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Binding of Semirigid Nicotinic Agonists to Nicotinic and Muscarinic Receptors

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SUMMARY

Isoarecolone methiodide (1-methyl-4-acetyl-1,2,3,6-tetrahydropyridine methiodide) was previously shown to be among the most potent agonists tested at the frog neuromuscular junction. Because nicotinic receptors from different sources vary in their selectivities, isoarecolone methiodide as well as 19 additional congeners, most of which were also previously tested at the frog neuromuscular junction, were studied in binding assays. *Torpedo nobiliana* was the tissue source for nicotinic receptors. Two types of experiments were conducted. The first evaluated the affinities of the agonists (including acetylcholine and carbamylcholine) for the recognition site by allowing the agonists to compete for that site with ^{125}I-α-bungarotoxin. The inhibition potencies obtained correlated strongly (Spearman's correlation coefficient, −0.91) with the potency obtained at the frog neuromuscular junction. The second type of experiment evaluated the agonists for their ability to activate the receptor. The binding of [^{3}H]perhydrohistrionicotoxin, which was employed as an indicator of the activation of the receptor, was measured in the presence of each of the agonists. Isoarecolone methiodide was the most potent of all. A few of the agonists (partial agonists) were incapable of fully enhancing this binding. For the full agonists, the concentration that produced half of the maximum binding of [^{3}H]perhydrohistrionicotoxin was defined as the EC_{50}. The correlation coefficient (Spearman's) for EC_{50} versus potency at the frog neuromuscular junction was −0.73, indicating innate differences between *Torpedo* and frog receptors. In addition, these compounds were tested for their affinity at muscarinic receptors from rat brain. Competition experiments were carried out using [^{3}H]*N*-methylscopolamine. The affinity of isoarecolone methiodide was only about 7-fold lower than that of acetylcholine and less than 2-fold lower than that of carbamylcholine. In contrast, 1-methyl-4-acetylpiperazine methiodide was much more selective for nicotinic receptors. Its activity was similar to isoarecolone methiodide at the nicotinic receptor, but it was among the weakest compounds in its affinity for the muscarinic receptor.

The nAChR was the first purified and the first sequenced and remains the best understood of all the receptors for drugs and neurotransmitters (reviewed in Refs. 1–6). Despite the scrutiny to which the nAChR has been subjected, the mechanism whereby it transduces the binding of an agonist into the opening of its ion channel remains obscure. One approach toward gaining an understanding of its transduction mechanism is to probe the recognition site with agonists sufficiently potent and rigid that one can identify the atoms and bonds essential for activity. In 1970 Beers and Reich (7) deduced a cogent model for a pharmacophore for this receptor. Since then, their pharmacophore has been supported by other studies (8–12). Recently, however, we showed that the Beers and Reich pharmacophore is incomplete. We introduced several new semi-rigid agonists, all of which bear the pharmacophore, but whose activities, evaluated at the frog neuromuscular junction, spanned 4 orders of magnitude (12–14). Additional factors, such as small deviations from the optimal conformation, electrostatic potentials about regions of the agonist, the presence of a methyl group α to the carbonyl carbon, and the degree of solvation, helped to explain the potencies of the agonists.

Among the new agonists was isoarecolone methiodide (**1**, Fig. 1), which was 50 times more potent than carbamylcholine at the frog neuromuscular junction (13). It is, therefore, one of the most potent agonists tested at this receptor. Several other related compounds also approached or exceeded carbamylcholine in activity (12–15). The analogous structures of these agonists make them useful in studies of how subtle differences in the agonist molecule alter the activation of the ion channel of the receptor (16, 17).

Part of the future utility of these new agonists depends upon the extent to which the activity seen at the frog neuromuscular junction generalizes to other ACh receptors. It has already been shown that **1** is highly active in displacing (−)-nicotine at

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor of electric organs or voluntary muscle; mAChR, muscarinic acetylcholine receptor of the central nervous system; ^{125}I-BGT, ^{125}I-α-bungarotoxin; [^{3}H]H_{12}-HTX, [^{3}H]perhydrohistrionicotoxin; [^{3}H]MS, [^{3}H]*N*-methylscopolamine; ACh, acetylcholine; PHT, pyrido[3,4-*b*]homotropane; CIMS, chemical ionization mass spectrometry, EIMS, electron impact mass spectrometry.

