Pharmacology

Guide to insight into ligand classes

Purpose and scope

Welcome to this pharmacology booklet, a document that helps scientists and researchers understand and navigate the diversity of ligands and ligand/receptor interactions associated with pharmacology studies. We hope the explanations and visuals provided in this document will contribute to shedding light on this area of research.

The document is organized around the distinction between the different classes of ligands and their characteristics. An introduction addresses the issues of pharmacological research, before reviewing the different ligand classes involved and the basic principles of ligand/receptor interaction models as well as the pharmacological parameters that are commonly used to study them. It is followed by four illustrated sections that present and explain the characteristics and properties of each class.

Introduction

Pharmacology is the science studying the effects of compounds on biological systems. It is a critical area of therapeutic research and a mandatory part of all drug development projects, as such processes have demanding requirements in terms of understanding, predicting and quantifying the ability of drug candidates to exert sought-after effects on the body. Pharmacology is therefore heavily involved in many steps of the drug development process, where it is part of early drug discovery steps and toxicology studies. It remains a significant aspect of all clinical trial stages from I through III.

Ligand-receptor relationships are especially relevant to applied pharmaceutical research, as the most important area of therapeutic research is that of G protein coupled receptors (GPCRs) which account for over 800 receptors and numerous functions in humans. In January 2018, the Food and Drug Administration (FDA) reported that a total of 475 drugs targeting 108 GPCRs had received its approval (34% of all FDA-approved drugs), while an undisclosed number of drugs targeting 66 other GPCRs were undergoing clinical trials *(Hauser A. S, 2018).*

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NOTIONS OF PHARMACOLOGY

Orthosteric and allosteric ligands

Depending on the receptor binding site they target, ligands are referred to as either orthosteric or allosteric (Fig. 1).

An orthosteric site is usually understood as the “natural” site of a receptor, which triggers the receptor biological effect upon binding of said ligand. It corresponds to the region of the receptor that binds its natural ligand. However, this definition remains somewhat incomplete as some receptors (especially GPCRs) can have several natural ligands that bind to different sites and trigger different effects. For that reason, an orthosteric site is best defined for a receptor/ligand couple as “the preferred binding site of a given natural ligand on its receptor”.

On the other hand, all other regions of a receptor that may bind a ligand are called allosteric sites. Even though most of them do not have the ability to generate biological effects on their own, these regions and the ligands that target them are still relevant to pharmacology as allosteric binding can induce conformational modifications in the receptor. Such modifications can sometimes lead to increases/decreases of the orthosteric ligand binding and/or signal strength, which indirectly affect the receptor’s biological activity *(Nussinov R, 2012)*.

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NOTIONS OF PHARMACOLOGY

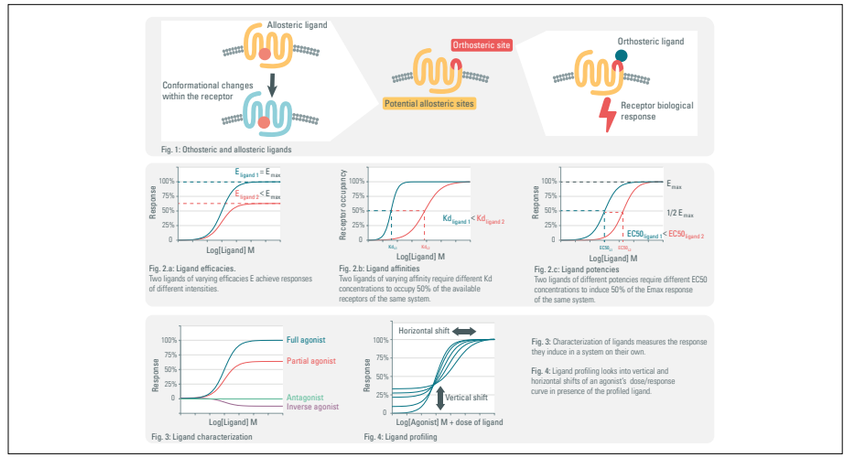
Affinity, efficacy and potency

Ligands are well-characterized in equilibrium contexts (equilibrium of receptor/ligand binding & equilibrium of the induced response) ***(Bdioui S, 2018)*** where three parameters determine their effects on receptors:

- Efficacy is noted E and refers to the property of different ligands to trigger responses of varying intensity when binding the same number of receptors. For every receptor, the maximal theorical response obtainable is induced by a ligand of efficacy E_{max}, which is usually the natural ligand (Fig. 2.a).
- Affinity indicates the strength of the interaction between a ligand and its receptor. It is usually described with the equilibrium dissociation constant Kd, which corresponds to the molar concentration of ligand required to bind 50% of all receptors of a system. The smaller the Kd, the stronger the interaction (Fig. 2.b).
- Potency is an indicator of the activity of a ligand in terms of the amount needed to produce a given effect. The International Union of Pharmacology Committee warns users against the imprecise nature of that term, which needs further definition to be practical. In fact, potency is often measured with the half maximal effective concentration EC_{50} of a ligand, or half maximal inhibitory concentration IC_{50} for an antagonist, which correspond to the molar concentration of said ligand to mediate 50% of its effects (Fig.2.c). ***(Neubig R.R, 2003)***

The potency and efficacy parameters are used to study ligands in two ways.

- Characterization is the study of a ligand on its own and yields information related to the ligand's class. It is usually only performed as an early step in drug discovery processes and investigations (Fig. 3).
- Profiling is the study of a ligand in addition to an agonist. These experiments provide information about how ligands behave in an environment featuring other compounds. Profiling steps are critical to properly describe and understand a ligand, as well as to determine its true *in-vivo* effects. They are also heavily involved in the determination of doses and toxicity studies in pharmaceutical development (Fig. 4).



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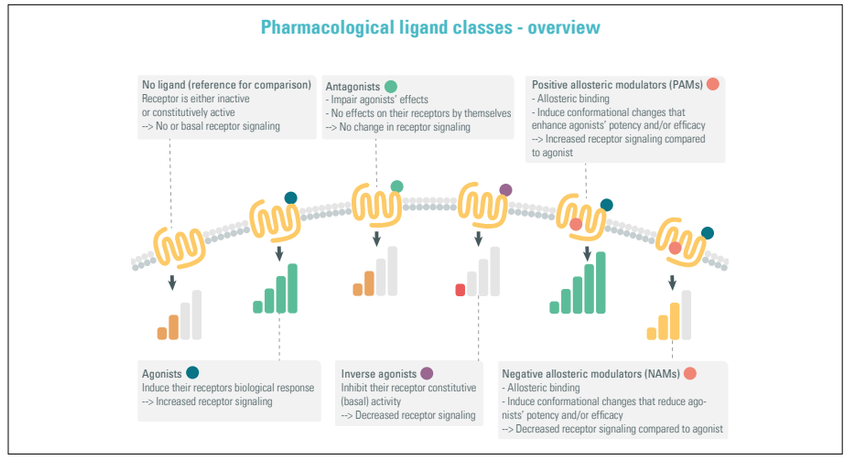

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Fig. 1: Othosteric and allosteric ligands

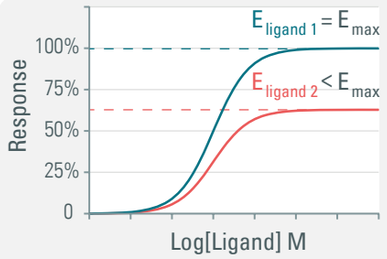

Fig. 2.a: Ligand efficacies.
Two ligands of varying efficacies E achieve responses of different intensities.

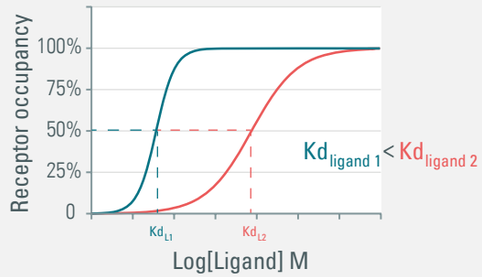

Fig. 2.b: Ligand affinities
Two ligands of varying affinity require different Kd concentrations to occupy 50% of the available receptors of the same system.

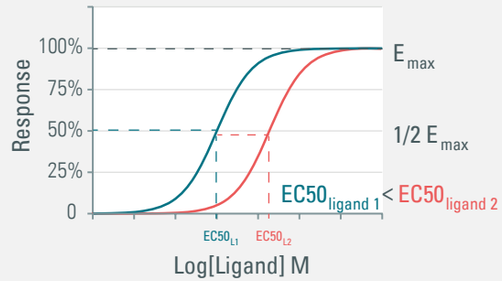

Fig. 2.c: Ligand potencies
Two ligands of different potencies require different EC50 concentrations to induce 50% of the Emax response of the same system.

