The International Journal of Biochemistry & Cell Biology 65 (2015) 81–90

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Coronaridine congeners inhibit human α3β4 nicotinic acetylcholine receptors by interacting with luminal and non-luminal sites

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ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 28 April 2015
Accepted 15 May 2015
Available online 27 May 2015

Keywords:
α3β4 Nicotinic acetylcholine receptor
Coronaridine congeners
Allosteric modulation
Molecular modeling
Conformational states
Structure–activity relationship
In silico mutations

ABSTRACT

To characterize the interaction of coronaridine congeners with human (h) α3β4 nicotinic acetylcholine receptors (AChRs), structural and functional approaches were used. The Ca^{2+} influx results established that coronaridine congeners noncompetitively inhibit hα3β4 AChRs with the following potency (IC_{50}'s in μM) sequence: (−)-ibogamine (0.62 ± 0.23) ~ (+)-catharanthine (0.68 ± 0.10) > (−)-ibogaine (0.95 ± 0.10) > (±)-18-methoxycoronaridine [(±)-18-MC] (1.47 ± 0.21) > (−)-voacangine (2.28 ± 0.33) > (±)-18-methylaminocoronaridine (2.62 ± 0.57 μM) ~ (±)-18-hydroxycoronaridine (2.81 ± 0.54) > (−)-noribogaine (6.82 ± 0.78). A good linear correlation (r^2 = 0.771) between the calculated IC_{50} values and their polar surface area was found, suggesting that this is an important structural feature for its activity. The radioligand competition results indicate that (±)-18-MC and (−)-ibogaine partially inhibit [^{3}H]imipramine binding by an allosteric mechanism. Molecular docking, molecular dynamics, and in silico mutation results suggest that protonated (−)-18-MC binds to luminal [i.e., β4-Phe255 (phenylalanine/valine ring; position 13′), and α3-Leu250 and β4-Leu251 (leucine ring; position 9′)], non-luminal, and intersubunit sites. The pharmacophore model suggests that nitrogens from the ibogamine core as well as methylamino, hydroxyl, and methoxyl moieties at position 18 form hydrogen bonds. Collectively our data indicate that coronaridine congeners inhibit hα3β4 AChRs by blocking the ion channel's lumen and probably by additional negative allosteric mechanisms by interacting with a series of non-luminal sites.

1. Introduction

Coronaridine congeners, including (−)-ibogaine [12-methoxyibogamine or 7-ethyl-6,2,7,8,9,10,12,13-octahydro-2-methoxy-6,9-methano 5*H*-pyrido(1′,2′:1,2-azepine(4,5-)indole)] and (±)-18-methoxycoronaridine [(±)-18-MC], decrease drug self-administration in animals (reviewed in Maisonneuve and Glick, 2003), interrupt drug dependence in humans (reviewed in Alper et al., 2008), and behave pharmacologically as noncompetitive antagonists (NCAs) of several nicotinic acetylcholine receptors (AChRs) (Badio et al., 1997; Fryer and Lukas, 1999; Glick et al., 2002a; Pace et al., 2004; Arias et al., 2010a,b,c, 2011). Previous studies support the hypothesis that the inhibition of α3β4 AChRs expressed at the habenulo-interpeduncular cholinergic pathway is the main mechanism underlying the anti-addictive properties of coronaridine congeners (McCallum et al., 2012; Glick et al., 2002b; reviewed in Maisonneuve and Glick, 2003; Ortells and Arias, 2010). In addition, the inhibition of the habenulo-interpeduncular

Abbreviations: AChR, nicotinic acetylcholine receptor; NCA, noncompetitive antagonist; RT, room temperature; K_i, inhibition constant; K_d, dissociation constant; IC_{50}, ligand concentration that produces 50% inhibition of binding (or of agonist activation); n_H, Hill coefficient; EC_{50}, agonist concentration that produces 50% AChR activation; r^2, goodness-of-fit for the linear regression; DMEM, Dulbecco's Modified Eagle Medium; BSA, bovine serum albumin; (−)-ibogaine (or 10-methoxyibogamine), 7-ethyl-6,2,7,8,9,10,12,13-octahydro-2-methoxy-6,9-methano 5*H*-pyrido(1′,2′:1,2-azepine(4,5-)indole; (±)-18-MC, (±)-18-methoxycoronaridine; (±)-18-MAC, (±)-18-methylaminocoronaridine; (±)-18-HC, (±)-18-hydroxycoronaridine (or albifloranine), (−)-ibogamine; (−)-voacangine, (−)-10-methoxyibogamine-18-carboxylic acid methyl ester; (−)-noribogaine, (−)-10-hydroxyibogamine; (+)-catharanthine, (+)-3,4-didehydroibogamine-18-carboxylic acid methyl ester; PHE/VAL, phenylalanine/valine; SER, serine; LEU, leucine; PSA, polar surface area.

