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Flux Detectors versus Concentration Detectors: Two Types of Chemoreceptors

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Abstract

Dose–response curves relating the external stimulus concentration to receptor occupancy differ in two types of chemoreceptor organs. In 'concentration detectors' the receptor molecules at the receptor cell membrane are directly exposed to the external stimulus concentration; these organs exhibit the well-known hyperbolic dose–response relationship reflecting the association–dissociation of stimulus and receptor molecules. In contrast, 'flux detectors' accumulate the stimulus molecules in a perireceptor compartment. In flux detectors, deactivation of stimulus molecules may be in balance with arrival, as a prerequisite for producing a constant effective stimulus concentration at constant adsorptive flux of stimulus molecules. In a simple model of a flux detector in which receptor molecules themselves catalyze the deactivation, the dose–response relationship is linear. It reflects the rate of stimulus deactivation. If the deactivation is catalyzed by a separate enzyme, the dose–response relationship can be close to hyperbolic, or linear. In all cases, the receptor molecules are maximally occupied if the adsorptive flux equals or exceeds the maximum rate of stimulus deactivation. The time course of the receptor potential recorded from moths' pheromone receptors depends on the odor compound, which suggests that a peripheral process, possibly the stimulus deactivation, is the slowest, rate-limiting process of the transduction cascade. Further evidence comes from experiments with stimuli oversaturating the mechanism responsible for the decline of the receptor potential.

Introduction

Two extreme types of chemoreceptor organs can be distinguished according to the way they encounter the external stimulus, 'concentration detectors' and 'flux detectors'. In concentration detectors the sensitive receptor cell membranes are directly exposed to the external stimulus concentration S_{ext} (Figure 1a). That is, the effective stimulus concentration S at the receptor cell membrane is identical to the external stimulus concentration S_{ext} within the surrounding medium; S is diminished as quickly as the external stimulus S_{ext} disappears. No further mechanism of stimulus deactivation is necessary. Many taste receptors may belong to the category of concentration detectors.

Chemoreceptor organs of the second type, however, when exposed to a given external stimulus concentration, accumulate stimulus molecules within a perireceptor compartment (Getchell *et al.*, 1984; Keil, 1984; Steinbrecht *et al.*, 1995), especially if the stimulus molecules are acquired from the adjacent air, as in odor detectors (Figure 1b). This is the case in insect antennae, where the odor molecules are adsorbed on the cuticular hairs of olfactory sensilla. Using radiolabeled pheromone, it has been shown that, during brief stimuli, up to several seconds in duration, removal of stimulus molecules from the hairs by desorption or diffusional transport is negligible (Kanaujia and Kaissling, 1985). Accumulation of stimulus molecules can also occur if the external medium is aqueous, as, for example, in bacteria (*Escherichia coli*), where the stimulus is enriched in the periplasmic space due to the presence of binding proteins (Silhavy *et al.*, 1975; Manson *et al.*, 1985). Enrichment of stimulus molecules might occur in chemoreceptor organs of aquatic animals such as Crustacea (Carr *et al.*, 1990) but also in algae that respond to lipophilic pheromone molecules (Boland *et al.*, 1995).

Since adsorption can diminish the stimulus concentration in the immediate vicinity of the adsorbing surface, this concentration may be kept constant by a relative movement of the organ and the external medium. Consequently, the rate of adsorptive uptake U of stimulus molecules by the chemoreceptor organ depends on the external stimulus concentration S_{ext}, but also on the relative velocity v of the organ and the external medium, i.e. on the external stimulus flux

$$\phi_{ext} = S_{ext} \cdot v \qquad (1)$$

(molecules per unit cross-sectional area and per unit time) (Kaissling, 1971, 1990). Thus, the chemoreceptor organs which adsorb but do not desorb stimulus molecules are here denoted as 'flux detectors'. They differ fundamentally from concentration detectors because they need to deactivate the accumulated stimulus molecules. The distinction between concentration and flux detectors is important for the

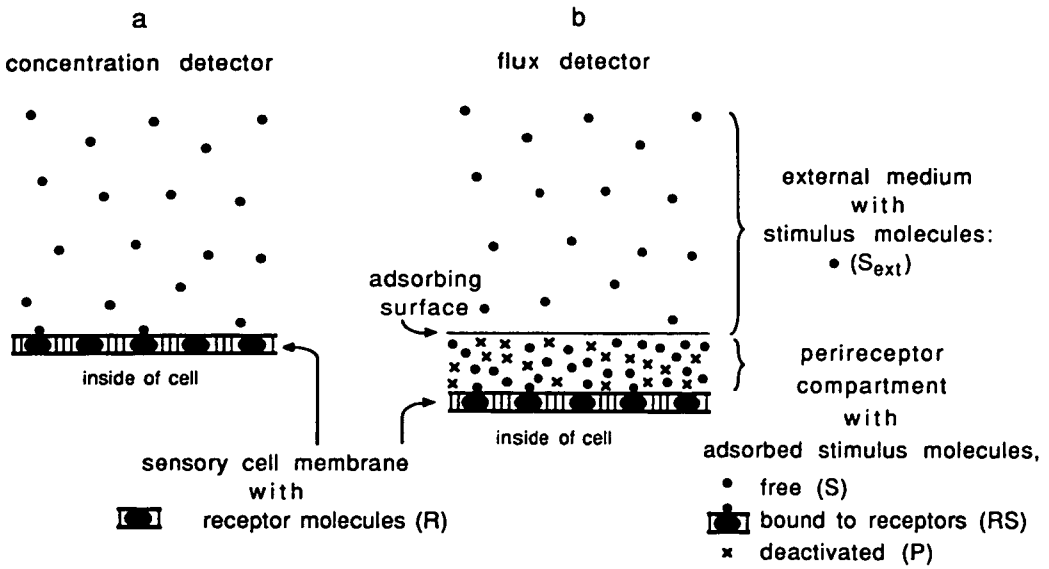

Figure 1 Schematic diagram of two theoretical types of chemoreceptor organs. The sensory cell membrane of a concentration detector **(a)** is directly exposed to the external stimulus concentration. In a flux detector **(b)** the stimulus molecules are adsorbed and accumulated within a perireceptor compartment. A constant concentration of stimulus molecules within the perireceptor compartment can be reached only if they are deactivated, with some delay, at a rate equal to the adsorption rate.

interpretation of dose–response relationships and response kinetics of chemoreceptors. This will be shown by discussing simple models of the two types of chemoreceptors.

Definitions

The terms and definitions used in this paper are listed in the following.

