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Catharanthine alkaloids are noncompetitive antagonists of muscle-type nicotinic acetylcholine receptors

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ABSTRACT

We compared the interaction of several catharanthine alkaloids including, ibogaine, vincristine, and vinblastine, with that for the noncompetitive antagonist phencyclidine (PCP) at muscle nicotinic acetylcholine receptors (AChRs) in different conformational states. The results established that catharanthine alkaloids: (a) inhibit, in a noncompetitive manner, (±)-epibatidine-induced Ca^{2+} influx in TE671-hα1β1γδ cells with similar potencies (IC_{50} = 17–25 μM), (b) inhibit [^{3}H]TCP binding to the desensitized *Torpedo* AChR with higher affinity compared to the resting AChR, and (c) enhance [^{3}H]cytisine binding to resting but activatable *Torpedo* AChRs, suggesting desensitizing properties. Interestingly, PCP inhibits [^{3}H]ibogaine binding to the AChR in a steric fashion. This is corroborated by additional docking experiments indicating that the amino groups of neutral ibogaine form hydrogen bonds with the serine ring (position 6′), a location shared with PCP. Since protonated ibogaine forms a salt bridge with one of the acidic residues at the outer ring (position 20′), this ligand could be first attracted to the entrance of the channel by electrostatic interactions. Our data indicate that the catharanthine moiety is a minimum structural requirement for AChR inhibition including, ion channel blocking and desensitization, and that ibogaine and PCP bind to overlapping sites in the desensitized AChR ion channel.

1. Introduction

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)] is an alkaloid obtained from the roots of the shrub *Tabernanthe iboga* (common name Black bugbane) that behaves pharmacologically as a noncompetitive antagonist (NCA) of both muscle- and neuronal-type nicotinic acetylcholine receptors (AChRs) (Glick et al., 2002; Badio et al., 1997; Fryer and Lukas, 1999; Arias et al., 2010a). AChRs are members of the Cys-loop ligand-gated ion channel superfamily that also includes types A and C γ-aminobutyric acid, type 3 5-hydroxytryptamine (serotonin), and glycine receptors (reviewed in Arias, 2006; Arias et al., 2006a; Albuquerque et al., 2009; Gotti et al., 2006). Furthermore, the inhibitory action elicited by ibogaine and its synthetic derivatives on neuronal AChRs seems to be related with its anti-addictive action (Glick et al., 2000, 2002; Glick and Maisonneuve, 1998). However, we do not have structural and functional information on the interaction of ibogaine with its binding site(s) when the AChR is in different conformational states, especially considering that AChR desensitization seems to play an important role in the process of drug addiction (reviewed in Ortells and Barrantes, 2010; Arias, 2009). As a first attempt to study this interaction we chose the muscle-type AChR because it is the archetype of the Cys-loop ligand-gated ion channel superfamily and because we can manipulate the different conformational states in a variety of *in vitro* assays. We also used this particular AChR subtype because the binding site locations for several NCAs have already been characterized, including that for phencyclidine (PCP) (Arias et al., 2003, 2006b; Sanghvi et al., 2008; Hamouda et al., 2008; Eaton et al., 2000; reviewed in Arias et al., 2006a; Arias, 2009). Taking advantage of this previous knowledge, the mutual interaction between ibogaine and PCP in the AChR will be compared. To further refine our understanding of the ibogaine interaction with the AChR, we will compare this interaction with

Abbreviations: AChR, nicotinic acetylcholine receptor; NCA, noncompetitive antagonist; PCP, phencyclidine; [^{3}H]TCP, piperidyl-3, 4-3H(*N*)]-(*N*-(1-(2 thienyl)-cyclohexyl)-3,4-piperidine; γ-BTx, γ-bungarotoxin; RT, room temperature; BS, binding saline; 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; DMEM, Dulbecco's Modified Eagle Medium; BSA, bovine serum albumin; FLIPR, fluorescent imaging plate reader.

