

# Suppressive Effect of $\gamma$ -Aminobutyric Acid (GABA) on Histamine Release in Rat Basophilic RBL-2H3 Cells

Ayumi HORI<sup>1</sup>, Takashi HARA<sup>2\*</sup>, Kaori HONMA<sup>1</sup> and Toshio JOH<sup>2</sup>

(Received August 7, 2008)

## 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  $\gamma$ -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  $\mu$ M. In the same condition, baclofen, a GABA<sub>B</sub> receptor agonist, also inhibited histamine release. At a concentration of 100  $\mu$ M, 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<sub>B</sub> receptor antagonist. These results suggest that GABA suppresses degranulation in basophils and mast cells via GABA<sub>B</sub> receptor.

*Bull. Facul. Agric. Niigata Univ.*, 61(1):47-51, 2008

**Key words** : degranulation,  $\gamma$ -aminobutyric acid (GABA), GABA<sub>B</sub> receptor, histamine, RBL-2H3 cells

$\gamma$ -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<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub> receptors (Bowery and Enna, 2000). GABA<sub>A</sub> and GABA<sub>C</sub> receptors are ligand gated Cl<sup>-</sup> channels activated by the binding of GABA, whereas GABA<sub>B</sub> 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<sub>B</sub> receptors at presynaptic or ganglionic sites (Kimura *et al.*, 2002). The existence of GABA<sub>A</sub> and GABA<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> receptor in RBL-2H3 cells was pharmacologically assessed by using baclofen, a GABA<sub>B</sub> receptor agonist, and CGP35348, a GABA<sub>B</sub> receptor antagonist.

## MATERIALS AND METHODS

### Reagents

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

<sup>1</sup>Graduate School of Science and Technology, Niigata University

<sup>2</sup>Faculty of Agriculture, Niigata University

\*Corresponding author: harata@agr.niigata-u.ac.jp

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°C in a humidified atmosphere with 5% CO<sub>2</sub>. The medium was changed in maintained cell cultures every 2 to 3 days.

### Histamine release

RBL-2H3 cells were washed with Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free Tyrode's buffer and resuspended in RPMI-1640 medium at the density of  $1.0 \times 10^7$  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 baclofen, stimulated with DNP-BSA at a final concentration of 1 µg/ml in Tyrode's buffer (including Ca<sup>2+</sup> and Mg<sup>2+</sup>). The final cell density and volume were  $1.0 \times 10^6$  cells/ml and 1.1 ml, respectively. These reaction mixtures were incubated at 37°C for 5 to 45 min and then, in order to terminate cellular reactions, cooled at 4°C for 5 min. After centrifugation at  $300 \times g$  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°C for 30 sec. After centrifugation at  $10000 \times g$  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  $300 \times g$  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  $300 \times g$  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<sub>2</sub>SO<sub>4</sub> 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

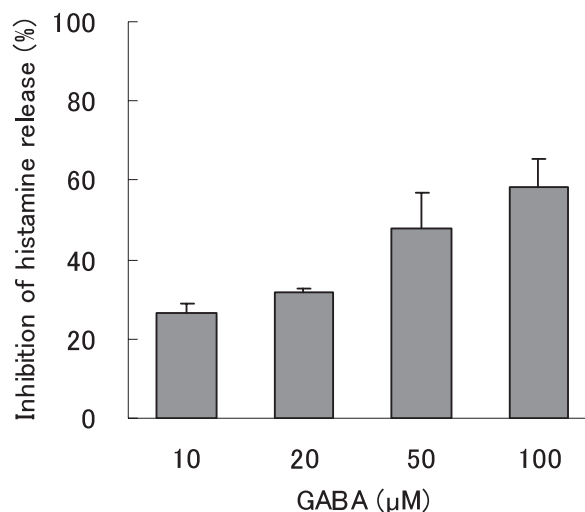
below.

$$\begin{aligned} & \text{Inhibition rate of histamine release (\%)} \\ & = \frac{(\text{Normal} - \text{Control}) - (\text{Sample} - \text{Control})}{(\text{Normal} - \text{Control})} \times 100 \\ & \text{Control : DNP-BSA (-), test sample (-)} \\ & \text{Normal : DNP-BSA (+), test sample (-)} \\ & \text{Sample : DNP-BSA (+), test sample (+)} \end{aligned}$$

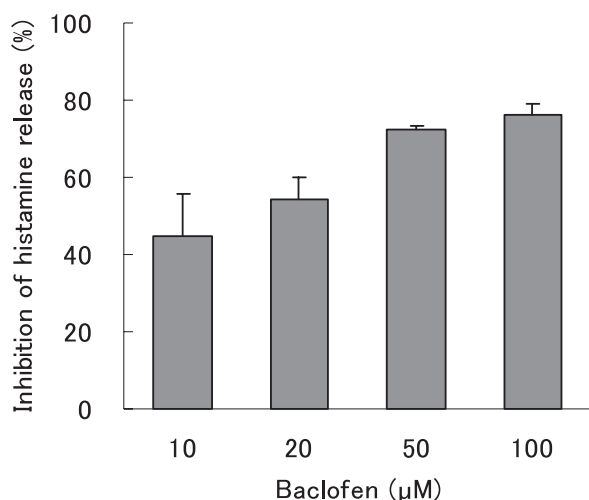
## RESULTS AND DISCUSSION

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<sub>B</sub> 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<sub>B</sub> 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.

