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A general molecular model for the effect of multicomponent mixtures on biological systems

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The purpose of this paper is to provide a general theoretical framework for the effect of multicomponent mixtures on biological systems when the components of these mixtures produce similar types of effects such as cardiac stimulation, sweet taste or a particular odor quality. These systems are assumed to involve receptor and, possibly, transducer entities with which the mixture components interact. In the general model, there are no constraints on the number of mixture components, receptors, transducers or binding activities in these systems. This means that substances may have receptors/transducers in common, they may have independent binding mechanisms or any combination of these two possibilities. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Chemosensory stimulation is rarely the result of the action of a single substance. Even when single substances are presented as odorants or tastants, they may undergo chemical or biological action leading to mixtures (Price, 1984). The development of mixture models is, therefore, of primary importance in chemosensory research. Mixture models capable of linking peripheral events with perceptual effects in humans would allow non invasive techniques to be used to quantify molecular events at the periphery. It has been suggested that the transduction mechanisms associated with adrenaline, photons, sweet taste and smell are similar (Lancet *et al.*, 1988). Black & Leff (1983) and Black (1989) discuss a mathematical model for β-receptor agonists involving a G protein transducer. Mixture models could usefully include a transducer component if they are to be applied to taste and smell.

The Law of Mass Action has played a central role in the development of equilibrium models capable of predicting the effect of substances on biological systems. The word 'effect' is used very generally to mean a measurable output in response to a particular concentration of an agonist, an antagonist or a dualist. Although hormones and drugs have been the primary targets of these models, they have also been applied to and developed for chemical sensing. Beidler's pioneering models of taste and taste mixtures (Beidler, 1954, 1962) are related to earlier receptor models such as those of Hill (1909) and Gaddum (1936). In modeling taste effects, either nerve responses or percepts, it is usually necessary to assume a relationship, often assumed to be linear, between the effect and the concentration of the activated receptors. An example is the linear relationship between nerve response and occupied receptors. In the case of the intensity of a percept, such as sweetness, it is highly unlikely that this relationship is linear and is, at present, unknown. This suggests that parameters characterizing peripheral events, such as receptor and transducer binding constants, cannot be based reliably on perceptual measurement. Is the inverse then also suggested? A knowledge of peripheral binding in humans will not lead to an assessment of how or how strongly a substance tastes or smells.

Since the relationship between activated receptors and percepts is so poorly defined, the task of linking perceptual measurement in humans to molecular events at the periphery seems hopeless. However, considerable relief from the linearity assumption mentioned earlier would be felt if this assumption was replaced with an assumption of monotonicity. It would then be necessary to assume only that a progressive increase in the concentration of the receptor–agonist, or receptor–transducer–agonist complex would lead to a progressive increase (or decrease) in the strength of the percept. It would not be necessary to know the form of this monotonic function, no matter how complex. In a paper on molecular models for binary mixtures, Ennis (1991) showed how receptor and transducer models could be developed based on receptor and transducer binding and assuming a *monotonic* connection between these events and the observed effects such as taste perception. Freedom from the linearity assumption was achieved

because the models were developed for the special situation of perceptual matching [reminiscent of the 'null' method in pharmacokinetic modeling (Black & Leff, 1983)]. To use Ennis' models, it is never necessary to know the functional relationship between a receptor-agonist complex concentration and the resulting perceptual strength. It is sufficient to know that the perceptual strength of a mixture has been *matched* to the equivalent strength of one of the components of the mixture. Ennis (1991) applied these models to published data on sweet tastant mixtures (De Graff & Frijters, 1986) and demonstrated that the model fits supported the existence of a transducer.

Taste and smell receptor systems may be capable of responding to more than one molecular species. Consequently, substances may have 'preferred' receptors while retaining the ability to bind to more than one type of receptor. Independence at the receptor level may occur, but competition for transducers may occur subsequent to the formation of agonist-receptor complexes. These different possibilities pose a significant challenge to the development of a comprehensive model that includes these as special cases. This general model is the focus of the present paper, and it will be shown that all six of the receptor and receptor–transducer models discussed by Ennis (1991) are special cases. The general model's real value, however, will probably lie in the identification of special cases not quite as extreme as those given in Ennis (1991) and in providing a general framework for mixture modeling in the chemical senses and pharmacology.

