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Interaction of Local Anaesthetics with Histamine H_1 Receptors in Guinea-pig Ileum

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Abstract

The interaction of amine local anaesthetics and related compounds with histamine H_1 receptors was investigated in guinea-pig ileal longitudinal muscle.

Quinacrine, chloroquine, tetracaine and procaine inhibited [^{3}H]mepyramine binding to solubilized membrane from ileal muscle with pK_i values of $5{\cdot}27 \pm 0{\cdot}11$, $5{\cdot}66 \pm 0{\cdot}01$, $4{\cdot}28 \pm 0{\cdot}08$ and $3{\cdot}97 \pm 0{\cdot}11$, respectively. The pK_B values obtained from the initial parallel shift of the dose–response curves for histamine in the presence of these drugs were $5{\cdot}49 \pm 0{\cdot}11$, $6{\cdot}14 \pm 0{\cdot}09$, $4{\cdot}86 \pm 0{\cdot}06$ and $4{\cdot}58 \pm 0{\cdot}06$, respectively, in reasonable agreement with the pK_i values. The combined dose-ratio test with both local anaesthetics and antagonist (mepyramine) present showed that tetracaine and procaine were competitive and chloroquine was partially competitive, but that quinacrine was not competitive at histamine H_1 receptors. These local anaesthetics inhibited histamine-induced desensitization in guinea-pig ileum. Receptor occupancy (%) by agonist decreased from 95·2 (without inhibitor) to 73·9, 42·8, 35·9 and 33·9 in the presence of quinacrine, chloroquine, tetracaine or procaine, respectively, under the conditions where each inhibitor drug induced half maximum inhibition of desensitization.

The results suggested that most of these local anaesthetics interacted competitively at histamine H_1 receptors and inhibited desensitization through their antagonizing actions, whereas quinacrine interacted allosterically and inhibited desensitization through a separate action.

Local anaesthetics such as tetracaine and procaine inhibit smooth muscle contractions induced by histamine (Bury & Mashford 1976; Ahn & Karaki 1988; Boyle et al 1988). Some related compounds, for example quinacrine and chloroquine, with a similar chemical structure to amine local anaesthetics also inhibit responses to histamine (Famaey et al 1977; Fontaine et al 1980). It has been well established that the inhibition of smooth muscle contractions by local anaesthetics is associated with the inhibition of Ca^{2+} influx into the cells (Feinstein & Paimre 1967; Ishii & Shimo 1984; Spedding & Berg 1985; Ahn & Karaki 1988), but other targeting sites of these drugs in histamine-stimulated cellular processes are not yet defined.

To examine the possibility that local anaesthetics interact with histamine H_1 receptors, we studied the effects of these drugs on contractile responses to histamine and the binding of [^{3}H]mepyramine to membrane preparations in guinea-pig ileal longitudinal muscle. The results indicated that most of these local anaesthetics bound to histamine H_1 receptors competitively, but that quinacrine bound non-competitively. On the basis of these results we examined whether the inhibition of histamine-induced desensitization by local anaesthetics was attributed to their blocking action on the receptors.

Materials and Methods

Drugs

[^{3}H]Mepyramine (28 Ci mmol^{-1}) was obtained from New England Nuclear. Quinacrine dihydrochloride, chloroquine diphosphate and mepyramine were purchased from Sigma, procaine hydrochloride from Daiichi Pharmaceutical, tetracaine hydrochloride from Kyorin Pharmaceutical, and promethazine hydrochloride, histamine dihydrochloride and digitonin from Wako Chemical Industry.

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Measurement of contractile responses

Guinea-pigs of either sex, 250–500 g, were killed by a blow on the head and cutting of 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 (composition (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·05, NaH_2PO_4 0·42, $NaHCO_3$ 11·9 and glucose 5·6) at 31°C and bubbled with air under a resting tension of approximately 0·5 g. Isotonic contractions were recorded with a lever on a smoked drum.

