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A Metactoid Sensitization Model to Describe Multiple Receptors Linked to a Common Response: Application to Histamine Receptors Coupled to [^{3}H]Cyclic AMP Accumulation in Guinea Pig Cortex

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SUMMARY

A metactoid sensitization model was developed to describe and resolve the activities of different receptor subtypes coupled to second messenger interactions. The model was formulated for a system in which activation of one receptor potentiates the measured response to activation of another receptor but does not produce the same direct response on its own. For a case in which both direct and indirect components of the response could be activated by the same agonist, simulations based on the model reveal that the overall agonist EC_{50} of the measured response cannot be less than the EC_{50} of the direct response. Unless the indirect receptor is fully activated before agonist occupancy of the direct receptor, the EC_{50} of the overall response must be greater than the EC_{50} at the direct receptor. Inhibition of the response by antagonists acting at the direct receptor may not reveal the indirect component, even if the entire response is dependent on simultaneous activation of both receptors. The pattern of inhibition seen with competitive antagonists acting at the indirect receptor would be critically dependent on the difference between agonist affinities for its two receptors. These conditions may thus lead to the misclassification of the receptors through conventional pharmacological techniques. The model was applied to pharmacological data of histamine (HA)-stimulated [^{3}H]cAMP accumulation in a vesicular preparation of guinea pig cerebral cortex. In this system, H_1 receptors potentiate the [^{3}H]cAMP response to H_2 and/or adenosine receptor activation but have no measurable effect alone. Fitted HA EC_{50} values at both H_1 and H_2 receptors agreed well with independent experimental observations in this system. Values were similar in both the presence and absence of an interaction between the response to adenosine and the action of HA on H_1 receptors. Similarly, affinity constants for both types of HA receptor antagonists, determined from fitting the pharmacological data to this model, were in agreement with literature values for these drugs. Thus, the metactoid sensitization model adequately describes this system, predicts the experimental data, and indicates experimental conditions for further testing and refinement of the model.

Because hormones and drugs exert their effects on cells through actions on receptors coupled to second messengers, an understanding of the coupling mechanisms and their consequences is required for complete characterization of receptor actions. Cellular activity can be regulated directly, by activation of effector systems, or indirectly, through the modulation of the synthesis or effects of second messengers (1, 2). For example, it has been proposed that cAMP indirectly modulates second messenger levels of phosphatidylinositol in peripheral tissues (1, 3). Similarly, activation of protein kinase C has been reported to potentiate the cAMP response to adenylate cyclase (EC 4.6.1.1) activation (e.g., Refs. 4–9). Such interactions complicate the characterization of the responses from receptor activation by specific hormones; their elucidation is required for understanding of receptor-mediated mechanisms and their control of the underlying physiological processes.

In many tissues, activation of specific receptors can alter the response to stimulation of other receptors, even if no direct effect is measurable from the activation of the first receptor itself. For example, in different nervous tissues, α adrenergic receptor (e.g., Refs. 10–13) and HA H_1 receptor (e.g., Refs. 14–18), as well as γ-aminobutyric acid$_B$ (19, 20) and vasopressin V_1 (21) receptors, can potentiate cAMP accumulation due to stimulation of β adrenergic, H_2, vasoactive intestinal polypep-

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ABBREVIATIONS: HA, histamine; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

tide, and adenosine receptors but produce no measurable cAMP response. Potentiation of such cAMP responses may involve receptor-mediated activation of phospholipase A2 (20) and/or protein kinase C (20, 22–24). The precise nature of these interactions varies between brain areas (11). In several regions, interactions between α/β adrenergic and HA H_1 and H_2 receptors appear to occur. In the adrenergic system, both direct and indirect receptor-mediated cAMP accumulation can be selectively modulated by physiological and/or pharmacological manipulations (e.g., Refs. 25–27). Not surprisingly, controversy exists as to the specific receptors responsible for these complex interactions.

In an attempt to help resolve the activities of receptors underlying second messenger interactions, we develop a model describing the theoretical effects of an indirectly acting stimulus on the response to a directly acting stimulus. Such an interaction has been termed metactoid sensitization (28), and the effects of an indirectly acting agonist on the response to a directly acting agonist have been modeled for systems in which neither agonist had affinity for the alternate receptor under study (28). Here, this model has been expanded to allow us to examine the general characteristics of metactoid sensitization, in a case in which both direct and indirect components of the response could be activated by the same agonist.

The model is probed by an application to the results obtained from measurements of HA stimulation of [^{3}H]cAMP accumulation in the vesicular preparation of guinea pig cortex (16). In this preparation, agonist actions on adenosine and H_2 receptors are considered to enhance the production of [^{3}H]cAMP by a direct coupling mechanism, whereas agonist actions on putative H_1 receptors indirectly potentiate these responses (16). The ability of the model to fit the responses of both agonists and antagonists of H_1 and H_2 receptors has been illustrated (29). We report here on the development of the model and its use in obtaining the values of parameters describing the actions on H_1 and H_2 receptors in the vesicular preparation from guinea pig cortex. The parameter values are compared with those obtained from simpler measures of other H_1 and H_2 receptor-mediated responses.