CLASSIFICATION OF BINDING MECHANISMS

In this paper, two broad categories of models are distinguished. The first of these involves agonist binding to a receptor or receptors without the participation of a transducer. These models will be referred to as *receptor* models. In the second category of models a transducer, such as a G–protein, is assumed to be involved in signal generation. These models will be referred to as *receptor–transducer* models. In principle, an agonist may bind to more than one type of receptor and the resulting agonist-receptor complex may bind to more than one type of transducer. Given two or more agonists, it is easy to imagine that particular agonists may have preferred receptors (receptors to which they have greatest affinity), but may also compete with other agonists in binding to non preferred receptors. Common and independent binding to receptors and/or transducers represent special cases of the general scheme just described. This occurs because binding constants can be assigned zero or non zero values consistent with the idea of common or independent receptors and/or transducers. Given any of these assumptions, binding to receptors and/or transducers may be simple or cooperative. Simple binding means that molecular interactions are one-to-one. Cooperative binding means that molecular interactions are not one-to-one.

MIXTURE MODELS

The *general mixture* model allows n agonists to bind to r receptors and involves t transducers. Special cases are the *general receptor model* and the *general receptor–transducer model.* Equations for these general models will be given. Further special cases within each of these categories are the *common* and *independent* binding models. In the common receptor and receptor–transducer models, it is assumed that agonists compete for a common receptor. The resulting agonist–receptor complexes compete for a common transducer, if a transducer is assumed to exist. In the specific independent receptor and receptor–transducer models discussed, all binding is assumed to be independent. It is possible that in the receptor–transducer models, there may be common receptors and independent transducers or the reverse. These will not be singled out as special cases although they are clearly derivable from the general mixture model. Finally, all of the models may be considered from the standpoint of *simple* or *cooperative* binding at the receptor and/or transducer levels. In the special cases considered, only cooperative binding at the receptor level will be discussed although other types of cooperative binding can be derived from the general receptor–transducer model.

In an earlier paper Ennis (1991) made the assumption that an agonist's effect was monotonically related to either an agonist–receptor complex (in receptor models) or to an agonist–receptor–transducer complex (in receptor-transducer models). Ennis assumed further that when two agonists produce the same type of effect (for example, both have the same type of percept, such as sweetness), then the same *post* receptor or *post* transducer monotonic function applies to both substances. This means that if a transducer does not exist or if two agonists have the same *efficacy*, then equivalence of effects implies that the agonist–receptor complex concentrations are equal. Similarly, if the two agonists require the involvement of one or more types of transducers (which means that their efficacies may not be equal), then equivalence of effects implies that the agonist–receptor–transducer complex concentrations are equal. This is a feature of a monotonic function.

If f is a monotonic function and if $f(x_1) = f(x_2)$, then $x_1 = x_2$. If two substances produce the same type of effect, such as sweetness, and if $f(x)$ is the percept of sweetness evaluated at some value of a variable x in the neural chain from the periphery to the brain, then the two substances matched perceptually are both presumed to involve an identical monotonic function of this variable. The variable may represent a peripheral event or a much later neural event. In this paper, it is assumed that this variable is the concentration of the agonist–receptor complex in receptor models and the concentration of the agonist–receptor–transducer complex in the receptor–transducer models. When, for instance, GTP replaces GDP on the α subunit of a G protein in response to the formation of an agonist-receptor complex, it is assumed that *subsequent* events do not further depend on the

nature of the agonist. In other words, the GTP-G protein does not 'remember' how it was created. Of course, the events leading to this stage may be very different for different substances. A difference between two different substances in their pharmacological or perceptual effects is modeled in terms of the concentrations of agonist–receptor–transducer complex formed which is a function of the agonist's affinity, efficacy and stoichiometry in binding receptors and transducers. In this paper, these ideas will be generalized considerably beyond Ennis (1991) by applying them to multicomponent mixtures and deriving the corresponding mixture equations.

