(±)-Octahydro-2-methyl-*trans*-5 (1*H*)-isoquinolone methiodide: A probe that reveals a partial map of the nicotinic receptor's recognition site

Charles E. Spivak

Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD, USA

James A. Waters

Laboratory of Bio-Organic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA

Janardan S. Yadav,* Wen-Chung Shang, Mark Hermsmeier,† Rong Fa Liang,‡ and Tamara M. Gund

Department of Chemistry and Chemical Engineering, New Jersey Institute of Technology, Newark NJ, USA

*A new, semirigid, nicotinic agonist (±)-octahydro-2-methyl-*trans*-5 (1H)-isoquinolone methiodide was synthesized. The disposition of this agonist's nitrogen and carbonyl group conforms well to the prevailing notion of a pharmacophore for the nicotinic receptor. Comparing its structure and electrostatic potential surfaces, we predicted that its activity would be similar to that of carbamylcholine at the frog neuromuscular junction. Instead, the potency of the isoquinolone was only 0.015 times as potent as (+)-carbamylcholine. We conclude, after eliminating other possibilities, that the vicinity of the carbonyl group of an agonist must be planar to fit a confined space within the receptor's recognition site. The isoquinolone is a weak agonist because its methylene group β to the carbonyl intrudes on this space.*

Keywords: nicotinic receptor, receptor map, nicotinic agonist, molecular modeling

*Present address: University Medical School of New Jersey, Newark, NJ 07102.
†Present address: Squibb Pharmaceutical Company, Princeton, NJ 08540
‡Present address: BASF, New Jersey

Address reprint requests to Dr. Spivak at the Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224, USA.
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INTRODUCTION

A major step in the design of drugs is to discover the pharmacophore. Potent drugs serve as increasingly better guides to the pharmacophore as their structures are made more nearly rigid through synthetic modification.

Semirigid, nicotinic agents led Beers and Reich to deduce a pharmacophore for the nicotinic acetylcholine receptor in 1970.[1] In essence, this pharmacophore consists of a cationic center (usually a quaternary amine) and a hydrogen-bond acceptor (usually a carbonyl oxygen). The distance between the quaternary nitrogen and the van der Waals surface of the carbonyl oxygen in the direction defined by the carbonyl bond is 5.9 Å. Although subsequent work has supported this view[2–6], we recently showed that the Beers–Reich concept of the pharmacophore is incomplete.[5,6] We introduced several new, semirigid agonists with structures that approximate the proper Beers–Reich pharmacophore, but whose activities span four orders of magnitude.[6] The most potent agonist, isoarecolone methiodide (1-methyl-4-acetyl-1,2,3,6-tetrahydropyridine methiodide, Figure 1, Color Plates 2 and 3), is 50 times more potent than carbamylcholine (2-[(aminocarbonyl)-oxy]-N,N,N-trimethylethanaminium chloride). We used the latter as a standard for comparison because it is a potent, readily available analogue of acetylcholine that is stable in the presence of acetylcholinesterase at the frog neuromuscular junction.[5,6]