U = rate of uptake of stimulus molecules, measured as number of stimulus molecules collected in a given volume of the perireceptor compartment per unit time
S_{ext} = concentration of stimulus molecules in the external medium
S = effective stimulus concentration, in flux detectors within the perireceptor compartment
S_0 = equilibrium or steady-state concentration of S
P = concentration of deactivated stimulus molecules within the perireceptor compartment
v = relative velocity of the organ and the external medium
ϕ_{ext} = external stimulus flux (molecules per unit cross-sectional area and per unit time)
R = concentration of free receptor molecules, in flux detectors number of free receptor molecules per unit volume of the perireceptor compartment
RS = concentration of occupied receptor molecules
RS_0 = equilibrium or steady-state concentration of RS
R_{tot} = total concentration of receptor molecules = $R + RS$
E = concentration of free deactivating enzyme per unit volume of the perireceptor compartment
ES = concentration of occupied enzyme molecules
E_{tot} = total concentration of enzyme molecules = $E + ES$
S_{upt} = total concentration of adsorbed stimulus molecules = $S + RS + P$
k_1 = rate constant of association of S and R ($M^{-1} \cdot s^{-1}$)
k_2 = rate constant of dissociation of S and R (s^{-1})
k_3 = rate constant of deactivation, i.e. of formation of P (s^{-1})
k_4, k_5 = rate constants of receptor activation and deactivation
K_d = dissociation constant = k_2/k_1
K_m = equilibrium constant = $(k_2 + k_3)/k_1$
a = U/S_{ext} (s^{-1}) = $v \cdot b$
b = U/ϕ_{ext} (cm^{-1})

The symbols S, R, RS, E, ES and P denote molecular species when used in the text and reaction schemes, or their concentrations when used in equations.

Concentration detectors

In the ideal case of concentration detectors the effective concentration S at the receptor molecules of the sensory cell is equal (or remains in a fixed proportion) to S_{ext}. The simplest case can be described as an adsorption–desorption process as proposed for taste receptors by Beidler (1954)

$$R + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RS$$

where k_1 and k_2 are the rate constants for the association and for the dissociation, respectively. R and RS are the concentrations of the receptor molecules and of the stimulus–receptor complex. The dose–response relation-

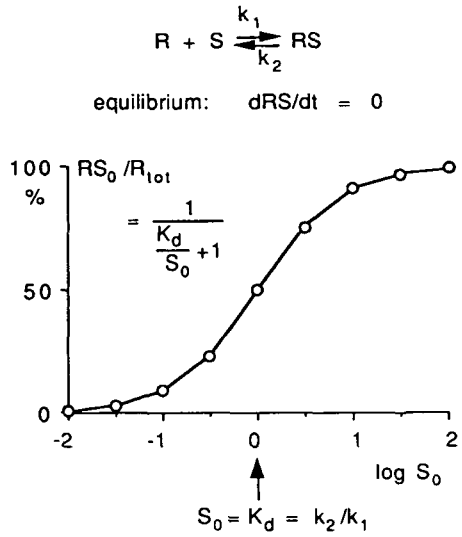

Figure 2 Hyperbolic relationship of the receptor occupancy RS/R_{tot} in a model concentration detector. The curve is shifted along the x-axis when the dissociation constant $K_d = k_2/k_1$ of the stimulus receptor complex is changed.

ships of RS/R_{tot} and S_{ext} are hyperbolic. For equilibrium conditions they are given by

$$RS_0/R_{tot} = 1/((K_d/S_0) + 1) \quad (2)$$

(adsorption isotherm), where the dissociation constant of the stimulus–receptor complex $K_d = k_2/k_1$ (Figure 2).

The kinetics of this reaction are given by

$$dRS/dt = k_1 \cdot R \,.\, S - k_2 \cdot RS \quad (3)$$

It is assumed that S instantly follows any changes of S_{ext}. If the stimulus concentration S suddenly drops to zero, the concentration of occupied receptor molecules will decline as

$$-dRS/dt = k_2 \cdot RS \quad (4)$$

Besides taste receptors, receptor organs for very volatile compounds might also function as concentration detectors, if the concentration of adsorbed volatiles equilibrates very rapidly with the external stimulus concentration. This might apply, for example, to CO_2 in insect CO_2 receptors (Stange, 1996). Ideal concentration detectors would be insensitive to the relative velocity v of the medium. Indeed, no dependence on v was found in moths' CO_2 receptors when v was changed tenfold (Stange and Diesendorf, 1973). If the CO_2 receptor acted as a flux detector, the responses to such a change should comprise the entire working range of the cell (Stange, 1992).

Flux detectors

For flux detectors, i.e. detector organs with negligible desorption, it is convenient to define the rate of adsorptive

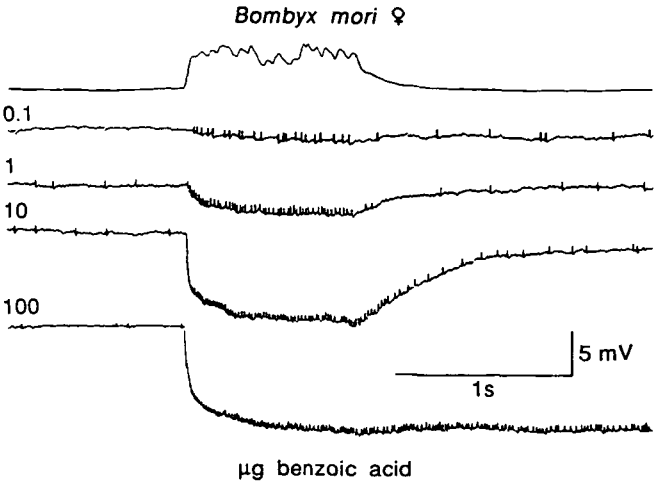

Figure 3 DC-coupled responses (receptor potential and nerve impulses) recorded from a single sensillum trichodeum of a female antenna of *B. mori*. One of the two receptor cells innervating the sensillum responds best to benzoic acid (Priesner, 1979). Upper trace: thermistor signal indicating the airstream velocity. Stimuli were presented at increasing intensity (0.1–100 µg of benzoic acid per filter paper). The receptor potential reaches a constant level during stimulation and decreases immediately after the end of stimulation. The highest intensity produces a prolonged depolarization accompanied by impulse discharge (Kaissling, 1987).

stimulus uptake U as the number of molecules adsorbed per unit volume of the perireceptor compartment and per unit time. The rate of uptake U is proportional to the external stimulus concentration S_{ext} with the factor 'a' (s^{-1}).

$$U = a \cdot S_{ext} \quad (5)$$

where $a = v \cdot b$, v (cm/s) is the velocity of the external medium relative to the receptor organ and b (cm^{-1}) depends on the geometry of the adsorptive structures, their adsorptive properties and the volume of the perireceptor compartment. With a constant value of b, the adsorptive stimulus uptake U is proportional to ϕ_{ext}, the external stimulus flux

$$U = b \cdot \phi_{ext} \quad (6)$$

The accumulation of stimulus molecules can also be described as an integration where the total concentration of stimulus molecules adsorbed S_{upt} is the integral of U

$$S_{upt}(t) = \int_0^t U \cdot dt \quad (7)$$

During stimulation, the effective concentration S ought to increase along with S_{upt}. In many insect olfactory cells, however, the receptor potential approaches steady levels dependent on stimulus intensity and declines immediately after the end of stimulation (Figure 3). Apparently the effective stimulus concentration S reaches constant levels,

which is possible if the stimulus molecules are deactivated as rapidly as they arrive (Kaissling, 1974). To account for this finding we assume an enzymatic deactivation process and consider two cases. In the first case, the receptor molecules themselves catalyze the deactivation as recently suggested by Ziegelberger (1995). In the second case stimulus deactivation is catalyzed by a separate enzyme E.