Definitions

$[A_1]$ is a particular concentration of an agonist A_1 (called the 'target' concentration of A_1),

$[A_i^{(m)}]$ is the concentration of agonist A_i in a mixture,

$[R_j]$ is the concentration of receptor R_j,

$[T_k]$ is the concentration of transducer T_k,

K_{ij} is the association constant for the binding of agonist A_i to receptor R_j,

$[A_iR_j]$ is the concentration of the complex formed when A_i binds to R_j,

$[A_iR_j^{(m)}]$ is the concentration of the complex formed *in a mixture* when A_i binds to R_j,

K_{ijk} is the association constant for the binding of the agonist-receptor complex A_iR_j to transducer T_k,

$[A_iR_jT_k]$ is the concentration of the complex formed when complex A_iR_j binds to transducer T_k,

$[A_iR_jT_k^{(m)}]$ is the concentration of the complex formed *in a mixture* when the complex A_iR_j binds to transducer T_k,

a_{ij} is the stoichiometric coefficient for A_i binding to R_j,

a_{ijk} is the stoichiometric coefficient for A_iR_j binding to T_k,

n is the number of agonists in a mixture,

r is the number of different receptor types,

t is the number of different transducer types.

Note that the target compound has been labeled A_1, but this does not affect the generality of what follows since any one of the A_i can be chosen as the target.

The general mixture model

When a mixture percept (or effect) equals a target percept, from the earlier comments this implies that

$$\sum_{i,j,k}\left[A_iR_jT_k^{(m)}\right] - \sum_{j,k}\left[A_1R_jT_k\right] = 0 \tag{1}$$

where $i = 1,2,...,n$, $j = 1,2,...,r$, and $k = 1,2,...,t$.

Gaddum's classic equation (1937) for competitive agonism applied to A_xR_j binding to T_k in the presence of A_yR_j (A_x and A_y are specific agonists with the general subscript i replaced by x and y) yields

$$\left[A_xR_jT_k^{(m)}\right] = \frac{K_{xjk}\left[A_xR_j^{(m)}\right][T_k]}{1 + K_{xjk}\left[A_xR_j^{(m)}\right] + K_{yjk}\left[A_yR_j^{(m)}\right]} \tag{2}$$

If cooperative binding occurs, then

$$\left[A_xR_jT_k^{(m)}\right] = \frac{K_{xjk}\left[A_xR_j^{(m)}\right]^{a_{xjk}}[T_k]}{1 + K_{xjk}\left[A_xR_j^{(m)}\right]^{a_{xjk}} + K_{yjk}\left[A_yR_j^{(m)}\right]^{a_{yjk}}} \tag{3}$$

In general when there are nr competitive agonist–receptor complexes,

$$\left[A_iR_jT_k^{(m)}\right] = \frac{K_{ijk}\left[A_iR_j\right]^{a_{ijk}}[T_k]}{1 + \sum_{ij} K_{ijk}\left[A_iR_j^{(m)}\right]^{a_{ijk}}} \tag{4}$$

From the Law of Mass Action, when A_1 alone binds to R_j and T_k,

$$\left[A_1R_jT_k\right] = \frac{K_{1jk}\left[A_1R_j\right]^{a_{1jk}}[T_k]}{1 + K_{1jk}\left[A_1R_j\right]^{a_{1ij}}} \tag{5}$$

Substituting into equation 1,

$$\sum_{ijk}\frac{K_{ijk}\left[A_iR_i^{(m)}\right]^{a_{ijk}}[T_k]}{1 + \sum_{ij} K_{ijk}\left[A_iR_j^{(m)}\right]^{a_{ijk}}} - \sum_{jk}\frac{K_{1jk}\left[A_1R_j\right]^{a_{1jk}}[T_k]}{1 + K_{1jk}\left[A_1R_j\right]^{a_{1ij}}} = 0 \tag{6}$$

