RBL-2H3　　　　　　　　-
(GABA)

Suppressive Effect of γ-Aminobutyric Acid (GABA) on Histamine Release in Rat Basophilic RBL-2H3 Cells

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Summary

Basophils and mast cells play a crucial role in immediate allergic reactions. These cells release various inflammatory mediators including histamine in response to binding of antigen to its specific IgE bound to the high affinity IgE receptor on the cell surface. In the present study, we examined the influence of γ-aminobutyric acid (GABA) on histamine release in rat basophilic leukemia RBL-2H3 cells. After sensitization with anti-dinitrophenyl (DNP) IgE, challenge of RBL-2H3 cells with DNP-conjugated bovine serum albumin (DNP-BSA) caused a release of histamine in the cell suspension. The IgE-mediated histamine release was suppressed by GABA, with about 60% inhibition at 100 μM. In the same condition, baclofen, a $GABA_B$ receptor agonist, also inhibited histamine release. At a concentration of 100 μM, the inhibition rate of baclofen was approximate 80% of histamine release induced by DNP-BSA stimulation. In addition, these inhibitory effects of GABA and baclofen were attenuated by the addition of CGP35348, a $GABA_B$ receptor antagonist. These results suggest that GABA suppresses degranulation in basophils and mast cells via $GABA_B$ receptor.

γ-Aminobutyric acid (GABA), which is synthesized by glutamic acid decarboxylase from glutamic acid, is the main inhibitory neurotransmitter and plays a key role in modulating neuronal activity in mammalian central nervous system. Additionally, GABA is considered to be a multifunctional molecule that has different situational functions in some nonneuronal tissues. GABA exerts its actions via three pharmacologically and structurally different classes of GABA receptors: $GABA_A$, $GABA_B$ and $GABA_C$ receptors (Bowery and Enna, 2000). $GABA_A$ and $GABA_C$ receptors are ligand gated Cl^- channels activated by the binding of GABA, whereas $GABA_B$ receptor belongs to members of the seven transmembrane G-protein-coupled receptor superfamily (Bowery *et al.*, 2002).

It is reported that oral administration of GABA to animal models for hypertension resulted in the decrease of their blood pressure without affecting their heart rates. In spontaneously hypertensive rats, low-dose (0.3 to 1.0 mg/kg, i.d.) GABA had a hypotensive effect, which may result from attenuation of sympathetic transmission through the activation of $GABA_B$ receptors at presynaptic or ganglionic sites (Kimura *et al.*, 2002). The existence of $GABA_A$ and $GABA_B$ receptors has been demonstrated in the mammalian intestine and respiratory tract (Gentilini, *et al.*, 1995, Luzzi. *et al.*, 1985, 1987). Recently, it is reported that the $GABA_B$ receptor is functionally expressed in neutrophils, and acts as a chemoattractant receptor (Rane *et al.*, 2005).

Previous study revealed that GABA, through an activation of $GABA_B$ receptors, inhibits antigen-induced contractions of tracheal strips isolated from sensitized guinea-pigs. Tracheal contractile response is closely related with histamine release in basophils and mast cells. Both types of cells appear to play an important role by producing and releasing mediators such as histamine, leukotriene and cytokines during anaphylactic responses. These mediators are secreted from basophils and mast cells in response to activation through the specific receptors against immunoglobulin (Ig)E, cytokines, complements or other functional molecules. It is therefore possible that GABA-mediated inhibition of tracheal contractions is directly due to inhibition of histamine release in mast cells. However, there is few information available for efficacies of GABA on basophils and mast cells.

In the present study, we examined the inhibitory effect of GABA on histamine release in rat basophilic RBL-2H3 cells. If so, the possibility that GABA inhibits the release of histamine via $GABA_B$ receptor in RBL-2H3 cells was pharmacologically assessed by using baclofen, a $GABA_B$ receptor agonist, and CGP35348, a $GABA_B$ receptor antagonist.

MATERIALS AND METHODS

Reagents

GABA is purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Baclofen, CGP35348, mouse

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anti-DNP IgE, DNP-BSA and ionomycin were bought from Sigma Chemical Co. (St Louis, MO).

Cells and cell culture

Rat basophilic leukemia RBL-2H3 cells, obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan), were maintained in RPMI-1640 medium (Sigma Chemical Co.) supplemented with 10% fetal bovine serum (Roche Diagnostics K.K.), 100 U/ml penicillin G, 100 mg/ml streptomycin and 15 mM HEPES buffer. Cell cultures were incubated at 37℃ in a humidified atmosphere with 5% CO_2. The medium was changed in maintained cell cultures every 2 to 3 days.

