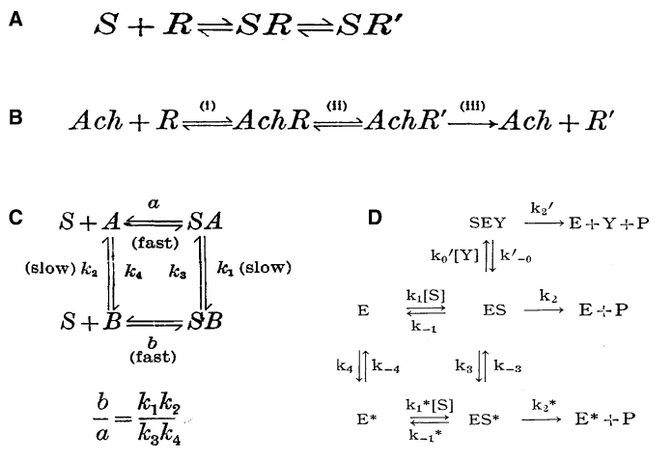

Figure 5.1. Four original reaction schemes. (A+B) DelCastillo and Katz (1957, p. 369 and p. 380) reaction schemes 2 and 3 (dC&K). Ach = S, R = initial reactive receptor, SR = intermediate reactive ('inactive') conformation, and SR′ = an active form (open), that may desensitize. In panel B a nearly irreversible step →S+R′ is added. R′ is the receptive unit in its un-liganded form, a non-reactive conformation, i.e., desensitized. (C) Katz and Thesleff (1957) reaction scheme 5 (K&T5). Note the balanced equilibrium condition in panel C: $b/a = k_1k_2/k_3k_4$. (D) Botts and Drain (1958) reaction scheme 14 (B&D14). The authors comment 'Free energy considerations also require that, for generality, the deformation steps in the cycle be reversible'.

explicit involvement of a conformational change for **un-liganded** 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 un-liganded receptor ran in parallel with observations of partial agonism and receptor reserve (Furchgott 1955; Nickerson 1956; Stephenson 1956). Ariëns (1954) introduced the parameter 'intrinsic activity' α, 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:

$$stimulus = occupancy \cdot efficacy\ constant, \qquad (5.1)$$

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*:

$$response = \text{unknown function of } (occupancy \cdot efficacy\ constant). \qquad (5.2)$$

Note in Eq. 5.2 that 'response' or 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-efficacy, that is, varying the efficacy constant in Eq. 5.2, Stephenson could explain the observation of response for partial agonism including a possible receptor reserve (Fig. 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:

$$r = f(y \cdot e), \tag{5.3}$$

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

$$r = f(y, e). \tag{5.4}$$

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, '$\cdot$' (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 of the receptive unit. In the Stephenson scheme, the effect of a conformational change is simply multiplied as an efficacy constant e onto the occupancy term y (Eq. 5.3), and the product is often taken as the response to other efficacy, not just the stimulus. That is, Eqs. 5.1 and 5.2 in sub-chapter 5.4 are combined, thus omitting the 'function of'. The misuse is exerted due to impatience with the lack of an explicit formulation for Stephenson's 'function of'.

There is a world of difference between this misconception of Stephenson's efficacy scheme due to its ambiguity, and the scheme by del Castillo & Katz. This difference will be made even clearer by the end of sub-chapter 5.10 on 'operational models'.

As mentioned, the temptation to ignore the 'function of' operator in Eqs. 5.2 and 5.3 often results in equations where binding and conformational changes appear as a simple product (see Section 1.3.4). Users of the Stephenson scheme are misled by Eq. 5.3, and as mentioned in Chapter 1, Colquhoun (1998) has referred to the temptation to misinterpret Stephenson's scheme as 'Stephenson's error'. With Stephenson's error, the efficacy coefficient easily becomes identical to Ariëns 'intrinsic activity' coefficient α (Ariëns 1954) which it is not supposed to be (Stephenson 1956; van Rossum 1966).

From conceptually being separable in the misconceived Stephenson scheme (Furchgott 1966; Venter 1997), the system entities y and e, insensibly, become inseparable in the del Castillo & Katz scheme (shown later in sub-chapter 5.8 and discussed further in sub-chapter 5.10) (Fig. 5.3B).

A Stephenson's

occupancy = $y = S/(S+K_S)$

stimulus = $e \cdot y$

relative response = $f\{e \cdot [S/(S+K_S)]\}$

B del Castillo & Katz's

relative response = $f\{L', [S/(S+K_S)]\}$

$$R + S \overset{A_S}{\rightleftharpoons} RS \overset{L'}{\rightleftharpoons} R^*S \qquad \begin{matrix} A_S = 1/K_S \\ L' = e \end{matrix}$$

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. Note that constant L' is homologous to parameter $a \cdot L$ in the cyclic two-state model (cTSM) in Fig. 5.4.

