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H_2 Histaminic Receptors in Rat Cerebral Cortex. 3. Inhibition of [^{3}H]Histamine by H_2 Agonists†

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ABSTRACT: The binding of [^{3}H]histamine to H_2 receptors in homogenates of rat cerebral cortex is inhibited by 11 H_2 agonists in a characteristic and unique manner. At low concentrations of the radioligand (<1.5 nM), the inhibitory profiles of individual agonists (A) are distinctly biphasic; specific binding is well described in most cases by the empirical expression $Y = F_1K_1/(K_1 + [A]) + F_2K_2/(K_2 + [A])$, in which F_1 and F_2 sum to 1. Maximal inhibition is the same for all agonists. Since values of F_2 vary from 0.42 to 0.90, the agonist appears to determine the equilibrium distribution of receptors between two states of affinity. Ratios of apparent affinity (K_2/K_1) vary from 204 to 3 090 000, and there is no correlation between values of K_1 and K_2. Compounds lacking H_2 activity, including structural analogues of histamine and dimaprit, reveal a Hill coefficient of 1 and inhibit the radioligand only weakly. For six agonists, values of K_2 agree and correlate well ($P = 0.00047$) with H_2 pharmacological potency (EC_{50}) in the guinea pig right atrium; for the others, K_2 is less than EC_{50} by 15–61-fold. Four observations suggest that the inhibition corresponding to F_1 is allosteric and cooperative: (a) the dissociation constant of the radioligand appears to vary in the presence of an unlabeled agonist, (b) absolute levels of binding corresponding to F_1, as defined by dimaprit, decrease at higher concentrations of [^{3}H]histamine, (c) F_1 for dimaprit is reduced from 0.48 to 0.32 by 2-methylhistamine ($F_1 = 0.27$) at a concentration of 20 nM ($\sim K_1^{0.5}K_2^{0.5}$ for 2-methylhistamine), but the increase in K_1 for dimprit is at least 100-fold less than expected from competitive effects, and (d) 1 equiv of some agonists appears to preclude access of [^{3}H]histamine to more than 1 equiv of receptors, with no evidence that an appreciable fraction of the unlabeled drug is bound. Noncompetitive effects also may account in part for the inhibition corresponding to F_2.

The sites labeled by [^{3}H]histamine in homogenates of rat cerebral cortex reveal a sensitivity to guanylyl nucleotides and magnesium that establishes their identity as neurohumoral receptors linked to a nucleotide-specific G/F protein (Barbin et al., 1980; Steinberg et al., 1985a). Evidence presented in the preceding paper indicates that the specific binding of [^{3}H]histamine is inhibited by H_2 antagonists in a complex manner characterized by Hill coefficients between 0.57 and 1.01 (Steinberg et al., 1985b). Since 50% inhibition is achieved at concentrations that agree well with reported estimates of pA_2 for blockade of the chronotropic effect of histamine in guinea pig right atrium, the sites labeled by [^{3}H]histamine

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Table I: Mean Parametric Values for the Inhibition of [^{3}H]Histamine by H_2 Agonists[a]

no.	H_2 agonist	pharmacological potency[b] (−log EC_{50})	eq 4 −log K_1	eq 4 −log K_2	eq 4 F_2	eq 4 log (K_2/K_1)	eq 4 ΔB_{min}[c] (%)	eq 4 $[R_1]_t/2K_1$[d]	eq 3 n_{H1}[e]
1	triazolylethylamine	5.11[h]	9.190 ± 0.010	6.880 ± 0.034	0.418 ± 0.003	2.31	0.6, 0.1	0.038, 0.022	0.83 ± 0.15
2	4-methylhistamine	5.51[i]	11.309 ± 0.029	5.049 ± 0.006	0.429 ± 0.002	6.26	−0.3, 0.2	7.2, 4.4	1.46 ± 0.44
3	N^{α},N^{α}-dimethylhistamine	5.67[j]	10.027 ± 0.004	7.079 ± 0.061	0.455 ± 0.001	2.95	−0.8, −0.8	0.21, 0.19	0.97 ± 0.02
4	nordimaprit	3.96[k]	11.410 ± 0.004	5.166 ± 0.077	0.480 ± 0.002	6.24	2.4, 0.4	3.3, 4.3	1.11 ± 0.16
5	N^{α}-methylhistamine	5.82[j]	11.384 ± 0.033	7.605 ± 0.006	0.497 ± 0.001	3.78	0.1, −0.4	3.9, 5.8	0.89 ± 0.05
6	imidazolylpropylguanidine	5.62[l]	10.111 ± 0.008	6.792 ± 0.013	0.502 ± 0.005	3.32	1.4, 0.1	0.25, 0.20	1.35 ± 0.06
7	dimaprit (1.33 and 1.34 nM)[f]	5.81[m]	10.689 ± 0.009	5.999 ± 0.009	0.521 ± 0.003	4.69	−1.0, −0.7	1.8, 0.90	0.98 ± 0.05
	dimaprit (3.73 and 3.75 nM)[f]		9.808 ± 0.000	5.954 ± 0.001	0.779 ± 0.001	3.85	−0.2, −0.1	0.043, 0.041	1.00 ± 0.00
	dimaprit (6.00 and 6.01 nM)[f]		9.355 ± 0.000	5.953 ± 0.008	0.876 ± 0.005	3.40	−0.1, −0.2	0.0047, 0.0045	0.92 ± 0.01
	dimaprit (10.2 and 11.4 nM)[f]			6.032 ± 0.030	1.0[g]		−3.4, −0.1		[g]
8	impromidine	7.64[n]	10.431 ± 0.023	7.237 ± 0.003	0.562 ± 0.004	3.19	−0.7, −0.5	0.75, 0.50	1.45 ± 0.03
9	2-methylhistamine	4.60[j]	10.654 ± 0.120	4.432 ± 0.005	0.732 ± 0.003	6.22	−2.9, −3.1	0.48, 0.54	1.58 ± 0.00
10	pyridylethylamine	4.36[o]	10.744 ± 0.020	4.249 ± 0.002	0.860 ± 0.000	6.50	−3.1, −0.6	0.43, 0.39	0.90 ± 0.01
11	thiazolylethylamine	4.30[o]	10.461 ± 0.017	3.968 ± 0.005	0.899 ± 0.003	6.49	−4.0, −3.0	0.16, 0.16	1.03 ± 0.02

[a] Values listed in the table reflect best fits of eq 3 or 4 (n = 1 or 2) to the data from individual experiments; two experiments were performed for each agonist. The concentration of [^{3}H]histamine was between 1.30 and 1.45 nM unless indicated otherwise. [b] Pharmacological potency is presented as the concentration of the agonist that elicits 50% of the maximal, H_2 chronotropic response in guinea pig right atrium; log EC_{50} for histamine has been taken as −5.96 (Black et al., 1972). [c] ΔB_{min} was calculated according to the expression $\Delta B_{min} = 100(B_{min} - B'_{min})/(B_{max} - B_{min})$, where B_{max} and B_{min} are taken from the best fit of eq 4; B'_{min} indicates binding in the presence of 1.0 mM unlabeled histamine and was measured in each experiment. The value for each of the duplicate experiments is shown. [d] $[R_1]_t$ was calculated according to the expression $[R_1]_t = [PR_1]([P] + K_{P1})/[P]$; $[PR_1]$ was calculated from the values of B_{max}, B_{min}, and F_1 derived from fitting eq 4, and K_{P1} was taken as 3.9 nM (Steinberg et al., 1985a). [P] was taken as the total concentration of [^{3}H]histamine. The value for each of the duplicate experiments is shown. [e] Except for dimaprit above 10 nM [^{3}H]-histamine, values listed in the table reflect best fits of eq 3 (n = 2) with both n_{H1} and n_{H2} determined by regression; the latter was near or equal to 1 in all experiments. [f] The total concentration of [^{3}H]histamine. [g] F_2 was fixed at 1. [h] Durant et al. (1975). [i] Black et al. (1972). [j] Ganellin et al. (1976). [k] C. R. Ganellin, Smith Kline and French Research Ltd., personal communication. [l] Parsons et al. (1975). [m] Parsons et al. (1977). [n] Durant et al. (1978). [o] Ganellin (1980).

reveal a pharmacological specificity typical of H_2 receptors. Antagonists appear to act noncompetitively, however, since their inhibitory behavior is insensitive to the concentration of [^{3}H]histamine, or even of other antagonists. H_2 agonists are reported to inhibit the specific binding of [^{3}H]histamine with Hill coefficients indistinguishable from 1 (Barbin et al., 1980). Although estimates of apparent affinity recall H_2 pharmacological potency with some agonists (Kandel et al., 1980; Barbin et al., 1980), marked differences have been found with others (Barbin et al., 1980). Agonists therefore have not revealed a consistent pattern or trend that reflects the identity of the labeled sites as H_2 receptors. In contrast, the present report describes evidence that H_2 agonists inhibit the specific binding of [^{3}H]histamine in a unique and highly characteristic manner. The results support the identification of the sites as H_2 receptors and suggest that inhibition of the radioligand is both allosteric and cooperative.

