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The Effect of Substance P on the Antigen-Induced Bronchoconstriction in Guinea Pigs

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The effect of substance P (SP) on the antigen-induced bronchoconstriction *in vitro* and *in vivo* was investigated in guinea pigs. SP caused the contraction of isolated non-sensitized guinea pig tracheal muscle at concentrations between 10^{-9} to 10^{-7} g/ml. The elimination of epithelium in the tracheal muscle produced a slight enhancement of SP response as compared to control. Antigen-induced contraction of isolated sensitized guinea pig tracheal muscle was slightly enhanced by the pretreatment with SP at a concentration of 10^{-9} g/ml. The SP-induced enhancement of antigen-induced contraction was siginificantly augmented by the elimination of epithelium of sensitized tracheal muscle. Moreover, the clear bronchoconstriction was observed by the infusion of SP (1 μg/min) intravenously, but not by the infusion of SP at the rates between 0.1 and 0.01 μg/min. Antigen-induced asthmatic respiratory disorder was accelerated temporarily by the infusion of subthreshold SP (0.1 μg/min). The reactivity of bronchial smooth muscle measured by repeated injection of acetylcholine was significantly potentiated by the infusion of subthreshold SP (0.1 μg/min). These results suggest that SP has dual actions in the contractile response of guinea pig airway smooth muscle. One is the direct contractile activity, and the other one is enhancing activity of antigen- and acetylcholine-induced bronchoconstriction.

Keywords — substance P; bronchoconstriction; asthma; allergy; hyper-reactivity

Introduction

It has been accepted for many years that the motor supply to the mammalian respiratory tract is autonomic, consisting of excitatory cholinergic and inhibitory adrenergic nerves.[1] However, it has recently become apparent that a third component to the autonomic nervous system exists in the respiratory tract of several species.[2,3] There is increasing evidence that neuropeptides, including substance P (SP), are the neurotransmitters of a third component.[4] SP causes a potent bronchoconstriction,[5] airway secretion[6,7] and increasing a microvascular permeability of bronchial walls *in vitro*.[8,9] These effects of SP on airways suggest the participation of SP in the etiology of bronchial asthma. In the present study, therefore, we examine the effect of SP on the antigen-induced airway smooth muscle contraction *in vitro* and *in vivo* in guinea pigs.

Materials and Methods

Animals — Male Hartley guinea pigs weighing 350—550 g were purchased from Japan SLC Inc. (Hamamatsu, Japan). The animals were housed in wire mesh cages in an air-conditioned room at 24 °C and fed the usual laboratory diet and water *ad libitum* as provided.

Materials — Substance P (Sigma Chemical Company, St. Louis, U.S.A.) and acetylcholine (Nakarai Chemicals, Ltd. Kyoto, Japan) were purchased commercially. Drugs were dissolved in saline.

Sensitization of Guinea Pigs — One hundred μg of mite antigen was dissolved in 1 ml of saline which included 1 mg of $Al(OH)_3$. Guinea pigs were actively sensitized by 4 i.p. injections of 1 ml of the antigen and $Al(OH)_3$ solution at 4 weeks interval. In order to make antiserum containing IgE antibody for experimental asthma, guinea pigs were immunized with benzylpenicilloyl-bovine-γ-globulin (BPO-BGG) and $Al(OH)_3$. Briefly, each guinea pig was immunized by i.p. injection of 1 ml of Tris-buffered saline (pH8.2) containing 2 μg BPO-BGG and 1 mg of $Al(OH)_3$ monthly for 8 to 12 months. The antiserum containing IgE antibody was obtained from blood withdrawn from the descending aorta 10 d after the last immunization.

