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What is the Opposite of a Receptor Reserve?

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Abstract: There are semilogarithmic (lg-) concentration-response sigmoids with slopes exactly representing the opposite asymmetry of that encountered in the case of a receptor reserve (reserve: large curvature in the left part of the curve and narrow curvature in the right part; opposite: narrow curvature in the left part of the curve and large curvature in the right part). In addition, the binding K_ds of agonists are smaller than their EC_{50} in such curves. Using experimental concentration-response data a non-linear fitting model of a receptor "antireserve" was developed which is the topic of the present paper. This model reflects the possibility of a subthreshold preactivation of a signaling system. Hypothetically, only after exceeding this threshold a receptor-mediated response takes place.

Key words: Receptor theory, model of receptor agonism, receptor reserve, receptor antireserve, semi-logarithmic concentration-response curve, nonlinear regression analysis

INTRODUCTION

In a pharmacological system, where an agonist A induces an effect E through a receptor R, a receptor reserve is defined as $E/E_{max} > [RA]/[R_t]$, with RA being the concentration of the agonist-receptor complex inducing E and R_t the concentration of all receptors. Consequently, the fractional receptor occupation necessary to induce the half-maximal effect (EC_{50}) is lower than the dissociation constant K_d of the agonist-receptor complex (location parameter of the concentration-response curve, EC_{50} < location parameter of the concentration-binding curve, K_d). Consequently, the position of the (usually semilogarithmic) concentration-binding curve is on the right of the (lg-) concentration-response curve. Apart from $EC_{50} < K_d$ it is typical for spare receptors that the semi-logarithmic concentration-response curve of a full agonist is asymmetric in its inflection point with a large curvature in the left part and a narrow curvature in the right part of the curve. The asymmetry induces a steepening of the lg-concentration-response sigmoid. In its mid-point (≠ its inflection point) the steepness is larger than 1n10/4 = 0.675. This isthe slope of the mid-point (= inflection point) of a corresponding bimolecular binding sigmoid and of a concentration-response curve with direct proportionality between fractional receptor occupation and relative respose[1,2]. Receptor reserve in terms of a single functional unit (a cell, a nerve terminal, a subcellular structure[1,3], endowed with n receptors means that the activation of less than n receptors would suffice to elicit the full maximum response of this unit, contributing to the overall response obtained from all functional units. In other words, a receptor reserve is characterized by an imbalance between the number of receptors and the availability of signal transduction molecules: Less than 100% of the receptors suffice to activate all transduction molecules with the ensuing maximum possible response.

According to this characterization the opposite of a receptor reserve means that $E/E_{max} < [RA]/[R_t]$ and the position of the (semilogarithmic) concentration-binding curve is on the left of the (lg-) concentration-response curve. The opposite asymmetry of such a response curve were a narrow curvature in the left part and a large curvature in the right part, also inducing a steepening especially in the mid-point. Then, on the level of a single functional unit, the opposite of a receptor reserve (receptor "antireserve") is the following: One or only some few activated receptors do not suffice to elicit any response of this unit; more receptors must be simultaneously activated in order to initiate a measurable response. However, agonist occupation of only some few receptors does not mean that in this case nothing happens than a pure binding reaction. In contrast, a subthreshold preactivation of the signaling system may occur, leading hypothetically to an increase in a (non-measurable) intracellular messenger which, after exceeding the above mentioned threshold, induces the (measurable) pharmacological response.

The question of this study was: Are there real pharmacological examples of this condition? In other words, experimental examples with K_d lower than the

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agonist concentration yielding the half-maximum effect (EC_{50}), to be distinguished from the case of "positive cooperativity" where K_d is also lower than EC_{50}? In the following we give a positive answer, both as real examples and in terms of a theoretical derivation of such an antireserve system.

The case of positive cooperativity is dealt with first since it differs from receptor antireserve with respect to the so-called molecularity. The most simple case of a bimolecular reaction between agonist and receptor molecule leading to a response through this receptor is compatible with both a receptor reserve and a receptor antireserve. However, such a bimolecular reaction is not the basis of positive cooperativity where two or more agonist molecules must bind to a single receptor to activate this receptor.

