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Review

Allosteric modulators of the α4β2 subtype of neuronal nicotinic acetylcholine receptors

Anshul Pandya, Jerrel. L. Yakel *

Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC 27709, USA

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ABSTRACT

Nicotinic acetylcholine receptors are ligand-gated ion conducting transmembrane channels from the Cys-loop receptor super-family. The α4β2 subtype is the predominant heteromeric subtype of nicotinic receptors found in the brain. Allosteric modulators for α4β2 receptors interact at a site other than the orthosteric site where acetylcholine binds. Many compounds which act as allosteric modulators of the α4β2 receptors have been identified, with both positive and negative effects. Such allosteric modulators either increase or decrease the response induced by agonist on the α4β2 receptors. Here we discuss the concept of allosterism as it pertains to the α4β2 receptors and summarize the important features of allosteric modulators for this nicotinic receptor subtype.

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1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are cation-conducting pentameric transmembrane proteins that are activated by the endogenous neurotransmitter acetylcholine (ACh) [1]. These receptors are formed by the assembly of a variety of the 17 different subunits (α1–α10, β1–β4, γ, δ, ε) thus far known to exist, resulting in different subtypes of functional nAChRs [2]. The nAChRs at the neuromuscular junctions are composed of α1, β1, γ, δ, ε subunits and referred to as the muscle type of nAChRs, while those present at the synapse or other tissues in the nervous system are composed of α (2–10) and β (2–4) subunits and referred to as the neuronal type of nAChRs [3,4]. The main subtypes of nAChRs widely expressed in the brain are the homomeric α7 and the heteromeric α4β2 receptors [5–10]. The α4β2 receptors are expressed in two different stoichiometries, either the $(\alpha4)_2(\beta2)_3$ stoichiometry which binds to ACh and nicotine with high affinity,

Abbreviations: ACh, acetylcholine; nAChRs, neuronal nicotinic acetylcholine receptors; dFBr, desformylflustrabromine; PAM, positive allosteric modulator; NAM, negative allosteric modulator; 17-BE, 17-β-Estradiol.

* Corresponding author at: National Institute of Environmental Health Sciences, MD F2-08, P.O. Box 12233, Research Triangle Park, NC 27709, USA.
Tel.: +1 919 541 1407; fax: +1 919 541 4611.
E-mail addresses: pandyaaa@niehs.nih.gov (A. Pandya), yakel@niehs.nih.gov (Jerrel. L. Yakel).

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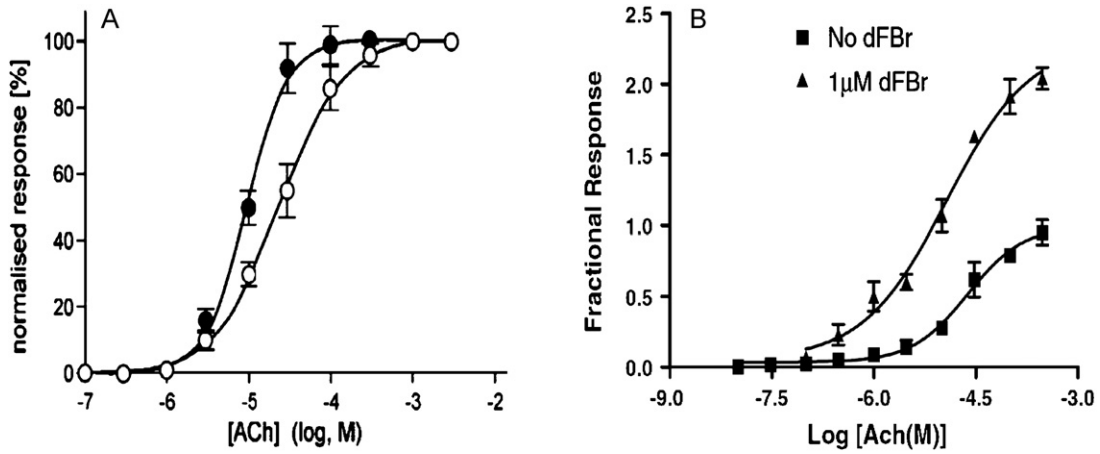

Fig. 1. Effect of α4β2 nAChRs PAMs, galantamine and dFBr on efficacy. (A) Galantamine does not change the maximal response obtained by Ach in α4β2 nAChR stably expressed in cultured HEK-293 cells [29]. (B) dFBr increases the maximal response obtained by Ach in α4β2 nAChR expressed in *Xenopus* oocytes [28].

or the $(\alpha4)_3(\beta2)_2$ stoichiometry which binds to ACh and nicotine with low affinity [11,12]. The α4β2 receptors have been implicated in a number of neurological conditions including (but not limited to) nicotine addiction [13], Alzheimer's disease [14], depression [15], impaired cognitive functions [16] and autism [17]. Positive allosteric modulators (PAMs) are rapidly being developed for neuronal nAChRs and they represent a promising new approach for treating disorders involving the α4β2 receptors [18–21].