Materials and Methods

[^{3}H]Histamine was obtained from Amersham Corp. (40–54 Ci/mmol) and New England Nuclear (32.2 Ci/mmol) and unlabeled histamine from Sigma. Tiotidine was kindly donated by ICI Americas Inc., Wilmington, DE. τ-Methylhistamine, N-methyldimaprit, cimetidine, and all H_2 agonists were the generous gift of Smith Kline and French Research Ltd., Welwyn Garden City, U.K. Dopamine hydrochloride, epinephrine bitartrate, isoproterenol bitartrate, serotonin hydrochloride, scopolamine hydrobromide, and atropine sulfate were purchased from Sigma. Experimental procedures are described in detail in the preceding papers (Steinberg et al., 1985a,b), and equations are numbered according to Steinberg et al. (1985a). In all experiments, the reaction mixture contained 100 mM tris(hydroxymethyl)aminomethane (Tris), 10 mM $MgCl_2$, and 1 mM ethylenediaminetetraacetic acid (EDTA) adjusted to pH 7.48 with sulfuric acid.

Results

H_2 agonists inhibit the specific binding of 1.30–1.45 nM [^{3}H]histamine in a biphasic manner than can be described in most cases by eq 4 assuming two classes of sites (Figure 1). The mean values of log K_1, log K_2, and F_2 from duplicate experiments with each of 11 agonists are listed in Table I. The reproducibility of the data is demonstrated by the effect on the variance of residuals when the two curves are analyzed simultaneously with common rather than separate values of K_1, K_2, and F_2: the increase is small (P_4 = 0.017) with 2-methylhistamine and negligible ($P_4 > 0.15$) with the other 10 agonists. Total binding in the presence of 1.0 mM unlabeled histamine differs from the asymptotic value of eq 4 (B_{min}) by less than 1.5% of specific binding in 21 of the 28 experiments summarized in Table I and by less than 5% in all experiments; each agonist thus appears to preclude access of the radioligand to the same population of sites.

Among those experiments performed at 1.30–1.45 nM [^{3}H]histamine, the mean value of F_2 varies from 0.418 with triazolylethylamine to 0.899 with thiazolylethylamine; the ratio of affinities varies from 204 with triazolylethylamine to about 3 000 000 with pyridylethylamine and thiazolylethylamine. Since K_2/K_1 is large for all agonists tested, F_2 is well-defined by the data and clearly differs for agonists at opposite ends of the observed range. It can be shown, however, that the differences also are significant for agonists with similar values of F_2. All pairs of agonists have been compared in the manner described by Steinberg et al. (1985b). The comparison is based on the difference in the variance of residuals when four sets of data from duplicate experiments with two agonists are fitted twice with eq 4, once assuming a single value of F_2 for both agonists and once with a separate value for each. Both

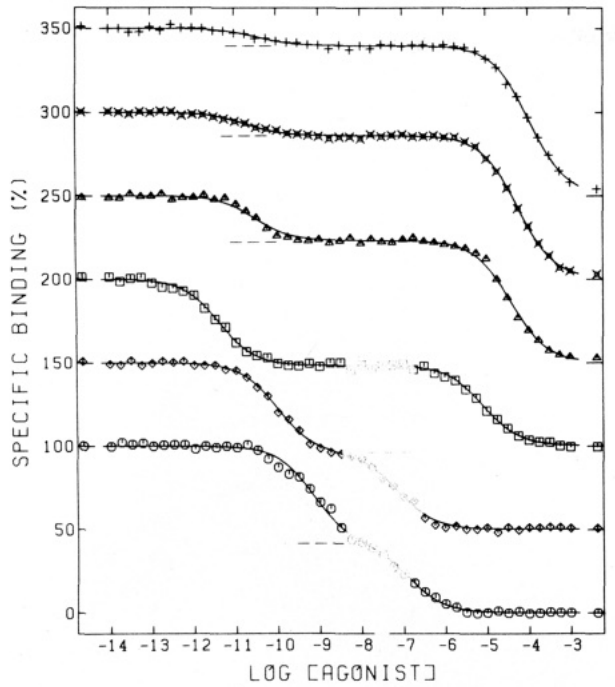

FIGURE 1: Inhibition of [^{3}H]histamine by H_2 agonists at low concentrations of the radioligand. Total binding was measured following incubation of the suspension with [^{3}H]histamine (1.30–1.37 nM) and with the agonist at the concentrations shown on the abscissa. The lines represent best fits of eq 4 ($n = 2$) to the experimental data; values of log K_1, log K_2, and F_2 obtained by regression are as follows: for triazolylethylamine (○), −9.18 ± 0.04, −6.91 ± 0.05, and 0.42 ± 0.01; for N^α,N^α-dimethylhistamine (◊), −10.03 ± 0.04, −7.14 ± 0.04, and 0.46 ± 0.01; for nordimaprit (□), −11.41 ± 0.03, −5.09 ± 0.02, and 0.48 ± 0.01; for 2-methylhistamine (△) −10.54 ± 0.06, −4.43 ± 0.02, and 0.73 ± 0.01; for pyridylethylamine (×), −10.72 ± 0.07, −4.25 ± 0.01, and 0.86 ± 0.00; for thiazolylethylamine (+), −10.44 ± 0.11, −3.96 ± 0.02, and 0.90 ± 0.00. Values plotted on the ordinate are normalized at 100% and 0% to the asymptotic values of eq 4 (B_{max} and B_{min}) obtained from the fitting procedure; successive curves are offset by 50% for clarity. Points at the upper and lower limits of the abscissa indicate binding in the absence of unlabeled agonist and in the presence of 1.0 mM unlabeled histamine, respectively; the latter was omitted from the regression. The value of F_2 is illustrated by the horizontal dashed lines; in subsequent figures, log K_S is indicated by arrows.

analyses employ two values of K_1 and K_2, one for each agonist, and four values of B_{max} and B_{min}, one for each experiment. As noted above, values of K_1, K_2, and F_2 are indistinguishable in duplicate experiments with the same agonist. Out of 55 possible combinations, the difference in F_2 is highly significant ($P < 0.00002$) in 46, significant ($0.0004 < P < 0.006$) in 3,[1] and marginally significant ($0.01 < P < 0.05$) in 4.[1] In only two combinations is the difference in F_2 found to be negligible: triazolylethylamine and 4-methylhistamine ($\Delta F_2 = 0.011$; $P = 0.076$) and N^α-methylhistamine and imidazolylpropylguanidine ($\Delta F_2 = 0.005$; $P = 0.41$). It thus is possible with the present data to discern differences in F_2 of as little as 0.017. Since the concentration of [^{3}H]histamine was within 3.7% of the mean value of 1.35 ± 0.01 nM in 21 of the 22 experiments summarized in Table I, the differences in F_2 rule out the possibility that the biphasic nature of the inhibition derives

[1] N^α-Methylhistamine and dimaprit ($\Delta F_2 = 0.024$; $P = 0.00048$), nordimaprit and imidazolylpropylguanidine ($\Delta F_2 = 0.022$; $P = 0.0055$), triazolylethylamine and N^α,N^α-dimethylhistamine ($\Delta F_2 = 0.037$; $P = 0.0057$), 4-methylhistamine and N^α,N^α-dimethylhistamine ($\Delta F_2 = 0.026$; $P = 0.012$), N^α,N^α-dimethylhistamine and nordimaprit ($\Delta F_2 = 0.025$; $P = 0.012$), imidazolylpropylguanidine and dimaprit ($\Delta F_2 = 0.019$; $P = 0.013$), and nordimaprit and N^α-methylhistamine ($\Delta F_2 = 0.017$; $P = 0.030$).

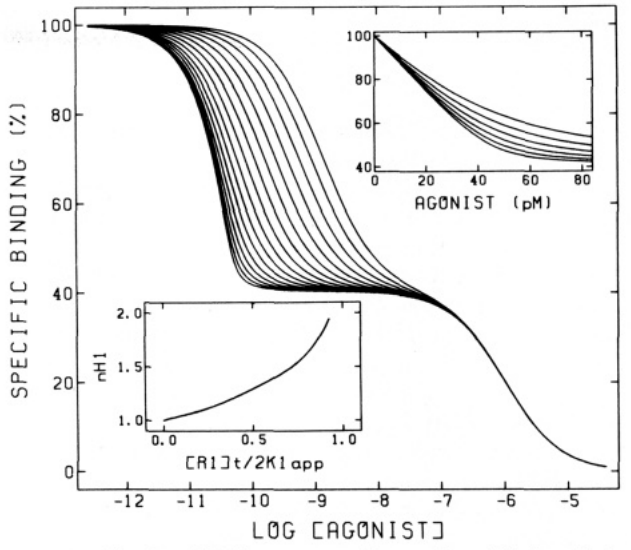

FIGURE 2: Simulated binding patterns for agonists with dissociation constants comparable to or less than the concentration of binding sites. The data were calculated according to eq 5 ($n = 2$) with $[R]_t = 1318$ pM, $F_2 = 0.9655$, log $K_{P1} = -8.409$, log $K_{P2} = -6.655$, and log $K_{A2} = -6.0$; the value of log K_{A1} increases by increments of 0.2 log unit from left (log $K_{A1} = -12.0$) to right (log $K_{A1} = -9.0$). Data in the upper inset are plotted on linear coordinates and illustrate the curves for values of log K_{A1} from −12.0 to −11.0 inclusive. Values of n_{H1} plotted in the lower inset were obtained by fitting eq 3 ($n = 2$) to the simulated data shown in the figure, with B_{max} and B_{min} fixed at 100% and 0%, respectively; $[R_1]_t$ was taken as 45.5 pM and K_{1app} as the total concentration of agonist that reduces binding corresponding to F_1 by 50%.

from two populations of noninterconverting sites.