Note that equation 6 requires that $A_iR_j^{(m)}$ and $[A_1R_j]$ are known. These concentrations are given by

$$\left[A_iR_j^{(m)}\right] = \frac{K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}[R_j]}{1 + \sum_{i} K_{ij}[A_i]^{a_{ij}}} \tag{7}$$

and

$$\left[A_1R_j\right] = \frac{K_{1j}[A_1]^{a_{1j}}[R_j]}{1 + K_{1j}[A_1]^{a_{1j}}} \tag{8}$$

Substituting into equation 6,

$$\sum_{ijk} \frac{K_{ijk}\left(\frac{K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}[R_j]}{1+\sum_i K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}}\right)^{a_{ijk}}[T_k]}{1+\sum_{ij} K_{ijk}\left(\frac{K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}[R_j]}{1+\sum_i K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}}\right)^{a_{ijk}}} - \sum_{jk} \frac{K_{1jk}\left(\frac{K_{1j}[A_1]^{a_{1j}}[R_j]}{1+K_{1j}[A_1]^{a_{1j}}}\right)^{a_{1jk}}[T_k]}{1+K_{1jk}\left(\frac{K_{1j}[A_1]^{a_{1j}}[R_j]}{1+K_{1j}[A_1]^{a_{ij}}}\right)^{a_{1jk}}} = 0 \quad (9)$$

It is assumed that the target concentration $[A_1]$ is a known constant. Estimates of the parameters or other variables based on them can be obtained by fitting specific models to data, as shown for the special cases discussed by Ennis (1991).

It is important to point out what equation 9 does and does not say. Equation 9 says that when a target concentration ($[A_1]$) of a substance is matched in its effect to a mixture of substances, then this equivalence implies an earlier equivalence in the neural chain. It is assumed that this earlier equivalence occurs after the formation of ligand-receptor-transducer complexes. A_1 may bind many receptors and may interact further with many types of transducers. The extent of this binding is determined by the affinities, efficacies, and stoichiometric coefficients for A_1 in these various reactions. These parameters, combined with receptor density, determine the concentration of the variety of ligand complexes produced by A_1. The same statements may be made about other agonists in the mixture found equivalent to A_1 in effect. In mixtures, of course, different compounds may have to compete for common receptors and/or transducers and the resulting ligand complex concentrations reflect this. The equivalence assumed by equation 9 occurs after the formation of the manifold ligand–receptor–transducer complexes formed in mixtures and with A_1 alone. Equation 9 does *not* say that the percept (or effect, generally) resulting from a mixture is the sum of the percepts resulting from the substances alone. Equation 9 does *not* imply additivity at the percept (response) level. It does *not* imply that dose-response functions for the components of mixtures must be equal. The components may involve cross-binding, differential affinity and efficacy and simple and/or cooperative binding of any type.

BINARY MIXTURE MODELS

The binary mixture models discussed by Ennis (1991) were somewhat extreme. Two compounds either shared a common receptor–transducer system (i.e. competed with each other up to the formation of the receptor–transducer complex) or they acted on independent receptor-transducer systems. These models did not allow for the possibility that two substances may bind differently to two or more receptor-transducer systems and, although they may have 'preferred' receptors, they may bind to some extent to other systems. Equation 9 allows for this possibility with respect to any number of agonist, receptor and transducer types. The generality of equation 9 may not be required if, for instance, a group of receptors interacts with a much more limited group of transducers. General models for binary mixtures will now be given and it will now be shown that the six binary mixture models discussed by Ennis (1991) are special cases of equation 9.

Receptor models

Let $r = n = 2$, which means that there are two agonists and two receptor types. The percept is assumed to be monotonically related to the agonist-receptor concentrations without the participation of a transducer. At equilibrium this implies that

$$2A_1 + 2A_2 + 2R_1 + 2R_2 \leftrightarrow A_1R_1 + A_1R_2 + A_2R_1 + A_2R_2$$

When a target concentration of A_1 has been matched perceptually to a mixture of A_1 and A_2 this means that

$$\sum_{ij}\left[A_iR_j^{(m)}\right] - \sum_j [A_1R_j] = 0 \quad (10)$$

where $i = 1,2$, and $j = 1,2$.