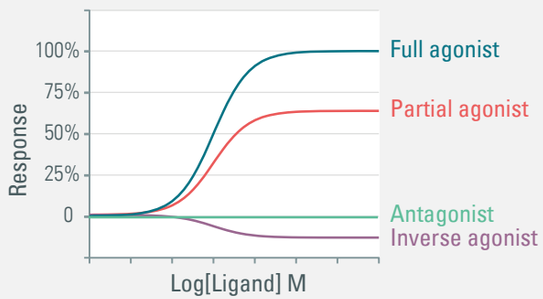

Fig. 3: Ligand characterization

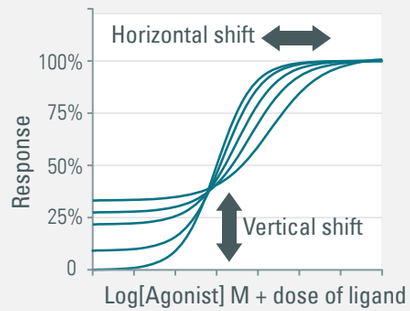

Fig. 4: Ligand profiling

Fig. 3: Characterization of ligands measures the response they induce in a system on their own.

Fig. 4: Ligand profiling looks into vertical and horizontal shifts of an agonist's dose/response curve in presence of the profiled ligand.

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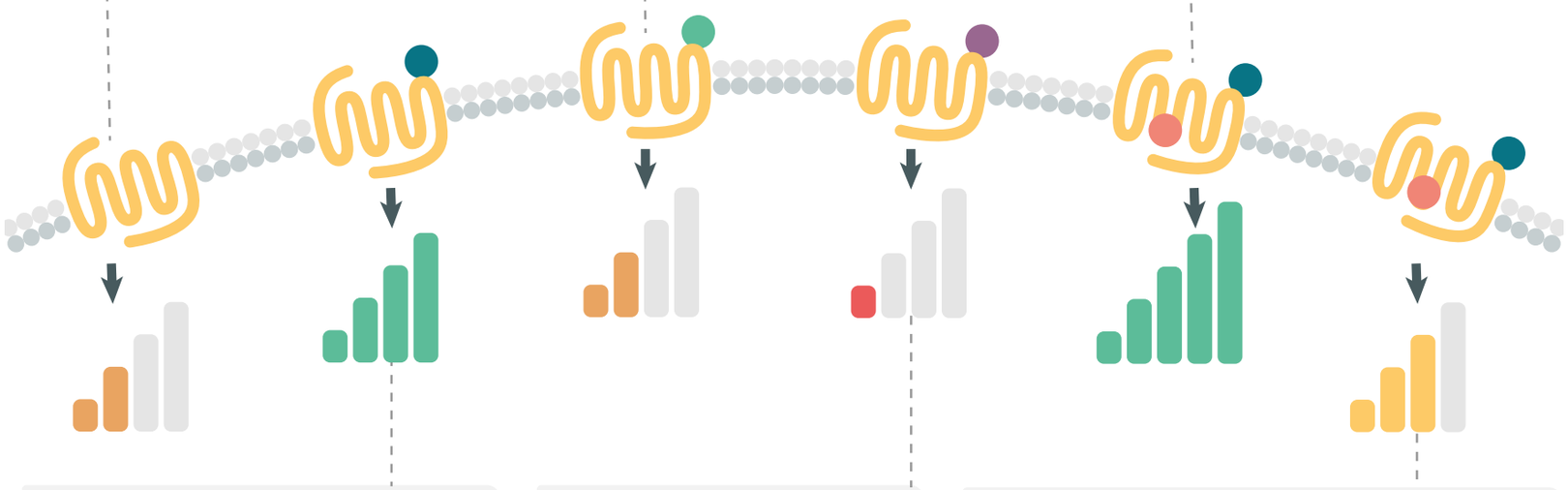
Pharmacological ligand classes - overview

No ligand (reference for comparison)
Receptor is either inactive
or constitutively active
--> No or basal receptor signaling

Antagonists
- Impair agonists' effects
- No effects on their receptors by themselves
--> No change in receptor signaling

Positive allosteric modulators (PAMs)
- Allosteric binding
- Induce conformational changes that enhance agonists' potency and/or efficacy
--> Increased receptor signaling compared to agonist



Agonists
Induce their receptors biological response
--> Increased receptor signaling

Inverse agonists
Inhibit their receptor constitutive (basal) activity
--> Decreased receptor signaling

Negative allosteric modulators (NAMs)
- Allosteric binding
- Induce conformational changes that reduce agonists' potency and/or efficacy
--> Decreased receptor signaling compared to agonist

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LIGAND CLASSES IN THERAPEUTIC APPLICATIONS

Ligand classes in therapeutic application

Agonists are commonly used in medicine to induce sought-after responses relevant to pathologies. Full agonists mimic the effects of natural ligands and are used to induce strong biological responses or support organisms lacking natural ligands. For instance, isoproterenol and morphine are full agonists of b-adrenoreceptors and m-opioid receptors in the CNS respectively, and are used to induce strong stimulation of those for bradycardia (low heart rate) and acute pain treatment purposes.

Partial agonists offer the opportunity to mediate beneficial biological responses of a lesser intensity than that of full agonists. Their therapeutic relevance lies in their ability to trigger a desired response while decreasing the risk for adverse effects.

A widely used example is that of salbutamol, better known as Ventolin. Being a fast and short-acting partial agonist of β2-adrenergic receptors, this ligand acts within 15 minutes and for 2-6 hours to induce moderate relaxation of airway smooth muscles, which results in bronchodilation and is an effective treatment for any at-risk airway constriction (asthma attacks but also allergies, chronic obstructive pulmonary or exercised-induced constriction). Another case is that of bupropion, a partial agonist of the acetylcholine nicotinic receptor used for nicotin withdrawal symptoms management. Its partial agonism allows for a reduction of these symptoms while avoiding to reinforce addiction pathways the way a full agonist such as nicotin does.

Antagonists are the counterpart of agonists and prevent their receptors from receiving activating signals. For this reason, they are widely used in therapy to obtain various results by blocking relevant signaling pathways. Atropine is a case of a competitive antagonist of the acetylcholine muscarinic receptors. It is used to treat bradycardia (low heart rates) and heart blocks by preventing vagal nerve signals (which result in acetylcholine release at synapses in cardiac tissues) from affecting the heart, thus increasing the firing rate of the sinoatrial node and resulting in accrued heart rates.

Inverse agonists have been mistaken for antagonists for a long time until the constitutive activity of GPCRs was identified. For this reason, many antagonists were re-evaluated and characterized as inverse agonists. This was the case for nearly all antihistaminics.

In current pharmacology, carvedilol is a relevant example of an inverse agonist of the b-adregnergic receptor. It is used as a beta-blocker in congestive heart failure and hypertension, and has repeatedly been a top US prescription *(Khilnani G, 2011)*. Thanks to its inverse agonism, carvedilol lowers the maximal activity of b-adrenergic receptors regardeless of the amount of natural ligand present, whereas a conventional antagonist could be rendered ineficient by an excess of natural igand. Inverse agonists also represent a significant hope for addiction medication as they lower the maximal response of systems to drugs, thus preventing risks of overdoses and promoting low addictive responses.

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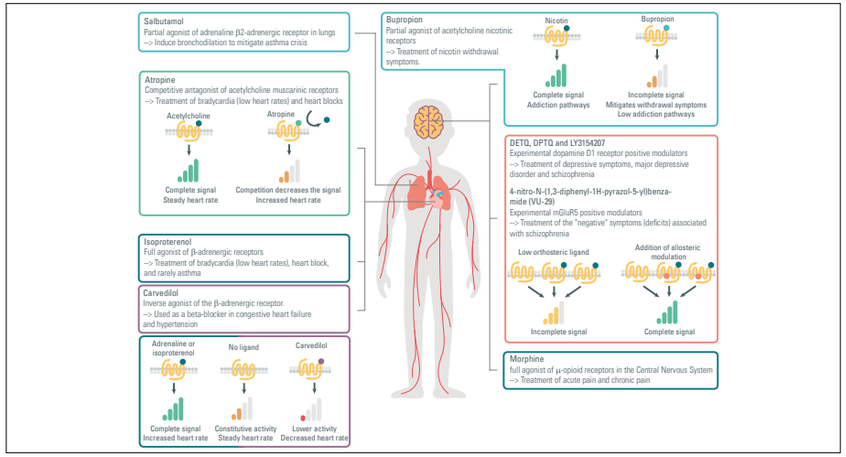
LIGAND CLASSES IN THERAPEUTIC APPLICATIONS

Ligand classes in therapeutic application

Allosteric modulators are not as present in therapeutic arsenals as the other ligand classes but have been increasingly investigated for the fine tuning of ligand-based therapies they could introduce to medicine. They have already been proven beneficial to condition previously challenging for other ligand classes. Unlike orthosteric ligands which tend to target their receptors across the whole body, allosteric modulators can have tissue-specific effects as they modulate already existing responses within tissues.

Some experimental positive modulators such as 4-nitro-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (VU-29) for mGluR5 receptors or DETQ, DPTQ and LY3154207 for dopamine D1 receptor have already demonstrated positive results in depressive disorders and schizophrenia *(J.E. Ayala, 2009) (K.A. Svensson, 2019)*.



