Isoproterenol</td>
<td>8</td>
<td>564 ± 34</td>
<td>298 ± 8</td>
<td>5 (5 mg/ml)</td>
<td>3 min: 20.4 ± 3.3*</td>
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<td>0.002</td>
<td>6 min: 22.2 ± 3.3*</td>
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<td>0.01</td>
<td>10 min: 22.0 ± 3.5*</td>
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<td>0.004</td>
<td>20 min: 16.1 ± 2.8*</td>
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<td>0.1</td>
<td>40 min: 6.6 ± 1.2</td>
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<td>0.071</td>
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<td>5 (35.4 mg/ml)</td>
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</tbody>
</table>

Each animal was exposed to aerosols of NKH477 solutions (0, 0.1, 0.71, 5 and 35.4 mg/ml) and isoproterenol solutions (0, 0.002, 0.014, 0.1, 0.71 and 5 mg/ml) for 2 min at 45-min intervals between the doses. Heart rate values are expressed as percent changes from values just before each test drug inhalation. Values represent the mean±S.E. *P<0.05: Significantly different from 0.9% saline (Dunnett method).
EC50 values of NKH477 and isoproterenol were 1.3 mM (0.71 mg/ml) and 0.089 mM (0.022 mg/ml), respectively. Thus, the bronchodilator effect of NKH477 was 15 times less potent than that of isoproterenol. The selectivity of NKH477 for bronchodilation vs an increase in HR was somewhat lower than that of procateterol, a highly selective β2-agonist (13), but considerably higher than that of isoproterenol and similar to that of aminophylline. The selectivity of NKH477 vs a decrease in DBP was somewhat higher than that of aminophylline. The selectivity of inhaled NKH477 for bronchodilation vs an increase in HR was similar to that of inhaled isoproterenol and was about 4 times higher than that of i.v. bolus injected NKH477. Furthermore, tachycardia and hypotension induced by NKH477 recovered after termination of i.v. infusion, whereas aminophylline-induced hypotension did not. These results suggest that NKH477 is a bronchodilator with few cardiovascular effects, and that the effects are easy to control.

Some β-adrenergic receptor agonists may increase airway reactivity (14). Although the mechanisms are not yet clear, i.v. infusion of isoproterenol has been shown to increase airway reactivity in guinea pigs (15, 16) and to produce rebound bronchospasm in some asthmatic patients (17). In our study, NKH477, like forskolin (18), did not enhance the bronchoconstriction induced by histamine after the infusion, in contrast to isoproterenol. Thus, NKH477 appears not to increase airway reactivity acutely.

From the results described above, the novel water-soluble forskolin derivative NKH477 appears to be useful as a bronchodilator in the treatment of bronchial asthma. Clinical trials with NKH477 for this purpose are now in progress.

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