For some agonists, considerations related to the stoichiometry of inhibition further demonstrate that eq 4 provides only an empirical description of the data. If 1.30–1.45 nM [^{3}H]-histamine and the unlabeled agonist are in equilibrium with a subpopulation of sites that is homogeneous with respect to the radioligand, the capacity corresponding to F_1 (i.e., $[R_1]_t$) can be calculated from the apparent affinity of [^{3}H]histamine [$K_{P1} = 3.9$ nM (Steinberg et al., 1985a)], the free concentration of [^{3}H]histamine, and the values of B_{max}, B_{min}, and F_1 derived from eq 4. Since the abscissa in Figure 1 and the independent variable in eq 4 are in units of total concentration of unlabeled agonist, there is an upper limit of 1 on the value of $[R_1]_t/2K_1$; that is, 50% of the sites cannot be blocked by less than a stoichiometrically equal quantity of agonist. The values listed in Table I indicate that this limit was exceeded with 4-methylhistamine, nordimaprit, N^α-methylhistamine, and, in one experiment, dimaprit; it is approached with several other agonists that reveal values between 0.1 and 1. The extrapolated value of $[R_1]_t$, and hence the value of $[R_1]_t/2K_1$, would be lower if the dissociation constant of [^{3}H]histamine at the sites corresponding to F_1 were less than 3.9 nM. The measured values of $[PR_1]$ constitute a minimal estimate of $[R_1]_t$, however, and the quantity $[PR_1]/2K_1$ exceeded 1 in experiments with both 4-methylhistamine (1.7) and N^α-methylhistamine (1.4). In other experiments, values of $[PR_1]/2K_1$ were between 0.1 and 1 for 4-methylhistamine (1.0), N^α-methylhistamine (1.0), nordimaprit (0.79 and 1.1), and dimaprit (0.47 and 0.22). If the capacity calculated for [^{3}H]histamine is correct, the data imply that 1 equiv of some agonists precludes the binding of [^{3}H]histamine at more than 1 equiv of receptor.

The Hill coefficients associated with F_1 provide further evidence that the corresponding inhibition is not strictly competitive. With those agonists for which $[R_1]_t/2K_1$ exceeds

about 0.1, the sites ostensibly of higher affinity are expected to bind a substantial fraction of the unlabeled agonist at concentrations near or below K_1. In a competitive model, substantial depletion of the free agonist gives rise to the effects illustrated in Figure 2, where the curves have been calculated according to eq 5 with parametric values chosen to reflect the present data. Total binding is to two classes of sites differing in affinity for the unlabeled agonist and for the radioligand. The values of log K_{P1} (−8.409) and log K_{P2} (−6.655) are taken from Steinberg et al. (1985a); while only the former is observed at low concentrations of histamine, results described below implicate the latter in that portion of the inhibition corresponding to F_2 with other H_2 agonists. Similar behavior would be observed, however, if all sites were to bind the radioligand with higher affinity (log K_{P1} = log K_{P2} = −8.409) and capacities were adjusted appropriately. The dissociation constant of the unlabeled agonist at the sites of higher affinity varies from 1 pM (log K_{A1} = −12.0) to 1 nM (log K_{A1} = −9.0) and thus encompasses the capacity associated with F_1 (log $[R_1]_t$ = −10.34). With values of K_{A1} much greater than $[R_1]_t$, the Hill coefficient is 1 for both inflections of the binding profile. As K_{A1} decreases below $[R_1]_t$, however, the upper inflection becomes progressively steeper, reflecting the developing linearity in the relationship between specific binding and the total concentration of unlabeled agonist (Figure 2, upper inset).

Analysis of the present data (eq 3, n = 2) reveals that Hill coefficients at the sites corresponding to F_2 are near or equal to 1 for the 11 agonists studied. At the sites corresponding to F_1, however, the mean Hill coefficient exceeds 1 for 4-methylhistamine, imidazolylpropylguanidine, impromidine, and 2-methylhistamine (Table I). When both n_{H1} and n_{H2} are fixed at 1, neighboring residuals are correlated ($P < 0.05$) at concentrations below $K_1^{0.5}K_2^{0.5}$ M in at least one experiment with each agonist; moreover, the corresponding variance of residuals is reduced significantly ($P < 0.01$) in six out of eight experiments when n_{H1} and n_{H2} are allowed to vary. The comparatively high values of n_{H1} found with some agonists suggest that an appreciable fraction of the unlabeled ligand may bind to the receptor at concentrations near or below K_1. This interpretation is consistent with the data for impromidine, which are well described by eq 5 with K_{P1} and K_{P2} set at 3.9 nM and 221 nM, respectively; neighboring residuals are not correlated below 1.5 nM, and the corresponding variance of residuals is reduced significantly from that obtained with eq 4 (n = 2). It is inconsistent, however, with the data for all agonists taken together. The lower inset in Figure 2 illustrates the expected relationship between n_{H1} and $[R_1]_t/2K_1$ for the computed curves shown in the figure. Although the Hill equation provides only a first approximation of the theoretical binding patterns at the lower values of K_{A1} (log K_{A1} < −10.6; fits not shown), the Hill coefficient is expected to increase appreciably with $[R_1]_t/2K_1$ at higher values of the latter. Values of $[R_1]_t/2K_1$ exceed 0.15 for compounds **2–11** in Table I, but a comparison of rank order among the 20 experiments indicates that there is no relationship between n_{H1} and $[R_1]_t/2K_1$ (ρ_s = 0.002; $P > 0.10$). This inconsistency is most pronounced with nordimaprit and N^{α}-methylhistamine. Values of K_1 for those compounds are the lowest found in the present investigation, but the corresponding Hill coefficients (n_{H1}) are indistinguishable from 1 ($P_1 > 0.5$; $P_2 > 0.1$).[2] Also, n_{H1} is indistinguishable from one in both experiments with dimaprit

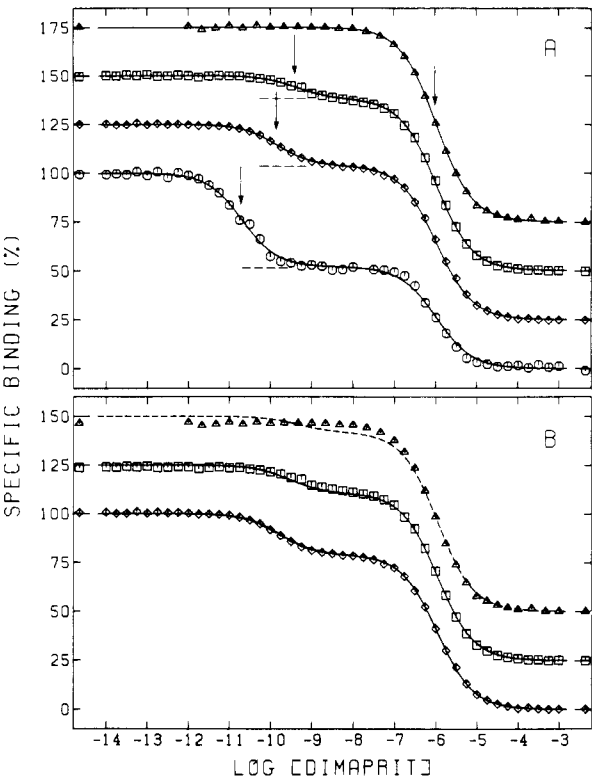

FIGURE 3: Inhibition of [^{3}H]histamine by dimaprit at four concentrations of the radioligand. Total binding was measured following incubation of the suspension with 1.33 (○), 3.73 (◇), 6.00 (□), or 11.4 nM (△) [^{3}H]histamine together with dimaprit at the concentrations shown on the abscissa. (A) The lines represent the best fit of eq 4 (n = 2) to the four sets of data taken together, with a common value of K_2 and separate values of K_1, F_2, B_{max}, and B_{min}. F_2 was set to 1 for the data at 11.4 nM [^{3}H]histamine. Parametric values obtained by regression are as follows: log K_2 for all curves, −5.98 ± 0.01; (○) log K_1 = −10.69 ± 0.02 and F_2 = 0.52 ± 0.00; (◇) log K_1 = −9.84 ± 0.01 and F_2 = 0.78 ± 0.01; (□) log K_1 = −9.38 ± 0.14 and F_2 = 0.88 ± 0.01. Further details are given in the text and in the legend to Figure 1. (B) The lines represent best fits of eq 5 (n = 2) to the data at 3.73 and 6.00 nM (—) or at 3.73 and 11.4 nM (---), taken together; the two lines at 3.73 nM are superimposable. In each analysis, both sets of data shared common values of K_{A1}, K_{A2}, K_{P1}, and F_2; values of $[R]_t$ and C were separate, and K_{P2} was fixed at 221 nM throughout. Parametric values obtained by regression are as follows: for 3.73 and 6.00 nM [^{3}H]histamine (◇, □), log K_{A1} = −14.03 ± 0.20, log K_{A2} = −5.97 ± 0.01, log K_{P1} = −12.70 ± 0.20, and F_2 = 0.9955 ± 0.0002; for 3.73 and 11.4 nM [^{3}H]histamine (◇, △), log K_{A1} = −13.72 ± 0.43, log K_{A2} = −5.97 ± 0.01, log K_{P1} = −12.34 ± 0.43, and F_2 = 0.9955 ± 0.0003. The correlation coefficient of neighboring residuals is as follows: (—) 0.439 (P = 0.0025) at 3.73 nM [^{3}H]histamine and 0.824 ($P < 0.00001$) at 6.00 nM; (---) 0.380 (P = 0.010) at 3.73 nM and 0.918 ($P < 0.00001$) at 11.4 nM. Values plotted on the ordinate are normalized at 100% and 0% to the asymptotic values of eq 5, and successive curves are offset by 25% for clarity.