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http://dx.doi.org/10.1016/j.biocel.2015.05.015
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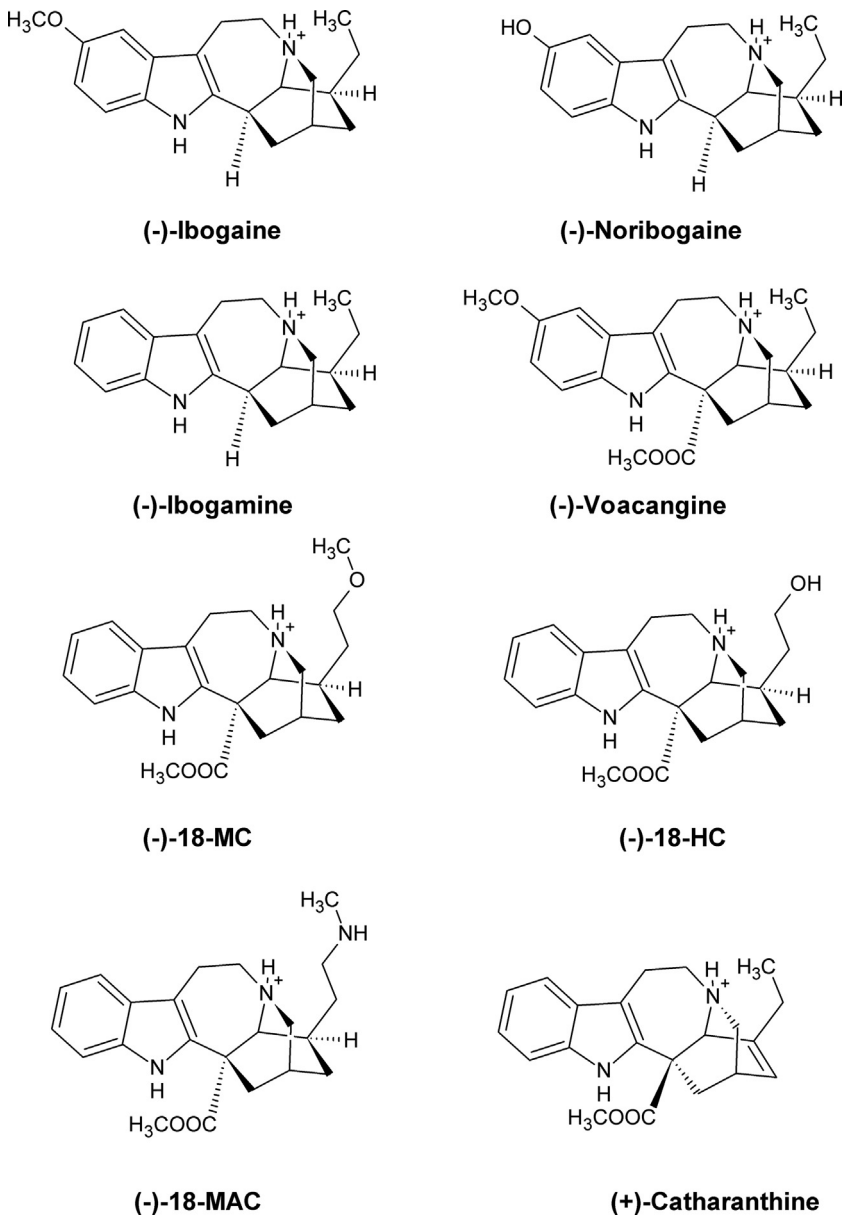

Fig. 1. Molecular structure of protonated coronaridine congeners, including (−)-ibogaine [10-methoxyibogamine or 7-ethyl-6,2,7,8,9,10,12,13-octahydro-2-methoxy-6,9-methano 5*H*-pyrido(1′,2′:1,2-azepine(4,5-)indole], (−)-noribogaine, (−)-ibogamine, (−)-voacangine, (−)-18-MC [(−)-18-methoxycoronaridine], (−)-18-HC [(−)-18-hydroxycoronaridine or albifloranine], (−)-18-MAC [(−)-18-methylaminocoronaridine], and (+)-catharanthine.

pathway modulates the dopaminergic brain reward circuitry located at the corticolimbic system.

Previous structure–activity relationship results from our laboratory suggest the existence of a threshold in the volume of the coronaridine congeners binding site within the *Torpedo* AChR ion channel, where ligands with molecular volumes significantly greater than 345 Å^3 do not fit the locus located between the serine and nonpolar rings (Arias et al., 2011). These results indicate that the size of the luminal binding site is an important structural feature for the pharmacological activity of these ligands. However, we do not know if this structural feature is also important for the human (h) α3β4 AChR, the most important target for these compounds. A better understanding of the functional and structural interaction of coronaridine congeners with the hα3β4 AChR is crucial to develop novel analogs for safer anti-addictive therapies. In this regard, a series of natural and synthetic coronaridine congeners (see molecular structures in Fig. 1) are tested by Ca^{2+} influx assays to determine the structure–activity relationship of these compounds at the hα3β4 AChR. Additional functional and structural studies are completed by combining radioligand binding assays as well as molecular docking and molecular dynamics approaches.

2. Materials and methods

2.1. Materials

[^{3}H]Imipramine hydrochloride (47.5 Ci/mmol) was obtained from PerkinElmer Life Sciences Products, Inc. (Boston, MA, USA), and stored in ethanol at −20 °C. (±)-18-Methoxycoronaridine hydrochloride [(±)-18-MC] was purchased from Obiter Research, LLC (Champaign, IL, USA). (−)-Ibogaine hydrochloride was obtained through the National Institute on Drug Abuse (NIDA) (NIH, Baltimore, USA). (±)-18-Methylaminocoronaridine [(±)-18-MAC], (+)-catharanthine, and (±)-18-hydroxycoronaridine [(±)-18-HC] were a gift from Dr. Kuehne (University of Vermont, VT, USA). (−)-Ibogamine (free base), (−)-voacangine (free base), and