1.1. Allosteric modulation of the α4β2 receptors

According to the allosteric theory, the α4β2 receptor protein has multiple conformations (states) [22]. The binding of ligand to the receptor reduces the free energy of the stabilized conformation and the energy from this ligand binding interaction provides the energy required to stabilize a certain conformation of the receptor [23]. Accordingly, an agonist for the α4β2 receptor is a ligand which stabilizes the open ion conducting conformation of the receptor. The binding site of ACh occurs at the α4(+)/β2(−) interface between the subunits forming the α4β2 receptor. For the purposes of this review, we have defined the ACh binding site on the α4β2 receptor as the orthosteric site. In addition to the orthosteric site, multiple distinct binding sites on the α4β2 receptor can be present where different ligands can bind. All such sites other than the orthosteric site are defined as allosteric sites. This is the situation in the related $GABA_A$ receptor which has multiple allosteric sites [24–26]. If an allosteric ligand stabilizes the open ion conducting conformation of the receptor channel that is induced by the agonist, such ligands are termed PAMs for the receptor [18,27,28].

On a macroscopic level for purposes of this review, we will define PAMs of the α4β2 receptors as ligands that increase potency (i.e. an increase in the apparent affinity; Fig. 1A and B) by acting at a site different from the orthosteric site [28]. PAMs for the α4β2 receptor can also increase efficacy (maximal response amplitude), however based on our working definition here, this is not a requirement for a PAM [28,29]. The change in efficacy caused by PAMs is rather varied and is discussed in Section 1.2. On a single channel level the effect of PAMs for α4β2 receptors translates into an increase in the number of openings or an increase in mean open times of the channel, indicating the stabilization of the open state of the receptor [18].

Some PAMs, in addition to being allosteric modulators by acting at an allosteric binding site, also act as partial or full agonists for the α4β2 receptor [20]. If such compounds are applied along with an agonist, then an increase in receptor response amplitude is observed over what would normally be achieved by the agonist alone. This is seen with some of the 2-amino-5-ketothiazole compounds when tested on the α4β2 receptors [20]. Such ligands may be classified as allosteric agonists or allosteric partial agonists; however for the purposes of this review, we do not consider these to be pure PAMs. As we have defined here, a pure PAM is one which binds to an allosteric site on the receptor, but fails to activate the channel by itself.

Negative allosteric modulators (NAMs) are ligands which *decrease* the maximal current (efficacy) or affinity of the agonist, and many NAMs have been discovered for the α4β2 receptor [30–35]. Similar to the PAMs, the NAMs bind to a site on the α4β2 receptors other than the orthosteric site, and inhibit receptor function by preferentially favoring a non-conducting conformation of the receptor. NAMs for the α4β2 receptor may bind in the lumen of the channel or at non-luminal sites on the receptor such as the extracellular-transmembrane interface, the intracellular loop or anywhere else on the protein [32,33]. There are several compounds for α4β2 receptors that are referred to as allosteric antagonists or noncompetitive antagonists [32,33,35–37], all of which would be considered to be NAMs as long as they bind to a site distinct from the orthosteric site and inhibit the function of the α4β2 receptors.

There may also be compounds yet to be discovered for α4β2 receptors which could potentially bind to the same allosteric site as a PAM on the receptor and competitively inhibit its binding and actions. For example for the $GABA_A$ receptors, one such compound is Ro15-1788 (Flumazenil) which binds to the benzodiazepine site and acts as an allosteric antagonist [25,38,39]. Such ligands for the α4β2 receptor, in particular or for other subtypes of nAChR, in general, may be consequential as they can inhibit the potentiation induced by PAMs by competing for the same allosteric binding site.

1.2. Efficacy changes in α4β2 receptors by PAMs

The effect of PAMs on potentiating the maximal response amplitudes of α4β2 receptors is varied. While a PAM increases the apparent affinity (potency) of the agonist for the α4β2 receptors, it may or may not change the maximal response amplitude (efficacy) elicited. Galantamine and desformylflustrabromine (dFBr) are both PAMs for the α4β2 receptors and display different effects on receptor efficacy. Galantamine when co-applied with ACh on human α4β2 nAChRs stably expressed in cultured HEK-293 cells causes an increase in apparent affinity without any change in efficacy (Fig. 1A) [29]. In contrast, dFBr (when co-applied with ACh) increases both apparent affinity and efficacy (Fig. 1B) [28]. This later effect is similar to that is seen with PNU-120596 on α7