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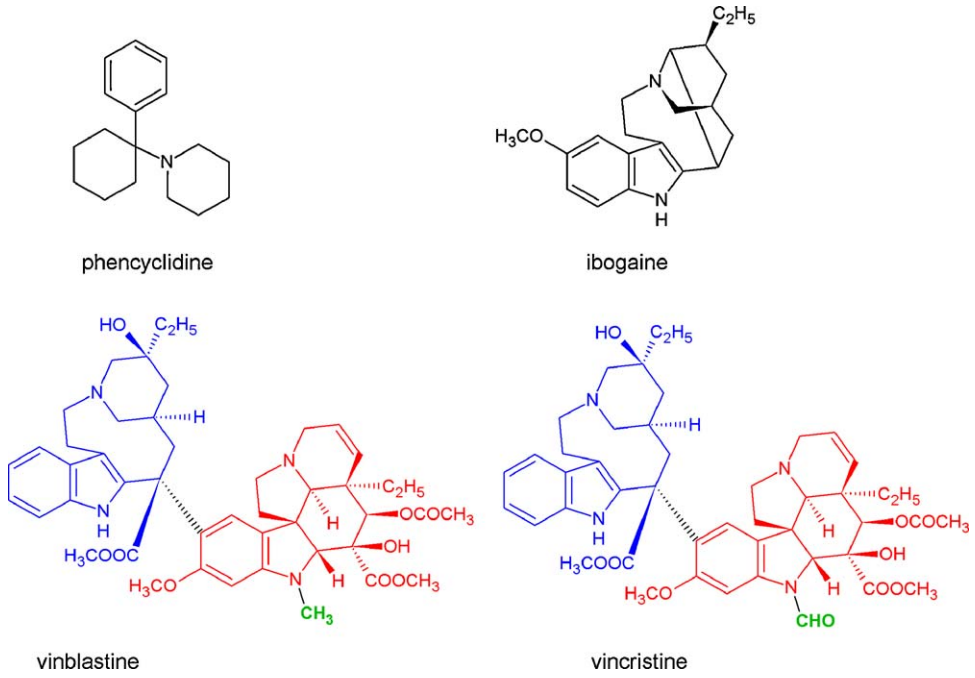

Fig. 1. Molecular structure of ibogaine, vinblastine, vincristine, and phencyclidine. The structures of vinblastine and vincristine, two vinca alkaloids, are formed by two polycyclic moieties, namely vindoline (red) and catharanthine (blue). The catharanthine portion is also the basic motif found in the ibogaine molecule. Vinblastine has a N-methyl group (green) in the indole ring, whereas vincristine has an aldehyde moiety (green) in the homologous position. Phencyclidine is a high-affinity noncompetitive antagonist with no structural resemblance with catharanthine alkaloids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

that determined for vinblastine and vincristine, two vinca alkaloids. Vinca alkaloids are antineoplastic agents whose structure is formed by two polycyclic moieties, namely vindoline and catharanthine (Roche, 2008), where the latter is also the basic motif found in the ibogaine molecule (see Fig. 1). To accomplish these objectives, we will use binding and functional approaches including radioligand binding assays using [^{3}H]ibogaine, the analog of PCP [^{3}H]TCP ([piperidyl-3, 4-^{3}H(*N*)]-*N*-(1-(2 thienyl)cyclohexyl)-3, 4-piperidine), and the agonist [^{3}H]cytisine, as well as Ca^{2+} influx assays, and molecular modeling and docking studies. Although this study does not intend to determine the anti-addictive properties of catharanthine alkaloids, the results from this work will pave the way for a better understanding of how these compounds interact with the AChR.

2. Materials and methods

2.1. Materials

[Piperidyl-3, 4-^{3}H(*N*)]-(*N*-(1-(2 thienyl)cyclohexyl)-3,4-piperidine) ([^{3}H]TCP; 45 Ci/mmol) and [^{3}H]cytisine hydrochloride (35.6 Ci/mmol) were obtained from PerkinElmer Life Sciences Products, Inc. (Boston, MA, USA), and stored in ethanol at −20 °C. [^{3}H]Ibogaine (23 Ci/mmol), ibogaine hydrochloride, and phencyclidine hydrochloride (PCP) were obtained through the National Institute on Drug Abuse (NIDA) (NIH, Baltimore, MA, USA). Vinblastine sulfate, vincristine sulfate, and (±)-epibatidine were purchased from Tocris Bioscience (Ellisville, MO, USA). Carbamylcholine chloride (CCh), suberyldicholine dichloride, and polyethylenimine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). α-Bungarotoxin (α-BTx) was obtained from Invitrogen Co. (Carlsbad, CA, USA). [1-(Dimethylamino) naphtalene-5-sulfonamido]ethyltrimethylammonium perchlorate (dansyltrimethylamine) was purchased from Pierce Chemical Co. (Rockford, IL, USA). Trypsin/EDTA was obtained from Gibco BRL (Paisley, UK). Salts were of analytical grade.

