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New Concept of Spare Receptors and Effectors

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Abstract. The present study provides a new concept of the spare receptor. Model [A]: 1) Several receptors connect with an effector; 2) if an agonist occupies one of the receptors connecting with one effector, the effector fully functions. When the number of receptors connecting with one effector is "m", the relationship between the functional effectors (E) and the concentration of agonists ([a]) is as follows:

$$E = \frac{R_t}{m}\left(1 - \left(1 - \frac{1}{\frac{1+K_d}{[a]}}\right)^m\right) \quad \text{(I)}$$

where R_t is the total number of receptors and K_d is the agonist dissociation constant from the receptor. Model [B]: 1) Several receptors connect with an effector; 2) only when agonists occupy all of the receptors connecting with one effector, the effector functions. The relationship between E and [a] is as follows:

$$E = \frac{R_t}{m}\left(1 - \left(1 - \frac{1}{1 + \frac{K_d}{[a]}}\right)^m\right) \quad \text{(II)}$$

If $m = 1$, equations (I) and (II) are exactly the same as the Michaelis-Menten equation. If m is larger than 1, the apparent saturation in the effector efficiency becomes larger in Model [A], and smaller in Model [B], respectively. The dissociation of the fractional efficiency of effectors from the fractional binding of agonists to receptors becomes larger as m becomes larger in both models. Further, we propose a variable model, including the concept of agonist-occupancy-dependent stability in the functional conformation change of the effector; only when more than j pieces of receptors connecting with one effector are occupied by agonists, the effector functions (Model [M]). The relationship between E and [a] is as follows:

$$E_{M>J} = \frac{R_t}{m}\left(1 - \sum_{i=0}^{j}\left(\frac{m!}{(m-j)!j!}\left(1 - \frac{1}{\frac{1+K_d}{[a]}}\right)^{(m-j)}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^j\right)\right) \quad \text{(III)}$$

Key words: Receptor — Effector — Spare — Model

Introduction

One effector, in general, connects with one receptor. When an agonist occupies one receptor, the agonist-occupied effector expresses its full function. This means that overall efficiency of effectors has a linear relation to the number of agonists binding to receptors. The Michaelis-Menten equation can express the number (saturation) of agonists binding to receptors as a function of the concentration of agonists. Using the same K_M as in the binding relation of agonists to receptors, the Michaelis-Menten equation can express the efficiency (saturation) of effectors as a function of the concentration of agonists.

However, in some cases, the relationship between the efficiency of effectors and the number of agonists binding to receptors is not linear. For example, human platelet aggregation has a non-linear relation between the number of agonists binding to receptors and the efficiency of effectors (Dorn & DeJesus, 1991). An agonist of the human platelet thromboxane A_2-prostaglandin H_2 (TxA_2/PGH_2) receptor, BOP, binding to TxA_2/PGH_2 receptors causes human platelet aggregation. The relationship

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between the number of BOP binding to TxA_2/PGH_2 receptors and aggregation of human platelets is not linear, but hyperbolic with half-maximal aggregation occurring at 25% of the receptors occupied by agonists. This suggests that there is some system other than just one effector connecting with one receptor. In the present study, we propose models of spare receptors, providing an explanation of a non-simple (non one-to-one) coupling of receptor and effector.

The model of spare receptors shown in the present study provides a completely new aspect of spare receptors, and explains various phenomena reported in previous studies that cannot be understood by conventional spare-receptor models. Thus, we have provided here for the first time a breakthrough of the understanding and modelling of spare receptors based upon a completely new, noble concept.

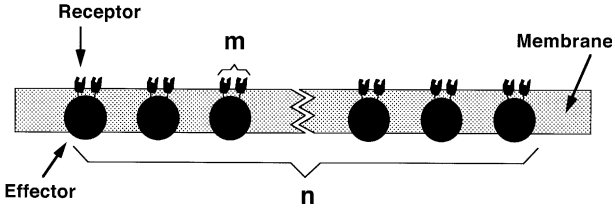

Fig. 1. A model of spare receptors and their effectors. In this case, the number "*m*" of receptors connecting to one effector is 2. The total number of effectors per cell or tissue is *n*. The following three assumptions are used in Model [A]: 1) several receptors connect with an effector; 2) if one receptor connecting with one effector is occupied by an agonist, the effector works fully; 3) even if more than one receptor connecting with the same effector is occupied by agonists, the efficiency of the effector does not change and is the same as that when only one of the receptors connecting with the effector is occupied by an agonist. On the other hand, in Model [B], we assume based on: 1) *k* pieces of agonists bind to receptors; *2*) only when all receptors connecting with one effector are occupied by agonists, the effector functions; 3) the effector has no function even when some parts (not all) of the receptors connected with the same effector are occupied by agonists.

Calculations

Figure 1 describes a model of the relationship between receptors and effectors; i.e., *m* pieces of receptors connect with one effector and the number of the receptor-effector complexes is *n* per one experimental sample (cell or tissue). In Fig. 1, the value of *m* is 2.