The effects of quinacrine, chloroquine, tetracaine and procaine on the contractile responses to histamine were examined as follows. Firstly, a cumulative dose–response curve to histamine was examined. Then the muscle strip was treated with one of the drugs for 10 min and the dose–response curve was re-examined in the continued presence of the drug. Essentially the same results were obtained when the pre-treatment was for 30 min. These drugs shifted the dose–response curve to the right at low concentrations, and the dissociation constant (K_B) was determined from the parallel shift of the curve according to Arunlakshana & Schild (1959). The dose-ratio method of Paton & Rang (1965) was employed to test whether these drugs were acting in a competitive manner.

Measurement of [^{3}H]mepyramine binding

The strips of guinea-pig ileal longitudinal muscle were cut into small pieces with scissors, and homogenized in 10 vols

50 mM sodium potassium phosphate buffer (pH 7·4) by means of a Polytron blender (setting 6) for three periods of 15 s at 1-min intervals. The homogenate was centrifuged at 50 000 *g* for 30 min and the pellet was resuspended in 5 mM phosphate buffer. Digitonin (1%) was added to the suspension and the mixture was stirred for 60 min at 4°C and then centrifuged at 90 000 g for 60 min. The supernatant was used for binding assay immediately. Incubations in 20 mM Tris-HCl (pH 7·4) contained 2 nM [^{3}H]mepyramine, the drugs (quinacrine, chloroquine, tetracaine or procaine) and the supernatant membrane fraction (0·8 mg protein) in a total volume of 0·5 mL. Equilibration was for 30 min at 25°C. Incubations were then cooled to 0°C and a 0·2-mL sample was applied in duplicate to a column of sephadex G-50 (pre-equilibrated with 20 mM Tris-HCl, pH 7·4) and then eluted with 1·1 mL buffer (Haga & Haga 1983). The whole elute was collected in a vial and the radioactivity was measured by liquid-scintillation spectrophotometry in a toluene-Triton X100-based scintillation fluid. The level of non-specific binding was defined as that insensitive to inhibition by 2 μM promethazine. It represented less than 15% of the total binding. Protein was determined by the method of Lowry et al (1951) with bovine serum albumin as standard. Inhibition curves were fitted to the equation:

$$B/B_{max} = I^n/(I^n + (IC50)^n) \quad (1)$$

where B represents specific [^{3}H]mepyramine binding, B_{max} maximum binding of [^{3}H]mepyramine, I the concentration of the inhibitor drug, IC50 the concentration of the inhibitor drug inducing half-maximum inhibition of [^{3}H]mepyramine binding, and n the Hill coefficient. The best-fit values of n were obtained by a non-linear least-squares curve-fitting procedure—the program was implemented on a PC-9800 (NEC, Japan) microcomputer system using the simplex method (Nelder & Need 1965) as previously described (Horio et al 1990b). The dissociation constants (K_i) were calculated from IC50 values according to the equation of Cheng & Prusoff (1973).

Measurement of desensitization

Cumulative dose–response curves to histamine were measured on a longitudinal muscle strip at intervals of approximately 1 h. The muscle strip was then treated with a desensitizing agent (10^{-4} M histamine) for 30 min. After washing of the muscle with Tyrode solution for 10 min, the dose–response curve for histamine was re-examined. The curve shifted almost in parallel to the right in the desensitized state. The dose-ratio for shifted dose–response curves was determined at 50% response to assess the extent of desensitization (Horio et al 1990a).

In our attempt to check the effect of the local anaesthetics on desensitization, the muscle strip was pretreated with each drug for 10 min, then treated with the desensitizing agent (10^{-4} M histamine) in the continued presence of the drug for 30 min, washed for 10 min, and the dose–response curve was examined.

When the concentrations of histamine used for the desensitizing treatment were varied between 10^{-6} M and 10^{-4} M, desensitization was reduced at low concentrations of histamine, compared with the control (10^{-4} M histamine). The concentration of histamine that induced 50% desensitization of the control was determined from the dose–response (desensitization) curves for histamine.

