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The Effect of Desensitization on the Antagonism of the Histamine Response by Phenoxybenzamine

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SUMMARY

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Blockade of the histamine response by phenoxybenzamine was studied in both desensitized and nondesensitized preparations of the longitudinal smooth muscle of the guinea pig ileum. It was found that desensitization to histamine had little effect on the parallel shift in the dose–response curve to histamine produced by phenoxybenzamine but did affect the antagonism of the maximum histamine response by this agent. Since there is evidence that this antagonist interacts at the receptor, these results suggest that desensitization of the histamine H_1 receptor in this preparation involves changes at the receptor level, possibly alterations in conformation. The results are described in terms of the cyclic model of desensitization proposed by Katz and Thesleff and the "metaphilic effect" described by Rang and Ritter.

INTRODUCTION

The determination of the binding site for irreversible agents, such as the β-haloalkylamines, often involves receptor-protection experiments using very high doses of agonist. As these concentrations of agonist are sufficient, in many cases, to produce significant desensitization, and since desensitization has been shown to affect the blockade of cholinergic receptors by irreversible agents in some preparations (1–4), it was felt that this effect required investigation prior to receptor-protection studies on the histamine H_1 receptor in guinea pig ileum.

Although desensitization to histamine is well known, it has not been established whether this is an effect at the level of the receptor or mediated by changes in the excitation–contraction mechanism. As well as the histamine-specific aspect of this effect (5), a nonspecific component of the desensitization has been characterized (5–8) which makes quantitative studies difficult. The action of phenoxybenzamine (POB),[1] at the concentrations employed in these experiments, appears to be confined to the receptor level. Thus, by using this agent, it becomes possible to derive information concerning the origins of desensitization.

METHODS

Adult male guinea pigs were killed by a blow on the head, and the terminal ileum was excised, cleared of contents, and placed in Tyrode solution at 37° and pH 7.4. The ileum was cut into 3-cm segments and the longitudinal muscle layer removed (9). The strips were suspended in Tyrode solution at 37° and pH 7.4 and gassed with 95% oxygen/5% carbon dioxide under a resting tension of 300–500 mg. Isotonic contractions were recorded by means of a Hewlett–Packard linear motion transducer Model 1000-7DCDT connected to a Grass Model 5P1 polygraph. The strips were allowed to equilibrate in the organ bath for 1 hr before a control dose–response curve to histamine was obtained. The drugs used were histamine phosphate (Sigma Chemical Co.), acetylcholine bromide (Eastman Organic Chemicals), and phenoxybenzamine hydrochloride (Smith, Kline and French Ltd.).

1. *Selectivity of Antagonism Produced by POB*

The responses to histamine, acetylcholine, and potassium chloride were obtained and the tissues were then treated with POB (2×10^{-5} M) for 5 min. The responses to each of the antagonists were then determined again after a 2-hr wash period.

2. *Effect of Desensitization on Blockade Produced by POB*

Recent studies in our laboratory suggest that the parallel shift in the dose–response curve to histamine caused by low doses of POB arises from a different mechanism from the depression of maximum response caused by higher doses of this agent (10, 11). It was therefore decided that the effects of desensitization on the shift and depression of maximum response should be studied separately.

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[1] The abbreviation used is: POB, phenoxybenzamine.

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(a) Effect of desensitization on the parallel shift induced by POB. After determination of control dose–response curves the experimental tissues were treated with Tyrode solution containing histamine (2×10^{-4} M) for a period for time varying from 5 to 30 min. The agonist was then removed by washing and the baseline regained. Test doses of histamine in the ED_{50} range were then used to determine the dose ratio for the shifted dose–response curve. The formula 100 × (dose ratio − 1)/(dose ratio) was then utilized to calculate ρ (12), defined for cholinergic preparations as the percentage of receptors in the desensitized form. Due to the nonspecific component of the desensitization to histamine in this preparation, it is uncertain whether ρ can be completely described in receptor terms and may represent both components of the process. Immediately after the dose ratio had been determined, the experimental and the control tissues were treated with POB (2×10^{-6} M) for 3 min. The preparations were then washed for 1 hr with Tyrode solution containing sodium thiosulfate (10^{-3} M). Thiosulfate ion reacts with aziridinium ion to form a pharmacologically inactive "Bunte" salt thereby facilitating the removal of the POB that has not interacted covalently with the tissue (13, 14). After the wash period, a second dose–response curve was obtained and the magnitude of the shift determined.

