Naunyn-Schmiedeberg's Arch Pharmacol (2014) 387:601–603
DOI 10.1007/s00210-014-0993-1

EDITORIAL

A pharmacological paradox: may a neutral antagonist shift an agonist concentration-response curve to the left?

Thomas J. Feuerstein

Received: 14 May 2014 / Accepted: 15 May 2014 / Published online: 28 May 2014

Abstract It is generally accepted that the presence of a competitive antagonist shifts an agonist concentration-response curve to the right. However, this may not always be the case: The concentration-response curve of an inverse receptor agonist may be shifted to the left by a neutral antagonist; a condition, which can be hypothetically explained by the assumption of both negative cooperativity of dimeric receptors plus a receptor reserve.

The presence of a competitive antagonist usually shifts an agonist concentration-response curve to the right. As it can be learned from the paper of Jergas et al. (2014), O-2050, assumingly a neutral CB_1 receptor antagonist, indeed shifts the concentration-response curve of the classical CB_1 receptor agonist WIN 55,212-2 to the right. The concentration-response curve of the inverse CB_1 receptor agonist rimonabant, however, is shifted to the left by O-2050. What is the explanation for this puzzling finding?

By definition, a neutral competitive antagonist only changes the affinity of an agonist. Nevertheless, under the following assumptions, such a sole decrease in the agonist affinity can explain an increase in the potency of an inverse agonist, as observed by Jergas et al. (2014), i.e., a shift to the left of its concentration-response curve.

T. J. Feuerstein
Freiburg Institute for Advanced Studies (FRIAS),
University of Freiburg, 79104 Freiburg, Germany

T. J. Feuerstein (✉)
Section of Clinical Neuropharmacology of the Department of Neurosurgery, University of Freiburg, Breisacher Strasse 64, 79106 Freiburg, Germany
e-mail: thomas.feuerstein@uniklinik-freiburg.de

1. A dimerization of two CB_1 receptors is the basis of ^{35}S-GTPγS binding (see, for instance: Wager-Miller et al. 2002, Turu and Hunyady 2010).
2. Constitutive activity of the dimers induces ^{35}S-GTPγS binding.
3. Inverse agonist activation of only one subunit of the dimer is already sufficient to maximally reduce the constitutive ^{35}S-GTPγS binding of a CB_1 receptor dimer (receptor reserve).
4. Cooperativity has to be assumed for inverse agonist activation of the dimer. This is suggested by the position on the right of the rimonabant concentration-response curve compared to its concentration-binding curve.

In the study of Jergas et al. (2014), the pK_d of rimonabant, 7.8, was much higher than its pEC_{50}, 5.0. To take this into consideration, the condition of cooperativity has to be assumed in addition to that of a receptor reserve (for quantification of the differences between pK_d and pEC_{50} see Feuerstein and Limberger 1999 and Feuerstein and Sauermann 2005). Cooperativity means that two inverse agonists bind "non-independently" of each other to the CB_1 receptor dimer. We don't know explicitly from the data of Jergas et al. (2014) whether this cooperativity is positive or negative or neutral with respect to the binding affinities at the first and the second receptor of the dimer, although negative cooperativity may occur more often (Franco et al. 2007). We may only say that two inverse agonist molecules bind simultaneously to one CB_1 receptor dimer, and that binding of only one inverse agonist molecule is also sufficient to maximally reduce the constitutive ^{35}S-GTPγS binding. Cooperativity results in a position of such a *four-molecular* (two inverse agonists plus two CB_1 receptors) concentration-response curve far on the right, as compared with a binding curve which reflects a *bimolecular* association of an inverse agonist to a single CB_1 receptor. The mentioned receptor reserve per se

shifts the inverse agonist concentration-response curve to the left; this left shift, however, is largely overcompensated by the cooperativity-induced right shift.

How can we now explain that the presence of the pure neutral antagonist O-2050 *enhanced* the *inhibitory* action of rimonabant (i.e., shifted its concentration-response curve to the left)? The pK_d of O-2050, 6.8, was assessed convincingly (note that this pK_d of 6.8 was very similar to the pA_2 of 7.0, Jergas et al. 2014).

First, some binomial considerations have to be made: these considerations may mathematically develop concentration-response functions from concentration-binding functions (e.g. Feuerstein and Limberger 1999).

The functional system, usually consisting of multiple functional units (cells or nerve terminals endowed with receptors), is currently represented by constitutively active CB_1 receptor dimers, each with receptors 1 and 2. As already said, inverse activation of both dimeric receptors does not result in a larger ^{35}S-GTPγS binding reduction than activation of only one.