Experiments with Airway Smooth Muscle Preparation *in Vitro* — After guinea pigs were stunned and exsanguinated, the tracheas

were excised, trimmed of excess tissue and cut vertically along the cartilage tissue area. In order to eliminate the epithelium, the surface of the tracheal smooth muscle was gently rubbed with a cotton tube. Each open trachea was then cut horizontally into 10 segments. Five segments were tied together to form a chain which was then placed in an organ bath containing Tyrode's solution. Change in tone of the preparation was recorded isotonically by strain gauge (MEC, ME-4013, World Medical, Co., Ltd., Nagoya, Japan). Contractile responses were expressed as a percentage of the contraction induced by 10^{-7} g/ml carbachol.

Schultz-Dale Reaction — Normal and epithelium-eliminated sensitized guinea pig tracheal smooth muscles were contracted by 10^{-7} g/ml carbachol and then challenged with mite antigen (10^{-10}—10^{-6} g/ml).

Experimental Asthma — Guinea pigs were passively sensitized with antiserum containing anti-BPO-BGG IgE antibody at a dose of 0.25 ml/animal. Forty eight hours later, tracheotomy was performed under urethane anesthesia (0.3 g/kg). The trachea was then connected to a transducer. Benzyl penicilloyl-bovine-serum-albumin (BPO-BSA) at a dose of 30 μg/kg was injected into the jugular vein as a challenging antigen. Changes in the number and the volume of respiration were recorded by both transducer (Nihon Kohden, MEP-1T, MP-24T and MFP-1100) and respirometer (RM-150 and RM-25). Determination of obstruction in the respiration was done by measuring the number and volume of respirations and the ratio of expiration time to inspiration time (Ex/In).

Measurement of the Reactivity of Airway Smooth Muscle *in Vivo* — To test the reactivity of airway smooth muscle *in vivo,* the magnitude of respiratory disorder due to acetylcholine was measured by the method described previously.[10] The amount of acetylcholine needed was determined to be 250 μg/kg from the results of preliminary experiments to obtain an appropriate respiratory disorder. Respiratory disorder was measured by the same method as described above. The reactivity of airway smooth muscle was measured by the repeated injection of acetylcholine at 5 min interval for 80 min.

Statistics — Results were statistically evaluated by the Student's *t*-test.

Results

Effect of SP on the Contraction of Guinea Pig Tracheal Muscle *in Vitro*

SP induced a contraction of non-sensitized guinea pig tracheal smooth muscle at concentrations between 10^{-9} and 10^{-7} g/ml (Fig. 1). This contraction was slightly accelerated by the elimination of epithelium of the trachea. Figure 2 indicates the effects of the elimination of epithelium and SP (10^{-9} g/ml) on the antigen (mite)-induced contraction of sensitized guinea pig tracheal muscle. SP at a dose of 10^{-9} g/ml caused a slight contraction (less than 6% of the contraction caused by 10^{-7} g/ml carbachol). The

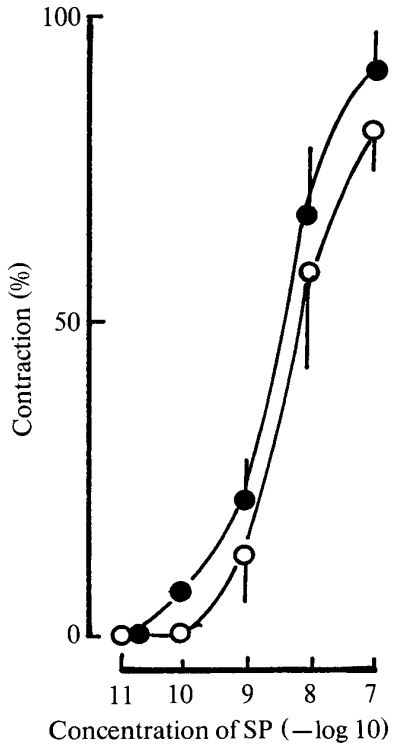

Fig. 1. Substance P (SP)-Induced Contraction of Guinea Pig Tracheal Muscle and the Effect of the Elimination of Epithelium of SP-Induced Contraction of Guinea Pig Tracheal Muscle

The contractile responses were expressed as a percentage of 10^{-7} g/ml carbachol-induced contraction. Each column consists of mean ±S.E. of 6 to 8 experiments.