($P_1 > 0.5$; $P_2 > 0.3$),[2] although there was a 1.8-fold difference in the concentration of protein, a 1.9-fold difference in maximal specific binding, and a corresponding, 2-fold difference in $[R_1]_t/2K_1$. The absence of a relationship among agonists between n_{H1} and $[R_1]_t/2K_1$ argues further that the inhibition corresponding to F_1 is not competitive. Moreover, the Hill coefficient of 1 found with nordimaprit and N^{α}-methylhistamine implies that the free concentration of those compounds is not reduced through binding to the sites associated with F_1. The apparent affinity is sufficiently high, however, that virtually all of the drug would be required simply to match the corresponding amount of bound [^{3}H]histamine (i.e., $[PR_1]/2K_1 \simeq 1.0$). The notion that 1 equiv of agonist precludes the binding of [^{3}H]histamine at several equivalents of receptor thus

[2] Equation 3 (n = 2) was fitted with both n_{H1} and n_{H2} either fixed at 1 or variable, and goodness of fit was tested only for concentrations of agonist below $K_1^{0.5}K_2^{0.5}$ M; P_1 and P_2 otherwise are as defined by Steinberg et al. (1985a).

Table II: Effect of 2-Methylhistamine and Cimetidine on the Inhibition of [^{3}H]Histamine by Dimaprit[a]

second unlabeled ligand	[[^{3}H]histamine] (nM)	$-\log K_1$ value	P_5[c]	$-\log K_2$ value	P_5[c]	F_2 value	P_5[c]	ΔB_{min}[b] (%)
none[d]	1.33, 1.34	10.69		6.00		0.52		
0.020 μM 2-methylhistamine	1.38	10.36 ± 0.02	<0.00001	5.98 ± 0.01	0.022	0.68 ± 0.00	<0.00001	−0.03
0.63 μM cimetidine	1.38	10.35 ± 0.01	<0.00001	5.94 ± 0.01	0.00002	0.49 ± 0.00	<0.00001	−0.3

[a] Values listed in the table reflect the best fit of eq 4 ($n = 2$) to the data illustrated in Figure 4. [b] ΔB_{min} was calculated as described in footnote *c* to Table I, except that B_{max} was taken as total binding measured in the absence of both unlabeled ligands. [c] Level of confidence for the difference in the variance of residuals between the best fit of eq 4 with five variable parameters (K_1, K_2, F_2, B_{max}, and B_{min}) and that with K_1, K_2, or F_2 fixed at the mean value measured in the absence of a second, unlabeled ligand. [d] Parametric values are taken from Table I.

is supported not only by the value of K_1 but also by the corresponding Hill coefficient.

The binding patterns of dimaprit at four concentrations of [^{3}H]histamine between 1.33 and 11.4 nM are illustrated in Figure 3. As the concentration of radioligand is increased, there is a concomitant increase in both log K_1 and F_2 while log K_2 remains constant (Table I, eq 4). At the highest concentration used, the biphasic nature of the inhibition is lost: the Hill coefficient is indistinguishable from 1 ($P_1 > 0.2$; $P_2 > 0.4$), and the data are well described by eq 4 assuming a single class of sites ($P_3 > 0.2$). The similarity in K_2 can be seen in Figure 3A, where the fitted curves have been obtained assuming one value common to the four sets of data. There is a small increase in the variance of residuals ($F = 3.8$; $P = 0.012$) over that obtained with four values of K_2, but the residuals themselves are small, and the lines from simultaneous and independent analyses are virtually superimposable. In contrast, the increase in the variance is substantial ($P < 0.00001$) when the four sets of data at 1.3 and 6.0 nM or at 6.0 and 11 nM (Table I) are fitted assuming a single value of K_2 and one rather than two values of either K_1 or F_2. The absence of any change in log K_2 is in accord with competitive inhibition if the radioligand binds weakly ($K_{P2} > 0.1$ μM) at the sites corresponding to F_2. As described in the accompanying paper (Steinberg et al., 1985a), weak binding is not observed at concentrations of the radioligand below 1.5 nM but appears at higher concentrations where histamine reveals an affinity (K_{P2}) of 221 nM.

Both log K_1 and F_2 are expected to increase at higher concentrations of [^{3}H]histamine if dimaprit and the radioligand compete for a single population of sites corresponding to F_1. Upon closer inspection, however, neither parameter is found to increase in a manner that is in quantitative agreement with competitive inhibition. The affinity of [^{3}H]histamine for the sites corresponding to F_1 can be estimated by using eq 5 ($n = 2$) if data at different concentrations of the radioligand are analyzed simultaneously assuming that all binding is controlled by single values of K_{A1}, K_{A2}, K_{P1}, K_{P2}, and F_2. Since K_{P2} is not implicit in the data, at least with dimaprit, it must be set arbitrarily, and the value of 221 nM reported by Steinberg et al. (1985a) has been used for this purpose. Six combinations are possible in principle if the four sets of data illustrated in Figure 3A are analyzed in pairs; in practice, however, combinations involving the data at 1.33 nM [^{3}H]-histamine are precluded by the anomalous stoichiometry described above. Two of the three remaining combinations are illustrated in Figure 3B, where the lines represent the best fit of eq 5 to the data at 3.7 nM [^{3}H]histamine and either 6.0 or 11 nM [^{3}H]histamine, taken together. The estimated affinity of the radioligand (K_{P1}) is 0.20 pM from the data at 3.7 and 6.0 nM and 0.46 pM from the data at 3.7 and 11 nM; a value of 1.1 pM is obtained from the data at 6.0 and 11 nM (fit not shown). The dissociation constant of [^{3}H]histamine revealed in the binding patterns of dimaprit thus is 3500–19 500-fold lower than that found with histamine alone [K_{P1} = 3.9 nM (Steinberg et al., 1985a)]. Similar differences are obtained if it is assumed arbitrarily that the free concentration of dimprit equals the total concentration and if the data acquired at all four concentrations of [^{3}H]histamine (Table I) then are analyzed simultaneously with the appropriate expression analogous to eq 5. The dissociation constant (K_{P1}) of [^{3}H]histamine is less than 1.0 pM irrespective of whether one assumes a single value of F_2 for all experiments or a separate value for that at each concentration of the radioligand. Such discrepancies between the value of K_{P1} revealed by dimaprit and by histamine alone are at variance with the competitive scheme underlying eq 5 and suggest that dimaprit and [^{3}H]histamine bind at different sites.

Further evidence against a competitive model derives from the values of F_1 or F_2 at different concentrations of the radioligand. Each of the three experiments illustrated in Figure 3B yields a poor correlation of neighboring residuals ($P > 0.10$) when the data are analyzed independently in terms of eq 4. In contrast, simultaneous analysis in terms of eq 5 yields correlations that are highly significant and fitted curves that deviate markedly from the experimental data (Figure 3B). This reflects the observed changes in absolute levels of binding corresponding to F_1 as defined by eq 4. Values of PR_1 calculated from B_{max}, B_{min}, and F_1 for the eight experiments listed in Table I are as follows: 13 pmol/g of protein at 1.4 nM [^{3}H]histamine, 5.7 and 7.6 pmol/g of protein at 3.7 nM [^{3}H]histamine, 4.3 and 4.9 pmol/g of protein at 6.0 nM [^{3}H]histamine, and zero at or above 10 nM [^{3}H]histamine. The decrease in PR_1 observed at increasing concentrations of the radioligand is opposite to the increase predicted by eq 5. The fitted curves thus tend to show larger values of F_1, as defined by eq 4, than are found experimentally at the higher concentration of [^{3}H]histamine in each of the two analyses depicted in Figure 3B. This difference is particularly obvious at 11 nM [^{3}H]histamine. Analysis with eq 4 shows that F_1 for the fitted curve is 0.08; in contrast, F_1 is indistinguishable from zero for the experimental data and clearly less than the value predicted by fitting eq 5. As noted in the accompanying paper (Steinberg et al., 1985a), absolute levels of binding have shown little variation in the present investigation when measured under the same conditions. The decrease in PR_1 at higher concentrations of [^{3}H]histamine therefore reflects a property of the system and not merely differences in the specific capacity of the homogenate for the radioligand.