We first propose two different models; Model [A] and Model [B]. Further, we propose another model explaining more general cases. Table 1 shows the definition of characters that are used in the present study. Calculation was performed by Jandel Sigma Plot (Aspire Software International, Leesburg, VA, USA).

Model [A]

We simulate a model based on the following assumptions: 1) *k* molecules of agonists bind to receptors; 2) when at least one of the receptors connecting with one effector is occupied by an agonist, the effector fully functions; 3) even though agonists occupy more than one receptor connecting with the same effector, the efficiency of the effector's function is the same as that when one agonist binds to only one of the receptors associated with the effector. The probability (P_A) that at least one of the receptors associated with an effector is occupied by agonist(s) is expressed as follows:

$$P_A = 1 - \frac{{}_{mn-m}C_k}{{}_{mn}C_k} \tag{1}$$

where *C* represents the combination: *mn* is the total number of receptors, while *mn*−*m* (i.e., *m*(*n*-1)) is the total number of receptors (*mn*) minus the number of receptors connecting to one effector (m).${}_{mn}C_k$represents the number of the combination that *k* pieces of things are chosen from *mn* pieces of things. Namely, ${}_{mn}C_k$ is as follows:

$${}_{mn}C_k = \frac{(mn)!}{(mn-k)!k!} \tag{2}$$

${}_{mn-m}C_k/{}_{mn}C_k$is the probability that all *k* pieces of agonists bind to receptors except *m* receptors connecting with one effector. In other words, ${}_{mn-m}C_k/{}_{mn}C_k$ is the probability that there is at least one effector whose receptors are not bound by agonists. P_A can be calculated by subtracting the probability, ${}_{mn-m}C_k/{}_{mn}C_k$ from 1. So, P_A is represented as follows.

$$\begin{aligned}
P_A &= 1 - \frac{{}_{mn-m}C_k}{{}_{mn}C_k} \\
&= 1 - \frac{\dfrac{(mn-m)!}{(mn-m-k)!k!}}{\dfrac{(mn)!}{(mn-k)!k!}} \\
&= 1 - \frac{(mn-m)!(mn-k)!}{(mn)!(mn-m-k)!} \\
&= 1 - \frac{(mn-k)(mn-k-1)\cdots(mn-k-(m-1))}{mn(mn-1)\cdots(mn-(m-1))} \\
&= 1 - (1-\frac{k}{mn})(1-\frac{k}{mn-1})\cdots(1-\frac{k}{mn-(m-1)})
\end{aligned} \tag{3}$$

Here, the following equation represents the relationship between the concentration of agonists and the number (concentration) of agonists binding to receptors:

Table 1. Definition of characters

Character	Definition
[*a*]	Concentration of agonists
mn *C* k	Number of combinations to choose *k* pieces from *mn* pieces: i.e, *mn* C *k* = *mn* ! / (*mn*-*k*) ! *k* !)
E_A	Expected number of effectors with at least one receptor occupied by agonist in Model [A]
E_B	Expected number of effectors with at least one receptor occupied by agonists in Model [B]
E_{Mj}	Expected number ot effectors with just *j* receptors occupied by agonists in Model [M]
$E_{M>j}$	Expected number of effectors with more than *j* receptors occupied by agonists in Model [M]
F_{RA}	Fractional occupation of receptors by agonists in Model [A]
F_{RB}	Fractional occupation of receptors by agonists in Model [B]
F_{EA}	Fractional efficiency of effectors in Model [A]
F_{EB}	Fractional efficiency of effectors in Model [B]
$F_{EM>j}$	Fractional efficiency of effectors in a case where the effector fully functions when more than *j* pieces of receptors connected to the same effector are occupied by agonists in Model [M]
k	Number of agonists bound to receptors
kd	Agonist dissociation constant from receptor
m	Number of receptors connected to effector
n	Number of effectors in an experimental sample (cell or tissue)
P_A	Probability that at least one agonist binds to receptors associated with one effector in Model [A]
P_B	Probability that at least one agonist binds to receptors associated with one effector in Model [B]
P_{Mj}	Probability that just *j* pieces of receptors connected to one effector are occupied by an agonist
$P_{M>j}$	Probability that more than *j* pieces of receptors connected to one effector are occupied by an agonist in Model [M]
R_t	Total number of receptors in an experimental sample (cell or tissue)

$$k = \frac{mn}{1 + \frac{K_d}{[a]}} \tag{4}$$

where k is the number of agonists binding to the receptor, m is the total number of receptors connecting with one effector, n is the total number of effectors, K_d is the dissociation constant of agonist from the receptor, and $[a]$ is the concentration of agonist. From Eqs. 3 and 4, the following equation is induced.