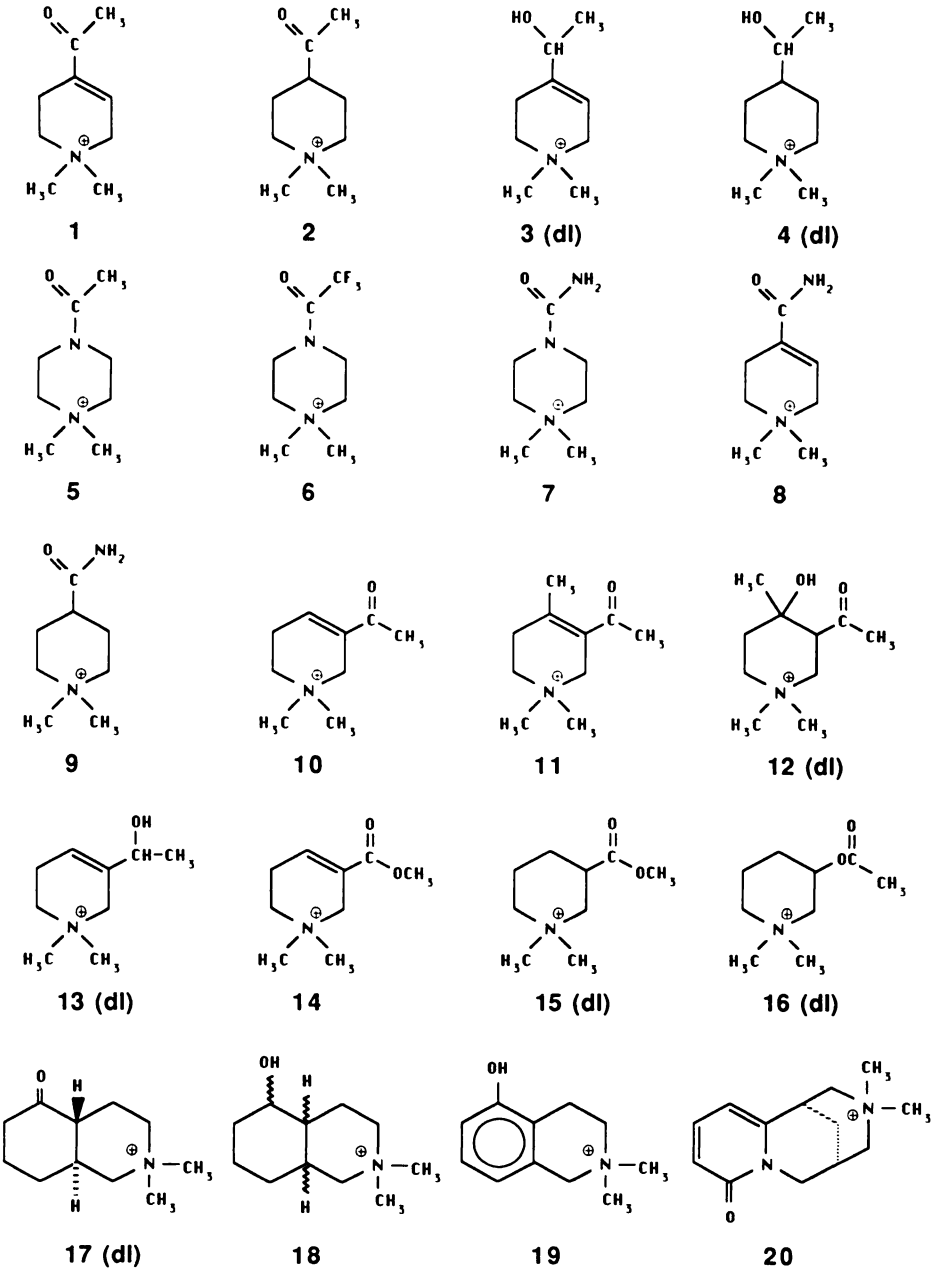

Fig. 1. Chemical structures of all of the agonists tested in the binding experiments.

nAChRs from rat brain (18). One of the most useful tissues for studying the nAChR is the electric organ from *Torpedo*, because the density of the nAChR is higher in this tissue than in other sources (19). However, the activity of the new agonists in *Torpedo* may not parallel that observed at the frog neuromuscular junction or at ganglia (6). An example of how activity depends on the source of the nAChR is the photoisomerizable agonist *trans*-bis-Q, which is 30 times more potent at *Electrophorus* receptors than it is at myoball receptors (20). A second area of interest is how specific these agents are for nicotinic, as opposed to muscarinic, receptors. In this paper we characterize 20 of the nicotinic agonists (Fig. 1) by their ability to bind to and activate the nAChR from *Torpedo* and by their ability to bind to central muscarinic receptors from rat brain.

Materials and Methods

Chemistry. Melting points were taken on a Kofler hot stage and are corrected. CIMS was on a Finnigan 1015 spectrometer. EIMS was on a VG Micromass 7070F spectrometer. IR spectra were recorded on a Beckmann 4230 spectrophotometer. NMR spectra were recorded on a JOEL FX-100 spectrometer. Elemental microanalyses were performed by Atlantic Microlab, Inc. (Atlanta, GA). Starting materials for syntheses were purchased from Aldrich Chemical Co. (Milwaukee, WI); cytisine was obtained from ICN Pharmaceutical, Inc. (Plainview, NY).

The syntheses of compounds **1** (isoarecolone methiodide), **2** (dihydroisoarecolone methiodide), **3** (isoarecolol methiodide), **4** (dihydroisoarecolol methiodide), and **5** (1-methyl-4-acetylpiperazine methiodide) were described previously (12). Compounds **6**, **7**, **8**, and **9** have also been described (12, 14).

Arecolone (**10**, free base) was synthesized in low yield by the reaction of arecaidine, lithium salt, with methyl lithium, by the procedure of Campbell *et al.* (21). IR ($CHCl_3$): 1665 (conjugated C═O), 1635 (inflection), 1620 (weak, conjugated C═C), 1355, 1310, 1290, 1255, 1135, 1120, 1080, 1060, 1042, and 1010 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.84 (unresolved t, 1, C-4 H), 3.12 (s, 2, C-2 CH_2), 2.6–2.4 (m, 4, C-5,6 CH_2), 2.36 (s, 3, CH_3), 2.28 (s, 3, CH_3 C═O). Arecolone methiodide (**10**) had m.p. 231–

232°. ^{1}H NMR (D_2O): δ 7.32 (unresolved t, 1, C-4 H), 4.78 (s, 2, C-2 CH_2), 4.1 (2, C-6 CH_2), 3.5 (t, 2, C-5 CH_2), 3.16 (s, 6, $N(CH_3)_2$), 2.40 (s, 3, CH_3 C═O). CIMS (NH_3): *m/z* 140 ($M^+ + 1 - CH_3I$). Analysis, calculated for C_9H_{16} INO: C, 38.45, H, 5.74, N, 4.98; found: C, 38.28, H, 5.47, N, 4.95.

dl-1-Methyl-4-hydroxy-4-methyl-3-acetylpiperidine (**12**, free base) was prepared by the Mannich reaction of methylamine hydrochloride, formaldehyde, and acetone, according to the procedure of Mannich and Ball (22). The *dl*-methiodide salt (12) had m.p. 202.5–203° (from absolute ethanol); reported (22), m.p. 205° (decomposed α form) and m.p. 217° (decomposed β form).