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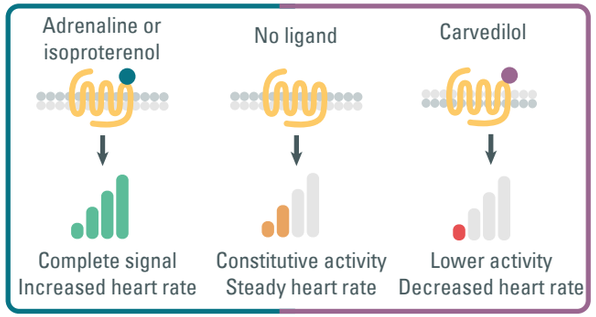
Salbutamol
Partial agonist of adrenaline β2-adrenergic receptor in lungs
--> Induce bronchodilation to mitigate asthma crisis

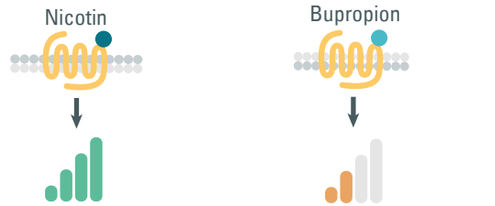
Atropine
Competitive antagonist of acetylcholine muscarinic receptors
--> Treatment of bradycardia (low heart rates) and heart blocks

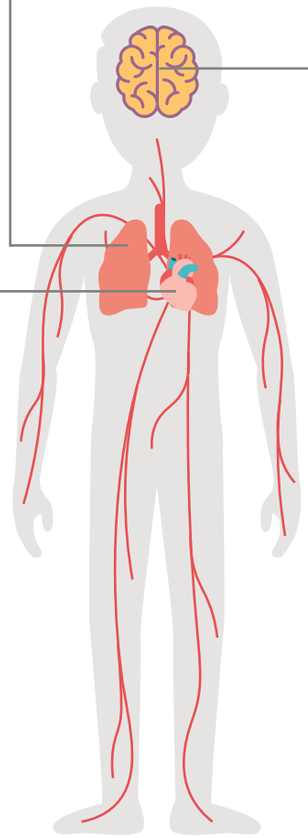
Isoproterenol
Full agonist of β-adrenergic receptors
--> Treatment of bradycardia (low heart rates), heart block, and rarely asthma

Carvedilol
Inverse agonist of the β-adrenergic receptor.
--> Used as a beta-blocker in congestive heart failure and hypertension



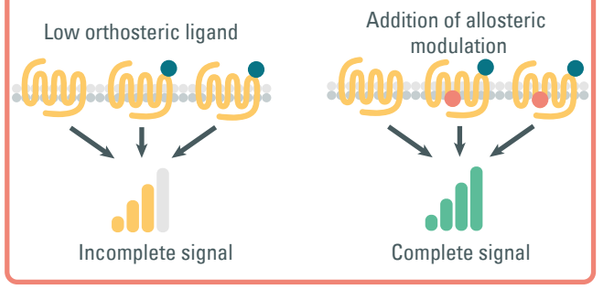
Bupropion
Partial agonist of acetylcholine nicotinic receptors
--> Treatment of nicotin withdrawal symptoms.





DETQ, DPTQ and LY3154207
Experimental dopamine D1 receptor positive modulators
--> Treatment of depressive symptoms, major depressive disorder and schizophrenia

4-nitro-N-(1,3-diphenyl-1H-pyrazol-5-yl)benza-mide (VU-29)
Experimental mGluR5 positive modulators
--> Treatment of the "negative" symptoms (deficits) associated with schizophrenia



Morphine
full agonist of μ-opioid receptors in the Central Nervous System
--> Treatment of acute pain and chronic pain

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AGONISTS
Agonists

"A ligand that binds to a receptor and alters the receptor state resulting in a biological response" – International Union of Pharmacology Committee

The term agonist refers to any ligand which may induce a receptor's biological response upon binding to said receptor's orthosteric site. Agonists are characterized by their affinity for their receptors (Kd), potency (EC_{50}), and maximal response or efficacy (E).

Agonists are split into two categories depending on how their efficacy compares to that of the target receptor's natural ligand: full agonists and partial agonists.

Full agonists

"When the receptor stimulus induced by an agonist reaches the maximal response capability of the system (tissue), then it will produce the system maximal response and be a full agonist in that system" – International Union of Pharmacology Committee

As this extract indicates, full agonists may induce the full or maximal response of the receptor they bind to, which is usually measured against the receptor's natural ligand. They are characterized by the equation $E_{full\ agonist} = E_{max} = 100\%$ (ligand efficacy is equivalent to that of the natural ligand). Although they all induce maximal response, all full agonists are not equal and can differ in potency. Potent full agonists have lower EC_{50} and reach maximal efficacy sooner than their less potent kin (Fig. 5).

For a biological system such as a cell or an organ, full agonism and maximal response do not necessarily require all receptors of said system to be bound. Often, full agonists trigger the maximal response in humans without binding a portion of receptors known as the spare receptors, or receptor reserve which exists in excess to the amount necessary for maximal response. In pharmacology, these discrepancies indicate a non-linear relationship between the receptor occupancy rate of a system and its biological response, and can be characterized by the inequation $Kd > EC_{50}$ (affinity < potency) (Fig. 6). The greater the receptor reserve, the lower the EC_{50} is for a given receptor occupancy *(Neubig R.R, 2003) (Gomperts B.D, 2009).*

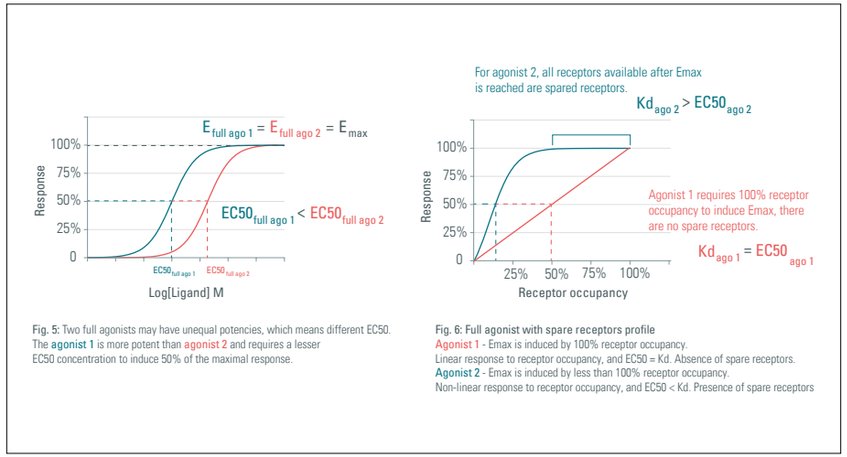

Fig. 5: Two full agonists may have unequal potencies, which means different EC50. The **agonist 1** is more potent than **agonist 2** and requires a lesser EC50 concentration to induce 50% of the maximal response.

Fig. 6: Full agonist with spare receptors profile
Agonist 1 - Emax is induced by 100% receptor occupancy.
Linear response to receptor occupancy, and EC50 = Kd. Absence of spare receptors.
Agonist 2 - Emax is induced by less than 100% receptor occupancy.
Non-linear response to receptor occupancy, and EC50 < Kd. Presence of spare receptors

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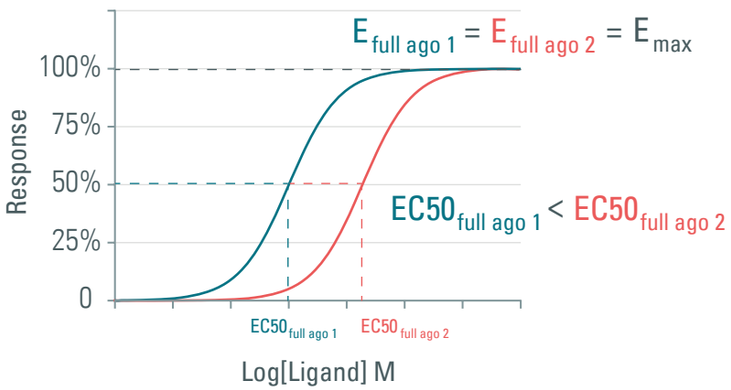

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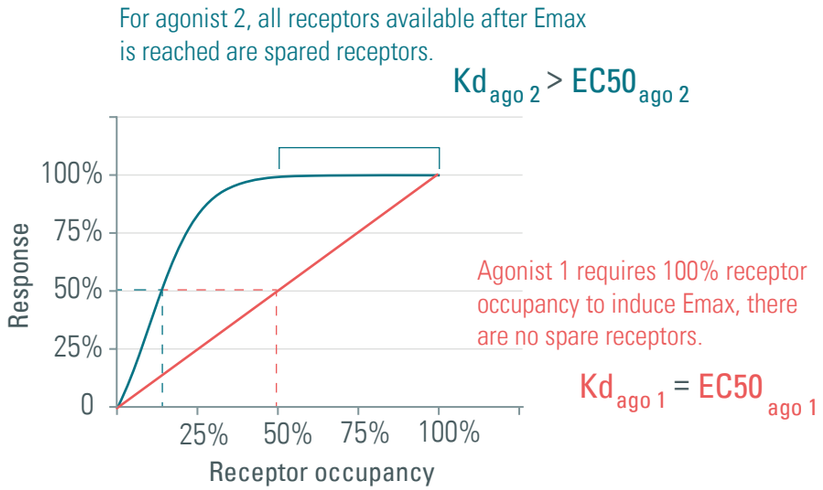

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Non-linear response to receptor occupancy, and EC50 < Kd. Presence of spare receptors

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AGONISTS
Partial agonists

"in a given tissue, under specified conditions, cannot elicit as large an effect (even when applied at high concentration, so that all the receptors should be occupied) as can another agonist acting through the same receptors in the same tissue" – International Union of Pharmacology Committee

Partial agonists are agonists that fail to induce the maximal response of a system compared to that system's natural ligand or full agonists, even at full receptor occupancy. Their maximal efficacy is inferior to that of the natural ligand or full agonists. In pharmacology they are characterized by the inequation $E_{partial\ agonist} < E_{max}$ (efficacy is inferior to that of the natural ligand) (Fig. 7).

