Inhibitory Effect of KW-4679, an Anti-allergic Drug, on Tachykinin-Mediated Airway Response Induced by Electric Vagal Stimulation in Guinea Pigs

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ABSTRACT—We examined the effect of KW-4679 ((Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride), an anti-allergic drug, on the tachykinin-mediated airway response in guinea pigs. Electrical stimulation of the vagus nerve in atropine-treated and propranolol treated guinea pigs caused a 38.1% decrease in dynamic compliance (CdYn), which was suppressed by the combination of the tachykinin NK1-receptor antagonist (−)CP-96345 and NK2-receptor antagonist SR 48968. KW-4679 at a dose of 3 mg/kg significantly reduced the decrease in CdYn (P<0.05). On the other hand, KW-4679 did not inhibit substance P or neurokinin A-induced decrease in CdYn. These results suggest that KW-4679 may inhibit the tachykininergic airway response by prejunctional inhibition of peripheral sensory nerves.

Keywords: KW-4679, Tachykinin, Vagal stimulation

Tachykinin, which contains substance P (SP) and neurokinin A (NKA), is localized in the airway sensory nerves of several species. Exogenous SP produces several features of asthma, including bronchoconstriction (1) and microvascular leakage (2). Recently, it was revealed that the tachykinins contribute to antigen-induced bronchoconstriction, airway inflammation and airway hyperresponsiveness in guinea pigs (3–5). Therefore, modulation of tachykinin action in the airways should attenuate neurogenic inflammation and hyperreactivity of the airway.

KW-4679 ((Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride, synthesized in our laboratories) is an anti-allergic drug that inhibits the antigen-induced airway hyperresponsiveness and late asthmatic response in actively sensitized guinea pigs (6). Recently, it was demonstrated that KW-4679 inhibits the tachykinin-mediated contractile responses in isolated guinea pig bronchial preparations in a prejunctional manner (7). This study was designed to reveal the effect of KW-4679 on tachykinins-mediated bronchoconstriction in vivo by studying its action on the bronchoconstriction induced by the electrical stimulation of the vagus nerve in guinea pigs.

Male Hartley guinea pigs (350–500 g in weight; Charles River, Yokohama) were anesthetized with urethane (1.4 g/kg, i.p.) and placed in a plethysmobox (Model PYLAN; Buxco Electronics, Sharon, CT, USA). The breathing of the animals was then stopped with gallamine (10 mg/kg, i.v.), and then they were ventilated (1 ml air for 100 g body weight, 60 breaths/min) with a tracheal cannula using a constant-volume ventilator (rodent ventilator 7025; Ugo Basile, Varese, Italy). The airflow signal and the pressure signal measured using a differential pressure transducer (Model DP45; Validyne Engineering Co., Northridge, CA, USA) were electronically integrated using a computerized pulmonary function mechanical analyzer (Model 6, Buxco). Lung resistance (RL) and dynamic compliance (Cdyn) were simultaneously calculated by this system according to the method of Aoki et al. (8). The noncholinergic and nonadrenergic component of the bronchospasm was evaluated after the intravenous administration of 1 mg/kg of atropine (Wako Chemicals, Tokyo) and 1 mg/kg of propranolol (Sigma Chemical Co., St. Louis, MO, USA) (9). The jugular vein was cannulated for injection of test compounds. An electrical stimulation of the unilateral vagi was made in the cervical region by means of an electrode connected to a stimulator (PST-001; Star Medical, Tokyo) with an isolator (ISS-011, Star Medical). Pulse
trains were delivered for 30 sec at a frequency of 8 Hz (10 V, 5-msec duration). At 10 to 20 min after the airway response induced by the first stimulation and the $C_{\text{dyn}}$ was returned to basal levels, the second bronchoconstriction was induced by vagus stimulation. To reveal the effect of drugs, the first bronchoconstriction and the second bronchoconstriction in the presence of drugs were compared. ($\pm$)-CP-96345 (dihydrochloride salt of racemic mixture containing both (2S,3S-cis- and (2R,3R-cis-2(diphenylmethyl)-N-(2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]octan-3amine) and SR 48968 ((S)-N-methyl-N-[4-(4-acethylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl) buthyl] benzamide) were synthesized in our laboratory and dissolved in dimethyl sulfoxide (DMSO) at 5 mg/ml and injected into the animals at 0.2 ml/kg. KW-4679 was dissolved in saline.

