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A pH-Dependent Model of the Activation Mechanism of the Histamine H_2 Receptor

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ABSTRACT. A pH-dependent model of agonist action for the histamine H_2 receptor was developed by taking into account the different ionic states of the amino acid residues that constitute the agonist-binding pocket of the receptor. The model offers the possibility of examining diverse mechanistic pathways to yield the active form of the receptor according to the molecular structure of the ligand. The rationale is valid for either tautomeric or non-tautomeric agonists and provides new insight into the mechanism of receptor activation. The subsequent application of the operational model of agonism allows one to derive agonist concentration–effect relationships that may prove useful for both the simulation of agonist profiles under different physiological conditions and the estimation of the pharmacologic parameters of efficacy and potency. General principles involved in the formulation are expected to be valid for other G-protein-coupled receptors. BIOCHEM PHARMACOL **58**;2: 343–353, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. Histamine H_2 receptor; receptor activation mechanism; operational model of agonism; efficacy; potency; pH

There are two general approaches for understanding how pharmacologic receptors translate extracellular data embodied in the chemical structure of agonists into intracellular information: selection or induction of receptor-active states [1–4]. The first approach, in its simplest form, assumes that receptors exist in two non-interconvertible states, one inactive (R) and the other active (R*). Those ligands that are able to discriminate between these forms by binding preferentially to the active state are denoted agonists, whereas those that do not distinguish between them are designated antagonists. The second approach focuses on the molecular processes by which an inactive receptor is transformed into an active one. In this approach, both agonists and antagonists have the ability to bind to the inactive receptor (receptor recognition), but only agonists can promote the generation of the active state by inducing a chemical alteration on the bound receptor (receptor activation).

GPCR† form one of the major cellular mechanisms of signal transduction [5–7]. The inherent intricacy of the regulation mechanisms of biological information associated with GPCR makes it necessary to increase the complexity of pharmacologic models. A ternary complex model was initially suggested to explain agonist binding to the β-adrenergic receptor [1]. This model is composed of two binding steps: the binding of the agonist to the receptor (AR), followed by the binding of the G-protein to this binary agonist–receptor complex to form a ternary complex (ARG). The ternary complex model posits the existence of two receptor states, one of low and the other of high affinity, with the latter involving the binding of the G-protein. Moreover, the possibility exists of receptor binding to G-protein in the absence of agonist (RG). The ternary complex model had proved instrumental in reproducing marked GPCR pharmacological activity until the constitutively active mutant receptors appeared [8]. A distinctive feature of constitutively active mutant receptors, i.e. their higher affinity for agonists but not for antagonists when compared with their native forms, cannot be accommodated within the framework of the ternary complex model. Moreover, the observed correlation between the increased affinity of a ligand for a constitutively active mutant receptor relative to the wild-type receptor and the intrinsic efficacy of the ligand cannot be adequately rationalized by the ternary complex model [8]. However, the explicit inclusion of a receptor isomerization step in an active state previous to G-protein coupling (the allosteric ternary complex model) [8, 9] has been shown to be an effective condition capable of accounting for constitutively active receptors and the ensuing behavior of inverse agonists [10].

Recently, additional progress in pharmacologic modeling has been made. Weiss *et al.* [11] completed the allosteric ternary complex model by allowing the G-protein to bind

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† *Abbreviations:* GPCR, G-protein-coupled receptors; HA, histamine; TA, 5-(2-aminoethyl)4-methylthiazole, K_L, ligand–receptor binding constant; K_T, occupancy–response transducer constant; $[R_0]$, total receptor concentration; E_m, maximum possible effect; K_1, first proton transfer reaction constant; K_2, second proton transfer reaction constant; K_{LR}, ternary ligand-activated receptor–G-protein complex binding constant; and E/[L], agonist concentration effect.

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the inactive receptor (the cubic ternary complex model). Leff *et al.* [12] derived a three-state receptor model by including one inactive and two active receptor states, whereas a complementary model considering *m* inactive and one active receptor states was developed by Pardo *et al.* [13]. A final point worth mentioning in this regard is that the foregoing models are all operational inasmuch as no specific molecular mechanisms are proposed for the processes of ligand recognition and receptor activation. However, there are situations in which the inclusion of molecular structure-related variables in the mathematical modeling may provide new strategies for a better understanding of the mechanisms of drug action. Within this scope, we aimed to explore in this paper different mechanistic routes for the activation of the histamine H_2 receptor with the ultimate goal that the general principles involved might be applicable to a wide variety of other GPCR.

The histamine H_2 receptor is one of the best characterized GPCR both experimentally and theoretically. Structure–activity relationships [14 and references therein], site-directed mutagenesis [15], and theoretical studies [16–27] have made it possible to characterize the molecular determinants of the ligands and the receptor associated with agonism and antagonism. On the basis of quantum chemical calculations on the HA molecule, the natural agonist, a mechanistic model of action (induction approach), was proposed [16, 17]. The model assumes that HA comes into the receptor as the N(3)-H tautomer (teleposition relative to the side chain) of the monocationic form, the most abundant species at the physiological pH of 7.4. The interaction of the HA cationic side chain with a putative negative region of the receptor (site I) causes an electron charge redistribution of the imidazole ring, which shifts the tautomeric stability towards N(1)-H (proximal position relative to the side chain). As the authors suggested, a proton acceptor group (site II), matching the imidazole N(3)-H moiety, and a proton donor group (site III), matching the imidazole N(1) nitrogen, would allow, in principle, the HA N(3)-H tautomer to be converted into N(1)-H, the energetically most stable form in the agonist-binding pocket. The process involves two proton transfers, the first from site III to HA N(1) to give the imidazolium cation and the second from N(3)-H to site II to give the N(1)-H tautomer.

The elucidation of the amino acid sequence of the HA H_2 receptor [28] permitted the investigation of the residues responsible for pharmacologic action. Comparison with other known GPCR and mutational studies [15] identified Asp^{98} in the third transmembrane domain as the counter ion for positively charged side chain amines (site I in the aforementioned receptor model). In addition, a non-conserved Asp^{186} in the fifth helix was found to be related to both ligand recognition and receptor activation. Mutations of Asp^{186} to Ala^{186} or Asn^{186} resulted in a complete loss of binding of the [methyl-^{3}H]tiotidine antagonist and a decrease in the HA maximum response to 35% of that observed with the wild-type receptor. The authors proposed Asp^{186} as the residue corresponding to supposed site II [15]. The same authors also suggested Thr^{190} as the proton donor residue (site III). Ala^{190} mutants retained the ability to transduce the HA signal, but the maximum physiologic response was 50% of that observed with the wild-type receptor. Based on receptor sequence comparisons [29] and quantum-chemical calculations, Arg^{257} in the sixth helix was recently identified as the putative site III [30].