Calculation of receptor occupancy

Receptor occupancy in the presence of the inhibitor drug was calculated by use of a one-site model with the equation:

$$Y = (A/K_A)/[1 + (A/K_A) + (B/K_B)] \quad (2)$$

where Y represents percentage of receptors occupied by agonist, A the concentration of agonist, K_A its dissociation constant, B the concentration of the inhibitor drug and K_B its dissociation constant. In the calculation, we used $K_A = 5{\cdot}0 \times 10^{-6}$ M for histamine (Horio et al 1990b). The values of K_B were obtained from functional studies as described in the section 'Measurement of contractile responses'.

Statistics

Statistical evaluation of significant differences was performed with Student's *t*-test. Differences with *P* values less than 0·05 were considered statistically significant.

Results

Interaction of local anaesthetics with receptors

Local anaesthetics and related compounds, quinacrine, chloroquine, tetracaine and procaine inhibited the binding of histamine H_1-antagonist ([^{3}H]mepyramine) to solubilized membranes from guinea-pig ileal longitudinal muscle (Fig. 1).

The values of the Hill coefficient obtained from the inhibition curves are shown in Table 1. These values were all close to unity, indicating that these drugs bound to a single site on the receptors. The dissociation constants (pK_i) are shown in Table 1. In this study we used solubilized receptors instead of membrane preparations to eliminate the indirect effects of the local anaesthetics through their action on membrane lipids (Seeman 1972). Essentially the same results were obtained by the binding experiments with membrane preparations (data not shown).

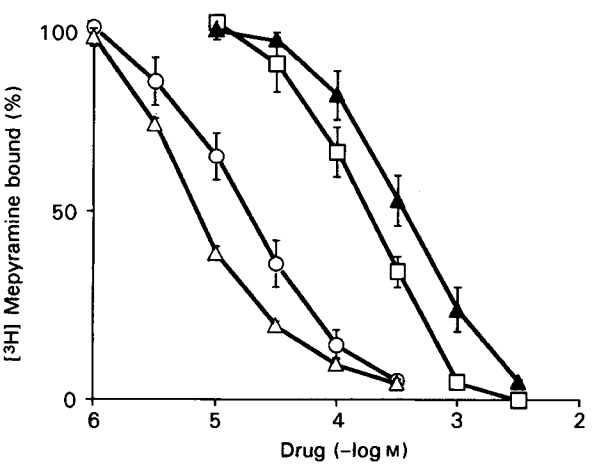

FIG. 1. Inhibition by quinacrine (○), chloroquine (△), tetracaine (□), and procaine (▲) of [^{3}H]mepyramine binding to solubilized membranes prepared from guinea-pig ileal longitudinal muscle. Measurement of the inhibition of the binding of 2 nM [^{3}H]mepyramine was performed in 20 mM Tris-HCl, pH 7·4. Each point is the mean of results from three or four experiments, each performed in duplicate, and s.e.m. are indicated by vertical bars.

Table 1. An index of inhibitory potency of local anaesthetics on histamine H_1 receptors in guinea-pig ileal longitudinal muscle.

Drug	Inhibition of [^{3}H]mepyramine binding (pK_i)	Hill coefficient	Competitive antagonism (pK_B)	Inhibition of desensitization (pIC50)
Quinacrine	5·27 ± 0·11	1·05 ± 0·08	5·49 ± 0·11	4·64 ± 0·16
Chloroquine	5·66 ± 0·01	1·07 ± 0·04	6·14 ± 0·09	4·73 ± 0·08
Tetracaine	4·28 ± 0·08	1·30 ± 0·11	4·86 ± 0·06	3·32 ± 0·22
Procaine	3·97 ± 0·11	1·19 ± 0·10	4·58 ± 0·06	<3·0

Hill coefficients were obtained by non-linear least-squares regression. Dissociation constants (pK_i) were calculated from concentrations giving 50% inhibition of the binding. Dissociation constants (pK_B) were calculated from the parallel shift of the dose–response curves obtained in the presence of 5×10^{-6} M quinacrine, 10^{-5} M chloroquine, 3×10^{-5} M tetracaine or 10^{-4} M procaine after pre-exposure for 30 min, under which condition maximum response was not suppressed (> 95%). Values are means ± s.e.m. (n = 3–5). Values of pIC50 for desensitization were obtained from the results shown in Fig. 3. The IC50 of procaine could not be determined because this drug did not inhibit desensitization > 50% in this experiment.