(b) Effect of desensitization on the depression of maximum response by POB. Paired tissues were also used to determine the effects of desensitization on the depression of maximum response produced by POB. In experimental tissues, desensitization was induced and measured as above, and then both experimental and control preparations were treated with POB (5×10^{-6} M) for 3 min. The preparations were washed for 1 hr and second dose–response curves were determined. The difference between the percentage maximum response of the desensitized and control tissues was calculated and it is this difference (Δ_{max}) that reflects the effect of desensitization on the ability of POB to cause depression of maximum response to histamine.

3. Possible Receptor-Protection Effects

One obvious methodological problem is the possible protection of the binding site by a subthreshold amount of histamine present in the bath fluid of desensitized tissues and arising from incomplete washout of previous doses. This subthreshold concentration theoretically can be relatively high (up to 10^{-6} M) since a highly desensitized tissue would not respond to such a dose. A second control was therefore included in which the protecting effect of low concentrations of histamine (10^{-6} M) on the antagonism by POB was observed. The protecting effect of 10^{-4} M histamine was also determined.

4. Specificity of Desensitization

The specificity of the desensitization was determined by observing the effect of exposure of the tissue to histamine (2×10^{-4} M) on the response to acetylcholine.

The shift in a dose–response curve to acetylcholine produced by desensitization to histamine reflects the amount of nonspecific desensitization produced by this dose of histamine.

5. Recovery from Desensitization

Tissues which had been desensitized to histamine (2×10^{-4} M for 30 min) were tested with histamine (10^{-6} M) at 10-min intervals until no further change in response was observed.

RESULTS

1. Selectivity of the Blockade Induced by POB

POB at a concentration of 2×10^{-5} M and an exposure time of 3 min produced a profound blockade of the histamine response as shown in Fig. 1. It can be seen, however, that the response to acetylcholine is affected to a lesser extent. The dose–response curve to potassium chloride (not shown) is not significantly different after 2×10^{-5} M POB.

2. Effect of Desensitization on Shift in Dose–Response Curve

Differences in the parallel shift induced by POB in maximally desensitized ($\rho = 100$) and nondesensitized tissues were determined. Under these circumstances, a small decrease of 0.39 ± 0.07 log unit was observed (paired *t*-test, $P < 0.05$). There is, however, no correlation between differences in parallel shift and ρ, as shown in Fig. 2.

3. Desensitization and Depression of Maximum Response

As shown in Fig. 3, POB depresses the maximum response of a nondesensitized tissue to 32% of the control value. A tissue which had been maximally desensitized to histamine was depressed to 82%. Figure 4 shows the correlation between ρ and Δ_{max} and it can be seen that desensitization reduces the depression of maximum response by POB (correlation coefficient 0.86, $P < 0.05$).

