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Review

The Nemertine Toxin Anabaseine and Its Derivative DMXBA (GTS-21): Chemical and Pharmacological Properties

William Kem [1,*], Ferenc Soti [1], Kristin Wildeboer [1], Susan LeFrancois [1], Kelly MacDougall [1], Dong-Qing Wei [3], Kuo-Chen Chou [4] and Hugo R. Arias [2]

[1] Department of Pharmacology and Therapeutics, University of Florida, College of Medicine, Gainesville, FL 32610-0267, USA

[2] Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766-1854, USA

[3] College of Life Science and Technology, Shanghai Jiaotong University, Minhang District Shanghai, 200240, China

[4] Gordon Life Science Institute, San Diego, CA 92130, USA

* Authors to whom correspondence should be addressed; Tel. (352) 392-3541; Fax (352) 392-9696; E-mail: Kem@pharmacology.ufl.edu

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Abstract: Nemertines are a phylum of carnivorous marine worms that possess a variety of alkaloidal, peptidic or proteinaceous toxins that serve as chemical defenses against potential predators. The hoplonemertines additionally envenomate their prey with a mixture of proboscis alkaloids delivered with the help of a calcareous stylet that punctures the skin of the victim. Anabaseine, the first of these alkaloids to be identified, stimulates a wide variety of animal nicotinic acetylcholine receptors (AChRs), especially the neuromuscular [e.g., $\alpha 1_2\beta 1\gamma\delta$ (embryogenic) or $\alpha 1_2\beta 1\gamma\varepsilon$ (adult)] and $\alpha 7$ AChRs that are inhibited by the snake peptide α-bungarotoxin. A synthetic derivative, 3-(2,4-Dimethoxybenzylidene)-Anabaseine (DMXBA; also called GTS-21), improves memory in experimental animals and humans and is currently in clinical trials to determine whether it can ameliorate cognitive problems associated with schizophrenia. Here we summarize present knowledge concerning the chemistry and mechanisms of action of these two substances (anabaseine and DMXBA) on AChRs, especially those found in the mammalian brain.

Keywords: Anabaseine, Cognition, DMXBA, GTS-21, Nemertine, Nicotinic receptors.

Abbreviations: ACh, acetylcholine; AChR, nicotinic acetylcholine receptor; α-BTx, α-bungarotoxin; DMXBA (or GTS-21), 3-(2,4-dimethoxybenzylidene)-anabaseine; LTP, long-term potentiation; NMDA; CNS, central nervous system; N-methyl-D-aspartate; 5-HT; 5-hydroxytryptamine or serotonin; AD, Alzheimer's disease; Aβ, β-amyloid; PTHP, 2-(3,4,5,6-tetrahydropyrimidinyl)-3-pyridine; PCP, phencyclidine; K_i, inhibition constant.

Introduction

The Belgian pharmacologist Bacq discovered the existence of toxins in nemertines during the mid-1930s while searching for invertebrate neurotransmitters. An aqueous homogenate of a small intertidal hoplonemertine species (*Amphiporus lactifloreus*), like acetylcholine (ACh), contracted isolated frog skeletal muscle and stimulated the cat cervical autonomic ganglion. Unlike ACh the cholinergic activity of the homogenate was stable in highly alkaline solution and was soluble in organic solvents under basic conditions. On the basis of this somewhat limited profile Bacq [15,16] inferred that "amphiporine" was an alkaloid similar to nicotine. King [52] showed that amphiporine acted as an organic base and attempted its further purification by crystallization with standard alkaloidal precipitants, however a crystalline salt was not obtained.

Nemertines are a phylum of carnivorous, mainly marine worms [32]. While over a 1,000 species have been described, the actual number of species in this inconspicuous phylum is likely to be several times this figure. Being soft-bodied and relatively vulnerable to predators, they contain integumentary toxins which serve as chemical defenses against predators [39,42,43,45,50]. The phylum is roughly divided into two large groups, the Enoplans (hoplonemertines) bearing a mineralized proboscis stylet and the Anoplans (paleo- and heteronemertines) lacking a stylet. The relatively small size of most nemertines makes them more difficult to collect than many aquatic animals. Another problem is species identification, as the external morphologies of some species may be so similar that the preparation of fixed and stained tissue sections for histological examination may be necessary for unequivocal identification. Nonetheless, the phylum undoubtedly represents an unusually rich source of alkaloid, peptide and protein toxins, most of them still awaiting investigation.