Next, to obtain the functional affinities (K_B) we examined the effects of the local anaesthetics on the dose–response curves for histamine (Fig. 2). Quinacrine, chloroquine and tetracaine shifted the dose–response curves to the right at low concentrations (up to 5×10^{-6} M, 10^{-5} M and 3×10^{-5} M, respectively), but suppressed the maximum responses at higher concentrations. Procaine (at 10^{-4} M) did not suppress the maximum response. The dissociation constants (pK_B) calculated from the initial parallel shift of the curves are shown in Table 1. The pK_B values for each drug were in reasonable agreement with the pK_i values, suggesting competitive interaction at the receptor site.

The combined dose-ratio method of Paton & Rang (1965) was employed to test further for competitive interaction. If two antagonists (e.g. mepyramine and quinacrine) giving dose-ratios DR_1 and DR_2 are competitive with each other, then $DR_{1+2} = DR_1 + DR_2 - 1$ (DR_{1+2} represents the dose-ratio obtained when both antagonists are present simultaneously). If the two antagonists are not competitive with each other, then $DR_{1+2} = DR_1 DR_2$. The results are summarized in Table 2. The data showed that tetracaine and procaine interacted as a competitive antagonist at histamine H_1 receptors. Chloroquine, which gave values between the expected values for competition and non-competition, could be partly competitive. Quinacrine was, however, not competitive with mepyramine and probably interacted at a separate site on the receptors and inhibited mepyramine binding to the receptor site.

Effect of local anaesthetics on desensitization

These local anaesthetics inhibited histamine-induced desensitization in guinea-pig ileal longitudinal muscle (Fig. 3). Here the degree of desensitization was assessed by dose-ratios as described previously (Horio et al 1990a). The order of potency was quinacrine = chloroquine > tetracaine > procaine. Values of pIC50, which denotes negative logarithm of IC50 for desensitization, are shown in Table 1.

Blockade of receptors by local anaesthetics

The rank order of pK_i and pK_B values agreed well with the order of pIC50 values for desensitization, suggesting that the blockade of the receptors by the local anaesthetics was responsible for the inhibition of desensitization. To clarify this point, we calculated the receptor occupancy by agonist in the presence of these inhibitor drugs. For simplicity we used a one-binding-site model, as described in the 'Materials and Methods' section, although histamine H_1 receptors of guinea-pig ileal muscle have been shown to be best fitted by a two-binding-sites model (Hill & Young 1981; Horio et al 1990b). Here, we must note that quinacrine probably interacted at an allosteric site, but not at the receptor site. The Schild plot for such a negative allosteric ligand is shown to be curvilinear and the inhibiting effect of the ligand reaches a plateau at higher concentrations (Stockton et al 1983; Ehlert 1988). Therefore when we calculated receptor occupancy by using the above equation, we might overestimate the blocking action of quinacrine. The results are summarized in Table 3.

Receptor occupancy was calculated for the conditions when the desensitizing treatment (coexistence of agonist and inhibitor) gave 50% desensitization. For comparison, receptor occupancy was also calculated for conditions in which desensitizing treatment was performed at a low concentration of histamine (2·45 ± 0·23 μM, n = 9) in the absence of any inhibitor. This treatment gave 50% desensitization for the control (10^{-4} M histamine). Chloroquine, tetracaine and procaine effectively inhibited agonist binding to histamine H_1 receptors, but quinacrine was not fully effective in inhibiting it.