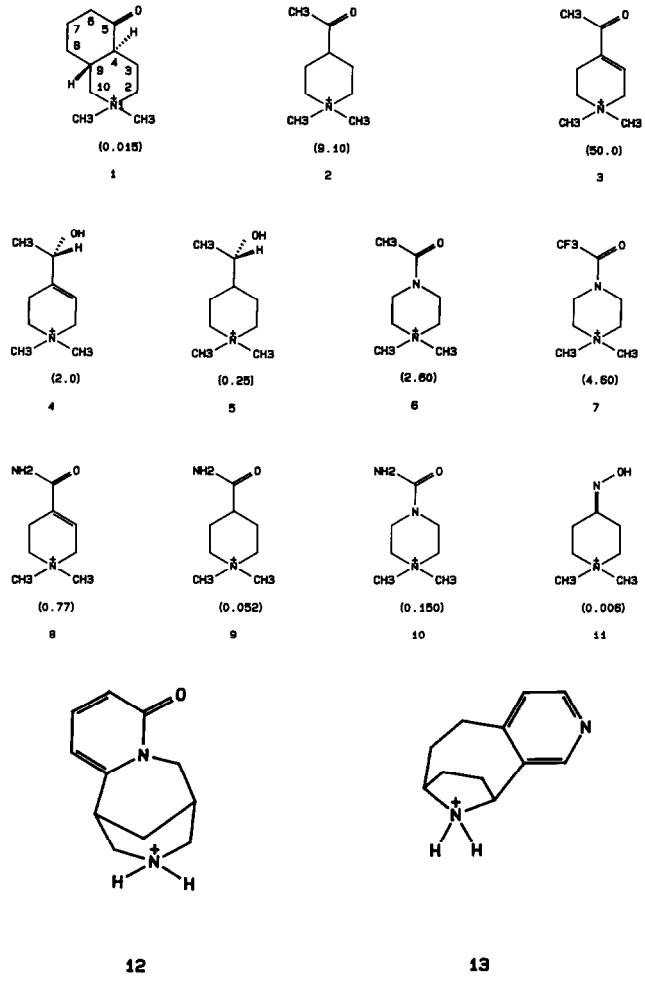

*Figure 1. Nicotinic Agonists: (±)-octahydro-2-methyl-(*trans*)-(1H)-isoquinolone methiodide (1), dihydroisoarecolone methiodide (2), isoarecolone methiodide (3), isoarecolol methiodide (4), dihydroisoarecolol methiodide (5), 1-methyl-4-acetylpiperazine methiodide (6), 1-methyl-4-(trifluoroacetyl)piperazine methiodide (7), 1-methyl-4-carbamyl-1,2,3,6-tetrahydropyridine methiodide (8), 1-methyl-4-carbamylpiperidine methiodide (9), 1-methyl-4-carbamylpiperazine methiodide (10), 1-methyl-4-piperidone oxime methiodide (11), cytisine (12), pyrido[2,3-b]-homotropane (13). Values in parentheses are measured potencies relative to carbamylcholine*

Because isoarecolone is one of the most potent agonists tested at this muscle, we used it as a template against which we compared analogues. All of the analogues consisted of a requisite hydrogen-bond acceptor (usually a carbonyl group) joined to a six-membered ring. The rings may flip from one chair conformation to the other, but torsion of the carbonyl group can compensate to yield identical, or very similar, structures. Thus, the only variable in the conformation of isoarecolone is the dihedral angle between the planes defined by C=C–C(O) and (ring) >C–C=O. For isoarecolone, the energy minima occur at 0° and 180° dihedral angles, with the 180° angle being lower in energy by 0.8 kcal/mol. Although both positions fit the Beers–Reich pharmacophore and the gauche conformation of acetylcholine, believed to be bioactive,[2,4] we took the 0° conformation (*s-cis*) as the bioactive one in analogy to anatoxin-*a* and to pyrido[2,3-*b*]-homotropane.[7,8] We used this conformation as a template for gauging the recognition site of the receptor and for comparing other potential agonists. The potencies of all of our analogues were rationalized under the hypothesis that this was indeed the bioactive conformation. We attributed the lower activities of the analogues to small deviations of their ground state conformations from the conformation of isoarecolone, to differences in electrostatic potential about both the cationic head and the region of the carbonyl group, and to the presence of a methyl group α to the carbonyl carbon.[6] These factors constitute an extended, testable Beers–Reich pharmacophore.

The extended pharmacophore was tested in this paper by locking the hydrogen bond acceptor into an equivalent to the *s-cis* position by forming it into a second, fused ring. The resulting compound, (±)-octahydro-2-methyl-*trans*-5(1*H*)-isoquinolone methiodide, was a direct, nearly rigid, analogue of dihydroisoarecolone methiodide, which is 9.1 times as potent as carbamyl-choline (it is second ranking in the series of analogues).[6] In contrast to predictions of the extended pharmacophore, isoquinolone was nearly inert. We conclude, by eliminating other possibilities, that a steric protrusion of the methylene group β to the carbonyl carbon impinges on a confined space in the recognition site.