The Metactoid Sensitization Model

Results from measurement of HA-stimulated [^{3}H]cAMP accumulation in guinea pig cortex were fitted to a metactoid sensitization model. This was the simplest model that predicts the experimental data. A model of two independent sites (30), i.e., representing independent H_1 and H_2 receptors that are separately and directly coupled to [^{3}H]cAMP accumulation, was previously considered for these data (16). It was rejected based on the degree of inhibition of the response to HA seen in the presence of selective antagonists at H_1 and H_2 receptors. Thus, in the absence of endogenous adenosine, the degree of inhibition of the HA response by H_2 and H_1 antagonists was 100% and 31%, respectively. Because the combined inhibition cannot be greater than 100%, these results indicated that 31% of the HA response was attributable to the simultaneous activation of H_1 and H_2 receptors, and 69% to direct activation of H_2 receptors. Such observations can be accommodated by the original formulation of the metactoid sensitization model (28), in which an agonist, D, is assumed to combine with its receptors (R_a and R_b) according to the law of mass action, with equilibrium dissociation constants K_a and K_b, respectively, and another agonist, C, is also assumed to combine with its receptor (R_c), with an equilibrium dissociation constant K_c (shown schematically is Fig. 1). The present formulation of the model is constructed for the case in which only activation of R_a and/or R_c will directly stimulate adenylate cyclase (transducers T_a and T_c, respectively, in Fig. 1) and, hence, cAMP synthesis (responses E_a and E_c, respectively). Stimulation of R_b does not find direct expression in the measured effect (i.e., stimulation of cAMP production) but acts indirectly (via transducer T_b) to generate the measured response E_b, which is the potentiation of the responses E_a and E_c.

The responses to activation of the receptors interacting through the mechanisms described by the metactoid model were simulated conformationally from the functional forms in Eqs. 1–6, as described below. Curve fitting of experimental results obtained from HA-stimulated [^{3}H]cAMP accumulation in guinea pig cortex (16) to the metactoid model, as well as the simulations, were performed on the PROPHET computer system.

Potentiation of the Response to R_a by R_b.

The response from a system in which a metactoid sensitizer (D) acts on a receptor R_b to potentiate the response to a single directly acting agonist (in this instance also D, but acting on R_a) can be described [after Van Den Brink (28)] as:

$$\text{Response} = \frac{E_{ab}}{E_{ab}^{\max}} = \frac{E_a(1 + (p - 1)E_b)}{E_{ab}^{\max}} \quad (1)$$

where: E_a = response to D acting at R_a; E_b = response to D acting at R_b; E_{ab} = the combined observable response; $E_{ab}^{\max}$ = maximum response of E_a in the presence of the maximum response E_b; and p = system constant to describe the nature of interaction of E_a and E_b, e.g., $p = 1$ signifies that the response is due to E_a alone; $p > 1$ signifies that E_b potentiates the response to E_a (E_b = metactoid sensitizer); $p < 1$ signifies that E_b inhibits the response to E_a (E_b = metactoid inhibitor).

Note that the equation describing the response in a system with either a metactoid sensitizer or a metactoid inhibitor remains the same, and only the value of p determines the effect of the metactoid agent. In the model formulated below, only metactoid sensitization is considered, i.e., $p > 1$.

Eq. 1 can be modified to describe the relationship of the concentration of agonist D to the response in the presence of competitive antagonists A and B, acting exclusively at R_a and R_b, respectively, assuming the following: 1) the responses E_a and E_b are directly proportional to occupancy of R_a and R_b, respectively, i.e., the system has no receptor reserve; 2) the responses E_a and E_b occur as separate events, i.e., the response E_b is not influenced by E_a; 3) D acts as a full agonist at both E_a and E_b; and 4) all reacting species are at equilibrium. With these assumptions, the response in the presence of antagonists A and B becomes:

$$\text{Response} = \left[\frac{[D]}{[D] + K_a(1 + [A]/K_i)}\right] \cdot \left[1 + \frac{(p - 1)[D]}{[D] + K_b(1 + [B]/K_{i'})}\right] \quad (2)$$

where: $[D]$ = agonist concentration; K_a = dissociation constant of D at R_a; K_b = dissociation constant of D at R_b; $[A]$ = concentration of selective antagonist for R_a, with dissociation constant K_i; and $[B]$ = concentration of selective antagonist for R_b, with dissociation constant K_i'.

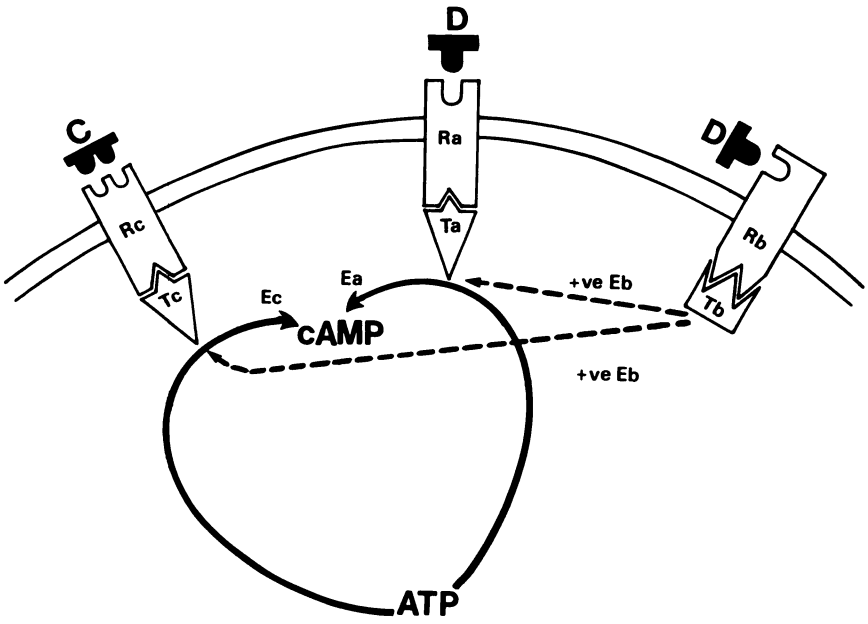

Fig. 1. Schematic representation of a model exhibiting metactoid sensitization. Occupancy of extracellular receptors R_a, R_b, and R_c, through transducers T_a, T_b, and T_c, produce responses E_a, E_b, and E_c, measured as increases of intracellular cAMP. *D* acts as an agonist at both R_a and R_b, but not R_c. *C* acts as an agonist only at R_c. E_a and E_c produce direct increases in cAMP. Occupancy of R_b by agonist *D* does not directly increase cyclic AMP but acts to potentiate the cyclic AMP response to directly acting stimuli (i.e., E_a and E_c). For the present application of the model to HA-stimulated [^{3}H]cAMP levels in guinea pig cortex (16), *D* = HA; R_a = H_2 receptors; R_b = H_1 receptors; *C* = adenosine; and R_c = adenosine receptors.