Approximately thirty years elapsed after Bacq's discovery before another study of nemertine toxins was reported [46]. Extracts of most species were found to be toxic to crustaceans, but only those of hoplonemertines displayed nicotinic agonist properties and contained pyridyl alkaloids [39,40]. The heteronemertines were shown to contain basic peptide and protein neurotoxins and cytotoxins [41]. Since this article focuses on the alkaloids, those interested in the peptide toxins should consult a recent review [45].

Anabaseine

This first nemertine alkaloid to be isolated and identified, anabaseine, occurs in relatively large concentrations in the intertidal Pacific nemertine Paranemertes peregrina [46]. The "peregrine" (wandering) designation refers to the relatively unique foraging behavior of this moderately large (>15

cm) species: it glides along the exposed surface of mud flats at low tide searching for annelid worms in full view of potential predators such as seagulls, raccoons and other large predators. Several thousand worms were collected and an alkaloid fraction was obtained from the ethanolic extract, much as described by King [52]. Because more than a gram of alkaloid was isolated, it was possible to obtain a homogeneous picrate salt, even though in relatively small yield [46]. After conversion back to the free base, nuclear magnetic resonance and mass spectrometric analyses indicated that the alkaloid was anabaseine, a previously synthesized compound that had not been reported as a natural product. This was corroborated by comparison of the chemical and toxicological properties of natural and synthetic samples. A decade later anabaseine was also found in certain ants [91].

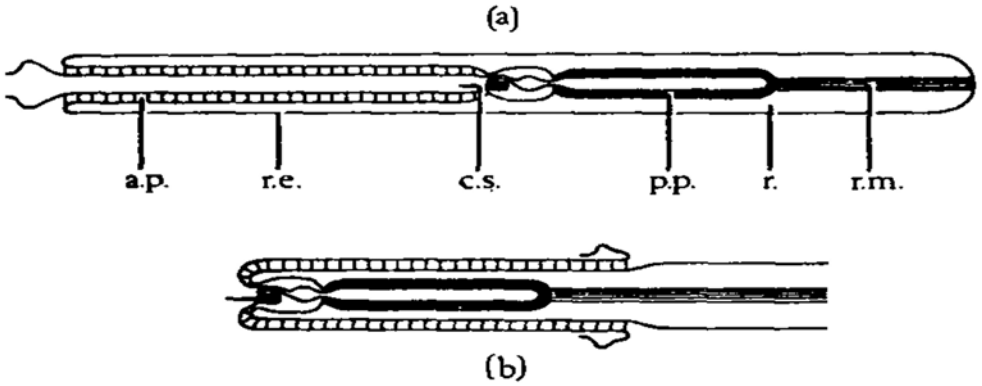

Figure 1. Anatomy of the proboscis apparatus in a hoplonemertine worm. **(a)** The apparatus is in the resting (retracted) position. **(b)** The apparatus is in the protruded position, the anterior part has been everted so that the stylet can puncture the surface of the prey and thus facilitate entry of the venom. Key: a.p., anterior proboscis epithelium; c.s., central stylet; p.p., posterior proboscis epithelium; r., rhynchocoel; r.e., rhynchocoel endothelium; r.m., proboscis retractor muscle (modified from Gibson [32]).

Anabaseine is chemically similar to the tobacco alkaloid, anabasine, but possesses an imine double bond in the otherwise saturated piperidine ring (Fig. 2). Imine-enamine tautomerism constrains the β-carbon to lie within the same plane as the α-carbon and the imine nitrogen. This tautomeric system is conjugated with the π electrons of the pyridyl ring. This electronic conjugation strongly favors the two rings of anabaseine being approximately co-planar with respect to each other. This contrasts with nicotine and anabasine, whose respective pyrrolidine and piperidine rings are oriented approximately at right angles in their preferred conformations. Anabaseine was first prepared as an intermediate in the synthesis of anabasine by two Austrian tobacco chemists [77]. A mixed aldol-like condensation reaction between nicotinic acid ethyl ester and N-benzoyl piperidone yielded the expected diketone, which rearranged in the presence of concentrated hydrochloride acid at high temperature to anabaseine hydrochloride. Conversion of the salt to the free base, extraction of the free base with organic solvent and purification by distillation, the method reported by Spath and Mamoli [77] or column chromatography generally provided anabaseine in relatively low yields [40,46]. Subsequent modifications have provided a more efficient synthesis and isolation in much higher yields [18]. Synthetic anabaseine dihydrochloride (M.W. 251) obtained in this manner exists as the ammonium-

ketone form and contains one molecule of water. While stable as the dried salt, aqueous solutions of anabaseine hydrochloride should be refrigerated when not in use and replaced after several weeks. The cationic forms of anabaseine are quite soluble in protic solvents such as water, methanol and ethanol, but the more lipophilic free base is best dissolved in non-aqueous solvents such as alcohols, acetone, or ethyl acetate.