CHEMISTRY

Synthesis

The intermediates to *trans*-2-methyloctahydro-5(1*H*)-isoquinolone methiodide have been described previously. We carried out low-pressure catalytic hydrogenation of 5-nitro-2-methylisoquinolinium *p*-toluenesulfonate over PtO_2 for 5 days using the procedure of Mathison and Gueldner.[9] Nitrous acid deamination[10] of the isomeric isoquinoline amines gave the isomeric 5-hydroxy-2-methyl-decahydroisoquinoline. Oxidation of the isomeric 5-hydroxy compounds with CrO_3 using the procedure of Kimoto and Okamoto[11] gave the known *trans*-2-methyloctahydro-5(1*H*)-isoquinolone exclusively, because oxidation of alcohols adjacent to a ring junction leads exclusively to the formation of the more stable *trans* fused ketone.[12] After removing impurities from the 5(1*H*)-ketone using column chromatography on silica gel, we converted the free base to the methiodide salt under the usual conditions.

Computer assisted molecular modeling

Details of the programs, parameterization and strategy have been given previously.[6,14] An abbreviated version follows.

We performed molecular modeling studies using an Evans and Sutherland PS330 color vector graphics terminal coupled to a VAX 11/785 computer. We generated conformations using the program Ring-Conformation-Generator (RCG5)[15] operating on a Cyber 205 supercomputer at the

John von Neuman Center in Princeton, NJ, USA. We calculated the conformation energies using a revised version of Allinger's MM2 program,[16] modified by T. Halgren[17] to handle formally charged molecules, and parameterized for ammonium salts in collaboration with J. Snyder.[18] Molecules were superimposed, displayed and manipulated using the Sybyl and Chem-X programs.[19,20] We calculated the partial charges using Dewar's MNDO program from MOPAC;[21] we derived the electrostatic potential (ESP) energies on the van der Waals surfaces from the partial charges and a contouring program, ARCHEM.[22] The dielectric constant was set to unity. The points selected for ESP determination make a uniform distribution on the van der Waals surfaces, and the atomic radii determine the extent of this surface. The areas of ESP ranges are determined by counting points within a range and multiplying by the area per point. This must be done separately for each atom type, as the area per point differs slightly among elements. The areas are very dependent on the atomic radii, which we took to be (Å): C, sp^3 = 1.70; C, sp^2 = 1.74; O = 1.50; N = 1.60; H = 1.30; and hydroxyl = 1.00. Electrostatic potentials are calculated[23] relative to an incoming, positive charge and are repulsive because the molecules have net positive charge. We used ARCHEM to calculate areas of electrostatic potentials on the van der Waals surfaces. Electrostatic potentials, projected as isopotential contours, were produced by Chem-X from the partial charges calculated from MNDO.

RESULTS AND DISCUSSION

The agonist isoquinolone (Figure 1) was subjected to an extensive conformation search. We obtained nine conformations after performing a filtering and minimization procedure and selected the four conformations with the lowest energies. These conformations are shown in Color Plate 1; their energies and conformation parameters are given in Table 1. We selected conformation *a* as the bioactive one because it has the least energy by 8.5 kcal/mol; it has a proper Beers–Reich distance of about 6 Å (the distance between the quaternary nitrogen and the van der Waals extension of the carbonyl oxygen); and it superimposes closely on the potent agonist dihydroisoarecolone.[6] We used isoarecolone and dihydroisoarecolone as templates in this study because they are semirigid and because they were shown previously[5,6] to be among the most potent nicotinic agonists (50 and 9.1 times, respectively, more potent than carbamylcholine). Color Plate 2 shows that the atoms of isoquinolone critical to the Beers–Reich pharmacophore (i.e., the quaternary N, and the C and O of the carbonyl group) can superimpose onto the same atoms of dihydroisoarecolone and isoarecolone, though in the latter the rest of the corresponding atoms deviate somewhat from each other. However, as Color Plate 2 shows, the superposition of isoquinolone onto dihydroisoarecolone is nearly perfect. Therefore, based on stereochemistry alone, one would expect isoquinolone to be about as effective as dihydroisoarecolone. However, the nicotinic acetylcholine receptor of the frog neuromuscular junction revealed that recognition was poor. Its potency with respect to carbamylcholine (defined as unity) was 0.015 (7 frogs; 95% confidence interval, 0.013–0.017). Thus, agonist isoquinolone was only 0.0016 times as potent as dihydroisoarecolone.