Substituting from equations 7 and 8,

$$\sum_{ij} \frac{K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}[R_i]}{1+\sum_i K_{ij}\left[A_i^{(m)}\right]^{a_{ij}}} - \sum_j \frac{K_{1J}[A_1]^{a_{1J}}[R_j]}{1+K_{1j}[A_1]^{a_{1j}}} = 0 \quad (11)$$

Equation 11 is a logical consequence of the assumptions being made, but can also be obtained from equation 9 by assuming that $r = n = 2$, that $t = 1$, and that the common binding constant for all A_iR_j is zero. These assumptions exclude the participation of a transducer. From equation 11 and assuming $a_{ij} = 1$ it can be shown that

$$\begin{aligned}
&[[A_2^{(m)}]^2]\{K_{21}K_{22}(2 + [A_1]K_{11} + [A_1]K_{12})\} + \\
&[A_2^{(m)}]\Big\{K_{21} + K_{22} + K_{21}K_{12}(2[A_1^{(m)}] + \\
&[A_1][A_1^{(m)}]K_{12}) + K_{11}K_{22}(2[A_1^{(m)}] + \\
&[A_1][A_1^{(m)}]K_{11}) + [A_1]K_{11}K_{12}\Big(K_{22}([A_1^{(m)}] - \\
&[A_1]) + K_{21}([A_1^{(m)}] - [A_1])\Big)\Big\} + ([A_1^{(m)}] - [A_1])\times \\
&(K_{11} + K_{12} + 2K_{11}K_{12}[A_1^{(m)}] + 2K_{11}K_{12}[A_1] + \\
&[A_1][A_1^{(m)}]K_{11}^2K_{12} + [A_1][A_1^{(m)}]K_{11}K_{12}^2) = 0
\end{aligned} \quad (12)$$

Equation 12, a special case of equation 9, is the general receptor model for binary mixtures assuming that $r = n = 2$. A more general model would not assume $n = r$,

such as when there are more receptor types than agonists. This type of model is a special case of equation 9, but will not be discussed further in this paper. The common and independent receptor models should be special cases of equation 12. It will now be shown that Ennis' (1991) receptor models, common and independent, simple and cooperative binding, are special cases of equation 9.

Common receptors

If there is a common receptor (R_1 and R_2 are equivalent), then $K_{11} = K_{12}$ and $K_{21} = K_{22}$.

Equation 12 now reduces to

$$\left\{K_{21}[A_2^{(m)}] - K_{11}([A_1] - [A_1^{(m)}])\right\}\left\{(2K_{21}+ 2[A_1]K_{11}K_{21})[A_2^{(m)}] - \left(-2 - 2K_{11}([A_1] + [A_1^{(m)}])- 2[A_1][A_1^{(m)}]K_{11}^2\right)\right\} = 0 \quad (13)$$

Equation 13 gives two roots for $[A_2^{(m)}]$, only one of which is positive. Note that the expression in the second set of curly brackets gives a negative $[A_2^{(m)}]$ when set to zero. Hence

$$K_{21}[A_2^{(m)}] - K_{11}([A_1] - [A_1^{(m)}]) = 0,$$

from which

$$[A_2^{(m)}] = k([A_1] - [A_1^{(m)}]), \quad (14)$$

where $k = \frac{K_{11}}{K_{21}}$

If a_{11} and a_{21} are the stoichiometric coefficients for A_1 and A_2 binding to the common receptor (cooperative binding)

$$[A_2^{(m)}] = \left(k([A_1)^{a_{11}} - [A_1^{(m)}]^{a_{11}})\right)^{\frac{1}{a_{21}}} \quad (15)$$

Equation 15 is identical to Equation 13 in Ennis (1991) for cooperative binding of two substances to a common receptor. Equation 14 is the corresponding simple binding model. Equations 14 and 15 are, therefore, two of the six models derived in that paper.