Discussion

This study clearly demonstrated that local anaesthetics and related compounds, quinacrine, chloroquine, tetracaine and procaine interacted with histamine H_1 receptors in guinea-pig ileal longitudinal muscle. Tetracaine and procaine were competitive and chloroquine was partially competitive at this type of receptor, as shown by the good agreement of the pK_i values of each drug with the corresponding pK_B values, and the results from the combined dose-ratio test of Paton & Rang (1965). In the presence of these drugs the dose–response curves shifted rightwards at low doses but levelled off at higher concentrations (except for procaine). This flattening was probably a result of their inhibitory action on Ca^{2+} channels as reported previously (Ishii & Shimo 1984; Spedding & Berg 1985; Ahn & Karaki 1988). There are no reports either of the binding of local anaesthetics to histamine H_1 receptors or of a functional study showing competitive interaction of these drugs with the receptors. Thus our results can be compared with those obtained with other types of receptor, i.e. muscarinic receptors, where tetracaine and procaine interact competitively (Aguilar et al 1980; Fairhurst et al 1980; Taylor

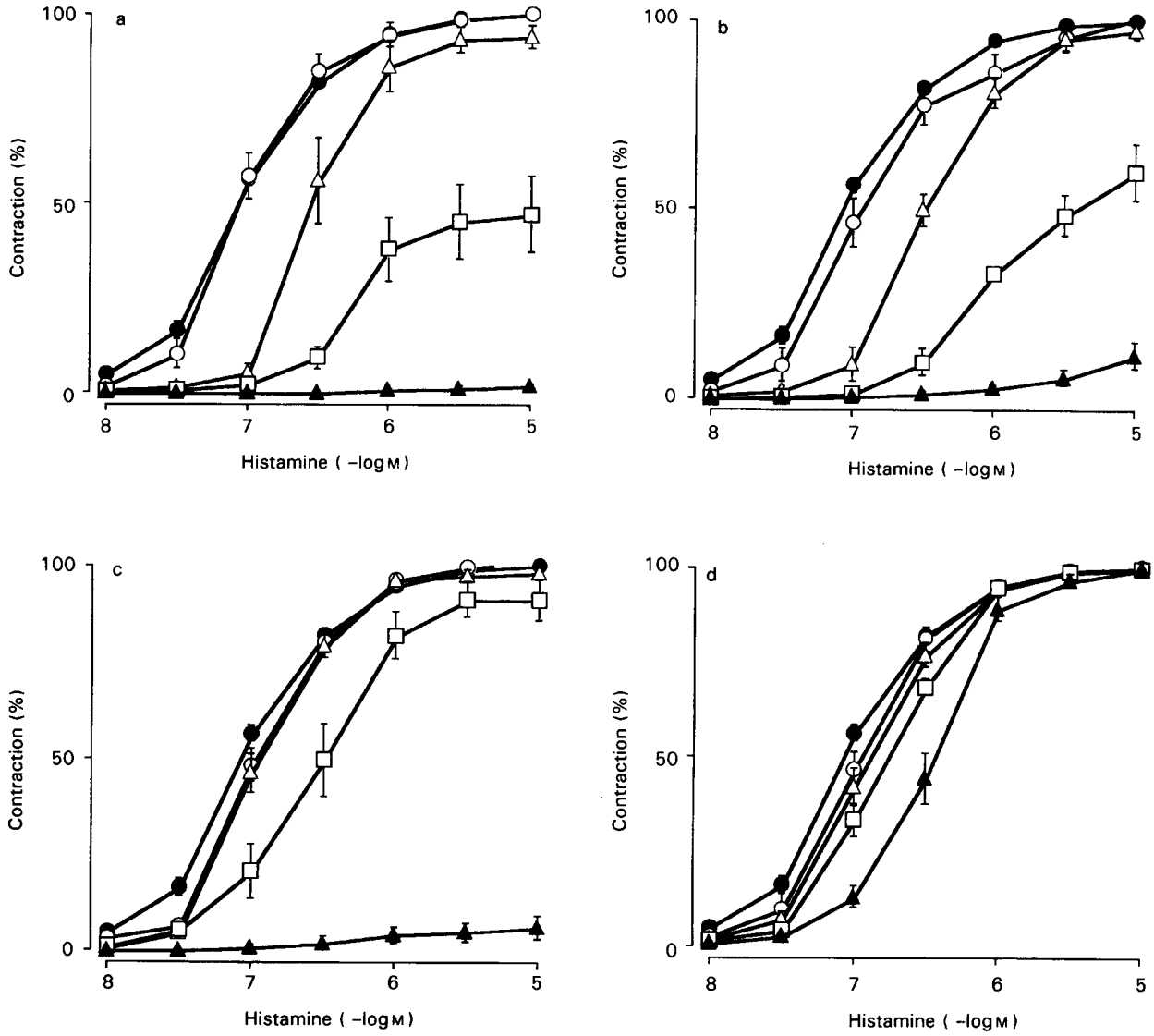

FIG. 2. Effects of four drugs, quinacrine (a), chloroquine (b), tetracaine (c) and procaine (d) on the dose–response curves for histamine in guinea-pig ileal longitudinal muscle, showing control dose–response curves (●) and dose–response curves in the presence of each drug at (○) 3 μM, (△) 10 μM, (□) 30 μM and (▲) 100 μM. Each point is the mean of five experiments and s.e.m. are indicated by vertical bars.

et al 1980; Hisayama et al 1989). These studies on muscarinic receptors show pK_i values for each drug to be around 5·0, a little larger than the values obtained for histamine H_1 receptors in our study (Table 1). These data suggested that these local anaesthetics had antagonizing effects on both muscarinic and histamine H_1 receptors at concentrations usually used for studies on cellular responses.

In contrast with these local anaesthetics, the combined dose-ratio test showed quinacrine to be non-competitive at histamine H_1 receptors. It was evident from our binding studies that quinacrine bound to this receptor. These results indicated that quinacrine bound to a distinct site (an allosteric site) on the receptor and led to the inhibition of binding of agonist and antagonist to the receptor site. Similar allosteric interaction has been reported for gallamine against muscarinic receptors (Stockton et al 1983; Lee & El-Fakahany 1991). Here, the pK_B value obtained from the functional study can be a good approximation of the dissociation constant of the allosteric drug for its site on the receptor, as described by Ehlert (1988).