When in presence of a full agonist targeting the same receptors, partial agonists compete with them for a finite number of receptors. In this context, some receptors are bound by full agonists while others are bound by the partial ones, and the overall system is therefore prevented from reaching the maximal response as long as this competition persists. The competition can however be overpowered by an excessive concentration of full agonist, in which case the system maximal response is still achieved. For this reason, partial agonists are described as acting as antagonists when in presence of a full agonist, because they compromise the full agonist's ability to induce the maximal response (see competitive antagonism). In pharmacology, partial agonists are profiled in presence of a full agonists by a rightward shift of the dose-response curve (Fig. 8) and the following equation and inequation *(Neubig R.R, 2003):*

- $E_{full+partial} = E_{max}$; maximal efficacy is unaltered, as the maximal response can be achieved by a significant enough excess of full agonist.
- $EC_{50\ full\ +\ Partial} > EC_{50\ full}$; potency is inferior to that of the full agonist alone (Fig. 8)

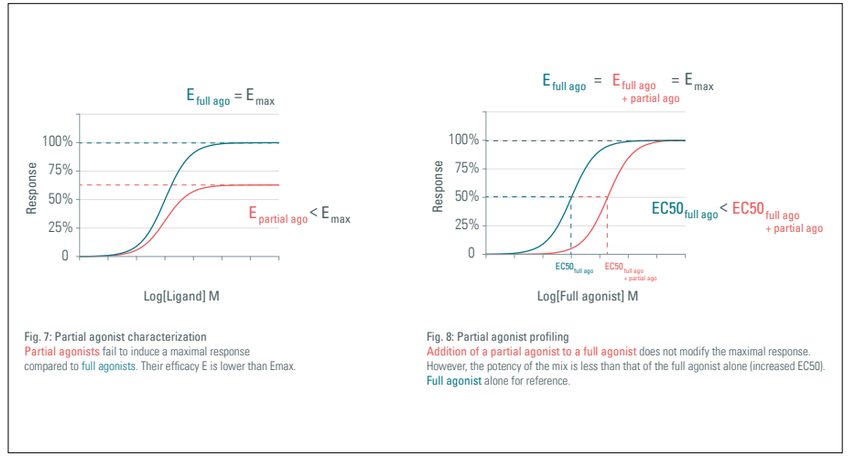

Fig. 7: Partial agonist characterization
Partial agonists fail to induce a maximal response compared to full agonists. Their efficacy E is lower than Emax.

Fig. 8: Partial agonist profiling
Addition of a partial agonist to a full agonist does not modify the maximal response. However, the potency of the mix is less than that of the full agonist alone (increased EC50). Full agonist alone for reference.

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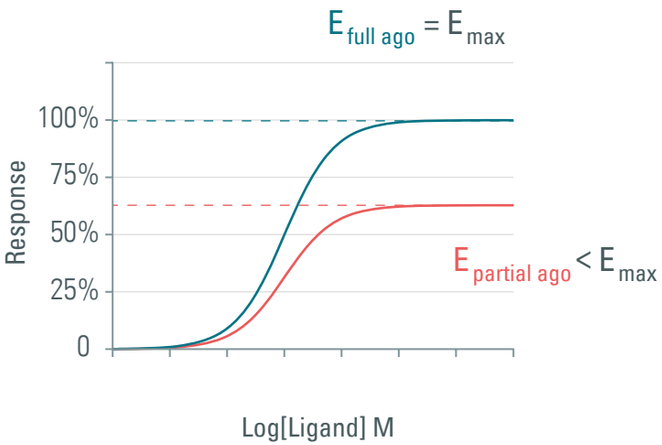

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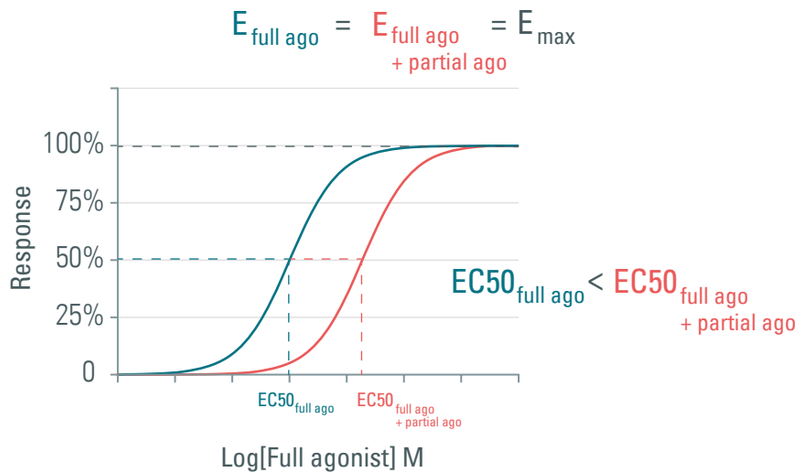

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ANTAGONISTS
Antagonists

"A drug that reduces the action of another drug" – International Union of Pharmacology Committee

The term antagonist refers to a ligand which may impair the biological response induced by a receptor's agonist. Depending on the strength of the impairment induced and the mechanisms by which they induce it, antagonists can be characterized in several subclasses *(Neubig R.R, 2003).* Whatever their mechanisms of action, antagonists are generally characterized by a lack of activity on their own (Fig. 9.a), and profiled in presence of an agonist by a rightward parallel shift of the dose/response curve and the equation $EC_{50Full\ ago} < EC_{50Full\ ago+\ antago}$ (Fig. 9.b). The overall efficacy E of a full agonist in presence of an antagonist can be altered or preserved, and is relevant to the antagonist subclasses. The reversibility of that decrease by introducing an excess of agonist is the key to determining the subclass of antagonists.

Additionally, antagonists are often described in relation to a full agonist of their receptors by their IC_{50} value, which corresponds to the amount of antagonist required to reduce the effects of the agonist by 50%. This value therefore serves as a read out of the antagonist potency (Fig. 9.c).

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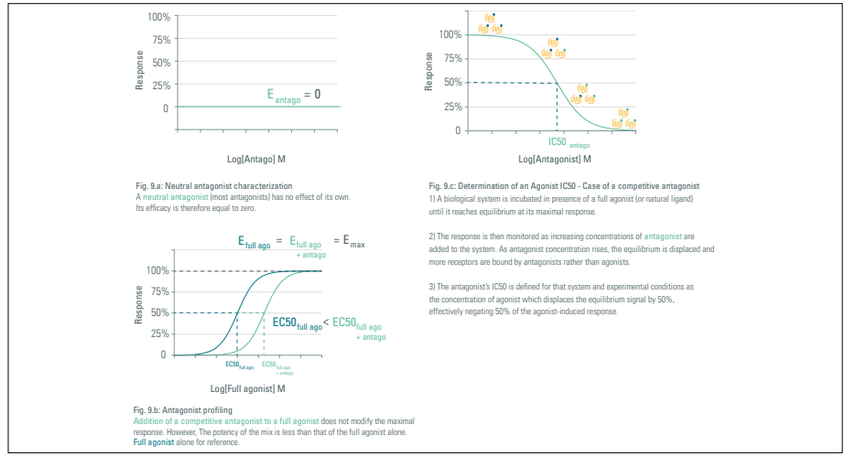
ANTAGONISTS
Competitive antagonism

Most ligands that are usually referred to as simple antagonists are competitive antagonists, meaning they bind to the same orthosteric site on their receptor as agonists. Consequently, the agonist and antagonist bindings are mutually exclusive, hence the term “competitive”. Unless they exhibit additional features (see insurmountable antagonism and long-lasting effects), competitive antagonists do not affect the efficacy E_{max} of agonists, which can still trigger the maximal response provided that they are present in a large enough excess to overpower antagonists’ competition. However competition does decrease the potency of agonists, as a larger amount of said agonists is required to produce the same effect as they would alone. This makes competitive antagonists recognizable by the rightward parallel shift of the dose response curve they induce when in presence of agonists (Fig. 9.b). In pharmacology, competitive antagonists are profiled in relation to full agonists by the following equation and inequation *(Wyllie D.J.A, 2007)*:

- $E_{max} = E_{full\ ago+compet\ antago}$; maximal efficacy is unaltered, as the maximal response can be achieved by a significant enough excess of full agonist.
- $EC_{50\ full\ ago\ +\ compet\ antago} > EC_{50\ full\ ago}$; potency is inferior to that of a full agonist alone (Fig. 9.b).

