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Role of substance P in allergic nasal symptoms in rats

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Abstract

The present study was undertaken to investigate the pathological role of substance P in allergic nasal symptoms in rats. The topical application of substance P caused an increase in the incidence of sneezing and nasal rubbing in a dose-dependent fashion, and at a dose of 30 nM/site it showed a significant effect. L-732,138, a tachykinin NK_1 receptor antagonist, at doses of 3 and 10 mg/kg showed a significant inhibition of the nasal signs induced by exogenous substance P in rats. In addition, L-732,138 also showed a significant inhibition of nasal behavior induced by antigen in actively sensitized rats at the same dose. On the other hand, histamine H_1 receptor antagonists, such as cyproheptadine, epinastine and olopatadine had no effect on the nasal behaviors induced by exogenous substance P, even at higher doses, indicating that exogenous substance P does not cause the degranulation of mucosal mast cells in the rat. Moreover, all the histamine H_1 receptor antagonists showed the dose-dependent inhibition of the nasal signs induced by antigen in actively sensitized rats, which revealed that the inhibition of these drugs was exhibited through the antagonistic effect on histamine H_1 receptors. Therefore, from these results, it is reasonable to conclude that substance P released from the nasal mucosa through the activation of tachykinin NK_1 receptors during the antigen antibody reaction plays an important role in allergic nasal symptoms.

Keywords: Tachykinin NK_1 receptor antagonist; L-732,138; Substance P; Antigen; Sensitization; EEG (Electroencephalogram) spike; Olfactory bulb; Cyproheptadine; Olopatadine; Epinastine

1. Introduction

The main symptoms of allergic rhinitis are sneezing, nasal rubbing, nasal blockage and rhinorrhea, which are caused by the interaction between the chemical mediators and the sensory nerves through the activation of specific receptors. Histamine has been shown to contribute substantially in causing allergic disease, but histamine H_1 receptor antagonists caused no clear amelioration of the symptoms (White, 1990; Howarth et al., 2000). Therefore, it is essential to give insight into the role of other receptors present at the nasal mucosa in allergic diseases. Several studies have been conducted to clarify the pathology of allergic disease, but very few studies are available on the exact role of substance P in nasal symptoms in animal models. Moreover, protein sequencing of human and rat tachykinin NK_1 receptor showed a 92% similarity between these two species, which has also driven us to investigate the nasal symptoms in rats induced by substance P (Gerard et al., 1991; Sachais et al., 1993). The tachykinins are a family of structurally related peptides, including substance P, neurokinin A and neurokinin B. There are three types of tachykinin receptors i.e., tachykinin NK_1, NK_2 and NK_3, which have been found in the nasal mucosa of humans, and they have shown a preferential affinity towards substance P, neurokinin A and neurokinin B, respectively (Harrison and Geppetti, 2001). There is some evidence suggesting the involvement of neuropeptides in the pathogenesis of allergic diseases, and several efforts are underway to find an effective antagonist against the neuropeptides due to its plurality of roles in many diseases. Nerve fibers containing substance P are found around the nasal arterial and venous vessels, gland acini and in the epithelium (Baraniuk et al., 1991). Substance P is released from the nociceptive nerves by the stimulation of histamine, bradykinin, prostaglandins, leukotrienes and antigens, and causes neurogenic inflammation, such as plasma exudation, vasodilation and mucus secretion (Baraniuk et al., 1991; Mosimann et al., 1993; Saria et al., 1988). Kaise et al. (2001) also demonstrated that substance P causes nasal obstruction in guinea pigs via the tachykinin NK_1 receptor. On the other hand, Regoli et al. (1994) reported that

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endogenous tachykinins are not highly selective, and substance P not only activates the tachykinin NK_1 receptor, but also tachykinin NK_2 and NK_3 receptors. Therefore, the involvement of the tachykinin NK_1 receptor in nasal allergic symptoms in animal models remains unclear. Substance P is found in the nasal secretions of patients with allergic rhinitis, (Baraniuk et al., 1991) and causes plasma exudation in guinea pig airways (Rogers et al., 1988). In addition, substance P is found in the nasal mucosa after topical antigen challenge in OVA sensitized guinea pigs (Imamura and Kambara, 1992). In in vitro studies, substance P caused the degranulation of the mucosal mast cells to release histamine from lung mast cells in humans (Schierhorn et al., 1995). The intestinal mucosa and peritoneal cavities of rats have also been shown to release histamine following stimulation with substance P (Ali et al., 1986; Shanahan et al., 1985). On the other hand, in an in vivo experiment in humans, histamine was not found in the nasal lavage fluid after the topical application of substance P, indicating the heterogeneity of mast cells in the nasal mucosa (Braunstein et al., 1994). These findings prompted us to clarify the pathological role of substance P in the nasal symptoms in the animal model. Therefore, in this experiment, we investigated the pathological role of substance P in allergic nasal symptoms in rats using substance P, tachykinin NK_1 or histamine H_1 receptor antagonists.