Concurrently with these findings, a new H_2 receptor activation model appeared to justify the agonistic activity of some non-tautomeric thiazoles [31]. These compounds, which behave as full agonists, are structurally capable of accepting a proton from a receptor site but not of donating a proton to a proton-accepting site of the receptor. Consequently, the requirement of the second proton transfer proposed in the original activation mechanism [16] was questioned [31]. This objection joined some others concerning the necessity of ligand tautomerism to activate the HA H_2 receptor [14, 32, 33]. Alternatively, the authors suggested a mechanistic model in which the aromatic ring of the agonist, after anchoring the positively charged side chain onto receptor site I, is protonated by a proton transfer from the proton-donating site III of the receptor. The resulting positive charge, which spreads over the ring atoms according to the probability of the different mesomeric forms, yields a pure electrostatic interaction between a partially positively charged atom of the ligand ring and a negatively charged residue of the receptor (site II). This electrostatic interaction, which is structurally likely for either tautomeric or non-tautomeric ligands, becomes one of the determinants of the receptor activation model [31] and apparently renders unnecessary the suggested early proton transfer between the protonated ligand and the receptor site II for the understanding of the mechanism. More recently, in order to justify the activity of the histamine H_2 receptor after $Thr^{190} \rightarrow Ala^{190}$ mutation, the same authors suggested a tyrosine residue (Tyr^{182}) as the proton donor site [26, 27].

In the present paper, we aimed to show that the two current mechanistic models of HA H_2 receptor activation [16, 31] are compatible if the amino acid residues which constitute the recognition center of the receptor (sites I, II, and III) are present in different acid–base conjugate forms. This assumption leads to a dynamic pharmacologic system comprising an ensemble of various ligand, receptor, and ligand–receptor ionic populations whose relative proportions are governed by a set of equilibrium constants. The rationale of the allosteric ternary complex model [8, 9] admits the inclusion of the aforementioned mechanistic hypothesis for the agonist-induced receptor isomerization step (receptor activation). Quantitative pharmacologic relationships are derived by applying the operational model of agonism [34].

MATERIALS AND METHODS

The Operational Model of Agonism

The operational model of agonism was developed to provide an explicit description of agonist action [34]. This model furnishes an agonist concentration–effect, E/[L] equation (Eqn 1) which can be used either to simulate

theoretical E/[L] curves under different physiological conditions or to fit experimental E/[L] curve data with the aim of estimating the pharmacologic parameters associated with agonist affinity and efficacy [35–38].

$$R + L \underset{}{\overset{K_L}{\rightleftarrows}} L \cdot R \overset{K_T}{\cdots} \rightarrow \text{Effect}$$
$$E = \frac{E_m \cdot [R_0] \cdot K_L \cdot K_T \cdot [L]}{1 + K_L \cdot (1 + K_T \cdot [R_0]) \cdot [L]} \quad (1)$$

In Eqn 1, an agonist, L, binds to a receptor molecule, R, to form the agonist–receptor complex, LR. Suspension points embody the sequence of biochemical processes linking receptor occupation to physiologic effect. By applying the law of mass action for receptor conservation and the necessity of saturation for the transducer function, a theoretical E/[L] equation is derived. K_L is the ligand–receptor binding constant, K_T is a constant modulating the efficiency of transduction of receptor occupancy into response, E_m is the maximum possible effect in a given system, and $[R_0]$ is the total concentration of receptors.

Algebraic exploitation of Eqn 1 leads one to deduce meaningful expressions for the geometric properties which characterize the E/[L] curves, α and $[L_{50}]$ (asymptote and location parameters; see Eqn 2).

$$\alpha = \lim_{[L]\to\infty} E = \frac{E_m \cdot [R_0] \cdot K_T}{1 + K_T \cdot [R_0]} = \frac{E_m \cdot \tau}{1 + \tau}, \text{ with } \tau = [R_0] \cdot K_T$$
$$[L_{50}] = \frac{1}{K_L \cdot (1 + \tau)} \quad (2)$$
$$pD_2 = -\log[L_{50}] = \log(K_L \cdot (1 + \tau))$$

We see that τ embodies those pharmacologic properties that make it possible to distinguish between full agonists, partial agonists, and antagonists. For $\tau \gg 1$, the asymptote of the E/[L] curve, α, approaches E_m and the ligand behaves as a full agonist; for $\tau \approx 1$, $\alpha \approx E_m/2$ and the ligand behaves as a partial agonist; and for $\tau \ll 1$, $\alpha \approx 0$ and the ligand behaves as an antagonist. The value of τ is dictated by two terms, one tissue-dependent $[R_0]$ and the other ligand and receptor structure-dependent, K_T. Finally, the potency of an agonist measured by the $[L_{50}]$ value ([L] for $E = \alpha/2$) relies on both the affinity, K_L, and the efficacy, τ, of the agonist–receptor pair [34].

The operational model of agonism is a suitable methodology for GPCR. Substitution of suspension points in Eqn 1 by the process of formation of a ternary ligand-receptor–G-protein complex provides K_T with a chemical meaning: the binding constant of the G-protein to the ligand–receptor pair [34]. Moreover, this general model incorporates the mechanisms of receptor activation by including the corresponding chemical processes and associated equilibrium constants in the basic pharmacologic model depicted in Eqn 1. Validation of a putative mechanism or comparison of the agonist actions of various ligands can be carried out by simulating the derived E/[L] equations or by estimating the pharmacologic parameters of interest. Thus, the operational model is a useful technique for investigating each of the pharmacologic profiles resulting from the presumed mechanisms of activation of the HA H_2 receptor.

Computation of Structural and Electronic Properties

Quantum-chemical calculations were performed to gain insight into the structural features of the two molecular mechanisms under consideration. All the calculations were carried out at the restricted Hartree–Fock level with the 6-31G* basis set in the Gaussian 94 [39] suite of programs. 5-Methylimidazole and 4,5-dimethylthiazole were used as molecular models of HA (tautomeric) and TA (non-tautomeric) ligands, respectively. Formate anion and formic acid were used as molecular models of receptor site II for the interaction with HA and TA, respectively. The molecular structures of both the free ligands and the ligand–receptor complexes were fully optimized. Net atomic charges were derived by least-square fitting of calculated electrostatic potentials to quantum mechanically determined electrostatic potentials according to the Singh–Kollman procedure [40].