Although anabaseine appears to be chemically simple, it actually occurs in several different forms under physiological conditions [95,96]. At neutral pH there are three forms in roughly equal concentrations: the unprotonated cyclic imine, the monocationic cyclic iminium and the monocationic ammonium-ketone (Fig. 3). This multiplicity complicated our initial attempts to determine which forms interact with AChRs based upon the pH dependence of anabaseine potency, so stable analogs of each form were prepared so that the pharmacological properties of the different forms could be inferred. 2,3'-Bipyridyl [47], which can be prepared by oxidation of anabaseine or anabasine, is predicted to possess a chemical conformation similar to the cyclic imine, so it was selected as an analog of the unprotonated form, while 2-(3,4,5,6-tetrahydropyrimidinyl)-3-pyridine (PTHP) was selected as a stable permanently ionized analog of the cyclic iminium form. Two stable analogs of the open-chain ammonium-ketone form were prepared by di- or tri-methylation of the ammonium group. Amongst the various stable analogs, only PTHP potently stimulated skeletal muscle and brain nicotinic acetylcholine receptor (AChR)-expressing cells (Kem et al., in preparation). Thus, we conclude that only the cyclic iminium form of anabaseine is active on these mammalian AChRs.

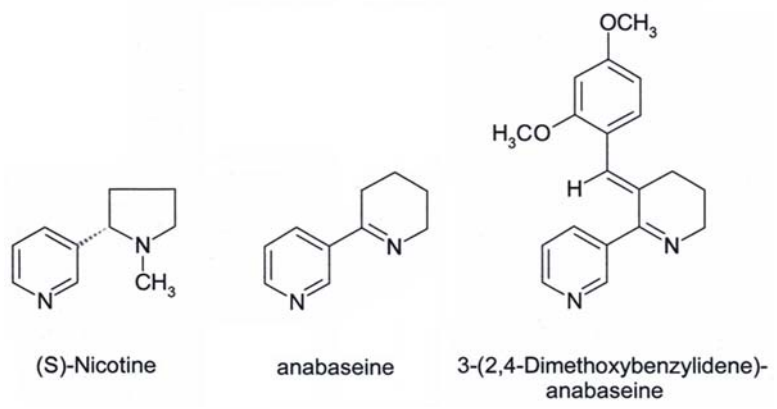

Figure 2. Structure of (S)-nicotine, anabaseine and its 2,4-dimethoxybenzylidene derivative, DMXBA. While nicotine and anabaseine stimulate a wide variety of vertebrate AChRs, DMXBA selectively stimulates α7-type AChRs.

AChRs are a family of receptors that belong to the Cys-loop ligand-gated ion channel superfamily that includes types A and C γ-amminobutyric acid, glycine, and type 3 serotonin (5-hydroxytryptamine; 5-HT) receptors (reviewed in [3,4]). In the peripheral nervous system, AChRs can be subdivided into muscle-type, that have the stoichiometry $\alpha 1_2\beta 1\delta\gamma$ (embryonic or *Torpedo*) or $\alpha 1_2\beta 1\delta\varepsilon$ (adult), and ganglionic AChRs (e.g., α3β4). In the central nervous system (CNS), AChRs are of two main subclasses: receptors that bind the competitive antagonist α-bungarotoxin (α-BTx) with high affinity but the agonist nicotine with low affinity (e.g., α7-containing receptors), and AChRs that bind nicotine with high affinity but α-BTx with low affinity (e.g., α4β2-containing receptors).

The physiological and pharmacological effects of anabaseine on a variety of vertebrate AChRs were previously reported [49]. Like nicotine, anabaseine stimulates all AChRs to some degree and thus must be classified as a non-selective nicotinic agonist. However, it preferentially stimulates the same AChRs (e.g., skeletal muscle and brain α7 subtypes) that display high affinities for the snake toxin α-BTx. In contrast, nicotine preferentially and almost fully stimulates α4β2 (brain) and α3β4 (predominantly autonomic) receptors. Anabaseine is a full agonist at the α7 receptor but only a very weak (low efficacy) agonist at the α4β2 subtype. The maximal effect of nicotine on the latter receptor is much greater than its maximal effect on the α7 receptor. Since nicotine also binds to α4β2 receptors at much lower (about 100-fold) concentrations than to the α7 receptor, its *in vivo* effects at "smoking" concentrations are most likely mediated through β2 subunit-containing receptors. Anabaseine stimulates PC12 cell and guinea pig ileum AChRs thought to contain α3β4 (and probably other) autonomic receptors. A more recent study of anabaseine action on rat AChRs expressed in *Xenopus* oocytes indicates that anabaseine is a rather weak partial agonist on the α3β4 receptor subtype [72].