4-Methylarecolone (**11**, free base) was prepared by the dehydration of **12** (free base) with ethanolic HCl. The methiodide salt (**11**) had m.p. 177–178.5° (from ethanol); reported (22), m.p. 173°. *dl*-1-Methyl-3-(2-hydroxyethyl)-1,2,3,6-tetrahydropyridine methiodide (arecolol methiodide, **13**) was prepared by the $NaBH_4$ reduction of 1-methyl-3-acetylpyridinium iodide in methanol, according to the procedure described by Waters *et al.* (12). For the free base of **13**, EIMS: *m/z* 141 (M^+); IR ($CHCl_3$): 3605 (OH), 1290, 1120, 1070, and 948 cm^{-1}. The methiodide salt (**13**) had m.p. of 156–158° (from absolute ethanol). CIMS (NH_3): *m/z* 142 ($M^+ + 1 - CH_3I$).

Arecoline methiodide (**14**) was prepared by refluxing arecoline with iodomethane in ethanol for 2 hr; m.p. 176–177° (from absolute ethanol); reported, m.p. 173–174° (23) and 167–170° (24). *dl*-3,4-Dihydroarecoline (**15**, free base) (1-methyl-nipecotate) was prepared by the catalytic hydrogenation (PtO_2) of arecoline in glacial acetic acid at 16 psi for 22 hr. CIMS (NH_3): *m/z* 158 ($M^+ + 1$). The methiodide salt (**15**) had m.p. 200–201.5° (from acetone/ethanol); reported (25), m.p. 199–200°.

dl-3-Acetoxypiperidine (**16**, free base) was prepared by the acetylation of 1-methyl-3-hydroxypiperidine with acetic anhydride in pyridine, by the method of Lambrecht (26). The methiodide salt (**16**) had m.p. 157.5–159° (from acetone/ethanol); reported, m.p. 153–154° (26) and 150–152° (27).

The synthesis of *dl*-octahydro-2-methyl-(*trans*)-5-(1*H*)-isoquinolone methiodide (**17**) and its 5-hydroxy analogue (**18**) was similar to the procedure of Mathison and co-workers (28, 29). 1,2,3,4-Tetrahydro-2-methyl-5-isoquinolol methiodide (**19**) was synthesized through $NaBH_4$ reduction of 5-hydroxyisoquinoline methiodide by a procedure similar to that of Durand *et al.* (30). M.p. (ethanol), 235.0–235.5°. IR (Nujol): 3200 (broad, O-H) cm^{-1}. Analysis, calculated for $C_{11}H_{16}$ INO: C, 43.30, H, 5.28, O, 4.59; found: C, 43.32, H, 5.33, N, 4.57.

Caulophylline (**20**, free base) was prepared by the *N*-methylation of cytisine using iodomethane and anhydrous K_2CO_3 in refluxing dry dimethylformamide for 16 hr. The free base of **20** had m.p. of 141–142°; reported, m.p. 128° (31) and 134° (32). EIMS: *m/z* 204 (M^+). The methiodide salt (**20**) had m.p. 285° (decomposed); reported, m.p. 265.5° (31) and 276° (decomposed) (32). CIMS (NH_3): *m/z* 205 ($M^+ + 1 - CH_3I$).

Tissue preparations. Electric organs from *Torpedo nobiliana* were purchased from Biofish Associates (Georgetown, MA) and were stored at −70° for up to 4 months before use. Electric organ was minced and then homogenized in a blender in 5 volumes of 50 mM Tris·HCl, pH 7.4, that contained 1 mM phenylmethylsulfonyl fluoride to prevent proteolysis. The homogenate was filtered through four layers of cheese cloth to remove undisrupted material and then centrifuged at 20,000 × *g* for 20 min. The pellets were resuspended in 50 mM Tris·HCl buffer and used without further treatment.

Neuronal membranes were prepared from the cerebral cortex of adult male Wistar rats (Harlan-Sprague Dawley; Indianapolis, IN) by homogenization in 5 volumes of 50 mM Tris·HCl, pH 7.4. After centrifugation at 1,000 × *g* for 5 min, the supernatants were spun at 20,000 × *g* for 20 min. The pellets were resuspended in 50 mM Tris·HCl buffer and used without further treatment.

Binding measurements. ^{125}I-BGT (10–20 Ci/g; DuPont NEN, Boston, MA) was used as a probe for nAChR binding sites. *Torpedo* membranes (5–15 μg of protein) were incubated with competing ligands for 15 min at room temperature. ^{125}I-BGT (5 nM) was then added and the incubation was continued for 20 min. The reaction was then quenched by the addition of 0.5 volumes of a solution containing 5 mg/ml methylated bovine serum albumin (Sigma Chemical Co., St. Louis, MO). The suspensions were filtered through glass fiber filters (Whatman GF/B) that had been soaked in the albumin solution. The filters were washed once with 5 ml of buffer, and their radioactivity content was determined by liquid scintillation counting. Nonspecific binding was determined in the presence of 30 μM *d*-tubocurarine.

