explicit involvement of a conformational change for unliganded receptive units between a reactive and an active state in a fully reversible reaction scheme (Fig. 5.1D).

5.4. Transition Away from Classic Receptor Theory

5.4.1. Stephenson’s Reaction Scheme

Recognition of a need to explicitly express the existence of a conformational change for the unliganded receptor ran in parallel with observations of partial agonism and receptor reserve (Furchgott 1955; Nickerson 1956; Stephenson 1956). Arieëns (1954) introduced the parameter ‘intrinsic activity’ \( a \), a system constant between 0 and 1, that could account for partial agonism. However, to incorporate both partial agonism and receptor reserve, Stephenson (1956) gave a simple approach for the formulation of response as a function of a stimulus.

Stephenson wrote the stimulus expressed as adsorption in the form of a Langmurian occupancy, the load, times activation in the form of an efficacy constant, \( e \) (Eqs. 5.1 and 1.17), i.e. not the property ‘efficacy’ as used in relation to response (see also the following discussion and sub-chapter 1.3). Furthermore, the actual response or activity was now an unknown function of this stimulus (see Eq. 5.2).

Stephenson’s stimulus, consisting of a binding process and an activation process, was equated as:

\[
\text{stimulus} = \text{occupancy} \cdot \text{efficacy constant}. \tag{5.1}
\]

and a response, related to the concept termed ‘efficacy’ as a property, was then imagined by Stephenson as a yet unknown function of the stimulus:

\[
\text{response} = \text{unknown function of } (\text{occupancy} \cdot \text{efficacy constant}). \tag{5.2}
\]

Note in Eq. 5.2 that ‘response’ to its related property efficacy is not the efficacy constant, but an unknown function of the efficacy constant.

Thus for partial agonists, dialing on a nob-of-effficacy, i.e., varying the efficacy constant in Eq. 5.2, Stephenson could explain the observation of response for partial agonism including a possible receptor reserve (Eq. 1.12A + B) (Stephenson 1956, Figs. 1 and 9). Similar plots are generated later from the functional form of the dC&K model (Fig. 5.6).

5.4.2. Efficacy and Efficacy Constant Starts to Merge

Although, the two terms ‘efficacy’ as a property and ‘efficacy’ as a constant were intended as separate concepts, a slip of the mind due to wording later brought the two together as equal. Thus, the relative response due to an agonist ‘property’, equal to ‘efficacy’ (Stephenson 1956, p. 380), was unfortunately not clearly differentiated from ‘efficacy’ as a constant used throughout most of the paper by Stephenson (cf. for instance the legend to Stephenson’s Fig. 9 (1956), reproduced in Fig. 1.12B, and his Table V). See also Sections 1.3.4 – 1.3.8 and more details on this subject in Section 5.6.2.

In the intervening years, Stephenson’s model for efficacy has been refined by several authors in an attempt to experimentally isolate and determine an entity such as the efficacy (Furchgott 1966; MacKay 1966, 1977; Black & Leff 1983; Clarke & Bond 1998; Clark et al. 1999). Why the effort? Well, if one can obtain experimental data for the activation process per se, i.e., the efficacy in absolute...
5.6.2. The Misconception Induced by Stephenson’s Efficacy Scheme

At this point in our development of models, it would be beneficial to compare the scheme by Stephenson, described in Chapter 1 and Eqs. 5.1 and 5.2, with the scheme by del Castillo & Katz.

Let efficacy parameter for conformational change be given by the symbol ‘e’ and occupancy by the symbol ‘y’. Then we can abbreviate Stephenson’s formula (Eq. 5.2) into:

\[ r = f(y-e), \] (5.3)

while the del Castillo & Katz hypothesis may be written by a near-identical expression, in an abstract form, as:

\[ r = f(y, e). \] (5.4)

In these two equations, r is the response and f (·) means ‘function of’.