5.6.3. Formulations of the Two-step dC&K model

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 Langmurian sense, we can write the fraction of receptors in an active form for a dC&K response as:

$$\frac{\text{response}}{\text{total}} = \frac{R^*S}{R + RS + R^*S}, \tag{5.5}$$

and this represents the actual *functional* level.

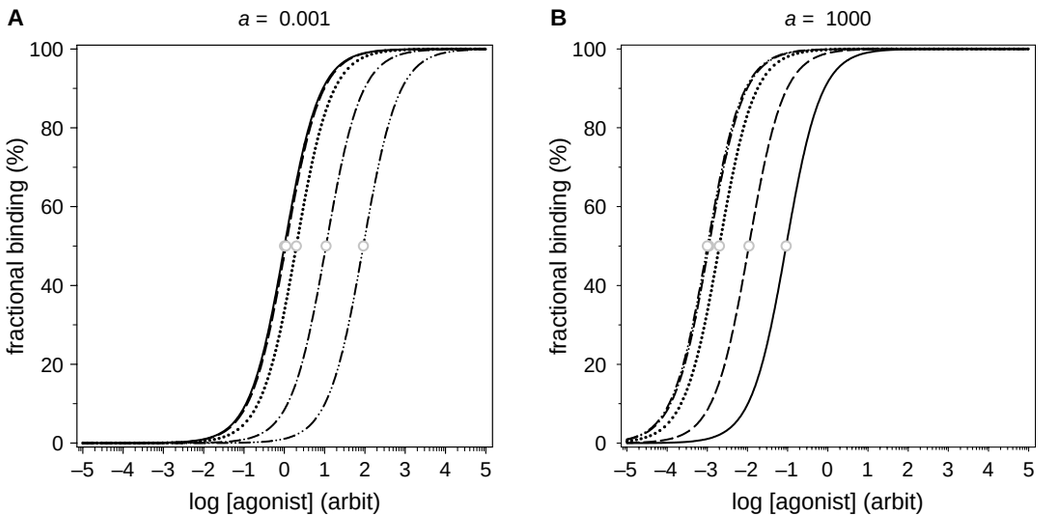

Figure 5.7. Examples of plots for the **binding** in the cTSM. The association constant A_s for binding of ligand S to the receptive unit is 1 in panels A and B, while parameter *a* (see Fig. 5.4), is 0.001 in panel A and 1000 in panel B. The isomerization parameter *L* varies in five steps from 10^{-2} (——) 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).

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 $a < 1$, increasing *L* displaces the c-o curves to the right (Fig. 5.7A), while for $a > 1$, 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* = 1 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} (see Fig. 5.12).

As before, A_s moves the dose-effect curve in a proportional fashion along the concentration axis (Fig. 5.8B). With *L* fixed, it is parameter *a* that determines the maximal response of the functional cTSM (Fig. 5.8). The appK$_s$s described in Box 5.1 are indicated by circles at each curve.

A detail discussion of the implications of changing cTSM parameters is presented in Section 5.9.4.