○, control; ●, epithelium eliminated preparation.

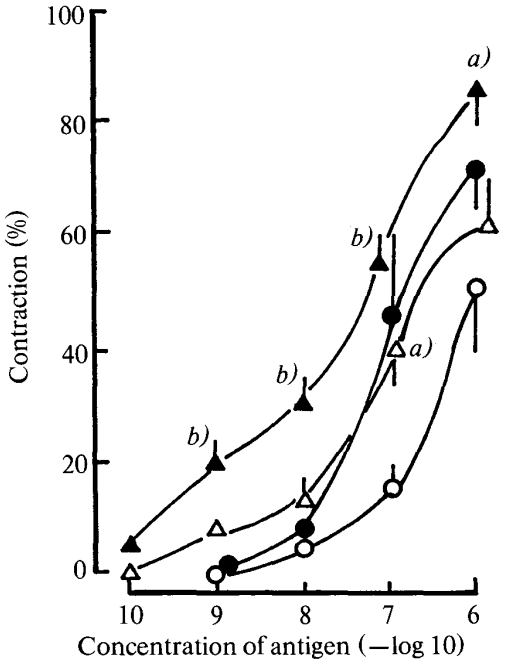

Fig. 2. Effects of the Elimination of Epithelium and Substance P (SP) on Antigen-Induced Contraction of Guinea Pig Tracheal Muscle

Each value was expressed as a percentage of 10^{-7} g/ml carbachol-induced contraction. Each experiment consists of mean ±S.E. of 4 to 8.

○, control; ●, epithelium eliminated preparation (EpEP); △, SP (10^{-9} g/ml); ▲, EpEP+SP. *a)* $p < 0.05$, *b)* $p < 0.01$.

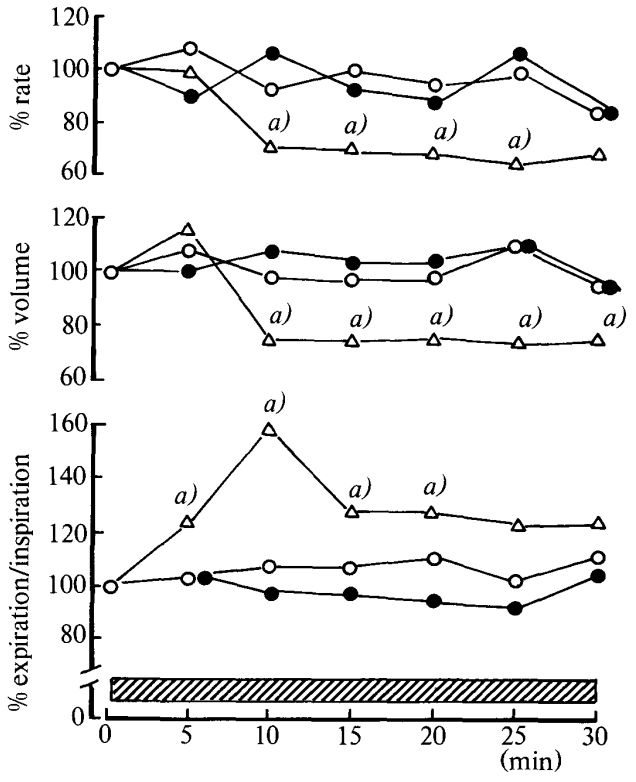

Fig. 3. Effect of Substance P on Respiration in Guinea Pigs

Substance P was infused at the rates of 0.01 (○), 0.1 (●) and 1 (△) μg/min for 30 min. Each group consists of 4 animals. *a)* $p < 0.05$.

antigen-induced contraction was potentiated by either the elimination of epithelium or the pretreatment with SP. When SP was treated on the epithelium eliminated preparation, antigen-induced contraction was significantly enhanced as compared to control.