Non-competitive antagonists are characterized by their ability to impair agonists’ effects without substituting for the agonist on the receptor’s orthosteric site. In practice, they are split between functional antagonists and Negative Allosteric Modulators, or NAMs, that are part of the larger ligand class of Allosteric modulators.



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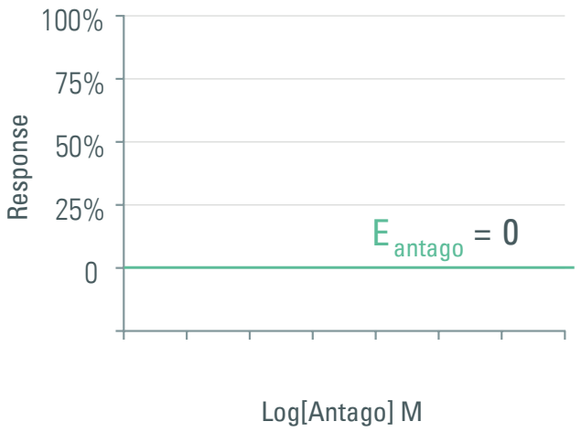

Fig. 9.a: Neutral antagonist characterization
A neutral antagonist (most antagonists) has no effect of its own. Its efficacy is therefore equal to zero.

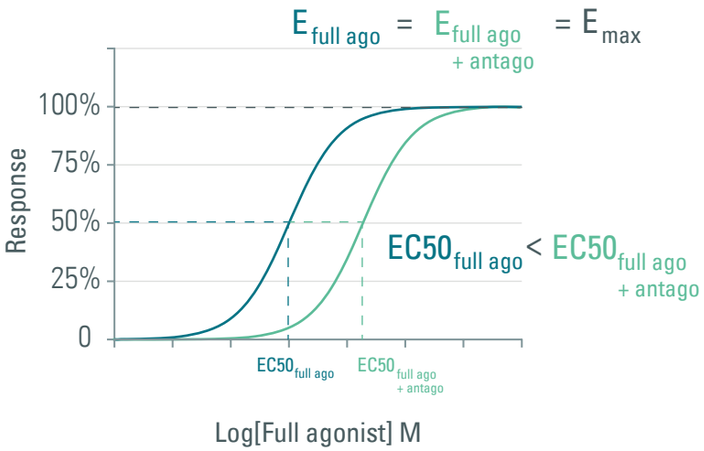

Fig. 9.b: Antagonist profiling
Addition of a competitive antagonist to a full agonist does not modify the maximal response. However, The potency of the mix is less than that of the full agonist alone. Full agonist alone for reference.

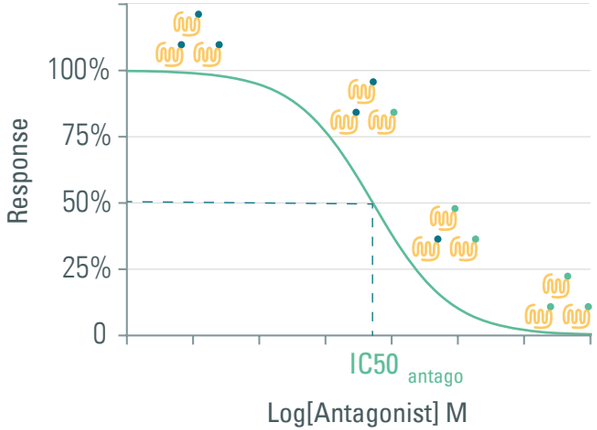

Fig. 9.c: Determination of an Agonist IC50 - Case of a competitive antagonist
1) A biological system is incubated in presence of a full agonist (or natural ligand) until it reaches equilibrium at its maximal response.

2) The response is then monitored as increasing concentrations of antagonist are added to the system. As antagonist concentration rises, the equilibrium is displaced and more receptors are bound by antagonists rather than agonists.

3) The antagonist's IC50 is defined for that system and experimental conditions as the concentration of agonist which displaces the equilibrium signal by 50%, effectively negating 50% of the agonist-induced response.

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ANTAGONISTS
Insurmountable antagonism

As a result of antagonists' ability to decrease agonists' potency, the biological response induced by an agonist in presence of an antagonist is less than that of the same amount of agonist alone (Fig. 10). In many cases, this phenomenon does not prevent an agonist from inducing its maximal response, provided a high enough concentration is added to the system. When such requirements are met, the antagonist is called surmountable or reversible, and is characterized in presence of a full agonist by the competitive antagonist equations

- $E_{ago+ antago} = E_{max}$; maximal efficacy is unaltered, as the maximal response can be achieved by a significant enough excess of full agonist.
- $EC_{50\ ago\ +\ antago} > EC_{50\ ago}$; potency is inferior to that of a full agonist alone (Fig. 10).

However, the terms insurmountable or irreversible refer to antagonists whose impact on an agonist's maximal efficacy E_{max} cannot be made up for by a large excess of said agonists, and is therefore "insurmountable". Such antagonists can yield these results through different molecular behaviors including long-lasting effects (irreversible binding, slow dissociation, fast dissociation & association rates), allosteric modulation, functional antagonism, or receptor internalization induction. In pharmacology, insurmountable antagonists are characterized in presence of a full agonist by a rightward shift of the dose/response curve, a decreased maximal response (Fig. 10), and the following inequations *(Neubig R.R, 2003):*

- $E_{full\ ago+antago} < E_{max}$; maximal efficacy is lower than that of the full agonist alone.
- $EC_{50\ full\ ago+antago} > EC_{50\ full\ ago}$; potency is inferior to that of the full agonist alone. *(Vauquelin G, 2002)*

The terms reversible and irreversible that are sometimes used in place of surmountable and insurmountable refer to the antagonists' effects on agonists' efficacy. They are not to be confused with reversible and irreversible binding, which come into play for long-lasting effect ligands (Fig. 11).

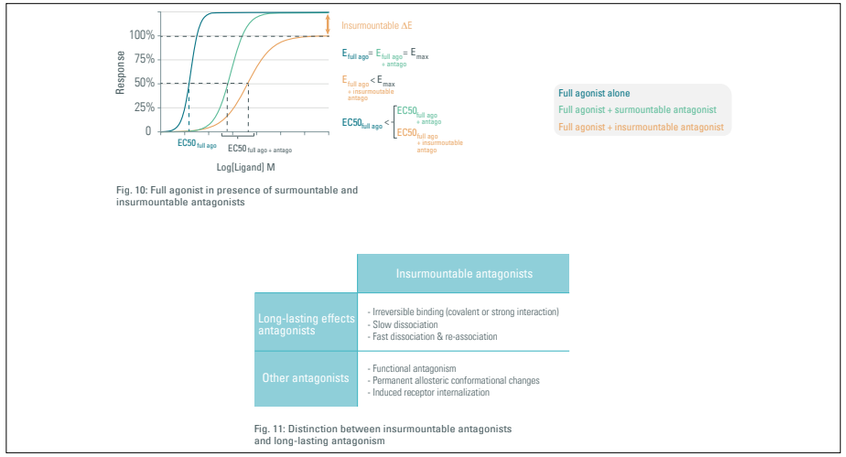

Fig. 10: Full agonist in presence of surmountable and insurmountable antagonists

Fig. 11: Distinction between insurmountable antagonists and long-lasting antagonism

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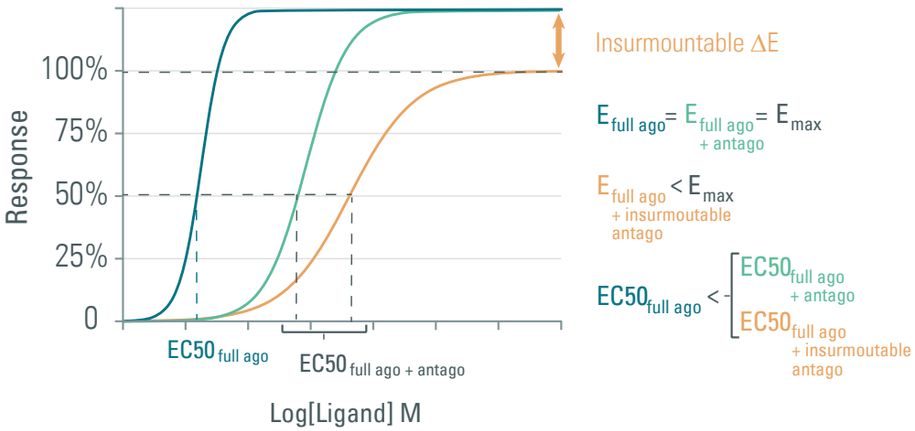

Fig. 10: Full agonist in presence of surmountable and insurmountable antagonists

	Insurmountable antagonists
Long-lasting effects antagonists	- Irreversible binding (covalent or strong interaction) - Slow dissociation - Fast dissociation & re-association
Other antagonists	- Functional antagonism - Permanent allosteric conformational changes - Induced receptor internalization

Fig. 11: Distinction between insurmountable antagonists and long-lasting antagonism

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ANTAGONISTS

Functional antagonism

Functional antagonists differ from all the previously mentioned antagonists as they do not decrease agonists' effects by interacting with the agonist receptor directly, but rather through opposite cell or tissue-mediated effects. In short, they are molecules which have effects of their own that negate those of an agonist (Kim J, 2005). A classic example of functional antagonism is that of epinephrine, which mediates higher arterial pressure through the A1-adrenergic receptor, and is a functional antagonist of histamine, mediating lower arterial pressure through the histamine receptor *(Keeney, 1950)*. Consequently, epinephrine is a common treatment for anaphylaxis, along with antihistamines (Fig.12).