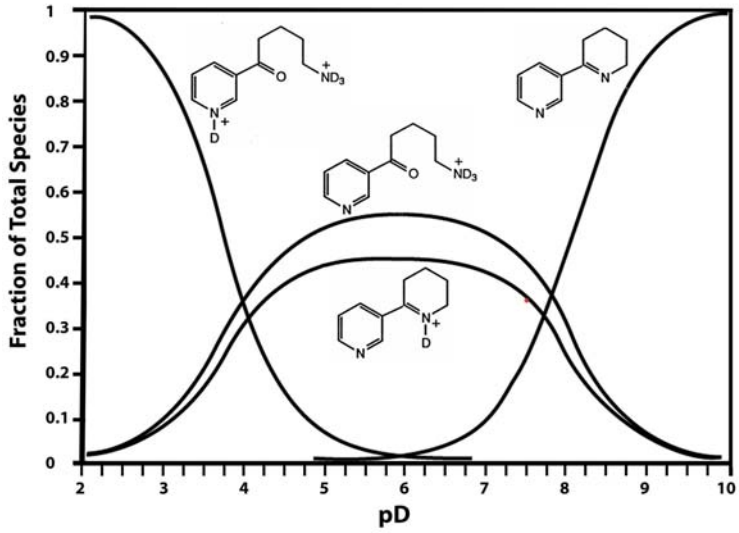

Figure 3. Equilibrium between the four major forms of anabaseine in aqueous solution as a function of pD [95]. Note that pD = pH + 0.4.

The whole animal (mouse) toxicity of anabaseine is very similar to that of nicotine and is significantly higher than for anabasine [47,54]. Nicotine toxicity is likely due to convulsions mediated by stimulation of CNS α4β2 AChRs. In contrast, anabaseine has very weak partial agonist activity at this receptor and probably exerts its toxicity by causing peripheral neuromuscular block and respiratory arrest. Because of its high toxicity and relative lack of receptor selectivity, few *in vivo* studies have been carried out with anabaseine. The significantly higher potency of nicotine relative to anabaseine in causing prostration is consistent with the notion that α4β2 receptors primarily mediate this characteristic behavior [49].

Anabaseine also affects a variety of invertebrate AChRs. Marine annelids which are the usual prey for *Paranemertes* are paralyzed, as are crustaceans and insects. Nicotinic cholinergic receptors primarily reside on central neurons in arthropods, but are also found in their cardiac pacemaker ganglion. 2,3'-Bipyridyl, a largely unionized analog of anabaseine, is even more active than anabaseine

in paralyzing crustaceans [47]. While it does not cause paralysis, nemertelline (a tetrapyridyl found in *Amphiporus angulatus*), like anabaseine and 2,3'-bipyridyl, stimulates an unusual receptor in the stomatogastric muscle of the crayfish which is apparently a chloride channel [50]. At present this is the only known action of this alkaloid, which is the most abundant pyridine in this species of *Amphiporus*. A variety of pyridine compounds including anabaseine and 2,3'-bipyridyl stimulate chemoreceptor present in sensory neurons present at the surfaces of crayfish and lobster walking legs that influence feeding behavior [35]. Anabaseine and 2,3'-bipyridyl were found to be two of the most active compounds in stimulating similar pyridine receptors on spiny lobster sensory nerves [50]. The nemertine alkaloids, by acting upon these chemoreceptors, may act as repellants against certain predators. Some of these compounds are also able to inhibit the settlement of barnacle larvae to marine surfaces and thus might be useful "antifouling" additives to marine paints.

DMXB-Anabaseine (DMXBA), A Synthetic Anabaseine Derivative

While anabaseine is a broad spectrum nicotinic agonist, a large variety (>200) of substituted anabaseines that have been synthesized over the past two decades displayed selective agonistic effects on the α7 AChR. The 3-arylidene-anabaseines are of special potential therapeutic interest because they have been shown to possess neuroprotective as well as cognition enhancing properties. Here we shall only consider 3-(2,4-dimethoxybenzylidene)-anabaseine (DMXBA), whose pharmaceutical code name GTS-21 refers to its origination as the 21st compound generated in a joint project by Gainesville (University of Florida) and Tokushima (Taiho Pharmaceuticals) Scientists. DMXBA was the first nicotinic agonist reported to selectively stimulate α7 AChRs; it was also the first α7 agonist to enter clinical tests for possible treatment of cognition problems such as occur in schizophrenia, Parkinsonism and Alzheimer's disease (AD).