[^{3}H]H_{12}-HTX (54.5 Ci/mmol) was prepared by reduction of 5.5 mg of *dl*-octahydrohistrionicotoxin with 25 Ci of tritium gas, by DuPont NEN. *Torpedo* membranes (20–50 μg of protein) were incubated with 2 nM [^{3}H]H_{12}-HTX, in the absence or presence of competing ligands, in 50 mM Tris·HCl, pH 7.4, in a final volume of 1 ml at 20° for 60 min. The suspensions were filtered through glass fiber filters (Whatman GF/B), the filters were washed with 5 ml of buffer, and their radioactivity content was determined by liquid scintillation counting. The filters were pretreated with a 1% organosilane solution (Sigmacote; Sigma) to eliminate [^{3}H]H_{12}-HTX binding to the filters. Nonspecific binding was determined in the presence of 100 μM unlabeled histrionicotoxin.

mAChR were labeled using [^{3}H]MS (60–80 Ci/mmol; DuPont NEN), as previously described (33). Assay conditions were as follows: incubation time, 90 min; buffer, 50 mM Tris·HCl, pH 7.4, with 2 mM $MgCl_2$; temperature, 20°; volume, 1 or 2 ml; [^{3}H]MS concentration, 0.1 nM; protein content, 50 μg. Nonspecific binding was determined in the presence of 10 μM atropine.

Binding values were determined in triplicate in each experiment, and each experiment was performed three times using tissue prepared from different animals.

It should be noted that the binding of ligands to the nAChR is complex. There are two interacting sites for agonists, and desensitization (with concomitant assumption of a higher affinity conformation) would be expected to occur under the present assay conditions. Moreover, ^{125}I-BGT is an essentially irreversible probe under these conditions. Accordingly, these values should be treated as indications of relative affinity of the receptor (i.e., relative to ACh and carbamylcholine), not true, thermodynamic affinity constants.

Results

Fig. 2 shows that most of the nicotinic agonists were capable of inhibiting the binding of ^{125}I-BGT nearly completely at high concentrations. Thus, they were recognized by the nAChR of *Torpedo*. The IC_{50} values for the agonists are given in Table 1.

The inhibition of the binding of BGT monitors the initial recognition of the agonist but not the subsequent conformation changes that progress to the opening of the ion channel. In contrast, when the receptor is activated by an agonist, the binding of H_{12}-HTX is enhanced (34). Although the conformational state of the receptor that permits enhanced binding of H_{12}-HTX and its relationship to the train of states leading to the opening of the ion channel are unknown, we empirically employed this enhanced binding to define operationally the activation of the receptor. Fig. 3 shows that all of the agonists but one (**12**) could enhance H_{12}-HTX binding. Several, however, (**4**, **9**, **11**, **13**, **15**, **17**, **18**, and **20**) caused incomplete activation in the concentration range used. The binding of [^{3}H]H_{12}-HTX in response to some agonists (**9**, **11**, **15**, and **18**, in particular) showed strong signs of approaching asymptotes below the maxima induced by other, more potent, agonists; that is, these compounds behaved as partial agonists in this preparation. For full agonists, the concentration that caused 50% activation of the receptor is defined as the EC_{50}. These values are listed in Table 1.

It has been shown that activation of the nAChR is a multistep

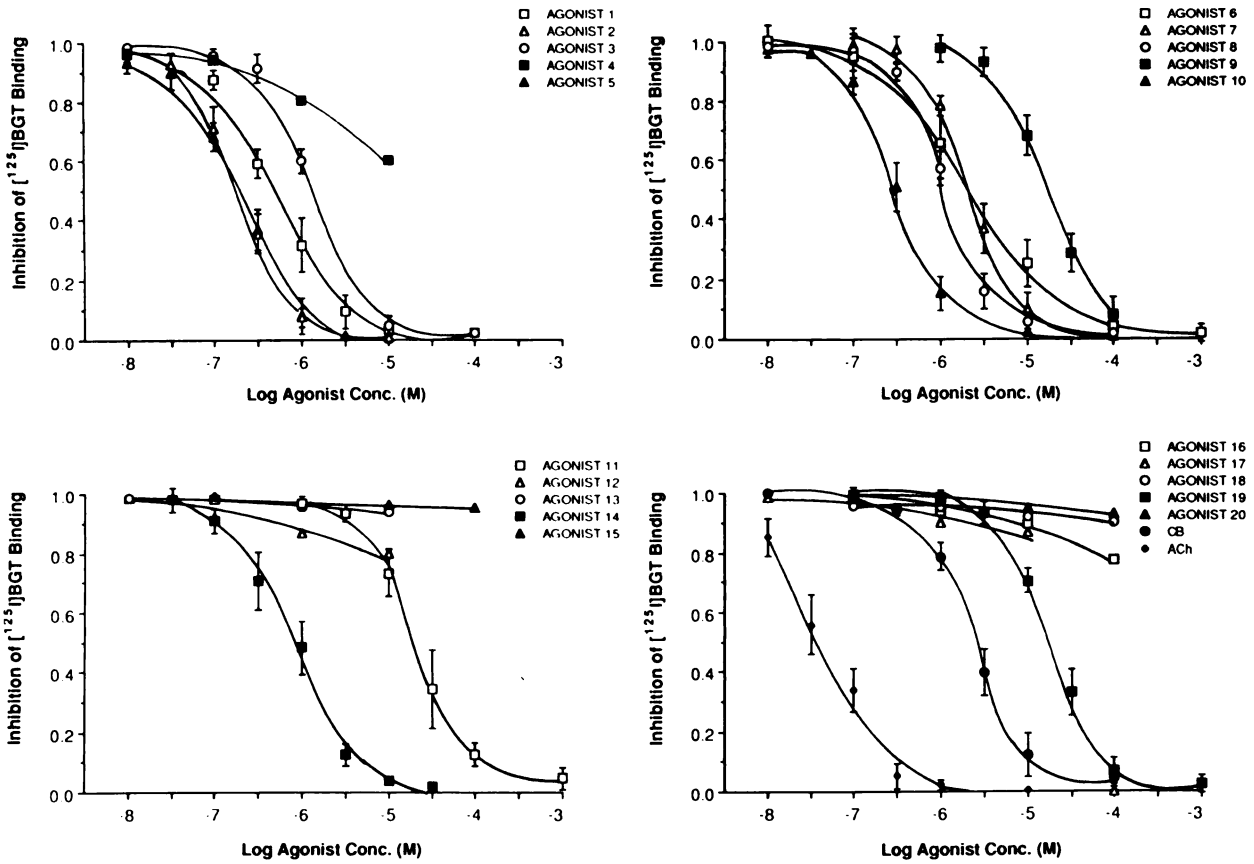

Fig. 2. Binding of nicotinic agonists to the nAChR in electric organ from *T. nobiliana*. Binding was determined in competition studies using ^{125}I-BGT. Each *point* and *bar* represent the mean ± standard deviation from four or five experiments. *CB*, carbamylcholine.