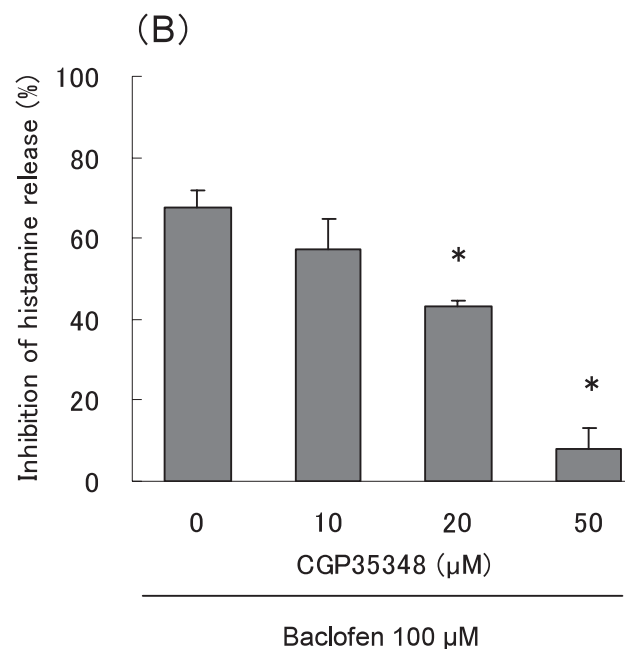
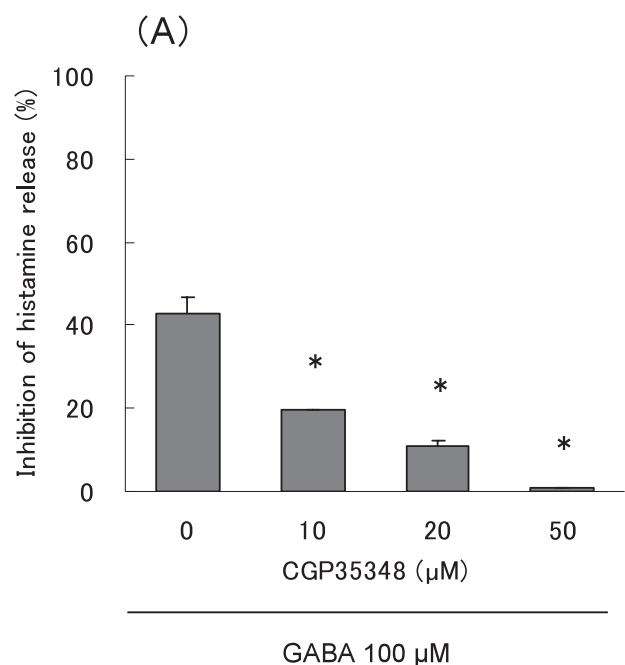


**Fig.2.** Effect of Baclofen on histamine release in RBL-2H3 cells. In the presence of baclofen, cells were stimulated by DNP-BSA. Results were expressed as inhibition percentage of histamine release (mean  $\pm$  SE) and are representative of four different experiments.

effect of CGP35348, a GABA<sub>B</sub> receptor antagonist, was examined. In the presence of CGP35348 at 50  $\mu$ M, the inhibitory action of GABA and baclofen were significantly reduced (Fig. 3). In agreement with our data, RBL-2H3 cells might express functional GABA<sub>B</sub> receptor on the cell surface. It is well known that several cellular processes are influenced by GABA<sub>B</sub> 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<sub>B</sub> 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  $\beta$ -cells (Brice et al., 2002), cardiomyocytes (Lorente et al., 2000), and chondrocytes (Tamayama et al., 2005). However, the expression of GABA<sub>B</sub> receptors in mast cells and basophils has never been described. Further experiments are necessary to detect GABA<sub>B</sub> receptor expression and to determine the role of G-protein in Fc $\epsilon$ RI-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  $\beta$ -cells by G-protein-dependent activation of calcineurin (Braun et al., 2004). It is likely that GABA<sub>B</sub> 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 degranulation 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. reported 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  $\pm$  SE) and are representative of four different experiments. The asterisk represents a  $P < 0.05$  compared to 100  $\mu$ M GABA or baclofen treatment without CGP35348.

serosal mast cells isolated from ovalbumin-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<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> receptor-mediated-signals, especially for the role of G-protein signaling versus FcεRI-mediated signaling in basophils and mast cells.