DMXBA is prepared by reaction of 2,4-dimethoxybenzaldehyde with anabaseine in acidic alcohol at approximately 70^{o}C, in a manner similar to the preparation of 3-(4-dimethylaminobenzylidene)-anabaseine [39,40]. The resulting product can be precipitated and recrystallized using less polar solvents. Whereas the two rings of anabaseine have been shown to be electronically conjugated and thus nearly coplanar, all three rings of DMXBA are predicted to lie in different planes. Unlike anabaseine, 3-arylidene-anabaseines do not readily hydrolyze to open-chain forms at physiological pHs like anabaseine. In principle, these compounds can adopt two possible conformations with respect to the vinyl portion of the arylidene ring, namely E- or Z. By NMR we have shown that only the E form occurs in aqueous solution when the synthetic DMXBA dihydrochloride is dissolved in water [97]. Only after intense or maintained light exposure does the E to Z conversion become significant (Kem et al., unpublished data). Thus, the synthetic compound solid and stock solutions of the compound must be stored in containers that exclude light and stock. While photosensitivity of DMXBA was observed in the laboratory, when plasma samples from animal and human tests were prepared in an unlighted fume hood and subsequently determined by HPLC with a photodiode array detector, no Z-isomeric product was observed [71].

DMXBA is a lipophilic compound which readily passes across biological membranes including the gastrointestinal wall and the blood-brain barrier and reaches peak concentrations in the blood and brain within a very short time [14,48,51,59]. It is O-demethylated primarily at the *p*-position of the

benzylidene ring, but demethylation at the o-methoxy group also occurs to a much lesser extent. While the resulting hydroxy metabolites are actually more efficacious at the α7 receptor *in vitro*, their peak brain concentrations are much less than for DMXBA [51]. They are efficiently glucuronidated and excreted. Other anabaseine compounds in development are much less readily metabolized and possess better bioavailability.

Based on the crystal structure of acetylcholine-binding protein (AChBP) [19], monomer, homodimer, and homopentamer models of the α7 AChR were derived [23]. Since the agonist binding sites are located at the subunit interface (reviewed in [3,4]), a detailed analysis about the interface, as well as its interaction with the Hepes molecule that has been observed in the AChBP crystal, was performed. Furthermore, a ligand-binding pocket was defined providing useful information for conducting various mutagenesis studies to get clues for drug design. Although computer-predicted protein structures are still not as accurate as X-ray structures, the three modeled structures can at least serve as a basis for designing new ligands [22].

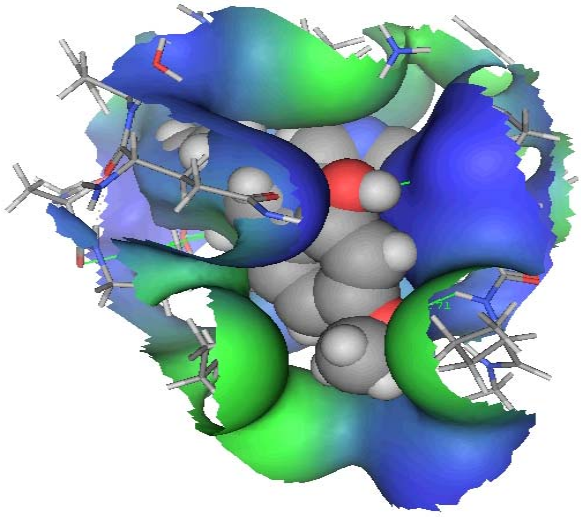

Figure 4. Close view of the α7 AChR binding pocket for 2OH-MBA (modified from [90]). The molecule is in the protonated form. Green and blue areas represent the hydrophobic and the hydrophilic surfaces, respectively, found in the binding pocket. The ligand is colored according to the atomic types: red (oxygen), gray (carbon), blue (nitrogen), and light-grey (hydrogen).

Theoretical and molecular modeling studies were done to better understand the details of how DMXBA and its two primary O-demethylated metabolites (2OH- and 4OH-MBA) might bind to α7 AChRs [90]. Figure 4 depicts a model of the binding pocket of 2OH-MBA at the extracellular domain of the α7 AChR. There was rather good accordance of the calculated preferred energies with the observed binding affinities [51]. Van der Waals repulsions made the dominated contribution to the predicted binding energy for the receptor. However, hydrophobic contacts were also observed. DMXBA and its metabolites seemed larger than the optimal size for fitting into the agonist binding site. Thus, one possible approach to improving the effectiveness of benzylidene-anabaseine binding might be to reduce the molecular volume while retaining the active groups. We are optimistic that

molecular modeling, in combination with experimental studies on model proteins such as AChBP [83], may provide a useful basis for rational design of nicotinic drug candidates for treating neurodegenerative and possibly other diseases.

