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Short communication

Olopatadine attenuates the enhancement of capsaicin-evoked substance P release by bradykinin from cultured dorsal root ganglion neurons

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Abstract

Olopatadine, a second-generation antihistamine, has recently been suggested to have an inhibitory effect on the tachykinin release from guinea-pig tracheobronchial smooth muscle preparation. In the present study, using a highly sensitive radioimmunoassay for substance P, we observed that olopatadine attenuated the enhancement of capsaicin-induced substance P release by bradykinin with an IC_{50} value of 12.5 μM, without any inhibitory effect on the substance P release induced by capsaicin, potassium or bradykinin from cultured dorsal root ganglion neurons. These data suggest that olopatadine may therefore be involved in the bradykinin-induced sensitization of the transient receptor potential vanilloid 1 in cultured dorsal root ganglion neurons.

Keywords: Olopatadine; Substance P release; Dorsal root ganglion; Bradykinin; Capsaicin; Transient receptor potential vanilloid 1

1. Introduction

Olopatadine ((Z)-11-[(3-dimethylamino) propylidene]-6,11-dihydro-dibenz[b,e]oxepin-2-acetic acid monohydrochloride), a second-generation antihistamine, is prescribed for allergic disorders such as rhinitis, urticaria and eczema dermatitis (Bielory et al., 2004). Olopatadine has been shown to suppress the release of inflammatory mediators, such as leukotrienes, thromboxane and tumor necrosis factor-alpha from various inflammatory cells (Sharif et al., 1996; Ohmori et al., 2002; Brockman et al., 2003), and to inhibit the release of tachykinins (substance P and/or neurokinin A)-regulated slow phase contraction caused by an electrical field stimulation in the guinea pig bronchi (Ikemura et al., 1996), to reduce the number of capsaicin-induced sneezing responses correlated with the tachykinin release in guinea-pig (Kaise et al., 2001). However, no report has so far shown that olopatadine could inhibit the substance P release from the central nervous tissue.

The substance P release from primary afferent neurons is a very complex process which often involves some important intracellular effectors, such as extracellular calcium influx, inositol 1,4,5-triphosphate (IP_3)-induced calcium release, the activation of extracellular signal-regulated kinase (ERK), cyclooxygenases (COXs) and prostaglandins, and the cyclic-AMP-dependent protein kinase A (Premkumar and Ahern, 2000; Purikiss et al., 2000; Oshita et al., 2005; Tang et al., 2005; Tang et al., 2006). We recently reported that the mechanism of substance P release evoked by capsaicin or potassium is conversely modulated with bradykinin in cultured rat dorsal root ganglion neurons (Tang et al., 2006), such as a rapid substance P release evoked by a short-term treatment (10 min) with capsaicin or a high concentration of potassium, and a slow substance P release induced by a long-term treatment (3 h) with bradykinin. The mechanisms of substance P release by different stimuli are suggested to be distinct from the tissues which come from the periphery to the central nerve system.

Bradykinin is a nonapeptide generated locally from plasma during tissue damage and inflammation. It is a powerful inflammatory mediator, while also stimulating the production of a number of endogenous chemicals to be involved in neurogenic inflammation and inflammation-induced hyperalgesia. We previously demonstrated that the long-term exposure of dorsal root ganglion neurons to bradykinin resulted in the enhancement of capsaicin-evoked substance P release due to the activation of the inositol 1,4,5-triphosphate (IP_3)-induced calcium release (Tang et al., 2006), and COX-2 protein was thus induced to cause prostaglandin E_2 generation during the bradykinin stimulation to dorsal root ganglion neurons (Inoue et al., 2006). These findings indicated that the long-term exposure of dorsal root ganglion neurons to bradykinin could mimic a chronic inflammatory condition in vitro, thereby setting off a series of reactions inside the neurons that ultimately make transient receptor potential vanilloid 1 (TRPV1) more sensitive to normal stimuli. Therefore, it is considered important to investigate the effect of the pretreatment with olopatadine on the attenuation of the substance P release from primary afferent neurons induced by the application of various stimuli (capsaicin, potassium or/and bradykinin).