Catalysis of deactivation by receptor molecules acting as enzymes

In the following we assume that the adsorbed molecules instantly become distributed within the perireceptor compartment, i.e. that diffusion from the site of adsorption to the receptor molecules is not rate-limiting (see Discussion). For convenience, we treat the receptor molecules as if they are distributed within the perireceptor compartment instead of being associated with the receptor cell membrane. This may be allowed in view of the small diameter of the hair lumen and if diffusion is not limiting. The reaction scheme for the first case, with receptor molecules as enzymes, is

$$S_{ext} \xrightarrow{a} S \quad \text{and} \quad R + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RS \xrightarrow{k_3} R + P$$

with the rate of adsorptive stimulus uptake

$$U = a \cdot S_{ext} = dS/dt + dRS/dt + dP/dt \qquad (8)$$

This reaction scheme corresponds to a system of nonlinear differential equations:

$$dS/dt = U - k_1 \cdot R \cdot S + k_2 \cdot RS \qquad (9)$$

$$dRS/dt = k_1 \cdot R \cdot S - k_2 \cdot RS - k_3 \cdot RS \qquad (10)$$

Transients

Figure 4 demonstrates the time course of the concentrations of S, RS and P (the deactivated stimulus molecules) for $S << K_m$, with $K_m = (k_2 + k_3)/k_1$, and for $R_{tot} << K_m$. At a constant rate of uptake the summed concentrations $S + RS + P = S_{upt}$ increase linearly. Under the above conditions S and RS decline after the end of stimulus exposure (when U is set to zero) with a rate constant of

$$-dS/dt \approx -dRS/dt \approx k_3 \cdot R_{tot}/K_m \qquad (11)$$

(see Appendix, equations XVIa and XVIb), which can be used for the determination of K_m (K.E. Kaissling, submitted for publication).

Steady state

The steady state for constant U is defined by $dS/dt = 0$ and $dRS/dt = 0$. From equations (9) and (10) we obtain for the dependence of receptor occupation RS_0/R_{tot} on S

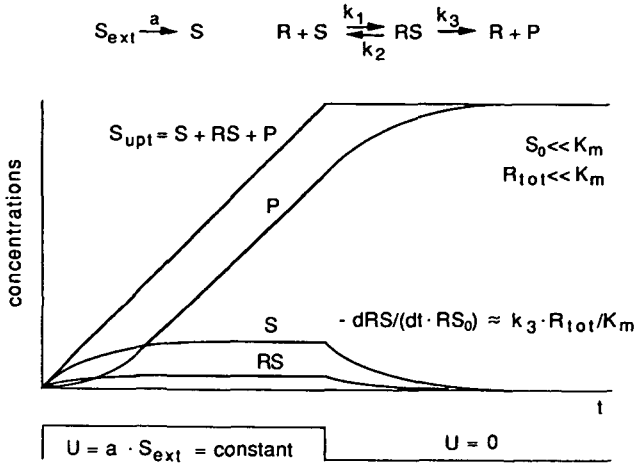

Figure 4 Concentration transients in a flux detector in which receptor molecules catalyze stimulus deactivation, produced by a computer model (J. Thorson, Oxford). Top: reaction scheme including the adsorption of the external stimulus S_{ext}. Schematic time courses of the effective stimulus concentration S, receptor occupancy RS and concentration of the deactivated stimulus molecules P, during stimulus uptake at a constant rate $U = a \cdot S_{ext}$, and after the end of stimulus exposure ($U = 0$). Conditions $S_0 << K_m$ and $R_{tot} << K_m$. For responses to stronger stimuli see Figure 6.

$$RS_0/R_{tot} = 1/((K_m/S) + 1) \qquad (12)$$

For the dependence of receptor occupation on U we obtain

$$dP/dt = k_3 \cdot RS_0 = U \qquad (13)$$

or

$$RS_0/R_{tot} = U/(k_3 \cdot R_{tot})$$

This means that flux detectors—if deactivation is catalyzed by the receptor molecules—exhibit a hyperbolic dependence of receptor occupancy on the effective concentration S_0 (as do concentration detectors) but a linear dependence of occupancy on the external stimulus concentration S_{ext} and also on the uptake U (see Figure 5).

At a just saturating uptake rate

$$U_{sat} = k_3 \cdot R_{tot} \qquad (14)$$

the receptors are maximally occupied. If U exceeds $k_3 \cdot R_{tot}$ the deactivation mechanism becomes overloaded, i.e. the effective stimulus concentration S cannot reach a steady state but increases indefinitely. The position of the dose–response curve can be characterized by the point of saturation, which is determined by the maximum deactivation velocity $k_3 \cdot R_{tot}$. After the termination of the external stimulus, the receptors remain near saturation until enough of the accumulated stimulus molecules have been

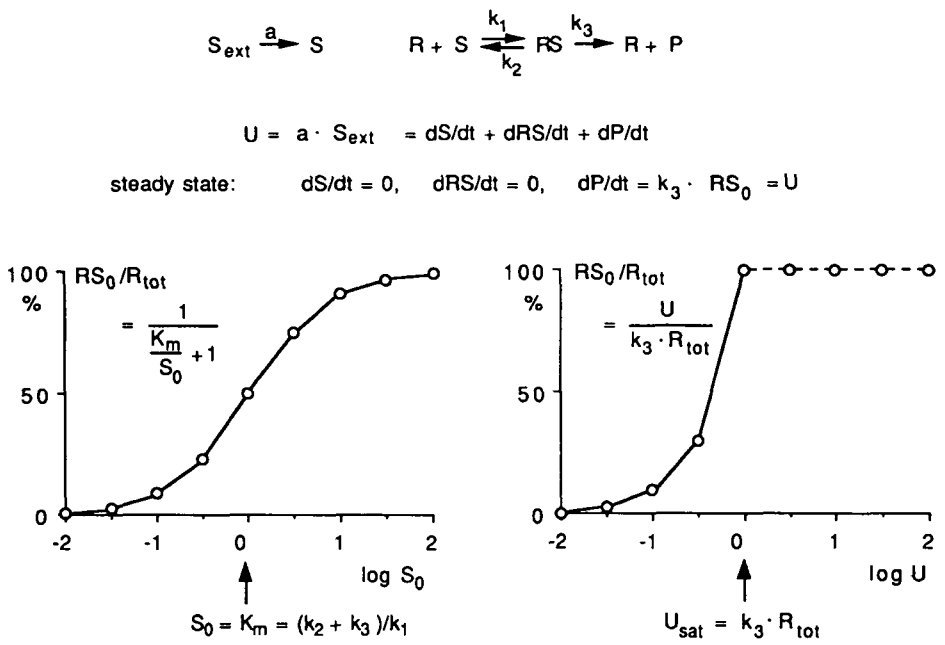

Figure 5 Dose–response curves at steady state of a model flux detector in which receptor molecules catalyze stimulus deactivation (see Figure 4). Dependence of receptor occupation on the effective stimulus concentration S (left graph, hyperbolic) and the rate of stimulus uptake U (right graph, linear), which is proportional to the external stimulus concentration ($U = a \cdot S_{ext}$). The uptake rate (U_{sat}) necessary for saturation of receptor occupation depends solely on the maximum velocity of stimulus deactivation $k_3 \cdot R_{tot}$.

deactivated (Figure 6, traces for $U = 10$). Surprisingly, the dose–response relation does not depend on the dissociation constant of the stimulus–receptor complex $K_d = k_2/k_1$ as it does in concentration detectors.

