CONSEQUENCES OF BINDING EQUILIBRIUM CONSTANT AND INTRINSIC ACTIVITY HETEROGENEITY ON LIGAND BIOLOGICAL ACTIVITY

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1. Introduction

A model to account for receptor-mediated biological effects of hormones, neurotransmitters and drugs was first proposed by Clark [1]. This model is based on the assumptions that the interaction between ligand and receptor is reversible and that biological activity is proportional both to the number of occupied receptors and to the intrinsic activity of the ligand. Despite its simplicity, there are many experimental systems which behave according to the predictions of Clark's theory. However, in many other systems the experimental data do not fit this model. Therefore, numerous additional models have been proposed to account for such observations. Most, if not all, models invoke mechanisms, described as complex multistep processes of coupling between ligand binding and biological activity (review [2]). By contrast, little attention has been paid to the ligand itself. Heterogeneity of ligand-binding equilibrium constant has been acknowledged but only as a possible cause of artefacts in binding studies [3,4]. In no case has the direct consequence of ligand heterogeneity on its biological activity been clearly established.

Here, we study, on a theoretical basis, the receptor-mediated effects of ligands heterogenous with respect to binding equilibrium constant and intrinsic biological activity. We demonstrate that, reacted with such ligands, systems for which the response to ligand is proportional to receptor occupancy are able to mimic systems for which coupling between ligand binding and biological effect is not linear.

2. Mathematical formulation of the model

Let assume that n different orders of univalent ligands denoted $P_1, \ldots, P_i, \ldots, P_n$, are reversibly bound by a single order of univalent and independent binding sites denoted R, according to the following reaction equation:

$$P_i + R \rightleftharpoons P_iR, i = 1, \ldots, n \quad (1)$$

At equilibrium the system is described by the law of mass action:

$$\frac{[P_iR]}{[P_i][R]} = K_i, i = 1, \ldots, n \quad (2)$$

where K_i is the binding equilibrium constant and $[P_i]$, $[R]$ and $[P_iR]$ are the concentrations of free P_i, free R and P_i bound to R, respectively. Conservation of reactants is expressed by:

$$[P_i]_T = [P_i] + [P_iR], i = 1, \ldots, n \quad (3)$$

$$[R]_T = [R] + \sum_{i=1}^{n} [P_iR] \quad (4)$$

where $[P_i]_T$ and $[R]_T$ are the total concentrations of P_i and R. Accordingly, the fractional receptor occupancy is:

$$r = \sum_{i=1}^{n} [P_iR]/[R]_T \quad (5)$$

If we assume that the biological activity, denoted A_i, displayed by P_i is proportional to the number of receptor sites occupied by P_i and to its intrinsic activity, denoted L_i:

$$A_i = L_i[P_iR], i = 1, \ldots, n \quad (6)$$

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the activity yielded by the heterogenous ligand is therefore:

$$A = \sum_{i=1}^{n} A_i \qquad (7)$$

If A_M is assumed to be the activity by the heterogenous ligand at infinite dose, the fractional biological activity is:

$$a = A/A_M = \sum_{i=1}^{n} A_i/A_M \qquad (8)$$

Calculations have been performed according to [5–7]. The computer program, written in BASIC has been executed on a Hewlett-Packard HP 30 desk-top calculator and the curves have been plotted automatically by a Hewlett-Packard plotting machine.

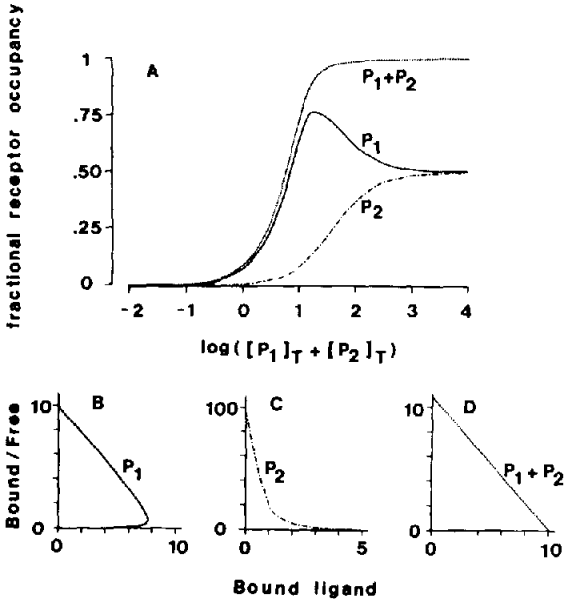

Fig.1. Binding isotherms and Scatchard plots of an heterogenous ligand and its components in the case of $n = 2$. The total receptor concentration is $[R]_T = 10$; the binding equilibrium constants are $K_1 = 1$ and $K_2 = 10$; the P_1 to P_2 concentration ratio is $[P_1]_T/[P_2]_T = 10$. (A) Fractional receptor occupancy by the heterogenous ligand ($P_1 + P_2$) and each of its two components (P_1 and P_2) as a function of the heterogenous ligand concentration ($[P_1]_T + [P_2]_T$). (B) Plot of P_1 bound to free ratio ($[P_1R]/[P_1]$) as a function of bound P_1 ($[P_1R]$). (C) Plot of P_2 bound to free ratio ($[P_2R]/[P_2]$) as a function of bound P_2 ($[P_2R]$). (D) Plot of the heterogenous ligand bound to free ratio ($([P_1R] + [P_2R])/([P_1] + [P_2])$) as a function of bound ligand ($[P_1R] + [P_2R]$)).