Independent receptors

If the receptors are independent, $K_{21} = K_{12} = 0$. Equation 12 now reduces to

$$[A_2^{(m)}](2[A_1^{(m)}]K_{11}K_{22} + K_{22}+ [A_1][A_1^{(m)}]K_{11}^2K_{22}) + [A_1^{(m)}]K_{11} - [A_1]K_{11} = 0 \quad (16)$$

from which,

$$[A_2^{(m)}] = \frac{k([A_1] - [A_1^{(m)}])}{1 + 2[A_1^{(m)}]K_{11} + [A_1][A_1^{(m)}]K_{11}^2} \quad (17)$$

where $k = \frac{K_{11}}{K_{22}}$.

Once again, under the assumption of cooperativity,

$$[A_2^{(m)}] = \left(\frac{k([A_1]^{a_{11}} - [A_1^{(m)}]^{a_{11}})}{1 + 2[A_1^{(m)}]^{a_{11}}K_{11} + [A_1]^{a_{11}}[A_1^{(m)}]^{a_{11}}K_{11}^2}\right)^{\frac{1}{a_{22}}} \quad (18)$$

where a_{11} and a_{22} are the stoichiometric coefficients for A_1 and A_2 binding to R_1 and R_2, respectively.

Equation 17 is identical to Equation 21 in Ennis (1991) and Equation 18, a simple extension, was not given. It is important to see that the binary mixture models based on common and independent binding are special cases of Equation 12, which itself is a special case of Equation 9, and that simple and cooperative binding models are further special cases of these.

Receptor-transducer models

Let $t = r = n = 2$. At equilibrium,

$$4A_1 + 4A_2 + 4R_1 + 4R_2 + 4T_1 + 4T_2 \Longleftrightarrow A_1R_1T_1 + A_1R_1T_2 + A_1R_2T_1 + A_1R_2T_2 + A_2R_1T_1 + A_2R_1T_2 + A_2R_2T_1 + A_2R_2T_2$$

Assuming that a target concentration of A_1 has been matched perceptually to a mixture of A_1 and A_2, then from Equation 1

$$\sum_{ijk}[A_iR_jT_k^{(m)}] - \sum_{jk}[A_1R_jT_k] = 0 \quad (19)$$

or, from equation 9

$$\sum_{ijk}\frac{K_{ijk}\left(\frac{K_{ij}[A_i^{(m)}]^{a_{ij}}[R_j]}{1+\sum_i K_{ij}[A_i^{(m)}]^{a_{ij}}}\right)^{a_{ijk}}[T_k]}{1 + \sum_{ij}K_{ijk}\left(\frac{K_{ij}[A_i^{(m)}]^{a_{ij}}[R_j]}{1+\sum_i K_{ij}[A_1^{(m)}]^{a}ij}\right)^{a_{ijk}}} - \sum_{jk}\frac{K_{1jk}\left(\frac{K_{1j}[A_1]^{a_{1j}}[R_j]}{1+K_{1j}[A_1]^{a_{1j}}}\right)^{a_{1jk}}[T_k]}{1 + K_{1jk}\left(\frac{K_{1j}[A_1]^{a_{1j}}[R_j]}{1+K_{1j}[A_1]^{a_{1j}}}\right)^{a_{1jk}}} = 0, \quad (20)$$

where $i = 1,2$ $j = 1,2$ and $k = 1,2$.

Equation 20 is the general receptor-transducer model for binary mixtures. As with the receptor models, Ennis' (1991) common and independent receptor-transducer models are special cases of Equation 20.