train of events and that potency of an agonist can depend on steps beyond recognition, the first step. Thus, potency can depend on how the agonist affects the kinetics for activation (e.g., see Ref. 35 and 36) and desensitization of the ion channel (see Discussion). These events subsequent to binding contribute to the efficacy of the agonist i.e., the propensity of steps subsequent to binding to yield the observed macroscopic response. If the efficacies were the same for all the agonists described here, a scatter plot of IC_{50} versus EC_{50} would be linear. Fig. 4, which shows some dispersion in such a plot, suggests variations in agonist efficacies. Exactly what these variations are can be obtained from patch-clamp experiments (which directly measure the kinetics of opening and shutting of single ion channels), which are in progress. Partial agonists, not shown in Fig. 4, bound weakly (had high IC_{50} values) at the ^{125}I-BGT site.

Because mAChR are believed to require a pharmacophore somewhat different from that which activates the nAChR (e.g., see Refs. 7 and 37–39), it was useful to know whether our nicotinic agonists had affinity for the muscarinic receptor. The extent to which the compounds inhibited the initial binding of [^{3}H]MS to central receptors from the cerebral cortex of rats yields IC_{50} values, which are tabulated for all of the compounds in Table 1. Arecoline methiodide (**14**) had higher affinity than did carbamylcholine, and (±)-3,4-dihydroarecoline methiodide (**15**) was more than one half as potent as carbamylcholine. These findings were not too surprising, because arecoline (the tertiary amine) is a well known muscarinic agonist. Isoarecolone methiodide (**1**) had moderately high muscarinic affinity, concomitant with its high nicotinic activity. The piperazinium compound **5**, a highly active nicotinic agonist (8, 13), was among the weakest in muscarinic affinity; it was selective for the nAChR. The high affinity of **18** for muscarinic receptors contrasted with its low nicotinic activity.

Discussion

Fig. 5 shows a scatter plot (on log-log axes) of IC_{50} at the ^{125}I-BGT site versus potency assayed by contracture of the frog (*Rana pipiens*) rectus abdominis muscle (data from Refs. 12–15). The correlation coefficient (Spearman's for ranked data; Ref. 40) is high, −0.909 ($p \ll 0.001$), but not perfect. A better correlation might be expected when comparing the ability of an agonist to trigger the conformation change that accompanies activation of the receptor (determined by the increased binding of [^{3}H]H_{12}-HTX) with the potency determined at the frog neuromuscular junction. Fig. 6 shows such a scatter plot; the correlation coefficient (Spearman's) was −0.727 ($p < 0.02$). These imperfect correlations (Figs. 5 and 6) may reflect differences between the nAChR from *Torpedo* and the frog. Indeed, agonists can even produce different results with different species of frog, such as *R. pipiens* versus *Rana temporaria* (41). Despite the variability, isoarecolone methiodide, **1**, remained the most potent in activating *Torpedo* receptor (Table 1 and Fig. 2). The assets of this drug include its high potency, its inertness to hydrolysis (because it is a ketone), and its restricted range of conformations (due to the conjugation between the ring C═C and the exocyclic C═O; see Ref. 12). This last point is being exploited to help define the nicotinic pharmacophore

TABLE 1

Binding parameters for agonists

Membranes from *T. nobiliana* served as the source for nAChR. The IC_{50} values are derived from competition experiments using ^{125}I-BGT. The EC_{50} values for channel interactions are derived from agonist-activated binding of [^{3}H]H_{12}-HTX. Membranes from rat brains served as the source for mAChR and the IC_{50} values are derived from competition experiments using [^{3}H]MS. Means ± standard deviations from three independent experiments are given. Parentheses denote that maximal stimulation was not achieved within the concentration range studied.

Compound	Nicotinic		Muscarinic Receptor IC_{50}
	Receptor IC_{50}	Ion channel EC_{50}	
		μM	
1	0.48 ± 0.06	0.021 ± 0.009	68 ± 23
2	0.21 ± 0.04	0.05 ± 0.01	215 ± 45
3	1.2 ± 0.5	0.11 ± 0.04	>300
4	15. ± 6.	(2.1 ± 0.4)	340 ± 110
5	0.19 ± 0.03	0.025 ± 0.005	560 ± 135
6	0.6 ± 0.4	0.39 ± 0.03	>100
7	2.5 ± 0.7	0.26 ± 0.08	>300
8	1.2 ± 0.3	0.23 ± 0.07	>300
9	15. ± 3.	(0.63 ± 1.2)	>300
10	0.32 ± 0.07	0.07 ± 0.02	126 ± 34
11	20. ± 7.	(0.56 ± 0.09)	>300
12	>50.		>600
13	>50.	7.8 ± 2.2	300 ± 110
14	0.9 ± 0.3	0.5 ± 0.1	20 ± 5
15	>50.	(20.)	65 ± 23
16	>50.	16. ± 5.	>300
17	>50.	3.6 ± 1.2	124 ± 36
18	>50.		79 ± 26
19	20. ± 5.	2.2 ± 0.5	135 ± 56
20	>50.		>600
ACh	0.04 ± 0.01	0.048 ± 0.013	68.9 ± 2.4
CB[a]	2.4 ± 0.3	0.25 ± 0.08	44. ± 14.