The total number of receptors per dimer is $n=2$; the number of occupied receptors is $i=0$, 1, or 2; the assumption of a receptor reserve defines $i=1$ for the (minimal) number of dimeric receptors which have to be activated to obtain a maximum agonist effect.

If $0<i\leq n$, or $0<1, 2\leq 2$, the number i of occupied receptors has a binomial distribution $B(n, q)$ with parameters $n=2$ and q. For $i=0$, no receptor of the dimer is occupied, for $i=1$ or 2, one or two receptors are occupied, $n=2$ is the maximum number of receptors occupied. q is the fractional receptor occupation, $\frac{L}{K_d+L}$, with L representing the concentration of a ligand, e.g., of the inverse agonist (rimonabant) or of the neutral antagonist (O-2050).

Occupancy by 1μM O-2050 alone

The pK_d of the pure antagonist O-2050 is 6.8. Then, at 1 μM, the fractional receptor occupation $q=\frac{10^{-6}}{10^{-6.8}+10^{-6}}=0.8632$. Here, O-2050 binds to 86.32 % of all receptors of the bulk of dimers. These 86.32 % are distributed in detail as follows:

The probability that no receptor is occupied means that $n=2$ and $i=0$, yielding $\binom{n}{i}q^i(1-q)^{n-i}$ or $\binom{2}{0}0.863^0(1–0.863)^{2-0}=1\times 1\times(0.137)^2=0.0187$. Thus, 1.87 % of all dimers have no occupied receptor and full constitutive activity for ^{35}S-GTPγS binding. These 1.87 %, for instance, can be inversely activated by rimonabant occupying at least one receptor of each dimer.

The probability that one of two receptors is occupied means that $n=2$ and $i=1$, yielding $\binom{2}{1}0.863^1(0.137)^1=0.2362$. Thus, 23.62 % of all dimers have one receptor occupied by O-2050. There is still full constitutive activity of the 23.62 % because the neutral antagonist does not interfere. These 23.62 % can be inversely activated by rimonabant, occupying the second receptor of the dimer. This may already result in inhibition of ^{35}S-GTPγS binding (only one of two receptors has to be inversely activated because of the assumed receptor reserve).

The probability that both receptors of a dimer are occupied means that $n=2$ and $i=2$ yielding $\binom{2}{2}0.863^2(0.137)^0=0.7451$. Thus, 74.51 % of all dimers have two receptors pre-occupied by O-2050. At first glance, these 74.51 % cannot be inversely activated by rimonabant, i.e., their ^{35}S-GTPγS binding remains unaffected (because the pure antagonist O-2050 by itself does not diminish the constitutive activity). This is in line with the finding of Jergas et al. (2014) that O-2050 does not influence the ^{35}S-GTPγS binding.

Note, however, that O-2050 and rimonabant compete for the same receptor sites, displacing each other (see below).

Occupancy by rimonabant alone and by rimonabant in the presence of 1μM O-2050

Absence of O-2050: $q=\frac{[\text{rimonabant}]}{10^{-7.8}+[\text{rimonabant}]}$; with $pK_d=7.8$, at [rimonabant]=10μM for instance, 99.84 % of all receptors are occupied by rimonabant).

Presence of O-2050: $q=\frac{[\text{rimonabant}]}{10^{-7.8}+10^{-6-7.8+6.8}+[\text{rimonabant}]}$; with 10^{-6} M=1 μM indicating the concentration of O-2050 and with $10^{-6.8}$ M reflecting the K_d of O-2050. For an explanation of the sum of the exponents in $10^{-6-7.8+6.8}$, reflecting that the summand increases the K_d of rimonabant due to the presence of O-2050 as a pure neutral antagonist, see Mantovani et al. (2009).

The probabilities that, at the different rimonabant concentrations, no receptor, one of two receptors, or both receptors are occupied are given in Table 1 below.

The probability that one of two receptors is occupied, for instance, at 10μM rimonabant, is 0.0032. Thus, 0.32 % of all dimers have only one of two receptor activated by rimonabant. These 0.32 % shows already maximally reduced constitutive activity to bind ^{35}S-GTPγS.

The probability that, at 10μM rimonabant, both receptors of a dimer are occupied, is 0.9968. Thus, 99.68 % of all dimers have two receptors activated by rimonabant which means that the ^{35}S-GTPγS binding of these 99.68 % is inhibited, but only to the same degree as if one of both receptors were occupied by rimonabant.