Common receptors/transducers

Assume that there is a common receptor and a common transducer for both A_1 and A_2. This means that

$$A_1 + A_2 + 2R_1 + 2T_1 \Longleftrightarrow A_1R_1T_1 + A_2R_1T_1,$$

since R_1 and R_2 are identical, as are T_1 and T_2. Note also that $K_{11} = K_{12}$, $K_{21} = K_{22}$, $K_{111} = K_{121} = K_{112} = K_{122}$, and $K_{211} = K_{221} = K_{212} = K_{222}$. Assume that

$a_{ijk} = a_{ij} = 1$. Under these assumptions, equation 20 simplifies to

$$[A_2^{(m)}](K_{21}K_{211} + K_{11}K_{21}K_{211}[A_1] - K_{11}K_{21}K_{111}[A_1]) = K_{11}K_{111}[A_1] - K_{11}K_{111}[A_1^{(m)}]. \quad (21)$$

Dividing by $K_{21}K_{211}$ and letting

$$\alpha = \frac{K_{11}K_{111}}{K_{21}K_{211}}$$

$$[A_2](1 + K_{11}[A_1] - K_{21}\alpha[A_1]) = \alpha([A_1] - [A_1^{(m)}]), \quad (22)$$

$$[A_2^{(m)}] = \frac{\alpha([A_1] - [A_1^{(m)}])}{1 + [A_1](K_{11} - \alpha K_{21})}, \quad (23)$$

$$[A_2^{(m)}] = \frac{\alpha([A_1] - [A_1^{(m)}])}{1 + \beta[A_1]}, \quad (24)$$

where $\beta = K_{11} - \alpha K_{21}$.

Under the assumption of cooperativity at the receptor level,

$$[A_2^{(m)}] = \left[\frac{\alpha([A_1]^{a_{11}} - [A_1^{(m)}]^{a_{11}}}{1 + \beta[A_1]^{a_{11}}}\right]^{\frac{1}{a_{21}}} \quad (25)$$

Equation 25 is identical to Equation 16 in Ennis (1991) for two agonists binding to common receptors and transducers. However, it can now be seen that Equation 25 is a special case of Equation 9.

Equations 14, 15, 17, 18, 24 and 25 are binary mixture models assuming common and independent receptors and common receptors and transducers (with and without cooperative binding). These models are all special cases of Equation 9. It will now be shown that Ennis' independent receptor-transducer model is also a special case of equation 9.

Independent receptors/transducers

In the case of independent receptors and transducers, at equilibrium

$$A_1 + A_2 + R_1 + R_2 + T_1 + T_2 \Longleftrightarrow A_1R_1T_1 + A_2R_2T_2$$

Note that

$$K_{12} = K_{21} = K_{112} = K_{211} = K_{121} = K_{211} = K_{212} = K_{122} = 0$$

These constants have zero values because it is assumed that A_1 and A_2 bind to exclusive receptor and transducer systems. Assume that $[T_1] = [T_2]$ and that $[R_1] = [R_2] = [R]$. Equation 20 then simplifies to

$$[A_2^{(m)}] = \frac{\alpha([A_1] - [A_1^{(m)}])}{\theta} \quad (26)$$

where $\theta = 1 + \beta[A_1] + [A_1^{(m)}]\{K_{11}(1 + 2\tau) + \alpha K_{22}\} + [A_1][A_1^{(m)}]\{K_{11}^2 + (1 + \tau)^2\}$

where

$\alpha = \frac{K_{11}K_{111}}{K_{22}K_{222}}$,

$\beta = K_{11} - \alpha K_{22}$, and

$\tau = K_{111}[R]$.

The common receptor–transducer model, equation 24, and the independent receptor–transducer model, equation 26, differ in the denominator. Equation 26 contains an expression in the denominator, missing in equation 24, involving $[A_1^{(m)}]$. Equation 24 is a linear function of $[A_1^{(m)}]$ leading to linear isoboles under the assumption of common receptors and transducers. This means that equation 24 can never explain synergistic or antagonistic effects. Of course, if cooperative binding is allowed, as shown in equation 25, nonlinear isoboles can be constructed under the common binding assumption. Equation 26, however, is nonlinear in $[A_1^{(m)}]$ and an appeal to cooperative binding is not necessary to explain effects like synergism. Ennis (1991) did not present the independent receptor–transducer model in the form of equation 26. However, it is not difficult to show that equation 26 can be rewritten as