Studies using two agonists in concert indicate that the inhibition corresponding to F_1 is mutually exclusive and additive but apparently not competitive. The data presented in Figure 4 illustrate the inhibitory patterns of dimaprit alone and in the presence of 20 nM 2-methylhistamine. The concentration of the latter approximates $K_1^{0.5}K_2^{0.5}$ M (eq 4) and was chosen to achieve complete inhibition at the sites corresponding to F_1 while having no effect at the sites corresponding to F_2. 2-Methylhistamine reduces the value of F_1 for dimaprit from 0.48 to 0.32 (Table II); the decrease corresponds to 26% of specific binding and agrees well with the 27% of specific

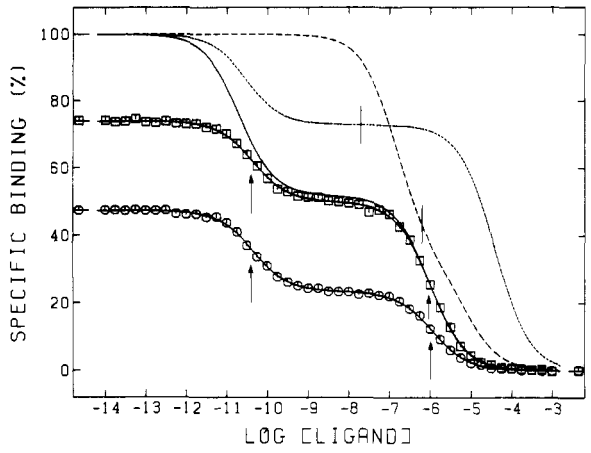

FIGURE 4: Effect of 2-methylhistamine and cimetidine on the inhibition of [^{3}H]histamine by dimaprit. Total binding was measured following incubation of the suspension with 1.38 nM [^{3}H]histamine, either 20 nM 2-methylhistamine (□) or 0.63 μM cimetidine (○), and dimaprit at the concentrations shown on the abscissa. The solid lines through the experimental data represent best fits of eq 4 (n = 2); parametric values obtained by regression are listed in Table II. Other lines represent the inhibitory patterns revealed by dimaprit (—), 2-methylhistamine (···), and cimetidine (- - -) when present as the only unlabeled ligand; each was calculated according to eq 4 (n = 2) by using the mean, parametric values listed in Table I of the present and preceding reports (Steinberg et al., 1985b). The vertical lines indicate the concentration of 2-methylhistamine (log [A] = −7.70) or cimetidine (log [A] = −6.20) that was present in the experiment. The data for dimaprit in the presence of either 2-methylhistamine or cimetidine are normalized at 100% to a control in which total binding was measured in the absence of both unlabeled ligands. Further details are given in the legend to Figure 1.

binding that corresponds to F_1 for 2-methylhistamine. There is virtually no effect of 2-methylhistamine on the value of K_2 for dimaprit (Table II), as expected if the concentration of the ligand is 3 orders of magnitude below its apparent binding constant. In contrast, the 2.1-fold increase in K_1 (Δ log K_1 = 0.33; $P_5 < 0.00001$) is negligible when compared with the increase expected from competitive effects. If the dissociation constants of 2-methylhistamine and [^{3}H]histamine are taken as 22.2 pM (Table I) and 3.9 nM (Steinberg et al., 1985a), respectively, a 660-fold increase is expected in K_1 for dimaprit; if K_{P1} for [^{3}H]histamine is in the subpicomolar range (Figure 3B) and K_{A1} for 2-methylhistamine is about 1500-fold lower than K_1, in analogy with the observed effect of the radioligand on dimaprit (Figure 3B), the expected increase in K_1 for dimaprit is about 300-fold.

The antagonist cimetidine differs markedly from 2-methylhistamine in its effect on the inhibitory behavior of dimaprit (Figure 4). As described in the preceding paper, the Hill coefficient for cimetidine alone is 0.59; the data are well described assuming two classes of sites (eq 4, n = 2) with values of K_1 and K_2 of 0.15 μM (log K_1 = −6.83) and 7.4 μM (log K_2 = −5.13), respectively (Steinberg et al., 1985b). A concentration of 0.63 μM thus achieves 81% of the inhibition corresponding to F_1 and 7.9% of that corresponding to F_2. Since F_1 for cimetidine is 0.67, the antagonist is expected to cause a substantial increase or decrease in F_2 for dimaprit if the two drugs inhibit [^{3}H]histamine via a common pathway. As illustrated in Figure 4, however, there is little change in the overall behavior of dimaprit, and F_2 has undergone only a small decrease, from 0.52 to 0.49 (Table II). This anomalous effect of cimetidine on dimaprit may be related to the observation that F_2 is higher with dimaprit alone (0.52) than with cimetidine alone (0.33), a difference that in itself is at variance with eq 5. It therefore appears that cimetidine and dimaprit may inhibit [^{3}H]histamine via different pathways, possibly

Table III: Mean Parametric Values for the Inhibition of [^{3}H]Histamine by Compounds Lacking H_2 Pharmacological Activity[a]

compound	eq 3	eq 4	
	n_{H1}	−log K_1	ΔB_{min}[b] (%)
τ-methylhistamine (2)	1.00 ± 0.02	4.86 ± 0.00	−3.0, −2.2
N-methyldimaprit (2)	1.03 ± 0.03	3.49 ± 0.01	−2.3, −2.7
dopamine (1)	1.00 ± 0.01	3.63 ± 0.00	0.5
(−)-epinephrine (1)	1.09 ± 0.02	3.53 ± 0.01	2.0
isoproterenol (1)	0.98 ± 0.00	3.12 ± 0.00	[c]
serotonin (1)	1.00 ± 0.01	3.04 ± 0.00	[c]
scopolamine (1)	0.99 ± 0.03	3.45 ± 0.01	−0.4
(±)-atropine (1)	0.99 ± 0.02	2.95 ± 0.01	[c]

[a] Values listed in the table reflect best fits of eq 3 (n = 1) and 4 (n = 1) to the data from individual experiments; the number of experiments is indicated in parentheses. The concentration of [^{3}H]histamine was between 1.34 and 1.38 nM; the highest concentration of unlabeled ligand was 1.0 mM in each case. [b] ΔB_{min} was calculated as described in footnote c to Table I. [c] In fitting either equation, B_{min} was fixed at the value measured in the presence of 1.0 mM unlabeled histamine.

reflecting the identity of the former as an H_2 antagonist and of the latter as an H_2 agonist. The noncompetitive nature of cimetidine's effect on the binding of dimaprit also is evident in the apparent affinity of the latter. As described in the preceding paper (Steinberg et al., 1985b), the apparent dissociation constants of cimetidine at 1.36 nM [^{3}H]histamine are given by eq 5 as either 0.11 or 0.15 μM for K_{A1} and either 5.5 or 7.3 μM for K_{A2}, depending upon the affinity and the effect of the radioligand. If cimetidine and dimaprit are competitive with respect to each other, 0.63 μM cimetidine is expected to increase either K_1 or K_2 (eq 4) for dimaprit by at least 4.2-fold (Δ log K = 0.62) irrespective of whether cimetidine is competitive or noncompetitive with respect to [^{3}H]histamine. In contrast, the largest increase observed experimentally is a 2.2-fold change in K_1 (Table II).

The specific binding of 1.34–1.38 nM [^{3}H]histamine is inhibited weakly by several compounds known or presumed to be inactive at H_2 receptors (Table III). τ-Methylhistamine (Ganellin et al., 1976) and N-methyldimaprit are structural analogues of histamine and dimaprit and thus have been proposed as controls for the H_2 histaminic nature of a system or a response (Ganellin et al., 1976; C. R. Ganellin, personal communication). Both compounds reveal Hill coefficients indistinguishable from 1, and the data are well described by eq 4 assuming a single class of sites. Agonists and antagonists for other neurohumoral receptors behave similarly, although some inhibit only about 50% of specific binding at the highest concentration used and the lower half of the curve is not defined by the present data.

Discussion

H_2 agonists are unique in their inhibitory behavior at low concentrations of [^{3}H]histamine. The distinctly biphasic profiles are remarkable for the large differences observed between estimates of affinity at the two classes of sites: no agonist tested in the present investigation has shown a difference of less than 200-fold (Table I). The shape of the curve thus appears to be characteristic of H_2 agonists. Conversely, monophasic binding profiles are obtained with ligands to other neurohumoral receptors and with two compounds structurally similar to H_2 agonists but lacking H_2 activity (Table III). The biphasic patterns thus appear to occur only with H_2 agonists. This apparently absolute distinction between compounds with and without H_2 activity complements the H_2 pharmacological specificity found among H_2 antagonists (Steinberg et al., 1985b) and serves further to identify the sites labeled by [^{3}H]histamine as H_2 receptors.

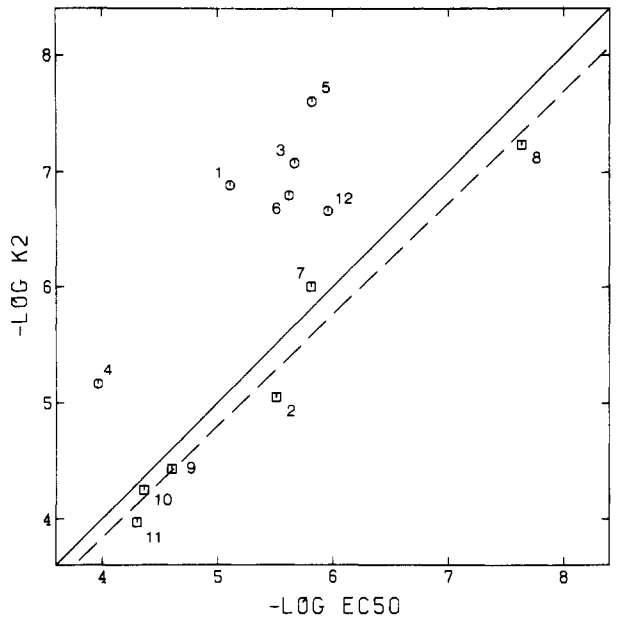

FIGURE 5: Comparison of apparent affinity and H_2 pharmacological potency for H_2 agonists. Agonists are identified according to their numbers in Table I; compound **12** is histamine. Estimates of H_2 pharmacological potency in the guinea pig right atrium (log EC_{50}) and apparent affinity in the binding assay (log K_2, eq 4) are listed in Table I for compounds **1–11**; the apparent affinity of histamine is taken from the preceding paper [log K_{P2} = −6.655 (Steinberg et al., 1985a)]. The solid line indicates numerical equivalence. The dashed line indicates the result of linear regression for the six compounds represented by squares (r = 0.982; P = 0.00047); each point was assigned a weight of 1.