We sought an explanation for the low activity of the bicyclic agonists in the electrostatic field calculations. We calculated partial charges after choosing the bioactive conformation of isoquinolone. We projected electrostatic potentials, using ARCHEM,[22] onto the van der Waals surfaces of the agonists (Color Plate 3). Some differences between isoquinolone and the more potent agonists dihydroisoarecolone and isoarecolone are apparent. Table 2 lists the areas we found for each calculated range of potential. The most apparent difference appears in the 120–140-kcal range where isoquinolone shows less brown in Color Plate 3 than dihydroisoarecolone or isoarecolone and less calculated area in Table 2. The most potent agonist, isoarecolone, shows electrostatic potentials even in the 140–160-kcal range. If one

Table 1. Conformational parameters and energies for the four lowest energy conformations of isoquinolone

Conformation	Energy (kcal/mol)	Beers–Reich distance (Å)	Dihedral Angle (O=C–CH–CH, deg.)
a	18.4	6.2	−2.0
b	26.9	5.8	8.5
c	27.4	6.3	−7.4
d	28.8	6.3	−1.6

Table 2. Areas (square Angstroms) of electrostatic potentials on the van der Waals surfaces

	Energy range (kcal/mol)						
Agonist	0–40	40–60	60–80	80–100	100–120	120–140	140–160
Isoquinolone	7.66	16.02	69.89	66.93	77.02	14.73	0.00
Dihydroisoarecolone	7.46	17.14	39.16	63.69	84.52	18.37	0.00
Isoarecolone	7.99	19.24	33.52	45.58	88.39	22.85	0.78

sums the most negative ranges (0–60 kcal) and the most positive (100–160 kcal) for the three compounds, isoquinolone has the lowest area in the 0–60-kcal range (23.68) and in the 100–160-kcal range (91.75). Dihydroisoarecolone follows next, with an area of 24.68 and 102.89 in the 0–60-kcal and 100–160-kcal ranges, respectively. The most potent agonist isoarecolone has the highest area in both ranges (27.23 and 112.02). Thus from electrostatic considerations, ordering occurs that matches the observed order of potency. The ARCHEM projections of other, weak agonists, however, differ more from the more potent ones than isoquinolone, especially in the vicinity of the carbonyl oxygen.[6a] Steric and electrostatic considerations both predict, therefore, that isoquinolone should occupy the recognition site with a much higher affinity than the potency studies indicate.

A possible explanation is that isoquinolone does occupy the receptor's recognition site with high affinity but fails to activate or only weakly activates the cation channel; i.e., it is an antagonist or a partial agonist. However, binding experiments using various concentrations of the agonist to block the binding of the receptor-specific toxin [^{125}I]α-bungarotoxin, showed that isoquinolone is less than 1/100 as potent as dihydroisoarecolone and isoarecolone at the nicotinic receptor of the *Torpedo nobiliana* electric organ.[24]

By elimination, only three explanations for the low activity of isoquinolone remain: Either the pharmacophore is wrong; the agonist is too rigid; or isoquinolone contains a steric obstruction that prevents binding. The first explanation opposes the weight of accumulated evidence.[1–6,8] We reject the second as well because cytisine[26,27] and pyrido[2,3-*b*]-homotropane,[8] both of which are highly constrained by fused, tricyclic structures, are potent agonists.

A steric obstacle, therefore, seemed the only plausible explanation for the low activity of isoquinolone. The general shape of the most potent nicotinic agonists (best seen in space-filling models or stereo diagrams) is a planar region bearing the hydrogen-bond acceptor (the carbonyl oxygen here) adjoined to a bulky cationic head. The template isoarecolone displays this form nicely due to the double bond in the ring conjugated to the carbonyl group (Color Plates 2 and 3). Cytisine and pyridohomotropane both contain systems of conjugated double bonds that enforce planarity of the corresponding regions. However, isoquinolone bears a potential obstruction due to the methylene group in the 7 position (see modified numbering scheme, Figure 1), as revealed in Color Plates 2 and 3. We suggest that the axial hydrogen of this methylene imposes on a confined space within the receptor's recognition site. Thus, through this repulsive interaction, isoquinolone provides strong evidence for a particular topographical constraint in the native receptor.

