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Histamine Develops Homologous Desensitization under Ca^{2+}-free Conditions with Increase in Basal Tone in Smooth Muscle of Guinea Pig Taenia Caeci

Shigeru HISHINUMA* and Masaru SHOJI

Department of Pharmacodynamics, Meiji Pharmaceutical University, 2–522–1 Noshio, Kiyose, Tokyo 204–8588, Japan

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Histamine regulates a variety of physiological or pathophysiological processes *via* the activation of $G_{q/11}$ protein-coupled and Ca^{2+}-mobilizing histamine H_1 receptors, including smooth muscle contraction. We have found that histamine induces progression from heterologous to homologous desensitization of contraction under normal physiological conditions in smooth muscle of guinea pig taenia caeci. In this study, we characterized the development of histamine-induced desensitization under Ca^{2+}-free conditions and we found that histamine developed only a homologous phase of desensitization to histamine with an increase in EC_{50} values for histamine and basal tone. In contrast, histamine treatment reduced EC_{50} values for a muscarinic agonist, carbachol, and depolarizing high K^+. These results suggest that the failure of excess histamine to induce a normal Ca^{2+} response under Ca^{2+}-free conditions may lead to homologous desensitization to histamine with apparent hyper-reactivity of smooth muscles to cholinergic and depolarizing stimuli. We estimate that this characteristic of histamine to change smooth muscle contractility may be potentially involved in its physiological and pathophysiological aspects, including histamine-induced allergic conditions, depending on cellular circumstances.

Key words——G protein-coupled receptor; histamine H_1 receptor; homologous desensitization; heterologous desensitization; allergy; smooth muscle

INTRODUCTION

Cells or tissues undergo a process of desensitization upon stimulation with a receptor agonist for cellular adaptation or homeostasis.[1–4] Desensitization is divided into two types:[5–7] homologous desensitization, where only the signal from the stimulated receptor becomes attenuated, and heterologous desensitization, where not only the signal from the stimulated receptor but also that from the unstimulated receptor is attenuated. It is important to clarify the type of desensitization, *i.e.*, homologous or heterologous, with respect to the selection of stimulation that should be refused. In the smooth muscle of guinea pig taenia caeci, histamine transiently induces heterologous desensitization in the initial stage followed by the predominant development of homologous desensitization to histamine under normal physiological conditions.[8] Thus, histamine is revealed to induce progression from heterologous to homologous desensitization in this smooth muscle preparation. Although Ca^{2+} is known to play a crucial role in determining the development of carbachol-induced desensitization in this smooth muscle,[9–10] it is unclear how such processes of histamine-induced desensitization are modified by deprivation of Ca^{2+}. We therefore examined development of histamine-induced desensitization under Ca^{2+}-free conditions.

*e-mail: hishi@my-pharm.ac.jp

MATERIALS AND METHODS

Measurement of Control Responses The experimental protocols of the present research were approved by the Institutional Review Board, Meiji Pharmaceutical University. Strips of taenia caeci were prepared from guinea pigs, weighing 250–400 g, as described previously.[8] The muscle strips were maintained in normal Locke-Ringer solution (normal solution), pH 7.8 at 30°C, with the following composition (mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 2.1, $NaHCO_3$ 6 and glucose 5.6. Contractions were induced cumulatively with histamine (10^{-8} to 10^{-4} M), carbachol (10^{-9} to 10^{-4} M) or isotonic K^+-rich solution (high K^+ solution; 10 to 42 mM of K^+) at 30°C. High K^+ solution was prepared by increasing the concentration of KCl and correspondingly decreasing the concentration of NaCl.

Histamine Pretreatment in the Absence of Extracellular Ca^{2+} One hour after the control response

was recorded, the muscle was preincubated with Ca^{2+}-free solution (EGTA was added to normal solution at a final concentration of 0.2 mM, instead of adding Ca^{2+}) for 30 min. The muscle was then treated with 10^{-5} M histamine for 15 s, 30 s, 1 min, 2 min, 10 min or 30 min in Ca^{2+}-free solution.