The described differences between concentration and flux detectors have to be considered when interpreting dose–response curves, at least on the basis of the simple reaction schemes discussed here. Stimulus compounds producing dose–response curves in the same receptor cell which differ with respect to their position along the intensity axis might have different dissociation constants K_d in concentration detectors. In flux detectors, such compounds would differ in the maximum deactivation rate $k_3 \cdot R_{tot}$. Consequently, saturation of receptor occupancy at higher stimulus concentrations S_{ext} indicates either a larger number of receptor molecules R_{tot} or a higher rate of stimulus deactivation k_3. Thus, a stimulus compound is less effective in exciting the receptor cell if it is more quickly deactivated.

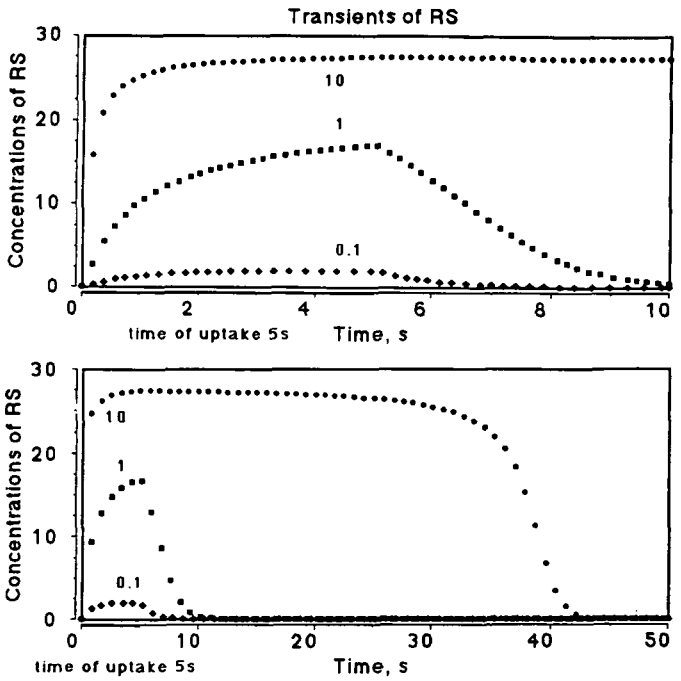

Figure 6 Kinetics of the receptor occupancy RS for a flux detector in which receptor molecules catalyze stimulus deactivation (cf. Figure 4). Schematic time course of $RS \cdot 100/R_{tot}$ (ordinate) was produced by a computer model (J. Thorson, Oxford) for three rates of stimulus uptake U at 10-fold increments (0.1, 1 and 10). Upper and lower graphs differ in time scale. The uptake rate was constant for 5 s and zero after the end of stimulus exposure. $U = 1$ corresponds to an uptake rate of K_m/s. Further conditions $R_{tot} = 0.28\ K_m$ and $k_3 \cdot R_{tot} = 1.4 \cdot K_m$/s (see K.E. Kaissling, submitted fpr publication).

Catalysis of deactivation by a separate enzyme

The situation is a little more complicated in flux detectors where a separate enzyme is responsible for the stimulus deactivation. The reaction scheme is

$$S_{ext} \xrightarrow{a} S \qquad R + S \underset{k_{2R}}{\overset{k_{1R}}{\rightleftharpoons}} RS$$

and

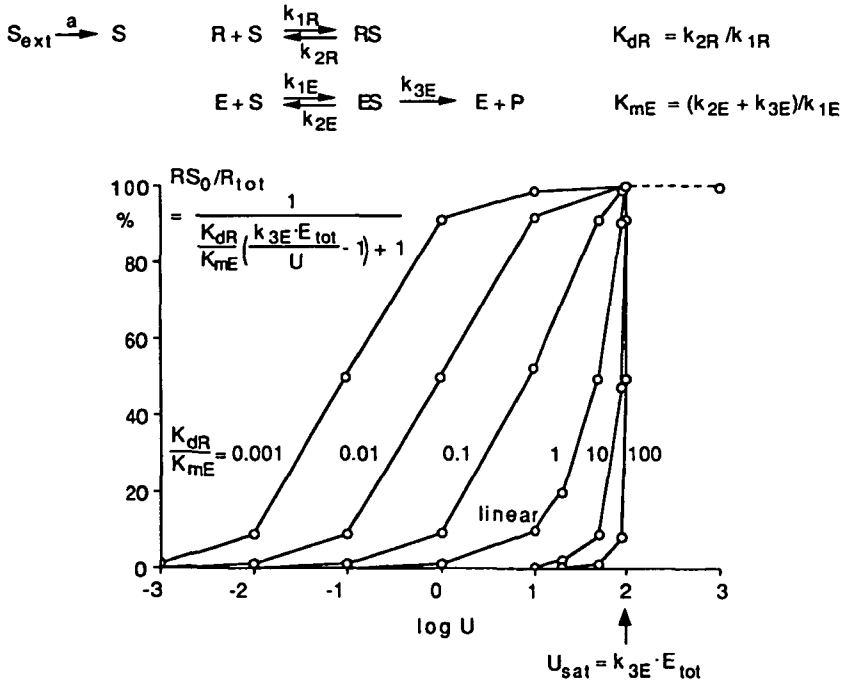

Figure 7 Dose–response curves of a model flux detector in which a separate enzyme (E) catalyzes stimulus deactivation. **Top**: reaction scheme. **Bottom**: dependence of receptor occupation on the rate of stimulus uptake U, which is proportional to the external stimulus concentration ($U = a \cdot S_{ext}$). The relationship is linear at $K_{dR} = K_{mE}$, hyperbolic with $K_{dR} < K_{mE}$ (with half saturation of the receptors at $U_{1/2occ} >> k_{3E} \cdot E_{tot} \cdot K_{dR}/K_{mE}$), and steeper than linear with $K_{dR} > K_{mE}$.

$$E + S \underset{k_{2E}}{\overset{k_{1E}}{\rightleftharpoons}} ES \xrightarrow{k_{3E}} E + P$$

For the steady-state state we have

$$dS/dt = U - k_1R \cdot R \cdot S + k_{2R} \cdot RS - k_{1E} \cdot E \cdot S + k_{2E} \cdot ES = 0 \quad (15)$$

$$dRS/dt = k_{1R} \cdot R \cdot S - k_{2R} \cdot RS = 0 \quad (16)$$

$$dES/dt = k_{1E} \cdot E \cdot S - k_{2E} \cdot ES - k_{3E} \cdot ES = 0 \quad (17)$$

From these equations we find

$$RS_0/R_{tot} = 1/((K_{dR}/K_{mE})\,((k_{3E} \cdot E_{tot}/U) - 1) + 1) \quad (18)$$

where $K_{dR} = k_{2R}/k_{1R}$ and $K_{mE} = (k_{2E} + k_{3E})/k_{1E}$.