2. Materials and methods

2.1. Animals

Six-week-old male Wistar rats (body weight 180–190 g) were obtained from Japan SLC, Shizuoka, Japan. The animals were housed in an air-conditioned room maintained at 24±2 °C with a relative humidity of 55±15%. They were kept in aluminum cages with sawdust. The rats were given standard laboratory rodent chow (Oriental Yeast, Tokyo, Japan) and water ad libitum. All procedures involving the animals were conducted in accordance with Guidelines for Animal Experiments at Okayama University Advanced Science Research Centers, and all procedures were licensed by the Animal Research Control Committee of Okayama University.

2.2. Reagents and drugs

The following reagents and drugs were obtained from the sources shown in parentheses: substance P (Sigma, St. Louis, MO, USA), egg albumin (Grade VII; crystallized and lyophilized, essentially salt-free, Sigma), aluminum hydroxide hydrate gel (alum, LSL, Tokyo, Japan), dimethyl sulfoxide (Wako, Osaka, Japan), cyproheptadine hydrochloride (Sigma), L-732,138, *n*-acetyl-L-tryptophan 3,5-bis (trifluoromethyl)-benzyl ester (Sigma), epinastine hydrochloride (Nippon Boehringer Ingelheim, Hyogo, Japan) olopatadine hydrochloride (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan). *Bordetella pertussis* inactive microorganism suspension (*B. pertussis*) was kindly provided by Kitasato Institute Research Center for Biologicals, Saitama, Japan. Substance P and egg albumin were dissolved in saline and administered intranasally. L-732,138 was dissolved in 70% dimethyl sulfoxide (DMSO) and administered intravenously (tail vein) 5 min before substance P and antigen challenge. Other drugs were suspended in 5% gum arabic solution and administered orally 1 h before the topical application of substance P. Nasal behavior and electroencephalogram (EEG) were measured for 30 min after the instillation of substance P into the bilateral nasal cavities using a micropipette.

2.3. Implantation of electrodes

The animals were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg, i.p., Abbott Laboratories, North Chicago, IL, USA), then fixed to a stereotaxic apparatus (Narishige, Type SR-5, Tokyo, Japan). For EEG recording, two stainless steel screw electrodes were chronically implanted

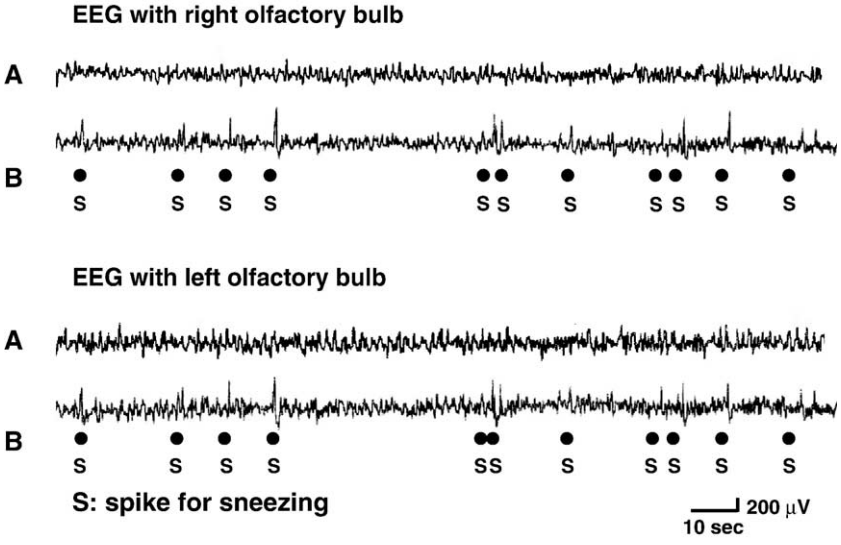

Fig. 1. Representative example of EEG changes at the bilateral olfactory bulb induced by substance P (30 nM/site) application. A: EEG of the bilateral olfactory bulb recorded from the control rat, B: EEG of the bilateral olfactory bulb recorded from the rat treated with substance P (30 nM/site). S: spike for sneezing.