4. Receptor Protection by Subthreshold Concentrations of Histamine

The possibility exists that some measure of receptor protection could be produced by histamine remaining in the tissue after exposure to the high desensitizing dose. The upper limit of such a concentration would be about

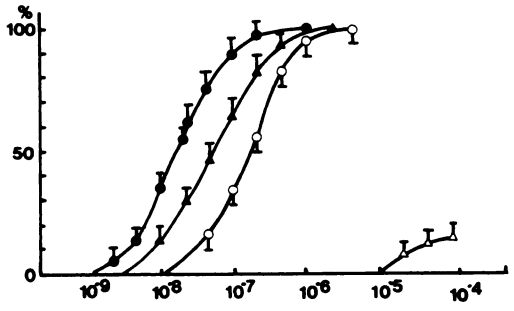

FIG. 1. *Effects of phenoxybenzamine on responses to histamine and acetylcholine*

Concentration of agonist as abscissa, percentage maximum response as ordinate. Bars represent standard errors. ●—●, control response to acetylcholine; ○—○, response to acetylcholine after 2×10^{-5} M POB; ▲—▲, control response to histamine; △—△, response to histamine after 2×10^{-5} M POB.

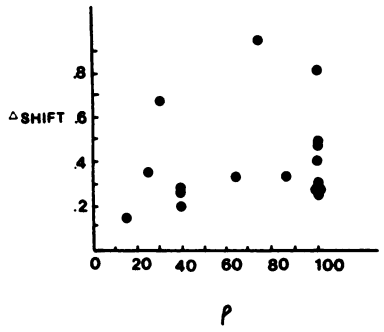

FIG. 2. *Effect of changes in ρ, a measure of desensitization (abscissa) on changes in the size of shift in log units in dose-response curve to histamine produced by 2×10^{-6} M POB (ordinate)*

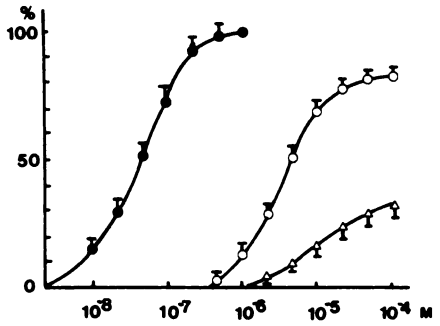

FIG. 3. *Effects of phenoxybenzamine on responses to histamine in desensitized and control tissues*

Concentration of agonist as abscissa, percentage maximum response as ordinate. Bars represent standard errors. ●—●, control response to histamine; Δ—Δ, response after 5×10^{-6} M POB, blockade being carried out in normal tissues; ○—○, response after 5×10^{-6} M POB blockade being carried out in desensitized tissues.

10^{-6} M since higher concentrations would produce detectable responses even in maximally desensitized tissue. In experiments designed to examine this possibility, no effects of this dose of histamine on the shift in dose response were observed. A small protection of the maximum response was observed in depressed tissues ($\Delta_{max} = 8.3 \pm 3$, $N = 6$) in contrast to the major effects caused by desensitization ($\Delta_{max} = 50.5 \pm 4.6$). It thus seems that the observed effects arise largely or completely from desensitization rather than receptor protection by residual histamine. Twofold reduction in the desensitizing dose of histamine to 10^{-4} M decreases Δ_{max} to 39.2 ± 9.6.

5. *Specificity of Desensitization*

Figure 5 shows the effects of prior desensitization on the dose–response curve to histamine. The concomitant desensitization to acetylcholine is also shown in Fig. 5 illustrating the nonspecific component of blockade.

6. *Recovery from Desensitization*

Desensitized tissues recover slowly; after half an hour only a 50% recovery had occurred. In tissues that did not show the control response to 10^{-6} M histamine after 2 hr, the response could be elicited by increasing the dose, and thus no permanent depression of response had occurred.

DISCUSSION

In the study of desensitization irreversible agents possess certain advantages over reversibly acting drugs. The antagonism produced by phenoxybenzamine in a highly desensitized preparation can be studied after sufficient time has elapsed for the complicating effects of desensitization to have disappeared.

If these antagonists are to be used to determine whether or not desensitization is associated with changes at the receptor level, certain basic criteria must be satisfied.