These local anaesthetics inhibit histamine-induced desensitization in smooth muscle (Siegel et al 1984; Hishinuma & Uchida 1987). The order of potency of these drugs in inhibiting desensitization agreed well with the rank order of affinity for the receptors (Table 1), suggesting that blockade of receptors was responsible for the inhibition of desensitization. To confirm this point we examined the effects of these drugs on receptor occupancy by agonist. Tetracaine, procaine and chloroquine reduced receptor occupancy from 95·2% (control) to 33·9–42·8% under conditions when the desensitizing treatment (coexistence of agonist and the inhibitor) gave half-maximum inhibition of desensitization. Similarly, a low concentration of histamine (2·45 μM) that led to 50% desensitization occupied 32·9% of the receptors. These results were consistent with the idea that these drugs inhibited desensitization through their antagonizing action on the receptors.

Table 2. Dose-ratio test for competitive antagonism of local anaesthetics at histamine H_1 receptors.

Drug	Quinacrine (5×10^{-6} M)	Chloroquine (10^{-5} M)	Tetracaine (3×10^{-5} M)	Procaine (10^{-4} M)
Dose-ratio [mepyramine (10^{-8} M)]	51·3 ± 3·3	49·6 ± 3·3	49·7 ± 5·1	44·4 ± 2·9
Dose-ratio [drug]	2·9 ± 0·7	15·7 ± 2·4	3·2 ± 0·3	5·1 ± 0·3
Dose-ratio [mepyramine + drug]	125·7 ± 7·9*	111·5 ± 17·0†	62·2 ± 7·1‡	44·8 ± 2·6‡
Dose-ratio [mepyramine] + dose-ratio [drug] (expected value for competition)−1	53·2 ± 3·1	64·3 ± 6·4	51·9 ± 5·4	48·4 ± 3·1
Dose-ratio [mepyramine] × dose-ratio [drug] (expected value for non-competition)	143·0 ± 27·3	772·8 ± 127·2	161·6 ± 33·2	225·4 ± 25·3

The guinea-pig ileal longitudinal muscle strips were pre-treated with each drug or a combination of the drugs for 30 min and the dose–response curves for acetylcholine or histamine were examined in the presence of the drugs. Dose-ratios were determined from the parallel shift of the curves. Values are means ± s.e.m. (n = 3–5). *$P < 0.001$ and †$P < 0.05$ compared with the expected dose-ratio value for competition. ‡Not significantly different from the expected dose-ratio value for competition ($P > 0.05$).