As mentioned in the corresponding section, functional antagonists can be insurmountable if their effect is too strong to be overpowered a large amount of agonist (reference).

Long-Lasting effects

Some ligands, and especially antagonists, are qualified as long-lasting to describe their ability to induce effects in a permanent-like fashion. They are usually defined by observable dissociation rates equivalent to zero at relevant time scales. There can be several reasons for these ligands to behave the way they do, and the most common ones include:

- Irreversible binding to the receptor, either covalent or extremely tight,
- Slow dissociation from the receptor,
- Fast dissociation & re-association rates.

Due to their long-lasting effects, competitive antagonists that qualify in this category cannot be effectively displaced by agonists when competing for receptors, even at high agonist concentrations. Consequently, all long-lasting antagonists are also insurmountable (Fig. 11) and are characterized in presence of a full agonist by a rightward shift of the dose/response curve, a decreased maximal response (Fig. 10), and the same inequations as insurmountable antagonists *(Neubig R.R, 2003) (Dougall I.G, 2015)*.

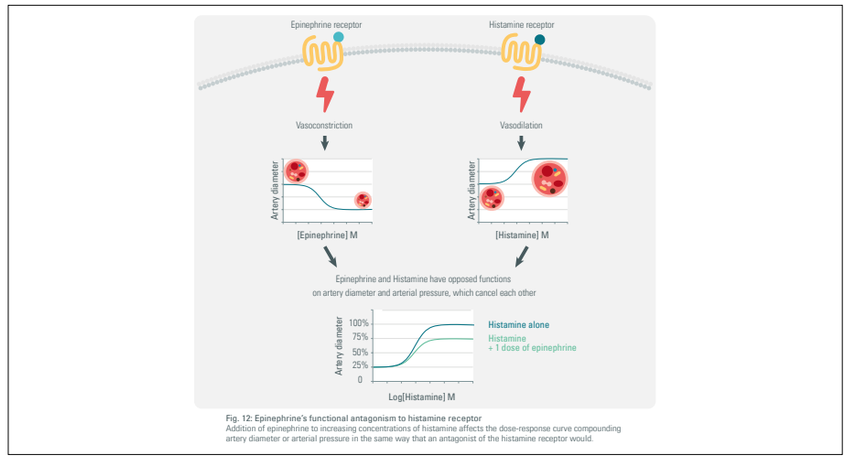

Fig. 12: Epinephrine's functional antagonism to histamine receptor
Addition of epinephrine to increasing concentrations of histamine affects the dose-response curve compounding artery diameter or arterial pressure in the same way that an antagonist of the histamine receptor would.

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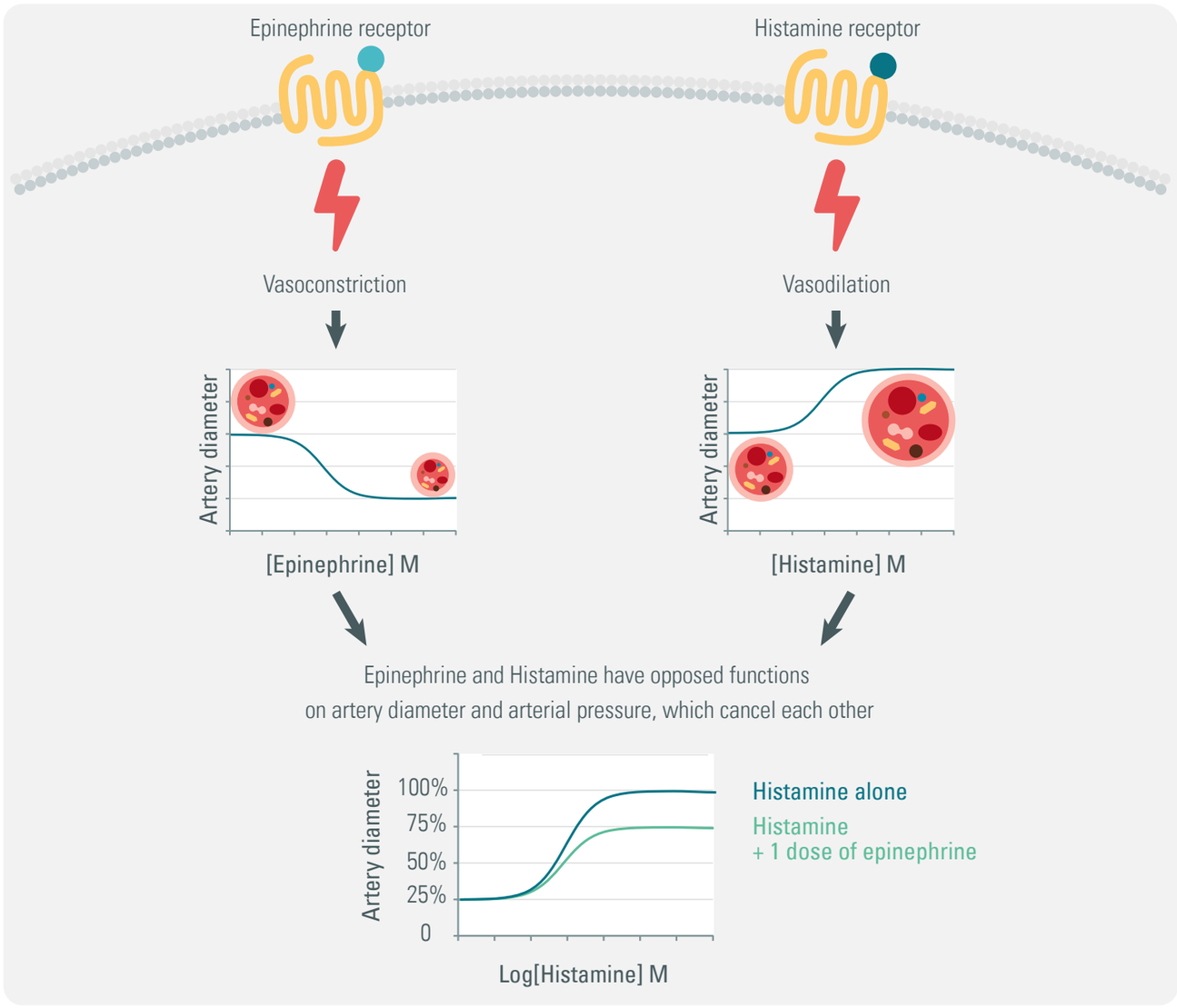

Fig. 12: Epinephrine's functional antagonism to histamine receptor
Addition of epinephrine to increasing concentrations of histamine affects the dose-response curve compounding artery diameter or arterial pressure in the same way that an antagonist of the histamine receptor would.

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INVERSE AGONISTS
Inverse agonists

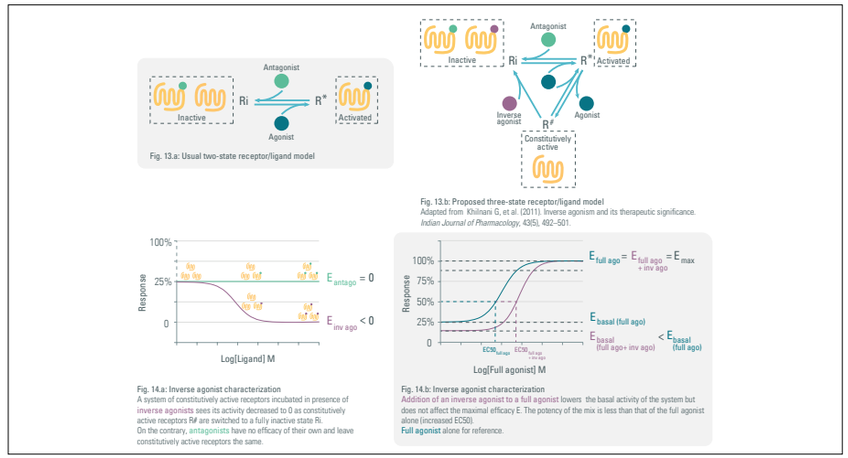
"A ligand that by binding to receptors reduces the fraction of them in an active conformation" – International Union of Pharmacology Committee

For a ligand to qualify as an inverse agonist, it requires its target receptor to exhibit some constitutive and spontaneous activity in the absence of agonist. This is a situation often observed in GPCRs, which is why inverse agonism tends to be relevant only for that family of receptors.