The objective of the present study is to clarify the possible anti-inflammatory effect of olopatadine on the substance P release evoked by potassium, capsaicin or bradykinin, and on the potassium- or capsaicin-evoked substance P release from cultured adult rat dorsal root ganglion neurons pre-exposured to bradykinin for 3 h. We herein show that olopatadine can attenuate the enhancement of capsaicin-induced substance P release by bradykinin, thus suggesting that olopatadine may be a promising anti-inflammatory drug for the treatment of itching or/and pain for clinical use.

2. Materials and Methods

2.1. Materials

The following drugs were used: bradykinin (Peptide Institute, Inc., Osaka, Japan);

Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Tokyo, Japan); mouse laminin (Upstate Biotechnology, Lake Placid, NY, USA); Olopatadine ((Z)-11-[(3-dimethylamino) propylidene]-6,11-dihydro-dibenz[b,e]oxepin-2-acetic acid monohydrochloride) (Kyowa Hakko Kogyo Co., Ltd , Tokyo, Japan); [^{125}I]Tyr^{8}-Substance P (New England Nuclear, Boston, MA, USA); polyethylenemine and capsaicin, potassium chloride (Sigma Chemical Co., St Louis, MO, USA).

2.2. Isolation and culture of dorsal root ganglion neurons

Dorsal root ganglion neurons were prepared according to the method of Tang et al. (2004). All procedures used in these experiments were approved by the Animal Care and Use Committee of Hiroshima University School of Medicine, Hiroshima, Japan (A05-119). The isolated dorsal root ganglion neurons (2-3 dorsal root ganglions/dish) from adult Wistar rats (6-9 weeks) were plated on polyethyleneimine- and laminin-coated dishes and incubated in Dulbecco's modified Eagle's medium (DMEM). The cultures were maintained at 37°C in a water-saturated atmosphere with 5% CO_2 for 5 days before the experiment.

2.3. Measurement of substance P content in the culture medium

Except for some cells treated only by a control or olopatadine alone, all other cells were exposed to bradykinin, either alone or together with olopatadine in DMEM (serum free) containing peptidase inhibitors for 3 h at 37°C in a water-saturated atmosphere with 5% CO_2, or were exposed to potassium or capsaicin, either alone or together with olopatadine in Krebs-HEPES buffer containing peptidase inhibitors (10 μM phosphoramidon, 40 μg/ml bacitracin and 10 μM captopril) for 10 min at 37°C. After being washed with Krebs-HEPES buffer, those cells pretreated with bradykinin were continuously stimulated by potassium or capsaicin plus peptidase inhibitors or by peptidase inhibitors only in Krebs-HEPES buffer for 10 min at 37°C. Thereafter, the substance P content collected from the culture medium

(DMEM or Krebs-HEPES buffer) was measured by a highly sensitive radioimmunoassay (Tang et al., 2005).

2.4. Statistical analysis

The data are presented as the mean ± S.E.M. Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Bonferroni's test. The differences between two groups were considered to be statistically significant when $P< 0.05$.

3. Results

3.1. Effects of olopatadine on the substance P release evoked by various chemicals from cultured adult rat dorsal root ganglion neurons

We examined whether pretreatment with olopatadine has any significant influence on the substance P release evoked by capsaicin or a high concentration of potassium from cultured dorsal root ganglion neurons. After a 30 min-pretreatment with various concentrations of olopatadine (0.1–50 μM), those cultured dorsal root ganglion neurons were exposed to Krebs-HEPES buffer containing potassium (30 mM) or capsaicin (100 nM) for 10 min. The substance P content in Krebs-HEPES buffer was collected and measured by a radioimmunoassay. As shown in Figs. 1A and B, both the short-term treatment with capsaicin and potassium caused significant increases in the amount of substance P release from cultured dorsal root ganglion neurons, however, olopatadine did not influence the increase in the substance P release by them under these experimental conditions. In contrast to the short-term treatment with capsaicin or potassium, we found that a long-term treatment (3 h) with bradykinin evoked an increase in the substance P release from the cultured dorsal root ganglion neurons (Tang et al., 2005). As shown in Fig. 1C, a 3-h treatment with bradykinin (1μM) caused a significant increase in the substance P release from cultured dorsal root ganglion neurons. However, the substance P release in response to bradykinin stimulation was

not significantly inhibited by the pretreatment with olopatadine. In comparison to the control group, olopatadine alone seemed to have little effect on the substance P release from cultured dorsal root ganglion neurons.