Table 3. Receptor occupancy by agonist under the various desensitizing conditions at histamine H_1-receptors.

Desensitizing drug	Receptor occupancy (%)	Desensitization (%)
10^{-4} M Histamine	95·2	100·0
10^{-4} M Histamine + 2.29×10^{-5} M Quinacrine	73·9	50·0
10^{-4} M Histamine + 1.86×10^{-5} M Chloroquine	42·8	50·0
10^{-4} M Histamine + 4.78×10^{-4} M Tetracaine	35·9	50·0
10^{-4} M Histamine + 1.00×10^{-3} M Procaine	33·9	> 50·0
2.45×10^{-6} M Histamine	32·9	50·0

Receptor occupancy was calculated by use of a one-site model. Desensitization induced by 10^{-4} M histamine was used as control. Receptor occupancy was calculated for the conditions that induced 50% desensitization, which was obtained either by reducing the concentration of desensitizing agent (2.45×10^{-6} M histamine) or by adding local anaesthetic (at a concentration of IC50 for desensitization).

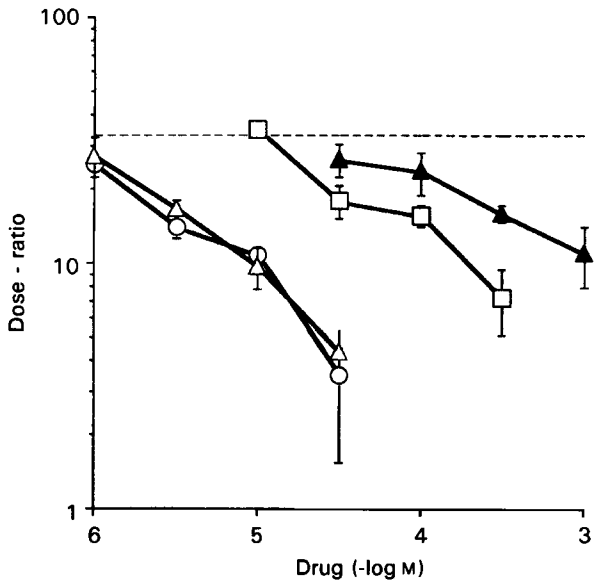

FIG. 3. Inhibition by four drugs, quinacrine (○), chloroquine (△), tetracaine (□), and procaine (▲) of desensitization in guinea-pig ileal longitudinal muscle. Desensitization was induced by treatment with 10^{-4} M histamine for 30 min. Dose-ratios on the ordinate scale were determined as described in 'Materials and Methods' to express the extent of desensitization. Control desensitization was measured by desensitizing the tissue in the absence of any drug, and is represented by a dotted line (dose-ratio was 45·8 ± 0·7). Desensitization in the presence of the drugs was measured by treating the tissue with the drugs for 10 min before the desensitizing treatment with histamine in the continued presence of the drugs. Each point is the mean of three experiments and s.e.m. are indicated by vertical bars.

Under the same conditions quinacrine reduced receptor occupancy to 73·9% only. Here, it should be noted that we calculated the receptor occupancy by assuming competitive antagonism by the inhibitor. For allosteric interaction the Schild plot is curvilinear and the effect of the drug becomes saturated at higher concentrations (Ehlert 1988). Therefore our calculation of receptor occupancy gave a good approximation at low concentrations of quinacrine, but overestimated the value at higher concentrations. Thus the receptor occupancy should be > 73·9% for quinacrine. This result indicated that quinacrine exerted only slight blocking action on the receptor, which was insufficient for inhibition of desensitization. Another action of this drug, such as phospholipase A_2 inhibition, could have participated in inhibiting desensitization (Mallorga et al 1980; Siegel et al 1984).

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