When this is the case, inverse agonists are ligands that produce the opposite effect to that of agonists upon binding their receptors' orthosteric site. They differ from antagonists in that they do not simply prevent the agonists' biological response to take place, but also induce an opposite response. Effectively, this means decreasing the constitutive activity of their receptor. For this reason, inverse agonists are understood to have a negative efficacy. Much like agonists, their effects can be opposed and impaired by antagonists likely to compete with them for receptors or that alter receptors. Inverse agonists have been challenging the usual 2-state model for receptors, which states that a receptor is either inactive (Ri) and free or antagonist-bound, or active and agonist-bound (Fig. 13.a). New models have been proposed to account for a third "constitutively active" state (Fig. 13.b) *(Khilnani G, 2011) (Berg K.A, 2018) (Neubig R.R, 2003).*

On their own, inverse agonists are recognizable for their ability to decrease biological activity without the presence of an agonist (as opposed to antagonists, which have no efficacy of their own) (Fig. 14.a). Inverse agonists are profiled in presence of full agonists by a decrease in basal activity and a rightward shift of the dose/response curve, which translates as a decrease in potency (Fig. 14.b).

- $E_{basal\ full\ ago+\ inv\ ago} < E_{basal\ full\ ago}$; basal activity is lower than that of the full agonist alone.
- $E_{full\ ago+\ inv\ ago} = E_{max}$; maximal efficacy is equal to that of the full agonist alone, provided the latter is present in a significant excess.
- $EC_{50\ full\ ago+inv\ ago} > EC_{50\ full\ ago}$; potency is inferior to that of the full agonist alone.



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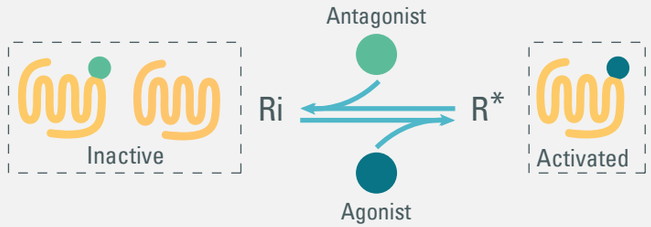

Fig. 13.a: Usual two-state receptor/ligand model

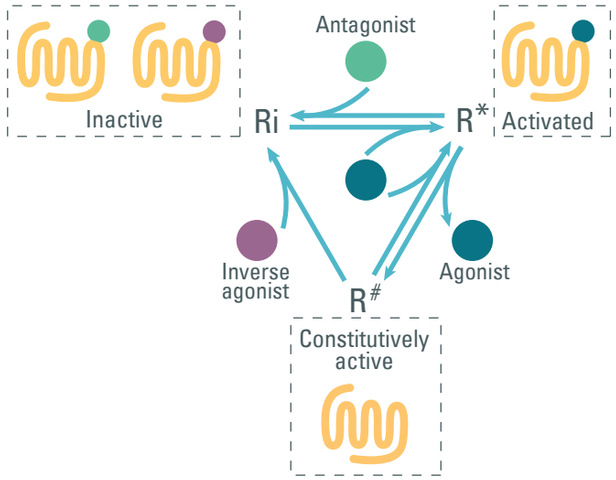

Fig. 13.b: Proposed three-state receptor/ligand model
Adapted from Khilnani G, et al. (2011). Inverse agonism and its therapeutic significance. *Indian Journal of Pharmacology*, 43(5), 492–501.

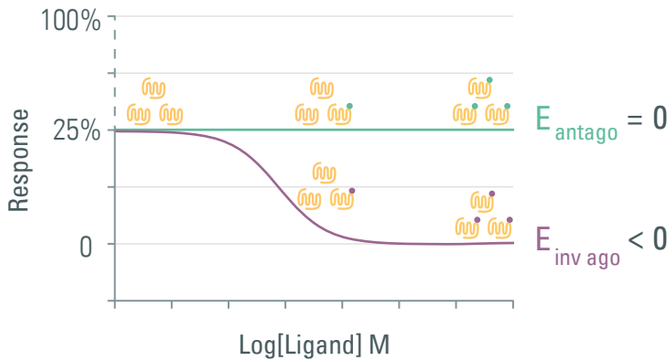

Fig. 14.a: Inverse agonist characterization
A system of constitutively active receptors incubated in presence of inverse agonists sees its activity decreased to 0 as constitutively active receptors R# are switched to a fully inactive state Ri.
On the contrary, antagonists have no efficacy of their own and leave constitutively active receptors the same.

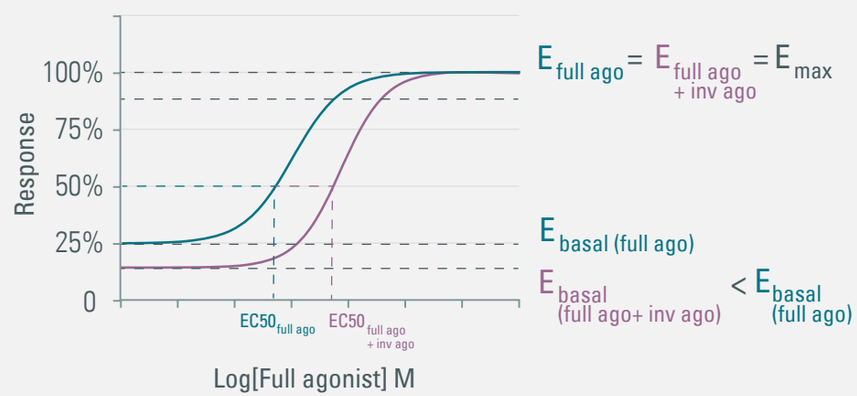

Fig. 14.b: Inverse agonist characterization
Addition of an inverse agonist to a full agonist lowers the basal activity of the system but does not affect the maximal efficacy E. The potency of the mix is less than that of the full agonist alone (increased EC50).
Full agonist alone for reference.

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ALLOSTERIC MODULATORS

Allosteric modulators (AM)

"A ligand that increases or decreases the action of an (primary or orthosteric) agonist or antagonist by combining with a distinct (allosteric or allotopic) site on the receptor macromolecule." – International Union of Pharmacology Committee

Compounds likely to bind a receptor on a site different from the orthosteric one are qualified as allosteric ligands. Among such ligands, modulators are those capable of inducing conformational changes in the receptors they bind, in a way that modulates said receptors' effects upon orthosteric agonist binding. Therefore, allosteric modulators are said to have indirect effects upon a receptor's signaling activity. Allosteric modulators have parameters of their own that characterize their properties:

A few allosteric ligands induce low levels of biological response on their own, on top of their modulation potential. The τ factor refers to their intrinsic efficacy in the same way that the efficacy E does for orthosteric ligands.

The α factor refers to the propensity of an allosteric modulator to affect the affinity of agonists for their receptors. Consequently, it also affects the potency of said agonists, as changes in affinity modify the amount of agonist necessary to induce a response similar to that of a given amount of agonist alone. In pharmacology the α factor of an AM is characterized in presence of agonists by a parallel horizontal shift of the dose/response curve and an increase or decrease in the agonist EC_{50} (Fig. 15.a).

The β factor corresponds to the ability of an allosteric modulator to affect the efficacy of agonists. In pharmacology, the β factor of an AM is characterized in presence of agonists by an increase or decrease in the agonist maximal response E_{max} (Fig. 15.b).

The α and β factors are independent numerical objects whose values indicate the direction of allosteric-induced modulations *(Revvity, 2016).*

α antd β	Cooperativity between the agonist and the modulator
Superior to 1	Positive cooperativity – The modulator enhances the agonist's parameters
Between 0 and 1	Negative cooperativity – The modulator decreases the agonist's parameters
Equal to 0	Neutral cooperativity – The modulator has no effect on the agonist's parameters

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ALLOSTERIC MODULATORS

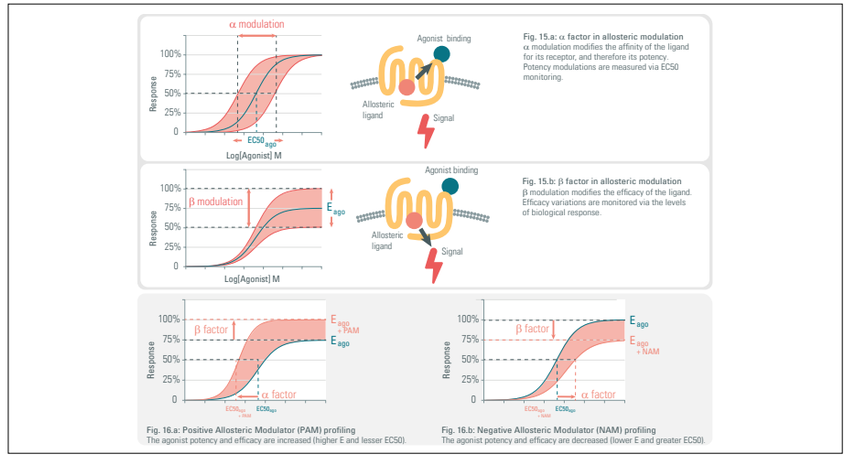
Allosteric enhancers and antagonists And neutral allosteric ligands

Depending on the direction of their effects on agonists, allosteric modulators are either allosteric enhancers - or antagonists - and are most often referred to as

- Positive Allosteric Modulators (PAM): Increase the affinity and/or efficacy of an agonist (Fig. 16.a).