The parameters F_1 and F_2 derived from eq 4 are of uncertain significance, as discussed below, but each seems to reflect a common phenomenon for all agonists, including histamine. The increase in K_1 found with dimaprit at higher concentrations of [^{3}H]histamine implies that the effective dissociation constant of the radioligand is well below 1.0 nM; the absence of any change in K_2 implies that the corresponding dissociation constant of the radioligand exceeds 0.1 μM. These predictions show qualitative agreement with the values of 3.9 nM and 221 nM found for histamine (Steinberg et al., 1985a), although the former is too high to account quantitatively for the reduction in the apparent affinity of dimaprit at the sites corresponding to F_1. Also, the value of F_1 obtained with dimaprit is decreased in the presence of 0.1 mM guanylyl imidodiphosphate (GMP-PNP),[3] in accord with the decrease in apparent capacity at the sites of higher affinity for histamine (Steinberg et al., 1985a). For both dimaprit and unlabeled histamine, the value of F_1 decreases at higher concentrations of the radioligand. Finally, the inclusion of 20 nM 2-methylhistamine reduces the value of F_1 for dimaprit by an amount equivalent to the value of F_1 for 2-methylhistamine. It therefore seems appropriate to compare values of K_1 or K_2 among different agonists.

Attempts to correlate binding with response are problematic with agonists in view of the presumably complex relationship that exists between the two events. The comparison may be particularly dubious when different animals and different tissues are involved. Values obtained for K_2 nevertheless recall published estimates of pharmacological potency (EC_{50}) for several agonists studied in the present investigation (Figure 5). With six compounds,[4] K_2 and EC_{50} agree numerically to within a factor of 3 (Table I) and are highly correlated over a 1000-fold range of potency (P = 0.00047). Other agonists are relatively more potent in the binding assay, with ratios of EC_{50} to K_2 varying from 5 with histamine to about 60 with triazolylethylamine and N^{α}-methylhistamine. The pharmacological potency thus appears to represent a lower limit on the apparent affinity that is expressed in the binding assay. Values of K_1 seeem unrelated, at least at the lowest concentrations of [^{3}H]histamine, to pharmacological potency in the guinea pig atrium. Differences between K_1 and EC_{50} range from 600-fold with impromidine to 28 million-fold with nordimaprit, and there is no correlation between the two parameters. At higher concentrations of [^{3}H]histamine, however, the value of K_1 obtained with dimaprit is found to increase. Since F_1 decreases to 0, it is not clear whether K_1 remains sensitive to the radioligand at all concentrations of the latter, or whether there is an upper limit, possibly represented by K_2. In either event, the rank order could change at higher concentrations of [^{3}H]histamine if all agonists are not affected equally.

Differences between K_1 and pharmacological potency notwithstanding, several observations suggest that binding at the sites corresponding to F_1 is related to H_2 activity. First, the binding of higher affinity gives rise to the distinctly biphasic pattern characteristic of H_2 agonists; compounds lacking H_2 activity seem to be uniformly weak inhibitors of [^{3}H]histamine, are monophasic in their behavior, and reveal none of the anomalies associated with most agonists. Second, H_2 receptors are known to stimulate adenylate cyclase in some tissues (Green et al., 1977; Johnson et al., 1979; Gajtkowski, et al., 1983), albeit not in homogenates of rat cortex (Hegstrand et al., 1976); it is the binding of histamine associated with F_1 that is affected by guanylyl nucleotides and magnesium in the manner characteristic of cyclase-linked receptors (Steinberg et al., 1985a). Third, F_1 constitutes between 40% and 60% of specific binding with those compounds that are more potent as H_2 agonists than as H_1 agonists, taking the potency of histamine as the basis for comparison. Lower values are obtained only for those compounds that are predominantly H_1 in their activity. This trend suggests not only that F_1 is related to the H_2 activity of the agonist but also that the H_1 potency of the agonist is reflected in its binding to H_2 receptors. The relative potencies[5] of thiazolylethylamine, pyridylethylamine, and 2-methylhistamine in H_2 and H_1 systems are 0.012, 0.036, and 0.12, respectively; corresponding values of F_1 are 0.10, 0.14, and 0.27 (Table I). As noted above, the differences in F_1 are small but highly significant ($P < 0.00001$) in each case. The observation of a common rank order for relative potency (H_2/H_1) and F_1 raises the possibility that H_1 and H_2 receptors interact in a manner that influences the binding of agonists to the latter. It will be of interest if this trend can be confirmed with other agonists that exhibit comparable, relative potency in H_1 and H_2 systems. Should there be a link between H_1 and H_2 receptors, it appears not to be sensitive to H_1 antagonists at concentrations that block the pharmacological response in H_1 systems. Mepyramine inhibits the histamine-induced contraction of guinea pig ileum and binds to homogenates of rat brain with apparent dissociation constants of 1.2 nM (Hill et al., 1977) and 3–10 nM (Trans et al., 1978; Kandel et al.,

[3] G. H. Steinberg, M. Kandel, S. I. Kandel, and J. W. Wells, unpublished observations.

[4] 4-Methylhistamine, dimaprit, impromidine, 2-methylhistamine, pyridylethylamine, and thiazolylethylamine.

[5] H_1 potencies relative to that of histamine (D_1) were taken from Durant et al. (1975); H_2 potencies relative to that of histamine (D_2) were calculated from the values shown in Table I. The values cited in the text represent the ratio D_2/D_1.

1980; Hill & Young, 1980), respectively; in contrast, it inhibits the specific binding of [^{3}H]histamine with an apparent dissociation constant of 17 μM (Steinberg et al., 1985b). A concentration of 0.1 μM therefore is sufficient to block at least 91% of the H_1 response while having virtually no effect (<1%) on the specific binding of 1.4 nM [^{3}H]histamine. The binding patterns of 2-methylhistamine in the presence and absence of 0.1 μM mepyramine are indistinguishable (data not shown).

Agreement between experimental data and a mathematical model is not in itself evidence that the model is correct. An uncertainty arising with H_2 antagonists relates to the degree of multiplicity that can be read from the binding patterns: one or two classes of sites are sufficient to describe the data for individual compounds, but the possible involvement of three or more classes cannot be ruled out (Steinberg et al., 1985b). With agonists, however, the differences between K_1 and K_2 are large and the two components of the inhibition are exceptionally well-defined. It therefore seems unlikely that more than two processes are involved, although that associated with K_1 appears cooperative with some drugs and may involve several steps. It follows that the observed variation in F_2 reflects some action of the agonist on the properties of the receptor at equilibrium and not a degree of multiplicity beyond the resolution of the data. The nature of that action is unclear, however, apart from the implication that the agonist controls the equilibrium distribution of receptors among different states. Further efforts to rationalize the binding patterns in terms of models commonly applied to neurohumoral receptors are confronted with anomalies similar to those found in the binding patterns of H_2 antagonists (Steinberg et al., 1985b) and of histamine itself (Steinberg et al., 1985a). Since the model implied by eq 4 is not consistent with data for more than one agonist at a time, the physical significance of F_2 is speculative; it does not necessarily follow, however, that other parameters are similarly obscure. Among the 27 Hill coefficients that characterize the data summarized in Table I, four may exceed 1 but none is significantly lower. This close adherence of the data to a rectangular hyperbola, together with the absence of any evidence that more than two classes of sites are involved, suggests that K_1 and K_2 either represent or are directly related to the equilibrium dissociation constants of the agonists for sites of each class. Interestingly, there is no correlation between K_1 and K_2 when the binding of agonists is measured at 1.30–1.45 nM [^{3}H]histamine. As noted above, the possibility of a correlation at higher concentrations of the radioligand cannot be ruled out; the observed difference in pharmacological specificity suggests, however, that the biphasic nature of the inhibition may reflect separate and distinct sites and not differences induced within an otherwise homogeneous population.

The inhibition corresponding to F_1 reveals at least four features that suggest an allosteric process. First, the dependence of K_1 for dimaprit on the concentration of [^{3}H]histamine is qualitatively in accord with competitive inhibition but implies that K_{P1} for the radioligand is below 1 pM; since Steinberg et al. (1985a) report that K_{P1} is 3.9 nM, the affinity of histamine appears to vary in the presence of another agonist. Second, absolute levels of binding corresponding to F_1 decrease from about 13 pmol/g of protein to zero as the concentration of [^{3}H]histamine is increased from 1.4 to 11 nM. The capacity for [^{3}H]histamine is expected to vary somewhat from preparation to preparation; the quality of the data is such, however, that the component of higher affinity for dimaprit ought to be clearly visible at 11 nM [^{3}H]histamine if the inhibition is competitive. Third, 2-methylhistamine reduces the value of F_1 for dimaprit, but the concomitant increase in K_1 is at least 100-fold less than that expected were the two drugs to inhibit [^{3}H]histamine competitively. Fourth, the apparent stoichiometry of inhibition ($[R_1]_t/2K_1$) equals or exceeds 1 for 4-methylhistamine, nordimaprit, N^α-methylhistamine, and dimaprit. The stoichiometry is reduced if the capacity is recalculated assuming that the relevant sites are saturated with radioligand (i.e., $K_{P1} \ll 3.9$ nM) but still equals or exceeds 1 for 4-methylhistamine, nordimaprit, and N^α-methylhistamine. A stoichiometry greater than 1 implies that the inhibition is not only allosteric but also highly cooperative, since 1 equiv of agonist is able to block access of the radioligand to more than 1 equiv of receptor. The notion that relatively few sites bind the unlabeled drug is supported by the observation that compounds such as nordimaprit and N^α-methylhistamine, for which the stoichiometry exceeds or equals 1 at any value of K_{P1}, show no evidence in their Hill coefficients that the free concentration is reduced through binding to the receptor. The inhibition thus appears to reflect an amplified effect of one class of sites upon binding of the radioligand at another, much larger class.