[a] CB, carbamylcholine.

with greater precision (12, 13). Although care must be exercised in its synthesis and yields are low (12, 13), it is easier to obtain than anatoxin-a (21, 42–46) and PHT (47), the only semirigid agonists whose potencies exceed that of **1**. The moderate affinity of **1** for the muscarinic receptor was unexpected.

Other agonists notable for their high activities at the nAChR of *Torpedo* are agonists **2, 3, 6, 8, 10,** and **14**. Also among the most potent of these agonists was **5**. It was employed previously to support the hypothesis that ACh is active in its *gauche* conformation (8, 13). Its high activity, its high selectivity for the nAChR over the mAChR (Table 1), its resistance to hydrolysis (because it is an amide), its restricted range of conformations, and the ease with which it is synthesized (8, 13) could make it a valuable tool in studies of the nAChR. It is noteworthy that the trifluoro derivative of **5**, agonist **6**, was more potent than **5** (by 1.8-fold) at the frog neuromuscular junction (12) but less potent at *Torpedo* receptors.

One of the least active of the agonists was the isoquinolone, **17**. Despite the deployment of its amine and carbonyl functional groups in a conformation that favorably fits the putative pharmacophore,[1] it is among the least active compounds tested at both the frog neuromuscular junction and *Torpedo* electric organ. We believe that the low activity of **17** was due to steric hindrance arising from the methylene group β to the carbonyl carbon.[1] Its phenolic analogue, **19**, was much more effective than **17** at activating the receptor (Fig. 3), where it behaved as a full agonist. At the frog neuromuscular junction, its activity is low, equal to that of **17**.[12]

[1] C. E. Spivak, and T. M. Gund, unpublished observation.
[2] C. E. Spivak, unpublished observation.

Cytisine is among the very few [namely, nicotine, anatoxin-a (9, 10, 48), PHT (47), and isoarecolone (18)] potent nicotinic agonists that are not quaternary amines. The explanation that we proposed (1, 15) was that an agonist must possess steric bulk above the plane defined by the carbonyl bond and its substituents. Cytisine, nicotine, PHT, and anatoxin-a all derive this bulk from their bi- or tricyclic ring structures. Barlow and McLoed (31) showed that the *N*-methyl derivative to cytisine was active at the frog neuromuscular junction but that the quaternary *N,N*-dimethyl derivative (**20** here) was virtually inert. The present binding data agree with this finding (Table 1 and Figs. 2 and 3). We believe that the explanation is as follows. Whereas the *N*-methyl derivative can place the methyl group more nearly above the plane of the other two rings *or* parallel to them according to whether the third ring is in a chair or boat conformation, the quaternary amine has no options. After adding the second *N*-methyl group, one of them *must* extend the bulk of the agonist further in the direction perpendicular to the plane of the first two rings. We believe that the agonist is then simply too big to fit.

The receptor activation curves (Fig. 3) revealed a qualitative difference among the agonists. Most can fully activate the receptor, but some, **9, 11, 15,** and **18** in particular and perhaps **4, 13, 17,** and **20**, induce incomplete activation. We have referred to these as partial agonists. Three mechanisms may account for this observation. First, the agonist may plug the ion channel of the receptor. Agonists as small as carbamylcholine can block the channel when the concentration is sufficiently high (e.g., see Ref. 49). Most of the agonists that behave as partial agonists here have low affinity for the receptor. This means that a high concentration is required just to occupy the recognition site. When the channel opens, it does so in an environment rich in potential blockers, namely the agonist itself. One agonist, however, **16**, contains the ACh moiety. It is, nevertheless, among the weakest agonists at occupying the recognition site (Fig. 2), although it is a full agonist (Fig. 3). It is not apparently smaller than the rest of the agonists (Fig. 1). Either the channel blockade mechanism does not account for partial agonism here, or **16**, for obscure reasons, singularly permeates the channel.

The second mechanism that could account for the partial agonism is that these agonists, while occupying the recognition site, fail to switch the receptor to its open channel conformation. Using an oversimplified model, we can express this sequence as:

$$A + R \rightleftharpoons AR \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AR^*$$

where A represents agonist, R the receptor, AR an agonist-receptor complex, and AR^* the open channel conformation. Because the maximum fraction of open channels is $\beta/(\alpha + \beta)$, the maximum depolarization or contracture is limited by the rate constants of the second step, not by the affinity of the agonist for the recognition site alone. Patch-clamp experiments have provided examples in which potencies of agonists are dissected into component rate constants (e.g., Refs. 35 and 36).

A third explanation invokes desensitization that varies among the agonists. If an agonist is, for obscure reasons, more prone than others to trip the receptor into a desensitized state, its potency will be curtailed. Electrophysiological studies could clarify the role of desensitization in delimiting potencies of these drugs.