In this model the dissociation constant of the stimulus–receptor interaction K_{dR} is relevant to the measured dose–response curve. It influences the rate of stimulus uptake $U_{1/2occ}$ necessary for half saturation of the receptors, and thus determines the position of the dose– response curve.

The curve relating RS_0/R_{tot} to stimulus uptake U differs in shape depending on the ratio K_{dR}/K_{mE} (see Figure 7). When $K_{dR} = K_{mE}$, receptors and enzyme half-saturate at the same concentration of S. We find a linear relationship with

$$RS_0/R_{tot} = U/(k_{3E} \cdot E_{tot}) \quad (19)$$

as in the previously discussed case with receptors as catalysts (equation 13).

For $K_{dR} < K_{mE}$ the receptors saturate at lower concentrations of S than the enzyme; the relationship between RS/R_{tot} and U is about hyperbolic.

$$RS/R_{tot} \approx 1/((K_{dR}/K_{mE})\,(k_{3E} \cdot E_{tot}/U) + 1) \quad (20)$$

The rate of stimulus uptake necessary for half occupation of receptors is

$$U_{1/2occ} \approx k_{3E} \cdot E_{tot} \cdot K_{dR}/K_{mE} \quad (21)$$

For $K_{dR} >> K_{mE}$ the receptors saturate at higher concentrations of S than the enzyme; the relationship between RS/R_{tot} and U is again hyperbolic, but of a different type than the usual adsorption isotherm. The part of the curve near saturation is steeper than a linear curve (Figure 7). In all cases the receptors are quasi-saturated at and above $U = k_{3E} \cdot E_{tot}$.

Receptor activation

Usually, chemoreceptor reaction schemes include a step of receptor activation consequent upon the binding between stimulus and receptor:

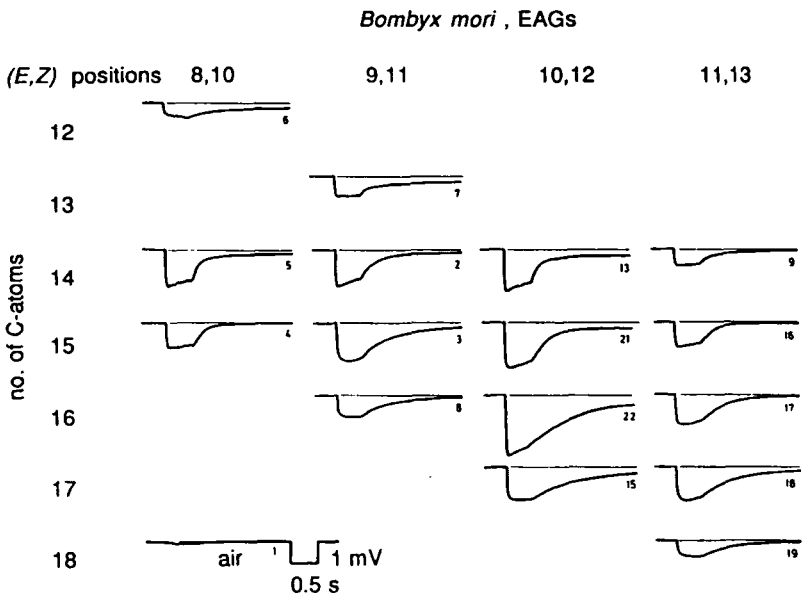

Figure 8 Electro-antennograms obtained from a single antenna of a male moth stimulated with bombykol (response no. 22) and related (*E,Z*)-dienols of different chain lengths and positions of conjugated double bonds. Stimulus load: 10 μg of each stimulus compound per odor source (filter paper), stimulus duration 0.5 s. Small numbers indicate the sequence of stimulation (not all responses are shown). Compounds with the same position of double bonds but with shorter chain length mostly produce a more rapid decline of EAG. Moving the position of double bonds produces an alternating pattern of decline times (cf. compounds 5, 2 and 13; 4, 3 and 21; or 8, 22 and 17). Stimulus compounds were kindly provided by H.J. Bestmann, Erlangen (from Kaissling, 1987). In a different set of experiments six of the compounds were tested in n = 7 or 9 animals (no. of compound, average half time of EAG decline ± SD, n =): no. 2, 0.23 ± 0.07 s, n = 7; no. 3, 0.49 ± 0.18 s, n = 7; no. 8, 0.43 ± 0.15 s, n = 9; no. 21, 0.28 ± 0.07 s, n = 7; no. 22, 0.45 ± 0.17 s, n = 9; no. 15, 0.65 ± 0.27 s, n = 9.

$$RS \underset{k_{5R}}{\overset{k_{4R}}{\rightleftharpoons}} R'S$$

The activated complex $R'S$ triggers the cell response. It follows that even with saturating stimuli only a fraction of the occupied receptors

$$RS'/(RS + RS') = k_{4R}/(k_{4R} + k_{5R}) \qquad (22)$$

can be activated and can lead to cell excitation, since $R'S$ is always smaller than R_{tot}. The velocity constants k_{4R} and k_{5R} very probably depend on the stimulus compound. Consequently, their ratio may be responsible for different maximum values of $R'S/R_{tot}$ obtained with different ligands (see Kaissling, 1977, 1987).

Dose dependence of the receptor potential

So far, dose–response relations have been discussed for the occupation of olfactory receptor molecules, which has not been measured directly. When an electrophysiological response, e.g. the receptor potential amplitude, is plotted over the external stimulus concentration S_{ext} or the rate of stimulus uptake U, the dose–response curves may be hyperbolic even if the receptor occupation is linear. This is due to the nonlinear relationship between increase of membrane conductance and membrane depolarization. Thus, the electrical response can saturate at a relatively low level of receptor occupation, i.e. at $RS << R_{tot}$. As demonstrated by Kaissling (1977, 1987; see also Rospars *et al.*, 1996), the electrophysiologically measured dose–response curve can reach half-saturation at stimulus concentrations far below those necessary for half-occupation of the receptor molecules (see also K.E. Kaissling, submitted for publication). However, the stimulus intensity necessary for half-saturation of the receptor potential must shift along the stimulus axis if the maximum rate of deactivation $k_3 \cdot R_{tot}$ changes. Thus, compounds with different dose–response curves may differ with respect to k_3 or R_{tot}. As shown here for flux detectors, the shift of the dose–response curve does not reflect a change in the rate constants for association (k_1) and dissociation (k_2) of stimulus and receptor molecules.