The shape of the resulting curve can be seen (from Eq. 2) to be a function of variations in p and of the relationship between the dissociation constants of *D* at R_a (K_a) and R_b (K_b). It can be seen from Eq. 2 that, when R_b is unoccupied by *D* ($K_b \gg K_a$), the measured response will be primarily E_a (see Fig. 2A, $K_b = \infty$) i.e., there is no metactoid interaction. When R_b is fully occupied before R_a ($K_b \ll K_a$), the effect E_b is only an increase in the maximum effect of *D* (see Fig. 2A, $K_b = 0.01K_a$), determined by the value of p. Thus, at either of these two extremes, 50% of the maximum effect occurs at $[D] = K_a$.

The ratio of K_b/K_a also characterizes the expected pattern of inhibition of a competitive antagonist *B* acting at R_b (Fig. 2). As noted above, because the response to *D* is obtained from occupancy of R_a, the minimum EC_{50} of the overall response to *D* is limited to K_a, i.e., occupancy of R_b before R_a does not shift the concentration response curve to *D* to the left (Fig. 2A, $K_b = 0.01K_a$ versus $K_b = 0.1K_a$). Consequently, higher concentrations of a competitive antagonist *B*, acting at R_b, are required to inhibit the observed response from R_b when K_b/K_a is small (Fig. 2A, $K_b = 0.01K_a$ versus $K_b = 0.1K_a$) than when K_b equals or is greater than K_a (Fig. 2A, $K_b = 1$, 10, or $100K_a$, respectively).

The values of K_b and K_a also affect the pattern of inhibition of a competitive antagonist *A* acting at R_a (Fig. 3). When $K_b \ll K_a$ (*D* will fully occupy R_b before R_a), increasing concentrations of *A* will cause a rightward shift of the concentration-response curve of *D*, in a fashion indistinguishable from simple competitive antagonism, i.e., neither the response E_a alone nor the sensitization of E_a by E_b will be readily revealed (see Fig. 3, $K_b = 0.01K_a$). In actuality, when $K_b = 0.01K_a$, the theoretical curves do deviate from parallelism, because *D* will occupy R_b before full occupancy of R_a), but it is unlikely that these deviations, which are slight, could actually be determined experimentally. Indeed, only when K_b is much larger than K_a (*D* will occupy R_a before R_b) will the complex interactions of this model be revealed by increasing concentrations of *A* (Fig. 3C, $K_b = 100K_a$). In the example given in Fig. 3C, low concentrations of *A* shift the E_a response to the right. With increasing concentrations of *D*, the response attributable to the stimulating effect of the response from R_b on the response from R_a (Fig. 1) will also be expressed. At this point, the effect of *A* at R_a is already surmounted, such that this response is not influenced by *A* and the curves are close together (Fig. 3C, $[A]/K_i = 0$, 3, and 10). With yet higher concentrations of *A*, the response to *D* at R_a is inhibited, the stimulating effect of action of R_b is not observable, and the curves are again shifted to the right (Fig. 3C).

Potentiation of the Response to R_a and R_c by R_b

Concentration-response curves obtained from the simulations described above resemble the [^{3}H]cAMP response to HA observed in the presence of adenosine deaminase (16) but deviate from our observations obtained in the absence of this enzyme (Fig. 2B versus Fig. 4B). This deviation is probably due to the absence of an explicit role for adenosine receptors in the simplified model given in Eq. 2. Adenosine has been shown to stimulate cAMP accumulation in guinea pig cortex, and this response is potentiated by H_1 receptor stimulation (e.g., Refs. 14 and 16). This effect is reduced by adenosine deaminase, which removes the active adenosine. To account for the effects of adenosine, another receptor (R_c), directly coupled (T_c) to cAMP accumulation (E_c), was added to the model (Fig. 1); stimulation of R_b results in metactoid sensitization of the response E_c from this receptor. According to this scheme, the following additional assumptions are introduced in the metactoid model: 1) occupancy of another receptor R_c by agonist *C* can directly stimulate cAMP accumulation; 2) *D* has no affinity for R_c and *C* has no affinity for R_a or R_b; 3) stimulation of R_b can act as a metactoid sensitizer by potentiating both the E_a and E_c responses; 4) the responses E_a and E_c are additive; and 5) the result of the interaction of E_b and E_a is not altered by the interaction of E_b and E_c and vice versa.

The overall response (from Eq. 1) becomes:

$$\text{Overall response} = \frac{E_{abc}}{E_{abc}^{max}} = \frac{E_{ab} + E_{bc}}{E_{abc}^{max}} \qquad (3)$$

where: E_{bc} = response from any combination of E_c and E_b; E_{abc} = response from any combination of E_a, E_b, and E_c; and E_{abc}^{max} = maximum response of E_a and E_c in the presence of the maximum response E_b.