While DMXBA selectively stimulates α7 AChRs, at significantly higher concentrations it also is an antagonist of α4β2 AChRs and related type 3 5-HT receptors [51,58,66,94]. At even higher concentrations DMXBA also is a weak antagonist at other AChRs. In at least some AChR subtypes, DMXBA and its metabolites may actually exert a noncompetitive inhibition of channel activation (Table 1). For instance, it has been found that DMXBA at micromolar concentrations [inhibition constant (K_i) = 13 ± 1 μM] displaces the binding of [^{3}H]thienylcyclohexylpiperidine ([^{3}H]TCP) within the channel of the neuromuscular-type AChR [7,9]. While the 4-hydroxy metabolite also displayed this inhibitory binding, it occurred at a higher concentration (K_i = 48 ± 5 μM). Schild-type analyses of these experiments indicated that these competitions are mediated by a steric mechanism. Thus, considering that [^{3}H]TCP is a structural and functional analog of the dissociative general anesthetic and potent noncompetitive antagonist phencyclidine (PCP), we suggest that the anabaseine analog binding site overlaps the PCP locus in the desensitized ion channel [7]. In this regard, photoaffinity labeling studies using [^{3}H]ethidium diazide, which binds with high affinity to the PCP locus, helped to determine the structural components of this site in the desensitized state [73] (reviewed in [5,6,10]). The results indicated that residues Leu^{251} at position 9' (e.g., the leucine ring) and Ser^{252} at position 10' from the α1-M2 transmembrane segment as well as other unknown amino acids located in the M1 and M2 transmembrane segments from the δ subunit are structurally involved in the PCP binding site.

In the resting state (in the presence of α-BTx), anabaseine analogs modulate either [^{3}H]TCP, [^{3}H]tetracaine, or [^{14}C]amobarbital binding to the *Torpedo* AChR in an allosteric fashion. These results indicate that the anabaseine analog binding site overlaps neither the PCP, the tetracaine, nor the amobarbital binding domain in the resting ion channel. We suggested that the PCP binding site in the resting state is located more extracellularly than that in the desensitized state, probably close to the mouth of the external vestibule (probably after position 13' and closer to position 20') [8,11,13], whereas the barbiturate locus is located practically in the middle of the resting ion channel (between position 9' and 13') [12] (reviewed in [5,6,10]). In addition, the tetracaine binding domain bridges both the PCP and the amobarbital loci in the resting ion channel (probably between position 5' and 20') [31,67].

Interestingly, anabaseine analogs enhance [^{3}H]TCP binding to the *Torpedo* AChR when the receptor is in the resting but activatable state (in the absence of α-BTx) [7,9]. We consider that this enhancement is due to an anabaseine analog-induced AChR desensitization process. This hypothesis was supported by the fact that anabaseine analogs also increase the binding of the agonist [^{3}H]cytisine to the resting but activatable AChR. In this regard, AChR desensitization seems to be another mechanism by which anabaseine analogs produce the noncompetitive inhibition of AChRs, which in turn, might account for the partial agonistic effect of these compounds in α7 AChRs [26].

Considering this new experimental evidence it is plausible that the maximal channel activation observed in conventional voltage-clamp electrophysiological recordings might be influenced by the propensity of anabaseine analogs for causing channel block and/or desensitization as well as the probability of the bound agonist to trigger the conformational changes associated with moving from a

resting (closed but activatable) to an activated (open) channel state, to finally a desensitized (closed) conformation.

What makes DMXBA of considerable scientific as well as potential clinical interest is its selective stimulation of α7 AChRs. The physiological function of this receptor had been very difficult to investigate in the past due to its propensity to rapidly desensitize when high concentrations of agonist are applied. Initially this receptor was only recognized by its ability to bind α-BTx. Later, after cloning and expression in cultured cells, it was found to be physiologically active as a ligand-gated ion channel with unusually high permeability for calcium ions. α7 AChRs occur at presynaptic as well as on postsynaptic sites at densities that are sometimes as high as that of glutamate receptors [28]. By causing an influx of calcium ions even at normal membrane resting potentials, when most voltage-gated calcium channels are closed, these AChRs are able to stimulate a variety of second messenger systems responsive to elevations in intracellular calcium [24], including nitric oxide synthesis [1].

