Alterations in histamine receptors of guinea-pig ileal smooth muscle produced during agonist-induced desensitization

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1 The effects of prolonged treatment with histamine (10^{-4} M, 30 min) on desensitization at histamine H_1 -receptors of guinea-pig ileal longitudinal muscle were investigated.

2 This treatment did not change either the maximum amount or dissociation constant (K_d) of [3H]-mepyramine binding to membranes of guinea-pig ileal muscle.

3 In contrast, this treatment shifted the histamine inhibition curves of [3H]-mepyramine binding to the right both in the presence and absence of 0.5 mM guanosine-5'-triphosphate (GTP). This rightward shift of the curves occurred rapidly in the first 10 min of exposure to histamine.

4 The histamine inhibition curves were analyzed with a two binding sites model. It was shown that the histamine-induced affinity change of the receptor for the agonist occurred with the high affinity binding component (which comprise about 80% of the total), whereas no significant change occurred with the low affinity component. The GTP-dependent decrease in the affinity of the receptor for the agonist also occurred with the high affinity component both in control and histamine-treated preparations.

5 These studies suggest that histamine-induced desensitization was caused by alteration in the affinity of the receptor for the agonist rather than reduction in the number of the receptors and that the interaction of the receptor with a guanine nucleotide regulatory protein was retained in the desensitized state.

Introduction

The administration of a large dose of histamine to guinea-pig ileal smooth muscle is followed by a period of desensitization, during which the responses to histamine are reduced. This desensitization phenomenon has been shown to be non-specific, that is, the responses to other agonists such as acetylcholine may also be reduced (Paton, 1961; Gosselin & Gosselin, 1973; Aboulafia *et al.*, 1987). This situation is currently explained by assuming that steps common to the excitation-contraction coupling pathway used by these agonists have been modified in the desensitization process (Siegel *et al.*, 1984). However, a specific component of desensitization is also proposed as one of the mechanisms of histamine-induced desensitization (Kenakin & Cook, 1980; Bielkiewicz & Cook, 1984). These authors suggested that histamine-induced desensitization involved changes at the receptor level. Our previous study (Horio *et al.*, 1990) confirmed this proposition. At present, however, there is no direct evidence which indicates the involvement of the receptor molecule in the histamine-induced desensitization processes. Thus, in the present study, we used the method of receptor binding assay using [3H]-mepyramine as a selective ligand to determine the changes in the histamine H_1 -receptor which might occur in the process of desensitization of guinea-pig ileum.

Methods

Preparation of the membrane fraction

Guinea-pigs of either sex, weighing 300–500 g, were killed by a blow on the head and cutting the throat. The ileum was removed and strips of longitudinal muscle were obtained according to the method of Rang (1964). The strips were suspended in Tyrode solution at 31°C and bubbled with air under a resting tension of about 0.5 g. The Tyrode solution had the following composition (mM): NaCl 136.9, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.05, NaH₂PO₄ 0.42, NaHCO₃ 11.9 and glucose 5.6. The strips were then treated with 10^{-4} M histamine for 30 min in Tyrode solution, washed for 5 min with Tyrode solution, and transferred to ice-cold 50 mM sodium-potassium phosphate buffer (pH 7.4). Strips were cut into small pieces with scissors, and homogenized in 10 volumes of 50 mM phosphate buffer with a Polytron blender (setting 6) for three periods of 15 s at 1 min intervals. In control experiments, the strips were incubated for 35 min in Tyrode solution without histamine, and transferred to ice-cold 50 mM phosphate buffer and treated by the same procedure as above. In the experiments on time course, strips were treated with 10^{-4} M histamine for 5, 10, and 30 min, washed for 5 min with Tyrode solution and transferred to ice-cold 50 mM phosphate buffer and then treated by the same procedure as above. The homogenate was centrifuged at 50,000 *g* for 30 min; the pellet was resuspended in the same buffer and then centrifuged at 50,000 *g* for 30 min. The final pellet was resuspended in 5 mM phosphate buffer. The suspension was used immediately.