The inhibition corresponding to F_2 also reveals a number of anomalies that argue against a strictly competitive process. If both dimaprit and [^{3}H]histamine compete for the same sites, the failure of K_2 for the former to increase with the concentration of the latter implies that K_{P2} for histamine exceeds 100–150 nM. This prediction is in good agreement with the value of 221 nM that emerges from isotopic dilution (Steinberg et al., 1985a), but dimaprit and histamine differ in the relative number of sites ostensibly of lower affinity at equal concentrations of the radioligand. For dimaprit, mean values of F_2 are 0.52, 0.78, 0.88, and 1.0 at 1.3, 3.7, 6.0, and about 11 nM [^{3}H]histamine, respectively (Table I); for histamine, however, corresponding values of F_2 are 0.0, 0.18, 0.26, and 0.62 at similar concentrations of [^{3}H]histamine [Table III in Steinberg et al. (1985a)]. The relative number of sites corresponding to F_2 thus is consistently less with unlabeled histamine than with dimaprit. The difference is similar to the variation in F_2 among H_2 agonists at about 1.4 nM [^{3}H]histamine (Table I) and suggests that agonists control the affinity of the labeled sites for the radioligand. Another estimate of K_{P2} can be obtained from the relationship between the concentration of [^{3}H]histamine and the absolute level of binding corresponding to F_2 as defined by dimaprit (PR_2). The normalized values of PR_2 plotted in Figure 6 were calculated as described by Steinberg et al. (1985a) for the concentrations of the radioligand listed in Table I. The increase in PR_2 can be described assuming a single class of sites and a Hill coefficient of 1 (Figure 6, inset), although the variance of residuals is somewhat lower ($P = 0.0016$) if the Hill coefficient is allowed to exceed 1. The apparent dissociation constant of 12 ± 1 nM is about 18-fold lower than that of 221 nM found by Steinberg et al. (1985a) and at least 6-fold lower than is consistent with the failure of [^{3}H]histamine to influence the value of K_2 (eq 4) for dimaprit. The discrepancy among estimates of K_{P2} may reflect the limitations of the data, particularly in the analysis of PR_2 where a normalization is required and the resolution is low. On the other hand, reproducibility has been excellent throughout the investigation, and in the experiments with dimaprit, conflicting estimates of K_{P2} are obtained from two different approaches to the same data. Since a competitive model is apparently at variance with much if not all of the data presented here and in the preceding papers (Steinberg et al., 1985a,b), the inhibition corresponding to F_2 also may reflect noncompetitive effects.

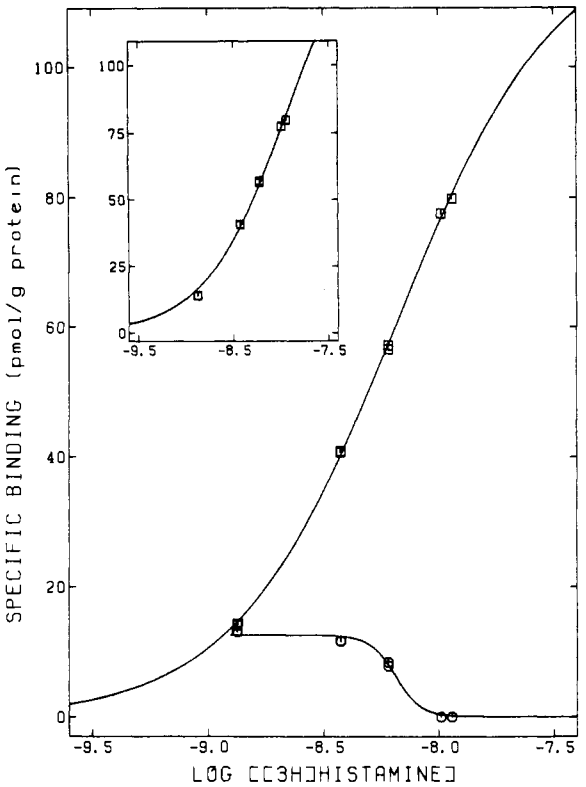

FIGURE 6: Absolute levels of binding corresponding to F_1 and F_2 for dimaprit at different concentrations of [^{3}H]histamine. Total specific binding of [^{3}H]histamine (PR_1 + PR_2) was calculated according to the expression ($[PR_1] + [PR_2]$) = $[R]_t[P]/([P] + K_P)$ for each concentration of [^{3}H]histamine ([P]) listed for dimaprit in Table I; $[R]_t$ and K_P were taken as 107 pmol/g of protein and 3.9 nM, respectively. Values of PR_1 (○) and PR_2 (□) were calculated from total specific binding and the appropriate value of F_2 (Table I). The lines represent the best fit of eq 7 to the normalized data, taken together with K_β common to both PR_1 and PR_2 and with other parameters separate; the reduction in the variance of residuals is negligible (P = 0.50) when PR_1 and PR_2 are assigned separate values of K_β. Parametric values obtained by regression are as follows: for PR_1 and PR_2, log $K_\beta = -8.19 \pm 0.01$; for PR_1, $n_\beta = 7.8 \pm 2.7$ and $B_{max} = 12.5 \pm 0.4$ pmol/g of protein; for PR_2, $n_\beta = 1.26 \pm 0.02$ and $B_{max} = 120 \pm 2$ pmol/g of protein. The values of K_α and n_α for PR_1 were chosen arbitrarily to permit the attainment of B_{max} at 1 nM [^{3}H]histamine. $I_{\alpha a}$, $I_{\alpha b}$, $I_{\beta a}$, and $I_{\beta b}$ were set to 0, 1, 1, and −1 for PR_1 and to 1, 0, 0, and 1 for PR_2. The line in the inset represents the best fit of the expression $[PR_2] = [R_2]_t[P]/([P] + K_P)$ to the data for PR_2; values of $[R_2]_t$ and K_P obtained by regression are 166 ± 11 pmol/g of protein and 11.8 ± 1.2 nM, respectively. All points were weighted equally during the fitting procedures.

The difference in pharmacological specificity between K_1 and K_2 suggests that each component of the inhibition reflects an interaction of the unlabeled agonist with a different site. Moreover, the arguments related to stoichiometry suggest that binding of the radioligand at the sites corresponding to F_1 is inhibited by H_2 agonists via sites of a separate and much smaller population. Three lines of evidence suggest, however, that the sites labeled by the radioligand and identified as either F_1 or F_2 by unlabeled agonists may be different conformers of the same site. First, the labeled sites appear homogeneous with respect to unlabeled histamine at low concentrations of the radioligand (Steinberg et al., 1985a) and with respect to dimaprit at higher concentrations (Figure 3); the sites thus appear to be exclusively of higher affinity for [^{3}H]histamine in the absence of agonists and exclusively of lower affinity at sufficient concentrations of agonist. Second, saturating concentrations of all agonists reduce total binding to the same level at the same concentration of [^{3}H]histamine; all agonists thus appear to block access of the radioligand to the same sites irrespective of the value of F_2. Third, the increase in PR_2 revealed by dimaprit at higher concentrations of [^{3}H]histamine is concomitant with an anomalous decrease in PR_1. The lines in Figure 6 represent the best fit of eq 7 to the two sets of data, taken together in a manner analogous to that described by Steinberg et al. (1985a). Of the two processes that comprise eq 7, the first (α) is assigned to the unobserved increase in PR_1 that presumably occurs at concentrations of the radioligand below those used in the present investigation; the second (β) is assigned to the increase in PR_2 and to the decrease in PR_1, both of which can be observed in the figure. The value of K_β was common to both sets of data, whereas the values of n_β and B_{max} were separate. The fitted curves agree well with the data assuming a common value of K_β; the same process therefore seems to control the changes in both PR_1 and PR_2, with the attendant implication that the latter is achieved at the expense of the former. Unlike the patterns observed with unlabeled histamine (Steinberg et al., 1985a), however, common values of n_β or B_{max} cause the fitted curves to deviate markedly from the data. The rate of decrease in PR_1 ($n_\beta = 7.8 \pm 2.7$) thus exceeds the rate of increase in PR_2 ($n_\beta = 1.26 \pm 0.02$), and the apparent capacities are 12 and 120 pmol/g of protein, respectively. It follows that the changes in PR_1 and PR_2 cannot reflect a direct interconversion in which a loss of sites from one class is matched by an equal gain to another. Also, the curve for total binding, computed by summing eq 7 for PR_1 and PR_2, contains a shoulder that is not observed experimentally. These discrepancies may reflect the low resolution of the data in Figure 6, the inadequacies of eq 7, or both.

It is of interest to compare the binding patterns of histamine (Steinberg et al., 1985a) and other H_2 agonists with those of H_2 antagonists (Steinberg et al., 1985b). Apart from the general inference that more than one state of affinity is involved, there appears to be little in common between the two classes of drugs. The difference in the shape of the binding profiles is evident from an inspection of the data. Whereas the ratio K_2/K_1 varies among agonists from 204 to over 3 000 000 (Table I), the corresponding range among antagonists is from 1 to 60. A 3.4-fold margin (Δ log K = 0.53) thus exists between the minimum value of K_2/K_1 found with agonists other than histamine and the maximum value found with antagonists. With histamine and dimaprit, an increase in the concentration of the radioligand markedly alters the value of K_1 for the two agonists, albeit in opposite directions; with tiotidine and cimetidine, however, the value of K_1 is independent of the concentration of the radioligand, at least between 1.3 nM and 10 nM. One agonist is observed to reduce the value of F_1 revealed by another in an additive manner; in contrast, one antagonist is without effect on the value of F_1 revealed by another. Effects of agonists on the binding of antagonists, and vice versa, are small and apparently noncompetitive. Among agonists, there is no relationship between estimates of K_1 and K_2; the difference in pharmacological specificity suggests that the inhibition corresponding to F_1 and F_2 is mediated by different binding sites. Among antagonists, however, estimates of K_1 and K_2 correlate well (P = 0.00001); both components of the inhibition thus may reflect binding of the antagonist at the same sites. The differences between agonists and antagonists suggest that different phenomena determine the shape of the binding curves for each class of drugs. This interpretation finds support in the observation that the binding properties in solution and suspension show some similarities with respect to agonists, as noted below, but appear completely different with respect to antagonists (Wells et al., 1985).