Histamine release

RBL-2H3 cells were washed with Ca^{2+}- and Mg^{2+}-free Tyrode's buffer and resuspended in RPMI-1640 medium at the density of 1.0×10^7 cells/ml. After sensitization with mouse anti-DNP IgE at 10 μg/ml for 2 h, cells were washed twice, and, in the presence or absence of GABA or bclofen, stimulated with DNP-BSA at a final concentration of 1 μg/ml in Tyrode's buffer (including Ca^{2+} and Mg^{2+}).The final cell density and volume were 1.0×10^6 cells/ml and 1.1 ml, respectively. These reaction mixtures were incubated at 37℃ for 5 to 45 min and then, in order to terminate cellular reactions, cooled at 4℃ for 5 min. After centrifugation at 300 × g for 5 min, 1 ml of the supernatant was recovered and the amount of histamine was measured by fluorometric assay as described below. In addition, to determine the amounts of total cellular histamine, RBL-2H3 cells were sonicated in Tyrode's buffer at 4℃ for 30 sec. After centrifugation at 10000 × g for 20 min, the supernatant was recovered and subjected to histamine measurement.

Histamine measurement

Histamine content was measured by fluorometric assay (Shore et al., 1959). After mixing 500 μl of the supernatant with 0.19 g of NaCl and 125 μl of 1 N NaOH, 1.25 ml of the 3 : 2 (v/v) mixture of 1-butanol and chloroform was added, and mixed for 5 min. The solution was centrifuged for 5 min at 300 × g and 1 ml of the upper organic solvent layer was recovered. The recovered layer was mixed with 500 μl of heptane and 375 μl of 0.1 N HCl. After centrifugation at 300 × g for 5 min, 250 μl of the lower HCl layer was recovered, mixed with 37.5 μl of 1 N NaOH and the subsequent product was added with 25 μl of 0.2% o-phthalaldehyde dissolved in methanol for 5 min at room temperature to yield a fluorescent product. The reaction was terminated by adding 35 μl of 0.5 N H_2SO_4 and fluorescence intensity was measured using a spectrofluorophotometer (F3010, HITACHI, Tokyo, Japan) with the excitation set at 360 nm and the emission set at 450 nm. Histamine content was quantitated by comparison to a standard curve generated by coupling histamine dihydrochloride to o-phthalaldehyde. The inhibition rate of histamine release (%) was calculated as a formula showed below.

Inhibition rate of histamine release (%)

$$= |(Normal - Control) - (Sample - Control)|/(Normal - Control) \times 100$$

Control：DNP-BSA （−）, test sample （−）
Normal：DNP-BSA （＋）, test sample （−）
Sample：DNP-BSA （＋）, test sample （＋）

RESULTS AND DISCUSSION

Basophils and mast cells are critical effector cells in allergic diseases. At type I allergy, binding of antigen to its specific IgE bound to the high affinity IgE receptor (FcεRI) on the surface of basophils and mast cells leads to the release of chemical mediators, such as histamine and arachidonic acid metabolites. In this study, we examined the effect of GABA on histamine release in RBL-2H3 cells, an available model for studying IgE-mediated degranulation. The cells express FcεRI and release histamine upon stimulation with antigen and its specific IgE. After sensitization with anti DNP-IgE, stimulation with DNP-BSA caused histamine release with an average value of 18% (data not shown). DNP-BSA-induced histamine release was inhibited by GABA (10 - 100 μM) in a dose-dependent manner (Fig. 1). In addition, the inhibitory effect of GABA was mimicked by baclofen, a $GABA_B$ receptor agonist (Fig. 2). In this experiment, GABA and baclofen at the concentration of 100 μM showed about 60% and 80% inhibition of histamine release, respectively. These results suggest that GABA has a potential to suppress degranulation in basophils and mast cells and serves as effective therapeutic agent for allergic diseases.

Next, in order to investigate whether GABA modulates histamine release via $GABA_B$ receptor in RBL-2H3 cells, the
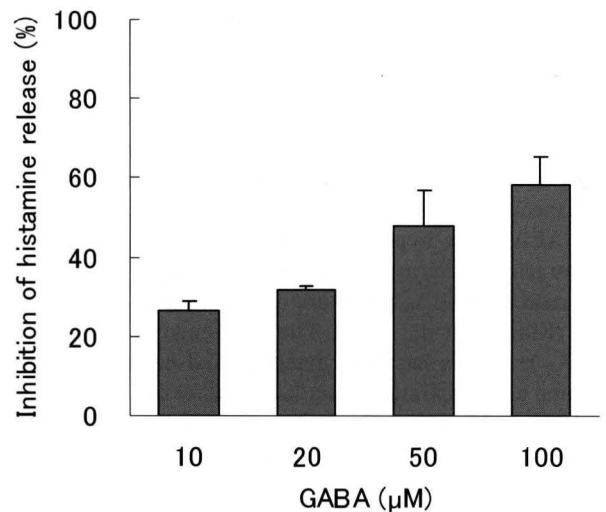