Receptor mapping studies

To test our hypothesis we performed receptor mapping studies using the excluded volume technique of Marshall.[28–30] In this method active agonists in their bioactive conformations are identified and superimposed and the sum of the active volume is calculated. Inactive analogs are identified and treated in the same way. The union of the active and inactive volumes is found, and the active volume is then subtracted from the inactive volume. The result is a receptor topography that gives a measure of the amount of steric repulsion afforded by the inactive analogs.

The active analogs for this study included isoarecolone and its congeners, i.e., agonists 2–8 and 10 in Figure 1. The inactive analogs included the isoquinolone, the amide derivative of saturated isoarecolone, and 4-piperidoxime. Color Plate 4 depicts the map with the active analogs. Color Plate 5 depicts the map with the inactive analogs inside the cavity. The portion of the acetyl methyl group that is responsible for the steric hindrance is clearly seen. The cavity may be fitted with other ligands, and Color Plate 6 shows a fitting with cytisine,[26,27] an agonist of moderate activity. The good fit indicates that cytisine fits the geometric constraints that are associated with active agonists, but provides some steric obstruction, as seen by the white region of the map.

We conclude that the low activity of isoquinolone must be due mainly to a steric obstruction between position 7 in the isoquinolone ring and the receptor's recognition site.

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APPENDIX: EXPERIMENTAL SECTION

Chemistry

Melting points were taken on a Kofler hot stage and are corrected. We recorded the NMR spectrum on a Varian XL-300 spectrometer; CIMS on a Finnigan 1015 spectrometer; and infrared spectra on a Beckman 4230 spectrophotometer. Elemental microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA, USA and are within 0.4% of the theoretical values. We purchased 5-nitroisoquinoline and methyl-*p*-toluene-sulfonate from Aldrich Chemical Co., Milwaukee, WI, USA.

5-nitro-2-methylisoquinolinium *p*-Toluenesulfonate

We prepared this compound from 5-nitroisoquinoline and methyl *p*-toluenesulfonate according to the procedure of Mathison and Gueldner.[9]

5-Amino-2-methyldecahydroisoquinoline

We prepared this compound in a manner similar to that described by Mathison and Gueldner.[9] We hydrogenated 5-nitro-2-methylisoquinolinium *p*-toluenesulfonate (12.5 g, 0.035 mol) in 75 mL of glacial acetic acid containing 0.3 mL

conc. H_2SO_4 over PtO_2 (2.0 g) at 48 psi for 5 days. After removing the acetic acid *in vacuo*, we made the isomeric mixture alkaline with 28 mL of 6N NaOH. Extraction with ether and chloroform gave 2.6 g of an oily mixture of the desired isomeric amines and a by product (mol. wt. 162, CIMS) in ca. 0.7:1.0 ratio. Saturation of the aqueous layer with NaCl, addition of 4 mL of 6 N NaOH, and extraction with $CHCl_3$ (dried over $MgSO_4$) gave 3.1 g (54%) of the desired isomeric 5-amino-2-methyldecahydroisoquinolines as a tan solid (pure by tlc, $CHCl_3$:MeOH, 6:4). Spectral analysis gave ir ($CHCl_3$): 2790 (N–CH_3), 1625 and 1580 (NH deform.), 1460, 1375, 1160, 1115 (C–N str.), 1005 cm^{-1}, CIMS (NH_3): m/e 169 (M + 1).

