短　報

Different effects of a bradykinin B_1 selective antagonist and B_2 selective antagonist on Mas-related G protein-coupled receptors in rat mast cells, and inhibitory effects of lithium carbonate

Tamiko Suzuki-Nishimura, Shiori Otani, Arisa Yamamoto,
Tomokazu Nishida
Department of Pharmacology II, School of Pharmacy, Shujitsu University
(Received Oct 10, 2019，accepted Dec 17, 2019)

Abstract Mas-related G protein-coupled receptors (MRGPRs) in mast cells are the targets of pseudoallergic drug reactions. Bradykinin (BK) and its analogues exert agonistic effects on mast cells; however, it currently remains unclear whether these effects depend on the activation of MRGPRs. In the present study, we examined the effects of the BK analogues on rat mast cells in order to clarify the influence of amino acid sequences on mast cell activation via MRGPRs. The B1 selective agonist released histamine, whereas the B1 selective antagonist did not inhibit histamine release induced by BK and the B1 agonist. The B2 antagonist did not exert inhibi1tory effects. The B2 antagonist exerted agonistic effects, whereas the B1 selective antagonist did not. The agonistic effects of the B1 agonist and B2 antagonist depended on the number of cationic amino acids in the amino acid sequence. The effects of lithium carbonate were investigated, and it was found to inhibit histamine release induced by the B2 antagonist, suggesting that the B2 antagonist is a MRGPR activator. The MRGPRs activated by selective B1 agonist and selective B2 antagonist are one of the pathways that induce pseudoallergic drug reactions. Lithium carbonate will contribute to the identification of candidates that control pseudoallergic drug reactions.

Key words: Mas-related G protein-coupled receptor, mast cell activation, bradykinin, bradykinin agonist, bradykinin antagonist

Introduction

Mast cells express Mas-related G protein-coupled receptors (MRGPRs), mouse Mrgprb2, rat MrgprC, and human MRGPRX2, which are the targets of allergic, anaphylactic, and pseudoallergic drug reactions[1-3]. Previous studies demonstrated that substance P (SP)[4] and bradykinin (BK)[5] released histamine from rat mast cells in an IgE-independent manner prior to the discovery of MRGPRs in mast cells. BK has 2 classes of receptors, BK B_1 receptor (B1) and BK B_2 receptor (B2). Devillier et al. reported that B2 selective antagonists were potent releasers of histamine from rat mast cells and did not exert any antagonistic effects[6]. Azimi et al. recently showed that the neurokinin-1 antagonist aprepitant did not inhibit the activation of human MRGPRX2, but tripeptide

QWF (Boc-Gln-D-Trp (Formyl)-Phe benzyl ester trifluoroacetate) inhibited β-hexosaminidase release from a human mast cell line activated by SP after blocking the binding of SP to MRGPRX2[7]. On the other hand, the B2 antagonist icatibant did not exert inhibitory effects on human mast cell line, suggesting that it caused pseudoallergic drug reaction on MRGPRX2[8,9]. In the present study, we examined the effects of a B1 selective agonist, B1 selective antagonist, and B2 selective antagonist (Table 1) on rat peritoneal mast cells in order to clarify the influence of amino acid sequences of bradykinin analogues on mast cell activation via MRGPRs. The effects of lithium carbonate, which is an inhibitor of histamine release induced by compound 48/80, SP, BK, mastoparan and melittin[10] were also investigated.

Materials and Methods

Chemicals

BK acetate salt, the B1 selective agonist [Lys-des-Arg^9]-BK, and lithium carbonate (Li_2CO_3) were purchased from Sigma-Aldrich (U.S.A.). The B1 selective antagonist des-Arg^9-[Leu^8]-BK and B2 selective antagonist [Thi^{5,8}-D-Phe^7]-BK were from Peptide Institute Inc. (Osaka, Japan). Thi indicates thienylalanine.

Table 1 Amino acid sequences of bradykinin, the B1 selective agonist, B1 selective antagonist, and B2 selective antagonist used in the present study.

Bradykinin and analogues	Amino acid sequence
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
B1 selective agonist	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe
B1 selective antagonist	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu
B2 selective antagonist	Arg-Pro-Pro-Gly-Thi-Ser-D-Phe-Thi-Arg

*Thi indicates thienylalanine.