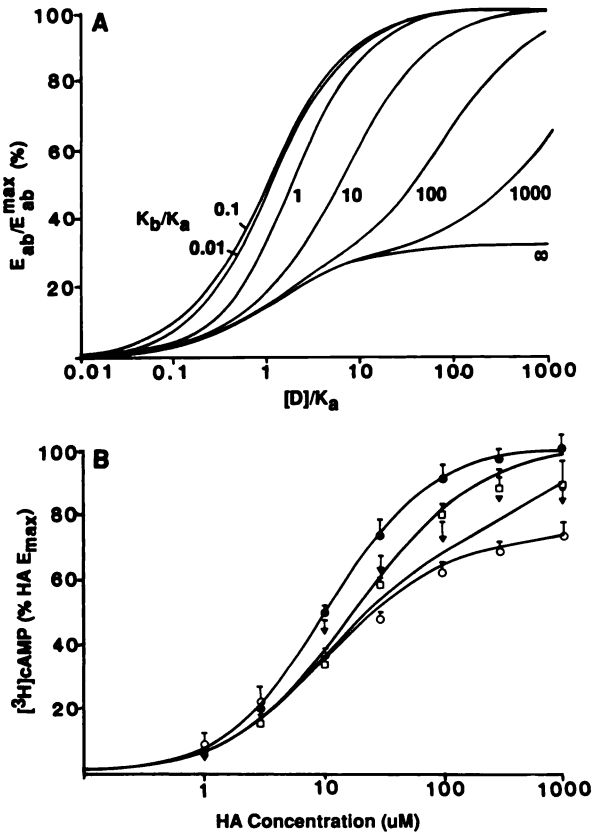

Fig. 2. A. Simulation of metactoid sensitization: effect of varying K_b/K_a on the response to D when only R_a can serve as the direct stimulus. Theoretical concentration-response curves to an agonist D are drawn, based on Eq. 2 of the metactoid model, in which D initiates the response through activation of two receptors (R_a and R_b). Occupancy of E_a by D directly initiates a response (E_a), whereas occupancy of R_b potentiates the response to E_a, via E_b. The effect is expressed in units of K_a on the x axis and is normalized to 100% of the response to D, with $p = 3.333$. Each *curve* in A is labeled with the value K_b/K_a. B. Fit to the metactoid model of mepyramine antagonism to HA in the presence of adenosine deaminase. The fit of mepyramine antagonism of HA-stimulated [^{3}H] cAMP accumulation, in the absence of adenosine, to Eq. 2 of the metactoid model was carried out with $p = 1.45$, $K_a = 5.60$ μM, $K_b = 2.67$ μM and $[A] = 0$. Mepyramine concentrations were 0 (●), 0.01 (□), 0.1 (▼), and 1.0 (○) μM. Experimental data are normalized to the maximum HA response obtained in the absence of antagonists. The fitted K_i' for mepyramine was 0.74 ± 0.2 nM. See text and Ref. 16 for further details.

The equation describing the metactoid interaction of E_b and E_c (analogous to Eq. 1) is:

$$\frac{E_{bc}}{E^{max}_{bc}} = \frac{E_c(1 + (q - 1)E_b)}{E^{max}_{bc}} \qquad (4)$$

where: E_c = response to C at R_c; and q = system constant to describe the nature of the interaction of E_c and E_b (analogous to p in Eq. 1).

In this model, the response to D in the presence of a fixed concentration of C can be written as:

$$\text{Response} = \frac{E_a(1 + (p - 1)E_b) + E_c(q - 1)E_b}{E^{max}_{abc}} \qquad (5)$$

Incorporating previously defined assumptions, we obtain:

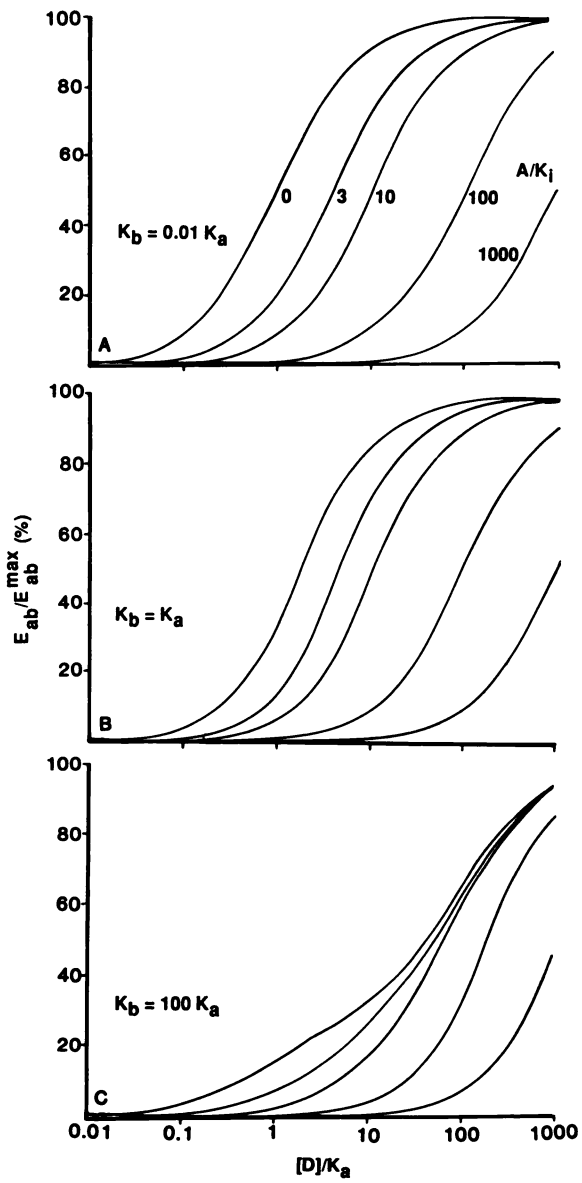

Fig. 3. Simulation of metactoid sensitization: effect of varying K_a/K_b on the pattern of inhibition seen with a competitive antagonist A acting at R_a, when only R_a can serve as the direct stimulus. Theoretical concentration-response curves to an agonist, D, are drawn based on Eq. 2 of the metactoid model, with $p = 1.43$. D initiates the response through activation of R_a and R_b. Occupancy of R_a by D initiates the response E_a; occupancy of R_b potentiates, but cannot directly elicit, this response. An antagonist A (with dissociation constant K_i) can act to inhibit the response to D at R_a, but not R_b. The effect of increasing concentrations of A (in units of A/K_i) on the response to D is shown for selected K_a/K_b ratios, expressed in units of K_a on the x axis and normalized to 100%.

$$\text{Response} = \left[\frac{[D]}{[D] + K_a(1 + [A]/K_i)}\right] \cdot \left[1 + \frac{(p-1)[D]}{[D] + K_b(1 + [B]/K_{i'})}\right] + \left[\frac{r[D]}{[D] + K_b(1 + [B]/K_i')}\right] \qquad (6)$$

where: $r = E_c(q - 1)$.