A comparison of the results from our own laboratory with those reported by Barbin et al. (1980) indicates that the sites of nanomolar affinity for [^{3}H]histamine are probably the same in both investigations (Steinberg et al., 1985a) but that the inhibitory behavior of H_2 antagonists differs markedly (Steinberg et al., 1985b). H_2 agonists were found by Barbin et al. (1980) to inhibit [^{3}H]histamine with Hill coefficients indistinguishable from 1. Since the concentration of the radioligand was 10 nM in their experiments, the reported pattern is in good agreement with the results obtained with dimaprit in the present investigation. Moreover, estimates of affinity reported by Barbin et al. (1980) correlate with the values of K_2 listed in Table I ($P = 0.00028$, $n = 8$). The sites identified in both investigations therefore show some similarities in the binding of agonists. In the report of Steinberg et al. (1985a), however, unlabeled histamine is shown to inhibit 10 nM [^{3}H]histamine with a Hill coefficient of 0.46, a value markedly lower than that of 1 reported by Barbin et al. (1980). Furthermore, the properties described by Barbin et al. (1980) resemble those found with a solubilized preparation wherein all binding appears fully competitive and the anomalies found in suspension are not observed (Wells et al., 1985; see footnote 6). First, the sites appear homogeneous toward all ligands. Second, when compared with the present results, affinities correspond to K_{P1} for histamine and to K_2 for all other agonists. Third, antagonists do not reveal a pharmacological specificity typical of H_2 receptors. It is suggested in the preceding paper (Steinberg et al., 1985b) that H_2 antagonists are allosteric inhibitors of [^{3}H]histamine and that the antagonist-specific site had become "uncoupled" from the receptor in the preparations studied by Barbin et al. (1980). A similar uncoupling may account for the changes that occur in the binding of agonists upon solubilization of the receptors in digitonin (Wells et al., 1985). The Hill coefficient of 1 found by Barbin et al. (1980) for all H_2 agonists thus may reflect some disruption of the receptor comparable to that achieved by solubilization and not the relatively high concentration of [^{3}H]histamine used in their investigation.

The apparent dissocation constant reported for [^{3}H]histamine at gastric mucosal cells [$K = 4$–20 μM (Batzri, 1981)] is at least 18-fold greater than the larger of the two values found in the present investigation [$K_{P2} = 221$ nM (Steinberg et al., 1985a)] and implies an exceptionally large capacity. Moreover, seven other H_2 agonists inhibit binding of the radioligand with Hill coefficients that appear to be near 1 (Batzri et al., 1982b); estimates of the dissociation constant are 4–80-fold larger than the corresponding values of K_2 listed in Table I. Although these differences suggest that [^{3}H]histamine labels different sites in the two preparations, there nevertheless is a correlation between the two estimates of affinity ($P = 0.0019$, $n = 7$). The agreement may be fortuitous; alternatively, [^{3}H]histamine may label H_2 receptors on mucosal cells, and the generally weaker binding may reflect differences between one tissue and another. Batzri et al. (1982a,b) were unable to observe a consistent relationship, either among agonists or among antagonists, between binding affinity and pharmacological potency. If the sites on mucosal cells are H_2 receptors, the absence of a pharmacological specificity identifiable as H_2 may reflect a change similar to that which occurs upon solubilization of rat cerebral cortex (Wells et al., 1985) or which may have occurred in the preparations studied by Barbin et al. (1980).

[6] D. L. Cybulsky, S. I. Kandel, and J. W. Wells, unpublished observations.

Gajtkowski et al. (1983) have investigated the inhibitory activity of four H_2 agonists at the sites labeled by [^{3}H]tiotidine in homogenates of guinea pig cortex. Hill coefficients reportedly are near 1, in contrast to the biphasic patterns found with [^{3}H]histamine in the present investigation. Moreover, estimates of the dissociation constants (K_i) for histamine, 4-methylhistamine, and dimaprit are 40–200-fold higher than the corresponding values of K_{P2} (Steinberg et al., 1985a) or K_2 (Table I). While these differences support the suggestion that [^{3}H]histamine and [^{3}H]tiotidine label different sites (Steinberg et al., 1985b), the values of K_i (0.63 nM) and K_2 (0.58 μM) for impromidine are almost identical. Comparative studies with a larger number of agonists are required to determine whether or not the agreement found with impromidine is fortuitous.

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Registry No. **2**, 36507-31-0; **3**, 673-46-1; **4**, 16111-27-6; **5**, 673-50-7; **7**, 65119-89-3; **8**, 55273-05-7; **9**, 34392-54-6; **10**, 69206-07-1; **11**, 98064-98-3; histamine, 51-45-6; τ-methylhistamine, 501-75-7; *N*-methyldimaprit, 70674-98-5; dopamine, 51-61-6; (−)-epinephrine, 51-43-4; isoproterenol, 7683-59-2; serotonin, 50-67-9; scopolamine, 51-34-3; (±)-atropine, 51-55-8.

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Diverse Properties of External and Internal Forms of Yeast Invertase Derived from the Same Gene†

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ABSTRACT: It has been shown by genetic analysis that the external and internal invertases from *Saccharomyces cerevisiae* share a common structural gene [Taussig, R., & Carlson, M. (1983) *Nucleic Acids Res. 11*, 1943–1954]. However, the only amino acid composition of these two forms of invertase reported to date has revealed extensive differences [Gascon, S., Neumann, N. P., & Lampen, J. O. (1968) *J. Biol. Chem. 243*, 1573–1577]. We have found from amino acid analyses of both enzymes and sodium dodecyl sulfate–polyacrylamide gel analysis of their cyanogen bromide peptides that they are most likely identical in their amino acid sequence. However, the invertases exhibit dramatically different physical properties, particularly in their stability. The most striking difference was in their renaturation following guanidine treatment where it was shown that inactivated external invertase could be renatured completely. Endo-β-*N*-acetylglucosaminidase H treated external invertase was restored to 40% of its original activity while internal invertase remained completely inactive. The observed differences may be attributed to the presence and absence of the oligosaccharide moiety in the external and internal invertases, respectively.

Yeast invertase (EC 3.2.1.26) exists in two forms (Gascon et al., 1968): a major glycosylated form (external) found mainly in the periplasmic space and secreted into the medium; and a minor nonglycosylated form (internal) found only in the cytosol. Of considerable interest are the structural and functional relationships between the external secreted invertase and its internal form. Although the two were shown to differ considerably in their amino acid compositions (Gascon et al., 1968), suggesting that they are products of different genes, recent genetic data indicate that their respective mRNA species are transcribed from a single gene, the SUC2 locus (Grossmann & Zimmermann, 1979; Rodriguez et al., 1981), but at different transcriptional starts so that translation of the mRNA for the external enzyme results in an amino-terminal signal peptide which is absent in the nascent internal peptide (Perlman & Halvorson, 1981; Carlson & Botstein, 1982; Taussig & Carlson, 1983). The signal peptide containing 19 amino acids is removed subsequently on transport of the external invertase to the periplasmic space, resulting in a subunit chain that is two amino acids (Ser-Met) longer than the corresponding internal invertase subunit (Carlson et al., 1983). However, a careful structural comparison of the mature forms of these two proteins has not been conducted and is necessary to establish that external and internal invertases are derived from the same gene.

Although external invertase contains up to 50% of its mass in the form of nine high-mannose oligosaccharide chains (Trimble & Maley, 1977), removal of the carbohydrate portion of the glycoenzyme with endo-β-*N*-acetylglucosaminidase H (Endo H)[1] (Tarentino & Maley, 1974) did not affect enzyme activity (Tarentino et al., 1974). Subsequent studies on the effects of Endo H deglycosylation of external invertase demonstrated not only that the invertase oligosaccharide moiety enhanced the enzyme's renaturation efficiency and stabilized its activity under a variety of conditions (Chu et al., 1978) but also that the carbohydrate component facilitated subunit interaction to form active oligomers (Chu et al., 1983). However, the use of Endo H in such studies raises possible objections in that removal of high-mannose oligosaccharide by Endo H is not quantitative unless the glycoprotein substrate is denatured by S-carboxymethylation (Trimble & Maley, 1977) or by boiling in sodium dodecyl sulfate (Chu & Maley, 1980). In addition, modifications which affect enzyme activity other than deglycosylation may occur, due to contaminating components such as proteases often found in Endo H prepa-

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[1] Abbreviations: Endo H, endo-β-*N*-acetylglucosaminidase H; Con A, concanavalin A; WGA, wheat germ agglutinin; βMSH, β-mercaptoethanol; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; UV, ultraviolet; CNBr, cyanogen bromide; PAGE, polyacrylamide gel electrophoresis; GdmCl, guanidinium chloride; CD, circular dichroism; Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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