RESULTS

The pH Dependence of the HA H_2 Receptor

Recently, various works have stressed the importance of considering the different protonation states of the ionizable residues of an enzyme to provide a correct description of a catalytic mechanism [41–43]. The arguments made for the catalytic mechanism of an enzyme are also expected to be valid for the activation mechanism of a pharmacologic receptor. Thus, in the case of the HA H_2 receptor, the different possible ionic states for the key residues involved in ligand recognition should be included in the model (see Appendix 1). Therefore, if we denote by Z^- the anchoring center (site I) for protonated amine moieties either in agonist or in antagonist molecules, by Y^- the general base located in site II, and by XH^+ the general acid located in site III, then conjugate acids ZH and YH and a conjugate base X should be contemplated as well. Experimental and theoretical approaches have suggested an aspartate residue for both the Z^- and Y^- moieties [15] and either a threonine [15], tyrosine [26], or arginine [30] for XH^+. Obviously, if an alcoholic residue is chosen as the putative proton donor, a neutral XH and a negative X^- should be used for the protonated and deprotonated states, respectively. Guanidinium ions and alcohols present similar pK_a values and, consequently, are equivalent within the scope of the present paper. Simply as a matter of notation, we have assumed that site III bears an arginine residue and the XH^+ and X symbols are thus used throughout the text.

Figure 1 depicts the pH profiles of the proportions of the HA H_2 receptor ionic states given by Appendix 1. A simulation for $pK_{a_{1R}} = 5$ (characteristic value for proposed sites I and II) and $pK_{a_{2R}} = 14$ (typical value for proposed site III) was performed.

The f_{a_R} (long dashed line) fraction, which corresponds to a receptor species with all three of the considered residues

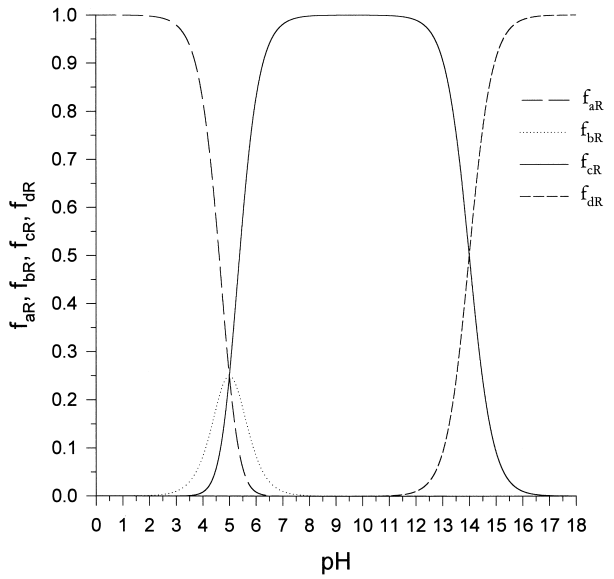

FIG. 1. A simulation of the pH dependence of the proportions of the HA H_2 receptor ionic states. ZH, YH, and XH^+ symbolize the protonated forms of receptor sites I, II, and III, respectively (see Appendix 1). $f_{a_R} = [R - ZH - YH - XH^+]/[R_0]$ (long dashed line), $f_{b_R} = [R - Z^- - YH - XH^+]/[R_0] = [R - ZH - Y^- - XH^+]/[R_0]$ (dotted line), $f_{c_R} = [R - Z^- - Y^- - XH^+]/[R_0]$ (solid line), and $f_{d_R} = [R - Z^- - Y^- - X]/[R_0]$ (short dashed line). pK_a values of 5 for both ZH and YH (aspartic acid residues) and 14 for XH^+ (threonine, tyrosine, or arginine, see introduction) were assumed. Note that in the case of threonine and tyrosine, the proper symbols should be XH and X^- for protonated and deprotonated alcohol moieties, respectively.

protonated (see Appendix 1), displays an asymptote towards one when the pH decreases and an asymptote towards zero when the pH increases. f_{b_R} (dotted line), which corresponds to receptor molecules in which arginine and one of the two aspartic acid residues are protonated, describes a bell-shaped curve with a maximum value of ~0.25 at pH = 5. We see that at this pH value the f_{a_R}, f_{b_R}, and f_{c_R} curves cross. Thus, at pH = $pK_{a_{1R}}$, $[R - ZH - YH - XH^+] = [R - Z^- - YH - XH^+] = [R - ZH - Y^- - XH^+] = [R - Z^- - Y^- - XH^+] \approx [R_0]/4$, and $[R - Z^- - Y^- - X]$ is negligible. f_{c_R} (solid line) displays a bell-shaped curve with a maximum value of approximately 1 broad spanning between 7 and 12 because of the great difference between $pK_{a_{1R}}$ and $pK_{a_{2R}}$ values. f_{d_R} (short dashed line) exhibits an asymptote towards zero when the pH decreases and an asymptote towards one when the pH increases. Figure 1 makes clear that at the physiological pH of 7.4 the receptor is mostly in the $R - Z^- - Y^- - XH^+$ state of ionization. Nevertheless, the consequences of the relative proportions of receptor ionic states for the observed physiologic response will depend on the mechanism of receptor activation.

Molecular Structure and Mechanism of Activation

Two pharmacologic paths for two structurally different H_2 agonists, namely HA (imidazol-like) and TA (thiazole-like), are addressed (see Fig. 2). We follow the rationale of the allosteric ternary complex model [8, 9] with different mechanistic proposals for the agonist-induced receptor isomerization step.