The difference between Stephenson and dC&K is a product operator in Stephenson’s expression, ‘.’ (Eq. 5.3) versus a separation or listing operator, ‘,’ (Eq. 5.4), for the del Castillo & Katz interpretation of receptor states (Fig. 5.3). The conceptual difference between a ‘Stephenson’ and a ‘Katz-et-al.’ formulation is subtle, but the influence on the outcome of the formulated equations is more than dramatic. It is mind-blowing.

For the dC&K model, the derivation of formulas takes in its origin including explicitly a ‘new’ conformation as the receptive unit. In the Stephenson scheme, the concept of a conformational change is simply implied as an efficacy constant or isomerization constant. S is ligand concentration, L is a dissociation constant, equal to Stephenson’s efficacy constant e. Note that constant L’ is homologous to parameter a in the cyclic two-state model (cTSM) in Fig. 5.4.

**Figure 5.3.** Formulation of the Stephenson and dC&K reaction schemes. (A) Occupancy, stimulus, and relative response efficacy as formulated by Stephenson. (B) The dC&K schemes were published without indication of rate constants, dissociation constant or isomerization constant. S is ligand concentration, \( K_s \) is a dissociation constant, and \( e \) an efficacy constant. \( A_s \) is an association constant for S, and \( L' \) an isomerization constant, equal to Stephenson’s efficacy constant e. The expression in Eq. 5.4 is open to many interpretations, one of which is the formulation of del Castillo-Katz’s reaction scheme with an intermediate receptor conformation, RS, and an additional conformation of the bound receptor in an active form, R*S.

Although not originally formulated by dC&K, their two-step reaction scheme may be equipped with an association constant, \( A_s \), for the first step, and governed by an equilibrium isomerization constant, \( L' \), for the second step. Thus:

\[ R + S \overset{A_s}{\rightleftharpoons} RS \overset{L'}{\rightleftharpoons} R*S, \]

(Fig. 5.3B). At a glance, this dC&K scheme may suddenly look as if we have separated binding and efficacy. Meanwhile, when equating this reaction scheme, the system constants \( A_s \) and \( L' \) become microscopic constants, i.e., they become inseparable (see Sections 5.8.1–5.8.3). Inseparable system constants are the reverse of ‘Stephenson’s error’.

In terms of a distribution formulation in the Langmuirian sense, we can write the fraction of receptors in an active form for a dC&K response as:

\[
\text{response} = \frac{R*S}{R + RS + R*S}, \quad (5.5)
\]

and this represents the actual functional level.
In Fig. 5.7 of the cTSM for binding, parameter $A_s$ is kept constant at 1. Changing this parameter moves the concentration-occupancy (c-o) relations in a proportional fashion along the concentration axis to the left. For parameter $a$, increasing $L$ displaces the c-o curves to the right (Fig. 5.7A), while for $a/C$, increasing $L$ pushes the c-o relations to the left (Fig. 5.7B).

In the functional aspect of the cTSM (Fig. 5.8), the basal response is solely dependent on parameter $L$. In Fig. 5.8A, $L$ is 1/1000 and the dose-effect curve starts at near zero. For $L$, the initial level starts at 50% of the maximal response (Fig. 5.8B). For higher values of $L$, the initial response approximates the initial $R_{max}$.

As before, $A_s$ moves the dose-effect curve in a proportional fashion along the concentration axis (not shown). $L = 0.001$ and $A_s = 1$, $L = 1$ and $A_s = 100$ or 0.01 as indicated by color code. The five plots in both panels vary with values of parameter $a$, which changes in five steps from $10^{-2}$ to $10^2$ by a factor 10 between steps. Circles indicate the EC$_{50}$. Compare panel A with Fig. 5.6.

**Figure 5.7.** Examples of plots for the binding in the cTSM. The association constant $A_s$ for binding of ligand S to the receptive unit is 1 in panels A and B, while parameter $a$ (see Fig. 5.4), is 0.001 in panel A and 1000 in panel B. The isomerization parameter $L$ varies in five steps from $10^{-2}$ to $10^2$ by a factor 10 between steps. Circles indicate the EC$_{50}$. Changes in $A_s$ move the assembly of plots in a proportional manner along the concentration axis (not shown).