It has previously been shown (Kaissling, 1977, 1987) that the stimulus concentration for half-maximum receptor potential can also be altered by changing the ratio $R'S/RS$ or by changing the increment of membrane conductance per activated complex $R'S$. In both ways, the maximum amplitude of depolarization may also be influenced.

Does odor deactivation determine the response kinetics?

Besides the stimulus molecules accumulated in the perireceptor compartment of flux detectors, each of the activated states of the transduction cascade needs to be

deactivated. This applies to the receptor molecules, G-proteins, enzymes, second messenger molecules and ion channels. Termination processes of the transduction cascade in mammalian olfactory receptors have been studied (Boekhoff and Breer, 1992; Breer *et al.*, 1994; Restrepo *et al.*, 1996), but their kinetics *in situ* and their relation to processes of odor removal or deactivation are unknown. The question of interest here is which one of the various terminating processes is the slowest one and thus determines the time course of the electrical receptor cell response, the receptor potential.

Response kinetics depend on stimulus compound

Evidence suggesting that a peripheral process, such as odorant deactivation, is the rate-limiting process comes from the observation that the decline of the receptor potential depends strongly on the chemical structure of the stimulus molecule. Such observations are presented here for olfactory sensilla of the moth *Bombyx mori*, which are innervated by two highly specific pheromone receptor cells, sensitive to the pheromone components bombykol and bombykal respectively. Figure 8 shows electro-antennograms (EAGs) elicited by various double-unsaturated alcohols (dienols) that excite the bombykol receptor cell of male *B. mori*. Most of the derivatives of bombykol produce receptor potentials that decline more rapidly than those produced by the pheromone itself. Furthermore, the decline time varies depending on both the chain length of the molecule and the position of the conjugated double bonds. Similar results have been shown for recordings from single sensilla in which the responses of a single receptor cell can be measured (Kaissling, 1977). EAGs with time courses depending on the stimulus compound were observed also by Roelofs and Comeau (1971) and Dickens *et al.* (1993).

Clearly, the decline time is not correlated with the response amplitude. In the context of the model discussed, these effects indicate that the rate constants k_1, k_2 and k_3 differ in their dependence on the stimulus compounds. According to equation (13), the steady-state response amplitude reflecting the receptor occupation RS/R_{tot} depends on the ratio $U/k_3 \cdot R_{tot}$. A smaller amplitude might therefore reflect a larger k_3. The decline time with weak stimuli ($S << K_m$) and for $R_{tot} << K_m$ depends on $k_3 \cdot R_{tot}/K_m$ (equation 11). We find for

$$k_3 >> k_2 \qquad dRS/dt \approx k_1 \cdot R_{tot} \tag{23}$$

$$k_3 = k_2 \qquad dRS/dt \approx k_1 \cdot R_{tot}/2 \tag{24}$$

$$k_3 < k_2 \qquad dRS/dt \approx k_1 \cdot R_{tot} \cdot k_3/k_2 \tag{25}$$

Thus, according to equation (25), a change of k_3 could influence both the response amplitude and the rate of decline. In this case a (reciprocal) correlation of these two parameters would be expected. The lack of this correlation

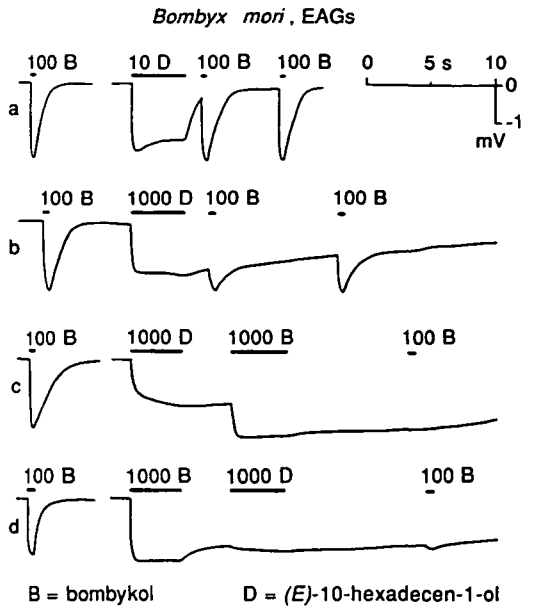

Figure 9 EAGs recorded from four antennae of *B. mori* stimulated with (*E,Z*)-10,12-hexadecadianol (bombykol, B) and (*E*)-10-hexadecenol (dihydrobombykol, D) at stimulus loads of 10–1000 μg per filter paper. The stronger stimuli (1000 μg) of both compounds produce a prolonged decline, apparently over-saturating the stimulus deactivating process **(b–d)**. The EAG elicited by D saturated at an intermediate amplitude (b, c). A higher EAG amplitude is reached by bombykol, even at its weaker concentration **(a–c)**. These effects could indicate that bombykol competes with the related compound D for the excitatory process as well as for the deactivating mechanism. (Graphs a and b from Kaissling, 1974.)

(Figure 8) shows that besides k_3 another constant must be changed for the various bombykol analogs. The conclusion that more than one parameter is involved in the specificity of the olfactory response has also been drawn from the interpretation of fluctuations of the receptor potential which depend on the stimulus compound (Kaissling, 1977).

In principle, the time course of the EAG or the receptor potential could be governed by the transport velocity of the stimulus compound from the site of adsorption at the surface of the hair cuticle to the receptor cell membrane. However, the receptor potential kinetics is much slower than expected from the measured velocity of pheromone transport along the olfactory sensillum [diffusion coefficient 5×10^{-7} cm^2/s in *Bombyx* (Steinbrecht, 1973; Kaissling, 1987)].

Receptor potential kinetics at saturating stimuli

An argument in favor of pheromone deactivation as the determinant of the kinetics of the receptor potential is based on responses to saturating stimuli (of bombykol, B, and a derivative, D) which produce a prolonged decline after the end of a stimulus (Figure 9b–d). Very strong stimulation by the less effective pheromone derivative can produce a prolonged decline of the receptor potential even though the amplitude of the response does not reach the maximum

obtainable with the pheromone itself (Kaissling, 1971, 1972) (Figure 9b,c). This prolongation may indicate that the uptake of the pheromone derivative exceeded the value necessary for saturation of the receptors and, according to the model presented, exceeded the maximum deactivation rate. Apparently, the stimulus oversaturated the deactivation mechanism but not the subsequent processes of cellular transduction, since the cell response to the derivative was submaximal.

Furthermore, a much smaller (in terms of number of molecules) but more potent bombykol stimulus, presented during the prolonged response to the derivative, produces an increase of the receptor potential to a level much higher than the previous level of prolonged depolarization (Figure 9b). After the end of the 'weaker' bombykol stimulus the receptor potential rapidly declined to the previous level caused by the derivative. This might indicate that the bombykol stimulus competed with the derivative for the receptors and also for the deactivation. It seems that, compared with the derivative, the relatively small amount of the pheromone was preferentially deactivated.