To learn something about the molecularity of the agonist/receptor interaction the Hill equation is often used to describe pharmacological responses directly proportional to receptor occupancy. According to Barlow and Blake[4] it may be advantageous to replace the Hill equation by a logistic equation which is as capable of assessing molecularity. For instance, when molecularity can be assumed as a reaction of two agonist molecules with one receptor molecule (trimolecular reaction) inducing a response, the Law of Mass Action, reflecting receptor binding of two agonist molecules, may be written as:

$$A + A + R \leftrightharpoons RAA$$

or

$$\frac{R^{*}A^{*}A}{RAA} = K_d$$

with $R = R_t - RAA = R_t - RA^2$ since free receptors equal total receptors minus bound receptors. Then, the equilibrium dissociation constant can be expressed as:

$$\frac{R_tA^2 - RA^4}{RA^2} = K_d \text{ or } \frac{R_tA^2}{RA^2} = A^2 + K_d \text{ or } \frac{RA^2}{R_t} = \frac{A^2}{K_d + A^2}$$

Thus, the fraction of bound receptors RA^2 to total receptors R_t reflects the relative response if proportionality between receptor activation -in this case by two agonist molecules- and response can be assumed. In the usual semi-logarithmic form, i.e. agonist concentration $[A] = 10^{lg[A]}$, this can be written as:

$$\frac{E}{E_{max}} = \frac{10^{2\,lg[A]}}{K_d + 10^{2\,lg[A]}} \text{ or, more generally, } \frac{E}{E_{max}} = \frac{10^{c\,lg[A]}}{K_d + 10^{c\,lg[A]}} \qquad \text{function (I)}$$

The abscissa value of the point of half-maximum effect (HP), HP_{abs}, corresponds to $K_d^{1/c}$, or, as K_d is often expressed as 10^{-pKd}, $HP_{abs} = 10^{-pKd/c}$. Since function (I) is a symmetrical sigmoid (see below) HP is the inflection point IP. HP_{abs} is different from $-pK_d$, the negative logarithm of the dissociation constant. The logistic function (I) is steeper than that obtained from the Law of Mass Action reflecting receptor binding of only one agonist molecule, $A + R \leftrightharpoons RA$, which yields:

$$\frac{E}{E_{max}} = \frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \qquad \text{function (II)}$$

In this case, HP_{abs} is $-pK_d$. Both concentration-response functions, (I) and (II), are symmetrical sigmoids with the inflection point being HP. This can be easily demonstrated by analysing the second and third derivatives of functions (I) and (II). Figure 1 shows examples to (I) and (II):

Note that the position of the exemplary curve to (I) is largely on the right of that reflecting function (II), despite the fact that the K_d of the example to (I) is 10^{-9} M whereas that of the example to (II) is 10^{-6} M. The inflection point meets $-pK_d$ only in case (II). Only then, the concentration-response curve of this agonist may coincide with its binding curve (which as such is mostly bimolecular: reversible binding of a single ligand to a single binding site).

The shape of experimental concentration-response curves can be assessed by fitting a common logistic function to an observed data cloud reflecting the form of function (I) or (II). Such a descriptive function could be:

$$\frac{E}{E_{max}} = \frac{10^{lg[A]^{*}c}}{10^{-pIP^{*}c} + 10^{lg[A]^{*}c}} \qquad \text{function (III)}$$