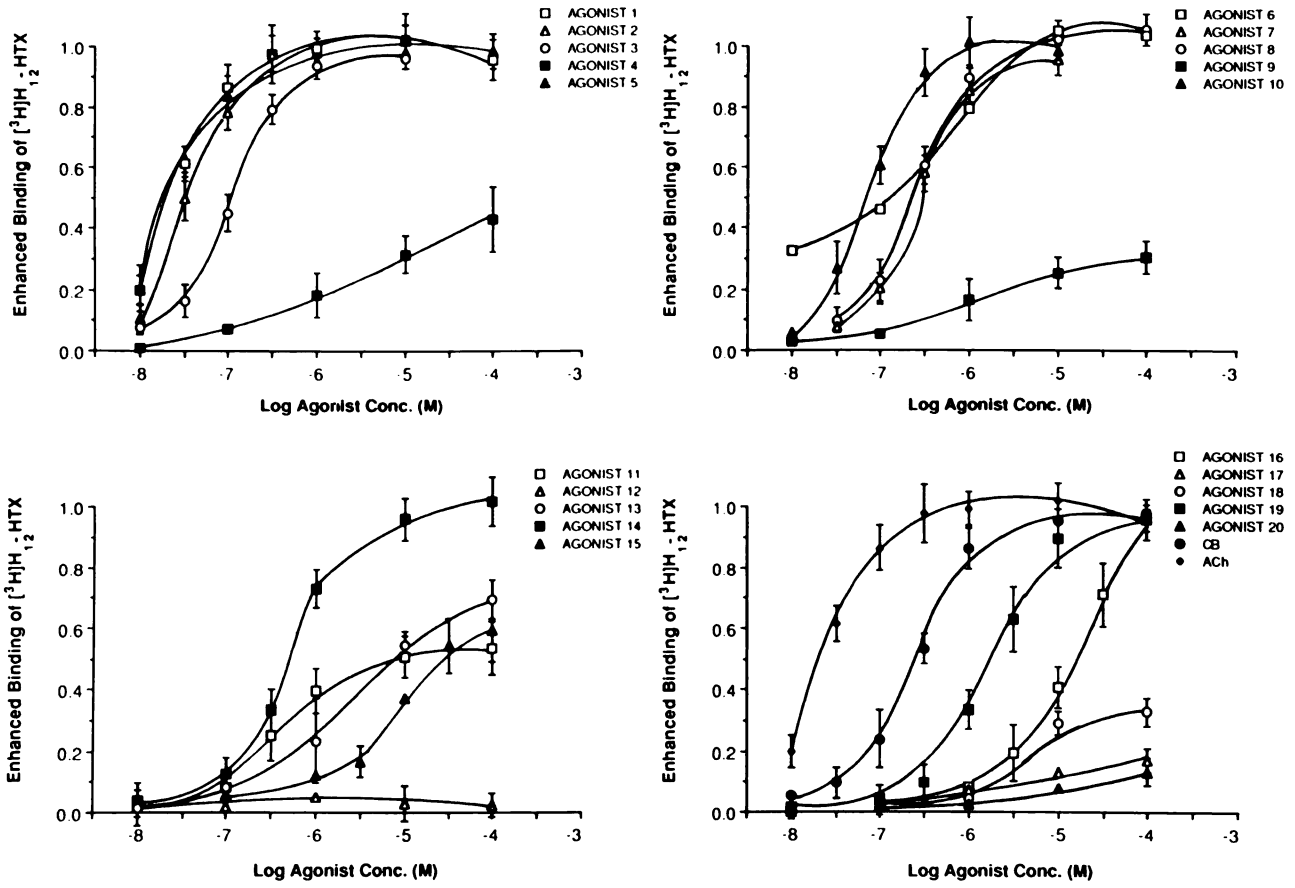

Fig. 3. Activation of the receptor by nicotinic agonists. The activated conformation is monitored by the enhanced binding of $[^3H]H_{12}$-HTX. Stimulation of 1 nM $[^3H]H_{12}$-HTX binding is expressed relative to the maximal stimulation produced by ACh (=1.0). Maximal stimulation was 2–4-fold, depending on the tissue preparation. Means ± standard deviations from three experiments are shown. *CB*, carbamylcholine.

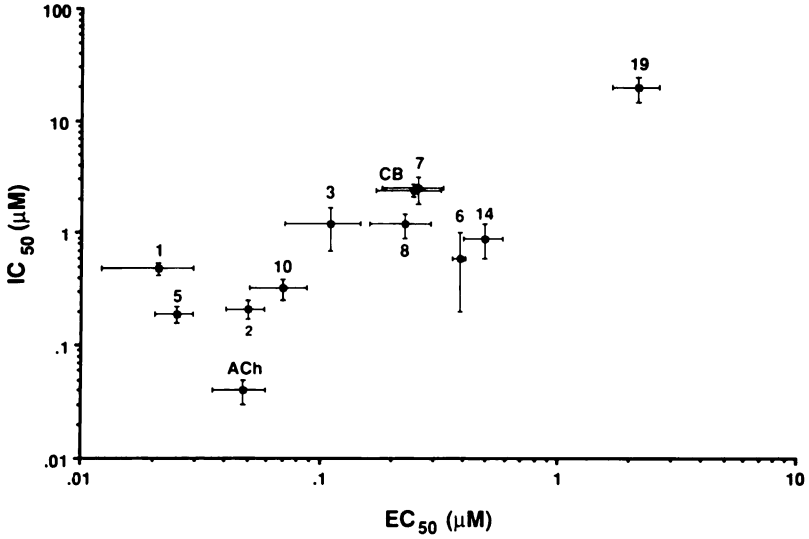

Fig. 4. Scatter plot of IC_{50} versus EC_{50} for full nicotinic agonists tested at *Torpedo* electric organ. The *numerals* refer to agonist numbers assigned in Fig. 1. The data are from Table 1; *error bars* represent standard deviations. *CB*, carbamylcholine.