Measurements of [3H]-mepyramine binding

Aliquots of the membrane suspension (450 μ l, 0.4 mg of protein in final concentrations of 50 mM Tris-HCl, pH 7.4) were incubated with 50 μ l of a [3H]-mepyramine solution. After 30 min at 25°C, the reaction was stopped by dilution with 4 ml of ice-cold buffer (50 mM Tris-HCl, pH 7.4) containing 10 μ M mepyramine, followed by rapid filtration over Whatman GF/B filters under vacuum (according to Daum *et al.*, 1982). Filters were washed once more with 4 ml of the same buffer containing 10 μ M mepyramine, and twice with 5 ml of buffer. Radioactivity trapped on the filters was measured by liquid scintillation spectrophotometry in a toluene-based scintillation cocktail. Specific binding was evaluated as the difference between radioactivity bound in the absence and in the presence of 2 μ M promethazine. For experiments on the inhibition of [3H]-mepyramine binding by histamine, the concentration of [3H]-mepyramine was fixed at 2 nM and the concentration of histamine varied. In this case, non-specific binding was evaluated as the binding in the presence of 2 μ M promethazine and varying concentrations of histamine, specific binding representing about 80% of the total. Protein was determined by the method of Lowry *et al.* (1951) with bovine

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serum albumin as a standard. All binding measurements were done in duplicate or triplicate.

Data analysis

Values for maximal binding ($B_{\max}$) and dissociation constants (K_d) of [3 H]-mepyramine were obtained by Scatchard analysis (Scatchard, 1952) by linear regression of least squares. The inhibition curves of [3 H]-mepyramine binding by histamine were analyzed with a model for either one or two binding sites by a nonlinear least squares curve-fitting procedure – the programme was implemented on the microcomputer system PC-9800 (NEC, Japan) using the SIMPLEX method (Nelder & Need, 1965) as previously described (Zhou *et al.*, 1988). The equations used for curve fitting were

$$\frac{B}{B_{\max}} = \frac{L^n}{L^n + IC_{50}^n}$$

for one-site model, and

$$\frac{B}{B_{\max}} = \frac{N_1 \times L}{L + (IC_{50})_1} + \frac{N_2 \times L}{L + (IC_{50})_2}$$

for two-site model, where B represents specific [3 H]-mepyramine binding, $B_{\max}$ maximum binding of [3 H]-mepyramine, L the concentration of histamine, IC_{50} the histamine concentration giving half maximal inhibition of [3 H]-mepyramine binding, n the Hill coefficient, N_1 and N_2 the respective contributions of the two components expressed as percentages of the total population, $(IC_{50})_1$ and $(IC_{50})_2$ the respective IC_{50} values of the two components. The equilibrium dissociation constants (K_d) were calculated using the relationship

$$K_d = \frac{IC_{50}}{C_{\text{mep}}/K_{\text{mep}} + 1}$$

where C_{mep} is the concentration of mepyramine and K_{mep} its dissociation constant. Statistical evaluation of significant differences was performed with Student's t test.

Drugs

[3 H]-mepyramine (28 and 32 Ci mmol $^{-1}$) was obtained from New England Nuclear. Mepyramine maleate was purchased from Sigma, and histamine dihydrochloride and promethazine hydrochloride were from Wako Chemical Industry. Guanosine-5'-triphosphate (GTP) was from Boehringer Mannheim. All other chemicals were from Wako Chemical Industry.

Results

Effects of agonist exposure on [3 H]-mepyramine binding

We previously demonstrated (Horio *et al.*, 1990) that a 30-min exposure to 10^{-4} – 10^{-1} M histamine caused substantial desensitization of guinea-pig ileal smooth muscle. In the present study, we examined whether such agonist treatment which led to desensitization of the histamine response also caused alterations in the histamine receptors. First, we measured the binding of the histamine H_1 -antagonist, [3 H]-mepyramine, to membranes prepared either from control or histamine-pretreated guinea-pig ileal muscle. In control membranes, the maximum binding ($B_{\max}$) of [3 H]-mepyramine varied between membrane preparations ranging from 102 to 335 fmol mg $^{-1}$ protein as was pointed out by Hill & Young (1981). Therefore, in the present study, we used the membranes prepared from the same animal for both control and histamine-pretreated experiments. Figure 1 shows the result obtained from one of three such experiments. Scatchard analysis of the data gave a single affinity of [3 H]-mepyramine binding both for control and histamine-pretreated membranes. The dissociation constant (K_d) was 0.91 ± 0.03 nM for control membranes and 0.81 ± 0.04 nM for histamine-pretreated membranes ($n = 3$). The maximum binding ($B_{\max}$) was 240 ± 71 fmol mg $^{-1}$ protein