Under these conditions, the effect E_b is no longer dependent solely on E_a for expression but can also be mediated by interaction with E_c. Thus, the contribution of C to the response to

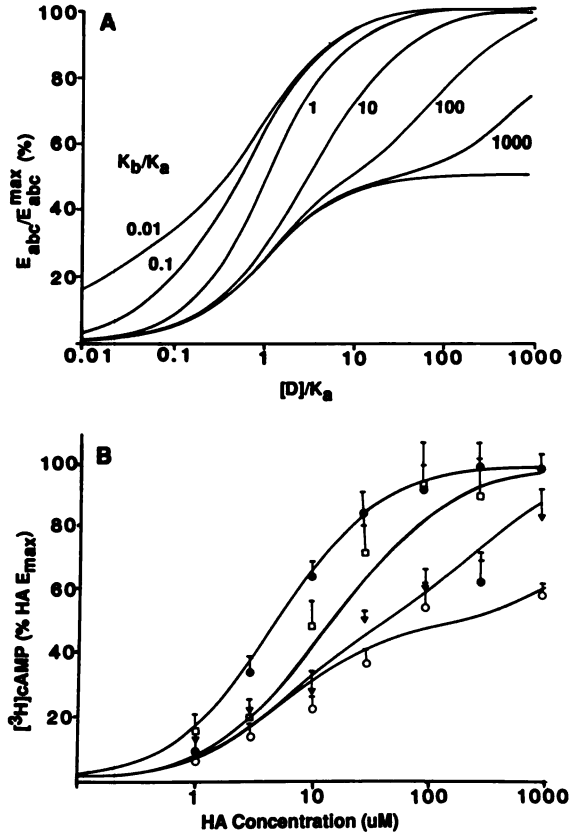

Fig. 4. A. Simulation of metactoid sensitization: effect of varying K_a/K_b on the response to D when both R_a and R_c can serve as the direct stimulus. Theoretical concentration-response curves to agonist D are drawn, based on Eq. 6 of the metactoid model, in which D initiates the response through activation of R_a and R_b. Occupancy of R_a by D directly initiates the response E_a. Occupancy of R_b potentiates the response E_a and the response E_c to another directly acting agonist, C. The effect of varying K_b/K_a is expressed in units of K_a on the x axis and is normalized to 100%, with p = 1.4 and r = 0.6. B. Fit to the metactoid model of mepyramine antagonism to HA in the absence of adenosine deaminase. The fit of mepyramine antagonism of the [^{3}H]cAMP response to HA, in the presence of endogenous adenosine, to Eq. 6 of the metactoid model was carried out with p = 1.45, r = 0.36, K_a = 5.60 μM, K_b = 2.67 μM, and [A] = 0. Mepyramine concentrations were 0 (●), 0.01 (□), 0.1 (▼), and 1.0 (○) μM. Experimental data are normalized to the maximum HA response obtained in the absence of antagonists. The fitted K_i' for mepyramine was 0.51 ± 0.15 nM. See text and Ref. 16 for further details.

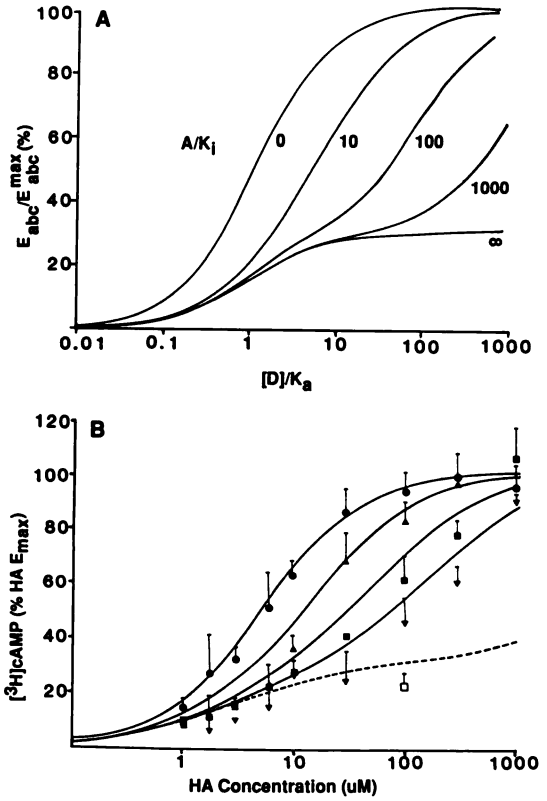

Fig. 5. A. Simulation of metactoid sensitization: effect of varying K_a/K_b on the pattern of inhibition of the response to D by a competitive antagonist A, acting at R_a, when both R_a and R_c can serve as the direct stimulus. The effect of varying A/K_i on the response to D, based on Eq. 6 of the metactoid model, is expressed in units of K_a on the x axis and is normalized to 100%, with K_a/K_b = 1, p = 1.40, and r = 0.6. See legend to Fig. 4 and text for further details. B. Fit to the metactoid model of cimetidine antagonism to HA in the absence of adenosine deaminase. The fit of cimetidine antagonism of the [^{3}H]cAMP response to HA, in the presence of endogenous adenosine, to Eq. 6 of the metactoid model was carried out with p = 1.45, r = 0.36, K_a = 5.60 μM, K_b = 2.67 μM, and [B] = 0. The *dashed curve* for 300 μM cimetidine was derived from the fitted values. Cimetidine concentrations were 0 (●), 1 (▲), 3 (■), 10 (▼), and 300 (□) μM. The experimental results are normalized to the maximum response to HA obtained in the absence of antagonists. The fitted K_i for cimetidine was 0.27 ± 0.03 μM. See text and Ref. 16 for further details.