Assay of histamine release

Histamine release from rat peritoneal mast cells was measured as described previously[11]. Mast cells from the peritoneal cavity of male Sprague Dawley rats (300-450 g) were purified using Percoll and suspended in HEPES-buffered Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 12 mM HEPES, 1 mM $MgC1_2$, 0.3 mM $CaC1_2$, 5.6 mM dextrose, and 0.03% bovine serum albumin, pH 7.4). Mast cells were preincubated with the B1 selective antagonist or Li_2CO_3 in HEPES-buffered Tyrode's solution at 37°C for 5 min, and 50 μM BK or 50 μM of the B2 selective antagonist was then added. After a 5 min incubation, the amount of histamine released was analyzed using a fluorometric assay[12].

Mast cells were also preincubated with 3 mM Li_2CO_3 at 37°C for 5 min before the incubation with 50 μM of the B2 selective antagonist for 5 min.

Animal studies

All animal studies were previously approved by the Animal Care Committee of Shujitsu University (#018-001 and #18-002) and conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication #85-23). Sprague-Dawley male rats were used in animal studies.

Statistical analysis

Values are box-and-whisker plots of the replicated experiments. Statistical analyses were performed using repeated measures ANOVA with Dunnett's test employing P=0.05 as the upper limit of significance.

Results

1. The B1 selective antagonist did not inhibit histamine release induced by BK.

At concentrations at 10, 50, and 100 μM, the B1 selective antagonist did not enhance spontaneous histamine release (Fig. 1). The effects of the B1 selective antagonist on histamine release induced by 50 and 100 μM BK were examined. The B1 selective

antagonist did not inhibit histamine release induced by 50 or 100 μM BK (Fig. 2). Therefore, we examined the effects of the B1 selective antagonist on histamine release induced by the B1 selective agonist. The B1 selective antagonist did not enhance or inhibit histamine release induced by the B1 selective agonist (Fig. 3).

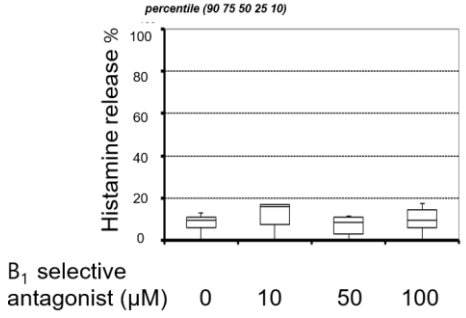

Fig. 1 Histamine release induced by the B1 selective antagonist. Mast cells were preincubated with 10, 50, and 100 μM of the B1 selective antagonist in HEPES-buffered Tyrode's solution at 37°C for 10 min. The amount of histamine released was analyzed, n=6, Dunnett's analysis, **P<0.01 Repeated measures ANOVA.

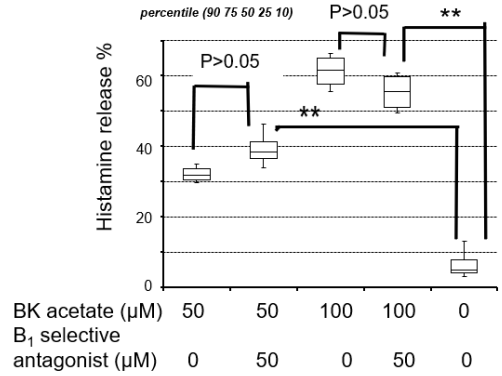

Fig. 2 Effects of the B1 selective antagonist on histamine release induced by 50 μM BK. Mast cells were preincubated with 50 μM of the B1 selective antagonist in HEPES-buffered Tyrode's solution at 37°C for 5 min, and 50 μM BK was then added. After a 5 min incubation, the amount of histamine released was analyzed, n=4, Dunnett's analysis, **P<0.01 Repeated measures ANOVA.

2. The B2 selective antagonist released histamine from rat mast cells, and lithium carbonate inhibited this release.

Since the B2 selective antagonist induced the release of histamine, it was not possible to examine the inhibitory effects of the B2 selective antagonist on histamine release induced by BK (data not shown). Lithium carbonate dose-dependently inhibited histamine release induced by BK, with IC_{50} of 1.50 mM[10], therefore, the effects of lithium carbonate on histamine release induced by the B2 selective antagonist were examined. Lithium carbonate inhibited histamine release induced by the B2 selective antagonist, with an IC_{50} of approximately 2.25 mM (Fig. 4).