$$\begin{aligned} P_A &= 1 - (1 - \tfrac{1}{1+\frac{K_d}{[a]}})(1 - \tfrac{mn}{mn-1}\tfrac{1}{1+\frac{K_d}{[a]}}) \cdots (1 - \tfrac{mn}{mn-(m-1)}\tfrac{1}{1+\frac{K_d}{[a]}}) \\ &= 1 - (1 - \tfrac{1}{1+\frac{K_d}{[a]}})(1 - \tfrac{1}{1-\frac{1}{mn}}\tfrac{1}{1+\frac{K_d}{[a]}}) \cdots (1 - \tfrac{1}{1-\frac{m-1}{mn}}\tfrac{1}{1+\frac{K_d}{[a]}}) \end{aligned} \tag{5}$$

Here, the value of n is much larger than 1 and the value of m is much smaller than that of mn; i.e., 1/mn,⋯, are (m-1)/mn nearly equal to 0. So, $P_{A'}$ defined below is nearly equal to P_A.

$$P_{A'} = 1 - (1 - \frac{1}{1 + \frac{K_d}{[a]}})^m \tag{6}$$

The following equation expresses the expected number (E_A) of effectors with at least one of their receptors occupied by agonists:

$$E_A = n\,P_A \tag{7}$$

Therefore, the following equation can be useful:

$$E_A = n\,P_{A'} \tag{8}$$

From equations (6) and (8), the following equation is induced:

$$E_A = n\,(1 - (1 - \frac{1}{1 + \frac{K_d}{[a]}})^m) \tag{9}$$

When the total number of receptors is expressed as R_t, the relation between n and m is as follows:

$$n = \frac{R_t}{m} \tag{10}$$

The following equation is induced from Eqs. 9 and 10:

$$E_A = \frac{R_t}{m}(1 - (1 - \frac{1}{1 + \frac{K_d}{[a]}})^m) \tag{11}$$

If m is one, Eq.11 is exactly the same as the Michaelis-Menten equation.

Further, we calculated the relationship between the fractional occupation of receptors by agonists (F_{R_A}) and the fractional efficiency of effectors(F_{E_A}). (F_{R_A}) and (F_{E_A}) are, respectively, expressed as follows:

$$F_{R_A} = \frac{1}{1 + \frac{K_d}{[a]}} \tag{12}$$

follows:

$$F_{E_A} = 1 - (1 - \frac{1}{1 + \frac{K_d}{[a]}})^m \tag{13}$$

where Eqs. 12 and 13 are induced from Eqs. 4 and 11, respectively. The relationship between F_{R_A} and

F_{E_A} obtained from Eqs. 12 and 13 is expressed as follows:

$$F_{E_A} = 1 - (1 - F_{R_A})^m \tag{14}$$

Model [B]

Further, we should consider another model, which can explain another case in which all receptors connecting with one effector are required to be occupied by agonists for the effector to be functional. We simulate a model based on the following assumptions: 1) k pieces of agonists bind to receptors; 2) only when all receptors connecting with one effector are occupied by agonists, the effector functions. The agonists (k–m) that do not bind to m receptors connecting with one effector bind to other receptors (mn–m) connecting with the effector, all receptors (m) of which are occupied by m agonists. The probability (P_B) that all receptors connecting with one effector are occupied by agonists is as follows:

$$\begin{aligned} P_B &= \frac{{}_{mn-m}C_{k-m}}{{}_{mn}C_k} \\ &= \frac{\frac{(mn-m)!}{(mn-m-(k-m))!(k-m)!}}{\frac{mn!}{(mn-m)!k!}} \\ &= \frac{(mn-m)!k!}{mn!(k-m)!} \\ &= \frac{k}{mn}\frac{k-1}{mn-1}\cdots\frac{k-(m-1)}{mn-(m-1)} \\ &= \left(\frac{k}{mn}\right)^m\left(\frac{1-\frac{1}{k}}{1-\frac{1}{mn}}\right)\left(\frac{1-\frac{2}{k}}{1-\frac{2}{mn}}\right)\cdots\left(\frac{1-\frac{m-1}{k}}{1-\frac{(m-1)}{mn}}\right) \end{aligned} \tag{15}$$

Equation (15) can be expressed as follows, since mn is much larger than m (in other words, n is much larger than 1):

$$P_B = \left(\frac{k}{mn}\right)^m\left(1-\frac{1}{k}\right)\left(1-\frac{2}{k}\right).....\left(1-\frac{m-1}{k}\right) \tag{16}$$

So, using Eq. 4, P_B can be expressed as follows:

$$P_B = \left(\frac{1}{1+\frac{K_d}{[a]}}\right)^m\left(1-\frac{1}{k}\right)\left(1-\frac{2}{k}\right).....\left(1-\frac{m-1}{k}\right) \tag{17}$$

Here, k is generally much larger than m except when $[a]$ is very small. Therefore, $P_{B'}$ defined below is nearly equal to P_B.

$$P_{B'} = \left(\frac{1}{1+\frac{K_d}{[a]}}\right)^m \tag{18}$$

The expected number (E_B) of effectors, all receptors of which are occupied by agonists, is as follows:

$$E_B = nP_B \tag{19}$$

Therefore, the following equation can be useful.

$$E_B = nP_{B'} \tag{20}$$

From Eqs. 18 and 20, the following equation is induced:

$$E_B = n\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^m \tag{21}$$

The following equation is induced from Eqs. 10 and 21.