MECHANISM A. IMIDAZOLE-LIKE LIGANDS: RECEPTOR ACTIVATION BY TWO PROTON TRANSFERS. The chemical structure of the imidazole ring (Fig. 2a, Appendix 2.1) causes imidazole-like ligands to bind preferentially to a deprotonated (negatively charged) site II and to a protonated (positively charged) site III, i.e. $R - Z^- - Y^- - XH^+$ form of the receptor, in accordance with early activation mechanism [16]. Thus, $f_{c_R} = [R - Z^- - Y^- - XH^+]/[R_0]$ renders the proportion of free receptors in the proper ionic state to produce the pharmacologic response (see Appendix 1 and Fig. 1, solid line) and can be considered, in some sense, the pH-dependent receptor intrinsic activity under this mechanism. The value of f_{c_R} will depend on the pH and the receptor dissociation acidic constants $K_{a_{1R}}$ and $K_{a_{2R}}$. Agonist binding yields the activated form of the receptor ($R - Z^- - YH - X$) by two proton transfers, the first from site III to nitrogen N(1) of the imidazole ring and the second from N(3)-H of the imidazole ring to site II.

MECHANISM B. THIAZOLE-LIKE LIGANDS: RECEPTOR ACTIVATION BY ONE PROTON TRANSFER. The chemical structure of the non-tautomeric thiazole ring (Fig. 2b, Appendix 3.1) suggests that thiazole-like ligands could bind preferentially to a protonated (neutral) site II and to a protonated (positively charged) site III, i.e. $R - Z^- - YH - XH^+$ form of the receptor. This alternative mechanism would result in the same assumed active form of the receptor ($R - Z^- - YH - X$) as that resulting from Mechanism a, but by a single proton transfer: from site III to the nitrogen atom of the thiazole ring. Analogously to f_{c_R} in Mechanism a, $f_{b_R} = [R - Z^- - YH - XH^+]/[R_0]$ (Appendix 1 and Fig. 1, dotted line) can be considered the pH-dependent receptor intrinsic activity under Mechanism b.

To test the likeness of the structural proposals for HA and TA receptor binding, potential based atomic charges on 5-methylimidazole and 4,5-dimethylthiazole (molecular models of HA and TA, respectively) were calculated. In both systems, a high negative charge over the nitrogen atom acting as the proton acceptor (−0.58 for N_1 in HA and −0.55 for N_3 in TA, see Fig. 2 for atom notation) was found. Interestingly, where a positive charge (0.34) appears on the hydrogen N(3)-H of the imidazole ring, a slightly negative charge (−0.07) is observed on the sulphur atom of the thiazole ring. Although the charge on the S-atom is low, it will become presumably more negative after binding because of receptor polarization. The opposite sign of the partial charges on these two ligand moieties is in qualitative agreement with our hypothesis of two different modes of binding. Figure 3 shows the optimized geometries of the molecular models representing the interactions of HA and TA ligands with the two proposed ionic forms of receptor site II: $-COO^-$ for HA and −COOH for TA. The charged

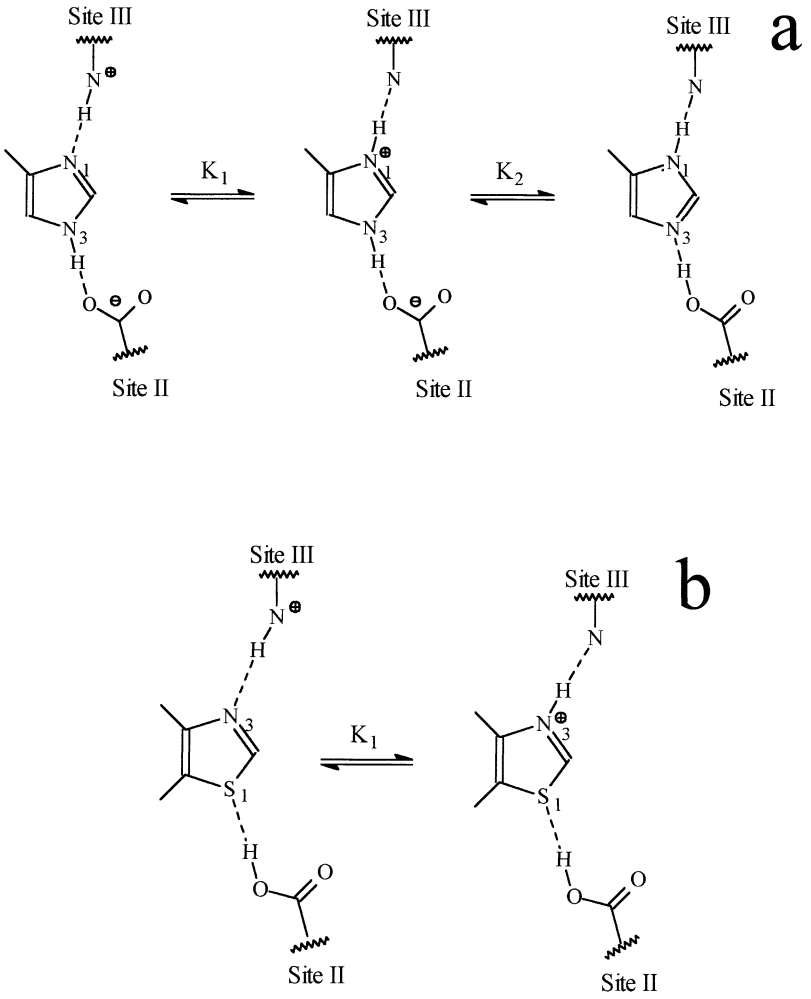

FIG. 2. A mechanistic model for HA H_2 receptor activation by either tautomeric or non-tautomeric ligands. The ligand side chain and the receptor anchoring site I, which remain constant in both mechanisms, are represented by a methyl group. (a) Mechanism a: imidazole-like (tautomeric) ligands. The imidazole ring binds preferentially to the positively charged site III and negatively charged site II. A double proton transfer from site III to ring nitrogen N(1) and from ring N(3)-H moiety to site II activates the receptor. (b) Mechanism b: thiazole-like (non-tautomeric) ligands. The thiazole ring binds preferentially to the positively charged site III and neutral site II. A single proton transfer from site III to the ring nitrogen activates the receptor. In both mechanisms, the active form of the receptor is that with protonated site II and deprotonated site III.

5-methylimidazole–formate complex is characterized by a plain structure with a linear N−H · · · O hydrogen bond (H · · · O distance is 1.78 Å), whereas the neutral 4,5-dimethylthiazole–formic acid complex is characterized by a non-plain structure with a weaker S · · · H-O hydrogen bond (S · · · H distance is 2.82 Å, about 0.2 Å below the sum of the van der Waals radii).