Table 1 shows the probabilities for occupations by rimonabant of one of two dimeric receptors in the absence and presence of 1 μM O-2050. These probabilities P correspond

Table 1 Probabilities for occupations by rimonabant of one of two dimer receptors in the absence and presence of 1-μM O-2050

Rimonabant μM	Rimonabant alone *P* (1 of 2 occupied) %	Rimonabant/O-2050 1 μM *P* (1 of 2 occupied) %
0.1	23.62	49.73
0.32	9.09	39.25
1	3.07	18.61
3.2	0.99	6.82
10	0.32	2.26
32	0.10	0.73

to the frequencies of occurrence of dimers with one of two receptors occupied by rimonabant.

With the assumption of negative cooperativity as intramolecular cross-talk in the CB_1 receptor dimer, we can now explain that the concentration-response curve of the inverse CB_1 receptor agonist rimonabant is shifted to the left by O-2050. The binding of one rimonabant molecule to one receptor negatively affects the binding of the second rimonabant molecule to the partner receptor. This implies the lack of involvement of intracellular signaling and suggests some kind of cooperative interaction between adjacent receptors (Franco et al. 2007).

Table 1 clearly shows that the frequency of the condition "only one of the two dimer receptors is occupied" is always higher in the presence of O-2050. This means that negative cooperativity, i.e., a decrease in the binding affinity for rimonabant, occurs more rarely in the presence of the neutral antagonist. Since, according to the assumed receptor reserve, the inverse activation of only one receptor of the dimer is sufficient to maximally inhibit the constitutive activity of this dimer, the inverse agonist effect occurs at lower agonist concentrations, due to the relatively increased (i.e., not decreased) inverse agonist affinity to only one of the two dimer receptors. This results in an increased inhibitory effect of rimonabant, i.e., a shift to the left of its concentration-response curve in the presence of a neutral antagonist.

We do not need to assume other than pure neutral antagonist actions of O-2050 to explain this shift to the left. Admittedly, other assumptions had to be made, i.e., a receptor reserve in the rimonabant action and negative cooperativity in the rimonabant binding to the dimer receptors. These other assumptions seem possible or even probable. However, they are not yet experimentally verified, at least not in the paper of Jergas et al. (2014) under consideration.

Whether the approach of this editorial, to make two additional assumptions (receptor reserve and negative cooperativity) and to deny the assumption of Jergas et al. (2014, O-2050 being a positive allosteric modulator), are closer to the maxim of William of Ockham that the simplest explanation is probably the correct explanation remains open. This question of the correct explanation should be answered experimentally.

In summary, the pure neutral antagonist O-2050 increases the inverse agonistic activity of rimonabant. Under the assumptions made, there is no need to attribute other qualities to O-2050 than pure neutral antagonist properties.

References

Feuerstein TJ, Limberger N (1999) Mathematical analysis of the control of neurotransmitter release by presynaptic receptors as a supplement to experimental data. Naunyn-Schmiedebergs Arch Pharmacol 359: 349–359

Feuerstein TJ, Sauermann W (2005) What is the opposite of a receptor reserve? Intl J Pharmacol 1:195–202

Franco R, Casadó V, Cortés A, Ferrada C, Mallol J, Woods A, Lluis C, Canela EI, Ferré S (2007) Basic concepts in G-protein-coupled receptor homo- and heterodimerization. Sci World J 7:48–57

Jergas B, Schulte K, Bindila L, Lutz B, Schlicker E (2014) O-2050 facilitates noradrenaline release and increases the CB1 receptor inverse agonistic effect of rimonabant in the guinea-pig hippocampus. Naunyn-Schmiedeberg's Arch Pharmacol, in press

Mantovani M, Dooley DJ, Weyerbrock A, Jackisch R, Feuerstein TJ (2009) Differential inhibitory effects of drugs acting at the norepinephrine and serotonin transporters in rat and human neocortical synaptosomes. Br J Pharmacol 158:1848–1856

Steffens M, Zentner J, Honegger J, Feuerstein TJ (2005) Binding affinity and agonist activity of putative endogenous cannabinoids at the human neocortical CB_1 receptor. Biochem Pharmacol 69:169–178

Turu G, Hunyady L (2010) Signal transduction of the CB_1 cannabinoid receptor. J Mol Endocrinol 44:75–85

Wager-Miller J, Westenbroek R, Mackie K (2002) Dimerization of G protein-coupled receptors: CB_1 cannabinoid receptors as an example. Chem Phys Lipids 121:83–89