Effect of SP on the Respiration in Guinea Pigs *in Vivo*

When SP was infused into the aorta of guinea pigs at the rates of 0.01, 0.1 and 1 μg/min, respectively, clear asthematic respiration was observed at 1 μg/min infusion (Fig. 3). Therefore, the effect of SP on antigen- and acetylcholine-induced respiratory disorder was examined under the infusion of 0.1 μg/min SP. The infusion of SP at the rats of 0.1 μg/min produced an enhancement of asthematic respiration at 0 to 6 min after the injection of antigen (BPO-BSA) (Fig. 4). Figure 5 shows the effect of SP (0.1 μg/min) on the reactivity of airway smooth muscle by measuring the response of acetylcholine (250 μg/kg). Acetylcholine-induced respiratory disorder was obviously accelerated by the infusion of SP.

Discussion

The present study indicates that SP has dual actions on the contractile response of guinea pig airway smooth muscle. One is the direct contractile activity, and the other is the enhancing activity of antigen- or acetylcholine-induced bronchoconstriction.

The direct contractile activity of SP on tracheal and bronchial smooth muscles has already been reported.[11-14] The concentration required for producing the contraction of guinea pig trachea is similar to that in those studies. At high concentrations of SP, the contractile response was clearly enhanced by the elimination of epithelium from tracheal muscle. Similar acceleration of histamine- or acethylcholine-induced contraction due to the loss of epithelium is

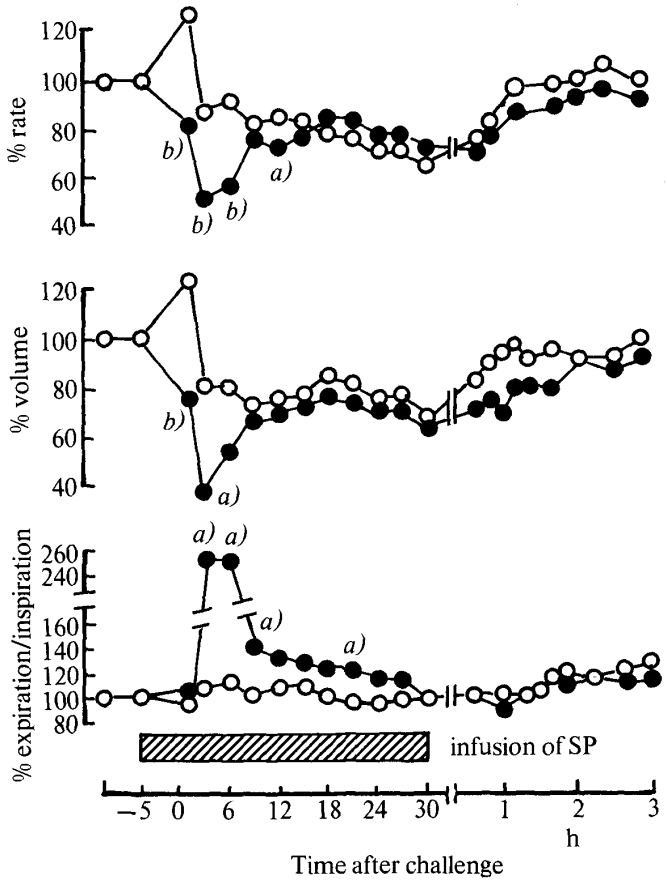

Fig. 4. Effect of Substance P on Experimental Asthma in Guinea Pigs

Guinea pigs were passively sensitized with anti-BPO-BGG-IgE serum and challenged with BPO-BSA. Substance P was infused at the rate of 0.1 μg/min from 5 min prior to challenge for 35 min. Each group consists of 4 animals. The standard error is not shown for clarity, but it is less than 12.6% of the mean value in all points.

○, control; ●, SP (0.1 μg/min). *a)* $p < 0.05$, *b)* $p < 0.01$.