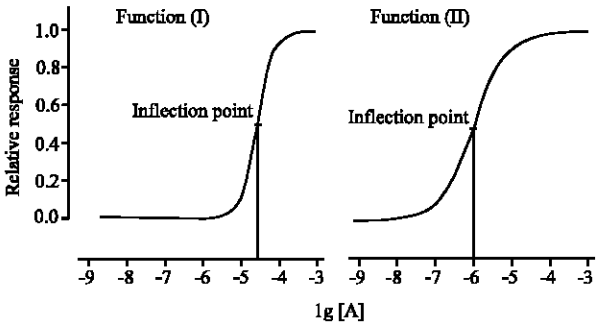

Fig. 1: Theoretical concentration-response curves, reflecting either direct proportionality between relative (bimolecular) binding and relative response [function (II)] or positive cooperativity, reflecting the binding of two agonist molecules to one receptor molecule (trimolecular), also with direct proportionality between this binding and the ensuing response [function (I)]. These curves are symmetric in their inflection point

with the independent variable being the lg-concentration of agonist A and the dependent variable being the relative response and with parameters pIP and c to be estimated, yielding information on (the negative logarithm of) the inflection point and the so-called slope parameter c of the curves (pIP = pK_d if c = 1, according to function (II); compare also the scale model of Sauermann and Feuerstein[3] and function (3) of Feuerstein and Limberger[1]. The estimate of c may give information on the molecularity of the agonist/receptor interaction[4]. For instance and according to the development of function (I), an estimate of c ~ 2 of an experimental concentration-response curve could indicate a trimolecular agonist/receptor interaction yielding a much steeper, but still symmetrical curve than a bimolecular process which is reflected by function (II).

What is the impact of c as factor of –pIP in function (III) which is absent as factor of $-pK_d$ in function (I)? Obviously, the derivatives of (I) and (III) are identical. Thus, c as factor of –pIP only influences the position of the curve on the abscissa. The meaning of c is obvious when we consider HP of (I) and (III). The relative response in function (I) is 0.5 if $-pK_d$ = lg[A] c and the relative response in function (III) is 0.5 if –pIP = lg[A]. Thus, pK_d = pIP c if function (III) describes a concentration-response relationsship with positive cooperativity.

When an experimental concentration-response curve is evaluated using function (III), for instance, with estimates pIP = 5 and c = 2, this could mean that two agonist molecules have to bind to one receptor molecule with a dissociation constant of $K_d = 10^{-5*2}$ M = 0.1 nM in order to elicit cooperatively a response. The location of this concentration-response curve would be far on the right of the binding curve (with HP = IP, reflecting K_d) of such an agonist. When binding is considered only, cooperativity does not play any role.

However, the possibility of such an interpretation does not at all mean that there is a high probability to interpret pIP of 5 and c of 2 as a pharmacological response on the basis of a trimolecular reaction. In the following, we will deal with steep experimental concentration-response curves, lying far on the right of the corresponding binding curves, which must not be interpreted as direct proportionality between receptor occupation and response on the basis of a reaction of more than one agonist molecule with a single receptor molecule.

Asymmetric concentration-response curves

First example: Evoked [^{3}H]–acetylcholine release through strychnine-sensitive glycine receptors: Recently we investigated the glycine-evoked [^{3}H]-acetylcholine ([^{3}H]-ACh) release in striatal slices of the rat which is mediated by strychnine-sensitive glycine receptors inducing chloride efflux from cholinergic interneurons[5,6]. A very steep concentration-effect curve was observed lying on the right the curve of the glycine binding curve. The experimental procedures of the release experiments are described by Darstein *et al.*[5] and can be summarized as follows:

Slices of the rat caudatoputamen were incubated with [^{3}H]-choline, superfused with physiological buffer and the release of [^{3}H]-ACh was elicited three times by addition of glycine (S_1, S_2, S_3). A concentration-response curve of the effects of glycine, quantified as S_x/S_1 ratios, x = 2, 3, was obtained when at S_1 always the same glycine concentration (100 μM) was used, whereas glycine concentrations varied at S_2 and S_3. The parameters E_{max}, pIP and c yielding the best nonlinear fit to the individual concentration-response data points were estimated according to function (III) with the dependent variable $E = S_x/S_1$ (Fig. 2).

The dashed binding curve on the left was drawn according to the literature (K_d = 2-40 μM, corresponding to an assumed pK_d of 4.7[7,8]). It is obviously flatter than the concentration-response curve on the right.