Measurement of Test Responses After histamine pretreatment, the muscle was washed with Ca^{2+}-free solution and then with normal solution, and 10 min later contractions were recorded in normal solution. Contractions, without histamine pretreatment, were also measured after control responses were first recorded to statistically test the differences between responses with and without histamine pretreatment (shown as ''none'' or ''0 min'' in Figures). Concentration-response curves for histamine, carbachol or high K^+ were expressed as percentages of the maximal contraction of the control response, taking 0% as the baseline in the resting state measured just before the addition of stimulants for the control response. To assess histamine-induced changes in contractility, we measured changes in contractile responses to mid- and maximal concentrations of histamine, carbachol and K^+ as well as changes in the baseline (basal tone) and EC_{50} values of these stimulants.

Statistical Analyses The results are presented as the mean±S.E.M. Statistical significance was evaluated by ANOVA (Stat View 5; Abacus Concepts, Inc., CA, USA). $p<0.05$ was considered significant.

Materials We purchased histamine dihydrochloride, carbachol, potassium chloride and EGTA from Wako Pure Chemical Industries (Osaka, Japan).

RESULTS

Contractile Responses to Histamine Figure 1 (a) shows concentration-response curves for histamine before (control) and after pretreatment without (none) or with 10^{-5} M histamine for the indicated times in Ca^{2+}-free solution. Changes in contractile responses to mid- and maximal concentrations of 3×10^{-7} M and 10^{-4} M histamine as well as baseline and EC_{50} values for histamine are shown in Fig. 1 (b) and 1 (c). Desensitization to histamine was observed as a reduction in contractile responses to 3×10^{-7} M and 10^{-4} M histamine concomitantly with increases in EC_{50} values for histamine. Thus, histamine induced desensitization to histamine under Ca^{2+}-free conditions. It is noted that histamine pretreatment tended to increase the baseline in a time-dependent manner.

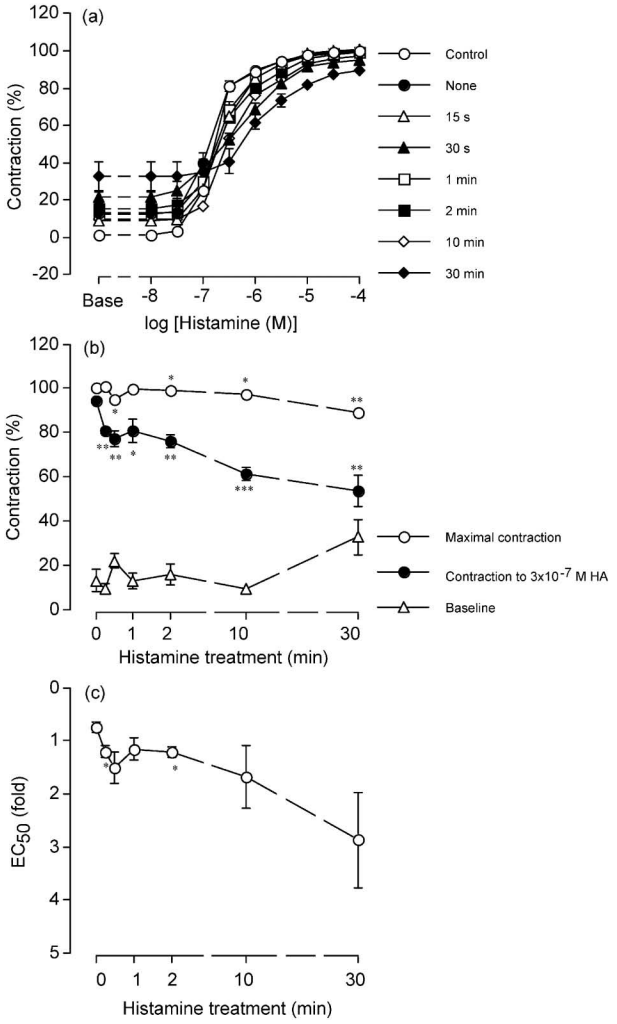

Fig. 1. Changes in Contractile Responses to Histamine after Histamine Pretreatment in Ca^{2+}-free Solution