3.2. Effects of olopatadine on the substance P release evoked by potassium or capsaicin from cultured adult rat dorsal root ganglion neurons pretreated with bradykinin

As demonstrated in a previous work (Oshita et al. 2005; Tang et al. 2006), a 3-h pretreatment with bradykinin (100 nM) caused a decrease in the substance P release induced by a high concentration of potassium, and also produced a significant increase in the substance P release induced by capsaicin from cultured dorsal root ganglion neurons. In the present study, we examined the effects of olopatadine (0.1–50 μM) on the inhibitory effect of potassium-evoked substance P release by long-term pretreatment with bradykinin (3 h). As shown in Fig. 2A, the pretreatment with olopatadine (0.1–50 μM) was not observed to show any significant change in the suppression of the potassium-evoked substance P release by bradykinin from cultured dorsal root ganglion neurons.

To further study whether the enhancement of capsaicin-induced substance P release by bradykinin is regulated by the pretreatment with olopatadine, we examined the effect of olopatadine (0.1–50 μM) on the enhancement of the effect by the long-term pretreatment with bradykinin. We calculated a 50% inhibitory concentration (IC_{50}) value for olopatadine using the Hill plot method with linear regression, and thus found that olopatadine (12.5 μM) exhibited a significant inhibition in the enhancement of capsaicin-induced substance P release by bradykinin from cultured dorsal root ganglion neurons (Fig. 2B).

4. Discussion

The present experiments provide evidence that the second-generation antihistamine olopatadine exhibits a significant inhibitory effect on the enhancement of capsaicin-induced

substance P release by bradykinin from cultured dorsal root ganglion neurons. As far as we know, the present study provides the first direct evidence for the pharmacological effects of olopatadine on the substance P release induced by various noxious stimuli from primary afferent neurons.

Ikemura et al. (1996) reported that olopatadine has been suggested to inhibit the substance P release from the evoked contractile responses in a tracheobronchial smooth muscle preparation of guinea-pig by electrical field stimulation. However, even though the electrical field stimulation appeared to be similar to that of capsaicin or a high concentration of potassium, we could not demonstrate olopatadine inhibited the substance P release from cultured dorsal root ganglion neurons. The discrepancies between the data from Ikemura et al. (1996) and ours in this study might be attributable to several factors, including the cell type and the stimulation parameters, thus suggesting that the mechanisms of substance P release depends on the specific tissue that the neurons innervate. We have previously demonstrated that the substance P release evoked by potassium is dependent of the IP_3-sensitive calcium release, but the capsaicin-evoked substance P release through the activation of TRPV1 is independent of it. These data therefore suggest that olopatadine is unlikely to involve in potassium channel block and the inhibition of TRPV1 activation, and it is also unlikely to exert an inhibitory effect on the IP_3-sensitive calcium release in cultured dorsal root ganglion neurons.

In contrast to the short-term treatment with potassium or capsaicin, the long-term treatment with bradykinin can induce the slow substance P release through the activation of the bradykinin B_2 receptor, ERK-1 and COX1/2 (Tang et al., 2005; Inoue et al., 2006; Tang et al., 2006). The 30-min pretreatment with olopatadine (0.1-50 μM) did not exhibit an inhibitory effect on the slow substance P release from cultured dorsal root ganglion neurons. This finding is partly consistent with the observation by Yanni et al. (1996) who showed that olopatadine did not display an inhibitory effect on the activation of COX in human

conjunctival mast cell preparations in vitro. We therefore suppose that olopatadine should neither inhibit the induction of COX1/2 nor the activation of ERK-1 by bradykinin in cultured dorsal root ganglion neurons.