It must first be established that at the concentrations and exposure times employed, phenoxybenzamine is binding to the extracellular surface of the receptor macromolecule. At much higher concentrations and longer exposure times than used in the present experiments, the uncyclized form of POB enters the cells of the pancreas (15) and vas deferens (16). The available evidence indicates, however, that under our experimental conditions this antagonist binds to the extracellular surface of the smooth muscle membrane. Furthermore, the blockade is largely confined to the histamine receptor as antagonism of other agonists is much less marked than that of histamine (Fig. 1). These results strongly imply that phenoxybenzamine, under these conditions, binds to the H_1 receptor macromolecule. There is no reason to suppose, however, that the irreversible agent binds to the histamine binding site. Recent studies suggest that this may not be the case (10) and thus the definition of "receptor phenomenon" may refer to binding of POB to some site on the receptor protein either allosterically linked, or in some other way intimately associated with the histamine-receptive active site.

If desensitization involves conformational changes in the receptor macromolecule then it might be supposed that these conformational changes would affect binding of POB as well. Maximum desensitization was found to produce a statistically significant difference in the ability of POB both to shift a dose–response curve and to depress the maximum response. If, however, these differences are a result of the desensitization, then some correlation should exist between ρ and the measured difference in blockade. No such correlation can be demon-

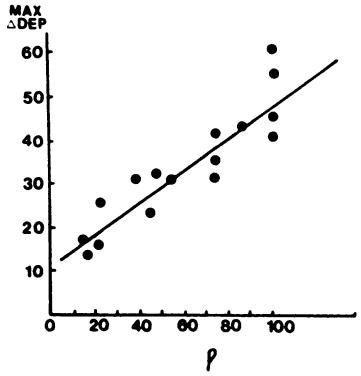

FIG. 4. *Effect of changes in ρ, a measure of desensitization (abscissa) on changes in the depression of maximum response, in percentage units, of the dose-response curve to histamine produced by 5 $\times 10^{-6}$ M POB (ordinate)*

strated for the differences in parallel shift of the dose response curve. Thus, it is difficult to interpret the significance of the 0.39-log-unit value of Δ shift. It is possible that desensitization produces minimal effects on the binding of the POB responsible for the shift in dose–response curve and therefore differences become apparent only in highly desensitized tissues.

The situation is somewhat less complicated when one considers the depression of maximum response to histamine by POB. Figure 4 shows that there is a correlation between ρ and Δ_{max} and thus, it appears that desensitization involves a change at the receptor level. Due to the nonspecific component of desensitization observed (Fig. 5), the magnitude of ρ cannot be considered accurate. Furthermore, it is impossible to assign a unique value of ρ to a tissue blocked with POB, as the preparation is recovering from the desensitization during the 3-min exposure to antagonist (ρ changes with time). Thus, the actual percentage values of ρ, shown on the correlation plot, can only be regarded as estimates.

These results suggest that a desensitized receptor does not bind irreversible antagonist with quite the facility of a nondesensitized receptor. This finding is similar to those of Lester (3) in studies with cobra toxin on cholinergic receptors, Miledi and Potter (2) with α-bungarotoxin on nicotinic receptors, and Dryden and Harvey (4) with α-bungarotoxin on skeletal muscle cells in culture. Rang and Ritter (1) found that desensitized cholinergic receptors were more easily antagonized by certain β-haloalkylamines and subsequently termed the phenomenon the "metaphilic effect." As the correlation between ρ and measure of irreversible blockade in these studies is opposite that observed by Rang (17), the H_1 receptor may be thought to display a "reverse metaphilic effect" with respect to POB.