(a) Concentration-response curves for histamine were obtained before (control: open circles) and 10 min after pretreatment without (none: closed circles) or with 10^{-5} M histamine for 15 s–30 min in Ca^{2+}-free solution. ''Base'' represents the baseline just before the addition of histamine indicated by the abscissa. (b) Changes in the baseline (open triangles) and contractile responses to histamine (HA) of 3×10^{-7} M (closed circles) and 10^{-4} M (open circles) after pretreatment with 10^{-5} M histamine for the indicated times by the abscissa. Contractile responses to 3×10^{-7} M and 10^{-4} M histamine after histamine pretreatment are expressed as percentages of control responses to 3×10^{-7} M and 10^{-4} M histamine before this pretreatment, respectively. (c) Changes in EC_{50} values for histamine after pretreatment with 10^{-5} M histamine for the indicated times by abscissa are expressed as ratios to EC_{50} values for histamine before this pretreatment. Values at 0 min represent the values obtained from contractions without histamine pretreatment. Values represent the means±S.E.M. of 4 experiments. Error bar lies within the dimension of the symbol where error bars are not shown. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared with the value for 0 min.

Contractile Responses to Carbachol We then examined how contractile responses to carbachol, which is known to induce smooth muscle contraction *via* the activation of $G_{q/11}$ protein-coupled and Ca^{2+}-

mobilizing muscarinic M_3 receptors, might be modulated by histamine pretreatment in Ca^{2+}-free solution (Fig. 2(a)). Histamine pretreatment induced no significant changes in contractile responses to mid- and maximal concentrations of 10^{-7} M and 10^{-4} M carbachol (Fig. 2(b)) but reduced EC_{50} values for carbachol from 1 min to 30 min (Fig. 2(c)).

Contractile Responses to High K^+ We then examined how contractile responses to depolarizing high K^+, which is thought to induce smooth muscle contraction *via* the non-receptor-mediated activation of voltage-dependent Ca^{2+} channels, might be modu-

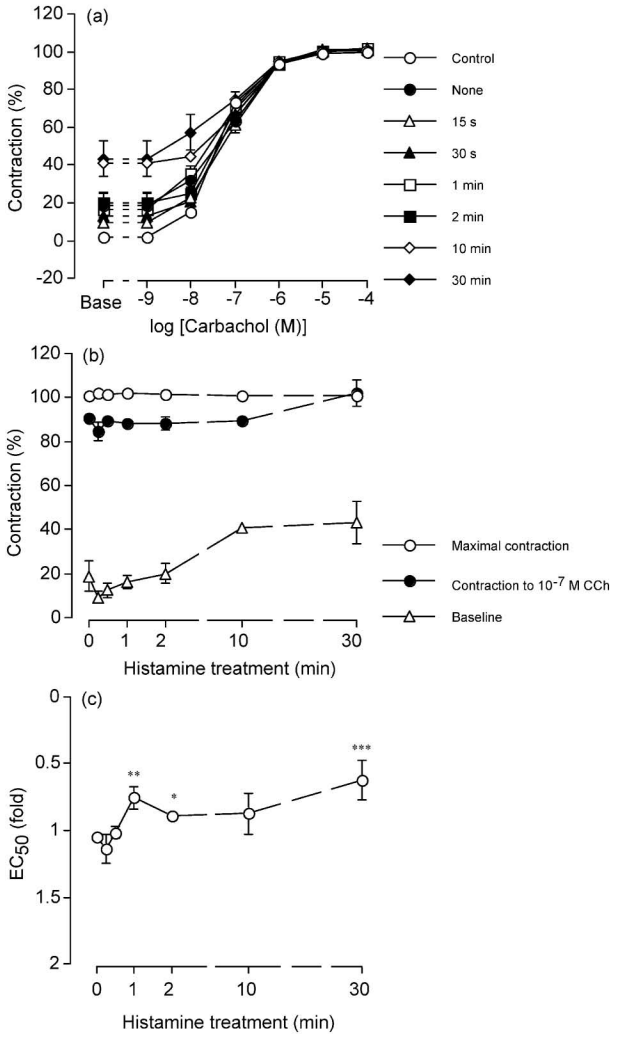

Fig. 2. Changes in Contractile Responses to Carbachol after Histamine Pretreatment in Ca^{2+}-free Solution