Prolonged depolarizations have been observed in insect visual receptor cells (Hamdorf *et al.*, 1989). They were caused by an excessive accumulation of activated rhodopsin (metarhodopsin), which can be considered as the functional equivalent of active odor molecules accumulated in the perireceptor compartment of olfactory organs. In fact, the visual system can be classified as a flux detector since, in proportion to the quantum flux, it 'produces' and also deactivates metarhodopsin, the first excitatory compound of the transduction chain.

Possible mechanisms of odor deactivation

There are few studies on possible mechanisms of stimulus deactivation in chemoreceptors, most of which presumably belong to the flux detector type. For the vertebrate nose it is assumed that the odor molecules adsorbed by the mucosa are removed by blood flow (Hahn *et al.*, 1994). Enzymatic odor degradation has been found in the olfactory epithelium (Dahl, 1988; Longo *et al.*, 1988; Persaud *et al.*, 1988; Nef *et al.*, 1989; Ding *et al.*, 1991; Rama-Krishna *et al.*, 1992) and a possible role of odorant-binding proteins has been envisaged (Pelosi, 1996). The stimulant AMP is deactivated by extracellular enzymes in the spiny lobster (Trapido-Rosenthal *et al.*, 1990). Stimulus deactivation is accomplished in *E. coli* by the transport of the stimulant from the periplasmic space into the cell, across the cell membrane (Bohl *et al.*, 1995).

It should be noted that the mechanism of stimulus deactivation postulated for the insect antenna (Kaissling, 1972) cannot be identical with the enzymatic pheromone degradation studied by Kasang and co-workers (Kasang, 1971; Kasang and Kaissling, 1972; Kasang *et al.*, 1988), Ferkovich *et al.* (1973) and Vogt *et al.* (1985), because the latter process, measured in living antennae, takes minutes. The postulated rapid deactivation discussed here is a process responsible for the decline of the receptor potential, which occurs in seconds (Zack, 1979; Kodadová and Kaissling, 1996).

The molecular mechanism of pheromone deactivation recently proposed by Ziegelberger (1995) does not involve a chemical degradation of the stimulus molecule. It is based on the redox shift of the pheromone-binding protein (PBP; Ziegelberger, 1995) measured in homogenates of isolated olfactory hairs of the moth *Antheraea polyphemus*. A subsequent paper offers a quantitative determination of the model constants based on electrophysiological, biochemical and radiometric studies (K.E. Kaissling, submitted for publication; see also Kaissling, 1996). In this model S is the complex of the pheromone molecule and the reduced form of the PBP, whereas P is the pheromone bound to the oxidized PBP. The receptor molecules are assumed to catalyze the redox shift of the complex.

Concluding remarks

A simple method to distinguish between concentration and flux detectors is to test their sensitivity to the relative velocity of the medium, for instance using electrophysiological methods. Besides taste and carbon dioxide receptors, humidity receptors may well prove to be concentration detectors, which in extreme cases are insensitive to velocity changes. Most olfactory organs that adsorb but do not desorb stimulus molecules may in fact be flux detectors, which depend not only on the external stimulus concentration but also on the relative velocity of the medium. They are likely to have a mechanism for rapid stimulus deactivation which governs the kinetics of the olfactory responses.

The simple models of flux detectors discussed here show that interpretation of the dose–response relationships is different from that in concentration detectors. Most importantly, the stimulus flux necessary for maximum occupation of receptor molecules in flux detectors depends on the maximum rate of stimulus deactivation.

The functional advantage of the proposed reaction model for flux detectors in which the receptor molecules act as deactivating enzymes is its one-way character: the pheromone molecules are not deactivated unless they hit a receptor molecule. This would help to ensure maximum sensitivity to odors of a flux detector organ like the moth antennae. Odorant-specific effects on the time course of the receptor potential indeed suggest that odor deactivation is the slowest, rate-limiting process of the transduction cascade in moth pheromone receptors.

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Appendix: Exponential approximations of the time course of effective stimulus concentration and receptor occupancy elicited by weak stimulation

The model of receptor molecules as enzymes will be considered below for the case of small uptake rates. The effective stimulus concentration $S = S(t)$ and the occupancy $RS = RS(t)$ in the presence of a constant rate U of stimulus uptake are determined by a system of nonlinear differential equations (9) and (10). According to (13) if one assumes a restriction for uptake

$$U << k_3 \cdot R_{tot} \quad \text{(I)}$$

This yields

$$RS_0 << R_{tot} \quad \text{(II)}$$

where RS_0 is the value of RS at steady state.

If

$$RS \leq RS_0 \quad \text{(III)}$$

therefore

$$R = R_{tot} - RS \approx R_{tot}$$

The system of nonlinear differential equations (9) and (10) can be approximated by a linear system

$$dS/dt = U - k_1 \cdot R_{tot} \cdot S + k_2 \cdot RS \quad \text{(IVa)}$$

$$dRS/dt = k_1 \cdot R_{tot} \cdot S - k_2 \cdot RS - k_3 \cdot RS \quad \text{(IVb)}$$

where coefficients k_1, k_2, k_3 and R_{tot} take positive values.

By setting the derivatives in equations (IVa) and (IVb) to zero [or by combining the steady-state values of equations (12) and (13) with equation (II)], the equilibrium values S_0 and RS_0 for linear approximation can be obtained:

$$S_0 = U \cdot K_m/(k_3 \cdot R_{tot}),\ RS_0 = U/k_3 \quad \text{(V)}$$

where

$$K_m = (k_2 + k_3)/k_1$$

Therefore

$$S_0/RS_0 = K_m/R_{tot} \quad \text{(VI)}$$

and then equation (II) gives

$$S_0 << K_m$$

If the stimulation with a constant uptake U stops at time $t = 0$, RS and S would decline from their initial values at zero time to zero values at infinite time. These functions are specified by a system of two homogeneous linear differential equations where the second one coincides with equation (IVb)

$$dS/dt = -k_1 \cdot R_{tot} \cdot S + k_2 \cdot RS \quad \text{(VIIa)}$$

$$dRS/dt = k_1 \cdot R_{tot} \cdot S - k_2 \cdot RS - k_3 \cdot RS \quad \text{(VIIb)}$$

The solution of this system is a sum of two functions (Murphy, 1960)

$$A_1 \cdot \exp(\gamma_1 \cdot t) + A_2 \cdot \exp(\gamma_2 \cdot t)$$

where, in general case, the constants γ_1 and γ_2 are complex numbers.

For the system, equations (VIIa) and (VIIb), these constants are defined as

$$\gamma_{1,2} = -(1/2) \cdot k_1 \cdot (K_m + R_{tot}) \cdot [1 \pm \sqrt{(1 - 4\alpha \cdot \beta)}] \quad \text{(VIII)}$$

where

$$\alpha = k_3/(k_2 + k_3) \qquad \beta = K_m \cdot R_{tot} \cdot (K_m + R_{tot})^{-2} \qquad \text{(IX)}$$

Since for all values of the coefficients in equations (IVa) and (IVb) $\alpha < 1$ and $\beta \leq 1/4$, the values in equation (VIII) are always real and negative and represent two rate constants that describe the exponential decline of RS or S. This means that the condition (III) is satisfied.