That DMXBA enhances performance in cognitive tasks indicates that α7 AChRs play a significant role in learning and memory [51,75]. That DMXBA and other nicotinic agonists primarily exert their cognition-enhancing actions through AChR stimulation rather than desensitization follows from the finding that their pro-cognitive effects are inhibited by administering nicotinic antagonists like mecamylamine, α-BTx and methyllycaconitine [55]. DMXBA and nicotine both enhance long-term potentiation (LTP) in the hippocampus [37,64]. One hypothesis is that stimulation of α7 receptors by released ACh or choline (an endogenous weak agonist) enhances the action of synaptically liberated glutamate on nearby N-methyl-D-aspartate (NMDA)-type glutamate receptors, as the depolarization resulting from α7 channel opening would eliminate the resting block of NMDA receptors by intracellular magnesium ions [60]. This could be a postsynaptic mechanism for nicotinic stimulation of LTP.

DMXBA, like nicotine, enhances auditory gating in mice [78] and in humans [71]. The DMXBA enhancement displays less acute tolerance (i.e., reduced response with successive applications) than does the nicotine effect. Since the auditory gating effects of both compounds in mice are prevented by prior administration of α-BTx, α7 receptors are the dominant AChRs mediating this action. Schizophrenics suffer from a relative inability to filter or gate repetitive sensory stimuli, particularly auditory and visual stimuli [29,62]. This gating defect probably contributes to the negative symptoms of the disease, which are not well treated by neuroleptic drugs which in general are dopamine receptor antagonists. A recent recommendation by an expert panel of psychiatrists recommended development of new therapies for treating the cognitive problems associated with this disease, since they are particularly problematic in preventing schizophrenics from holding jobs and functioning in society. The University of Colorado and University of Florida labs have recently collaborated on a phase 1 test of DMXBA in schizophrenics. The results of this study, both regarding safety and initial assessments of efficacy, encourage further tests [71]. It has also been shown that the deleterious effects of cocaine on auditory gating can be counteracted by DMXBA [79]. Thus, α7 nicotinic agonists may also be useful in treating psychoses resulting from use of these stimulants.

Table 1. Comparison of the relative activities of anabaseine, nicotine and DMXBA on several vertebrate AChRs.

Receptor Type	Anabaseine	Nicotine	DMXBA
CNS			
α7	Full Agonist	Weak Partial Agonist	Partial Agonist
α4β2	Weak Partial Agonist	Strong Partial Agonist	Competitive Antagonist
Sympathetic			
PC12 Cell	Partial Agonist	Full Agonist	Noncompetitive Antagonist
α3β4 (oocyte)	Partial Agonist	Full Agonist	Noncompetitive Antagonist
Muscle-type			
α1β1εδ	Full Agonist	Full Agonist	[1]Competitive Antagonist
α1β1γδ (*Torpedo***)**	Full Agonist	Full Agonist	[2]Noncompetitive Antagonist

[1] Weak potency; [2] Moderate affinity (data from refs. [7,9]).

Data summarized from refs. [26,49,72].

Two decades ago a drastic decrease in AChRs was first reported in Alzheimer's patients and some Parkinson's patients [92]. This finding stimulated considerable academic and pharmaceutical interest in the development of nicotinic agonists that could selectively stimulate the remaining brain AChRs involved in cognitive and other critical mental functions. At that time it was already apparent that cholinesterase inhibitors and non-selective muscarinic agonists were relatively weak therapeutic agents for counteracting the neurodegeneration and dementia associated with AD. DMXBA is a relatively unique drug candidate which readily enters the brain and acts as an α7 AChR partial agonist. Its effects upon cognitive behavior have been investigated by many laboratories using a variety of mammalian species (reviewed in [44]). Nucleus basalis-lesioned rats or aging rats, mice and rabbits were often used to simulate a cholinergic deficit. Initially it was observed that the compound enhanced passive avoidance performance in rats [65] and active avoidance in mice [2] and acquisition of conditioned eye-blink reflex in aging rabbits [93]. Memory of more intricate learning tasks such as water and radial maze performance by rats [17] and delayed matching by monkeys [20,21] was also enhanced, suggesting that the compound may be able to enhance cognition in aging humans, particularly AD patients. The latter paper is noteworthy in providing compelling evidence that single doses of relatively short plasma half-life (hours) nicotinic agonists are capable of enhancing cognition for relatively long periods of time (days). Generally, cognition enhancement is more readily demonstrated under conditions where cognitive function is deficient, as in chemically lesioned or aging animals. In the case of DMXBA performance on several cognitive tasks was even enhanced in a

phase 1 trial with healthy young male adults [53]. Thus, α7 nicotinic agonists may be useful in treating deficits in cognition, regardless of age.

One advantage of targeting α7 receptors for therapeutic enhancement of cognition, instead of α4β2 receptors, is that modulation of the former receptor does not seem to affect activities associated with nicotine dependence, namely hyperlocomotion, nicotine discrimination and nicotine self-administration, whereas the latter receptor is thought to be a major mediator of the euphoric and anxiolytic effects of nicotine [34,86,89].