(a) Concentration-response curves for carbachol were obtained before (control: open circles) and 10 min after pretreatment without (none: closed circles) or with 10^{-5} M histamine for 15 s–30 min in Ca^{2+}-free solution. ''Base'' represents the baseline just before the addition of carbachol indicated by abscissa. (b) Changes in the baseline (open triangles) and contractile responses to carbachol (CCh) of 10^{-7} M (closed circles) and 10^{-4} M (open circles) after pretreatment with 10^{-5} M histamine for the indicated times by abscissa. Contractile responses to 10^{-7} M and 10^{-4} M carbachol after histamine pretreatment are expressed as percentages of control responses to 10^{-7} M and 10^{-4} M carbachol before histamine pretreatment, respectively. (c) Changes in EC_{50} values for carbachol after pretreatment with 10^{-5} M histamine for the indicated times by abscissa are expressed as ratios to EC_{50} values for carbachol before histamine pretreatment. Values at 0 min represent the values obtained from contractions without histamine pretreatment. Values represent the means±S.E.M. of 4–12 experiments. The error bar lies within the dimension of the symbol where error bars are not shown. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared with the value at 0 min.

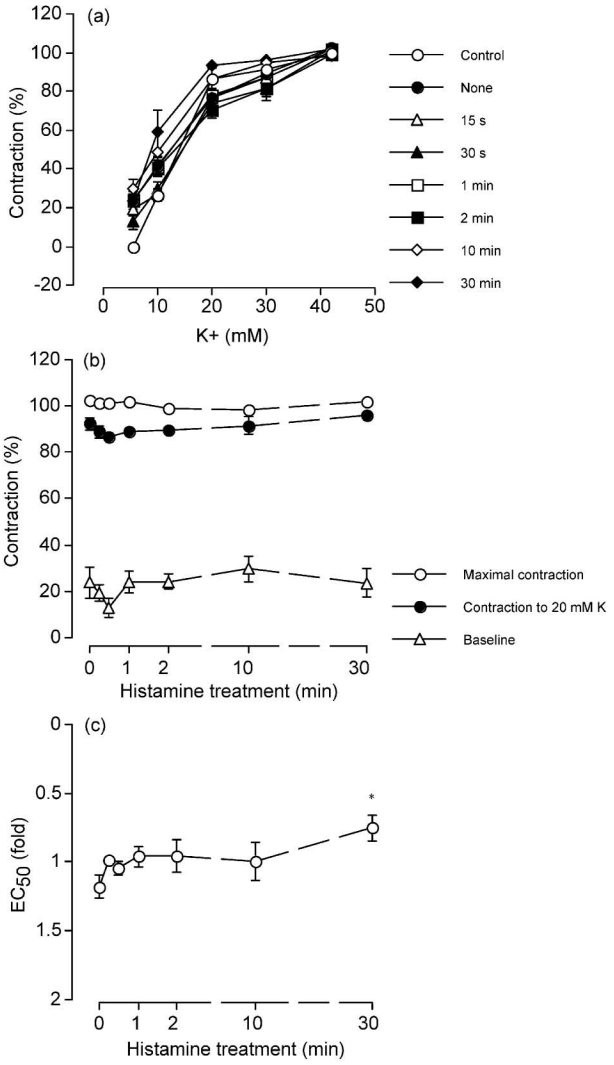

Fig. 3. Changes in Contractile Responses to K^+ after Histamine Pretreatment in Ca^{2+}-free Solution