Fig.1. Effect of GABA on histamine release in RBL-2H3 cells. In the presence of GABA, cells were stimulated by DNP-BSA. Results were expressed as inhibition percentage of histamine release (mean ± SE) and are representative of four different experiments.
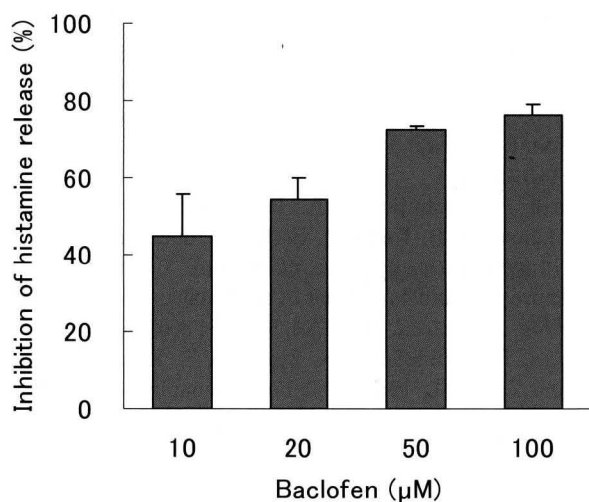
Fig.2. Effect of Baclofen on histamine release in RBL-2H3 cells. In the presence of baclofen, cells were stimulated by DNP-BSA. Results were expressed as inhibition percentage of histamine release (mean ± SE) and are representative of four different experiments.

effect of CGP35348, a GABA_B receptor antagonist, was examined. In the presence of CGP35348 at 50 μM, the inhibitory action of GABA and baclofen were significantly reduced (Fig. 3). In agreement with our data, RBL-2H3 cells might express functional GABA_B receptor on the cell surface. It is well known that several cellular processes are influenced by GABA_B receptor, which is a member of the large group of G-protein-coupled membrane receptors. Therefore, the particular G-protein might be involved in the GABA-mediated inhibition of histamine release.

Although GABA_B receptors play a critical role in the central nervous system, the molecules have been detected in peripheral tissues such as airway smooth muscle(Osawa et al., 2006), pancreatic β-cells(Brice et al., 2002), cardiomyocytes(Lorente et al., 2000), and chondrocytes (Tamayama et al., 2005). However, the expression of GABA_B receptors in mast cells and basophils has never been described. Further experiments are necessary to detect GABA_B receptor expression and to determine the role of G-protein in FcεRI-mediated signal transduction in basophils and mast cells.

Previous study revealed that baclofen reduces synaptic transmission by inhibition of vesicle priming (Sakaba and Neher, 2003). It has been reported that baclofen inhibits exocytosis in rat pancreatic β-cells by G-protein-dependent activation of calcineurin (Braun et al., 2004). It is likely that GABA_B receptor activation could be able to suppress exocytosis or vesicle trafficking in various cell types. However, there are few information concerning the effect of GABA on degranuration in basophils and mast cells. As shown here, we demonstrated GABA and baclofen inhibited antigen/IgE-induced histamine release by 40 to 80%. In contrast with our results, Gentilini et al. repoted that, in
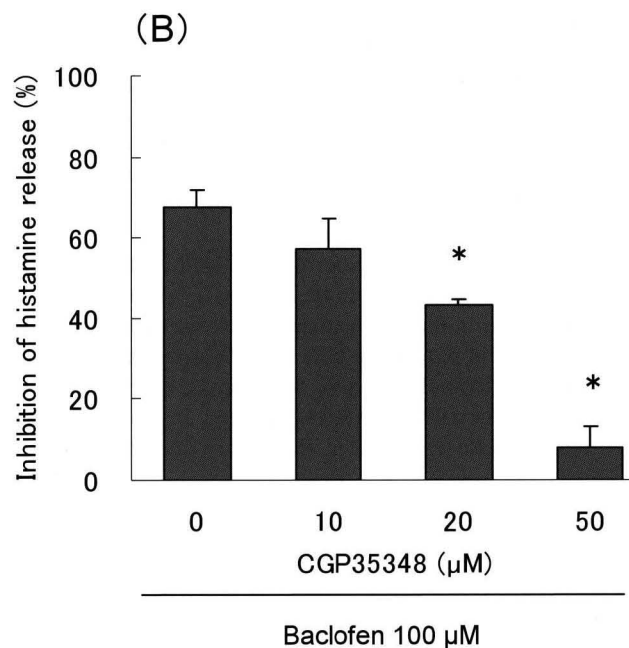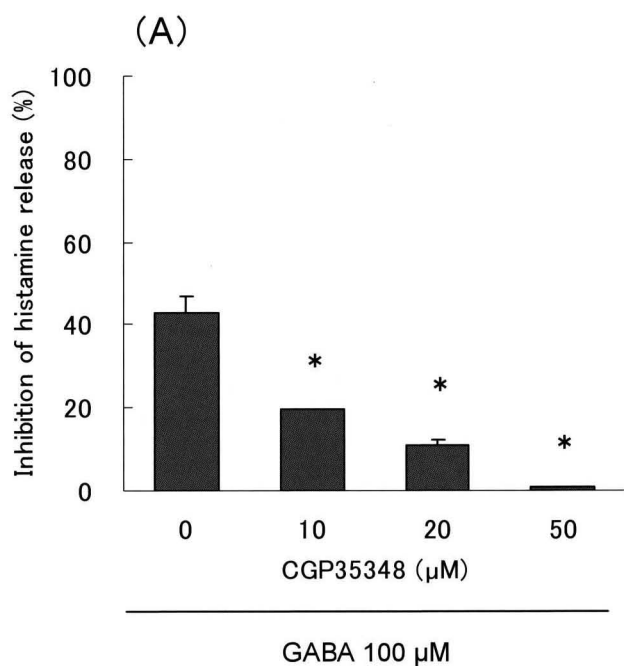