The pH Dependence of the HA H_2 Receptor Agonists

HA and, in general, all H_2 agonists have two protonable moieties, the side chain amine and the aromatic ring imine. If, as is generally accepted, only the monocationic species protonated on the side chain activates the receptor, the pH of the milieu and the pK_a values of the ligand will determine the proportion of ligand in the active form, $f_L = [L]/[L_0] = 1/(1 + [H^+]/K_{a_{1L}} + K_{a_{2L}}/[H^+])$. f_L/pH is a bell-shaped curve with a maximum for $pH = (pK_{a_{1L}} + pK_{a_{2L}})/2$. [L] is the concentration of monocationic ligand protonated on the side chain amine and $[L_0]$ is the total ligand concentration. $K_{a_{1L}}$ and $K_{a_{2L}}$ are the dissociation acidic constants of the ring imine and the side chain amine, respectively. Thus, f_L can be considered as the pH-dependent intrinsic activity of the ligand.

Figure 4 displays the f_L/pH curves for the two agonists we are analyzing. pK_a values of (5.93, 9.32) for HA and (3.23, 8.97) for TA were assumed [31]. The maximum for the imidazole compound is located at a pH of 7.6, whereas the maximum for the thiazole compound is at pH value 6.1. However, because of the great difference between the thiazole pK_as, the peak of the curve broad spans around the maximum. For the physiological pH of 7.4, f_{HA} is 0.96, whereas f_{TA} is 0.97. Thus, no direct differences coming from the ligands are found. However, by examining Fig. 1, we see that the pH-dependent intrinsic activity of the receptor (f_{c_R} for Mechanism a and f_{b_R} for Mechanism b) is more favorable for HA than for TA at physiological pH. To express the combined effect of f_R and f_L properties on the pharmacologic parameters, we need to derive E/[L] relationships.

Mechanism of Activation and Physiologic Effect

The aim of the present study is the analysis of different routes for agonist-induced histamine H_2 receptor activation. For simplicity, no basal response or precoupling was considered. This simplification is consistent with the likely negligible concentration of active receptor ($[R - Z^- - YH - X]$) in the absence of agonist on the basis of the acid–base properties of the proposed residues located on sites I, II, and III. The

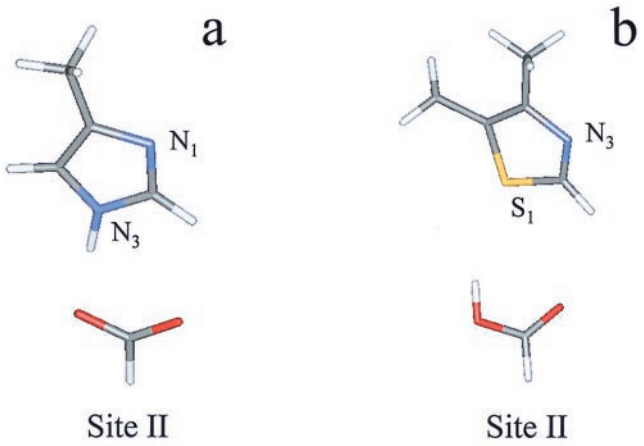

FIG. 3. Optimized geometries (6-31G*, see Methods) of the molecular models representing the interaction of HA and TA ligands with receptor site II: (a) 5-methylimidazole–formate anion and (b) 4,5-dimethylthiazole–formic acid. The recognition of both ligands is by the formation of an H-bond: N_3-H · · · O for HA and S_1 · · · H-O for TA.

pharmacologic response corresponding to each of the two mechanisms under discussion can be quantified by using the operational model of agonism [34, 44].

By applying the law of mass action (Appendix 2.2 and 3.2), concentration–effect equations are derived (Appendix 2.3 and 3.3). These equations express quantitatively the molecular processes by which agonist binding to HA H_2 receptor promotes biological response. It can be seen that τ maintains its operational definition of efficacy. The asymptote of the E/[L] relationship, α, is i) equal to the maximum response of the system, E_m, for high values of τ (full agonists); ii) equal to zero when τ is also equal to zero

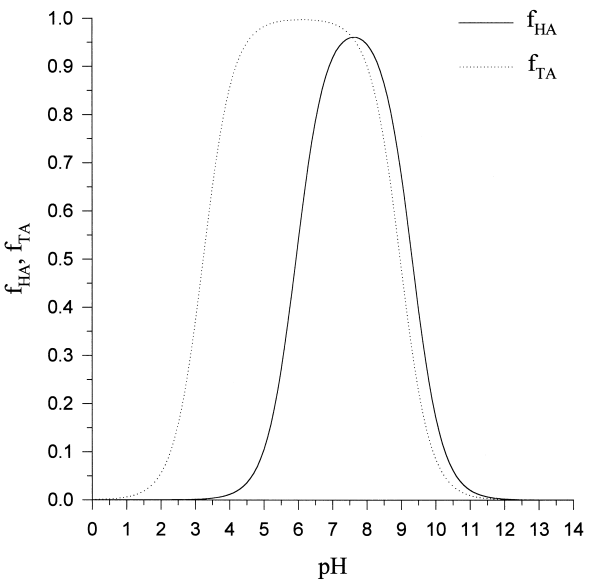

FIG. 4. A simulation of the pH dependence of the proportions of the ionic active forms of HA and TA. $f_{HA} = [HA]/[HA_O] = 1/(1 + [H^+]/K_{a_{1HA}} + K_{a_{2HA}}/[H^+])$ (solid line) and $f_{TA} = [TA]/[TA_O] = 1/(1 + [H^+]/K_{a_{1TA}} + K_{a_{2TA}}/[H^+])$ (dotted line); $pK_{a_{1HA}} = 5.93$, $pK_{a_{2HA}} = 9.32$, $pK_{a_{1TA}} = 3.23$, and $pK_{a_{2TA}} = 8.97$ [31].