- Negative Allosteric Modulators (NAM): Decrease the affinity and/or efficacy of an agonist (Fig 16.b).

Some allosteric ligands qualify as neither of these, and do not mediate any change in agonist effect. They are referred to as neutral allosteric ligands, which merely bind to their receptor away from the orthosteric site and do not carry any other properties *(Neubig R.R, 2003) (Burford N.T, 2011).*



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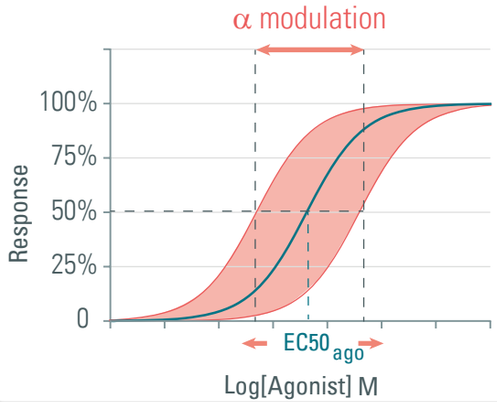


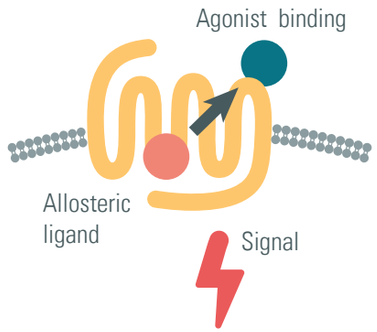
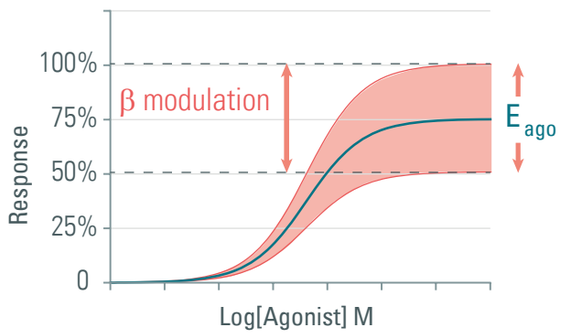


Fig. 15.a: α factor in allosteric modulation
α modulation modifies the affinity of the ligand for its receptor, and therefore its potency. Potency modulations are measured via EC50 monitoring.



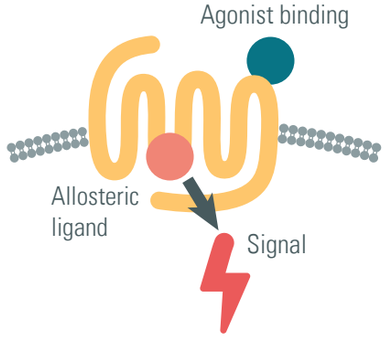


Fig. 15.b: β factor in allosteric modulation
β modulation modifies the efficacy of the ligand. Efficacy variations are monitored via the levels of biological response.

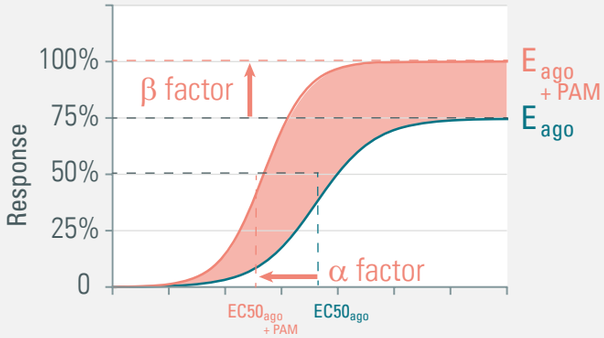


Fig. 16.a: Positive Allosteric Modulator (PAM) profiling
The agonist potency and efficacy are increased (higher E and lesser EC50).

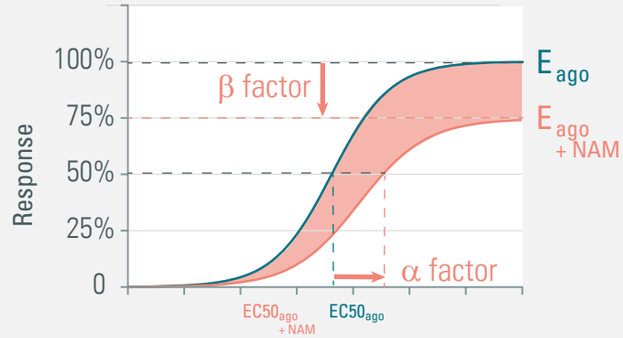


Fig. 16.b: Negative Allosteric Modulator (NAM) profiling
The agonist potency and efficacy are decreased (lower E and greater EC50).

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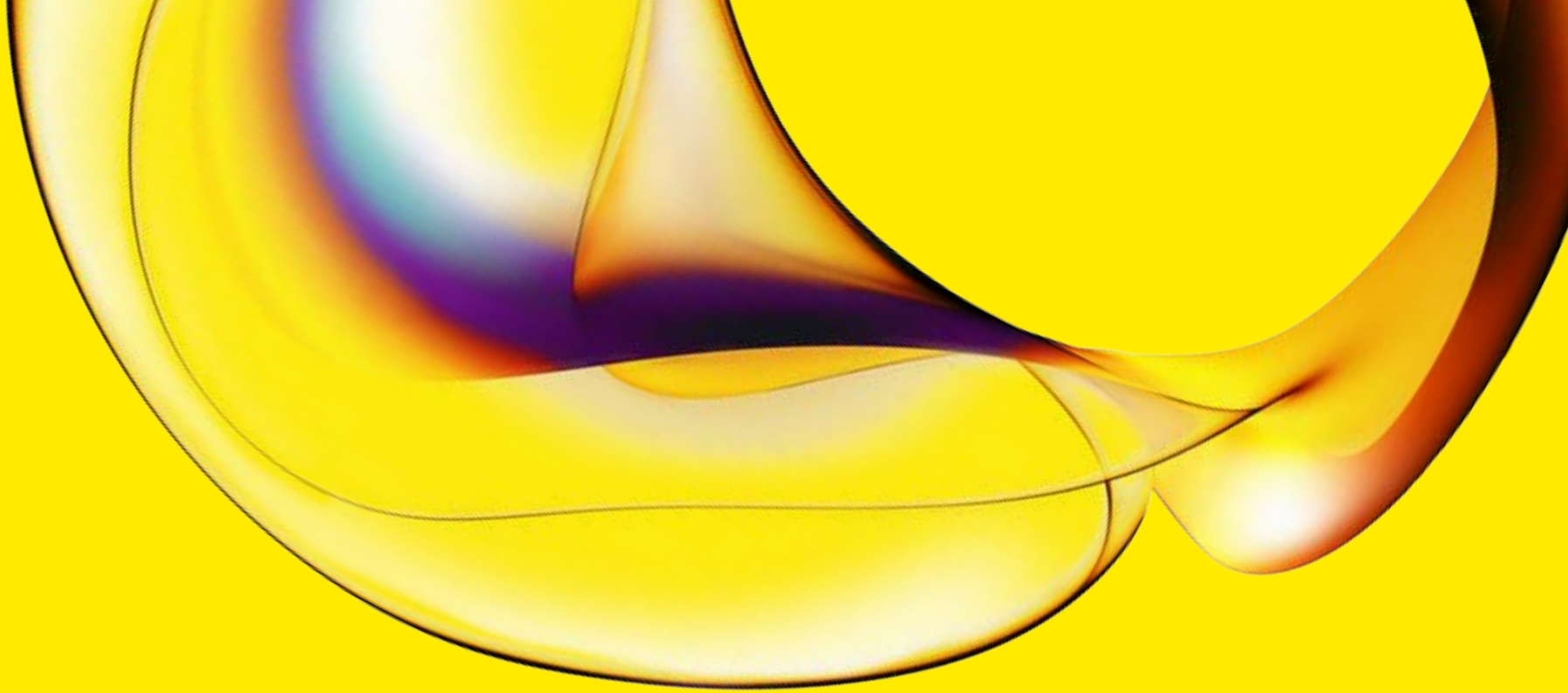

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