At first glance, this condition, i.e. a steep response curve with a c around 2 on the right of the binding curve, could be interpreted as trimolecular reaction, according to the consideration above: Two glycine molecules may activate one glycine receptor molecule, thereby shifting the concentration-response curve to the right of the binding curve. Similarly, Gundersen *et al.*[9] suggested that

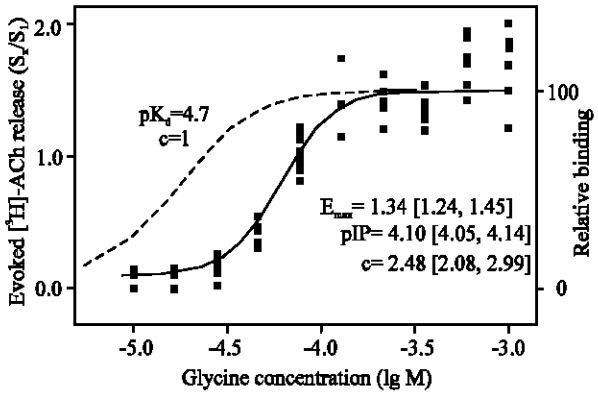

Fig. 2: Concentration-response data on glycine-evoked [^{3}H]-ACh release in slices of rat caudatoputamen. Data points were fitted with the logistic function (III) (solid line) yielding the parameter estimates indicated. For comparison, the binding curve of glycine to glycine receptor binding sites with a pK$_d$ according to the literature is shown (dashed line)

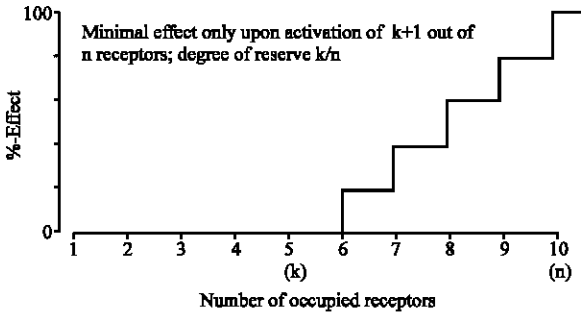

Fig. 3: Activation-effect relationship in a functional unit with a receptor antireserve. This functional unit contains ten receptors; a measurable response occurs only upon activation of more than five receptors

binding of three molecules of glycine may be required to open a single chloride channel (= effector of the glycine receptor) since – in their electrophysiological experiments on glycine receptors 'transplanted' into the Xenopus oocyte membrane – the glycine-evoked current, i.e. the response, increased with the glycine concentration following a 2.7th power relation.

However, the following refutations must not be ignored: (1) The pK$_d$ of 4.7 of the glycine binding curve in Fig. 2 does not at all match the expected pK$_d$ of 4.1* 2=8.2, according to pK$_d$ = pIP c: The difference between 8.2 and 4.7 is too large to be attributed, for instance, to different experimental assay conditions in binding and release experiments, yielding differing pK$_d$ and pIP values, respectively. (2) The fit with the symmetric function (III) of the data points in Fig. 2 seems insufficient, especially at the glycine concentrations $10^{-4.75}$ M to $10^{-4.25}$ M, where the bulk of data points lies below the regression curve and at 10^{-3} M and $10^{-2.75}$ M where the opposite is true. This suggests an asymmetric lg-concentration-response sigmoid with HP≠ IP. An asymmetric cloud of lg-concentration-response data points can indeed pretend a steep "symmetric" lg-concentration-response curve when fitted inappropriately with a purely symmetric model, like function (III)[1,3]. In addition, the use of an inappropriate model may lead to wrong conclusions about the receptor-effect coupling to be assumed. For instance, in the case of the model functions (I) and (II) direct proportionality between receptor occupancy and response is presupposed.

Thus, it was decided to apply another model which may adopt either symmetric or asymmetric properties, depending on the underlying lg-concentration-response data points to be fitted as concentration-response curve. This model was described by Sauermann and Feuerstein[3] and assumes that each cell or nerve terminal (i.e. a functional unit displaying a pharmacological response) has n receptors, that a minimal response from this functional unit occurs if k + 1 receptors are activated (k<n) and that otherwise response is proportional to receptor occupancy. Figure 3 displays schematically a functional unit of a antireserve system. The activation of the first five receptors leads to no measurable response (but may preactivate a subthreshold signaling system). A measurable effect occurs only when more than five receptors are activated.