Note that β is symmetrical with respect to K_m and R_{tot}, and when either R_{tot}/K_m or K_m/R_{tot} approaches zero, β is small; in both cases, considered below, serial approximation for square root

$$\sqrt{(1-x)} = 1 - x/2 - x^2/16^2 - \ldots$$

can be applied to square root in equation (VIII)

$$\sqrt{(1 - 4\alpha \cdot \beta)} = 1 - 2\alpha \cdot \beta - 2\,\alpha^2 \cdot \beta^2 - \ldots$$

Then equation (VIII) can be approximated as:

$$\gamma_{1,2} \approx -k_1 \cdot (K_m + R_{tot}) \pm k_1 \cdot (K_m + R_{tot}) \cdot (1 - 2\alpha \cdot \beta - 2\,\alpha^2 \cdot \beta^2) \qquad \text{(X)}$$

or, using the definitions of α and β in equation (IX), the following expressions for γ_1 and γ_2 can be found:

$$\gamma_1 \approx -k_3 \cdot R_{tot} \cdot (1 + \alpha \cdot \beta)/(K_m + R_{tot}) \qquad \text{(XIa)}$$

$$\gamma_2 \approx -k_1 \cdot (K_m + R_{tot}) + k_3 \cdot R_{tot} \cdot (1 + \alpha \cdot \beta)/(K_m + R_{tot}) \qquad \text{(XIb)}$$

Two limiting cases for the ratio R_{tot}/K_m will be considered below.

Case one

We consider

$$R_{tot} << K_m \qquad \text{(XII)}$$

From equation (IX) follows

$$\beta \approx R_{tot}/K_m \qquad \text{(XIII)}$$

From equations (VI) and (XII) one finds:

$$RS_0 << S_0$$

The estimations of γ_1, γ_2 can be found from equations (XIa), (XIb), (XII) and (XIII):

$$\gamma_1 \approx -(k_3 \cdot R_{tot}/K_m) \cdot (1 - (1-\alpha) \cdot R_{tot}/K_m) \qquad \text{(XIVa)}$$

$$\gamma_2 \approx -k_1 \cdot K_m \cdot (1 + (1-\alpha) \cdot R_{tot}/K_m) \qquad \text{(XIVb)}$$

Assuming that the initial values of S and RS are equal to their equilibrium values, S_0 and RS_0, the following solutions for RS and S can be derived from equations (IIa), (IIb), (XIIIa) and (XIIIb):

$$RS \approx RS_0 \cdot (1 + \alpha \cdot R_{tot}/K_m) \cdot \exp[-(k_3 \cdot R_{tot}/K_m) \cdot (1 - (1-\alpha) \cdot (R_{tot}/K_m)) \cdot t] - RS_0 \cdot (\alpha \cdot R_{tot}/K_m) \cdot \exp[-k_1 \cdot K_m \cdot (1 + (1-\alpha) \cdot (R_{tot}/K_m)) \cdot t] \qquad \text{(XVa)}$$

$$S \approx S_0 \cdot \exp[(-k_3 \cdot R_{tot}/K_m) \cdot (1 - (1-\alpha) \cdot (R_{tot}/K_m))] \cdot t \qquad \text{(XVb)}$$

or, as a further simplification:

$$RS \approx RS_0 \cdot \exp[(-k_3 \cdot R_{tot}/K_m)] \cdot t \qquad \text{(XVIa)}$$

$$S \approx S_0 \cdot \exp[(-k_3 \cdot R_{tot}/K_m)] \cdot t \qquad \text{(XVIb)}$$

As shown by the latter expressions, the restriction (XII) implies that occupancy $RS(t)$ closely reflects $S(t)$ and that both functions can be approximated by a single exponential with a common rate constant.

The same is true if a constant rate of stimulus uptake U is applied at zero time. The rising part of the response is governed by the same rate constants:

$$RS \approx RS_0 \cdot \{1 - \exp[(-k_3 \cdot R_{tot}/K_m)] \cdot t\} \qquad \text{(XVIIa)}$$

$$S \approx S_0 \cdot \{1 - \exp[(-k_3 \cdot R_{tot}/K_m)] \cdot t\} \qquad \text{(XVIIb)}$$

The decline function in equation (XVa) differs from the exact time-course of RS by the approximation error ε. The use of an approximate value of the rate constant and neglecting the second exponential both contribute to this error. As a function of time ε $(0) = \varepsilon\,(\infty) = 0$. Its maximum value is achieved at the beginning of decline and can be estimated by subtracting equation (XVIa) from equation (XVa):

$$\varepsilon_{max}/RS_0 < [\alpha + (1-\alpha) \cdot e^{-1}] \cdot R_{tot}/K_m \qquad \text{(XVIII)}$$

This estimation shows that if $k_3 < k_2$, then the error cannot be too large even if condition (XII) is not rigidly satisfied. For example, if $k_3/k_2 = 0.3$ and $R_{tot}/K_m = 0.3$ the relative error will still be below 10%.

Case two

We consider

$$R_{tot} >> K_m \qquad \text{(XIX)}$$

From equation (IX) follows

$$\beta \approx K_m/R_{tot} \qquad \text{(XX)}$$

when the main assumption (I) is kept unchanged.

From equations (VI) and (XIX) follows

$$RS_0 >> S_0$$

If conditions (XIX) and (XX) are applied to the series approximations (XIa) and (XIb) it gives for the rate constants:

$$\gamma_1 \approx -k_3 \cdot (1 - (1-\alpha) \cdot (K_m/R_{tot})) \approx -k_3 \qquad \text{(XXIa)}$$

$$\gamma_2 \approx -k_1 \cdot R_{tot} \cdot (1 + (1-\alpha) \cdot (K_m/R_{tot})) \approx -k_1 \cdot R_{tot} \qquad \text{(XXIb)}$$

and the solution for the functions RS and S, if the same initial values S_0 and RS_0 are assumed, can be derived as:

$$RS \approx RS_0 \cdot [1 + (\alpha \cdot K_m/R_{tot})] \cdot \exp(-k_3 \cdot t) - RS_o (\alpha \cdot K_m/R_{tot}) \cdot \exp(-k_1 \cdot R_{tot} \cdot t)$$

$$\approx RS_o \cdot \exp(-k_3 \cdot t) \qquad \text{(XXIIa)}$$

$$S \approx S_0 \cdot (1-\alpha) \cdot \exp(-k_3 \cdot t) + S_0 \cdot \alpha \exp(-k_1 \cdot R_{tot} \cdot t) \qquad \text{(XXIIb)}$$

In this case the constant k_3 should dominate the decline rate of RS, and RS can be approximated again by a single exponential with the rate constant $\gamma = -k_3$.