However, after we pretreated the cultured dorsal root ganglion neurons by bradykinin for 3 h, and then continuously stimulated the cultured dorsal root ganglion neurons using potassium (30 mM) or capsaicin (30 nM) in Krebs-HEPES buffer for 10 min at 37°C, we observed two different responses induced by bradykinin in the substance P release: the suppression of the potassium-evoked substance P release and the enhancement of the capsaicin-evoked substance P release. In this study, olopatadine exhibited an inhibitory effect on the enhancement of capsaicin-evoked substance P release by bradykinin in a dose-dependent manner with 12.5 μM of IC_{50} value, without any effect on the suppression of potassium-evoked substance P release (Figs. 2A and B). The observed inhibitory effect of olopatadine is quite similar to our previous finding that the enhancement of capsaicin-induced substance P release by bradykinin is completely reversed by the treatment of cycloheximide (a potent inhibitor of protein synthesis). The inhibitions of substance P release by olopatadine and by cycloheximide suggest that olopatadine might attenuate the sensitization of TRPV1 by preventing the de novo protein synthesis or the release of other intracellular effectors which are still as yet unidentified.

We do not yet know the exact target molecule(s) of olopatadine which induced the inhibitory effect on the enhancement of capsaicin-evoked substance P release by bradykinin in cultured dorsal root ganglion neurons. As a tentative explanation, we suppose that the pharmacological effect of olopatadine did not involve either the inhibition of the activation of bradykinin B_2 receptor, ERK-1, COX1/2 or cyclic-AMP-dependent protein kinase A by bradykinin, nor directly block the activation of TRPV1 by capsaicin, but olopatadine may have an indirect inhibitory effect on the substance P release by the sensitization of TRPV1 in dorsal root ganglion neurons. To clarify the possible target molecule(s) of olopatadine, we

will examine its pharmacological effect(s) on the activation of protein kinase C which have been shown to be involved in the sensitization of TRPV1 (Amadesi et al., 2004; Ferreria et al., 2005) in future experiments. However, olopatadine can inhibit the enhancement of capsaicin-evoked substance P release, we may therefore consider it effective for the treatment of chronic inflammatory conditions to suppress the overflow of the substance P release by the sensitization of the sensory neurons.

5. Conclusions

In conclusion, the present study performed a pharmacological investigation of olopatadine on the release of substance P induced by capsaicin, potassium or/and bradykinin from cultured adult rat dorsal root ganglion neurons. This compound significantly inhibited only the enhancement of capsaicin-induced substance P release by bradykinin. Although the substance P release from dorsal root ganglion neurons remains to be elucidated, olopatadine is therefore potentially considered to be a promising novel therapeutic agent for the treatment of allergic and inflammatory reactions of allergic diseases for clinical use.

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Figure legends Tang and Nakata

Fig. 1. Effects of olopatadine on the substance P release induced by potassium, capsaicin or bradykinin from cultured adult rat dorsal root ganglion neurons.

Except for some cells treated only by the control or olopatadine alone, all other cells were treated with 30 mM potassium (A), 100 nM capsaicin (B) and 1 μM bradykinin (C) with or without olopatadine (0.1-50 μM) in either Krebs-HEPES buffer for 10 min or in DMEM (serum free) for 3 h. The data are expressed as the means ± S.E.M. (bars) from 3 (A), 3 (B) or 3-4 (C) separate experiments. *** denotes $P < 0.001$.

Fig. 2. Effects of olopatadine on the potassium- or capsaicin-evoked substance P release from cultured adult rat dorsal root ganglion neurons pre-exposured to bradykinin for 3 h.

Except for some cells treated only by the control, all other cells were pretreated with bradykinin (100 nM), either alone or together with olopatadine (0.1-50 μM) in DMEM (serum free) for 3 h. Next, those cells were stimulated by either potassium (30 mM) or capsaicin (30 nM) plus peptidase inhibitors or by peptidase inhibitors only in Krebs-HEPES buffer for 10 min. The data are expressed as the means ± S.E.M. (bars) from 3 (A) or 6-15 (B) separate experiments. ** and *** denote $P < 0.01$ and 0.001, respectively.