The correlations between potencies at the frog neuromuscular junction and the relative binding affinities in *Torpedo* suggest a pharmacophore that, although not identical, is similar for the two species. A pharmacophore based on semirigid agents proposed by Beers and Reich (7) has been discussed (1), supported (e.g., Refs. 8–14), and expanded upon (12, 14). Recently, however, Behling *et al.* (50) determined, by proton NMR two-dimensional nuclear Overhauser effects, a conformation of ACh bound to the receptor that suggests a very different pharmacophore. For example, the Beers-Reich pharmacophore specifies a distance between the quaternary nitrogen and the van der Waals extension of the carbonyl oxygen of 5.9 Å. The corresponding distance found by Behling *et al.* was about 6.6 Å (based on measurements of a Corey-Pauling-Koltun model). A full discussion of the relative usefulness of the two conformations is beyond the scope of this paper, but we briefly note the following points. First, the finding of a bound conformation of ACh, although a remarkable achievement, was performed in

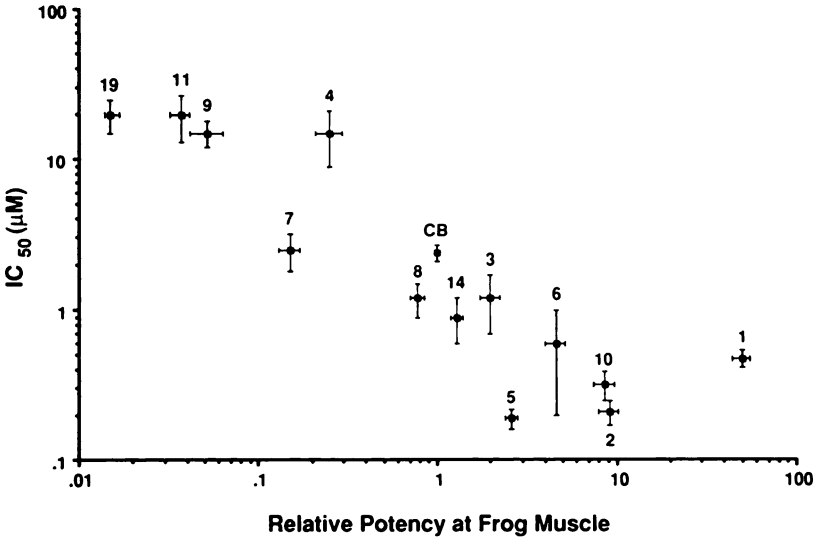

Fig. 5. Scatter plot of receptor affinity versus potency at the frog neuromuscular junction. The IC_{50} values for inhibition of ^{125}I-BGT binding are from Table 1. Potency is defined as the reciprocal of the equipotent molar ratio in comparison with carbamylcholine (*CB*). Potency data are taken from Refs. 12–15. The *numerals* refer to agonist numbers assigned in Fig. 1. *Vertical error bars* are standard deviations and *horizontal bars* are 95% confidence limits.

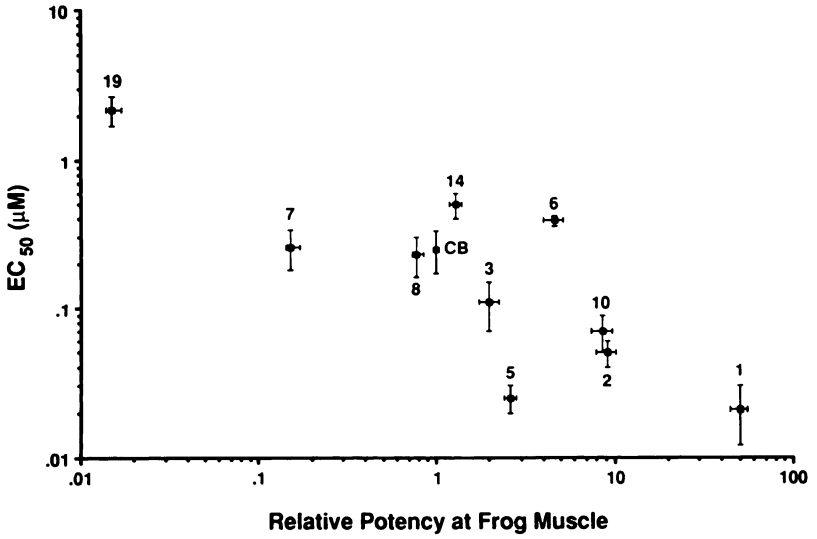

Fig. 6. Scatter plots of EC_{50} versus potency at the frog neuromuscular junction. The EC_{50} values are from Table 1. Potency is defined as the reciprocal of the equipotent molar ratio in comparison with carbamylcholine (*CB*). Potency data are taken from Refs. 12–15. The *numerals* refer to agonist numbers assigned in Fig. 1. *Vertical error bars* are standard deviations and *horizontal bars* are 95% confidence limits.

the presence of 10 mM ACh. The receptor must, then, have been nearly completely in a desensitized conformation, so that the conformation of the bound ACh bore no necessary relationship to the conformation of ACh that binds to and activates the resting receptor. Second, when ACh is in a *gauche* conformation that corresponds to the Beers-Reich pharmacophore (described in Ref. 1), the semirigid agonists described in this paper superimpose nicely on this ACh conformation. Thus, even if other atoms, neglected by Beers and Reich, contribute to the binding, the *gauche* model of ACh still has predictive value. By contrast, the conformation determined by Behling *et al.* bears no homology to the agonists we describe here. The quaternary nitrogens and carbonyl oxygens can be made to superimpose, but the rest of the structures are completely misaligned.

In summary, these experiments confirmed that the new semirigid agonist isoarecolone methiodide (**1**) is among the most potent nicotinic agonists known. It was more than twice as potent as ACh in activating the receptor of *Torpedo* electric organ. Agonist **5** was nearly as active as **1** and was also much more selective for the nAChR than the mAChR. The more rigid, bicyclic, agonists **17** and **19** were weak nicotinic agonists in *Torpedo*, as they are in the frog,[1] but the receptor activation studies revealed that **19**, in contrast to **17**, was a full agonist. Some agonists, **1**, **14**, **15**, and **18**, bound to muscarinic receptors with affinities comparable to that of ACh.

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