## REFERENCES

- Bowery, N. G. and Enna, S. J. 2000. Gamma-aminobutyric acid(B) receptors: first of the functional metabotropic heterodimers. *Pharmacol Exp Ther.*, **292**(1): 2-7.
- Bowery, N. G., Bettler, B., Froestl, W., Gallagher, J. P., Marshall, F., Raiteri, M., Bonner T. I. and Enna S. J. 2002. International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev.*, **54**(2): 247-264.
- Braun, M., Wendt, A., Buschard, K., Salehi, A., Sewing, S., Gromada, J. and Rorsman, P. 2004. GABAB receptor activation inhibits exocytosis in rat pancreatic beta-cells by G-protein-dependent activation of calcineurin. *J Physiol.*, **559**(Pt 2): 397-409.
- Brice, N. L. Varadi, A., Ashcroft, S. J. and Molnar, E. 2002. Metabotropic glutamate and GABA(B) receptors contribute to the modulation of glucose-stimulated insulin secretion in pancreatic beta cells. *Diabetologia*, **45**:242-252.
- Gentilini, G., Franchi-Micheli, S., Mugnai, S., Bindi, D. and Zilletti, L. 1995. GABA-mediated inhibition of the anaphylactic response in the guinea-pig trachea. *Br. J. Pharmacol.*, **115**: 389-394.
- Kimura, M., Hayakawa, K. and Sansawa, H. 2002. Involvement of gamma-aminobutyric acid (GABA) B receptors in the hypotensive effect of systemically administered GABA in spontaneously hypertensive rats. *Jpn. J. Pharmacol.*, **89**(4): 388-394.
- Lorente, P., Lacampagne, A., Pouzeratte, Y., Richards, S., Malitschek, B., Kuhn, R., Bettler, B. and Vassort, G. 2000. Gamma-aminobutyric acid type B receptors are expressed and functional in mammalian cardiomyocytes. *Proc. Natl. Acad. Sci. USA*, **97**: 8664-8669.
- Luzzi, S., Franchi-Micheli, S., Spagnesi, S., Rosi, E., Ciuffi, M. and Zilletti, L. 1985. Inhibition of anaphylactic histamine release in vitro by GABA. *Agents and Actions*, **16**: 3-4.
- Luzzi, S., Franchi-Micheli, S., Ciuffi, M., Rosi, E. and Zilletti, L. 1987. Effect of various GABA-receptor agonists and antagonists on anaphylactic histamine release in the guinea-pig ileum. *Agents and Actions*, **20**: 181-184.
- Osawa, Y., Xu, D., Sternberg, D., Sonett, J. R., D'Armiento, J., Panettieri, R. A. and Emala, C. W. 2006. Functional expression of the GABA<sub>B</sub> receptor in human airway smooth muscle. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **291**: 923-931.
- Rane, M. J., Gozal, D., Butt, W., Gozal, E., Pierce, W. M. Jr., Guo, S. Z., Wu, R., Goldbart, A. D., Thongboonkerd, V., McLeish, K. R. and Klein, J. B. 2005. Gamma-aminobutyric acid type B receptors stimulate neutrophil chemotaxis during ischemia-reperfusion. *J. Immunol.*, **174**(11): 7242-7249.
- Sakaba, T. and Neher, E. 2003. Direct modulation of synaptic vesicle priming by GABA(B) receptor activation at a glutamatergic synapse. *Nature*, **424**(6950): 775-8.
- Shore, P. A., Burkhalter, A. and Cohn, V. H. 1959. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.*, **127**: 182-186.
- Tamayama, T., Maemura, K., Kanbara, K., Hayasaki, H., Yabumoto, Y., Yuasa, M. and Watanabe, M. 2005. Expression of GABA(A) and GABA(B) receptors in rat growth plate chondrocytes: activation of the GABA receptors promotes proliferation of mouse chondrogenic ATDC5 cells. *Mol. Cell. Biochem.*, **273**: 117-126.

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

堀あゆみ<sup>1</sup>・原 崇<sup>2\*</sup>・本間香織<sup>1</sup>・城斗志夫<sup>2</sup>

(平成20年8月7日受付)

### 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にアレルギーが結合すると、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 $\gamma$ -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  $\mu$ M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<sub>B</sub>レセプター作動薬であるパクロフェンもまた、ヒスタミン遊離を抑制した。濃度100  $\mu$ Mにおいて、パ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およびパクロフェンのヒスタミン遊離抑制作用は、GABA<sub>B</sub>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<sub>B</sub>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。

新大農研報, 61(1):47-51, 2008

キーワード：脱顆粒、 $\gamma$ -アミノ酪酸 (GABA)、GABA<sub>B</sub>レセプター、ヒスタミン、RBL-2H3細胞

---

<sup>1</sup> 新潟大学自然科学研究科

<sup>2</sup> 新潟大学農学部

\* 代表著者：harata@agr.niigata-u.ac.jp