The model is actually the opposite of a receptor reserve model[3]. n and k are fixed unknown integer parameters which can be estimated from the experimental data. In precise terms, if i receptors, k<i≤n, are occupied, the response is assumed to be proportional to (I-k)/(n-k). At a single functional unit the number i of occupied receptors should have a binomial distribution B(n, q) with parameters n and q, since there may be one among the multitude of functional units which, despite a low probability for this condition, has no receptor occupied and another one where all receptors are occupied.

With q being the fraction of occupied receptors that is in binomial terms:

$$P(\text{i receptors occupied}) = \binom{n}{i} q^i (1-q)^{n-i}$$

If the number of functional units is large, the observed relative response is close to (I-k)/(n-k) which can be written as:

$$\frac{E}{E_{max}} = \sum_{i=k+1}^{n} \frac{i-k}{n-k} \binom{n}{i} \left(\frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^n \left(1 - \frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^{n-i}$$ function (IV)

since E = 0 for the occupancy of k or less receptors:

$$0 = \sum_{i=0}^{k} \binom{k}{i} \left(\frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^n \left(1 - \frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^{n-i}$$

As mentioned above, this possibly asymmetric model has a special symmetric case if k = 0. Then,

$$\frac{E}{E_{max}} = \sum_{i=0}^{n} \frac{i}{n} \binom{n}{i} \left(\frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^n \left(1 - \frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^{n-i}$$

$$\frac{E}{E_{max}} = \frac{1}{n} \sum_{i=0}^{n} i \binom{n}{i} \left(\frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^n \left(1 - \frac{10^{lg[A]}}{K_d + 10^{lg[A]}} \right)^{n-i}$$

since:

$$\frac{E}{E_{max}} = \frac{1}{n} n \frac{10^{lg[A]}}{K_d + 10^{lg[A]}} = 1 - \frac{10^{lg[A]}}{K_d + 10^{lg[A]}},$$ compare function (II)

$$\sum_{i=0}^{n} i \binom{n}{i} q^n (1-q)^{n-i} = nq, \text{ (B(n,q):expected value)}$$

The data points of Fig. 2 were re-fitted using function (IV), yielding the following parameters to be compared with those from function (III) and with the binding parameters shown in Fig. 2 and Table 1.

Figure 4 displays the graphs of function (III) and (IV). The fit with function (IV) was better than that with function (III): The sum of squares with (IV) was markedly lower (96.9 vs 117.8).

Only the asymmetric function (IV) allowed the estimation of a pK_d value, i.e. a mechanistic interpretation of the concentration-response data. This pK_d estimate approached the pK_d of glycine in binding studies[8,9], (Table 1), being markedly higher than the pIC_{50} estimate from function (III), but also markedly lower than a pK_d of 4.1*2 = 8.2, according to pK_d = pIP c (application of function (I) according to Gundersen *et al.*[9]. The estimates of the integer parameters k and n may be interpreted as follows: A functional unit, i.e. a cholinergic interneuron of the rat striatum in the present experimental model, may be endowed with 20 glycine receptors. A pharmacological response, i.e. transmitter release from these cholinergic interneurons, only occurs when more than 12 of these 20 receptors are activated. This is of course a very rough estimation of the absolute number of receptors per functional unit. We may call the proportion of 12/20 a receptor antireserve of 60% since function (IV) represents indeed the opposite of a receptor reserve, both in terms of the derivation[3] and with respect to the shape of the asymmetric antireserve sigmoid. Note that the E_{max}

Table 1: Glycine receptors: Comparison of binding parameters and corresponding functional parameters from concentration-response curves