$$E_B = \frac{R_t}{m}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^m \tag{22}$$

If m equals one, Eq. 22 is exactly the same as the Michaelis-Menten equation. Further, we calculated the relationship between F_{R_B} and F_{E_B}, F_{R_B} and F_{E_B} are, respectively, expressed as follows:

$$F_{R_B} = \frac{1}{1+\frac{K_d}{[a]}} \tag{23}$$

$$F_{E_B} = \left(\frac{1}{1+\frac{K_d}{[a]}}\right)^m \tag{24}$$

Equation 23 is induced from Eq. 4, since the binding process of agonists to receptors is identical in the case of Model [B] to that in the case of Model [A]. Equation 24 is induced from Eq. 22. From the Eqs. 23 and 24, the following equation is induced:

$$F_{E_B} = F_{R_B}^m \tag{25}$$

Results

Figure 2 shows the simulation curves of the relationship between the concentration of agonist and the fractional value of the effectors' efficiency; i.e., fractional E (F_{E_A} in Model [A] and F_{E_B} in Model [B]). The lines are drawn by Eq. 13 in Model [A] or Eq. 24 in Model [B], assuming R_t/m is constant ($R_t/m = n$; the total number of effectors). In Model [A], the fractional E (F_{E_A}) at a larger value of m shows higher saturation than that at a smaller value of m at the same concentration of agonists (*see* lines marked 2*A* and 3*A* in Fig. 2*A* and 2*B*). On the other hand, in Model [B] the fractional E (F_{E_B}) at a larger value of m shows lower saturation than that at a smaller value of m at the same concentration of agonists (*see* lines marked by *2B* and *3B* in Fig. 2*A* and 2*B*). Figure 3 shows the relationship between the value of m and the agonist concentration at half saturation of effectors' efficiency (EC_{50}; ref. $F_{E_A} = 0.5$ in Model [A] or $F_{E_B} = 0.5$ in Model [B] shown in Fig. 2*A* and 2*B*). In Model [A], EC_{50} becomes small as m increases (*squares* in Fig. 3). On