The basal level of RL and $C_{\text{dyn}}$ were $0.324 \pm 0.017$ cmH$_2$O/ml/sec and $0.436 \pm 0.018$ ml/cmH$_2$O, respectively (n = 28). Vagus stimulation evoked the 38.1% decrease in $C_{\text{dyn}}$ which peaked at 30 sec (minimum $C_{\text{dyn}}$: $0.270 \pm 0.021$ ml/cmH$_2$O, n = 28). However, vagus stimulation induced minor changes in RL (maximum RL: $0.554 \pm 0.043$ cmH$_2$O/ml/sec). So, we evaluated the airway response by using $C_{\text{dyn}}$. The similar observation that $C_{\text{dyn}}$ changes are more remarkable than RL changes was reported in aerosolized bradykinin-induced bronchoconstriction in guinea pigs (10). We confirmed the earlier findings reported by Boni et al. (11) that vagus nerve stimulation-induced noncholinergic bronchoconstriction was mediated by both the NK$_1$ and NK$_2$ receptors. Figure 1 indicates the effects of the tachykinin NK$_1$-receptor antagonist (+)-CP-96345 and the NK$_2$-receptor antagonist SR 48968 on the bronchoconstriction evoked by the vagus stimulation. The combination of (+)-CP-96345 (1 mg/kg) and SR 48968 (0.1 mg/kg) abolished the bronchoconstriction. The vehicle (DMSO) did not affect the bronchoconstriction (data not shown). This result indicates that the bronchoconstriction was mediated by tachykins. $C_{\text{dyn}}$ is considered to reflect changes in the

Fig. 1. Effect of the combination of the NK$_1$-receptor antagonist CP-96345 and NK$_2$-receptor antagonist SR 48968 on the airway response induced by electrical vagal stimulation (8 Hz, 5 msec, 10 V for 30 sec) in the atropine (1 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.) treated guinea pigs. Airway response is expressed as the decrease in dynamic compliance ($C_{\text{dyn}}$). Guinea pigs were intravenously administered with 1 mg/kg of (+)-CP-96345 and 0.1 mg/kg of SR 48968 20 min before the second vagal stimulation. Open circles indicate the first response, and closed circles indicate the second response. Data show the mean $\pm$ S.E.M., n = 5. ** indicates a significant difference from the first response (P < 0.01, paired t-test).

Fig. 2. Effect of KW-4679 on the airway response induced by electrical vagal stimulation (8 Hz, 5 msec, 10 V for 30 sec) in the atropine (1 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.)-treated guinea pigs. Guinea pigs were intravenously administered with 3 mg/kg of KW-4679 (A) or vehicle (B) 10 min before the second vagal stimulation. The airway response is expressed as the decrease in dynamic compliance ($C_{\text{dyn}}$). Open circles indicate the first response, and closed circles indicate the second response. Data show the mean $\pm$ S.E.M., n = 6. * indicates a significant difference from the first response (P < 0.05, paired t-test).
peripheral airways (12). So, the result also suggests that tachykinin released by vagus nerve stimulation acts preferentially on the smaller airways.

Against vagus stimulation-induced bronchoconstriction, KW-4679 at a dose of 1 mg/kg did not markedly affect Cdyn (data not shown), but a dose of 3 mg/kg reduced it (Fig. 2A). At 1 min, the inhibitory effect was 31.1% and significant (P<0.05) and at 30 sec, inhibition was 34.0% (P<0.1). On the other hand, administration of saline, the vehicle, had no effect (Fig. 2B). It was revealed that KW-4679 has no inhibitory effect on SP or NKA-induced bronchial contractile responses in isolated guinea pig bronchi. In addition, KW-4679 (3 mg/kg, i.v.) did not affect the decrease in Cdyn induced by intravenous administration of 10 µg/kg of SP (Sigma) nor did 1 µg/kg of NKA (Research Biochemicals International, Natick, MA, USA) (Table 1). Therefore, KW-4679 does not have a direct inhibitory effect on SP- or NKA-induced bronchial contractile responses in isolated guinea pig bronchi. In conclusion, the present study provides in vivo evidence indicating that KW-4679 can inhibit the tachykinin release from airway sensory nerves. This inhibitory effect on tachykinin release contributes to the suppression of inflammation and hyperresponsiveness of the airway.

### Table 1. Effects of KW-4679 on the decrease in dynamic compliance induced by substance P and neurokinin A

|                | Cdyn (mL/cmH2O) | pre | post |
|----------------|-----------------|-----|------|
| Vehicle        | substance P     | 0.315±0.019 | 0.346±0.019 |
|                | neurokinin A    | 0.322±0.025 | 0.328±0.029 |
| KW-4679 (3 mg/kg) | substance P     | 0.204±0.037 | 0.225±0.035 |
|                | neurokinin A    | 0.250±0.035 | 0.260±0.060 |

Substance P (10 µg/kg) and neurokinin A (1 µg/kg) were administered intravenously as a bolus before and 10 min after the treatment with the vehicle or KW-4679. Each value represents the mean±S.E.M. of the decrease in dynamic compliance (Cdyn) induced by substance P or neurokinin A. Statistical evaluation was performed with the paired t-test, n=5.

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