D is through the sensitizing component (E_{bc}). In the presence of a fixed concentration of C, the overall response to D will depend on the values of p, r, and K_a/K_b.

The addition of increasing concentrations of a competitive antagonist B (simulated by increasing values of K_b/K_a eventually reveals E_a^{max}, the component independent of E_b (Fig. 4). When $K_b < K_a$, the concentration-response curve to D is shifted to the left in the presence of C (Fig. 4A versus Fig. 2A, K_b/K_a = 0.01). Thus, the effect of E_b on E_c can be seen as a response obtained at concentrations of D that are too low to elicit a response in the absence of the response E_c, elicited by C on R_c.

The interaction between the response to D on R_b and the response to C on R_c can be used to quantitate the parameters of the actions of D on R_b. In the presence of a fixed concentration of C, increasing concentrations of A will reveal the extent of the interaction of E_b on E_c (Fig. 5). This is produced by the simultaneous shift to the right of the response to E_a and the contribution from the interaction of E_b and E_a. In the presence of high concentrations of A that block the direct response from R_a, i.e., when E_a and the interaction of E_b and E_a approach zero, K_b can be measured as the concentration of D at which the effect of E_b on E_c reaches half its maximum. This is valid for all values of K_a and K_b.

Fit of Histamine Concentration-Response Curves

Data from HA-stimulated [^{3}H]cAMP accumulation in guinea pig cortex (16) were fitted to the metactoid model, using the nonlinear least-squares curve-fitting procedure FITFUN (31), an interactive system for mathematical modeling. The program uses the Marquardt-Levenberg (32, 33) method to minimize the residual sum of squares of the deviation of the curves fitted according to the equations. In the present study, convergence was set at 0.1%, but other values may also be selected. Because coefficients of variation for each data point were not significantly different, the data were not weighted. The best fit of the data to the model was assessed by comparing the residual sum of squares of the fitted curves at equal degrees of freedom, an

index indicating the extent of deviation of the fitted values from the data (31).

Initial estimates of p and r were calculated from the proportions of the HA response insensitive to H_2 and H_1 antagonists under various conditions (16). Based on the experimental results in the presence of antagonists, 55% of the control response to HA was attributed to direct stimulation of H_2 receptors (i.e., E_a obtained directly from stimulation of R_a); 25% of the control HA response was attributed to putative H_1 receptor stimulation (R_b) that was dependent on H_2 receptor stimulation (E_{ab}); and 20% of the control HA response was attributed to putative H_1 receptor stimulation that was dependent on endogenous adenosine (E_{bc}). Results obtained in the presence of adenosine deaminase have a different distribution, with 69% of the HA response attributable to direct stimulation of H_2 receptors (E_a) and 31% to putative H_1 receptor stimulation dependent on H_2 receptor stimulation (E_{ab}). Thus, from Eq. 6, $p = 1.45$ in either the presence or absence of adenosine deaminase, and $r = 0$ or 0.36 in the presence or absence of adenosine deaminase, respectively. Fitted values of p and r, using the best fit estimates of K_a and K_b (see below), were not significantly different from the initial estimates.

Initial estimates of the HA dissociation constants at H_2 receptors, i.e., K_a, and at H_1 receptors, i.e., K_b, were obtained from fitting HA concentration-response curve parameters to Eq. 6 (Table 1). With p and r fixed, the fitting yields a series of K_b values obtained with various initial estimates of K_a (Table 1). A high K_a leads to a low K_b, whereas lowering of K_a led to high fitted K_b values (Table 1), in both the absence and presence of endogenous adenosine. The best fit to experimental data was obtained when K_a was set to 6 μM and a K_b of 2.62 μM was obtained from the fitting procedure. In the presence of adenosine deaminase, the HA concentration response was best fit when K_a was set to 10 μM, with a fitted K_b of 2.43 μM (Table 1). In experiments with antagonists, best fit estimates of K_b were obtained by varying K_a and simultaneously fitting values for K_b and antagonist dissociation constants (K_i and K_i'; Eq. 6) to data from individual experiments with cimetidine, mepyramine, and d-chlorpheniramine. Results from fits to experiments in the absence of adenosine deaminase (16) were similar to those obtained in the absence of antagonists (Table 1). These fits were significantly better at $K_a = 6$ μM, with a fitted K_b of 2.67 ± 0.30 μM, than at $K_a = 10$ μM, with a fitted K_b of 1.00 ± 0.12 μM ($p < 0.05$; t test on the residual sum of squares of the fitted curves at $K_a = 6$ versus 10 μM, four determinations).

Because the best fit estimates of K_b were similar in the presence and absence of adenosine deaminase, a mean K_b of 2.67 μM was used to refit the data to obtain a more accurate estimate of K_a (5.60 ± 0.26 μM) in the absence of adenosine deaminase; K_a was set at 10 μM in the presence of this enzyme. These best fit estimates were then used to obtain fitted dissociation constants for H_1 and H_2 antagonists.

The fitted dissociation constant for cimetidine on the HA response was 0.27 ± 0.03 μM (Fig. 5B), with $p = 1.45$, $r = 0.36$, $K_a = 5.60$ μM, and $K_b = 2.67$ μM. Note that the response to HA at higher concentrations of cimetidine (3 and 10 μM) fell to the right of the fit to these data (Fig. 5B). However, the fitted dissociation constant for cimetidine is identical to our independent estimate (16) from the pharmacological data (0.27 ± 0.5 μM) and is not significantly different from the dissociation constant (0.18 ± 0.05 μM) obtained in the presence of adenosine deaminase and EGTA (16). The latter experimental condition was shown to abolish the H_1 component of the HA response, without apparent effect on the H_2 component (16).