5-Hydroxy-2-methyldecahydroisoquinoline

We prepared this compound by the nitrous acid deamination procedure described by Mathison and Morgan.[10] From 1.0 g (6 mmol) of 5-amino-2-methyldecahydroisoquinoline (diastereoisomeric mixture) we obtained 516 mg (51%) of 5-hydroxy-2-methyldecahydroisoquinoline (isomeric mixture) as a clear, tan-colored oil. The product was homogeneous by tlc, $CHCl_3$:MeOH, 6:4. Spectral analysis gave ir ($CHCl_3$): 3550 cm^{-1} (OH); CIMS (NH_3): m/e 170 (M + 1).

The methiodide salt of the free base was obtained as follows: From 32 mg of the 5-hydroxy-2-methyldecahydroisoquinoline in 0.3 mL of acetone containing 0.02 mL of CH_3I (warmed at 50–55°C for 2 hours), we obtained 33 mg of crude solid. Recrystallization from absolute EtOH gave the methiodide salt of the isomeric 5-hydroxy compound as beige-colored flakes, double mp of 202–205 and 238–242°C. *Anal.* calcd. for $C_{11}H_{22}INO$: C, 42.45; H, 7.12; N, 4.50. Found: C, 42.53; H, 7.13; N, 4.47 (Atlantic Microlab, Inc., Atlanta, GA).

trans-2-Methyloctahydro-5 (1*H*)-isoquinolone

We prepared this compound by the oxidation procedure described by Kimoto and Okamoto.[11] A mixture of 889 mg (5.3 mmol) of 5-hydroxy-2-methyldecahydroisoquinoline (isomeric), 800 mg of CrO_3 in 1.5 mL of H_2O, and 3.0 mL of glacial AcOH was heated at 60–70° for 6 hours. After workup, the crude 5-keto compound, containing trace amounts of several impurities by tlc, was column chromatographed on silica gel (18 g, 230–400 mesh). Elution with 2–12% MeOH in $CHCl_3$ gave 242 mg (27%) of pure *trans*-2-methyloctahydro-5(1*H*)-isoquinolone, homogeneous by tlc, $CHCl_3$:MeOH, 6:4. Spectral analysis gave ir ($CHCl_3$): 1695 (C=O), 1358, 1305, 1140, 1078, 985, and 968 cm^{-1}. CIMS (NH_3): 168 (M + 1).

The picrate salt of the free base (yellow, shiny flakes from MeOH), gave mp 215–217.5° C; reported mp 211–213 and 213° C.[11,12]

trans-2-methyloctahydro-5 (1*H*)-isoquinolone methiodide

To 102 mg of the 5-keto free base in 1.0 mL of absolute EtOH we added 0.08 mL of CH_3I. We heated the mixture at 75–78° for 3.5 hours. Evaporation of the solvent with N_2 gave a brown semisolid. Recrystallization from acetone-ethyl acetate (3:1) gave 96 mg of tan crystals. A second recrystallization from the same solvent system gave 76 mg of *trans*-2-methyloctahydro-5(1*H*)-isoquinolone as beige-colored prisms, mp 196–197°C (dried at 56° C at 0.1 mm pressure, 2 hours). A third recrystallization gave the analytical sample, mp 198.5–199° C. Spectral analysis yielded CIMS (NH_3): m/e 168 (M + 1 − CH_3I); ^1H NMR (D_2O): 3.6-3.1 (m's, 4, C-1 and C-3 CH_2), 3.16 and 3.08 (singlets, 6, $N(CH_3)_2$), 2.54 (m, 2, C-6 CH_2), 2.40 (m, 1, C(=O)-*CH*), 2.21, 1.99, 1.76, and 1.57 (m's, 7, C-4, C-7, C-8 CH_2 and C-9 CH; unassigned). *Anal.* calcd. for $C_{11}H_{20}INO$: C, 42.73; H, 6.52; N, 4.53. Found: C, 42.79; H, 6.55; N, 4.53 (Atlantic Microlab, Inc., Atlanta, GA).

Pharmacology

We evaluated potency, expressed as the reciprocal of the equipotent molar ratio in reference to carbamylcholine, by isotonic contracture of the *rectus abdominis* muscle from the frog *Rana pipiens* exactly as described previously.[6] Because of the low activity of the isoquinolone, muscles were treated with agonists for 5 minutes before the response was read.

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