**Figure 5.8.** Examples of plots for function in the cTSM. Parameter $L$ is 0.001 in panel A and 1 in panel B. Parameter $A_s$ is 1 in panel A and is either 100 or 0.01 in panel B as indicated by color code. The five plots in both panels vary with values of parameter $a$, which changes in five steps from $10^{-2}$ to $10^2$ by a factor 10 between steps. Circles indicate the EC$_{50}$. Compare panel A with Fig. 5.6.
5.10.3. Formulating a Receptor Reserve

The models by Furchgott (1966) and Black and Leff (1983) were derived in an attempt to address the question of a receptor reserve. Contrary to these models, receptor reserve seems axiomatic in the B&L formulation (i.e., Section 4.10.3, Eq. 5.14), i.e., not only to reduce the maximal efficacy the same way as for 4-BrAMP mustard, \( \beta \)-haloalkylamines, and other irreversible inhibitors. Hence, manipulating the size of \( R_0 \) appears as a direct tuning of the receptor reserve. Meanwhile, it is possible to compensate for a reduction of \( R_0 \) by decreasing the value of coefficient \( K_b \). Thus, even with a receptor reserve less than sufficient for regular maximal response, according to the formulation, we can decrease \( K_b \), keep the parameter \( \tau \) constant, and regain a former maximal response. However, that is not the manner in which experimentation operates or how the coefficient \( K_b \) should be conceived. How should \( K_b \) be conceived?

In this context and to answer that question, parameter \( L' \) can be regarded as a product of an absolute number of receptors \( R_0 \) and a relative constant \( 1/K_b \), where \( K_b \) is a virtual factor while \( L' \) has a physical meaning. \( K_b \) emerges from a mere algebraic manipulation and therefore is not very useful. Efficacy coefficients \( e \) or \( L' \) are not mixed up with numbers of receptors, \( R_{tot} \) or \( R_0 \), as in the transducer ratio \( R_0/K_b \), which is also an efficacy coefficient.

Conversely, the concept efficacy is a mixture of both the number of receptors needed for maximal effect, \( R^*_{tot} \), and the efficacy coefficient \( e \). However, the portion of receptors that can be activated and the efficacy coefficient are kept separate in the expression for efficacy.

What does our derived dC&K formulation say about a receptor reserve? The present dC&K formulation simply states that if \( R_{tot} \) is larger than \( R^*_{tot} \) then there is a proportionally larger receptor reserve (RR). First, when \( R_{tot} \) is reduced to equal \( R^*_{tot} \), then there is no receptor reserve. Should \( R_{tot} \) be reduced further below the actual \( R^*_{tot} \), then \( R^*_{tot} \) is also reduced in parallel to a new \( R_{tot} \). Observing these conditions, we can formulate a relation between all receptive units and units needed for maximal response as: \( R^*_{tot} = R_{tot} - RR \). The B&L transducer ratio \( \tau \) is equal to \( L' \) or Stephenson’s \( e \) and determines the maximum fractional response by \( E/E_m = ar/TR = 1/(1+1/\tau) = 1/(1+1/L') \) (Fig. 5.13).

5.10.4. Furchgott’s Intrinsic Efficacy

Furchgott derived his formulation for spare receptors based on embedded load equations as later copied in the operational model by Black and Leff (Furchgott 1955, 1964; Black & Leff 1983). However, Furchgott (1966) paid more attention to tissue-dependent and ligand-dependent parameters by introducing his ‘intrinsic efficacy’ concept, wherein Stephenson’s efficacy


Tolkovsky AM & Levitzki A. Theories and predictions of models describing sequential interactions between the receptor, the GTP regulatory unit, and the catalytic unit of hormone dependent adenylate cyclases. *J Cyclic Nucleotide Res* 7: 139–150, 1981.