Dissociation constants for H_1 antagonists were also obtained by fitting the data to the best estimates of K_a and K_b. Antagonist dissociation constants obtained in the absence or presence of adenosine deaminase agreed within the experimental error (K_i' for mepyramine = 0.74 ± 0.20 versus 0.51 ± 0.15 nM; K_i' for d-chlorpheniramine = 0.40 ± 0.14 versus 0.79 ± 0.43 nM, respectively). The fits of the data from mepyramine antagonism of the HA response in the presence or absence of adenosine deaminase are shown in Figs. 2B and 4B, respectively. Fitting promethazine antagonism of the HA response obtained in the presence of adenosine deaminase (16) also generated a K_i' in the nanomolar range (1.05 ± 0.43 nM). Importantly, fitting to measurements of the HA response in the absence of adenosine deaminase was consistent with the stereospecific action of the H_1 antagonists; the dissociation constant for l-chlorpheniramine was approximately 100 times greater than that for d-chlorpheniramine (18.06 ± 3.74 versus 0.40 ± 0.14 nM, respectively).

TABLE 1

Stepwise fitting of HA concentration-response curves to the metactoid sensitization model: effect of varying K_a on fitted K_b values

Responses to HA were calculated for 21 data points evenly distributed over the concentration range 0.01–0.1 M, using EC_{50} values (5.91 and 11.36 μM in the absence and presence of adenosine deaminase, respectively) determined experimentally (16). These calculated responses were fit to Eq. 6 of the metactoid model with $p = 1.45$ and $r = 0$ or 0.36 in the presence or absence of adenosine deaminase, respectively (A/K_i and $B/K_{i'} = 0$).

K_a[a]	− Adenosine deaminase		+ Adenosine deaminase (2.5 units/ml)	
	K_b[b]	SS[c]	K_b[b]	SS[c]
μM	μM		μM	
1.00	20.10 ± 7.01	1050	88.50 ± 89.60	4346
3.00	8.68 ± 0.98	85	51.95 ± 25.80	924
6.00	2.62 ± 0.26	35	21.60 ± 3.72	76
10.00	0.89 ± 0.33	264	2.43 ± 0.13	1
30.00	0.35 ± 0.60	3313	−4.87 ± 0.46	2045

[a] Initial estimate.
[b] Fitted value; standard errors are standard deviations of the data from the fitted values.
[c] The residual sum of squares of the fitted curves.

Discussion

A useful mathematical model is expected to predict the experimental results with a minimum of *a priori* assumptions; it should also provoke further research and indicate the best experimental conditions for further testing and refinement of the model. Such criteria are fulfilled by the metactoid sensitization model presented here. From simulations and fits to experimental data under various conditions, the model is found to predict the complex characteristics of the experimental results, obtained in this preparation from guinea pig cortex. This is achieved in spite of oversimplifications of the true relationship(s) between stimulus and response represented by some of the basic assumptions. For example, because interactions between second messengers are common, it is unlikely that the response to the metactoid agonist (E_b) is not at all influenced by activation of direct stimuli (E_a and E_c), as assumed here. Indeed, adenosine receptor activation has been reported to potentiate H_1 receptor-mediated breakdown of inositol phospholipids in guinea pig cerebral cortical slices (34,

35). The products are thought to be involved in the indirect effects of H_1 receptor stimulation on cAMP levels. However, the lag period (10 min) for this interaction makes it unlikely that it would complicate the current application of the model to [^{3}H]cAMP accumulation, because agonist incubations were limited to 10 min (16). The assumption of linear stimulus-response relationships also merits scrutiny. Many receptor-linked second messenger systems act as amplifiers, commonly resulting in nonlinear stimulus-response relationships. In addition, the assumption that agonist D is a full agonist at both E_a and E_b may require modification in a general application of the model. It is not critical for the present application, because the response to only one endogenous agonist acting on both receptors was probed here. However, intrinsic activity can easily be factored into the metactoid equations if required (28). Given the likelihood that one, or more, of the assumptions used here will require modification, parameters for HA action at H_1 and H_2 receptors are conservatively given here as EC_{50} values, rather than as the dissociation constants used in the model.

Predictions of the metactoid model were in agreement with the pharmacological characteristics of an H_1 receptor-mediated potentiation of the [^{3}H]cAMP response to H_2 and adenosine receptor stimulation, obtained in a guinea pig vesicular preparation of cerebral cortex (16). Comparison between the theoretical simulations and experimental data (Figs. 2–5) revealed that the dissociation constants of HA for H_1 and H_2 receptors must be within 1 order of magnitude of each other; otherwise, biphasic HA concentration response curves would be expected. Best fit estimates indicated that the EC_{50} at H_1 receptors was approximately 3–4 times less than that at H_2 receptors, in either the presence or the absence of an adenosine H_1 receptor interaction. The observation that H_1 antagonists caused a shift to the right of the entire HA concentration response only in the presence of an adenosine H_1 receptor interaction (Fig. 4B) supports this difference in the fitted EC_{50} values. It indicates that the EC_{50} for HA on H_1 receptors potentiating the adenosine response is smaller than the EC_{50} of HA at H_2 receptors. In the absence of an interaction between the H_1 and adenosine receptors, simulations revealed that the overall EC_{50} of the measured response for HA could not be less than the EC_{50} of the direct response. Under these conditions, H_1 antagonists only shifted the upper part of the HA concentration response, which was dependent on simultaneous H_2 receptor activation (e.g., Fig. 2B). Concentrations of H_1 antagonists, calculated from binding data (36, 37) to cause a 10-fold shift in the H_1 response (E_b), actually produced a smaller shift in the HA response (Fig. 2B, mepyramine = 0.01 μM; see also Ref. 16). The shift requires higher antagonist concentrations because the curve reflects the H_2 response obtained at HA concentrations at which H_1 receptors have higher occupancy. This was predicted by simulations of the metactoid model and further supports the difference in the fitted EC_{50} values of HA at H_1 and H_2 receptors. The best fit estimate of the EC_{50} of HA at H_2 receptors (10 μM) also agrees well with our independent pharmacological estimates (16), in the absence of an interaction between the H_1 and the adenosine receptors. The independent results (16) were obtained either in the combined presence of adenosine deaminase and H_1 receptor blockade (EC_{50} = 11 μM) or with EGTA EC_{50} = 16 μM), which were used, respectively, to block or to eliminate the H_1 receptor-mediated component. Thus, fitted EC_{50} values for HA at H_1 and H_2 receptors adequately described the experimental data.