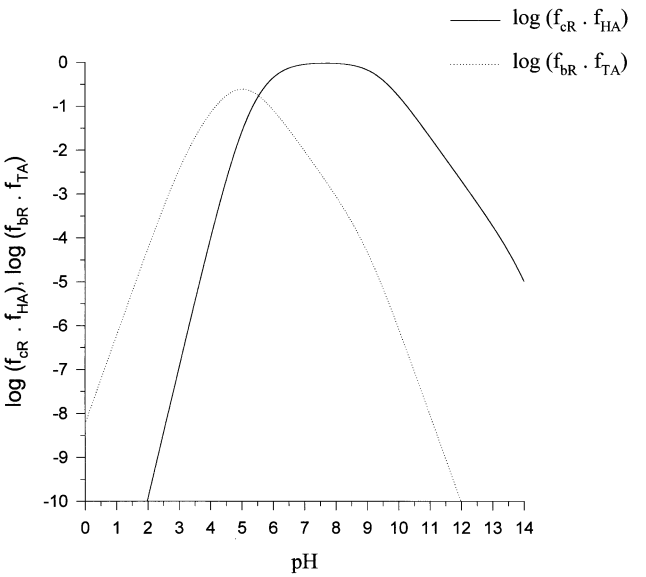

FIG. 5. A simulation of the pH-dependent term of pD_2. For HA, $\log(f_{c_R} \cdot f_{HA})$; for TA, $\log(f_{b_R} \cdot f_{TA})$. Algebraic expressions and numeric values can be found in the legends of Figs. 1 and 4.

(antagonists); and iii) lower than E_m for low values of τ (partial agonists). τ depends on the total concentration of receptors, $[R_0]$, the binding constant of the G-protein to the binary ligand-activated receptor complex, K_{LR}, and the constant K_1 or pair of constants $\{K_1, K_2\}$ that regulate(s) the activation of the receptor. In addition, pD_2, a measure of the potency of an agonist, is the sum of two terms. The first term depends on the pH of the local environment and on the acid–base properties of both the receptor and the ligand, while the second depends on the series of steps that lead from ligand binding to the formation of the ternary complex. Figure 5 displays the pH-dependent term of pD_2 for both ligands. The curves cross at pH 5.54. For a pH $<$ 5.54, TA is predicted to be more potent than HA; for a pH $>$ 5.54, HA is expected to be more potent than TA. The difference between the pH-dependent terms of $pD_2(\log(f_{c_R} \cdot f_{HA}) - \log(f_{b_R} \cdot f_{TA}))$ is 2.39 at the pH value of 7.4. Experimentally, the difference is 1.47 and with the same sign [31]. Thus, the second term of the equations from Appendix 2.3 and 3.3, which includes the equilibrium constants for the recognition and activation receptor processes, predicts a difference of 0.92 units of pD_2 favorable to TA. Note that the activity of TA appears to depend on the pH around its maximum more than the activity of HA.

DISCUSSION

The essential corollary of this paper is that even if only one ionic state of the receptor is expected to be the active form, there can exist different mechanistic paths leading to it depending on the molecular structure of the ligand. The pharmacologic effects of the hypothesized mechanisms can be evaluated if it is possible to incorporate the resulting chemical equations into the concentration–effect (E/[L]) relationships. Two activation mechanisms for the histamine

H_2 receptor for either tautomeric or non-tautomeric ligands have been considered. These mechanisms have been pharmacologically characterized by using the operational model of agonism. The explicit inclusion of chemical reactions in the operational model of agonism enlarges its classical framework, thereby building a conceptual bridge between molecular structure and physiologic function. In this regard, the present model can shed new light on some results from molecular biology and experimental pharmacology. The model assumes that the active form of the receptor is that with a negative site I, a neutral site II, and a deprotonated site III (i.e. $R - Z^- - YH - X$). G-proteins bind to the ligand-activated receptor complexes after ligand-mediated neutralization of sites II and III by either one or two proton transfers. Thus, if site II were previously mutated to a non-protonable neutral residue, it would be reasonable to expect that the mutated receptor could still be activated by HA, albeit with one single proton transfer (from site III to imidazole N(1)). This change in the receptor-binding pocket should induce a conformational change that is transmitted through the receptor molecule towards the intracellular portion where the G-protein binds with an affinity constant K_{LR}. If some of the amino acid residues corresponding to the receptor active sites are mutated, it is reasonable to suppose that the resulting conformational change after agonist binding will be different from the corresponding change provided by the native receptor. Consequently, the values of K_{LR} and τ would be different as well. This hypothesis is in qualitative agreement with the observation that mutation of Asp^{186} to Ala^{186} diminishes α for HA to 35% of that observed with the wild-type receptor [15]. In addition, the potency of an agonist, $[L_{50}]$, is determined by the aforementioned activation parameters and three additional ones: the proportion of receptors in the proper ionic state for activation, f_{b_R} or f_{c_R}; the proportion of ligand in the proper ionic state to activate the receptor, f_L; and the ligand–receptor binding constant, K_L. Note that the product $f_R \cdot f_L$ affects $[L_{50}]$, whose value determines the location of the E/[L] curve, but not α. This result could explain, in part, the different potency values of a given agonist on different receptor tissues when the total receptor concentrations are similar among the tissues. Likewise, equations from Appendix 2.3 and 3.3 show that apparent affinity constants, defined as

$$K_{app} = \lim_{[R_0]\to 0} \frac{1}{[L_{50}]},$$

depend on the mechanism and the efficiency of receptor activation and on the pH of the system.

Finally, we would like to remark that the formulation we have developed for the histamine H_2 receptor should have applicability to GPCR upon inclusion of the mechanistic hypothesis pertinent to each specific receptor.

APPENDIX

1. Protonic Equilibria among the Different Ionic Forms of the HA H_2 Receptor

Assuming that the ligand-binding pocket of the receptor consists of: ZH, aspartic acid (site I); YH, aspartic acid (site II); and XH^+, arginine (site III), the following equilibria and relative populations of the ionic forms of the receptor are obtained:

$$R - ZH - YH - XH^+ \underset{\rightleftarrows}{K_{a_{1R}}} R - Z^- - YH - XH^+ \underset{\rightleftarrows}{K_{a_{1R}}} R - Z^- - Y^- - XH^+ \underset{\rightleftarrows}{K_{a_{2R}}} R - Z^- - Y^- - X$$

$$K_{a_{1R}} = \frac{[R - Z^- - YH - XH^+] \cdot [H^+]}{[R - ZH - YH - XH^+]} = \frac{[R - ZH - Y^- - XH^+] \cdot [H^+]}{[R - ZH - YH - XH^+]}$$

$$= \frac{[R - Z^- - Y^- - XH^+] \cdot [H^+]}{[R - Z^- - YH - XH^+]} = \frac{[R - Z^- - Y^- - XH^+] \cdot [H^+]}{[R - ZH - Y^- - XH^+]};$$