3. Results

When the ligand is homogenous with respect to its binding equilibrium constant and intrinsic activity ($n = 1$) the system behaves strictly according to Clark's theory and the biological activity is proportional to receptor occupancy (not shown).

When $n = 2$, the binding isotherms (fig.1A) may be a bell-shaped curve as for P_1 or a sigmoid curve as for P_2 and $P_1 + P_2$. The Scatchard plots of the binding data show a downward concavity for P_1 (fig.1B) which could suggest positive cooperativity [2], and an upward concavity for P_2 (fig.1C) which could suggest either receptor heterogeneity or negative cooperativity [2]. In contrast, the Scatchard plot for binding of the heterogenous ligand ($P_1 + P_2$) is linear (fig.1D) as predicted by Clark's theory. Depending on the parameters assigned to the heterogenous ligand, the dose–activity profiles may appear as sigmoid or biphasic curves (fig.2). Biphasic curves are generally ascribed to sepa-

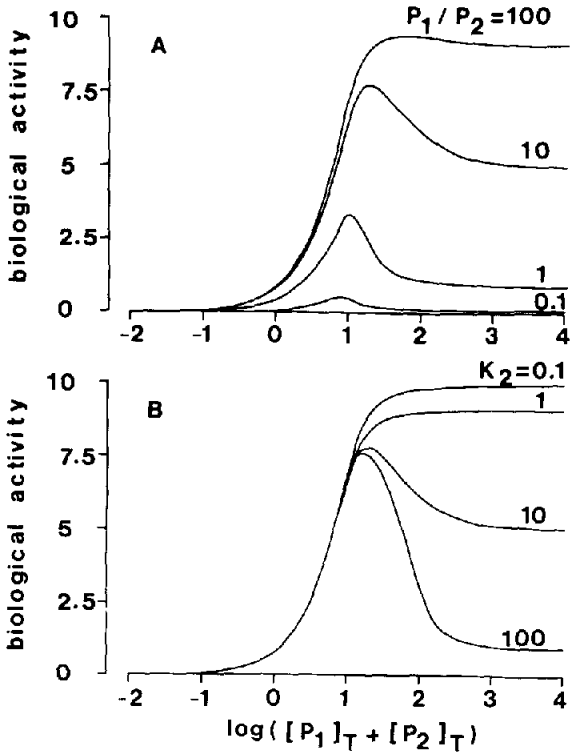

Fig.2. Dose–activity profiles of an heterogenous ligand in the case of $n = 2$. The total receptor concentration is $[R]_T = 10$; the intrinsic activities are $L_1 = 1$ and $L_2 = 0$. (A) Effect of variation of P_1 to P_2 concentration ratio; $K_1 = 1$ and $K_2 = 10$. (B) Effect of variation of K_2; $K_1 = 1$ and $[P_1]_T/[P_2]_T = 10$.