The agreement between the dissociation constants of HA receptor antagonists obtained by fitting the pharmacological data to the present model and the results of measurements in other systems with different HA receptor-mediated processes provides further support for the validity of the model. Derived (16) and fitted dissociation constants for the H_2 receptor antagonist cimetidine were identical and in agreement with those found in other H_2-mediated processes (38). Consonant with the model, the fitted dissociation constants for H_1 antagonists on the HA response were not significantly altered by the presence or absence of an interaction between H_1 and adenosine receptors. Fitted values were similar to those reported from H_1 receptor binding studies (14, 17, 36–38) and other H_1-mediated processes (14, 17, 39, 40). Thus, the observed pharmacological characteristics of the HA response are adequately described by the metactoid sensitization model.

If the stimulus-response relationships were nonlinear, the EC_{50} for HA acting at H_1 receptors to potentiate the response to adenosine would be different from the EC_{50} of its action at the same receptor potentiating the response to H_2 receptors. Our simulations indicated a possibility for an independent test of the model if only H_2 receptor stimulation permits expression of H_1 receptor involvement in the response; in this case, the EC_{50} of HA at H_1 receptors would be masked when the EC_{50} is considerably less than that at H_2 receptors. Thus, confirming that the EC_{50} of HA at H_1 receptors was *not* less than our fitted values, HA caused no significant potentiation of the [^{3}H]cAMP response to the H_2 agonist dimaprit (100 μM), at concentrations less than 3 μM [data not shown; experimental conditions, in the presence of adenosine deaminase, as described (16)]. The fitted value for the EC_{50} of HA at H_1 receptors also agrees with our independent estimate from HA potentiation of the [^{3}H]cAMP response to endogenous adenosine, obtained in the presence of H_2 receptor blockade (3 μM) (16). Thus, the EC_{50} for HA at H_1 receptors appears to be independent of the direct stimulus used, supporting the present model.

As noted above, the metactoid model presented here assumes a direct linear response relationship between all reacting components (i.e., all stimuli are directly related to receptor occupancy). However, if one step in the chain of events following a given receptor stimulus initiating [^{3}H]cAMP levels reaches saturation before full occupancy of that receptor, then the system exhibits a spare capacity, i.e., a phenomenological receptor reserve (28). Evidence suggests that the indirect effects of metactoid stimuli on cAMP levels may be limited (13, 41). In addition, although activation of protein kinase C has been implicated as a potential mechanism for the indirect action of agonists on the cAMP response to direct stimuli, the correlation between receptor-mediated phosphatidylinositol turnover and potentiation of the cAMP response is poor (23) and the exclusive involvement of protein kinase C on the cAMP response has been challenged (22, 42, 43). A receptor reserve for either direct or metactoid stimulation would alter somewhat the results of the simulations and data fits. Further definition of the effector mechanisms mediating these responses would be required to introduce such complications into the model.

The results of our simulations based on the metactoid sensitization model suggest how attempts to classify receptors may be confused by interactions of the kind described above. The

simulations reveal conditions ($K_b \ll K_a$) under which only one 'receptor' would be revealed by classical agonist and antagonist studies, if the measured response were entirely dependent on the simultaneous activation of both direct and metactoid receptors. Under these conditions, only nonselective receptor agonists working on both receptors would activate the response (at concentrations required to occupy the direct receptor), whereas selective agonists at either site would have no activity. Competitive antagonists at the direct receptor would appear to cause classical competitive antagonism through that receptor, and much higher concentrations of antagonists at the indirect receptor would be required to inhibit the response than those blocking the indirect receptor. In the worst case, such pharmacological characteristics might be presented as evidence for a new receptor subtype because the true agonist selectivity, as well as the relationships between receptor occupancy and response, would be obscured by the unresolved metactoid interaction. Analysis of the results according to the metactoid sensitization model presented here can resolve such complexities.

Application of the metactoid sensitization model should aid in elucidating the interactions between H_2 and β adrenergic receptor activation (direct stimuli) and H_1 and α adrenergic receptor activation (metactoid stimuli) on cAMP levels. These interactions appear to occur in differing degrees in various brain regions (11), and both components may be selectively modulated by physiological and pharmacological manipulations (25–27). For example, pituitary adrenal hormones alter the magnitude (i.e., p in Eq. 1) of α receptor potentiation of β receptor activation, without affecting agonist EC_{50} values (27). The model may also be useful in resolving the stimulus-response relationships between different subtypes of α adrenergic receptors, where selective agonists have been variously suggested to directly increase cAMP (e.g., Refs. 11 and 44), indirectly increase the cAMP response to different direct stimuli (e.g., Refs. 11–13 and 20), and/or to inhibit the indirect interaction of adrenergic receptors on cAMP responses (e.g., Refs. 20 and 45). In addition, detailed analyses of deviations of metactoid interactions from the assumed model may lead to further understanding of the interrelationship(s) governing direct and indirect receptor-mediated control of cAMP in brain.

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