Fig. 1A Tang and Nakata

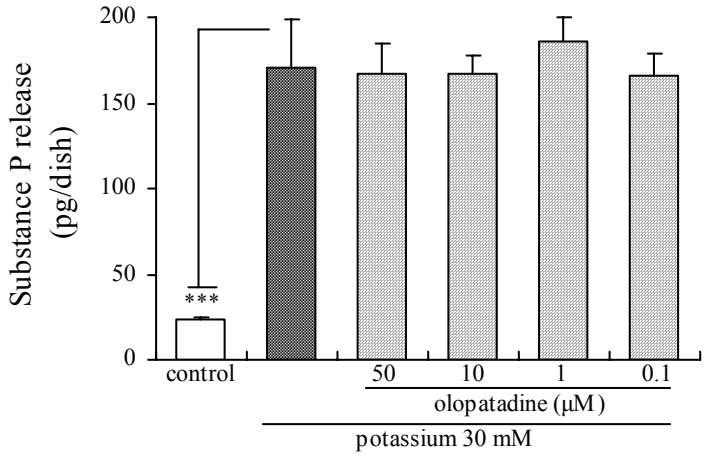
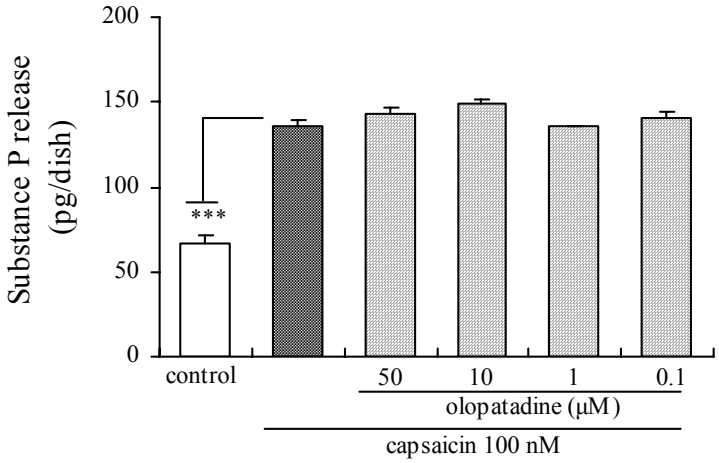


Fig. 1B



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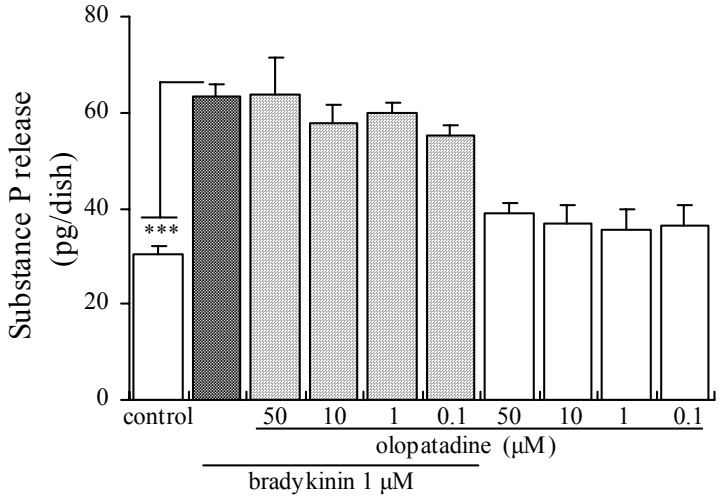

Fig. 2A Tang and Nakata

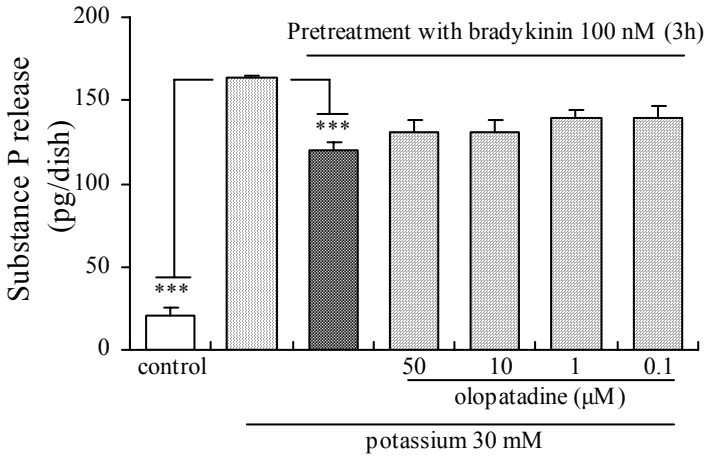

Fig. 2B