$$K_{a_{2R}} = \frac{[R - Z^- - Y^- - X] \cdot [H^+]}{[R - Z^- - Y^- - XH^+]};$$

$$f_{a_R} = \frac{[R - ZH - YH - XH^+]}{[R_0]} = \frac{1}{1 + \frac{2 \cdot K_{a_{1R}}}{[H^+]} + \frac{K_{a_{1R}}^2}{[H^+]^2} + \frac{K_{a_{1R}}^2 \cdot K_{a_{2R}}}{[H^+]^3}};$$

$$f_{b_R} = \frac{[R - Z^- - YH - XH^+]}{[R_0]} = \frac{[R - ZH - Y^- - XH^+]}{[R_0]} = \frac{1}{2 + \frac{[H^+]}{K_{a_{1R}}} + \frac{K_{a_{1R}}}{[H^+]} + \frac{K_{a_{1R}} \cdot K_{a_{2R}}}{[H^+]^2}};$$

$$f_{c_R} = \frac{[R - Z^- - Y^- - XH^+]}{[R_0]} = \frac{1}{1 + \frac{K_{a_{2R}}}{[H^+]} + \frac{2 \cdot [H^+]}{K_{a_{1R}}} + \frac{[H^+]^2}{K_{a_{1R}}^2}};$$

$$f_{d_R} = \frac{[R - Z^- - Y^- - X]}{[R_0]} = \frac{1}{1 + \frac{[H^+]}{K_{a_{2R}}} + \frac{2 \cdot [H^+]^2}{K_{a_{1R}} \cdot K_{a_{2R}}} + \frac{[H^+]^3}{K_{a_{1R}}^2 \cdot K_{a_{2R}}}}$$

The total concentration of receptors, $[R_0]$, can be decomposed into five subpopulations, $\{R - ZH - YH - XH^+, R - Z^- - YH - XH^+, R - ZH - Y^- - XH^+, R - Z^- - Y^- - XH^+, R - Z^- - Y^- - X\}$, whose proportions are determined by the pK_as of ZH, YH, and XH^+ acids and the pH of the local environment. Because of the expected relative pK_a values of the residues proposed for sites I, II, and III, we have assumed as negligible those species in which one or two of the aspartic residues are protonated and in which, in the same molecule, arginine is deprotonated. Thus, $[R - ZH - YH - X]$, $[R - ZH - Y^- - X]$, and $[R - Z^- - YH - X]$ have not been included in $[R_0]$. Moreover, we have assumed that the amino acid residues which constitute the ligand-binding pocket are independent of each other and that the ionic state of any one of them does not affect the ionic states of the others.

2. Activation of the HA H_2 Receptor by Imidazol-like Ligands: Two Proton Transfers

2.1. RECEPTOR-LIGAND EQUILIBRIA. The Weinstein activation model [16, 17] is assumed. Side chain protonated amine of the ligand binds to negatively charged site I (Z^-), whereas the neutral imidazole ring locates between deprotonated site II (Y^-) and protonated site III (XH^+). A proton transfer from site III to site II mediated by the ligand activates the receptor and allows the binding of the G-protein molecule:

$$R - Z^- - Y^- - XH^+ + L \underset{}{\overset{K_L}{\rightleftarrows}} L \cdot R - Z^- - Y^- - XH^+ \overset{K_1}{\rightleftarrows} LH^+ \cdot R - Z^- - Y^- - X \overset{K_2}{\rightleftarrows} L' \cdot R - Z^- - YH - X$$

$$L' \cdot R - Z^- - YH - X + G \overset{K_{LR}}{\rightleftarrows} L' \cdot R - Z^- - YH - X \cdot G \rightarrow \text{Effect}$$

$$K_L = \frac{[L \cdot R - Z^- - Y^- - XH^+]}{[R - Z^- - Y^- - XH^+] \cdot [L]}; K_1 = \frac{[LH^+ \cdot R - Z^- - Y^- - X]}{[L \cdot R - Z^- - Y^- - XH^+]};$$

$$K_2 = \frac{[L' \cdot R - Z^- - YH - X]}{[LH^+ \cdot R - Z^- - Y^- - X]}; K_{LR} = \frac{[L' \cdot R - Z^- - YH - X \cdot G]}{[L' \cdot R - Z^- - YH - X] \cdot [G]}$$

The pharmacologic path leading to the observed physiologic effect is governed by: i) a ligand–receptor binding constant (K_L); ii) a reaction constant (K_1) for a proton transfer from site III (XH^+) to imidazole (L) and a reaction constant (K_2) for a proton transfer from protonated imidazole (LH^+) to site II (Y^-); and iii) a binding constant (K_{LR}) for the formation of the ternary ligand-activated receptor–G-protein complex. Note that in these equations, L and L′ denote imidazole N(3)-H and N(1)-H tautomers, respectively, both protonated on the side chain amine. LH^+ represents the dicationic form of the ligand.

2.2. THE LAW OF MASS ACTION. To derive useful concentration–effect relationships, we first need to develop proper equations for total receptor and G-protein concentrations. By applying the law of mass action for receptor and G-protein conservation, the following equations are obtained:

$$\begin{aligned}
[R_0] = {} & [R - ZH - YH - XH^+] \\
& + [R - Z^- - YH - XH^+] \\
& + [R - ZH - Y^- - XH^+] \\
& + [R - Z^- - Y^- - XH^+] \\
& + [R - Z^- - Y^- - X] \\
& + [L \cdot R - Z^- - Y^- - XH^+] \\
& + [LH^+ \cdot R - Z^- - Y^- - X] \\
& + [L' \cdot R - Z^- - YH - X] \\
& + [L' \cdot R - Z^- - YH - X \cdot G] \\
\approx {} & [R - ZH - YH - XH^+] \\
& + [R - Z^- - YH - XH^+] \\
& + [R - ZH - Y^- - XH^+] \\
& + [R - Z^- - Y^- - XH^+] \\
& + [R - Z^- - Y^- - X] \\
& + [L \cdot R - Z^- - Y^- - XH^+] \\
& + [LH^+ \cdot R - Z^- - Y^- - X] \\
& + [L' \cdot R - Z^- - YH - X]
\end{aligned}$$

and

$$[G_0] = [G] + [L' \cdot R - Z^- - YH - X \cdot G]$$

$[R_0]$ and $[G_0]$ represent the total receptor and G-protein concentrations, respectively. (Note that in these equations, we make the simplifying assumption that $[R_0] \gg [G_0]$ [34]).