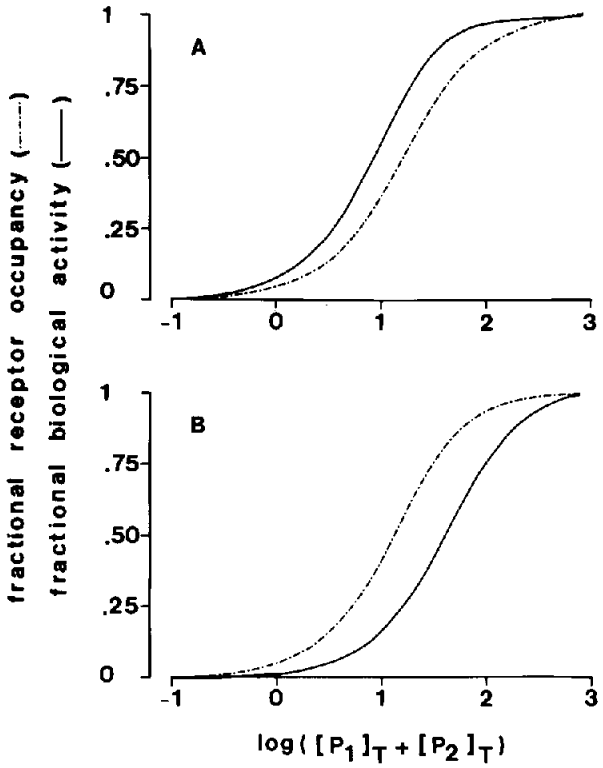

Fig.3. Fractional receptor occupancy (·—·) as defined by eq. (5) and fractional biological activity (——) as defined by eq. (8) as a function of total heterogenous ligand concentration in the case of $n = 2$. The total receptor concentration is $[R]_T = 10$; the intrinsic activities are $L_1 = 1$ and $L_2 = 0$; the P_1:P_2 concentration ratio is $[P_1]_T/[P_2]_T = 0.1$; the binding equilibrium constant for P_2 is $K_2 = 0.1$; (A) $K_1 = 0.01$; (B) $K_1 = 1$.

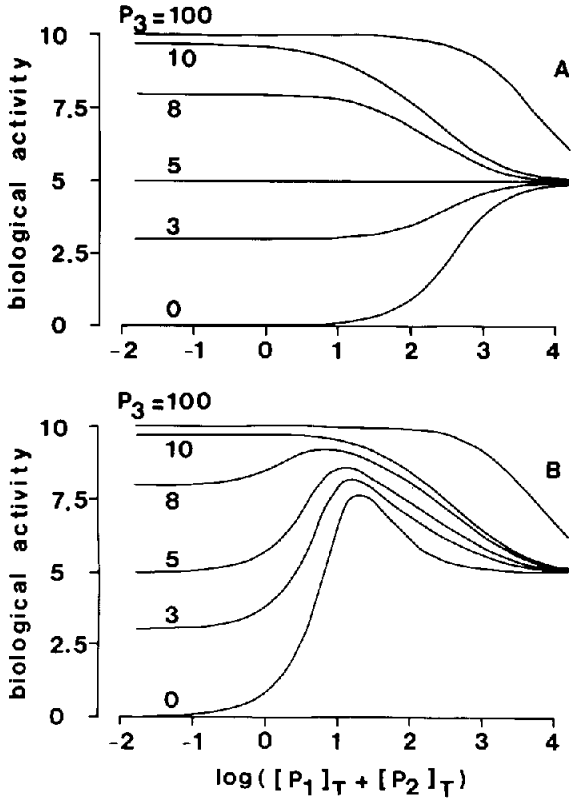

Fig.4. Dose–activity profiles of an heterogenous ligand ($n = 2$) in the presence of varying amount of an homogenous ligand P_3. The total receptor concentration is $[R]_T = 10$; the intrinsic activities are $L_1 = 1$, $L_2 = 0$ and $L_3 = 1$; the binding equilibrium constant of P_3 is $K_3 = 100$. (A) The P_1 to P_2 concentration ratio is $[P_1]_T/[P_2]_T = 0.01$, $K_1 = 100$ and $K_2 = 1$. (B) $[P_1]_T/[P_2]_T = 10$, $K_1 = 1$ and $K_2 = 10$.

rate receptors, multisubsite receptors, receptor cross-linking, receptor desensitization or exhaustion of the response system [2]. Considering the sigmoid curves, the absence of proportionality between receptor occupancy and biological activity as shown in fig.3, could be ascribed to positive or negative cooperativity or to complex allosteric effects [2].

In the presence of an homogenous ligand, the dose–activity profiles of an heterogenous ligand may mimic the effect of a partial agonist of the homogenous ligand (fig.4A). In other cases, the dose–activity profiles may exhibit a maximum (fig.4B). It is worth noting that the heterogenous ligand may exert stimulatory, inhibitory or no effect depending on the characteristics and relative concentrations of both the homogenous and the heterogenous ligand.

When the ligand heterogeneity increases, the geometric patterns of the binding isotherms and the dose–activity profiles may become very complex, showing various numbers of maxima, inflection points and plateaus (not shown).

4. Discussion

The main purpose of this paper is to demonstrate that ligands, heterogenous with respect to binding equilibrium constant and intrinsic biological activity, may generate complex binding isotherms and dose–activity profiles. Reacted with such ligands, systems which behave according to the very simply hypothesis of Clark's model [1], may mimic systems which

require far more sophisticated hypotheses for interpretation [2].

M_r and/or biological activity heterogeneity among glycoprotein hormones is now well documented [8–12]. Antibodies directed against hormone receptors are being extensively studied [13–16]. The heterogeneity of such polyclonal antibodies and hormone preparations is well recognized. Nevertheless, the direct consequences of ligand heterogeneity on binding and biological activity, are generally ignored in the interpretation of the data.

This model provides a simple and plausible mechanism which may explain many properties of ligands which can be reasonably suspected to be heterogenous. However, ligand heterogeneity does not preclude complex mechanisms of coupling between ligand binding and biological activity.

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