Figure 1 Saturation curves and Scatchard plots for [3 H]-mepyramine binding to membranes from (a) control and (b) histamine-treated preparations. Saturation curves for [3 H]-mepyramine binding were examined in 50 mM Tris-Cl (pH 7.4) as described under Methods. Specific binding ($\square$) was obtained by subtracting the binding in the presence of 2 μ M promethazine ($\blacktriangle$) from binding with no inhibitor present ($\triangle$). Scatchard plots were derived from these specific [3 H]-mepyramine binding data. The results shown are from a representative experiment. Mean K_d and $B_{\max}$ values are given in the text.

for control membranes and 297 ± 29 fmol mg $^{-1}$ protein for histamine-pretreated membranes ($n = 3$). Both the K_d values and the $B_{\max}$ values were not significantly different in control and pretreated membranes. Thus neither the total number nor the affinity of antagonist binding to histamine receptors was changed by histamine treatment.

Effects of agonist exposure on the inhibition of [3 H]-mepyramine binding by histamine

Next, we examined the affinity of the receptor for agonist in control and histamine-treated preparations by studying the inhibition curves of [3 H]-mepyramine binding by histamine. These assays were performed both in the presence and the absence of 0.5 mM GTP. The experiments summarized in Figure 2 demonstrated that histamine receptors in membranes from histamine-pretreated preparations had a decreased affinity for the agonist. This was shown by increases in the IC_{50} values and rightward shifts in the histamine inhibition curves. The IC_{50} values were increased from $16.0 \pm 2.1 \mu$ M to $26.6 \pm 2.8 \mu$ M (significantly different, $P < 0.05$). When assays were conducted in the presence of GTP, the inhibition curves were also shifted rightward both in control and histamine-treated preparations, that is, the IC_{50} values were increased from $16.0 \pm 2.1 \mu$ M to $25.7 \pm 1.4 \mu$ M in control preparations, and $26.6 \pm 2.8 \mu$ M to $45.4 \pm 5.6 \mu$ M in histamine-treated preparations. These changes of the IC_{50} values were significantly different ($P < 0.05$). Hill coefficients of the inhibition curves for control and histamine-treated membranes were 0.73 ± 0.06 and 0.79 ± 0.04 in the absence of GTP, and 0.78 ± 0.04 and 0.92 ± 0.03 in the presence of GTP. These values were significantly less than unity ($P < 0.05$) except for the last one. Each inhibition curve was analyzed on the assumption that histamine binding occurred at two independent sites (Hill & Young, 1981) using nonlinear least square regression. The best-fit values of dissociation constant (K_d) and the percentage of each site are shown in Table 1. These data indicate that histamine receptors are separated into two groups, a high-

Figure 2 Curves for inhibition by histamine of [^3H]-mepyramine binding to membranes prepared from control and histamine-treated preparations. The inhibition of the binding of 2 nM [^3H]-mepyramine by histamine in 50 mM Tris-Cl (pH 7.4) was measured both in the presence and the absence of 0.5 mM guanosine 5'-triphosphate (GTP) as described in Methods. (○) Control; (△) histamine-treated; (●) control, with GTP; (▲) histamine-treated, with GTP. Points are the mean calculated from six (control) or four (histamine-treated) experiments. Bars represent s.e.mean. Where no error bars are shown the error was within the size of the symbol.

affinity component and a low-affinity component, the former amounts to about 80%, the latter about 20%. The proportions of the two components were not changed by the prior treatment with histamine or in the presence of GTP. Rather, the K_d values for the high affinity component were changed by these treatments but not the K_d values for the low affinity component.

To examine whether changes in the affinity of the receptor for agonist may be related to histamine-induced desensi-

Figure 3 Time course of histamine-induced decrease in receptor affinity for agonist. Guinea-pig ileal strips were exposed to 10^{-4}M histamine for the times indicated on the abscissa scale, and membranes were prepared for binding assays as described under Methods. Dose-ratios on the ordinate scale were determined from the histamine inhibition curves of [^3H]-mepyramine binding to assess the extent of affinity change: the dose-ratio was calculated as the ratio of the IC_{50} value for histamine-treated membranes to that for control membranes. Each point is the mean of four experiments and the s.e.means are indicated by vertical lines.

tization of the histamine response, we determined the time course for histamine to cause a decrease in the receptor affinity. As shown in Figure 3, a decrease in the receptor affinity occurred within 10 min and remained at the maximal level for the next 20 min.