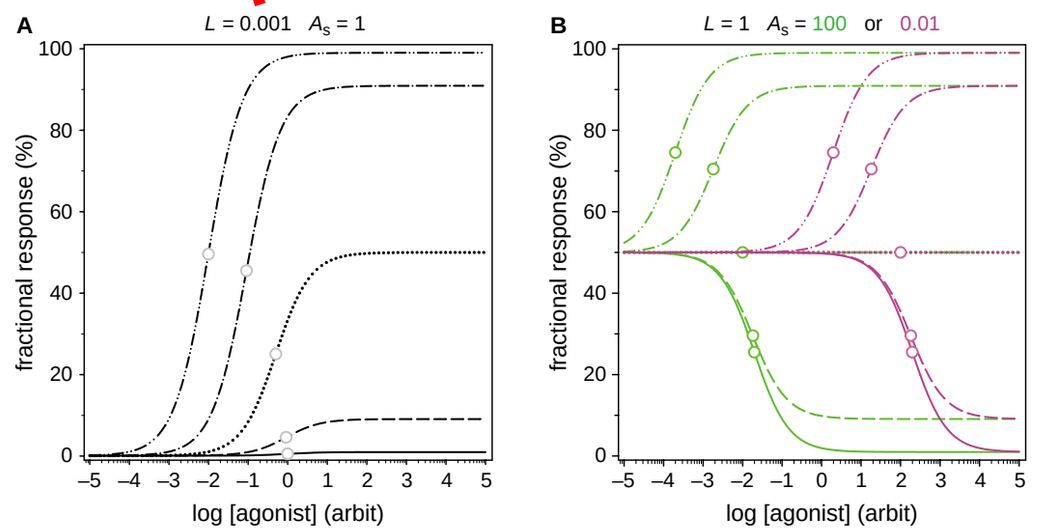

Figure 5.8. Examples of plots for **function** in the cTSM. Parameter *L* is 0.001 in panel A and 1 in panel B. Parameter A_s is 1 in panel A and is either 100 or 0.01 in panel B as indicated by color code. The five plots in both panels vary with values of parameter *a*, which changes in five steps from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

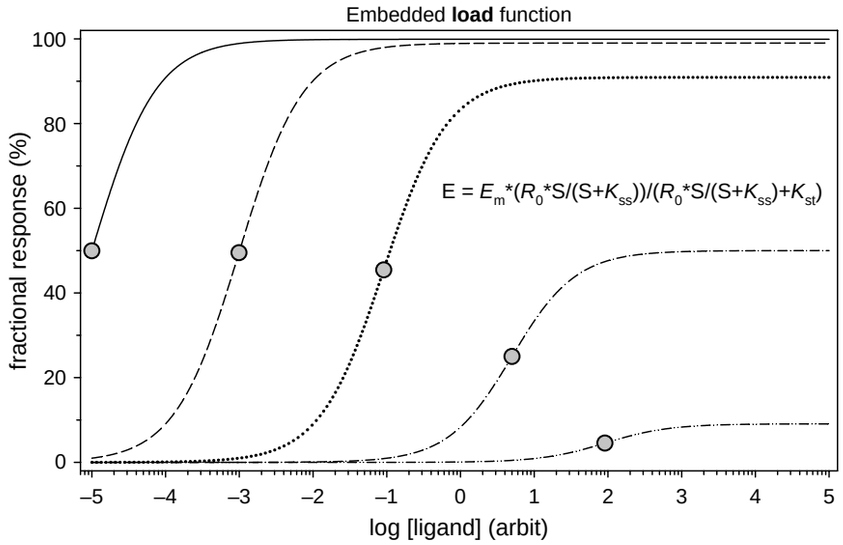

Figure 5.13. The embedded **load** function equal to the operational model, B&L. The embedded **load** formulation is indicated on the graph. The parameters E, E_m, R_0, $K_E = K_{st}$, and $K_A = K_{ss}$ are as defined in the original paper including the transducer ratio $\tau = R_0/K_E$ (Black & Leff 1983). The occupied receptors, here designated '*St*', is a fraction of the total receptors R_0 and formulated as: $St = R_0{*}S/(S + K_{ss})$ in which S is the ligand concentration. The total receptor concentration R_0 is fixed at 1, the maximum effect E_m at 100, while the maximum fractional response is $E_m{*}St/(St + K_{st})$ for $S \rightarrow \infty$. Dissociation constant K_{ss} is varied in 5 steps from 10^{-2} (——) to 10^{2} (— ·· —) by a factor 10 between steps, and dissociation constant K_{st} is varied at the same time in 5 steps from 10^{-3} (——) to 10^{1} (— ·· —) by a factor 10 between steps. Circles indicate the EC_{50}. The embedded **load** function is itself a **load** function (Paton & Rothschild 1965). See text on a comparison between embedded **load** and dC&K's regime.

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. On face value, receptor reserve seems axiomatic in the B&L formulation where by diminution of R_{tot} (R_0) in the transducer ratio τ (cf. Section 5.10.2 and Eq. 5.14), it is possible to reduce the maximal efficacy the same way as for 4-DAMP mustard, β-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_E. Thus, even with a receptor reserve less than sufficient for regular maximal response, according to the formulation, we can decrease K_E, keep the parameter τ constant, and regain a former maximal response. However, that is not the manner in which experimentation operates or how the coefficient K_E should be conceived. How should K_E be conceived?

In this context and to answer that question, parameter L' can be regarded as a product of an absolute parameter R_0 and a relative constant $1/K_E$, where K_E is a virtual factor while L' has a physical meaning. K_E 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_E, which is also an efficacy coefficient.

Conversely, the concept *efficacy* is a mixture of both the total number of receptors needed for maximal effect, R^*_{tot}, and the efficacy coefficient e. However, the pool 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 τ 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

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