Fig.3. Influence of CGP35348 on GABA-mediated and baclofen-mediated inhibition of histamine release in RBL-2H3 cells. In the presence of GABA (A) or baclofen (B), cells were stimulated by DNP-BSA with or without CGP35348. Results were expressed as inhibition percentage of histamine release (mean ± SE) and are representative of four different experiments. The asterisk represents a P < 0.05 compared to 100 μM GABA or baclofen treatment without CGP35348.

serosal mast cells isolated from ovoalbumin-sensitized guinea-pigs, GABA and baclofen inhibited antigen/IgE-induced histamine release by only 10% and 19% at 1 mM (Gentilini *et al.*, 1995). The reason for this discrepancy is not clear, but it may be due to a difference in the expression level of functional GABA$_B$ receptor, the state of cells and the experimental condition. Alternatively, GABA and baclofen affect contaminating cells, and these cells may exert some influences on histamine release in guinea-pig serosal mast cells.

Taken together, there raises the possibility that GABA might modulate basophils and mast cells to suppress histamine release through GABA$_B$ receptor, where G-protein plays an important role. Further investigations on this possibility are ongoing in our laboratory. RBL-2H3 cells could be an adequate model for evaluating GABA$_B$ receptor-mediated-signals, especially for the role of G-protein signaling versus FcεRI-mediated signaling in basophils and mast cells.

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ラット好塩基球様 RBL-2H3細胞における γ- アミノ酪酸（GABA）の ヒスタミン遊離抑制作用

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要　約

　　好塩基球やマスト細胞は、即時型アレルギー反応において重要な役割を担う。これらの細胞は、細胞表面上の高親和性 IgE レセプターに結合した特異的 IgE にアレルゲンが結合すると、脱顆粒によりヒスタミンなどの様々な炎症性メディエーターを遊離する。本研究では、ラット好塩基球様 RBL-2H3細胞を用い、ヒスタミン遊離に対する γ- アミノ酪酸（GABA）の影響を検討した。RBL-2H3細胞は、抗ジニトロフェニル IgE（DNP-IgE）により感作した後、抗原としてジニトロフェニル化ウシ血清アルブミン（DNP-BSA）を添加することによりヒスタミンを遊離した。この IgE を介したヒスタミン遊離は、GABA により抑制され、濃度100 μM において約60%の抑制率を示した。同様の実験条件において、$GABA_B$ レセプター作動薬であるバクロフェンもまた、ヒスタミン遊離を抑制した。濃度100 μM において、バクロフェンの抑制率は DNP-BSA によるヒスタミン遊離の約80%に達した。さらに、GABA およびバクロフェンのヒスタミン遊離抑制作用は、$GABA_B$ レセプター阻害剤である CGP35348 により減弱した。これらの結果は、GABA が $GABA_B$ レセプターを介し、マスト細胞および好塩基球のヒスタミン遊離を抑制することを示唆する。

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キーワード：脱顆粒、γ- アミノ酪酸（GABA）、$GABA_B$ レセプター、ヒスタミン、RBL-2H3細胞

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