The actions of subcutaneously administered anabaseine and DMXBA upon the brain levels of ACh and several biogenic amines have been investigated using cerebral (frontoparietal location) microdialysis methods [82]. Anabaseine, like nicotine [80,81] and other α4β2 agonists, elevated ACh levels (Table 2). However, an equimolar (3.6 μmol/kg) dose of DMXBA did not affect ACh levels at this cortical site or within the hippocampus [84]. Both anabaseine and DMXBA elevated dopamine and norepinephrine levels, but did not significantly affect serotonin levels. However, when mecamylamine, a noncompetitive AChR antagonist, was administered thirty minutes before administration of either compound, significant increases in ACh and 5-HT levels were observed. Explanations for these extraordinary mecamylamine effects are not yet at hand; one possible interpretation would be that at the mecamylamine dose administered, the more sensitive α4β2 receptors expressed on inhibitory (GABAergic) neurons innervating the basalis (cholinergic) and raphe (serotonergic) nuclei were preferentially inhibited, leaving the excitatory effects of anabaseine and DMXBA on the most resistant AChRs (α7) to be expressed without opposition from these inhibitory effects. Further investigation of the effects of α7 agonists upon brain neurotransmitter levels and their sensitivity to block by AChR antagonists is clearly warranted.

Table 2. Comparison of the relative effects of equimolar (3.6 μmol/kg) subcutaneous doses of anabaseine, nicotine and DMXBA on various neurotransmitter levels in the rat prefrontal cortex, as measured by microdialysis methods.

	Peak Effect (% Increase)		
Receptor Type	**[1]Nicotine**	**[2]Anabaseine**	**[2]DMXBA**
Acetylcholine	106	50	NE[3]
Dopamine	NE[3]	85	96
Norepinephrine	86	62	83
Serotonin	NE[3]	NE[3]	NE[3]

[1] Data taken from ref. [80]. [2] Data taken from ref. [82]. [3] No statistically significant effect.

Several laboratories have recently reported that β-amyloid (Aβ) binds to α7 receptors at very low concentrations. The peptide inhibits α-BTx binding to its ACh-binding site and has been reported to activate [27] or inhibit this AChR [27,33,87]. It was hypothesized that α7 receptors are a target of Aβ action on brain neurons. Electrophysiological analysis indicated a noncompetitive block of ACh activation of this receptor. The initially reported selective inhibition of α7 receptors was followed by reports from other laboratories that non-α7 AChRs are also affected by similarly low concentrations of the peptide [30,57]. If the α7 AChR were a major target for Aβ in generating AD, then one would predict that neurons bearing high concentrations of this receptor would be particularly susceptible and this would lead to a decrease in brain α7 receptors as the disease progresses. However, the loss of α7 receptors in AD brains is much smaller than the loss of α4β2 AchRs [63]. α7 Receptor levels in transgenic mice overexpressing Aβ may be reduced [70] although this may occur very early in life [85]. α7 AChR concentrations in cultured neurons were not affected by Aβ exposure [25]. Further investigations of the interaction of Aβ with AChRs are needed to determine whether α7 and/or other AChRs are directly involved in mediating neuronal destruction in AD.

Besides affecting cognitive functions, DMXBA and other nicotinic agonists also display neuroprotective properties such as inhibiton of the excitotoxic effects of Aβ [76] and high concentrations of ethanol [25,61,76]. In a stroke model, pre-administration of DMXBA was also able to reduce neuronal damage [68,69]. At very high concentrations (>10 μM), approximately 50 times higher than would occur under clinical conditions (200 nM), rapid addition of this compound to cultured neurons DMXBA was also excitotoxic [56]. If the compound was allowed to reach the cells gradually these high concentrations were not toxic. The neuroprotective effects of DMXBA were inhibited by reducing extracellular and intracellular calcium levels, and thus seem to be a consequence of calcium influx into the neuron [74].

Future Directions of Research

These are interesting times for the investigation of AChRs and their roles in health and disease. Besides the brain AChR targets discussed above, several peripherally expressed AChRs may also be useful therapeutic targets for treating other disease states. Examples which readily come to mind are acute inflammation [88] and controlling the growth (angiogenesis) of new blood vessels [36,38]. In these two examples considerable evidence already exists pointing to a major role of α7-type receptor involvement. In this article we hope to have convinced the reader that naturally occurring toxins acting on AChRs, besides being useful probes for particular nicotinic receptors, can also serve as molecular models for the design of nicotinic agonists and antagonists of possible therapeutic utility.

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