Discussion

It has been proposed that histamine-induced desensitization of guinea-pig ileal smooth muscle occurs at the receptor level (Kenakin & Cook, 1980; Bielkiewicz & Cook, 1984), and our previous study (Horio *et al.*, 1990) supported this view. The aim of the present study was, then, to determine if agonist treatments which led to desensitization of the histamine response were accompanied by alterations in histamine receptors. Our data showed that histamine pretreatment of the tissue did not change the total number of histamine receptors, but altered the affinity of the histamine receptor for the agonist. The affinity change observed in the desensitized state is not due to the effects of any residual histamine, since residual histamine in the histamine-treated preparations would produce a leftward shift in the histamine inhibition curves and in fact a rightward shift was observed in the present study. The receptor affinity for agonist was analyzed by the use of a two binding sites model as previously employed by Hill & Young (1981). This analysis showed that histamine treatment did not change the proportion of high and low affinity components but caused a decrease in the affinity of the receptor of the high affinity component. The high affinity component of the receptor comprised about 80% of the total and can be considered to take a physiologically significant role. It is not certain whether any change occurred in the receptors of the low affinity component because of the large standard errors of the parameter estimates in the case of the low affinity component (see Table 1). Previous studies (Quach *et al.*, 1981; Mitsuhashi & Payan, 1988) of histamine H_1 -receptor desensitization in slices from mouse cerebral cortex or cultured smooth muscle cell line have revealed a decrease in the number of [^3H]-mepyramine binding sites. These results differ from our results in that our data show that histamine treatment leads to a decrease in the affinity, but not in the numbers of the receptor. These differences probably come from differences in the preparations used, in which different mechanisms of desensitization may be operating. The decrease in the receptor affinity for the agonist should reflect a conformational change which occurred at the receptor molecules (Hoyer *et al.*, 1984). It is possible that such receptors are functionally impaired in their coupling to the effector component leading to a decreased response to the agonist, that is, desensitization. The time course of the affinity change of the receptor (see Figure 3) was similar to that of histamine-induced desensitization which was observed previously (Horio *et al.*, 1990), further supporting the view that the alteration in histamine receptors is the cause of desensitization.

There have been no previous reports indicating the alteration of the receptor molecule in the case of histamine-induced desensitization. The results of the present study

Table 1 Analysis of histamine inhibition curves as binding to two independent sites

Histamine pretreatment	Addition of GTP	High affinity component		Low affinity component	
		N (%)	K_d (μM)	N (%)	K_d (μM)
—	—	84.9 ± 3.4	3.62 ± 0.43	14.6 ± 3.8	205 ± 49
+	—	77.2 ± 5.2	$6.84 \pm 1.18^*$	24.1 ± 3.6	225 ± 164
—	+	84.1 ± 3.4	$6.05 \pm 0.66^*$	16.9 ± 3.4	200 ± 56
+	+	89.5 ± 6.3	$15.33 \pm 2.76^{**}$	8.1 ± 5.3	261 ± 213

The histamine inhibition curves of [^3H]-mepyramine binding to membranes from control and histamine-pretreated preparations were analysed by nonlinear least squares regression with two binding sites model as described in Methods. K_d is a dissociation constant and N is the percentage of receptors in each affinity state. Values are means $\pm$ s.e. ($n = 4-6$). * Significantly different from the value for control preparations ($P < 0.05$). ** Significantly different from the value for histamine-pretreated preparations and the value for control preparations examined in the presence of guanosine-5'-triphosphate (GTP) ($P < 0.05$).

concerning histamine receptors can be compared to results obtained with other receptors. A similar decrease in the affinity of the receptor for the agonist has been reported to occur in the process of desensitization in β -adrenoceptors (Harden *et al.*, 1979; Hoyer *et al.*, 1984) and in muscarinic receptors (Galper & Smith, 1980; Kagiya *et al.*, 1986). In these cases, phosphorylation of the receptor proteins correlated well with the process of desensitization (Sibley *et al.*, 1984; Kwatra *et al.*, 1987), leading to the proposition that the phosphorylation results in a functional modification of the receptor protein such that it is less efficacious in coupling to the effector system. It has not yet been shown that histamine receptors are phosphorylated in the process of desensitization but our data suggest it may be the case.

It has been considered that histamine H_1 -receptors interact with guanine nucleotide regulatory protein, since guanine nucleotide decreases the affinity of the receptors for the agonist (Chang & Snyder, 1980). Then the question was posed whether the interaction between histamine H_1 -receptors and a guanine nucleotide regulatory protein was altered by the desensitizing treatment. Our results showed that agonist binding to histamine H_1 -receptors was still regulated by guanine nucleotide in the desensitized membranes (Figure 2).

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