$$[A_2^{(m)}] = \frac{k([A_1] - [A_1^{(m)}])}{\phi}$$

where $\phi = k_t + [A_1](K_{11}k_t - K_{11}) + [A_1^{(m)}]K_{11}k_t(1 + 2\tau) + [A_1^{(m)}]K_{11} + [A_1][A_1^{(m)}]k_t(K_a\tau + K_a)^2$

where $k_t = K_{22}/K_{11}$ and $\tau = K_{11}[R]$ as given in Ennis (1991).

SCHILD REGRESSION AS A SPECIAL CASE OF EQUATION 24

Equation 24 gives the relationship between A_1 and A_2 required in a mixture of the two substances to match A_1 at concentration $[A_1]$. Suppose that A_2 is an antagonist in the presence of A_1. Then the expression

$$[A_2^{(m)}] = \frac{\alpha([A_1] - [A_1^{(m)}])}{1 + \beta[A_1]} \quad (27)$$

describes the concentration of A_1 needed in the mixture in the presence of A_2 at $[A_2^{(m)}]$ to match A_1 at $[A_1]$. Rearranging equation 28,

$$[A_1^{(m)}] = [A_1] - [A_2^{(m)}]\left(\frac{1 + \beta[A_1]}{\alpha}\right) \quad (28)$$

An antagonist is a substance with a positive affinity and zero efficacy [Black (1989)]. When $K_{211} = 0$,

$$\frac{1 + \beta[A_1]}{\alpha} = -K_{21}[A_1]. \quad (29)$$

Hence,

$$[A_1^{(m)}] = [A_1] - [A_2^{(m)}](-K_{21}[A_1])$$

$$= [A_1](1 + K_{21}[A_2^{(m)}]), \quad (30)$$

which is the equation for Schild regression [Arunlakshana & Schild (1959)].

A useful aspect of this derivation is to demonstrate the meaning of an antagonist in terms of affinity and efficacy. Affinity has been defined with respect to initial receptor binding and efficacy in terms of post receptor binding. By reducing the efficacy of A_2 to zero, equation 24 describes a mixture model of an antagonist and an agonist. Schild regression was not developed from this perspective and is not explicit about the participation of transducers (note that equation 30 has no terms for the involvement of A_1 with a transducer). The purpose of this section is to show that these two models intersect when an antagonist has been appropriately defined in the present theory.

Parameter estimation

Ennis (1991) showed that the parameters of the six models that he derived could be estimated using non linear least squares. The same techniques can be used to fit equation 9 and its special cases to mixture data. Ennis (1991) fit mixture models to the glucose–fructose mixture data of De Graff & Frijters (1986). These model fits supported the existence of a transducer entity for sweet taste in humans. They also supported the finding that fructose is sweeter than glucose in humans because its affinity (receptor binding) is stronger than glucose, although its efficacy (transducer binding) is weaker. The synergistic effects observed when glucose and fructose are mixed are predicted by the receptor–transducer model with simple binding.

CONCLUSION

One can imagine an almost infinite number of complex binding events when multicomponent mixtures interact with biological systems, even when the mixture components have the same type of effect (for example, a specific type of cardiac stimulation, sweet taste, or a particular odor quality). It is satisfying, then, that a great number of these events can be defined in such a way that mixture effects can be represented by a single model, equation 9 in this paper. Equations for simple or cooperative binding to common or independent receptors and transducers, and combinations of these, are all special cases of this model. The assumption that measured effects are only monotonically related to agonist concentrations makes it possible to determine peripheral binding parameters based on effects occurring at a considerable distance from the sites of action of these agents. The goal of predicting perceptual effects from biochemical parameters of odorants and tastants or estimating binding constants from psychophysical experiments may not be as elusive as intimated in the introduction. Since direct methods for estimating chemosensory binding constants in living humans is neither ethical or feasible, the modeling approach discussed in this paper should be helpful in achieving this goal.

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