Binding data	Symmetric function (III)	Asymmetric function (IV)
$pK_d = 4.70$	$pIC_{50} = 4.10$ [4.05, 4.14]	$pK_d = 4.57$ [4.47, 4.67]
c = 1	c = 2.48 [2.08, 2.99]	k = 12, n = 20
$B_{max} = 100\%$	$E_{max} = 1.34$ [1.24, 1.45]	$E_{max} = 1.74$ [1.59, 1.89]

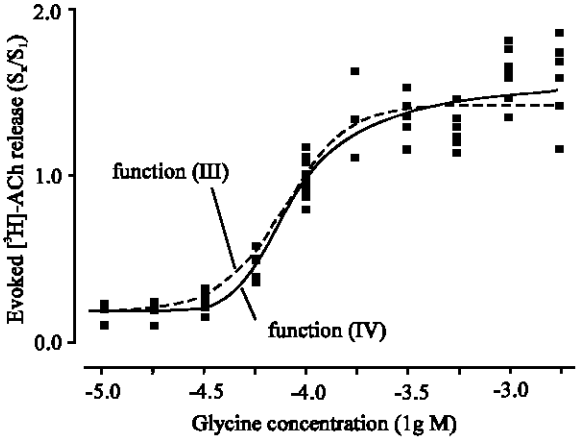

Fig. 4: Fits using function (III) (dashed line) and (IV) (solid line) to the concentration-response data on glycine-evoked [^{3}H]-ACh release in slices of rat caudatoputamen (see Fig. 2)

Table 2: 5-HT_{1A} receptors: Comparison of binding parameters and corresponding functional parameters from concentration-response curves

		Ca^{++} mobilization in HeLa cells	
Agonist	5-HT_{1A} binding to HeLa cell membranes	function (III)	function (IV)
5-HT	pK_d = 7.90 [7.68, 8.12]	pEC_{50} = 6.82 [6.75, 6.91], c = 1.43 [1.18, 1.76], E_{max} = 253 nM [238, 270]	pK_d = 7.43 [7.35, 7.52], k = 15, n = 29, E_{max} = 259 nM [245, 274]
Buspirone	pK_d = 7.11 [6.97, 7.25]	pEC_{50} = 5.99 [5.92, 6.05], c = 2.13 [1.68, 2.82], E_{max} = 160 nM [151, 170]	pK_d = 6.93 [6.86, 6.99], k = 32, n = 40, E_{max} = 171 nM [164, 180]

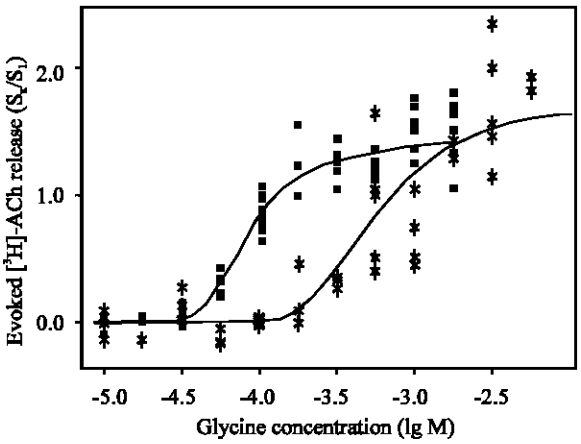

Fig. 5: Fits using function (IV) to the concentration-response data on glycine-evoked [^{3}H]-ACh release in slices of rat caudatoputamen in the absence (solid line, see also Fig. 4) and presence (dashed line) of strychnine (1 μM)

estimate using function (III) was significantly lower than that from function (IV), corresponding to the divergent lines at glycine concentrations higher than $10^{-3.25}$ M.

The presence of a competitive antagonist, like strychnine, at the glycine receptors under investigation should shift the (obviously asymmetric) concentration-response sigmoid to the right without changing the asymmetric shape of this curve, since – according to receptor theory – a pure competitive antagonist only decreases the agonist binding affinity of the receptor without an alteration of the intracellular receptor-effect coupling. Figure 5 shows the concentration-response curves of glycine on [^{3}H]-ACh release in rat striatal slices in the absence and presence of the competitive antagonist strychnine (1 μM), as fitted with function (IV).