2.3. CONCENTRATION–EFFECT (E/[L]) RELATIONSHIPS. Assuming a direct relationship between the effect and the concentration of ternary complex [34] and applying the law of mass action, the following E/[L] relationships are derived:

$$\frac{E}{E_m} = \frac{[L' \cdot R - Z^- - YH - X \cdot G]}{[G_0]};$$

$$E = \frac{E_m \cdot [R_0] \cdot f_{cR} \cdot f_L \cdot K_L \cdot K_1 \cdot K_2 \cdot K_{LR} \cdot [L_0]}{1 + f_{cR} \cdot f_L \cdot K_L \cdot (1 + K_1 + K_1 \cdot K_2 \cdot (1 + K_{LR} \cdot [R_0])) \cdot [L_0]};$$

$$\alpha = \lim_{[L_0]\to\infty} E = \frac{E_m \cdot [R_0] \cdot K_1 \cdot K_2 \cdot K_{LR}}{1 + K_1 + K_1 \cdot K_2 \cdot (1 + K_{LR} \cdot [R_0])} = \frac{E_m \cdot \tau}{1 + \tau}, \text{ with } \tau = \frac{[R_0] \cdot K_1 \cdot K_2 \cdot K_{LR}}{1 + K_1 + K_1 \cdot K_2};$$

$$[L_{50}] = \frac{1}{f_{cR} \cdot f_L \cdot K_L \cdot (1 + K_1 + K_1 \cdot K_2 \cdot (1 + K_{LR} \cdot [R_0]))};$$

$$pD_2 = -\log[L_{50}] = \log(f_{cR} \cdot f_L) + \log(K_L \cdot (1 + K_1 + K_1 \cdot K_2 \cdot (1 + K_{LR} \cdot [R_0])))$$

3. Activation of the HA H_2 Receptor by Thiazol-like Ligands: One Proton Transfer

3.1. RECEPTOR-LIGAND EQUILIBRIA. An alternative ionic form of the receptor for the binding of the ligand is proposed. Side chain protonated amine of the ligand binds to negatively charged site I (Z^-), whereas the neutral thiazole ring locates between protonated sites II (YH) and III (XH^+). A proton transfer from site III to thiazole nitrogen activates the receptor and allows the binding of the G-protein molecule:

$$R - Z^- - YH - XH^+ + L \underset{}{\overset{K_L}{\rightleftarrows}} L \cdot R - Z^- - YH - XH^+ \overset{K_1}{\rightleftarrows} LH^+ \cdot R - Z^- - YH - X$$

$$LH^+ \cdot R - Z^- - YH - X + G \overset{K_{LR}}{\rightleftarrows} LH^+ \cdot R - Z^- - YH - X \cdot G \rightarrow \text{Effect}$$

$$K_L = \frac{[L \cdot R - Z^- - YH - XH^+]}{[R - Z^- - YH - XH^+] \cdot [L]}; K_1 = \frac{[LH^+ \cdot R - Z^- - YH - X]}{[L \cdot R - Z^- - YH - XH^+]};$$

$$K_{LR} = \frac{[LH^+ \cdot R - Z^- - YH - X \cdot G]}{[LH^+ \cdot R - Z^- - YH - X] \cdot [G]}$$

The pharmacologic path leading to the observed physiologic effect is governed by i) a ligand–receptor binding constant (K_L); ii) a reaction constant (K_1) for a proton transfer from site III (XH^+) to thiazole (L); and iii) a binding constant (K_{LR}) for the formation of the ternary ligand-activated receptor–G-protein complex. Note that in these equations, L denotes the monocationic thiazole protonated on the side chain amine, whereas LH^+ represents the dicationic form of the ligand.

3.2. THE LAW OF MASS ACTION. The total receptor and G-protein concentrations can be expressed as:

$$\begin{aligned}[R_0] = {} & [R - ZH - YH - XH^+] \\ & + [R - Z^- - YH - XH^+] \\ & + [R - ZH - Y^- - XH^+] \\ & + [R - Z^- - Y^- - XH^+] \\ & + [R - Z^- - Y^- - X] \\ & + [L \cdot R - Z^- - YH - XH^+] \\ & + [LH^+ \cdot R - Z^- - YH - X] \\ & + [LH \cdot R - Z^- - YH - X \cdot G]\end{aligned}$$

$$\approx [R - ZH - YH - XH^+]$$
$$+ [R - Z^- - YH - XH^+]$$
$$+ [R - ZH - Y^- - XH^+]$$
$$+ [R - Z^- - Y^- - XH^+]$$
$$+ [R - Z^- - Y^- - X]$$
$$+ [L \cdot R - Z^- - YH - XH^+]$$
$$+ [LH^+ \cdot R - Z^- - YH - X]$$

and

$$[G_0] = [G] + [LH^+ \cdot R - Z^- - YH - X \cdot G]$$

(Note that in these equations, we make the simplifying assumption that $[R_0] \gg [G_0]$ [34]).

3.3. CONCENTRATION–EFFECT (E/[L]) RELATIONSHIPS. Assuming a direct relationship between the effect and the concentration of ternary complex [34] and applying the law of mass action, the following relationships are derived:

$$\frac{E}{E_m} = \frac{[LH^+ \cdot R - Z^- - YH - X \cdot G]}{[G_0]};$$

$$E = \frac{E_m \cdot [R_0] \cdot f_{b_R} \cdot f_L \cdot K_L \cdot K_1 \cdot K_{LR} \cdot [L_0]}{1 + f_{b_R} \cdot f_L \cdot K_L \cdot (1 + K_1 \cdot (1 + K_{LR} \cdot [R_0])) \cdot [L_0]};$$

$$\alpha = \lim_{[L_0]\to\infty} E = \frac{E_m \cdot [R_0] \cdot K_1 \cdot K_{LR}}{1 + K_1 \cdot (1 + K_{LR} \cdot [R_0])} = \frac{E_m \cdot \tau}{1 + \tau},$$

$$\text{with } \tau = \frac{[R_0] \cdot K_1 \cdot K_{LR}}{1 + K_1};$$

$$[L_{50}] = \frac{1}{f_{b_R} \cdot f_L \cdot K_L \cdot (1 + K_1 \cdot (1 + K_{LR} \cdot [R_0]))};$$

$$pD_2 = -\log[L_{50}]$$
$$= \log(f_{b_R} \cdot f_L) + \log(K_L \cdot (1 + K_1 \cdot (1 + K_{LR} \cdot [R_0])))$$

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