(a) Concentration-response curves for K^+ were obtained before (control: open circles) and 10 min after pretreatment without (none: closed circles) or with 10^{-5} M histamine 15 s–30 min in Ca^{2+}-free solution. Values at 5.6 mM K^+ correspond to the baseline just before the addition of high K^+ solution indicated by abscissa. (b) Changes in the baselines (open triangles) and contractile responses to K^+ of 20 mM (closed circles) and 42 mM (open circles) after pretreatment with 10^{-5} M histamine 20 mM and 42 mM K^+ after histamine pretreatment are expressed as percentages of control responses to 20 mM and 42 mM K^+ before this pretreatment, respectively. (c) Changes in EC_{50} values for K^+ after pretreatment with 10^{-5} M histamine for the indicated times by abscissa are expressed as ratios to EC_{50} values for K^+ before histamine pretreatment. Values at 0 min represent the values obtained from contractions without histamine pretreatment. Values represent the means± S.E.M. of 4–16 experiments. The error bar lies within the dimension of the symbol where error bars are not shown. $^*p<0.05$, compared with the value at 0 min.

lated by histamine pretreatment in Ca^{2+}-free solution (Fig. 3(a)). Histamine pretreatment induced no significant changes in contractile responses to mid- and maximal concentrations of 20 mM and 42 mM K^+ (Fig. 3(b)) but significantly reduced EC_{50} values for K^+ at 30 min (Fig. 3(c)). It is noted that reduction in EC_{50} values for K^+ was observed to be much slower than that for carbachol.

DISCUSSION

Histamine-induced Development of Homologous Desensitization under Ca^{2+}-free Conditions Histamine pretreatment under Ca^{2+}-free conditions resulted in the simple development of homologous desensitization to histamine. The general mechanisms for homologous desensitization of G protein-coupled receptors have been thought to involve G protein-coupled receptor kinases (GRKs) that phosphorylate the activated form of receptors.[1–7] Since histamine H_1 receptor is known to be a substrate for GRK2,[11–13] it is most likely that the Ca^{2+}-independent and simple development of homologous desensitization to histamine involves GRK-mediated processes.

In contrast, Ca^{2+} appears to play a crucial role in the induction of heterologous desensitization in this smooth muscle, since histamine failed to induce heterologous desensitization that was observed under Ca^{2+}-containing normal physiological conditions. This is consistent with our previous findings on Ca^{2+}-dependent development of carbachol-induced heterologous desensitization in this smooth muscle.[8] In astrocytoma cells, we have found that Ca^{2+}/calmodulin-mediated processes are involved in the initial process of histamine-induced desensitization to histamine.[14–15] It is possible that such Ca^{2+}-dependent mechanisms are involved in the initial development of histamine-induced heterologous desensitization under normal physiological conditions. We postulate that the development of desensitization of $G_{q/11}$ protein-coupled and Ca^{2+}-mobilizing receptors is dually regulated by Ca^{2+}-dependent heterologous and Ca^{2+}-independent homologous mechanisms.[15]

Histamine-induced Changes in Contractile Responses to Carbachol and High K^+ under Ca^{2+}-free Conditions Interestingly, histamine pretreatment under Ca^{2+}-free conditions reduced EC_{50} values for carbachol and high K^+. The rise in the baseline may be involved, at least in part, in these phenomena. It is possible that Ca^{2+} restoration in the extracellular medium after histamine pretreatment under Ca^{2+}-free conditions induced capacitative calcium entry so as to increase basal tone of the smooth muscle.[16] Since histamine-induced reduction in EC_{50} values for carbachol occurred more rapidly than that for high K^+, muscarinic receptor-mediated contractile pathways appeared to be affected more easily than non-receptor-mediated pathways. Although the precise mechanisms remain to be elucidated, we estimate that such properties of histamine to change contractility to cholinergic or depolarizing stimulation with the increase in basal tone may potentially contribute hyperreactivity under some allergic conditions, particularly when histamine fails to activate normal Ca^{2+} signaling pathways.

CONCLUSIONS

Under Ca^{2+}-free conditions, histamine was shown to develop homologous desensitization to histamine and reduce EC_{50} values for cholinergic or depolarizing agents with an increase in basal tone. We estimate that such histamine-induced changes in cellular responsibility may play a crucial role in physiological homeostasis as well as pathophysiological aspects, including immediate allergy under fluctuating circumstances.

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