In molecular terms, this effect is best described by the model for desensitization for cholinergic receptors described by Katz and Thesleff (8). Termed the cyclic model, it defines an equilibrium between normal and desensitized receptors indicated by R and R′, respectively.

$$\begin{array}{ccc} A + R & \overset{\text{fast}}{\rightleftharpoons} & AR \rightarrow \rightarrow \text{response} \\ \uparrow & \overset{\text{slow}}{\rightleftharpoons} & \downarrow \\ A + R & \underset{\text{fast}}{\rightleftharpoons} & AR' \end{array}$$

If the aziridinium ion of POB causes depression of maximum response by binding to the R form of the receptor, then desensitization of a portion of the receptors would essentially remove much of the binding site for the blocking agent. Recovery from desensitization would regenerate active receptor (R′ would convert to R); thus there would be a substantial amount of unblocked R and the maximum response in such a tissue would be much less depressed. The data indicate that the alkylation by POB to cause the parallel shift in dose–response curve is essentially independent of desensitization and it would thus appear that the site that binds phenoxybenzamine to cause the shift in dose–response curve is only minimally altered by the process of desensitization.

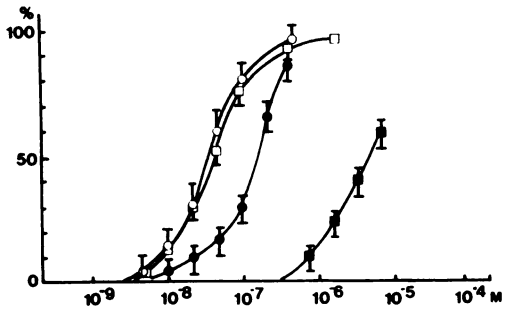

FIG. 5. *Effect of maximal desensitization to histamine on the dose–response curves to histamine and acetylcholine concentration of agonist as abscissa, percentage maximum response as ordinate*

Bars represent standard errors. □—□, control response to histamine; ■—■, response to histamine after desensitization; ○—○, response to acetylcholine; ●—●, response to acetylcholine after desensitization.

The Katz and Thesleff (18) model of desensitization describes a desensitized receptor that is conformationally different from a normal receptor for a considerable length of time thereby causing protection against POB binding. The experiments in which histamine (10^{-6} M) was present during exposure to antagonist and caused little difference in blockade would argue against a long-lasting drug–receptor complex as a representation of the desensitized receptor since this concentration of agonist is sufficient to cause maximum response in a nondesensitized tissue. This point of view is valid if a receptor reserve for histamine is not present in this preparation, as recent studies suggest (10, 11). Even if there is a significant receptor reserve, the possibility of a tightly bound histamine–receptor complex as a representation of desensitization appears unlikely as the presence of 100 times the concentration of agonist causes less difference in the blockade than does desensitization. This concentration of histamine (10^{-4} M) is sufficient to bind at almost 100% of the receptors, assuming a 99% receptor reserve.

A possible mechanism for desensitization to histamine in trachea involving prostaglandin release has recently been suggested (19). Exposure of this preparation to high doses of histamine causes release of a "prostaglandin E-like" substance that could either cause a relaxation of the smooth muscle, as found by Farmer and co-workers (20) (physiological antagonism) or a specific antagonism of the histamine receptor. Although spontaneous release of prostaglandin has been observed in guinea pig ileum (21, 22) and although prostaglandins have been proposed to be intimately involved with the histamine response in this preparation (23), no specific release, as a result of histamine stimulation, has been characterized. It is interesting to note that prostaglandin-like substances could possibly effect chemical inactivation of POB as the carboxylic acid moiety of prostaglandins could form an ester with the β-haloalkylamine and thereby reduce the antagonism. At present, the prostaglandins studied in guinea pig ileum have all caused contraction (24, 25) and thus could not participate in such a mechanism. Furthermore, to cause any noticeable effect in POB blockade, a great deal more prostaglandin-like material would have to be released than has been reported for our preparation (≃0.6 ng g^{-1} min^{-1}) (22).

It appears that receptor level binding of POB is affected by desensitization and that therefore this desensitization involves some kind of perturbation at the H_1 receptor. It is interesting to note that while desensitization profoundly affects the depression of maximum response by POB, it does not appear to have such effects on the parallel shift induced in the dose response curve by this agent, thereby further implying separate mechanisms for these processes.

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