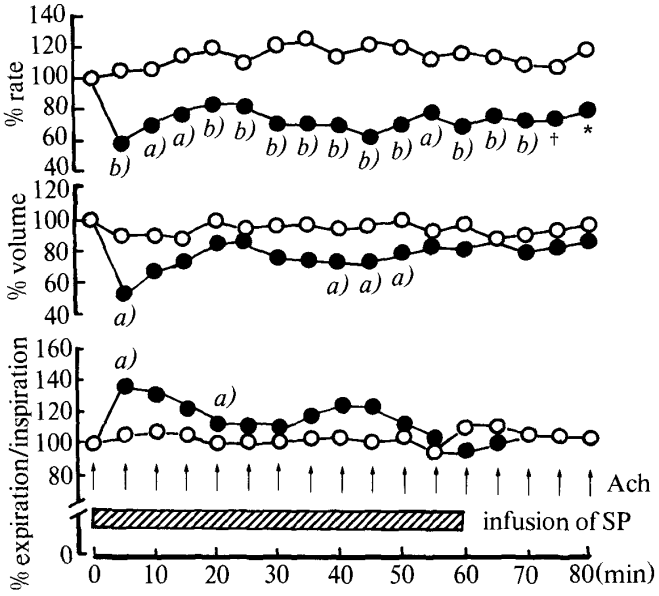

Fig. 5. Effect of Substance P on Acetylcholine-Induced Bronchoconstriction in Guinea Pigs

After the infusion of saline (○) or substance P (0.1 μg/min) (●), acetylcholine (250 μg/kg) was injected at 5 min interval. The acetylcholine-induced bronchoconstriction was expressed as a percentage of the previous value of the infusion of substance P. Each group consits of 5 to 6 animals. The standard error is not shown for clarity, but it is less than 10.8% of the mean value in all points.

a) $p < 0.05$, *b)* $p < 0.01$.

reported by Flavahan *et al.*[15] The reason for the acceleration is postulated to be loss of epithelium derived relaxing factor (EpDRF).[15,16] This potentiation of SP-induced contraction in the epithelium eliminated preparation is probably due to the same mechanism.

Regarding the antigen-induced contractile response of sensitized tracheal muscle *in vitro,* SP shows the potentiation of contractile response in the epithelium eliminated preparation. The acceleration of the antigen-induced contraction by the elimination of epithelium fron guinea pig tracheal muscle was already reported by Undem *et al.*[17] The present result indicates that SP shows a similar and additional synergistic effect on epithelium eliminating effects on the antigen-induced contraction. Whereas the precise mechanism for this synergistic effect is still obscure, the loss of EpDRF is postulated to be one of the main reasons for this enhancement. Additionally, since Aizawa *et al.*[18] reported that SP increases acetylcholine release from vagal nerves terminals without affecting smooth muscle sensitivity may be another reason for synergism.

In addition to *in vitro* study, SP causes a temporal acceleration of antigen-induced bronchoconstriction *in vivo.* Under the same condition, the reactivity of airway smooth muscle by measuring the responsiveness to acetylcholine is clearly enhanced. These obeservations suggest that SP changes an airway smooth muscle responsiveness to the contractile stimuli *in vivo.* This suggests that SP may play an important role for hyper-reactivity of airway smooth muscle in

guinea pigs. However, the reasons why SP accelerates the antigen- and acetylcholine-induced contraction *in vivo* are not yet clear. More detailed experiments will be necessary for clarifying the mechanism. Moreover, since SP infusion has clearly enhanced the reactivity to acetylcholine in guinea pig respiration, this model may be useful for investigating the mechanism and remedy for hyper-reactivity of the airways.

In conclusion, SP induced the contraction of guinea pig tracheal muscle *in vitro* and *in vivo*. This *in vitro* SP-induced contraction was accelerated by eliminating the epithelium of guinea pig tracheal muscle. And SP at a subthreshold dose enhanced the antigen-induced contraction *in vivo*. Under the same condition, the reactivity of airway smooth muscle by acetylcholine is enhanced by SP. These results suggest that SP shows dual actions in the contractile response of guinea pig tracheal muscle. One is the direct contractile activity and the other one is enhancing activity to the contractile response due to an antigen-antibody reaction.

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