The following parameter estimates were obtained in the presence of strychnine (those in the absence of an antagonist are included in Table 1): pK_d = 3.44 [3.30, 3.58], k = 8, n = 21, E_{max} = 1.97 [1.67, 2.27]. The integers k and n were not markedly dissimilar to those shown in Table 1, but their ratio only yielded a receptor antireserve of 38%. The potency (pA_2 value) of strychnine was calculated to 7.10 [6.70, 7.46] from the pK_d values obtained in its absence and presence according to:

$$pA_2 = \lg(10^{pK_{dAnt} - pK_d} - 1) - \lg[Ant]$$

with pK_{dAnt} being the pK_d in the presence of strychnine.

This pA_2 value is in agreement with that obtained earlier (6.86 [6.61, 7.08],[5]).

In the presence of another competitive antagonist, i.e. ethanol (68 mM=4 ‰), at the receptor under investigation, the fitting procedure of the right-shifted data cloud was unstable when the parameters k and n were to estimate together with pK_d and E_{max}, most probably due to an insufficient number of data points. Thus, k and n were fixed to 8 and 21 (according to the curve in the presence of strychnine). Then, re-fitting yielded the parameters pK_d = 4.01 [3.89, 4.13] and E_{max} = 1.76 [1.62, 1.89]. The corresponding pA_2 value was 1.10 [0.90, 1.26], again similar to the pA_2s of ethanol obtained earlier[5].

Second example: Intracellular Ca^{++} mobilization through recombinant human 5-HT_{1A} receptors: Boddeke *et al.*[10] obtained concentration-response data of 5-HT and of the partial agonist buspirone on intracellular Ca^{++} mobilization in HeLa cells mediated through 5-HT_{1A} receptors. The HeLa cells expressed 3000 fmol 5-HT_{1A} receptor protein/mg membrane. pEC_{50} values, the location parameters of symmetric concentration-response curves according to function (III) of 5-HT and buspirone, were compared with corresponding pK_d values determined in radioligand binding experiments (Table 2). pEC_{50} values were in all cases lower than binding pK_d values which is the opposite of the condition of spare receptors. In addition, for both agonists the slope parameter c was clearly and significantly higher than unity precluding the interpretation of direct proportionality between receptor occupation and mediated effect, i.e. interpretation of the concentration-response relationship according to function (II). However, the interpretation as a trimolecular reaction between two agonist molecules and one receptor molecule according to function (I) was also impossible since then a functional pK_d (pEC_{50} * c) of 6.82 * 1.43 = 9.75 for 5-HT would hardly fit the binding pK_d of 7.90 and the

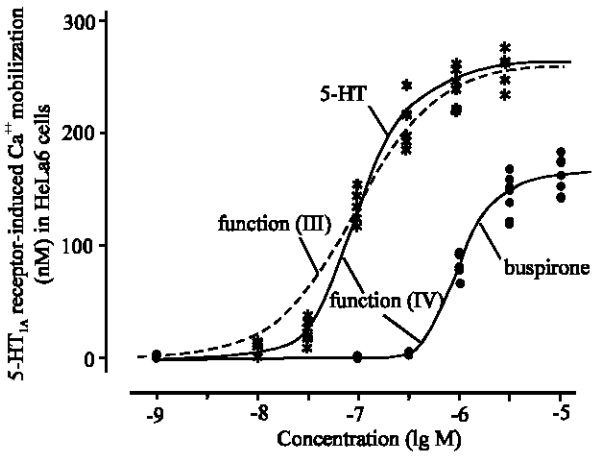

Fig. 6: Fits using function (III) (dashed line) and (IV) (solid lines) to the concentration-response data on 5-HT and buspirone-induced intracellular Ca^{++} mobilization in HeLa cells mediated through 5-HT_{1A} receptors

