Neurokinin A-Induced Bronchial Hyperresponsiveness to Methacholine in Japanese Monkeys

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Neurokinin A (NKA) and substance P (SP) are the mammalian peptides belonged to the tachykinin family. NKA is a more pronounced and long-lasting bronchoconstrictor than SP (Lundberg et al. 1985). In guinea-pig tracheal strips, NKA is a more potent bronchoconstrictor than leukotriene D₄, neurokinin B, or SP (Uchida et al. 1987). In addition, tachykinins have been reported to mediate the acute increase in airway responsiveness caused by toluene diisocianate in guinea-pigs (Thompson et al. 1987). In the present study, we have investigated whether NKA or SP increases bronchial responsiveness to inhaled MCh in Japanese monkey.

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Three male Japanese monkeys weighing 9-11 kg were anesthetized with ketamine hydrochloride (15 mg/kg, i.m.), intubated with an endotracheal tube (inner diameter: 6.5 mm, length: 15 cm), and inserted with an esophageal balloon. Then, the monkeys were paralyzed with pancuronium bromide (0.01 mg/kg, i.v.) and ventilated with a constant-volume respirator (Model 607E, Harvard Apparatus, South Natick, MA, USA) set at a tidal volume of 20 ml/kg and a frequency of 20 breaths per minute.

For measurement of pleural pressure, the esophageal balloon was inflated and positioned in the esophagus at the point of the most negative end-expiratory pressure. Trans-pulmonary pressure (Ptp) was measured as the difference between the pleural pressure and the pressure at the proximal end of the endotracheal tube with a differential pressure transducer (Model TP 603T, ±50 cmH2O; Nihon Kohden, Tokyo). Airflow at the opening of the endotracheal tube (V̇) was measured with a heated pneumotachograph (Fleish No. 2) connected to a differential pressure transducer (Validyne MP-54, ±5 cmH2O, Northridge, CA, USA). The pneumotachograph-transducer system was calibrated with a rotameter and was linear over the range measured. Tidal volume (VT) was obtained by electronic integration of the flow rate signal. Ptp, V̇, and VT were recorded on a 4-channel strip chart recorder (San-ei RECTI-HORIZ-8 K, NEC San-ei, Tokyo). Pulmonary flow resistance (Rl) was calculated as the ratio of Ptp to V̇ at points of equal volume (cmH2O/ml/sec) and dynamic lung compliance (Cdyn) as the ratio of VT to Ptp at points of zero flow (ml/cmH2O). Throughout the entire experiment, blood pressure and pulse rate were recorded with an automated sphygmomanometer (BP-103N, Nihon-kohrin, Tokyo).

NKA and SP were purchased from Peptide Institute Inc. (Osaka). 10-8 M NKA and 10-8 M SP were prepared with 0.1% acetic acid solution and saline immediately before use. Aerosols of NKA and SP were generated by a Bird nebulizer (Bird Co., Spring, CA, USA) which was driven by an air compressor (Iwaki Co., Tokyo) at a flow rate of 9 liter/min. Inhalation was obtained via a three-way valve connected to the proximal end of the endotracheal tube. NKA and SP were inhaled in 40 3-sec exposures (2 min total). After each 3 sec-inhalation of NKA, i.e., passive inspiration (about 400 ml of air), the three-way valve was opened to the atmosphere, i.e., passive expiration, which was repeated 40 times per about 4 min.

Inhalation challenges with MCh were performed before and 1, 2, 3 and 4 weeks after 2-min inhalation of 10-8 M NKA and 10-8 M SP. Two fold increasing concentrations of MCh (0.0625 to 4.0 mg/ml) were prepared with physiologic saline. Rl, Cdyn, pulse rate, and blood pressure were measured as control values, after 40 3-sec exposures to an aerosol of physiologic saline (2 min total). Immediately after an aerosol of each concentration of MCh was inhaled, each parameter was measured. Rl and Cdyn were expressed as percentages of their control values.

**RESULTS**

Fig. 1 shows Rl dose-response curves to inhaled MCh obtained before and 1, 2, 3 and 4 weeks after 2 min-inhalation of 10-8 M NKA. The dose-response curves were shifted to the left 1 to 4 weeks after NKA treatment, compared with that before NKA treatment. There were significant increases in Rl to 0.25 (from 44.3±33.5% to 122.7±33.7%; p<0.05) and 0.50 mg/ml (from 76.7±53.3% to 183.7±59.9%; p<0.01) of MCh 2 weeks after NKA treatment, compared with no treatment. However, Rl dose-response curves to MCh were not changed after SP treatment.
Neurokinin A and Bronchial Hyperresponsiveness

DISCUSSION

Bronchial hyperresponsiveness to various irritants is one of the important characteristics of bronchial asthma, even in the asymptomatic state. Various substances, such as allergen, ozone, sulphur dioxide, leukotriene C4, platelet activating factor, toluene diisocianate, C5A des arg, and endotoxin have been reported to increase nonspecific bronchial responsiveness to histamine or methacholine, although bronchial hyperresponsiveness induced by these substances disappears within 1 week. In the present study, we have demonstrated in Japanese monkeys that non-specific bronchial responsiveness to inhaled MCh was increased 1 to 4 weeks after only a 2 min-inhalation of $10^{-8}$ M NKA, compared with that obtained before the NKA exposure.

In clinical studies (Empey et al. 1976; Little et al. 1978), viral infection of
the upper respiratory tract was reported to induce long-lasting bronchial hyper-
reactivity to histamine in healthy subjects. Recently, it is reported that viral
infection decreases enkephalinase (Jacoby et al. 1988), which is one of the enzymes
which inactivates neuropeptides. Thus, from the combination of these two
findings, we may hypothesize that NKA increases non-specific bronchial re-
 sponsiveness to methacholine or histamine over a long period.

As shown in results, bronchial responsiveness to MCh was increased 1 to 4
weeks after 2 min-inhalation of 10^{-8} M NKA. However, it was only 2 weeks after
NKA exposure that the responsiveness was significantly increased. In the present
study, we used no inhibitors of neutral endopeptidase. In airways, neutral
endopeptidase activity exists in vagus nerve, tracheal smooth muscle, submucosal
gland and in the epithelium. In addition, enkephalinase inhibitors have been
reported to potentiate mammalian tachykinin-induced contraction in ferret tra-
chea (Sekizawa et al. 1987). Therefore, one of the reasons for the slight degree
of bronchial hyperresponsiveness may be inactivation of NKA by neutral endope-
ptidase.

As shown in results, non-specific bronchial hyperreactivity was not found
using Cdyn as the pulmonary function test, and % change in Cdyn to inhaled Mch
was smaller than that in R1. Since Cdyn is frequency dependent (Woolcock et al.
1969), it is possible that the faster the inspiratory flow rate is, the greater the Cdyn
decreases. During the period of this study, we measured Cdyn at mechanical
ventilation rates of 20, 30 and 40/min. Consequently, Cdyn decreased by 35%
from control values at a rate of 20/min of respiration, by 43% at a rate of 30/min,
and 48% at a rate of 40/min. These, therefore, uneven ventilation to inhaled MCh
might have been obscured due to the slow respiratory rate.

In conclusion, we have demonstrated that inhaled NKA induced long-lasting
bronchial hyperresponsiveness to inhaled MCh in Japanese monkeys. Further
investigation is needed to understand the mechanism.

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