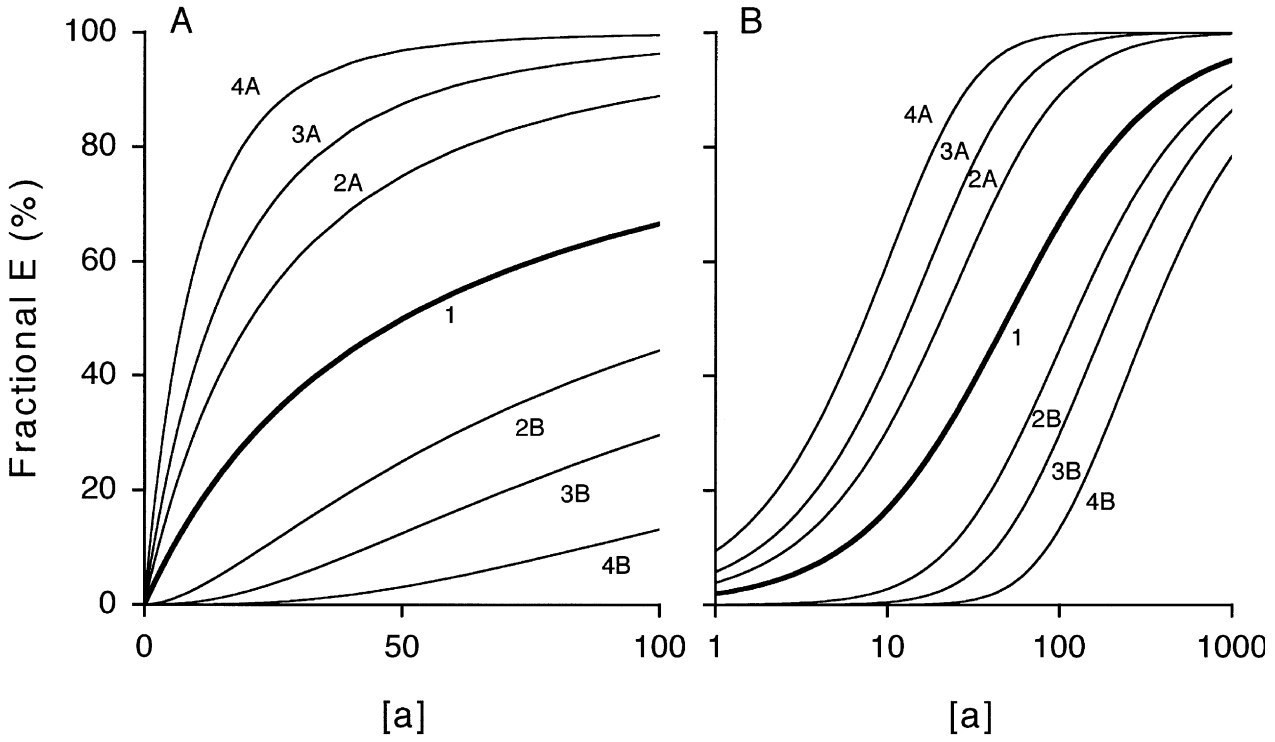

Fig. 2. The simulation of the relationship between the concentration of agonist and the relative value of effectors' efficiency: the concentration of agonist is represented in a linear (A) or logarithmical scale (*B*). In Model [A], the lines marked *1*, *2A* and *3A* were drawn by Eq. 14 which assumes that R_t ($R_t = mn$; total number of receptors) is constant; i.e., efficiency of effectors increases as the value of m increases. In Model [B], the lines marked *1*, *2B* and *3B* are drawn by Eq. 25, which assumes that R_t (R_t, $= mn$; total number of receptors) is constant; i.e., efficiency of effectors decreases as the value of m increases. The thick solid line marked with *1* indicates a model of one receptor connected to one effector. The line marked *2A* indicates a model of two receptors connected to one effector in Model [A]. The line marked *3A* indicates a model of three receptors connected to one effector in Model [A]. The line marked 2B indicates a model of two receptors connected to one effector in Model [B]. The line marked *3B* indicates a model of three receptors connected to one effector in Model [B].

the other hand, in Model [B], EC_{50} becomes large as m increases (*circles* in Fig. 3). Figure 4 shows the fractional binding of agonists to the receptors (*Fractional* B; F_{R_A} in Model [A] and F_{R_B} in Model [B]) and the fractional E (F_{E_A} in Model [A] and F_{E_B} in Model [B]). Figure 4 is obtained from Eq. 14 Model [A] and Eq. 25 in Model [B]. In Model [A], the fractional E (F_{E_A}; Eq. 13) is much higher than the fractional binding of agonists to the receptors (F_{R_A}; Eq. 12). On the other hand, in Model [B], the fractional E (F_{E_B}; Eq. 24) is much lower than the fractional binding of agonists to the receptors (F_{R_B}; Eq. 23). If m equals 1, the relationship is linear. In Model [A], the half maximal efficiency of effectors occurs at only 30 % of receptors occupied by agonists at $m = 2$. In the Model [B], the half maximal efficiency of effectors occurs at 70 % of receptors occupied by agonists at $m = 2$.

Discussion

The concept of spare receptors has been proposed by many investigators based upon experimental results. For example, Dorn and DeJesus (1991) have reported that the occupation of human platelet thromboxane A_2–prostaglandin H_2 (TxA_2/PGH_2) receptor by agonists causes platelet aggregation, and that an agonist TxA_2/PGH_2, BOP, binding to TxA_2/PGH_2 receptors causes human platelet aggregation. The relationship between the number of BOP binding to TxA_2/PGH_2 receptors and aggregation of human platelets is not linear, but hyperbolic with half-maximal aggregation occurring at 25% of receptors occupied by agonists (Dorn & DeJesus, 1991). These observations suggest some system other than one effector connecting with one receptor. As indicated in the report (Dorn & DeJesus, 1991), a non-linear system is proposed as a concept of spare receptor. In general, the concept of spare receptors is that some receptors do not connect with effectors. In other words, an agonist binding to a receptor not connecting with any effector does not show its action through the effector (McNeil & Evavold, 2002; Waelbroeck, 2001). This relationship shows a non-linear system between the agonist binding to the receptor and its action via effectors. A report (Bruheim et al., 2003) proposes a

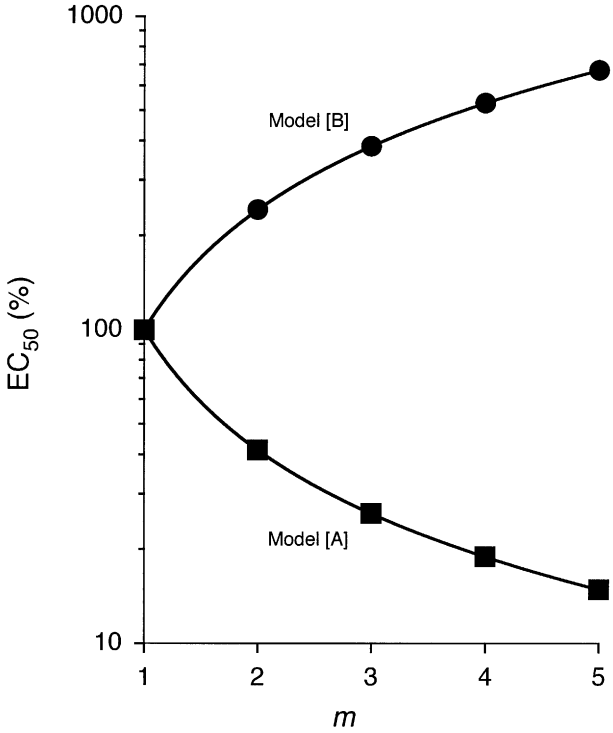

Fig. 3. The relationship between the value of m and the agonist concentration at half-maximal effector efficiency (EC_{50}). EC_{50} is represented as a normalized value since the value of EC_{50} is 100 % at $m = 1$. *Squares* and *circles*, respectively, represent the cases of Model [A] and Model [B]. The agonist concentration at the half maximal effector efficiency becomes smaller as the value of m increases in the case of Model [A]. On the other hand, the agonist concentration at half maximal effector efficiency becomes larger as the value of m increases in the case of Model [B].