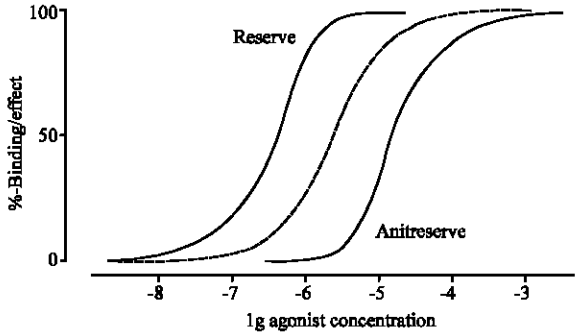

Fig. 7: Theoretical symmetric binding curve (dashed line) and corresponding asymmetric concentration-response curve in the case of spare receptors (left curve) or in the case of a receptor antireserve (right curve)

functional pK_d of 5.99 * 2.13 = 12.76 for buspirone were incommensurate with its binding pK_d of 7.11. Fitting of the concentration-response data of Ca^{++} mobilization with function (IV), however, yielded pK_d estimates close or similar to the pK_ds found in binding experiments (Table 2).

In addition, these concentration-response data were poorly fitted by a symmetric model function, like function (III) (Fig. 6). Again, as in the case of the glycine receptor-mediated [^{3}H]-ACh release mentioned above, the receptor antireserve model (function (IV)) yielded better fits for both 5-HT and buspirone data (Fig. 6).

Thus, the asymmetric shape of the concentration-response curves (steep onset at low concentrations followed by a more shallow progression) was better reflected by fitting with the antireserve model than with the logistic function.

The rough estimation of the absolute number of receptors per functional unit, i.e. per HeLa cell in the present case, yielded a receptor antireserve of 15/29 for 5-HT, corresponding to 52% and a proportion of 32/40 for the partial agonist buspirone, i.e. an antireserve of 80%. Although we cannot conclude from these ratios of the integer estimates k and n that the antireserve of 52% for 5-HT was significantly smaller than that of 80% for buspirone, the difference between 52 and 80% may point to another contradistinction between receptor reserve and receptor antireserve: Whereas the condition of a receptor reserve becomes less pronounced for a partial agonist[1], that of a receptor antireserve may become accentuated if a partial agonist is used.

In conclusion, the descriptive fit with a logistic function like (III) of concentration-response data yields an estimate of the slope factor c which, pursuant to its deviation from unity, may not be compatible with the interpretation of direct proportionality between receptor occupation and effect. When corresponding binding studies of the agonist used in the functional experiments yield a pK_d value higher than the agonist pEC_{50}, this condition may be interpreted as hint to a higher molecularity in the following case: If $pEC_{50} * c \approx pK_d$, i.e. if the negative logarithm of the location parameter of the curve times its slope factor approaches the negative logarithm of the dissociation constant, then a molecularity of about c + 1 may be assumed. For instance, an estimate of $c \sim 2$ of an experimental concentration-response curve could indicate a trimolecular agonist/receptor interaction, i.e. two agonist molecules interact with one receptor molecule. If, however, $pEC_{50} * c \neq pK_d$, or if the asymmetry of the concentration-response curve complies with the opposite of the asymmetry typical for spare receptor, the existence of a receptor antireserve condition should be considered.

The new antireserve model is characterized by the following combination: The (lg-) concentration-binding curve lies on the left of the concentration response curve ($pK_d > pEC_{50}$) and the concentration-response curve is asymmetric in its inflection point. This asymmetry is contrary to that seen in the case of a receptor reserve and, correspondingly, in the latter case the concentration-binding curve lies on the right of the concentration response curve ($pK_d < pEC_{50}$). Response curves based on positive cooperativity or binding curves, however, are typically symmetric in their inflection point. Mechanistically, positive cooperativity means that more

than one agonist molecules may bind to one receptor in order to elicit a response, the phenomenon of a receptor reserve is most probably due to a superior number of receptors as compared to signal transduction molecules and the need to preactivate a signaling system to exceed an intracellular threshold which then allows the measurable response may be the basis of a receptor antireserve. Receptor reserve and receptor antireserve are schematically opposed in Fig. 7.

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