2.2. Preparation of Torpedo AChR native membranes

AChR native membranes were prepared from frozen *Torpedo californica* electric organs obtained from Aquatic Research Consultants (San Pedro, CA, USA) by differential and sucrose density gradient centrifugation, as described previously (Pedersen et al., 1986). Total AChR membrane protein was determined by using the bicinchoninic acid protein assay (Thermo Fisher Scientific, Rockford, IL, USA). Specific activities of these membrane preparations were determined by the decrease in dansyltrimethylamine (6.6 μM) fluorescence produced by the titration of suberyldicholine into receptor suspensions (0.3 mg/mL) in the presence of 100 μM PCP and ranged from 1.0 to 1.2 nmol of suberyldicholine binding sites/mg total protein (0.5–0.6 nmol AChR/mg protein). The AChR membrane preparations were stored at −80 °C in 20% sucrose.

2.3. Ca^{2+} influx measurements in TE671 cells

2.3.1. TE671 cells expressing human fetal muscle AChRs

The TE671 cell line is a human rhabdomyosarcoma cell line (obtained from American Type Culture Collection, USA) that endogenously expresses the human fetal muscle AChR (i.e., α1β1γδ). This subunit composition is the same as that for the *Torpedo* AChR and thus, comparative correlations can be assessed. TE671 cells were cultured in a 1:1 mixture of Dulbecco's Modified Eagle Medium (DMEM) and Ham's F-12 Nutrient Mixture (Seromed Biochrom, Berlin, Germany), supplemented with 10% (v/v) fetal bovine serum (FBS), as previously described (Michelmore et al., 2002; Arias et al., 2009, 2010a,b). DMEM/Ham's F-12 contains 1.2 g/L $NaHCO_3$, 3.2 g/L sucrose, and stable glutamine (L-alanyl-L-glutamine, 524 mg/L). The cells were incubated at 37 °C, 5% CO_2, and 95% relative humidity. For passaging (every 3 days), the cells were detached from the cell culture flask by washing with phosphate-buffered saline and brief incubation (3–5 min) with trypsin (0.5 mg/mL)/EDTA (0.2 mg/mL).

2.3.2. Ca^{2+} influx measurements

Ca^{2+} influx was determined as previously described (Michelmore et al., 2002; Arias et al., 2009, 2010a,b). Briefly, 5×10^4 TE671 cells per well were seeded 72 h prior to the experiment on black 96-well plates (Costar, New York, USA) and incubated at 37 °C in a humidified atmosphere (5% CO_2/95% air). 16–24 h before the experiment, the medium was changed to 1% bovine serum albumin (BSA) in HEPES-buffered salt solution (HBSS) (130 mM NaCl, 5.4 mM KCl, 2 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.9 mM NaH_2PO_4, 25 mM glucose, 20 mM HEPES, pH 7.4). On the day of the experiment, the medium was removed by flicking the plates and replaced with 100 μL HBSS/1%BSA containing 2 μM Fluo-4 (Molecular Probes, Eugene, Oregon, USA) in the presence of 2.5 mM probenecid (Sigma, Buchs, Switzerland). The cells were then incubated at 37 °C in a humidified atmosphere (5% CO_2/95% air) for 1 h. Plates were flicked to remove excess of Fluo-4, washed twice with HBSS/1% BSA, and finally refilled with 100 μL of HBSS containing different concentrations of the catharanthine alkaloid and preincubated for 5 min. For the case of ibogaine, additional experiments were performed by pre-incubating the cells for 2 and 24 h, respectively. To determine the inhibitory mechanism for vinblastine, additional experiments were performed by pre-incubating the cells with 10, 30, and 100 μM vinblastine, respectively, before the (±)-epibatidine-induced Ca^{2+} influx determinations. Plates were then placed in the cell plate stage of the fluorimetric imaging plate