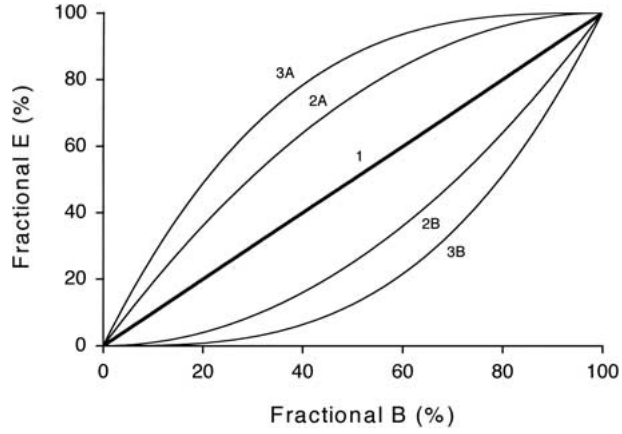

Fig. 4. The relationship between fractional occupation of receptors by agonists (*Fractional B*: F_{R_A} in Model [A] and F_{R_B} in Model [B]) and fractional efficiency of effectors (*Fractional E*: F_{E_A} in Model [A] and F_{E_B} in Model [B]). The lines in Models [A] and [B] were respectively, obtained by Eqs. 14 and 25. An increase in m value dissociates the relationship between F_{R_A} and F_{E_A} or F_{R_B} and F_{E_B} from a linear relationship.

model of spare receptors depending on the functional state of receptor coupling to the effector. Further, many studies from various laboratories (Erdmann et al., 1990; Nandagopal et al., 2001; Burns et al., 2002; Patacchini et al., 2002; Porter et al., 2002; Mansfield et al., 2003;) have reported results indicating the presence of spare receptors. However, the concept of spare receptors proposed in the previous studies (Patacchini et al., 2002; Porter et al., 2002; Mansfield et al., 2003) is very simple. Namely, the studies report the presence of some receptors that do not connect with any effectors, defining this type of receptor as a spare receptor. In this case, the effector efficiency is lower compared with the receptor occupation by agonist, since some receptors have no function in conducting their signaling to the effector even if the receptors are occupied by agonist. On the other hand, some studies report that the effector efficiency is much larger than the receptor occupation by agonist. For example, a study (Burris et al., 2002) reports that aripiprazole is an agonist for dopamine D_2, and that when 23 % of D_2 receptors are occupied by aripiprazole, an efficiency of 50% is observed. A similar relationship between the receptor occupation and the effector efficiency has been observed in another cell (Dorn & DeJesus, 1991). These phenomena are not explained by the type of proposal of spare receptors mentioned above; i.e., the presence of some receptors that do not connect with any effectors cannot explain those phenomena. On the other hand, the new model of spare receptor proposed in the present study (Model [A]) provides us with the relationship between fractional receptor occupation and fractional effector efficiency (Fig. 4). In Model [A], when two receptors connect with one effector ($m = 2$), agonist-occupation of 30 % receptors leads to 50 % effector efficiency (the line marked *2A* in Fig. 4). Similarly, in Model [A], when three receptors connect with one effector ($m = 3$), agonist occupation of 20 % of receptors by agonists leads to 50 % effector efficiency (the line marked *3A* in Fig. 4). These phenomena can explain the observations reported in the studies mentioned above (Dorn & DeJesus et al., 1991; Burris et al., 2002).

Burris et al. (2002) indicate that the relationship between receptor occupancy and effector efficiency is variable, depending on the kind of receptor agonist. For example, in a dopamine receptor system, dopamine presents 50 % effector efficiency at agonist-occupation of 2 % of receptors, while terguride shows 50 % effector efficiency at agonist-occupation of 13 % of receptors. In the same system, aripiprazole shows 50 % effector efficiency at agonist-occupation of 23 % of receptors. That is, in the same receptor system, the relationship between receptor occupancy and effector efficiency is changeable. In the present study, we indicate that the relationship between receptor

occupancy and effector efficiency is changeable with the number of receptors connected to one effector, or with transduction efficiency of receptor occupation vs. effector function. As a model for the latter case, we propose two different models, Model [A] and Model [B], in the present study. In Model [A] and Model [B], we can explain only two different states, but cannot explain variable states such as the dopamine system mentioned above (Burris et al., 2002). To clarify the problem in our models, we propose a modified model based upon Model [A] and Model [B]. We apply an assumption that: 1) in Model [A], when at least one of the receptors connected to one effector is occupied by an agonist, the effector functions, and 2) in Model [B], only when all receptors connecting with one effector are occupied by agonists, the effector functions. A modified model (Model [M]) is as follow. Only when more than j pieces of receptors connected to one effector are occupied by agonists, the effector functions. To calculate the probability ($P_{M>j}$) of Model [M], we first calculate the probability (P_{Mj}) that just j pieces of receptors connected to one effector are occupied by agonists.