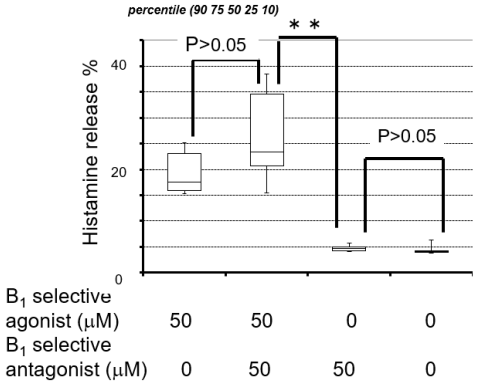

Fig. 3 Effects of the B1 selective antagonist on histamine release induced by 50 μM of the B1 selective agonist. Mast cells were preincubated with 50 μM of the B1 selective antagonist in HEPES-buffered Tyrode's solution at 37°C for 5 min, and 50 μM of the B1 selective agonist was then added. After a 5 min incubation, the amount of histamine released was analyzed, n=6, Dunnett's analysis, **P<0.01 Repeated measures ANOVA

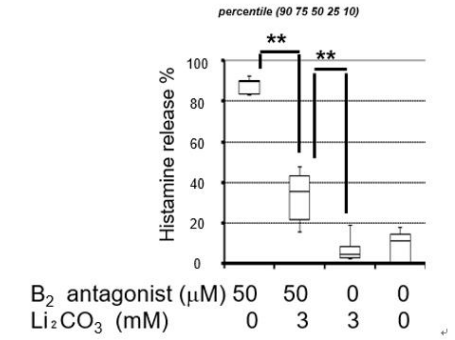

Fig. 4 Effects of 3 mM lithium carbonate on histamine release induced by 50 μM of the B2 selective antagonist. Mast cells were preincubated with 3.0 mM lithium carbonate in HEPES-buffered Tyrode's solution at 37°C for 5 min, and 50 μM of the B2 antagonist was then added. After a 5 min incubation, the amount of histamine released was analyzed, n=6, Dunnett's analysis, **P<0.01 Repeated measures ANOVA.

Discussion

Before the presence of MRGPRs on mast cells, which play roles in mast cell activation, was demonstrated, histamine release by kinins was either a non-specific effect not mediated by receptors or, if receptors were

involved, these sites were suggested to differ from other kinin receptors (i.e. B_1 or B_2)[6]. SP and BK are kinins that induce the release of histamine, and two B1 antagonists, des-Arg^{10}-[Leu^{9}]kallidin and des-Arg^{9}-[Leu^{8}]BK, were shown to promote histamine release and acted only as agonists[6]. The order of potency of these analogues was as follows: [Thi^{6,9,}D-Phe^{8}]kallidin > [Thi^{5,8,}D-Phe^{7}]BK > des-Arg^{10}-[Leu^{9}]kallidin > des-Arg^{9}-[Leu^{8}]BK [6]. In the present study, we examined the effects of a B1 selective agonist, B1 selective antagonist, and B2 selective antagonist. These peptides did not exert inhibitory effects. The agonistic effects observed depended on the number of cationic amino acids in the sequence, suggesting that at least 2 positively charged amino acids are needed to induce MRGPR activation (Table 1). The B1 selective antagonist des-Arg^{9}-[Leu^{8}]BK did not release histamine, suggesting that one Arg residue is insufficient to activate MRGPRs. Moreover, the B1 selective antagonist did not inhibit histamine release induced by BK and the B1 selective agonist. Devillier et al. previously reported that the agonistic effects of the B1 antagonist des-Arg^{9}-[Leu^{8}]BK were weak[6], which is inconsistent with the present results. Under our assay conditions that the B1 selective antagonist des-Arg^{9}-[Leu^{8}]BK did not affect spontaneous histamine release, we were able to examine the effects of the B1 selective antagonist on histamine release induced by BK and the B1 agonist (Figs. 1, 2, and 3). In the present study, the B2 antagonist [Thi^{5,8,}D-Phe^{7}]BK stimulated histamine release. Therefore, the inhibitory effects of lithium carbonate were investigated (Fig. 4). Lithium carbonate is one of the treatments for bipolar disorder; however, its mechanisms of action remain unclear. We previously reported that lithium carbonate inhibited histamine release from rat mast cells induced by compound 48/80, SP, BK, mastoparan, melittin, and GlcNAc-GlcNAc specific lectin *Datura stramonium* agglutinin, suggesting that it is one of the inhibitors of MRGPRs[10]. Since lithium carbonate inhibited histamine release induced by the B2 antagonist, the B2 antagonist appears to stimulate MRGPRs.

The high activity levels of BK and its analogues suggested that their potencies to induce the release of histamine from rat mast cells increases in parallel with the number of positively charged amino acids (Lys and Arg), and the N-terminal arginine appears to be important[13]. On the other hand, peptide sequences are not necessary to activate the MRGPRs of mast cells by BK because icatibant, a B2 antagonist with no peptide chain, promoted mast cell degranulation via MRGPRX2 and induced a pseudoallergic drug reaction[8,9]. It is important to note that the degranulation and intracellular calcium ion increases induced by icatibant were not observed in MRGPRX2 knockdown culture cells.