Based on the fact that n is much larger than 1, m, k or j and using Eq. 4, Eq. 26 can be expressed as follows:

$$P_{Mj} = \frac{m!}{(m-j)!j!}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^{(m-j)}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^{j} \tag{27}$$

The expected number (E_{Mj}) of effectors, just j pieces of receptors of which are occupied by agonist, is as follows:

$$E_{Mj} = n\frac{m!}{(m-j)!j!}\left(1-\frac{1}{1+\frac{K_d}{[a]}}\right)^{(m-j)}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^{j} \tag{28}$$

The following equation is induced from Eqs. 10 and

$$E_{Mj} = \frac{R_t}{m}\frac{m!}{(m-j)!j!}\left(1-\frac{1}{1+\frac{K_d}{[a]}}\right)^{(m-j)}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^{j} \tag{29}$$

Therefore, we calculated the expected number ($E_{M>j}$) of effectors, when more than j pieces of receptors connecting with one effector are occupied by agonists:

$$E_{M>j} = \frac{R_t}{m}\left(1-\sum_{i=0}^{j}\left(\frac{m!}{(m-j)!j!}\left(1-\frac{1}{1+\frac{K_d}{[a]}}\right)^{(m-j)}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^{j}\right)\right) \tag{30}$$

where 0! is defined as 1; namely, $j! = 1$ at $j = 0$ and $(m\text{-}j)! = 1$ at $m = j$. Further, we calculated the relationship between $F_{R_{M>j}}$ and $F_{E_{M>j}}$. $F_{R_{M>j}}$ and $F_{E_{M>j}}$ are, respectively, expressed as follows:

$$FR_{M>j} = \frac{1}{1+\frac{K_d}{[a]}} \tag{31}$$

$$F_{E_{M>j}} = 1-\sum_{i=0}^{j}\left(\frac{m!}{(m-j)!j!}\left(1-\frac{1}{1+\frac{K_d}{[a]}}\right)^{(m-j)}\left(\frac{1}{1+\frac{K_d}{[a]}}\right)^{j}\right) \tag{32}$$

where 0 is defined as 1; namely, $j! = 1$ at $j = 0$ and $(m\text{-}j)! = 1$ at $m = j$. From Eqs. 31 and 32, the relationship between $F_{R_{M>j}}$ and $F_{E_{M>j}}$ is as follows:

$$F_{E_{M>j}} = 1-\sum_{i=0}^{j}\left(\frac{m!}{(m-j)!j!}\left(1-F_{R_{M>j}}\right)^{(m-j)}\left(F_{R_{M>j}}\right)^{j}\right) \tag{33}$$

$$\begin{aligned}
P_{Mj} &= \frac{{}_{m}C_{jmn-m}\,{}_{C_{k-j}}}{{}_{mn}C_{k}} \\
&= \frac{\frac{m!}{(m-j)!j!}(mn-m)!}{\frac{(mn-m-(k-j))!(k-j)!}{mn!}{(mn-m)!k!}} \\
&= \frac{m!}{(m-j)!j!}\frac{(mn-m)!}{mn!}\frac{(mn-k)!}{(mn-m-(k-j))!}\frac{k!}{(k-j)!} \\
&= \frac{m}{(m-j)!j!}\frac{(mn-m)!}{mn!}\frac{(mn-k)!}{(mn-k-(m-j))!}\frac{k!}{(k-j)!} \\
&= \frac{m!}{(m-j)!j!}\frac{((mn-k)(mn-k-1)\cdots(mn-k-(m-j-1)))\ (k(k-1)\cdots(k-(j-1)))}{mn(mn-1)\cdots(mn-(m-1))} \\
&= \frac{m!}{(m-j)!j!}\frac{((1-\frac{k}{mn})(1-\frac{k}{mn})-\frac{1}{mn})\cdots(1-\frac{k}{mn}-\frac{m-j-1}{mn}))(\frac{k}{mn}(\frac{k}{mn}-\frac{1}{mn})\cdots(\frac{k}{mn}-\frac{j-1}{mn}))}{1(1-\frac{1}{mn})\cdots(1-\frac{m-1}{mn})}
\end{aligned} \tag{26}$$

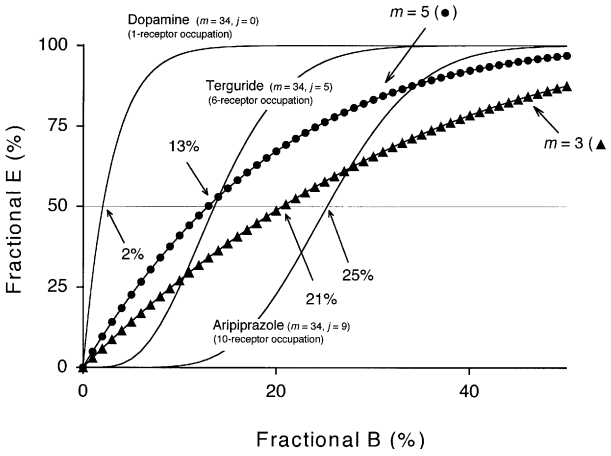

Fig. 5. The relationship between fractional binding of agonist to receptors (*Fractional B*: $FR_{M>j}$ in Model [M]) and fractional efficiency of effectors (*Fractional E*: $FE_{M>j}$ in Model [M]). In this case, $m = 34$. Three solid lines are, respectively, obtained by Eq. 33. The value of j is 0 for the top line (dopamine), 5 for the middle line (terguride) or 9 for the bottom line (aripiprazole) in each solid line: i.e., dopamine. $m = 34$ and $j = 0$; terguride. $m = 34$ and $j = 5$; aripiprazole. $m = 34$ and $j = 9$. When j is 0, the line is absolutely equal to the relationship obtained from Model [A]. When j is equal to m, the line is absolutely equal to the relationship obtained from Model [B]. *Filled circles* show the relationship between FE_A and FR_B in Model [A] (or Model [M] with j of 0) with m of 5, using Eq. 33. *Filled triangles* indicate the relationship between FE_A and FR_B in Model [A] (or Model [M] with j of 0) with m of 3, using Eq. 33.

where 0! is defined as 1; namely, $j\,! = 1$ at $j = 0$ and $(m\text{-}j)\,! = 1$ at $m = j$. As mentioned above (Burris et al., 2002), in a dopamine receptor system, dopamine presents 50 % effector efficiency at a receptor occupation of 2 %, while terguride shows 50 % effector efficiency at a receptor occupation of 13 %. In the same system, aripiprazole shows 50 % effector efficiency at a receptor occupation of 23 %. Using Eq. 33, we determined the value of m in the system. Based upon the observation on a previous study (Burris et al., 2002), we simulated the case of dopamine under an assumption (Model [A]) that the effector is fully functional when at least one of the receptors connecting to one effector should be occupied by dopamine. Our simulation indicated that m would be 34 in the case of dopamine (the top solid line in Fig. 5). That is, the simulation curve obtained from Model [A] with m of 34 (the top solid line in Fig. 5) indicates that an agonist (dopamine) shows 50 % effector efficiency at a receptor occupation of 2 %, and this result matches the experimental observation shown above (Burris et al., 2002). Model [A] is absolutely identical to Model [M] with $j = 0$. Using Model [M] with $m = 34$, we simulated the observation on terguride to calculate the value of j. In the case of terguride, terguride is required to bind to more than 5 pieces of receptors connecting with one effector for the effector to be fully functional; i.e., $j = 5$ (the middle solid line in Fig. 5). This simulation curve (the middle solid line in Fig. 5) indicates that the agonist terguride shows 50 % effector efficiency at a receptor occupation of 13 %, and this result matches the experimental observation shown above (Burris et al., 2002). We also simulated the case of aripiprazole. In the case of aripiprazole, aripiprazole is required to bind to more than 9 receptors connecting with one effector for the effector to be fully functional; i.e., $j = 9$ (the bottom solid line in Fig. 5). This simulation curve (the bottom solid line in Fig. 5) indicates that the agonist (aripiprazole) shows 50 % effector efficiency at a receptor occupation of 25 %, and this result matches the experimental observation shown above (Burris et al., 2002). We further simulated the observations on terguride and aripiprazole (Burris et al., 2002) using MODEL [A], which is identical to Model [M] at $j = 0$. In the case of terguride, the value of m was determined to be 5 in the case of the best fitting for the observation (Burris et al., 2002) (*closed circles* in Fig. 5). On the other hand, the value of m in the case of aripiprazole was determined to be 3 in the case of the best fitting for the observation (Burris et al., 2002) (*closed triangles* in Fig. 5). Thus, using these models we explain the phenomena containing a non-linear relationship between receptor occupancy and effector efficiency. Further, fixing the number of receptors connecting with one effector, we also explain that the phenomenon of the relationship being variable based on the kind of agonist is due to the fact that the strength of the receptor occupation causing a conformational change of the effector as an active form is dependent on the tightness of agonist-binding to receptor. In other words, the concept is as follows: 1) if the agonist-binding is strong, a small number of agonist-occupied receptors connecting to one effector is large enough for the effector to be fully functional, and 2) if the agonist-binding is weak, a large number of agonist-occupied receptors connecting to one effector is required for the effector to be fully functional.

Conclusion

The present study provides a noble, completely new concept of spare receptors. The concept proposed in the present report can explain a lot of phenomena concerning the relationship between agonists and efficiency of effectors that are not understood by conventional concepts of spare receptors reported by various investigators. Thus, the new concept reported here leads us to a breakthrough of understanding of spare receptors and provides us with new ideas for experimental procedures for in-

vestigating coupling of agonists, receptors and effectors.

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