Mast cells are activated in not only IgE-dependent, but also IgE-independent manners. The IgE-independent pathway is due to anaphylactic and pseudoallergic drug reactions because many polycationic chemicals stimulate this pathway. Regarding many polycationic chemicals, the IgE-independent pathway appears to have been misunderstood as a non-specific effect not mediated by receptors for a long time. MRGPRs are predominantly expressed in sensory neurons and mast cells, and are the target of polycationic chemicals to induce pseudoallergic drug reactions[14]. The selective B1 agonist and selective B2 antagonist are also activators. Furthermore, the inhibitory concentration of lithium carbonate on MRGPRs was shown to be close to its clinical and addictive doses[15]. Lithium carbonate as an inhibitor of mast cells has potential as a candidate to control anaphylactic and pseudoallergic drug reactions.

Conflicts of Interest: The authors declare no conflicts

of interest.

References

1) McNeil B D, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, Dong X: Identification of a mast cell specific receptor crucial for pseudo-allergic drug reactions, Nature, 519(7542), 237–241(2015).

2) Zylka MJ, Dong K, Southwell AL, Anderson DJ: Atypial expansion in mice of the sensory neuron-specific Mrg G protein-coupled receptor family. Proc Natl Acad Sci, 100: 10043-10048(2003)

3) Kashem SW, Subramanian H, Collington SJ, Magotti P, Lambris JD, Ali H: G protein coupled receptor specificity for C3a and compound 48/80 induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. Eur J Pharmacol, 668(1-2): 299-301(2011).

4) Heaney LG, Cross LIM, Stanford CF, Ennis M: Substance P induces histamine release from human pulmonary mast cells. Clin Exp Allergy 25: 179-186(1995).

5) Ito K, Sakamoto T, Hayashi Y, Morishita M, Shibata E, Sakai K, Takeuchi Y, Torii S: Role of tachykinin and bradykinin receptors and mast cells in gaseous formaldehyde-induced airway microvascular leakage in rats. Eur J Pharmacol. 307(3):291-398(1996).

6) Devillier P, Renoux M, Drapeau G, Regoli D: Histamine release from rat peritoneal mast cells by kinin antagonists. Eur J Pharmacol, 149(1-2):137-140(1988).

7) Azimi E, Reddy VB, K-T C Shade, Anthony RM, Talbot S, Pereira PJS, Lerner EA. Dual action of neurokinin-1 antagonists on Mas-related GPCRs. JCI Insight, 1(16): e89362(2016).

8) Alkanfari I, Gupta K, Jahan T, Ali H: Naturally occurring missense MRGRPX2 variants display loss of function phenotype for mast cell degranulation in response to substance P, hemokinin-1, human β-defensin-3 and Icatibant. J Immunol, 201:343-349(2018).

9) Roy S, Ganguly A, Hague M, Ali H: Angiogenic host defense peptide AG-30/5C and bradykinin B2 receptor antagonist Icatibant are G protein biased agonists for MRGPRX2 in mast cells. J Immunol, 202:1229-1238(2019).

10) Suzuki-Nishimura T, Baba M, Kodama Y, Otani S, Yamamoto A, Nishida T, Tanaka K: Histamine release from rat peritoneal mast cells induced by Mas-related G protein-coupled receptor activators was inhibited by lithium carbonate, whereas that by the calcium ionophore A23187 was not. Shujitsu Ronso, 49; 201-210 (2019).

11) Suzuki-Nishimura T, Uchida MK: Prostaglandin D_2 generation by rat peritoneal mast cells stimulated with *Datura stramonium* agglutinin and its inhibition by haptenic sugar and wheat germ agglutinin. Jpn J Pharmacol, 90(1): 77-80 (2002).

12) Shore PA, Burkhalter A, Cohn VH Jr: A method for the fluorometric assay of histamine in tissues, J Pharmacol Exp Ther, 127, 182-186(1959).

13) Devillier P, Drapeau G, Renoux M, Regoli D: Role of the N-terminal arginine in the histamine-releasing activity of substance P, bradykinin and related peptides. Eur J Pharmacol, 168(1):53-60 (1989).

14) Solinski HJ, Gudermann T, Breit A: Pharmacology and signaling of MAS-related G protein-coupled receptors. Pharmacol Rev, 66:570-597 (2014).

15) Prescribing information of LITHOB1D® (Lithium Carbonate, USP).